Vest, Lisa A. (DNREC)

From: Vest, Lisa A. (DNREC)
Sent: Thursday, December 10, 2015 9:09 AM
To: 'j.nichols87@yahoo.com'
Cc: Noyes, Thomas G. (DNREC)
Subject: FW: Proposed Regulation 102 - Public Comment
Attachments: Attachments_20151124.zip

Mr. Nichols:

This will acknowledge receipt of your email below, which offered additional public comment regarding the proposed regulations on Implementation of Renewable Energy Portfolio Standards Cost Cap Provisions. Please be advised that the same has been formally entered into the Hearing Record being compiled in this matter, and is at this time being forwarded to Department staff for their review.

As indicated previously, all public comment received while the record remains open bears the same weight, and will all be considered equally by Secretary Small prior to his making the final decision with regard to this proposed promulgation. Also, please note that the public comment period in this matter officially closed as of Tuesday, December 8, 2015.

As always, thank you for your participation in DNREC's public hearing process.

Lisa A. Vest
Public Hearing Officer
State of Delaware - DNREC
89 Kings Highway
Dover, DE 19901
(302) 739-9042
Fax: (302) 739-1174

When one tugs at a single thing in nature, he finds it attached to the rest of the world. - John Muir

From: John NIchols [mailto:j.nichols87@yahoo.com]
Sent: Tuesday, December 08, 2015 1:06 PM
To: Vest, Lisa A. (DNREC); Hartigan, Matthew (DOS); Hall-Long, Bethany (LegHall); Johnson, Quinton (LegHall); Kowalko, John (LegHall); Bonar, David L (DOS); Small, David (DNREC); Lawson, Dave (LegHall); Simpson, Gary (LegHall); Greg Lavelle; Knotts, Pamela (DOS); Todd Goodman; Amy Roe; Barrett Kidner; Richard Abbott; David Stevenson; Clint Laird
Subject: Proposed Regulation 102 - Public Comment

Attention - Lisa A. Vest, Hearing Officer.

ttached are four article that are representative of a growing body of academic research. They focus on the rising incidence of allergies and hay fever among children, explaining that the urbanization of our society is leading to asthma, over-sensitized allergic responses, and other respiratory problems in later life because urban children are shielded from the early childhood exposure to allergens common in rural environments.

Early exposure allows the human immune system to properly identify harmless background substances and respond less aggressively. Thus, it is not reduced particulate matter that improves health outcomes. Rather, it is the non-exposure of children to dust, dirt, pollen, and other natural and anthopogenic materials that primarily accounts for the rising number of diagnosed asthma cases, along with improved diagnostic techniques.

Outcomes based on a false underlying premise, and "black-box" models that present conclusions without full public disclosure of the underlying assumptions must not be used to formulate a regulation.

The regulation, as proposed, would further increase the cost of electricity to even more unafforadable levels, hurting the rich and poor alike, without any corresponding health benefits.

Further, the REPSA legislation that established the 3% cost-cap did not grant DNREC the authority to take into account "offsets" when calculating the cap-cap.

Moreover, REPSA specifically refers to the the "supply" charge as the base to be used when calculating the cost-cap provision, not all revenue received by Delmarva Power - including distribution charges and capacity charges, as calculated by DNREC. Using all revenue, instead of the supply charge only, dramatically increases the "allowable" subsidies for wind and solar energy electricity generation in a manner that the Delaware General Assembly surely never intended.

While REPSA uses the word "may" to determine the implementation of the 3% cost-cap, it is clear the legislative intent was to apply the cost-cap if/when the subsidies exceed 3% of the supply charge. DNREC's failure to apply the cost-cap as a firm spending limit will most likely lead to litigation and the corresponding expenses of defending an error in judgement.

Finally, DNREC erroneously excluded the Bloom Energy tariff when calculating the compliance costs under REPSA. Passage of the legislation that established the Bloom Energy feed-in tariff removed wind and solar generation subsidies from the supply charge calculation in order to make the Bloom Energy tariff non-bypassable. This does not mean it can be excluded as a compliance cost. In fact, the legislation acknowledges Bloom Energy is a compliance cost by reducing the amount of SREC's and REC's

Delmarva Power must purchase based on electricity generated by a "Qualified Fuel Cell Provider".

In Summary, the proposed regulation is deeply flawed for three reasons. (1) No "offset" can be used to reduce the cost-cap, which must be based only on the supply charge for electricity. (2) All compliance costs, including the Bloom Energy surcharge, must be taken into account. (3) The 3% cost-cap was intended to be applied as fixed spending limit.

John A. Nichols
406 Meadow Lane
Middletown, Delaware 19709
302-378-0683

Braun-Fahrlander et al 1999
von Mutius and Vercelli 2010
Eriksson et al 2010
Illi et al 2012

Clinical and Experimental Allergy, 1999, Volume 29, pages 28–34

Prevalence of hay fever and allergic sensitization in farmer's children and their peers living in the same rural community

CH. BRAUN-FAHRLÄNDER*, M. GASSNER†, L. GRIZE*, U. NEU‡, F. H. SENNHAUSER§, H. S. VARONIER¶, J. C. VUILLE**, B. WÜTHRICH† † and THE SCARPOL TEAM

**Institute of Social and Preventive Medicine, University of Basel, †School Health Service, Grabs, ‡Institute of Geography, University of Bern, §Paediatric Clinic of the University Hospital of Zürich, ¶Allergy Clinic, Paediatric University Hospital, Geneva, **Department of Public Health, City of Bern, † †Department of Dermatology, University Hospital, Zürich, Switzerland*

Summary

Introduction Lower prevalence rates of allergic diseases in rural as compared with urban populations have been interpreted as indicating an effect of air pollution. However, little is known about other factors of the rural environment which may determine the development of atopic sensitization and related diseases.

Objective The authors tested the hypothesis that children growing up on a farm were less likely to be sensitized to common aerollergens and to suffer from allergic diseases than children living in the same villages but in nonfarming families.

Materials and methods Three age groups of schoolchildren (6–7 years, 9–11 years, 13–15 years) living in three rural communities were included in the analyses. An exhaustive questionnaire was filled in by 1620 (86.0%) parents. A blood sample was provided by 404 (69.3%) of the 13–15 year olds to determine specific IgE antibodies against six common aeroallergens.

Results Farming as parental occupation was reported for 307 children (19.0%). After adjustment for potential covariates such as family history of asthma and allergies, parental education, number of siblings, maternal smoking, pet ownership, indoor humidity and heating fuels, farming as parental occupation was significantly associated with lower rates of sneezing attacks during pollen season (adjusted OR 0.34, 95% CI 0.12–0.89) and atopic sensitization (adjusted OR 0.31, 95% CI 0.13–0.73) whereas the association with wheeze (adjusted OR 0.77 95% CI 0.38–1.58) and itchy skin rash (adjusted OR 0.86, 95% CI 0.49–1.50) was not statistically significant. The risk of atopic sensitization was lower in children from full-time farmers (adjusted OR 0.24, 95% CI 0.09–0.66) than from part-time farmers (adjusted OR 0.54, 95% CI 0.15–1.96).

Conclusion Factors directly or indirectly related to farming as parental occupation decrease the risk of children becoming atopic and developing symptoms of allergic rhintis.

Keywords: hay fever, allergic sensitization, farming, children

Clinical and Experimental Allergy, Vol. 29, pp. 28–34. Submitted 29 June 1998; revised 4 August 1998; accepted 14 August 1998.

Introduction

Several recent population studies demonstrated lower prevalence rates of allergic diseases in rural as compared with urban populations [1–3], which has been interpreted as indicating an effect of air pollution. However, the role of air pollution in the development of allergic diseases and sensitization to inhalant allergens is still controversial. The principal literature arguing for a significant role of air

Correspondence: Dr med. Ch. Braun-Fahrländer, Institut für Sozial-und Präventivmedizin der Universität Basel, Steinengraben 49, CH-4051 Basel, Switzerland.

pollution comprises toxicological studies showing increased primary IgE responses in animals exposed acutely to relatively high levels of pollutants, concomitantly with allergen aerosols [4,5]. Several comparative epidemiological studies in Eastern and Western Europe [6,7], and the authors' own recently conducted Swiss Study on Childhood Allergy and Respiratory symptoms with respect to air Pollution (SCARPOL) could not demonstrate an association between long-term air pollution and the prevalence of allergic diseases [8,9].

The clinical observation of one of the SCARPOL investigators that allergic diseases such as hay fever were extremely rare among farmers' children [10] and a report from rural South Bavaria relating lower rates of allergic diseases to the use of coal and wood stoves for heating [11] led us to investigate whether factors of the rural environment other than air pollution were associated with lower rates of allergic disease and sensitization rates, and thus might explain the lower prevalence rates of allergies in rural communities.

The present study was part of the ongoing Swiss multicentre study SCARPOL. The analyses focused on schoolchildren living in three rural communities. Little is known about factors of the rural environment determining the development of atopic sensitization and related diseases. Therefore, the hypothesis that children growing up on a farm were less likely to be sensitized to common aerollergens and to suffer from allergic diseases than children living in the same villages but in nonfarming families was tested.

Methods

Study organization and participation rate

The Swiss Study on Childhood Allergy and Respiratory Symptoms with Respect to Air Pollution (SCARPOL) is a multicentre study designed to investigate associations between air pollution and respiratory and allergic symptoms in school children living in 10 communities [8]. It was first conducted during the school year 1992/1993 and repeated with identical methods as part of a environmental health monitoring program in 1995/96.

The study was organized within the framework of the School Health Services thereby taking advantage of an existing structure of the Health System. The children visit the School Health Services routinely at the age of 6–7 years (1st grade), 9–11 years (4th grade) and 13–15 years (8th grade).

The present analyses are restricted to the children living in the three rural SCARPOL communities (Grabs, Langnau, Payerne and surrounding villages). To avoid problems relating to language proficiency and literacy, the sample is restricted to Swiss nationals. Since there were no systematic differences in prevalence rates of respiratory and allergic symptoms or in atopic sensitization rates between the two surveys, data of both surveys were pooled. The second survey included only children who did not take part in the first survey.

All children visiting the School Health Services in their respective communities were invited to participate in the study. A total of 1620 (86.0%) parents filled in the questionnaire and allowed their children to participate in the study.

In addition, the 13–15 year olds were asked to provide a blood sample for specific IgE-testing, and 404 (69.3%) of the invited adolescents accepted. The prevalence rates of respiratory and allergic symptoms did not differ between those who provided blood samples and those who refused.

The study protocol was approved by the Ethics Committee of the University of Bern.

Questionnaire

The detailed questionnaire completed by the parents included questions on respiratory and allergic symptoms, family history of respiratory and allergic diseases, number of siblings, parental education, indoor fuels, passive smoking, indoor humidity, pet ownership, and study area. The core questions on asthma and allergy of the International Study on Childhood Asthma and Allergy (ISAAC) [12] were incorporated into the questionnaire.

Parents had to indicate whether the family was running a farm and if so, whether this was a full-time or part-time activity.

Eight respiratory and allergic illness and symptom responses obtained from the questionnaire were considered: repeated episodes of cough, and bronchitis occurring during the past year, current wheeze, asthma, sneezing attacks during pollen season, hay fever, itchy skin rash, and eczema.

Wheeze was defined as a positive response to the question: 'Has your child had wheezing or whistling in the chest in the last 12 months?' Sneezing during pollen season was an affirmative answer to the question: 'In the past 12 months, has your child had a problem with sneezing or a runny or blocked nose when he/she did not have a cold or the flu?' combined with the indication of the symptoms occurring during the months of April through September (pollen season), a symptom that showed high validity in the authors' recent validation study [13]. Itchy skin rash was defined as a positive response to the question: 'Has your child ever had an itchy rash which was coming and going for at least 6 months?' In addition, affirmative answers to the questions: 'Has your child ever had asthma, hay fever or eczema?' were considered.

Serological tests

Blood samples (10 cm^3) were taken and the serum separated by spinning 10 min at 1000 ***g***. The samples were kept frozen and sent to the allergy laboratory of the Department of Dermatology in Zürich.

The multiscreen test, SX_1 (screening test for eight common inhalative allergens, Pharmacia,) was performed with all serum samples by CAP-FEIA technology. If the SX_1 test was positive, the specific IgE levels to six allergens (timothy grass, birch, mugwort, house dust mite, cat and dog dander) were determined by CAP-FEIA. The cutoff for a positive result was defined as a specific IgE concentration ≥ 0.69 KU/L (CAP class ≥ 2). In addition to the SX_1 test, positive reactions to any of the outdoor allergens (timothy grass, birch, mugwort) and to any of the indoor allergens

Table 1. Characteristics of the study population according to farming as parental occupation

	Total study population $N = 1620$ (n,%)	Parental occupation		P value (χ^2 test)
		Farming $N = 307$ (n,%)	Non-Farming $N = 1313$ (n,%)	
Sex				
Boys	844/52.1	161/52.4	683/52.0	n.s.
Girls	776/47.9	146/47.6	630/48.0	
Age group				
6–7	555/34.3	108/35.3	447/34.0	
9–12	481/29.7	83/27.1	398/30.3	n.s.
13–15	583/36.0	115/37.6	468/35.6	
(Missing: 1)				
Parental education				
Low	135/8.3	66/21.5	69/5.3	
Medium	952/58.8	201/65.5	751/57.2	<0.001
High	469/29.0	33/10.7	436/33.2	
(Missing: 64)				
Number of siblings				
None	97/6.0	9/2.9	88/6.7	
1	677/41.8	55/17.9	622/47.4	
2	508/31.4	94/30.6	414/31.5	<0.001
3+	257/15.9	108/35.2	149/11.3	
(Missing: 81)				
Mother smoking	375/23.2	55/18.0	320/24.4	0.015
(Missing: 12)				
Father smoking	473/29.2	88/28.7	385/29.3	n.s.
(Missing: 12)				
Furred pets	1041/64.3	268/87.3	773/58.9	0.001
Pets in bedroom	430/26.5	56/18.2	374/28.5	0.001
Indoor humidity	353/21.8	87/28.3	266/20.3	0.02
Heating				
Central	1284/79.3	206/67.1	1078/82.1	
Single space gas/oil	30/1.9	6/2.0	24/1.8	
Electric	38/2.4	7/2.3	31/2.4	<0.001
Wood/coal	187/11.5	72/23.5	115/8.8	
(Missing: 81)				
Family history of asthma	265/16.4	38/12.4	227/17.3	0.05
(Missing: 27)				
Family history of hay fever	425/26.2	39/12.7	386/29.4	0.001
Family history of eczema	415/25.6	65/21.2	350/26.7	0.05

(house dust mite, cat and dog dander) were considered. Because of the small numbers, it was not possible to further analyse individual allergens.

Ambient monitoring

Air pollutants and meteorological parameters were monitored by local authorities and the Swiss Institute of Meteorology, Zürich, respectively. Annual mean concentrations of air pollutants such as NO_2, PM_{10} or ozone were similar in the three communities, ranging from 13.2 to 19.2 $\mu g/m^3$ for PM_{10}, 19.7–21.9 $\mu g/m^3$ for NO_2, and 43–50 $\mu g/m^3$ for ozone. Mean annual temperature ranged from 8 to 10 °C, and mean relative humidity from 73.3% to 81.1%. The study communities were situated between 451 and 557 m above sea-level.

Statistical analyses

Symptom prevalence and atopic sensitization rates were analysed for the total study population and stratified by farming as parental occupation. Since farming families may differ in many respects from nonfarming families, socioeconomic, personal, and indoor characteristics were analysed according to farming as parental occupation. Differences between categorical variables were assessed by the χ^2 test. Logistic regression analysis was used to evaluate whether the association between farming as a parental occupation and allergic symptoms, and sensitization rates persisted after adjustment for potential confounders. The models included age, sex, parental education, a family history of asthma, a family history of hay fever or eczema, number of siblings, maternal smoking, pet ownership, indoor humidity, and heating fuels. Crude and adjusted odds ratios (OR) and the corresponding 95% confidence intervals (CI) were computed.

Results

Study population

The study population consisted of three age groups of school children: 555 (34.3%) from 1st grade, 481 (29.7%) from 4th grade and 583 (36.0%) from 8th grade. Their respective mean ages were 6.5 years (SD ± 0.5), 10.0 years (SD ± 0.7) and 14.1 years (SD ± 0.7). Eight hundred and forty-four

Table 2. Association of respiratory and allergic symptoms[1] and allergic sensitization with farming as parental occupation

	Total study population (*n*,%)	Symptom prevalence according to parental occupation		Association with farming as parental occupation	
		Farming (*n*,%)	Non-Farming (*n*,%)	Crude OR (95% CI)	Adjusted[3] OR (95% CI)
Questionnaire (N = 1620)					
Repeated cough	594/36.7	103/33.6	491/37.4	0.85 (0.65–1.10)	0.90 (0.63–1.29)
Bronchitis	156/9.6	31/10.1	125/9.5	1.07 (0.71–1.62)	1.37 (0.77–2.40)
Wheeze	135/8.3	16/5.2	119/9.1	0.55 (0.33–0.94)	0.77 (0.38–1.58)
Asthma (ever)	150/9.3	24/7.8	126/9.6	0.80 (0.51–1.26)	1.17 (0.64–2.13)
Sneezing during pollen season	125/7.7	8/2.6	117/8.9	0.27 (0.14–0.54)	0.34 (0.12–0.89)
Hay fever (ever)	197/12.2	22/7.2	175/13.3	0.50 (0.32–0.79)	0.89 (0.49–1.59)
Itchy skin rash (ever)	193/12.0	27/8.9	166/12.7	0.67 (0.41–1.02)	0.86 (0.49–1.50)
Eczema (ever)	305/18.8	48/15.6	257/19.6	0.76 (0.54–1.07)	1.15 (0.74–1.81)
Serological tests[2] (N = 404)					
Positive SX_1 test (CAP-class ≥ 2)	139/34.4	16/18.6	123/38.7	0.33 (0.18–0.59)	0.31 (0.13–0.73)
Specific IgE's to outdoor allergens (CAP class ≥ 2)	119/29.5	15/17.4	104/32.7	0.43 (0.24–0.78)	0.38 (0.16–0.87)
Specific IgE's to indoor allergens (CAP class ≥ 2)	81/20.1	4/4.7	77/24.2	0.15 (0.06–0.38)	0.15 (0.04–0.57)

[1]during the past 12 months if not otherwise specified. [2]318 serological tests were done in children from nonfarming families, 86 in farmers' children. [3]The logistic regression model included the following variables: age, sex, parental education, a family history of asthma, hay fever, eczema, number of siblings, maternal smoking, pet ownership, indoor humidity, study area and heating fuels.

(52.1%) were boys and 776 (47.9%) were girls. Farming as parental occupation was reported for 307 (19.0%) children, 220 (13.6%) as a full-time activity, and 87 (5.4%) as a part-time activity.

The characteristics of the total study population according to farming as a parental occupation are given in Table 1. The socioeconomic and home characteristics of farming families differed in many respects from nonfarming families living in the same rural communities. Farming families were of lower socioeconomic status, had more children, often reported more humidity spots or visible molds in their home, heated their homes more often with traditional heating systems using mainly coal and wood and were more likely to keep furred pets, but these pets were less often allowed in the child's bedroom. Mothers in farming families were less likely to smoke and a family history of asthma, hay fever or eczema was reported less often in these families.

The bivariate analyses of respiratory and allergic symptoms and sensitization rates showed that significantly lower rates of current wheeze (5.2% vs 9.5%), sneezing attacks during the pollen season (2.6% vs 8.6%), and hay fever (7.2% vs 13.3%) were reported for farmers' children as compared with children from a nonagricultural environment, whereas the association with a reported diagnosis of asthma, itchy skin rash and eczema did not reach statistical significance (Table 2). The prevalence of bronchitis and repeated cough during the past year was similar in children from farming and nonfarming families. Children from farming families were significantly less likely to have a positive SX_1 test (18.6% vs 38.7%), and specific IgEs to outdoor allergens (17.4% vs 32.7%) and to indoor allergens (4.7% vs 24.2%) than children from nonfarming families.

Since many of the characteristics in which farming and nonfarming families differed have been associated with an increased risk for allergic disease (e.g. family history of asthma, hay fever or eczema), or a lower risk of allergies, such as the number of siblings and the use of wood for heating, the authors evaluated whether these potential confounders would affect the observed association of atopic sensitization and related diseases and farming as parental occupation. The covariates listed in Table 1 were incorporated into a logistic regression model (Table 2). Adjustment for covariates reduced the association of farming as parental occupation with current wheeze, hay fever and itchy skin rash, but was essentially unchanged and remained statistically significant for reported sneezing attacks during the pollen season (adjusted OR 0.34, 95% CI 0.12–0.89) and for a positive SX_1-test (adjusted OR 0.31, 95% CI 0.13–0.73). The low risk of a sensitization to outdoor and indoor allergens associated with farming as parental occupation was also unaffected by the adjustment of covariates.

The association between reported sneezing attacks and a positive SX_1 test with farming as parental occupation for children from full-time and part-time farmers as compared with nonfarming families was separately assessed. The adjusted ORs for sneezing attacks during the pollen season associated with part-time and full-time farming were 0.31 (95% CI 0.06–1.35) and 0.36 [0.10–1.22], respectively. For a positive SX_1 test the adjusted ORs were 0.54 [0.15–1.96] and 0.24 [0.09–0.66], respectively, indicating a gradient in atopic sensitization from nonfarming to full-time farming as parental occupation.

Discussion

The results of this analysis suggest that factors directly or indirectly related to farming as parental occupation reduce the risk of producing specific IgE antibodies to aeroallergens and of developing the clinical symptoms of allergic rhinitis. The amount of risk reduction for the development of atopic sensitization determined in this study is similar to the one observed in children from East Germany as compared with West Germany [6]. However, the children of the present study are living in the same villages.

Lower rates of sensitization to pollen and animal dander and self-reported hay fever have been observed in occupational studies of adult farmers [14–17]. The results of the present study are also supported by a study in Swedish conscripts [18] and a more recent study among Finnish university students demonstrating lower prevalence rates of self-reported allergic rhinitis in students who had been raised on a farm as compared with students from a nonagricultural environment [18,19].

Children from rural South Bavaria in Germany had lower rates of hay fever, atopic sensitization and bronchial hyperresponsiveness when living in a home where coal or wood was used for heating compared with children from homes with other heating systems [11]. In the present study, coal or wood as heating fuel was also associated with a lower risk of atopic sensitization (adjusted OR 0.77, 95% CI 0.35–1.64), but the effect of farming as a parental occupation was stronger. Although no information on parental occupation was available in the German study, it seems quite likely that most of the families with traditional heating systems were farmers.

A serious concern when interpreting the results of the present study is selection bias. If farmers suffering from allergic diseases such as hay fever move into other jobs a 'healthy or nonallergic' farmer effect would occur resulting in lower rates of atopic disease in the farming parents of this study population. This has indeed been observed in the authors' data. However, the association between atopic sensitization and farming as parental occupation remained significant after adjustment for family history of allergic diseases. In addition, the observation of a gradient in the prevalence of atopic sensitization from full-time to part-

time and nonfarming subjects argues against selection bias. Many farmers had to give up farming as a full-time activity and move to other jobs because of economic constraints during recent years, and not because one of the children developed hay fever. Usually, the family remains in the farm house and does some farming as part-time activity. Thus, full-time and part-time farming may indicate different degrees of exposure to a factor which interferes with the production of specific IgE antibodies to allergens.

Farms are rather small in these communities, usually managed by one family and farming activities include dairy farming and agriculture. Thus, one might speculate that children living in this environment may be more exposed to pollen when playing outdoors or in barns where hay for feeding cattle is kept. High exposure to allergens may thus contribute to the development of tolerance in these children, as has been shown in animal experiments [20]. Repeated exposure of the upper respiratory or gastrointestinal mucosae to microgram levels of protein antigen leads to the preferential suppression of TH2-dependent IgE production via immune deviation. Consequently, it has been suggested to expose children to a mixture comprising the 3–4 dominant inhalant allergens from a particular environment as a measure of primary prevention of allergic respiratory disease [21]. Thus, the possibility exists that living in a agricultural environment provides a model of such primary prevention.

Previous studies have pointed towards a protective role of early childhood exposure to infectious diseases for the development of atopic sensitization and hay fever [22]. Although the number of siblings was adjusted for, a factor which has been postulated to indicate increased childhood exposure to microbial infections [23,24], it is conceivable that the farming environment in itself (e.g. contact to cattle in barns) provides additional exposure to microbial antigens from both commensal organisms and pathogens, thus, stimulating the immune response of farmers' children to a TH1 immunity.

Alternatively, farming as a parental occupation may be an indicator of a more traditional lifestyle which has been proposed to explain the differences in atopic sensitization between Eastern and Western European populations [2,25]. Although the study assessed some of the most important socioeconomic and environmental factors by questionnaire and controlled for these in the analyses, it cannot be ruled out that uncontrolled confounding may still influence the results of this study. For example, dietary factors are potentially important and have not been assessed in the present study. It has recently been postulated that changes in the consumption of omega-3 fatty acids and fresh foods containing antioxidants and magnesium may be responsible for the higher prevalence of asthma observed in affluent countries [26–28]. All these factors may have a potential influence on inflammatory reactions. However, whether dietary factors interfere with the production of specific IgE antibodies against allergens has still to be shown. In addition, dietary factors during pregnancy may be important as the overall health of the mother through pregnancy and, therefore, the growth trajectory of the fetus may have particular relevance [29,30].

In summary, this study indicates that children from a farming population represent an informative population to study the role of environmental factors in the development of atopic sensitization. Whether the observed protective effect of the farming environment on atopic sensitization is explained by high exposure to microbial stimulation in childhood, by different exposures to dietary factors or other indicators of a 'traditional lifestyle', or whether it is the result of preferential suppression of TH2-dependent IgE production due to high allergen exposure remains to be elucidated.

Acknowledgements

SCARPOL team consists of the authors and: I. Gasser (Basel), Ch. E. Minder (Bern), Th. Küenzle (Bern) and A. Peeters (Zürich).

This study was supported by a grant of the Swiss National Research Foundation (#4026–033109). Ch. Braun-Fahrländer was supported by the grant #32–30252.90 from the same agency.

References

1 Brabäck L, Kälvesten L. Urban living as a risk factor for atopic sensitization in Swedish schoolchildren. Pediatr Allergy Immunol 1991; 2:14–9.
2 Björksten B. Risk factors in early childhood for the development of atopic diseases. Allergy 1994; 49:400–7.
3 Popp W, Zwick H, Steyrer K, Rauscher H, Wanke T. Sensitization to aeroallergens depends on environmental factors. Allergy 1989; 44:572–5.
4 Riedel F, Krämer M, Scheibenbogen C, Rieger CHL. Effects of SO_2 exposure on allergic sensitization in the guinea pig. J Allergy Clin Immunol 1988; 82:527–34.
5 Matsumura Y. The effects of ozone, nitrogen dioxide, and sulfur dioxide on the experimentally induced allergic respiratory disorders in guinea pigs. Am Rev Respir Dis 1970; 102:430–7.
6 von Mutius E, Martinez FD, Fritzsch C, Nicolai T, Röll G, Thiemann H-H. Prevalence of asthma and atopy in two areas of West and East Germany. Am J Respir Crit Care Med 1994; 149:358–64.
7 Brabäck L, Breborowicz A, Dreborg S, Knutsson A, Pieklik H, Björkstén B. Atopic sensitization and respiratory symptoms among Polish and Swedish schoolchildren. Clin Exp Allergy 1994; 24:826–35.
8 Braun-Fahrländer Ch, Vuille JC, Sennhauser FH et al. Respiratory health and long term exposure to air pollutants in

Swiss schoolchildren. Am J Respir Crit Care Med 1997; 155:1042–9.
9 Braun-Fahrländer Ch, Wüthrich B, Gassner M et al. Prävalenz und Risikofaktoren einer allergischen Sensibilisierung bei Schulkindern in der Schweiz. Allergologie 1998. In press.
10 Gassner M. Allergie und Umwelt. Allergologie 1989; 12:492–502.
11 Von Mutius E, Illi S, Nicolai T, Martinez F. Relation of indoor heating with asthma, allergic sensitization, and bronchial responsiveness: survey of children in South Bavaria. BMJ 1996; 312:1448–50.
12 Asher MI, Anderson HR, Beasley R et al. International study of asthma and allergies in childhood (ISAAC): rationale and methods. Eur Respir J 1995; 8:483–91.
13 Braun-Fahrländer Ch, Wüthrich B, Gassner M et al. Validation of a rhinitis symptom questionnaire (ISAAC core questions) in a population of Swiss school children visiting the School Health Services. Pediatr Allergy Immunol 1997; 8:75–82.
14 Kohler F, Kohler Ch, Patris A, Grillat JP. Fréquence de l'allergie pollinique chez les agriculteurs par rapport aux autres catégories socio-professionnelles. Rev Fr Allergol 1983; 23:119–24.
15 Rautalahti M, Terho EO, Vohlonen I, Husman K. Atopic sensitization of dairy farmers to work-related and common allergens. Eur J Respir Dis 1987; 71 (Suppl. 152):155–64.
16 Iversen M, Pedersen B. The prevalence of allergy in Danish farmers. Allergy 1990; 45:347–53.
17 Sigsgaard T, Hjort C, Omland O, Miller MR, Pedersen OF. Skin prick test to house dust mite, asthma and hyperreactivity in a cohort of young rurals. Eur Respir J 1996; 9 (Suppl.):379s.
18 Aberg N. Asthma and allergic rhinitis in Swedish conscripts. Clin Exp Allergy 1989; 19:59–63.
19 Kilpeläinen M, Terho EO, Koskenvuo M. Asthma and atopic diseases among Finish university students. Eur Respir J 1997; 10 (Suppl.):143s.
20 Holt PG. A potential vaccine strategy for asthma and allied atopic diseases during early childhood. Lancet 1994; 344:456–8.
21 Holt PG, Sly PD. Allergic respiratory disease: strategic targets for primary prevention during childhood. Thorax 1997; 52:1–4.
22 Martinez FD. Role of viral infections in the inception of asthma and allergies during childhood: could they be protective? Thorax 1994; 49:1189–91.
23 Strachan DP. Hay fever, hygiene, and household size. BMJ 1989; 299:1259–60.
24 Mutius E, Martinez FD, Fritzsch C, Nicolai T, Reitmair P, Thiemann HH. Skin test reactivity and number of siblings. BMJ 1994; 308:692–5.
25 Wichmann HE. Environment, life-style and allergy: the German answer. Allergo J 1995; 4:315–6.
26 Seaton A, Godden DJ, Brown K. Increase in asthma: a more toxic environment or a more susceptible population. Thorax 1994; 49:171–4.
27 Britton J, Pavord I, Richards K et al. Dietary magnesium, lung function, wheezing, and airway hyperreactivity in a random adult population sample. Lancet 1994; 344:357–62.
28 Black PN, Sharpe S. Dietary fat and asthma: is there a connection. Eur Respir J 1997; 10:6–12.
29 Godfrey KM, Barker DJP, Osmond C. Disproportionate fetal growth and raised IgE concentration in adult life. Clin Exp Allergy 1994; 24:641–8.
30 Fergusson DM, Crane J, Beasley R, Horwood LJ. Perinatal factors and atopic disease in childhood. Clin Exp Allergy 1997; 27:1394–401.

ORIGINAL ARTICLE EPIDEMIOLOGY AND GENETICS

Growing up on a farm leads to lifelong protection against allergic rhinitis

J. Eriksson[1], L. Ekerljung[1], J. Lötvall[1], T. Pullerits[1,2], G. Wennergren[1,3], E. Rönmark[1,5,6], K. Torén[1,4] & B. Lundbäck[1,5]

[1]Department of Internal Medicine/Krefting Research Centre, [2]Respiratory Medicine & Allergology, [3]Department of Paediatrics; and [4]Environmental & Occupational Medicine, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg; [5]The OLIN Studies, Department of Medicine, Sunderby Central Hospital of Norrbotten, Luleå; [6]Environmental & Occupational Medicine, Department of Medicine, University of Umeå, Umeå, Sweden

To cite this article: Eriksson J, Ekerljung L, Lötvall J, Pullerits T, Wennergren G, Rönmark E, Torén K, Lundbäck B. Growing up on a farm leads to lifelong protection against allergic rhinitis. *Allergy* 2010; **65**: 1397–1403.

Keywords
allergic rhinitis; allergy; epidemiology; population survey.

Correspondence
Jonas Eriksson, MB, Krefting Research Centre, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gröna stråket 5, SE - 413 45 Gothenburg, Sweden.
Tel.: +46 703 274729
Fax: +46 31 342 3827
E-mail: jonas.eriksson@lungall.gu.se

Accepted for publication 29 March 2010

DOI:10.1111/j.1398-9995.2010.02397.x

Edited by: Stephan Weidinger

Abstract

Background: Various studies have reported a low prevalence of allergic rhinitis in farmers and farmers' children. We sought to investigate whether the protective effect of childhood farm environment is conserved throughout adulthood and how it corresponds to different degrees of urbanization.

Methods: A questionnaire on respiratory health was mailed in 2008 to 30 000 randomly selected subjects aged 16–75 in West Sweden, 29 218 could be traced and 18 087 (62%) responded. The questionnaire included questions on allergic rhinitis, asthma, respiratory symptoms and possible determinants.

Results: When stratified into age groups of 15 years, subjects that lived on a farm during their first 5 years of life had a lower prevalence of allergic rhinitis in all groups, even among the oldest (61–75 years). The negative correlation between childhood farm living and prevalence of allergic rhinitis was similar in 46–75 years of age (OR 0.82; 95% CI 0.70–0.95) as in 16–45 years of age (OR 0.78; 0.64–0.95). There was a significant trend of increasing prevalence of allergic rhinitis with increasing degree of urbanization independent of the effect of childhood farm living.

Conclusions: We found a lifelong protective effect of childhood farm living on the prevalence of allergic rhinitis. In addition, we found an increasing prevalence of allergic rhinitis with increasing degree of urbanization both in those raised on a farm and those not, thus emphasizing the influence of both childhood and adult exposure for the development of allergic disease.

Allergic rhinitis is the most common immunologic disorder and it renders a considerable burden on both the affected individuals and the society (1). Rhinitis is further an important risk factor for asthma (2). Over the second half of the past century, the prevalence of allergic rhinitis has increased markedly in many countries including Sweden (1, 3). In a recent study, we found the prevalence of allergic rhinitis among adults in West Sweden to be high, 26.9%, and apparently on the increase (4).

Several environmental factors have been hypothesized to contribute to this increase, including allergen exposure, indoor and ambient air pollution, reduced early childhood infections and changes in dietary habits (5). However, none of these factors has convincingly explained the observed increase. In the past decade, various studies reported a lower prevalence of allergic rhinitis among children living on a farm (6–10). Furthermore, some studies indicated that the protective effect of childhood farm exposure continues into adulthood (9–12). However, it remains unclear whether this effect is present in the adult population including also middle aged and elderly.

Moreover, a number of studies have reported a greater prevalence of allergic rhinitis in urban compared to rural residents (13, 14). How the effect of urbanization relates to childhood farm environment and to age is yet to be elucidated.

The aim of this study was to assess the effect of childhood farm living and the degree of urbanization on the prevalence

of allergic rhinitis in a life-long perspective and their importance as independent risk factors for allergic rhinitis in a multivariate setting.

Material and method

Study population

In 2008, a self-administered postal questionnaire was mailed to 30 000 inhabitants, aged 16–75, in the Swedish region of West Gothia. By complete computerized randomization, a sample of 15 000 subjects was selected from the metropolitan area of Gothenburg. Likewise, 15 000 subjects of same ages were randomly selected from the rest of the region. The Swedish Population Register provided the names and addresses. The invited individuals were given the possibility to respond either by mail or over the internet. The selection of the study population is extensively described elsewhere (4). The ethical committee of the University of Gothenburg approved the studies.

Questionnaire

In this study, we used the Swedish OLIN study questionnaire (15) that has been used in several studies in northern Europe (16–19) and contains questions about obstructive respiratory diseases, rhinitis, respiratory symptoms and possible determinants of disease, such as smoking, occupation and family history of allergic disease. Further, additional questions about smoking habits and occupational and environmental exposures were included as well as the Swedish version of the GA^{2}LEN questionnaire (20).

Degree of urbanization

Localities of residence were classed into four categories based on their number of inhabitants. Metropolitan *Gothenburg*, with approximately 700 000 inhabitants, was used as a separate entity, while other localities with more than 10 000 inhabitants were considered *mid-sized towns* (all with < 100 000 inhabitants). Localities with 2000–10 000 inhabitants were considered *small towns* and those with < 2000 inhabitants *rural areas*. The classification was performed by matching the subjects' address information with official population data from Statistics Sweden (21).

Definitions

Allergic rhinitis: 'Have you ever had allergic eye or nose problems (hay fever)'; *Family history of allergy:* 'Has any of your parents or siblings ever had allergic eye or nose problems (hay fever)'; *Physician-diagnosed asthma:* 'Have you been diagnosed as having asthma by a doctor'; *Occupational exposure:* 'Have you been substantially exposed to dust, gases or fumes at work'; *Raised on farm:* 'Did your family live on a farm during your first 5 years of life'. *Smokers* reported smoking during the year preceding the survey; *Ex-smokers* reported having stopped smoking at least 12 months preceding the survey; *Nonsmokers* reported neither smoking nor ex-smoking.

Analyses

Ten percent of the data were computerized twice in purpose of quality control of the computerization. Errors amounted to 0.1–0.2% of the computerized data with only a few exceptions. Statistical analyses were performed using SPSS (SPSS Inc, Chicago, IL, USA) version 16.0. Comparisons of proportions were tested with Fishers' exact test. One-way analysis of variance (ANOVA) was used for testing for trends. A P-value of < 0.05 was regarded as statistically significant. Covariates used in the analyses included age, sex, family history of allergy, physician-diagnosed asthma, smoking habits, degree of urbanization, living on a farm during the first 5 years of life and occupational exposure to dust, gases and fumes. Multiple logistic regression models were performed using these independent variables as risk factors (odds ratios, OR, with 95% confidence intervals, CI) of allergic rhinitis. In order not to loose statistical power, two age groups were created and used in the multiple regression analyses.

Results

Demographics

The real study sample comprised of 29 218 subjects, 782 of the initial 30 000 could not be traced (22). Of the real study sample, 18 087 subjects (62%) participated. Participation was significantly higher among women than in men (69% vs. 55%, $P < 0.001$) as well as outside compared to inside the metropolitan area of Gothenburg (66% vs. 61%, $P < 0.001$). Participation increased significantly by age ($P < 0.001$), from 51% in 16–25 years of age to 77% in 66–75 years of age. The study population by age, sex, degree of urbanization and childhood living is presented in Table 1.

Prevalence

The prevalence of allergic rhinitis was considerably lower among subjects who lived on a farm during their first 5 years of life than among those that did not (20.1% vs. 28.0%, $P < 0.001$) (Table 2). The effect of growing up on a farm was present both in men (18.4% vs. 27.4%, $P < 0.001$) and in women (21.7% vs. 28.5%, $P < 0.001$). Further, the effect was present and significant in all age groups, being most pronounced in 16–30 years of age (19.5% vs. 30.5%, $P < 0.001$) and least so in 61–75 years of age (16.5% vs. 19.4%, $P = 0.045$).

Moreover, the prevalence of allergic rhinitis increased with increasing degree of urbanization. The highest prevalence was found in metropolitan Gothenburg (28.3%), the second highest in mid-sized towns (27.6%), the third highest in small towns (25.7%) and the lowest in rural areas (22.9%). This trend was present ($P < 0.001$) both in men and in women.

Table 1 Study population by age, sex, degree of urbanization and childhood farm living

		Age (years)				Sex		
		16–30	31–45	46–60	61–75	Men	Women	Total
By degree of urbanization								
Gothenburg (700 000 inh.)	*n*	2379	2416	2228	1634	3886	4771	8657
Mid-sized towns (>10 000 inh.)	*n*	893	1145	1203	1065	1963	2343	4306
Small towns (2000–10 000 inh.)	*n*	313	514	531	451	812	997	1809
Rural areas (<2000 inh.)	*n*	556	797	1055	907	1529	1786	3315
By childhood living								
Not raised on a farm	*n*	3766	4380	4125	3057	6904	8424	15 328
Raised on a farm	*n*	328	454	835	940	1186	1371	2557
Total	*n*	4141	4872	5017	4057	8190	9897	18 087

Table 2 Prevalence of allergic rhinitis by degree of urbanization, childhood farm living, age and sex

	Age (years)					Sex			
	16–30	31–45	46–60	61–75	*P*-value	Men	Women	*P*-value	Total
Degree of urbanization									
Gothenburg (700 000 inh.), %	30.7	33.7	26.5	19.3	<0.001	27.1	29.3	0.024	28.3
Mid-sized towns (>10 000 inh.), %	30.9	32.8	27.4	19.3	<0.001	27.8	27.4	0.76	27.6
Small towns (2000–10 000 inh.), %	27.8	31.5	26.4	16.9	<0.001	24.3	26.9	0.21	25.7
Rural areas (<2000 inh.), %	23.6	28.9	22.1	18.1	<0.001	21.9	23.7	0.23	22.9
P	0.002	0.012	0.013	0.32		<0.001	<0.001		<0.001
Childhood living									
Not raised on a farm, %	30.5	33.1	26.6	19.4	<0.001	27.4	28.5	0.13	28.0
Raised on a farm, %	19.5	26.0	21.3	16.5	0.006	18.4	21.7	0.043	20.1
P	<0.001	0.002	0.001	0.045		<0.001	<0.001		<0.001

The effect of degree of urbanization was strongest in 16–30 years of age ($P = 0.002$), where the difference between metropolitan Gothenburg and rural areas was 7% units, and decreased in magnitude with increasing age. The prevalence of allergic rhinitis was higher among women than in men in metropolitan Gothenburg (29.3% vs. 27.1%, $P = 0.024$), while no gender difference was found in the smaller localities. Independent of the degree of urbanization, the highest prevalence was found in 31–45 years of age (28.9–33.7%) and the lowest in 61–75 years of age (16.9–19.3%).

The effect of childhood farm living was present and significant in all degrees of urbanization (Fig. 1A). The effects of both variables were more pronounced in 16–30 years of age (Fig. 1B), yet present throughout adulthood (Fig. 2). However, among subjects raised on a farm, the effect of urbanization did not reach significance after 35 years of age.

Risk factors

Table 3 shows the difference in prevalence of potential confounders among subjects that either lived on a farm during the first 5 years of life or not. After adjusting for these variables in a multiple logistic regression model, growing up on a farm was significantly negatively associated with allergic rhinitis (OR 0.82; 95% CI 0.72–0.92) (Table 4). The risk was similar in 46–75 years of age (OR 0.82; 0.70–0.95) as in 16–45 years of age (OR 0.78; 0.64–0.95). Moreover, living in metropolitan Gothenburg (OR 1.29; 1.15–1.44) or in a mid-sized town (OR 1.28; 1.13–1.45) was associated with increased risk of allergic rhinitis. When removing family history of allergy from the multivariate model, the association between allergic rhinitis and childhood farm living did not alter significantly [OR 0.82 (95% CI 0.72–0.92) vs. OR 0.76 (95% CI 0.68–0.85)]. Likewise, when degree of urbanization was removed from the model, the OR of the other variables, including childhood farm living, changed only in the second decimal. Similarly, when removing childhood farm living from the model, the associations to other variables proved to be stable. Risk factors for allergic rhinitis are listed in Table 4.

When analyzing 16–30 years of age separately in the multiple logistic regression model, the negative association of being raised on a farm proved to be significantly stronger, OR 0.67 (95% CI 0.48–0.93).

Discussion

In this large-scale cross-sectional study, we found living on a farm during the first 5 years of life to be associated with a

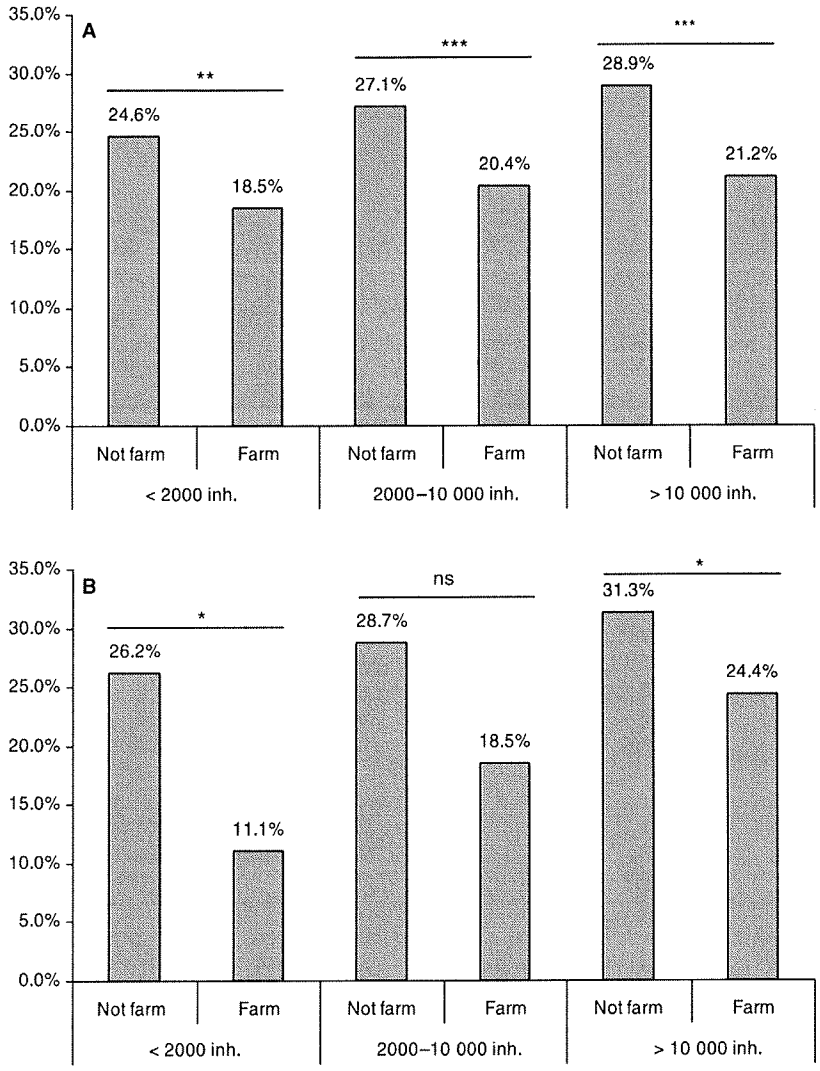

Figure 1 (A) Prevalence of allergic rhinitis by childhood farm living and degree of urbanization. (>10 000 inh includes metropolitan Gothenburg.) ***P*-value <0.01, ****P*-value <0.001. (B) Prevalence of allergic rhinitis in 16–30 years of age by childhood farm living and degree of urbanization. (>10 000 inh includes metropolitan Gothenburg.) **P*-value <0.05.

lower prevalence of allergic rhinitis. When stratified by age, this association was present and significant in all age groups. Further, we found a trend of increasing prevalence of allergic rhinitis with increasing degree of urbanization. In risk assessment adjusting for the effect of potential confounders such as sex, family history of allergic disease, smoking, degree of urbanization and occupational exposure, growing up on a farm was associated with a lower risk of allergic rhinitis in younger adults (16–45 years of age), as well as in older adults (46–75 years of age). Thus, our results are the first to demonstrate a lifelong protective effect of childhood farm living on the prevalence of allergic rhinitis.

The protective effect of childhood farm environment has been attributed to exposures such as animal sheds, hay lofts and consumption of unpasteurized milk (23). Particular interest has been given to endotoxin, a cell wall component from gram-negative bacteria. Significantly higher levels of endotoxin have been found in mattresses from farmers than from nonfarmers and high levels of endotoxin are inversely related to the occurrence of allergic rhinitis and atopy both among farmers and nonfarmers (24). Exposure to microbial components such as endotoxin is thought to promote a skewing of the Th1/Th2 balance toward nonallergic Th1 responses (23, 25). The immune deviation into either Th1 or Th2 polarization is further suggested to be established already at 5 years of age (26), thus indicating long-term effects of childhood environmental exposure on sensitization and allergic disease (27, 28). Accordingly, we found the protective effect of having lived on a farm during the first 5 years of life to be present throughout adulthood. Even so, our results suggest

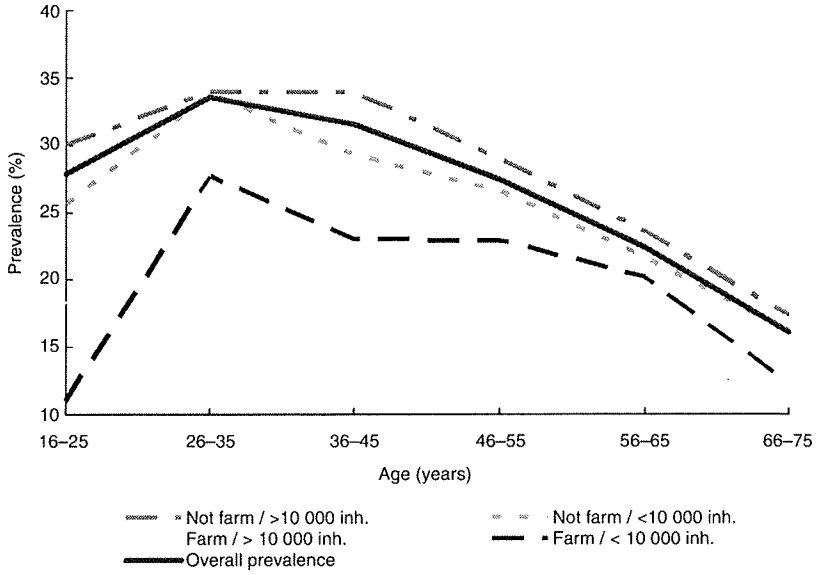

Figure 2 Prevalence of allergic rhinitis by age and childhood living on a farm and degree of urbanization. (>10 000 inh includes metropolitan Gothenburg.)

Table 3 Difference in distribution of background variables between subjects raised on a farm and those not raised on a farm

	Raised on a farm, %	Not raised on a farm, %	*P*-value
Family history of allergy			
Yes	20.2	30.2	<0.001
Smoking			
Current smoker	16.0	16.9	0.074
Ex-smoker	24.4	22.4	0.027
Never smoker	59.6	60.8	0.27
Sex			
Men	46.4	45.0	0.21
Exposed to dust, gases and fumes at work			
Yes	28.4	21.2	<0.001
Age			
16–30 years	12.8	24.6	<0.001
21–45 years	17.8	28.6	<0.001
46–60 years	32.7	26.9	<0.001
61–75 years	36.8	19.9	<0.001
Degree of urbanization			
Gothenburg	31.5	50.6	<0.001
Mid-sized towns	22.2	24.1	0.040
Small towns	11.3	9.8	0.016
Rural areas	35.0	15.5	<0.001

that the magnitude of the protection decreases with increasing age. It is, however, plausible that other protective factors were present in the nonfarming environment during the childhood of the older subjects, such as more childhood infections and different dietary habits, diminishing the sole effect of childhood farm living.

Furthermore, in agreement with a number of studies worldwide (13, 14), we found a trend of increasing prevalence of allergic rhinitis with increasing degree of urbanization. The trend was significant until 60 years of age, while no difference due the degree of urbanization was found in 61–75 years of age. It is likely that at the time critical for development of allergy in the older subjects, urban and rural environmental exposure diverged less than later on. Adjusting for potential confounders in a multivariate regression analysis, living both in a mid-sized town and in metropolitan Gothenburg was associated with an increased risk of allergic rhinitis. However, the odds ratio for allergic rhinitis for subjects living in Gothenburg was similar to that of subjects living in a mid-sized town. Thus, it is likely that the protective effect on the prevalence of allergic rhinitis is most pronounced in areas of low degree of urbanization. By means of multiple logistic regression analysis, we could confirm that the effect of urbanization could not be explained by a lower proportion of subjects growing up on a farm in the more populated localities. Hence, the trend of higher risk of allergic rhinitis associated with higher degree of urbanization is likely to be due to the environmental factors other than childhood farm environment. Traffic-related air pollution has been suggested to contribute to the increasing prevalence of allergic disease in urban cities (29), and toxicological studies have shown diesel exhaust particles to increase airway inflammation and induce a rise in production of total and specific IgE (30). Further, diesel exhaust particles may act as carriers of antigens and increase their allergenic potential in the airways (30). However, epidemiological studies that explicitly analyze the associations between traffic exposure and allergic rhinitis are few and their results are inconsistent (29). Another factor that has been hypothesized to contribute to

Table 4 Risk factors for allergic rhinitis by multiple logistic regression analysis

Independent variables		Dependent variable = Allergic rhinitis		
		16–45 years	46–75 years	16–75 years
Variables	Categories	OR (95% CI)	OR (95% CI)	OR (95% CI)
Sex	Women (reference: men)	**0.81 (0.73–0.90)**	0.98 (0.87–1.11)	**0.89 (0.82–0.96)**
Family history of allergy	Yes (reference: no)	**5.12 (4.61–5.68)**	**6.16 (5.41–7.00)**	**5.60 (5.16–6.07)**
Physician diagnosed asthma	Yes (reference: no)	**5.80 (4.87–6.92)**	**5.24 (4.30–6.39)**	**5.62 (4.93–6.41)**
Smoking	Non-smoker	1	1	1
	Ex-smoker	1.16 (0.99–1.35)	0.97 (0.85–1.10)	1.02 (0.92–1.12)
	Current smoker	0.92 (0.80–1.07)	**0.70 (0.59–0.83)**	**0.81 (0.73–0.91)**
Age	16–30 years			1
	31–45 years			**1.48 (1.33–1.64)**
	46–60 years			**1.31 (1.17–1.47)**
	61–75 years			1.03 (0.91–1.17)
Degree of urbanization	Rural areas	1	1	1
	Small towns	1.07 (0.86–1.35)	**1.26 (1.01–1.58)**	1.16 (0.99–1.36)
	Mid-sized towns	1.16 (0.97–1.38)	**1.39 (1.17—1.65)**	**1.28 (1.13–1.45)**
	Metropolitan Gothenburg	**1.22 (1.04–1.43)**	**1.29 (1.10–1.51)**	**1.29 (1.15–1.44)**
Occupational exposure to dust, gases and fumes	Yes (reference: no)	1.10 (0.97–1.25)	**1.18 (1.03–1.36)**	**1.13 (1.03–1.24)**
Raised on a farm	Yes (reference: no)	**0.78 (0.64–0.95)**	**0.82 (0.70–0.95)**	**0.82 (0.72–0.92)**

*All associations in each age group derive from a single multiple logistic regression analysis. Significant odd ratios are given in bold.

the urbanization effect is the decreased exposure to pollen in the urban environment, possibly leading to lower tolerance to pollens (31).

Our results suggest that living in a rural locality exerts an additive effect to that of growing up on a farm on the prevalence of allergic rhinitis, in particular among young adults (16–30 years). This finding is in line with studies demonstrating a greater risk reduction of allergic rhinitis among subjects living on a farm during both childhood and adulthood compared to those living on a farm during childhood only (9, 32).

When assessing the risk reduction for allergic rhinitis associated with childhood farm living, the influence of confounding factors needs to be carefully considered. We noted that subjects raised on a farm reported family history of allergy to a significantly lower extent than those not raised on a farm, suggesting a 'healthy farmer effect', i.e. selection out of farming because of the allergic disease. However, a Finnish study demonstrated that quitting or avoiding farming because of allergy was as common in parents and grandparents of nonfarmers' children as in those of farmers' children (33). Moreover, when adjusting for family history of allergy, the association between allergic rhinitis and childhood farm living remained statistically significant. In addition, removing family history of allergy from the multivariate model did not alter the association between allergic rhinitis and childhood farm living.

This study benefits from its population-based design and large scale. Further, it covers a large geographic area with a variety of types of locality, including rural areas and metropolitan Gothenburg, as well as a number of mid-sized and small towns, thus, representing well the population composition of Sweden. In addition, individuals from the full age span of adulthood were enrolled in the study rendering possible analyses with lifelong perspectives.

The response rate of the study is comparable to other recent European surveys. In addition, a study of nonresponse on the current study population showed no difference in the prevalence of allergic rhinitis between responders and nonresponders (22).

In risk factor assessment by multiple regression analysis, there is always a risk for type I-errors (e.g. significant association by chance). However, as the associations in our model did not alter when the analysis was performed in many different ways, such errors are less likely to have occurred. A limitation of the study is that childhood exposures others than living on a farm, such as number of siblings, parental smoking habits and childhood infections, were not asked for. However, a number of studies assessing the confounding effect of these variables found that they did not alter the association between allergic rhinitis and childhood farm environment (6–8, 11). Another limitation is that the question on allergic rhinitis is based on self-report. However, in an ongoing clinical follow-up study including skin prick testing, data so far show that a large majority, 82%, of subjects reporting allergic rhinitis was sensitized to common airborne antigens, thus confirming the validity of the question. The skin prick tests are performed following the EAACI guidelines (34). Moreover, among subjects reporting allergic rhinitis, 99% also reported symptoms of rhinitis including a large proportion reporting chronic nasal symptoms. Thus, the question on allergic rhinitis seems to represent subjects with both seasonal and chronic disease.

In conclusion, we found a protective effect of childhood farm living on the prevalence of allergic rhinitis and, as the first to date, we could show this effect to be conserved

throughout adulthood. Further, we demonstrated an increasing prevalence of allergic rhinitis with increasing degree of urbanization, present both in subjects raised on a farm and in those not, thus emphasizing the influence of both childhood and adult exposure for the development of allergic disease.

References

1. Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A et al. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). *Allergy* 2008;**63**(Suppl 86):8–160.
2. Toren K, Olin AC, Hellgren J, Hermansson BA. Rhinitis increase the risk for adult-onset asthma–a Swedish population-based case-control study (MAP-study). *Respir Med* 2002;**96**:635–641.
3. 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* 2004;**34**:38–43.
4. Lotvall J, Ekerljung L, Ronmark EP, Wennergren G, Linden A, Ronmark E et al. West Sweden Asthma Study: prevalence trends over the last 18 years argue no recent increase in asthma. *Respir Res* 2009;**10**:94, E-pub.
5. von Mutius E. The environmental predictors of allergic disease. *J Allergy Clin Immunol* 2000;**105**(1 Pt 1):9–19.
6. Braun-Fahrlander C, Gassner M, Grize L, Neu U, Sennhauser FH, Varonier HS 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* 1999;**29**:28–34.
7. 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.
8. 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.
9. 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.
10. Douwes J, Travier N, Huang K, Cheng S, McKenzie J, Le Gros G et al. Lifelong farm exposure may strongly reduce the risk of asthma in adults. *Allergy* 2007;**62**:1158–1165.
11. Leynaert B, Neukirch C, Jarvis D, Chinn S, Burney P, Neukirch F. Does living on a farm during childhood protect against asthma, allergic rhinitis, and atopy in adulthood? *Am J Respir Crit Care Med* 2001;**164**(10 Pt 1):1829–1834.
12. Portengen L, Preller L, Tielen M, Doekes G, Heederik D. Endotoxin exposure and atopic sensitization in adult pig farmers. *J Allergy Clin Immunol* 2005;**115**:797–802.
13. Filipiak B, Heinrich J, Schafer T, Ring J, Wichmann HE. Farming, rural lifestyle and atopy in adults from southern Germany–results from the MONICA/KORA study Augsburg. *Clin Exp Allergy* 2001;**31**: 1829–1838.
14. Nicolaou N, Siddique N, Custovic A. Allergic disease in urban and rural populations: increasing prevalence with increasing urbanization. *Allergy* 2005;**60**:1357–1360.
15. Lundback B, Nystrom L, Rosenhall L, Stjernberg N. Obstructive lung disease in northern Sweden: respiratory symptoms assessed in a postal survey. *Eur Respir J* 1991;**4**:257–266.
16. Ekerljung L, Ronmark E, Larsson K, Sundblad BM, Bjerg A, Ahlstedt S et al. No further increase of incidence of asthma: incidence, remission and relapse of adult asthma in Sweden. *Respir Med* 2008;**102**:1730–1736.
17. Lundback B. Epidemiology of rhinitis and asthma. *Clin Exp Allergy* 1998;**28**(Suppl 2):3–10.
18. Pallasaho P, Lundback B, Meren M, Kiviloog J, Loit HM, Larsson K et al. Prevalence and risk factors for asthma and chronic bronchitis in the capitals Helsinki, Stockholm, and Tallinn. *Respir Med* 2002;**96**:759–769.
19. Ronmark E, Lundback B, Jonsson E, Jonsson AC, Lindstrom M, Sandstrom T. Incidence of asthma in adults–report from the Obstructive Lung Disease in Northern Sweden Study. *Allergy* 1997;**52**:1071–1078.
20. Bousquet J, Fokkens W, Burney P, Durham SR, Bachert C, Akdis CA et al. Important research questions in allergy and related diseases: nonallergic rhinitis: a GA2LEN paper. *Allergy* 2008;**63**:842–853.
21. Population in Swedish localities 2005; Available from: http://www.scb.se/statistik/M1/M10810/2005A01/mi0810tab 5.xls [cited 02/06/09].
22. Rönmark EP, Ekerljung L, Lötvall J, Torén K, Rönmark E, Lundbäck B. Large scale questionnaire survey on respiratory health in West Sweden: effects of late- and non-response. *Respir Med* 2009;**103**:1807–1815, Epub 2009 August 19.
23. Schaub B, Lauener R, von Mutius E. The many faces of the hygiene hypothesis. *J Allergy Clin Immunol* 2006;**117**:969–977; quiz 978.
24. 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.
25. 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 TH2-dependent isotype switching in response to common inhalants. *J Allergy Clin Immunol* 2007;**119**:351–358.
26. Yabuhara A, Macaubas C, Prescott SL, Venaille TJ, Holt BJ, Habre W et al. TH2-polarized immunological memory to inhalant allergens in atopics is established during infancy and early childhood. *Clin Exp Allergy* 1997;**27**:1261–1269.
27. Strachan DP, Harkins LS, Johnston ID, Anderson HR. Childhood antecedents of allergic sensitization in young British adults. *J Allergy Clin Immunol* 1997;**99**(1 Pt 1):6–12.
28. Goksor E, Amark M, Alm B, Gustafsson PM, Wennergren G. The impact of pre- and post-natal smoke exposure on future asthma and bronchial hyper-responsiveness. *Acta Paediatr* 2007;**96**:1030–1035.
29. Heinrich J, Wichmann HE. Traffic related pollutants in Europe and their effect on allergic disease. *Curr Opin Allergy Clin Immunol* 2004;**4**:341–348.
30. Bartra J, Mullol J, del Cuvillo A, Davila I, Ferrer M, Jauregui I et al. Air pollution and allergens. *J Investig Allergol Clin Immunol* 2007;**17**(Suppl 2):3–8.
31. Linneberg A. Hypothesis: urbanization and the allergy epidemic – a reverse case of immunotherapy? *Allergy* 2005;**60**:538–539.
32. Koskela HO, Happonen KK, Remes ST, Pekkanen J. Effect of farming environment on sensitisation to allergens continues after childhood. *Occup Environ Med* 2005;**62**: 607–611.
33. 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.
34. Dreborg S, Frew AJ. Allergen Standardization and Skin Tests. *Allergy* 1993;**48**(Suppl 14):49–54.

Protection from childhood asthma and allergy in Alpine farm environments—the GABRIEL Advanced Studies

Sabina Illi, PhD,[a]* Martin Depner, PhD,[a]* Jon Genuneit, MD,[b] Elisabeth Horak, MD,[c] Georg Loss, MSc,[d,e] Christine Strunz-Lehner, MPH,[a] Gisela Büchele, PhD,[b] Andrzej Boznanski, MD, PhD,[f] Hanna Danielewicz, MD,[f] Paul Cullinan, MD,[g] Dick Heederik, PhD,[h] Charlotte Braun-Fahrländer, MD,[d,e] Erika von Mutius, MD, MSc,[a] and the GABRIELA Study Group‡ *Munich and Ulm, Germany, Innsbruck, Austria, Basel, Switzerland, Wroclaw, Poland, London, United Kingdom, and Utrecht, The Netherlands*

Background: Studies on the association of farm environments with asthma and atopy have repeatedly observed a protective effect of farming. However, no single specific farm-related exposure explaining this protective farm effect has consistently been identified.
Objective: We sought to determine distinct farm exposures that account for the protective effect of farming on asthma and atopy.
Methods: In rural regions of Austria, Germany, and Switzerland, 79,888 school-aged children answered a recruiting questionnaire (phase I). In phase II a stratified random subsample of 8,419 children answered a detailed questionnaire on farming environment. Blood samples and specific IgE levels were available for 7,682 of these children. A broad asthma definition was used, comprising symptoms, diagnosis, or treatment ever.
Results: Children living on a farm were at significantly reduced risk of asthma (adjusted odds ratio [aOR], 0.68; 95% CI, 0.59-0.78; $P < .001$), hay fever (aOR, 0.43; 95% CI, 0.36-0.52; $P < .001$), atopic dermatitis (aOR, 0.80; 95% CI, 0.69-0.93; $P = .004$), and atopic sensitization (aOR, 0.54; 95% CI, 0.48-0.61; $P < .001$) compared with nonfarm children. Whereas this overall farm effect could be explained by specific exposures to cows, straw, and farm milk for asthma and exposure to fodder storage rooms and manure for atopic dermatitis, the farm effect on hay fever and atopic sensitization could not be completely explained by the questionnaire items themselves or their diversity.
Conclusion: A specific type of farm typical for traditional farming (ie, with cows and cultivation) was protective against asthma, hay fever, and atopy. However, whereas the farm effect on asthma could be explained by specific farm characteristics, there is a link still missing for hay fever and atopy. (J Allergy Clin Immunol 2012;129:1470-7.)

Key words: *Asthma, hay fever, atopic dermatitis, atopic sensitization, childhood, farming, farm milk, early life*

Discuss this article on the JACI Journal Club blog: www.jaci-online.blogspot.com.

Asthma and allergies constitute complex diseases; their cause involves both genetic and environmental determinants. Moreover, both diseases frequently have their onset in childhood and thus appear to comanifest. However, recent results from the GABRIEL Surveys contradict this concept of interdependent phenotypes. The GABRIEL Surveys were designed to identify key factors in the development of asthma using the latest research across a variety of disciplines, including genetics, epidemiology, and immunology (see Table E1 in this article's Online Repository at www.jacionline.org).[1-6] A genome-wide association study within the GABRIEL Surveys found no overlap in genes associated with asthma and total IgE levels.[1] Furthermore, within the GABRIEL Surveys, discrepant results were also observed for the protective role of microbial diversity within a farming environment.[2] Whereas the protective farm effect on childhood asthma could be explained by the overall diversity of bacteria and fungi from dust of farm and nonfarm children, this did not hold for atopy.

Previous studies on the protective effect of growing up on a typical Central European farm were fairly consistent with respect to hay fever and atopy. In contrast, results for asthma were quite heterogeneous. This potentially indicates that not all farms are the same and that specific farm characteristics are possibly of greater effect than farm exposure in general.[7-10] These previous studies mainly used questionnaires assessing the farm's characteristics but not the child's exposure. The aim of the current epidemiologic GABRIEL Advanced Studies was an in-depth analysis of the protective exposures within a farming environment both on asthma

From [a]the Asthma and Allergy Research Group, University Children's Hospital, Munich; [b]the Institute of Epidemiology and Medical Biometry, University of Ulm; [c]the Department of Paediatrics and Adolescents, Innsbruck Medical University; [d]the Swiss Tropical and Public Health Institute, Basel; [e]the University of Basel; [f]the Department of Paediatrics, Allergology and Cardiology, Wroclaw Medical University; [g]Imperial College London, National Heart and Lung Institute, London; and [h]the Division of Environmental Epidemiology, Institute for Risk Assessment Sciences, Utrecht University.
*These authors contributed equally to this work.
‡For an alphabetical listing of the members of the GABRIELA study group, see this article's acknowledgments section.
Supported by the European Commission as part of GABRIEL (a multidisciplinary study to identify the genetic and environmental causes of asthma in the European Community), contract no. 018996 under the Integrated Program LSH-2004-1.2.5-1.
Disclosure of potential conflict of interest: M. Depner has received research support from the European Commission/European Research Council. A. Boznanski, H. Danielewicz, and D. Heederik have received research support from the European Commission. E. von Mutius has consulted for Novartis, GlaxoSmithKline, ALK-Abelló, and Protectimmune; has received a speaker's fee from InfectoPharm; has received research support from Airsonnett AB; and has served as a member of the Expert Panel for the UK Research Excellence Framework. The rest of the authors declare that they have no relevant conflicts of interest.
Received for publication August 31, 2011; revised March 15, 2012; accepted for publication March 16, 2012.
Available online April 24, 2012.
Corresponding author: Sabina Illi, PhD, University Children's Hospital, Lindwurmstrasse 4, 80337 Munich, Germany. E-mail: sabina.illi@med.uni-muenchen.de.
0091-6749/$36.00

doi:10.1016/j.jaci.2012.03.013

Abbreviations used
aOR: Adjusted odds ratio
LCA: Latent class analysis

and atopy. This was based on a newly designed questionnaire aiming at disentangling the protective effect of a child's distinct farm exposures.

METHODS

Study design and population

The GABRIEL Advanced Surveys were conducted by 5 study centers in rural areas of southern Germany (Bavaria and Baden-Württemberg), Switzerland (9 German-speaking cantons), Austria (Tyrol), and Poland (Silesia) from winter 2006 to spring 2008.[5] Because of differences in study design, the Polish data will be reported separately. In the population-based phase I study a short recruiting questionnaire was distributed to parents of all schoolchildren through their elementary schools. In phase II stratified random samples of all children whose parents had given written informed consent to blood sampling, genetic analyses, and dust sampling were studied. Three strata were defined: (1) farm children (ie, children living on a farm run by the family); (2) exposed nonfarm children (ie, children not living on a farm but regularly exposed to stables, barns, or cow's milk produced on a farm); and (3) unexposed nonfarm children.

In all centers the ethics committees of the respective universities and the data protection authorities approved the study.

Questionnaires

The recruitment questionnaire in phase I assessed the prevalence of respiratory and allergic symptoms and diagnoses, socioeconomic status, family history of atopy, maternal smoking, and farm characteristics comprising types of animal breeding, cultivation, and animal feeding. A comprehensive questionnaire was handed out to parents in phase II assessing characteristics of asthma and detailed information on the child's farm-related exposures. All farm-related exposures were assessed for 5 time periods (pregnancy; first, second to third, and fourth to fifth years of life; and past 12 months) and 5 frequency categories per time period (never/almost never, about once a month, about once a week, about once a day up to 15 minutes, and about once a day longer than 15 minutes). The following exposures were assessed: contact with animals (cats, dogs, cows, pigs, poultry, sheep, and horses), stay in animal sheds (cow, pig, and poultry), contact with animal feed (straw, hay, grain, corn, grass, silage, pellet feed, and sugar beet), presence during parental farming activities (harvesting/kibbling/ensiling corn, harvesting/handling hay, ensiling grass, harvesting/threshing/kibbling grain, fieldwork, manuring, and spraying pesticides), stay in barn or fodder storage room, and consumption of cow's milk produced on the farm.

Asthma and other allergic illnesses

Asthma was defined as either current wheeze (parental reporting of wheeze in the past 12 months), a positive answer to the question "Did your child ever use an asthma spray?," or a doctor's diagnosis of asthma at least once or of wheezy bronchitis more than once. Atopic and nonatopic current wheeze was defined as current wheeze with or without atopic sensitization (see the definition below), respectively, by using the children without current wheeze as a common reference group. Severe wheeze was defined as wheeze in the past 12 months with multiple triggers and asthma inhaler use ever.

Hay fever was defined as either nasal symptoms with itchy or watery eyes in the past 12 months or a doctor's diagnosis of hay fever ever. Atopic dermatitis was defined as a doctor's diagnosis ever.

All questionnaire-based outcomes were reported in phase I except for severe wheeze, which was assessed in phase II, and atopic and nonatopic current wheeze because atopic sensitization was also only assessed in phase II.

Atopic sensitization

Blood samples were collected, and serum IgE antibodies against inhalant (*Dermatophagoides pteronyssinus*, cat, grass mix [sweet vernal grass, rye grass, timothy grass, cultivated rye, and velvet grass], birch, and mugwort) and food (egg white, cow's milk, fish, wheat, peanut, and soybean) allergens were measured in one central laboratory at the Robert-Koch-Institute, Berlin, Germany, by using the UNICAP 1000 (Phadia AB, Uppsala, Sweden). Atopic sensitization was defined as specific IgE antibodies of at least 0.7 kU/L against *D pteronyssinus*, cat, or birch or a positive reaction (0.35 kU/L) to the grass mix.

Statistical analyses

For further information on statistical analyses, see the Methods section in this article's Online Repository at www.jacionline.org.

For phase I, categorical variables are presented as relative frequencies; P values are based on the Pearson χ^2 test. A latent class analysis (LCA) was used to derive different types of farming, the association of which with outcomes was then analyzed by using logistic regression analysis. For phase II, all questionnaire-based farm-related exposures were dichotomized into presence or absence of the exposure based on an exposure frequency of at least once a week in a specific time period. Early-life exposure was then defined as the presence of the exposure in pregnancy or the first 3 years of life. Correlation between these farm-related exposure variables was assessed by using the Kendall tau-b correlation coefficient. Diversity of farm exposures was defined by summing up all dichotomous farm exposures and division into quartiles based on the weighted distribution in the study sample. Categorical variables are presented as weighted relative frequencies and compared over categories by using the Rao-Scott χ^2 test. Weighted logistic regression models were used to calculate associations between outcomes and farm-related exposures. Stepwise logistic regression analyses were calculated to assess final models containing the most relevant exposures. Combined effects of all dichotomized farm-related exposure variables defined as 4-level categorical variables were included in this process. All models were adjusted for farming, center, and potential confounders (family atopy, ≥2 siblings, sex, maternal smoking in pregnancy, and parental education). Statistical analysis was performed with SAS 9.2 software (SAS Institute, Inc, Cary, NC), and a P value of .05 was considered significant. Because of the exploratory character of the analysis, corrections for multiple testing were not performed.

RESULTS

In phase I, 132,518 recruitment questionnaires were distributed, of which 79,888 (60.3%) were returned. Of those, 34,491 (43.2%) parents provided written informed consent for blood sampling, genetic testing, and dust sampling. Their children were eligible for phase II (Fig 1); mean age was 8.7 ± 1.4 years. Of these, 9,668 were randomly selected for phase II by exposure stratum (ie, farm children, exposed nonfarm children, and unexposed nonfarm children), and 8,419 (87%) returned the detailed phase II questionnaire. Of these participants, 7,682 (91%) provided blood samples for measurements of specific IgE levels. Families participating in phase II were of higher education and had more allergic illnesses in the family, as also observed in other studies.[11]

A lower prevalence of asthma, hay fever, atopic dermatitis, and atopic sensitization was found among farm children compared with nonfarm children in phases I and II (Table I), with the exposed nonfarm children having intermediate prevalences. After adjusting for confounding variables, the adjusted odds ratios (aORs) for asthma, hay fever, and atopic sensitization with farming status (farm vs nonfarm) were as follows: 0.68 (95% CI, 0.59-0.78; $P < .001$), 0.43 (95% CI, 0.36-0.52; $P < .001$), and 0.54 (95% CI, 0.48-0.61; $P < .001$), respectively. For atopic dermatitis, the farm effect only amounted to an aOR of 0.80 (95% CI,

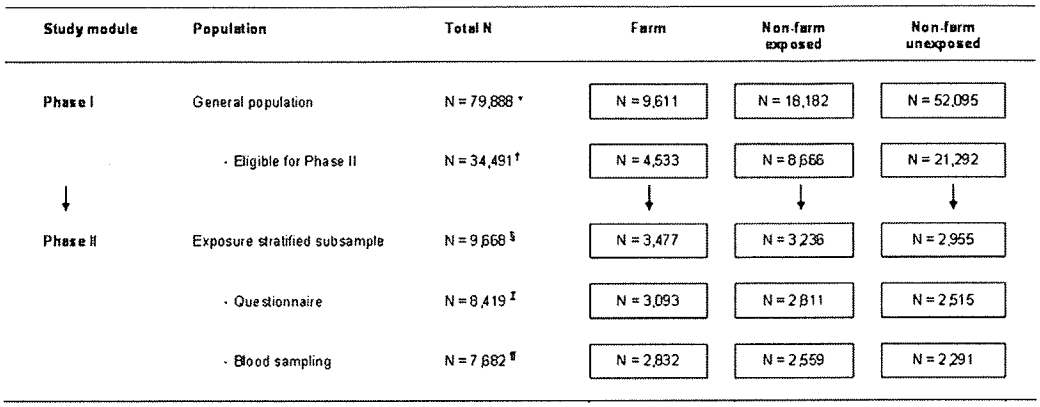

FIG 1. Study population and design. *Completed phase I recruiting questionnaire. †Completed phase I recruiting questionnaire and signed a consent form for analyses and all additional investigations in phase II. §Random selection stratified for exposure. ‡Completed phase II questionnaire. ¶Completed phase II questionnaire and participated in blood sampling and analysis of specific IgE levels.

TABLE I. Prevalence of asthma, other allergic illnesses, and atopic sensitization, as well as specific farm exposures among farm children compared with exposed and unexposed nonfarm children

		Nonfarm children		
	Farm children	Exposed	Unexposed	
Phase I*				
Atopic dermatitis	10.6%	14.4%	14.5%	†
Hay fever	4.8%	10.5%	14.7%	†
Asthma	11.4%	15.8%	18.3%	†
Current wheeze	6.7%	9.7%	11.7%	†
Phase II‡				
Atopic dermatitis	12.8%	17.3%	18.0%	‖
Hay fever	6.4%	11.6%	18.2%	‖
Atopic sensitization§	24.5%	35.5%	43.1%	‖
Asthma	14.1%	20.0%	22.2%	‖
Current wheeze	8.8%	12.6%	15.0%	‖
Atopic§	4.7%	7.5%	8.7%	‖
Nonatopic§	3.5%	5.2%	6.3%	‖
Severe wheeze	1.7%	2.9%	3.6%	‖
Phase II‡¶				
Contact with cows	70.7%	31.0%	5.2%	‖
Stay in cow shed	67.6%	24.3%	2.7%	‖
Contact with straw	64.9%	24.1%	3.8%	‖
Stay in barn	73.4%	28.5%	4.0%	‖
Stay in storage room	30.6%	7.0%	0.8%	‖
Consumption of farm milk	70.9%	51.1%	5.3%	‖

*Phase I population: n = 79,888.
†$P < .001$ of the Pearson χ^2 test for farm versus nonfarm children.
‡Phase II population: n = 8,419; analyses weighted to eligible subjects for phase II (n = 34,491).
§Reduced phase II population: n = 7,682 because of reduced sample size for blood sampling; analyses weighted to eligible subjects for phase II (n = 34,491).
‖$P < .001$ of the Rao-Scott χ^2 test for farm versus nonfarm children.
¶Farm exposures in pregnancy and the first 3 years of life assessed in the phase II questionnaire.

0.69-0.93; $P = .004$). The protective farm effect was seen for all asthma phenotypes: asthma, current wheeze, current atopic wheeze, current nonatopic wheeze, and severe wheeze.

In phase I farm characteristics with respect to animal breeding, cultivation, and animal feeding were assessed within the group of farm children. By using LCA, 3 types of farms were identified (Fig 2). The first type comprised farms without dairy cows or cattle breeding. These farms typically kept other animals, such as pigs, poultry, or horses, combined with cultivation of grain and feeding of grain shred. The second type of farming comprised farms with dairy cows and cattle breeding but nearly no cultivation. In contrast, the third farm type typically comprised those that kept dairy cows and bred cattle combined with cultivation, mostly of grain and corn. Farmers of the latter group also typically fed corn silage and grain shred to their animals. When assessing the association of the 3 types of farming with asthma, hay fever, atopic dermatitis, and atopic sensitization within the group of farm children, a protective effect of the third type of farming on asthma, hay fever, and atopic dermatitis was observed (Table II). For atopic sensitization, only a nonsignificant protective trend was observed, potentially because of the reduced sample size in phase II.

In contrast to phase I assessing farm characteristics irrespective of whether the child itself was actually exposed, in phase II the child's exposure to specific farm characteristics was assessed. First contact with these farm exposures typically occurred early in life, especially in pregnancy and the second to third year of life (Fig 3). Therefore in all subsequent analyses the timing of farm exposures relates to the period from pregnancy to the third year of life. Many of these exposures, such as contact with cows, other farm animals, or animal fodder, the consumption of cow's milk produced on a farm, and the child's presence in stables, barns, or fodder storage rooms, were inversely related to asthma, hay fever, atopic dermatitis, and atopic sensitization, even when adjusting for farming (Table III). Children were often exposed to several factors, although correlations between different factors were only moderate, with somewhat higher correlations for exposure to grass, hay, and straw (tau-b correlation coefficient, ≥0.7; data not shown). Still, many of the assessed exposures showed a strong overlap (eg, 75% of the children that "were present while the parents are manuring" also had contact with both cows and straw), requiring multivariate selection procedures to identify relevant exposures.

Therefore a stepwise variable selection process was performed. In the resulting final multivariate models, only few farm exposures remained inversely related to asthma, hay fever, atopic

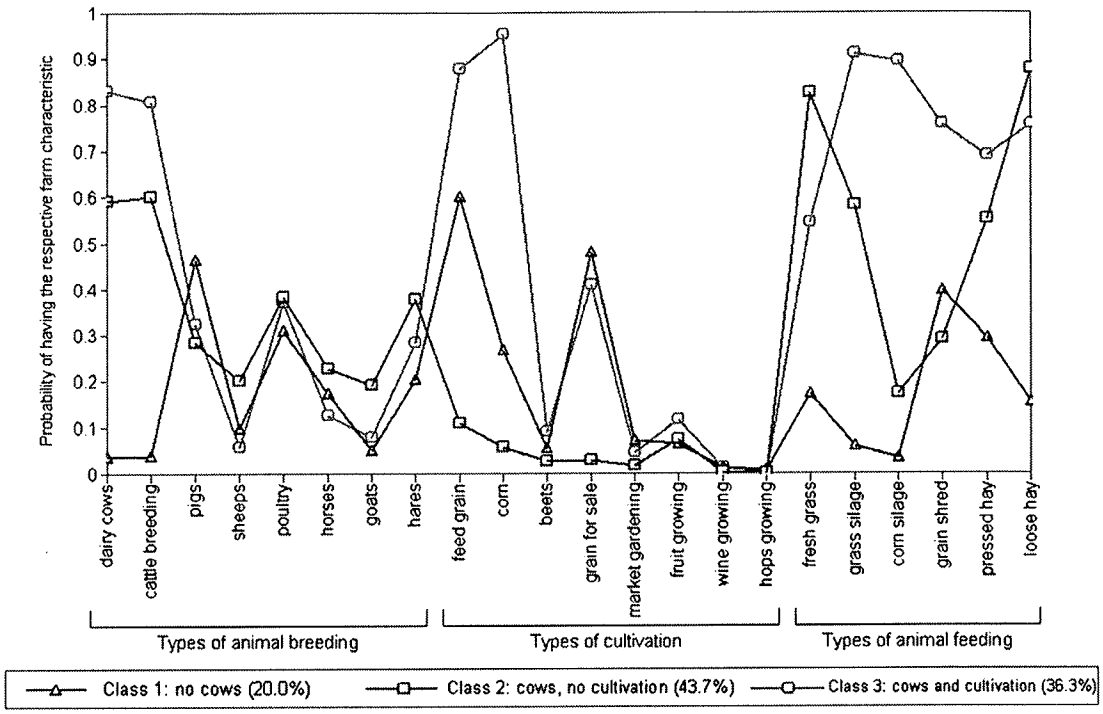

FIG 2. Types of farms based on farm characteristics. Results of LCA with 3-class solution are shown. Farm characteristics assessed in the phase I recruitment questionnaire are shown (n = 9611 farm children).

TABLE II. Types of farms and risk of asthma, hay fever, atopic dermatitis, and atopic sensitization

	Asthma*			Hay fever*			Atopic dermatitis*			Atopic sensitization†		
Farm type	aOR‡	95% CI	*P* value	aOR‡	95% CI	*P* value	aOR‡	95% CI	*P* value	aOR‡	95% CI	*P* value
No cows	1.00	—	—	1.00	—	—	1.00	—	—	1.00	—	—
Cows, no cultivation	0.84	0.69-1.03	.09	0.94	0.70-1.26	.68	0.78	0.64-0.96	.02	1.01	0.78-1.32	.94
Cows and cultivation	0.79	0.65-0.95	.01	0.70	0.53-0.94	.02	0.75	0.62-0.91	.004	0.82	0.64-1.06	.13

*Outcomes assessed in phase I recruitment questionnaire (n = 9611 farm children).
†Outcome assessed in phase II blood sampling (n = 2832 farm children).
‡Adjusted for center and potential confounders (family atopy, ≥2 siblings, sex, maternal smoking, and parental education).

dermatitis, and atopic sensitization (Fig 4; data are shown in Table E2 in this article's Online Repository at www.jacionline.org). Concurrent contact with cows and straw and the consumption of cow's milk produced on the farm were independent protective factors for asthma. The farm effect aORs increased from 0.68 (95% CI, 0.59-0.78) to 0.89 (95% CI, 0.75-1.06) after inclusion of the relevant farm exposures, suggesting that they accounted for most of the farm effect. When stratifying the analysis into atopic and nonatopic children, the variables selected into the final model remained unchanged in the group of nonatopic subjects, whereas only farm milk remained in the model as a significant protective factor for asthma among atopic children. "Being present while the parents are manuring" showed the lowest odds ratios for all outcomes except atopic sensitization. However, in the multivariate model, when including contact with cows and with straw, manuring was no longer significant.

Similarly to asthma, protective farm exposures remaining in the final multivariate model for hay fever were contact with cows and consumption of farm milk. However, in contrast to asthma, contact with straw was no longer significant in the final model, even in combination with concurrent contact with cows. The farm effect aOR increased from 0.43 (95% CI, 0.36-0.52) to only 0.68 (95% CI, 0.55-0.84), indicating the presence of additional undetected protective exposures in the farming environment.

For atopic sensitization, contact with straw and the consumption of cow's milk produced on the farm were significant independent protective determinants in the final model (Fig 4). Similarly to hay fever, the aOR for farming only increased from 0.54 (95% CI, 0.48-0.61) to 0.74 (95% CI, 0.64-0.86). Exposure to poultry and dogs early in life additionally contributed to the model when defining atopic sensitization at a higher cutoff (≥3.5 kU/L).

With respect to atopic dermatitis, only very few distinct questionnaire-based farm exposures were significantly protective after adjusting for farming and potential confounders. Of these, only staying in a fodder storage room remained in the final model, with the effect of farming being no longer significant. In contrast to the other phenotypes, onset of atopic dermatitis typically occurs in infancy, with an increased potential role of exposures in pregnancy. We thus repeated all analyses for exposures in pregnancy only. The maternal exposures inducing the greatest change in the effect of farming on atopic dermatitis were staying in a cow shed and manuring during pregnancy. In contrast, when

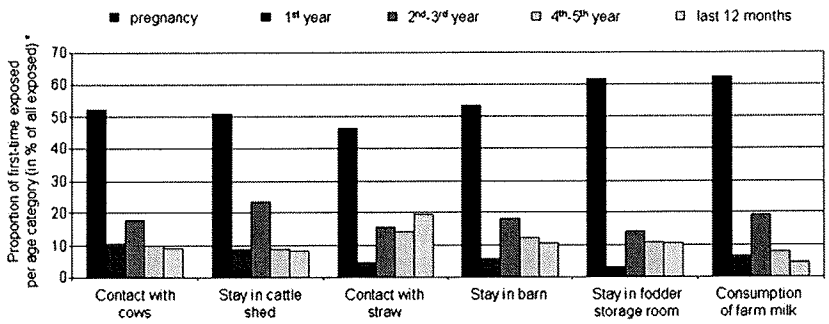

FIG 3. Timing of the first exposure to farm characteristics. Most children experienced their first exposure through their mothers in pregnancy. *Computations are based on 5 groups of children ever exposed to cows, cow sheds, straw, barns, fodder storage rooms, or farm milk. The *bars* show the proportion of children with first-time contact with the respective farm exposure per age category. Proportions of the 5 age categories add up to 100%.

including only maternal exposures during pregnancy for asthma, hay fever, and atopic sensitization, factors remaining in the final models were unchanged. Atopic dermatitis was merely defined as a doctor's diagnosis because this was assessed in phase I, whereas corresponding symptoms were only assessed in phase II. When using an outcome variable combining diagnosis and symptoms, the final multivariate models remained unchanged, except that manuring additionally remained in the model.

For the assessment of the diversity of exposures, a score was generated by summing up all dichotomous farm exposures. This diversity score was significantly associated with atopic sensitization: aORs of 0.79 (95% CI, 0.65-0.97; $P = .03$) for 2 to 4 exposures (third quartile) and 0.65 (95% CI, 0.52-0.80; $P < .001$) for 5 to 23 exposures (fourth quartile) versus no exposure (first quartile). However, when adjusting the final models for diversity, it was no longer significant, and the association of contact with straw and consumption of farm milk with the outcome remained basically unchanged (see Table E3 in this article's Online Repository at www.jacionline.org).

Sensitivity analyses investigating the individual contribution of prenatal and postnatal exposure for asthma, hay fever, and atopic sensitization showed some differences between factors but in general suggested that both periods were of importance, showing similar effects for exposure in pregnancy and in the first 3 years of life (data not shown). Furthermore, a dose-response relationship was seen (ie, stronger protection with increased frequency of exposures; see Fig E1 in this article's Online Repository at www.jacionline.org).

DISCUSSION

Children growing up on farms in Germany, Austria, and Switzerland are protected against asthma, hay fever, and atopic sensitization. Only 3 distinct farm exposures assessed by means of questionnaire (ie, the pregnant mother's and subsequently the toddler's exposure to cows and straw and the consumption of cow's milk produced on the farm) accounted for the farm effect on asthma and partially on hay fever and atopic sensitization.

The protective effect of a farm environment on atopic dermatitis was much less pronounced than for the other outcomes. This discrepancy has already been observed in previous studies on farming and is in line with results from the German International Study of Asthma and Allergies in Childhood, in which atopic dermatitis showed no strong associations with environmental factors, indicating that the hygiene hypothesis might not hold for atopic dermatitis as much as for respiratory allergic diseases.[10,12]

The definition of asthma for population-based studies has been vividly debated. We used a broader asthma definition, including diagnosis, symptoms, and treatment, to also include milder and nonatopic phenotypes, as well as more specific definitions of current, atopic and nonatopic, and severe wheeze. Farm children were at lower risk of any of these phenotypes compared with nonfarm children, potentially indicating antiviral properties of the protective exposures. When using the International Study of Asthma and Allergies in Childhood's definition (doctor's diagnosis of asthma or recurrent wheezy bronchitis), as in previous farm studies, the farm effect remained unchanged (6.5%, 9.0%, and 10.5% in farmers and exposed and unexposed nonfarmers, respectively; $P < .001$) and was of similar magnitude as in the previous farm studies ALEX (Allergy and Endotoxin) and PARSIFAL (Prevention of Allergy–Risk Factors for Sensitization In Children Related to Farming and Anthroposophic Lifestyle) (see Table E4 in this article's Online Repository at www.jacionline.org).[8,13,14]

In cooperation with farmers and field workers coming from a farm environment, we developed an extensive questionnaire to assess the large spectrum of potential exposures that a child might encounter on a farm over the first years of life. The most relevant farm exposures were then selected into a final multivariate model through a stepwise statistical procedure based on the change in estimate of farming: the closer to the null effect the overall farming effect became when a specific farm exposure was additionally included in the model, the more likely it was to account for this farm effect. This method was very robust with respect to the selection of the final set of exposure variables. The standard stepwise variable selection procedure that merely uses the P value as an inclusion criterion resulted in the same final models, irrespective of whether farming and potentially confounding variables were forced into the model in the selection process.

In this newly developed comprehensive questionnaire, the child's contact with all types of animal feeding was assessed. The strongest protective effect on all outcomes except atopic dermatitis was seen for contact with straw. Straw is an agricultural byproduct of cereal plants (ie, the dry stalks after the grain has been removed) and is mostly used as bedding material for animals

TABLE III. Farm exposures (pregnancy to age 3 years) associated with decreased risk of asthma, hay fever, atopic dermatitis, and atopic sensitization†

	Asthma‡				Hay fever‡				Atopic dermatitis‡				Atopic sensitization§			
	aOR‖	95% CI	*P* value		aOR‖	95% CI	*P* value		aOR‖	95% CI	*P* value		aOR‖	95% CI	*P* value	
Contact with animals																
Cat	0.90	0.77-1.04	.16	#	0.92	0.77-1.10	.37	#	0.85	0.72-1.01	.06	¶	**0.81**	**0.71-0.93**	**.003**	#
Dog	0.99	0.84-1.15	.86		0.90	0.74-1.10	.31	#	0.88	0.74-1.05	.15		**0.85**	**0.74-0.97**	**.02**	#
Cow	**0.74**	**0.62-0.89**	**.002**	*,¶,#	**0.52**	**0.41-0.66**	**<.001**	*,¶,#	0.87	0.71-1.06	0.17	¶,#	**0.75**	**0.65-0.88**	**<.001**	*,¶,#
Pig	0.89	0.70-1.14	.36	¶	0.76	0.53-1.07	.12	¶,#	0.98	0.77-1.26	.89		0.87	0.70-1.07	.18	
Poultry	0.95	0.77-1.17	.63		**0.72**	**0.54-0.95**	**.02**	*,¶,#	0.95	0.76-1.17	.61		**0.76**	**0.64-0.91**	**.003**	#
Sheep	0.79	0.62-1.02	.07		0.74	0.52-1.05	.09	#	0.91	0.69-1.21	.53		0.84	0.68-1.04	.12	
Horse	1.13	0.89-1.43	.30		0.95	0.69-1.29	.73		**1.33**	**1.05-1.70**	**.02**		**0.79**	**0.63-0.99**	**.04**	
Stay in animal sheds																
Cow	**0.79**	**0.65-0.95**	**.01**	*,¶	**0.66**	**0.52-0.85**	**.001**	*,¶,#	0.82	0.67-1.01	.06	¶,#	**0.78**	**0.67-0.92**	**.003**	*,¶,#
Pig	1.04	0.81-1.33	.78		0.72	0.51-1.02	.06	¶,#	0.99	0.76-1.29	.92		0.85	0.68-1.06	.14	
Poultry	0.92	0.74-1.15	.48	¶	0.89	0.66-1.20	.44	#	0.91	0.72-1.15	.42	¶	0.84	0.69-1.01	.06	#
Contact with animal feed																
Straw	**0.79**	**0.66-0.95**	**.01**	*,¶	**0.61**	**0.47-0.80**	**<.001**	*,¶,#	0.83	0.67-1.02	.07	¶,#	**0.61**	**0.52-0.72**	**<.001**	*,¶,#
Hay**	0.87	0.73-1.04	.14	¶,#	**0.78**	**0.63-0.98**	**.03**	*,¶,#	0.91	0.76-1.10	.35	¶,#	**0.74**	**0.63-0.86**	**<.001**	*,¶,#
Grain**	0.93	0.76-1.14	.49		0.91	0.68-1.21	.52	#	0.91	0.73-1.14	.43	¶,#	**0.72**	**0.61-0.86**	**<.001**	*,¶,#
Corn**	0.86	0.67-1.09	.21	¶,#	0.88	0.64-1.20	.41	#	0.84	0.66-1.06	.14	¶	**0.78**	**0.64-0.95**	**.01**	#
Corn silage**	0.81	0.61-1.07	.14	¶,#	0.72	0.47-1.09	.12	¶,#	**0.70**	**0.54-0.93**	**.01**	*,¶,#	0.84	0.67-1.04	.11	#
Grass	0.95	0.79-1.13	.56		0.82	0.65-1.03	.09	¶,#	0.86	0.70-1.04	.11	¶,#	**0.82**	**0.70-0.96**	**.01**	
Grass silage**	0.96	0.76-1.21	.72	#	0.73	0.52-1.02	.07	¶,#	0.79	0.62-1.01	.06	¶,#	**0.79**	**0.65-0.95**	**.01**	*,¶,#
Pellet feed	0.84	0.68-1.05	.13		0.77	0.54-1.09	.13	#	0.98	0.77-1.25	.85		**0.75**	**0.62-0.92**	**.005**	#
Sugar beet	1.19	0.82-1.73	.36		0.97	0.52-1.81	.92	#	0.95	0.62-1.46	.82		0.76	0.53-1.09	.14	
Stay in —																
Barn	0.87	0.72-1.04	.13		**0.62**	**0.48-0.80**	**<.001**	*,¶,#	0.86	0.70-1.05	.14	¶,#	**0.70**	**0.59-0.82**	**<.001**	*,¶,#
Fodder storage room	0.94	0.72-1.23	.65	#	0.72	0.49-1.07	.10	¶,#	**0.72**	**0.55-0.93**	**.01**	*,¶	**0.79**	**0.64-0.98**	**.03**	
Present while parents are —																
Doing field work	1.09	0.82-1.44	.57		0.91	0.59-1.41	.68		0.85	0.62-1.19	.35	¶	0.83	0.65-1.06	.14	
Manuring	**0.65**	**0.47-0.90**	**.01**	#	**0.51**	**0.33-0.80**	**.003**	*,¶	**0.66**	**0.45-0.96**	**.03**	*,¶,#	0.85	0.65-1.11	.23	
Spraying pesticides	1.22	0.32-4.62	.77		1.00	0.12-8.14	1.00		0.74	0.27-2.07	.57		1.45	0.52-4.02	.48	
Consumption of —																
Farm milk	**0.77**	**0.66-0.90**	**.001**	*,¶,#	**0.64**	**0.53-0.77**	**<.001**	*,¶,#	0.89	0.76-1.06	.18	¶	**0.73**	**0.64-0.84**	**<.001**	*,¶,#

Significant results are shown in boldface.

*Variable included in subsequent stepwise analyses for the respective outcome (criteria: significant results and ≥10% change in aOR of farming toward the null effect).

†Farm exposures assessed in phase II questionnaire.

‡Outcomes assessed in phase II questionnaire: n = 8,419; analyses weighted to eligible subjects for phase II (n = 34,491).

§Outcome assessed in phase II blood sampling: n = 7,682; analyses weighted to eligible subjects for phase II (n = 34,491).

‖Adjusted for center, farming, and potential confounders (family atopy, ≥2 siblings, sex, maternal smoking in pregnancy, and parental education).

¶Ten percent or greater change in aOR of farming toward the null effect.

#Significant aOR within strata of farmer's children.

**Combination of several questionnaire items: *Hay*, contact with forage hay or present while parents were harvesting or handling hay; *Grain*, contact with forage grain or present while parents were harvesting, threshing, or kibbling grain; *Corn*, contact with forage corn or present while parents were harvesting or kibbling corn; *Corn silage*, contact with forage corn silage or present while parents are ensiling corn; *Grass silage*, contact with forage grass silage or present while parents are ensiling grass.

in the study areas. Children are exposed either in barns or in the stable when litter is placed and aerosolized or removed. However, children exposed to straw were often also exposed to hay, grass, and manure. Therefore individual effects of grass, hay, manure, and straw could not be disentangled with certainty. Recent experimental studies have shown that the oligosaccharide arabinogalactan from grass and hay protects mice against allergic asthma.[15] Cereal, the source material of straw, also contains arabinogalactan, suggesting that exposure to this plant-derived oligosaccharide might protect children against asthma and atopy.[16] Alternatively or additionally, thus far unidentified microbial exposures associated with hay and straw might explain the effect. Straw has been shown to be contaminated with a high variety of fungi and bacteria.[17]

Consumption of cow's milk produced on the farm also showed a consistently strong inverse relation with 3 of the outcomes: asthma, hay fever, and atopy. This corroborates previous findings.[8,18,19] Refined analyses on the handling of milk samples by parents (boiling or skimming) and content of microbes, fat, protein, and various enzymes have been reported separately.[6] It is important to note that the effect of consumption of cow's milk produced on the farm was independent of the protective effect of contact with cows, potentially indicating different pathways: whereas milk exerts its effect through the gut, contact with cows might potentially be an inhaled exposure affecting the airway mucosa.

This notion of different pathways is supported by the fact that contact with cows only exerted a strong effect on outcomes involving the airways (ie, on asthma, including nonatopic asthma [data not shown] and on hay fever). No such effect was observed for atopy in the final model. The effect of contact with cows on hay fever was independent of other protective exposures, such as

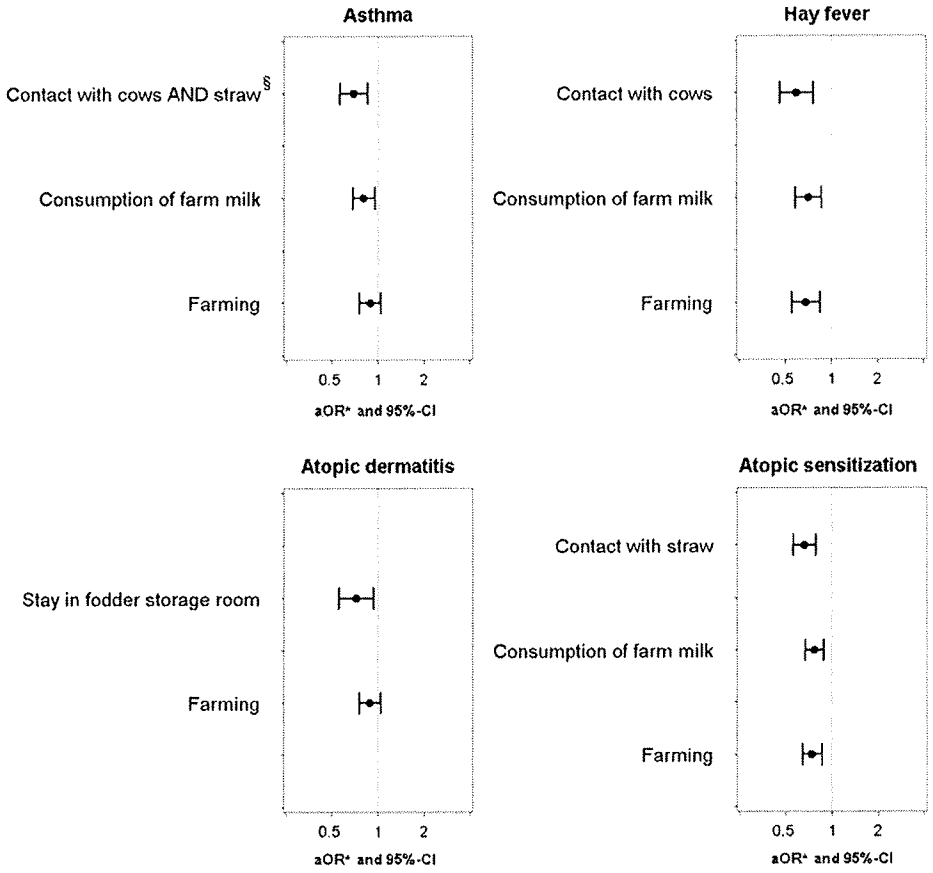

FIG 4. Specific farm exposures that best explain the overall effect of farming. Results of multivariate stepwise weighted regression models. *Mutually adjusted and additionally adjusted for center and potential confounders (family atopy, ≥2 siblings, sex, maternal smoking in pregnancy, and parental education). §Compared with the reference group (neither contact with cows nor straw). Odds ratios for intermediate categories (contact with cows or straw) are shown in Table E2.

contact with straw. This was in contrast to the effect on asthma: contact with cows was only inversely associated with asthma if the child also had contact with straw, potentially reflecting a specific type of farming accounting for this combined protective effect. No such interaction was observed for hay fever or atopy. This might explain why previous surveys on children from dairy farms have come to similar and homogeneous results for hay fever and atopy but have shown conflicting results with respect to protection against asthma[10,20]: perhaps specific combinations of exposures not investigated in previous studies are essential to exert a protective effect on asthma. Interestingly, similar effects were observed irrespective of whether characteristics of the farm were assessed or farm exposures of the child: both approaches resulted in a combination of cows and products of cultivation (eg, straw) as factors best explaining the overall farm effect. These results are not only observed in Alpine but also in other European areas, eg, in the Polish arm of the GABRIEL Advanced Studies (results will be reported separately). This points toward a protective effect of the traditional way of farming as it has been pursued for centuries, comprising cows, their products (eg, milk), and cultivation of grains both for alimentation and bedding material. From an evolutionary perspective, mankind has been exposed to these since settling down. Immune responses adapted to this prevailing environment might thus induce tolerance. Therefore it is not surprising that some of the farm effects observed in Central Europe are not seen in the United States because the type of farming differs greatly between continents.

The detailed questionnaire not only assessed the type of exposure but also both its time period and frequency. For most exposures, first contact in the child's life most frequently occurred during pregnancy and the second to third years of life, indicating mothers working on a farm. Furthermore, when analyzing the association of timing and outcomes, the effects of exposures early in life (ie, from pregnancy up to age 3 years, as shown in this article) showed much stronger effects than current exposure at the time of outcome assessment (data not shown). This correlates with findings from other studies that observed an effect of farm exposure in pregnancy on specific IgE levels and cytokine responses in cord blood, indicating a protective farm effect as early as *in utero*.[21-23]

Our results show that protective mechanisms differ for asthma and atopy. The exhaustive questionnaire assessed the child's farm exposures in as detailed a manner as possible. In contrast to asthma, the farm effect on atopy, although about half of it was explained by the questionnaire items, was not completely accounted for by these or their diversity, indicating a link was still missing. This is in line with previous results from the GABRIEL study group observing differing genes involved in the cause of asthma and atopy and discrepant results for the role of microbial diversity: whereas the diversity of bacteria and fungi from dust of farm and nonfarm children accounted for the protective farm effect

on asthma, this did not hold for atopy, indicating a potential role of an unknown, ubiquitous protective exposure on farms.[1,2] This unexpected finding is as puzzling as the very consistent protective effect of sibship size on atopy, which has not yet been completely explained by the hygiene hypothesis either.

The GABRIEL study group consisted of the following members listed in alphabetical order with affiliations:

Silvia Apprich, PhD,[g] Andrzej Boznanski, MD, PhD,[j] Charlotte Braun-Fahrländer, MD,[d,e] Gisela Büchele, PhD,[c] William Cookson, MD, DPhil,[a] Paul Cullinan, MD,[a] Hanna Danielewicz, MD,[j] Anna Dębińska,[j] Martin Depner, PhD,[b] Markus Ege, MD,[b] Urs Frey, MD, PhD,[q] Oliver Fuchs, MD,[k] Jon Genuneit, MD,[c] Dick Heederik, PhD,[f] Elisabeth Horak, MD,[l] Anne Hyvärinen, PhD,[h] Sabina Illi, PhD,[b] Michael Kabesch, MD,[m] Katalin Kovacs,[l] Aleksandra Kosmęda, PhD,[j] Wolfgang Kneifel, PhD,[g] Philipp Latzin, MD, PhD,[k] Roger Lauener, MD,[o] Georg Loss, MSc,[d,c] Stephanie MacNeill, MSc,[a] Bernhard Morass, MD,[l] Anne-Cécile Normand, PhD,[p] Ilka Noss, PhD,[f] Renaud Piarroux, MD, PhD,[p] Helena Rintala, PhD,[h] Mascha K. Rochat, MD,[b] Nikolaos Sitaridis,[c] Barbara Sozanska, MD,[j] David Strachan, MD,[n] Christine Strunz-Lehner, MPH,[b] Bertrand Sudre, MD, PhD,[i] Erika von Mutius, MD, MSc,[b] Marco Waser, PhD,[d,e] Juliane Weber, MD,[b] and Inge Wouters, PhD.[f]

From [a]Imperial College London, National Heart and Lung Institute, South Kensington Campus, London, United Kingdom; [b]LMU Munich, University Children's Hospital, Munich, Germany; [c]Ulm University, Institute of Epidemiology and Medical Biometry, Ulm, Germany; [d]the Swiss Tropical and Public Health Institute, Basel, Switzerland; [e]the University of Basel, Basel, Switzerland; [f]Utrecht University, Institute for Risk Assessment Sciences (IRAS), Division of Environmental Epidemiology, Utrecht, The Netherlands; [g]BOKU Vienna, University of Natural Resources and Life Sciences, Department of Food Science and Technology, Vienna, Austria; [h]THL Kuopio, National Institute for Health and Welfare, Kuopio, Finland; [i]Université de Franche-Comté, UMR 6249 Chrono-Environnement, Département de Parasitologie/Mycologie, UFR SMP, Besançon, France; [j]Wroclaw Medical University, 1st Department of Paediatrics, Allergology and Cardiology, Wroclaw, Poland; [k]the Division of Pulmonology, Department of Paediatrics, Bern University Hospital, Bern, Switzerland; [l]the Department of Pediatrics and Adolescents, Division of Cardiology and Pulmonology, Innsbruck Medical University, Innsbruck, Austria; [m]Hannover Medical School, Clinic for Paediatric Pneumology and Neonatology, Hannover, Germany; [n]St George's, University of London, London, United Kingdom; [o]High Mountain Hospital Davos, Davos-Wolfgang, Switzerland; [p]the Department of Parasitology and Mycology, Hôpital de la Timone, Assistance Publique-Hôpitaux de Marseille, Marseille, France; and [q]the University Children's Hospital (UKBB), University of Basel, Basel, Switzerland.

Key messages

- **Specific types of farms with cows and cultivation exerted a protective effect on asthma, hay fever, and atopic sensitization.**
- **This protective farm effect on asthma, hay fever, and atopic sensitization was determined by 3 specific early-life exposures of the child, namely contact with cows and straw and consumption of farm milk, thereby narrowing down the farm effect.**
- **Whereas the farm effect on asthma could be explained by contact with cows, straw, and farm milk, this was not the case for hay fever and atopic sensitization, indicating differing underlying protective mechanisms.**

REFERENCES

1. Moffatt MF, Gut IG, Demenais F, Strachan DP, Bouzigon E, Heath S, et al. A large-scale, consortium-based genomewide association study of asthma. N Engl J Med 2010;363:1211-21.
2. Ege MJ, Mayer M, Normand AC, Genuneit J, Cookson WO, Braun-Fahrlander C, et al. Exposure to environmental microorganisms and childhood asthma. N Engl J Med 2011;364:701-9.
3. Ege MJ, Strachan DP, Cookson WO, Moffatt MF, Gut I, Lathrop M, et al. Gene-environment interaction for childhood asthma and exposure to farming in Central Europe. J Allergy Clin Immunol 2011;127:138-44.
4. Normand AC, Sudre B, Vacheyrou M, Depner M, Wouters IM, Noss I, et al. Airborne cultivable microflora and microbial transfer in farm buildings and rural dwellings. Occup Environ Med 2011;68:849-55.
5. Genuneit J, Buchele G, Waser M, Kovacs K, Debinska A, Boznanski A, et al. The GABRIEL Advanced Surveys: study design, participation and evaluation of bias. Paediatr Perinat Epidemiol 2011;25:436-47.
6. Loss G, Apprich S, Waser M, Kneifel W, Genuneit J, Buchele G, et al. The protective effect of farm milk consumption on childhood asthma and atopy: the GABRIELA study. J Allergy Clin Immunol 2011;128:766-73.
7. 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;30:187-93.
8. Riedler J, Braun-Fahrlander C, Eder W, Schreuer M, Waser M, Maisch S, et al. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. Lancet 2001;358:1129-33.
9. Ege MJ, Frei R, Bieli C, Schram-Bijkerk D, Waser M, Benz MR, et al. Not all farming environments protect against the development of asthma and wheeze in children. J Allergy Clin Immunol 2007;119:1140-7.
10. von Mutius E, Vercelli D. Farm living: effects on childhood asthma and allergy. Nat Rev Immunol 2010;10:861-8.
11. Illi S, von Mutius E, Lau S, Niggemann B, Grüber C, Wahn U. Perennial allergen sensitisation early in life and chronic asthma in children: a birth cohort study. Lancet 2006;368:763-70.
12. Zutavern A, Hirsch T, Leupold W, Weiland S, Keil U, von Mutius E. Atopic dermatitis, extrinsic atopic dermatitis and the hygiene hypothesis: results from a cross-sectional study. Clin Exp Allergy 2005;35:1301-8.
13. 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;61:414-21.
14. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. Lancet 1998;351:1225-32.
15. Peters M, Kauth M, Scherner O, Gehlhar K, Steffen I, Wentker P, et al. Arabinogalactan isolated from cowshed dust extract protects mice from allergic airway inflammation and sensitization. J Allergy Clin Immunol 2010;126:648-56.
16. Muralikrishna G, Rao MV. Cereal non-cellulosic polysaccharides: structure and function relationship—an overview. Crit Rev Food Sci Nutr 2007;47:599-610.
17. Pfender WF, Fieland VP, Ganio LM, Seidler RJ. Microbial community structure and activity in wheat straw after inoculation with biological control organism. Appl Soil Ecol 1996;3:69-78.
18. Perkin MR, Strachan DP. Which aspects of the farming lifestyle explain the inverse association with childhood allergy? J Allergy Clin Immunol 2006;117:1374-81.
19. Bieli C, Eder W, Frei R, Braun-Fahrlander C, Klimecki W, Waser M, et al. A polymorphism in CD14 modifies the effect of farm milk consumption on allergic diseases and CD14 gene expression. J Allergy Clin Immunol 2007;120:1308-15.
20. von Mutius E, Radon K. Living on a farm: impact on asthma induction and clinical course. Immunol Allergy Clin North Am 2008;28:631-47, ix-x.
21. Ege MJ, Herzum I, Büchele G, Krauss-Etschmann S, Lauener RP, Roponen M, et al. Prenatal exposure to a farm environment modifies atopic sensitization at birth. J Allergy Clin Immunol 2008;122:407-12.
22. Pfefferle PI, Büchele G, Blümer N, Roponen M, Ege MJ, Krauss-Etschmann S, et al. Cord blood cytokines are modulated by maternal farming activities and consumption of farm dairy products during pregnancy: the PASTURE Study. J Allergy Clin Immunol 2010;125:108-15.
23. Schaub B, Liu J, Höppler S, Schleich I, Huehn J, Olek S, et al. Maternal farm exposure modulates neonatal immune mechanisms through regulatory T cells. J Allergy Clin Immunol 2009;123:774-82.

METHODS

Statistical analyses

For the analysis of the farm effect, exposed and unexposed nonfarm children were combined as nonfarm children and compared with farm children.

All questionnaire-based farm-related exposures were assessed for 5 time periods and 5 frequency categories per time period. For statistical analysis, these data were dichotomized into the presence or absence of the exposure based on an exposure frequency of at least once a week in a specific time period. Early-life exposure was then defined as the presence of the exposure in pregnancy or the first 3 years of life. The correlation between the dichotomized farm-related exposure variables was assessed by using the Kendall tau-b correlation coefficient. For assessment of the diversity of exposures, a score was generated by summing up all dichotomous farm exposures depicted in Table III and dividing the sum into quartiles based on the weighted distribution in the study sample.

Data from phase II were analyzed by using weighted statistical methods, taking the specific stratified sampling design into account. Fixed *a priori* weights were calculated as the inverse of the ratio of selected to eligible children per center and strata. All analyses were weighted to the total *n* value of the study population of phase I eligible for phase II. Missing values in selected children led to slightly diminished numbers per analysis. For the final logistic regression models, a sensitivity analysis was performed by using weights additionally adjusted for missing values in the variables included in the respective model, thus truly weighting the assessed data to the total *n* value. However, results remained unchanged (data not shown).

For phase I, categorical variables are presented as relative frequencies; *P* values are based on the Pearson χ^2 test. For phase II, categorical variables are presented as weighted relative frequencies and compared over categories by using the Rao-Scott χ^2 test, which applies a design effect correction to the Pearson χ^2 statistic computed from the weighted frequencies.

In phase I LCA was used to derive different types of farming.[E1] LCA is a statistical method for finding subtypes of related subjects (latent classes) from multivariable categorical data. Farmers of our study population were clustered into a number of discrete latent classes based on the pattern of response to various questions on farm characteristics (types of animal breeding, cultivation, and animal feeding), as assessed in the phase I questionnaire. The posterior probability of each subject belonging to a particular class was estimated, and from these data, logistic regression was used to estimate associations of the respective classes or "farm types" with asthma, hay fever, atopic dermatitis, and atopic sensitization.

In phase II, weighted logistic regression models using the Taylor series method to estimate variances were used to calculate associations between dichotomous outcomes and farm-related exposures. All models were adjusted for farming, center, and potential confounders differing between farm and nonfarm children (family atopy, ≥2 siblings, sex, maternal smoking in pregnancy, and parental education). Stepwise logistic regression analyses were calculated to assess final models containing the most relevant exposures to detect specific exposure variables underlying the overall farm effect. The aim of this procedure was to explain the farm effect, and thus all exposure variables that were significant and that induced a change of at least 10% in the effect of farming toward the null-effect in farm- and confounder-adjusted analysis were included in this process. At each forward step of this model-building procedure, the exposure inducing the largest change in estimate for farming was additionally included in the multivariate model if significant. In a backward step variables were removed from the model if no longer significant. The model building ended if no additional exposure was significant if included in the model. Combined effects of all dichotomized farm related exposure variables were defined as 4-level categorical variables to detect exposures that only exert an effect if occurring concurrently with another exposure: (− −), both variables negative (reference category for statistical analysis); (+ −)/(− +), 1 variable positive; and (+ +), both variables positive. If these combined exposures induced a change of 10% or greater in the farm effect and if only the (+ +) category was significant in farm- and confounder-adjusted analysis, as well as the overall type III *P* value, this categorical variable was included in the stepwise procedure based on the type III *P* value and the change in farm effect. For phase I and phase II analyses within the group of farm children, unweighted center- and confounder-adjusted logistic regression models using the same method to estimate variances as for weighted analyses were applied. aORs and 95% CIs are reported.

Statistical analysis was performed with SAS 9.2 software (SAS Institute, Inc); a *P* value of .05 was considered significant. Because of the exploratory character of the analysis, corrections for multiple testing were not performed.

REFERENCES

E1. McCutcheon AC. Latent class analysis. Beverly Hills: Sage Publications; 1987.
E2. Moffatt MF, Gut IG, Demenais F, Strachan DP, Bouzigon E, Heath S, et al. A large-scale, consortium-based genomewide association study of asthma. N Engl J Med 2010;363:1211-21.
E3. Ege MJ, Mayer M, Normand AC, Genuneit J, Cookson WO, Braun-Fahrlander C, et al. Exposure to environmental microorganisms and childhood asthma. N Engl J Med 2011;364:701-9.
E4. Ege MJ, Strachan DP, Cookson WO, Moffatt MF, Gut I, Lathrop M, et al. Gene-environment interaction for childhood asthma and exposure to farming in Central Europe. J Allergy Clin Immunol 2011;127:138-44.
E5. Normand AC, Sudre B, Vacheyrou M, Depner M, Wouters IM, Noss I, et al. Airborne cultivable microflora and microbial transfer in farm buildings and rural dwellings. Occup Environ Med 2011;68:849-55.
E6. Genuneit J, Buchele G, Waser M, Kovacs K, Debinska A, Boznanski A, et al. The GABRIEL Advanced Surveys: study design, participation and evaluation of bias. Paediatr Perinat Epidemiol 2011;25:436-47.
E7. Loss G, Apprich S, Waser M, Kneifel W, Genuneit J, Buchele G, et al. The protective effect of farm milk consumption on childhood asthma and atopy: the GABRIELA study. J Allergy Clin Immunol 2011;128:766-73.
E8. Riedler J, Braun-Fahrlander C, Eder W, Schreuer M, Waser M, Maisch S, et al. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. Lancet 2001;358:1129-33.
E9. 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;61:414-21.

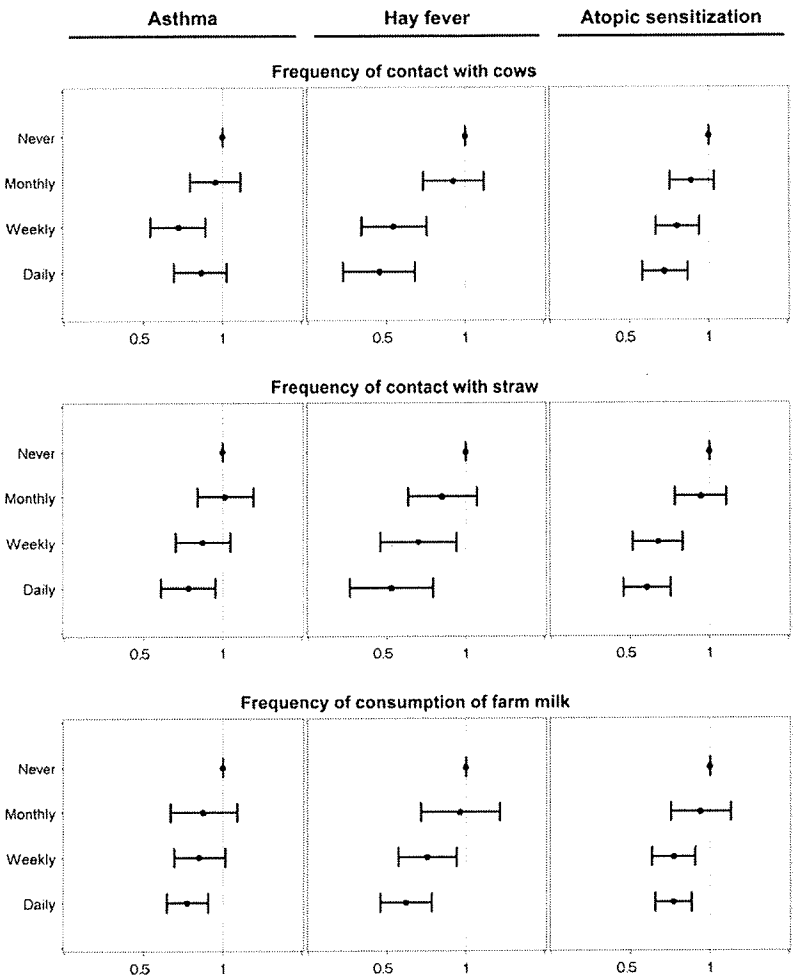

FIG E1. Frequency of exposure and risk of asthma, hay fever, and atopic sensitization. Frequency of exposure is defined as the maximum exposure of the 3 time periods (pregnancy, first year of life, and second to third year of life). aORs and 95% CIs are adjusted for farming and potential confounders.

TABLE E1. Previous publications from the GABRIEL Study Consortium

Publication	Title/key message
Moffatt et al,[E2] N Engl J Med 2010	A large-scale, consortium-based, genome-wide association study of asthma → This genome-wide association study found little overlap between the principal loci that confer susceptibility to asthma and those that regulate total serum IgE levels. This suggests that an increase in IgE level is probably an inconstant secondary effect of asthma rather than its cause.
Ege et al,[E3] N Engl J Med 2011	Exposure to environmental microorganisms and childhood asthma → Children living on farms were exposed to a wider range of microbes than were children in the reference group. This exposure explained a substantial fraction of the inverse relation between asthma and growing up on a farm. In contrast, atopy was only weakly associated with the diversity of microbes.
Ege et al,[E4] J Allergy Clin Immunol 2011	Gene-environment interaction for childhood asthma and exposure to farming in Central Europe → A genome-wide interaction analysis revealed several novel interaction candidate genes for asthma and atopy in a farming environment. In turn, the top single nucleotide polymorphisms of a meta-analysis for childhood asthma did not interact with farming. Previously published interactions with farming-related exposures for asthma and atopy were not replicated.
Normand et al,[E5] Occup Environ Med 2011	Airborne cultivable microflora and microbial transfer in farm buildings and rural dwellings → Microorganisms are transported from animal sheds and barns into farm dwellings. Therefore children living in these environments are exposed when indoors and when visiting animal sheds and barns. Indoor exposure might also contribute to the protective effect of the farm environment.
Genuneit et al,[E6] Paediatr Perinat Epidemiol 2011	The GABRIEL Advanced Surveys: study design, participation, and evaluation of bias → The GABRIEL Advanced Surveys are one of the largest studies to shed light on the protective "farm effect" on asthma and atopic disease. Bias with regard to the main study question was able to be ruled out by representativeness and high participation rates in phases 2 and 3. The GABRIEL Advanced Surveys have created extensive collections of questionnaire data, biomaterial, and environmental samples, promising new insights into this area of research.
Loss et al,[E7] J Allergy Clin Immunol 2011	The protective effect of farm milk consumption on childhood asthma and atopy: the GABRIELA study → Questionnaire-reported consumption of unboiled but not boiled farm milk was inversely associated with asthma, hay fever, and atopy. Higher levels of the whey proteins BSA, α-lactalbumin, and β-lactoglobulin in milk samples were associated with a reduced risk of asthma but not atopy. Neither total viable bacterial counts nor total fat content of milk were related to asthma or atopy.
MacNeill et al, Allergy 2011, submitted	Asthma and allergies: Is the farming environment (still) protective in Poland? The GABRIEL Advanced Studies → This cross-sectional survey of schoolchildren in rural Poland showed that living on certain types of farms is significantly protective against atopic sensitization. Early-life exposure to grain might explain part of this effect.
Fuchs et al, J Allergy Clin Immunol 2011, in revision	Farming environments and childhood atopy, wheeze, lung function, and exhaled nitric oxide → The protective farm effect on wheeze prevalence is independent of atopy and not attributable to improved airway size and lung mechanics. Underlying protective mechanisms include alterations of immune response and susceptibility to likely viral triggers of childhood airway disease also affecting airway inflammation.
Illi et al, J Allergy Clin Immunol 2012	Protection against childhood asthma and allergy in Alpine farm environments—the GABRIEL Advanced Studies → Specific types of farms with cows and cultivation exerted a protective effect on asthma, hay fever, and atopic sensitization. This protective farm effect on asthma, hay fever, and atopic sensitization was determined by 3 specific early-life exposures of the child, namely by contact with cows and straw and consumption of farm milk, thereby narrowing down the farm effect. However, whereas the farm effect on asthma could be completely explained by these, this was not the case for hay fever and atopic sensitization, indicating differing underlying mechanisms in spite of comanifestation of these outcomes.

TABLE E2. Specific farm exposures that best explain the overall effect of farming on asthma, hay fever, atopic dermatitis, and atopic sensitization, as identified in multivariate stepwise regression models*

	aOR§	95% CI	*P* value
Asthma†			
− Contact with cows, − contact with straw	1.00	—	—
− Contact with cows, + contact with straw	1.00	0.76-1.32	1.00
+ Contact with cows, − contact with straw	0.94	0.73-1.21	.63
+ Contact with cows, + contact with straw	0.68	0.54-0.85	<.001
Consumption of farm milk	0.81	0.68-0.96	.02
Farming	0.89	0.75-1.06	.20
Hay fever†			
Contact with cows	0.59	0.46-0.76	<.001
Consumption of farm milk	0.71	0.58-0.86	<.001
Farming	0.68	0.55-0.84	<.001
Atopic dermatitis†			
Stay in fodder storage room	0.72	0.55-0.93	.01
Farming	0.88	0.75-1.04	.12
Atopic sensitization‡			
Contact with straw	0.66	0.56-0.78	<.001
Consumption of farm milk	0.77	0.67-0.88	<.001
Farming	0.74	0.64-0.86	<.001

*Weighted logistic regression models with stepwise variable selection for asthma, hay fever, atopic dermatitis, and atopic sensitization based on the largest change in estimate for farming after adjusting for confounding variables. All significant exposure variables (pregnancy to age 3 years) from previous farm- and confounder-adjusted analyses that induced a change in estimate of farming of 10% or greater toward the null effect were included in the selection process.

†Outcomes assessed in phase II questionnaire: n = 8,419; analyses weighted to eligible subjects for phase II (n = 34,491).

‡Outcome assessed in phase II blood sampling: n = 7,682; analyses weighted to eligible subjects for phase II (n = 34,491).

§Mutually adjusted and additionally adjusted for center and potential confounders (family atopy, ≥2 siblings, sex, maternal smoking in pregnancy, and parental education).

TABLE E3. Final multivariate models for asthma, hay fever, atopic dermatitis, and atopic sensitization adjusted for diversity score*

	aOR§	95% CI	*P* value
Asthma†			
− Contact with cows, − contact with straw	1.00	—	.08
− Contact with cows, + contact with straw	0.90	0.60-1.37	.64
+ Contact with cows, − contact with straw	0.90	0.64-1.26	.53
+ Contact with cows, + contact with straw	0.66	0.46-0.96	.03
Consumption of farm milk	0.72	0.59-0.89	.002
Farming	0.97	0.80-1.18	.76
Diversity score*			
0	1.00	—	.66
1	1.09	0.86-1.37	.47
2-4	1.17	0.91-1.51	.21
≥5	1.13	0.77-1.66	.53
Hay fever†			
Contact with cows	0.57	0.42-0.77	<.001
Consumption of farm milk	0.62	0.50-0.78	<.001
Farming	0.71	0.55-0.92	.009
Diversity score*			
0	1.00	—	.42
1	1.11	0.85-1.44	.45
2-4	1.24	0.94-1.64	.13
≥5	1.28	0.90-1.82	.16
Atopic dermatitis†			
Stay in fodder storage room	0.72	0.52-0.98	.04
Farming	0.98	0.79-1.21	.84
Diversity score*			
0	1.00	—	.29
1	0.89	0.69-1.14	.35
2-4	1.02	0.80-1.31	.87
≥5	0.82	0.63-1.06	.14
Atopic sensitization‡			
Contact with straw	0.66	0.53-0.83	<.001
Consumption of farm milk	0.77	0.65-0.91	.002
Farming	0.79	0.67-0.94	.009
Diversity score*			
0	1.00	—	.57
1	0.96	0.78-1.18	.69
2-4	0.86	0.69-1.06	.17
≥5	0.92	0.70-1.20	.53

*The diversity score is defined as the number of exposures divided into quartiles based on the weighted distribution in the study sample.
†Outcomes assessed in phase II questionnaire: n = 8,419; analyses weighted to eligible subjects for phase II (n = 34,491).
‡Outcome assessed in phase II blood sampling: n = 7,682; analyses weighted to eligible subjects for phase II (n = 34,491).
§Mutually adjusted and additionally adjusted for center and potential confounders (family atopy, ≥2 siblings, sex, maternal smoking in pregnancy, and parental education).

TABLE E4. Prevalence of asthma, hay fever, and atopic dermatitis diagnoses and atopic sensitization in farm studies

	ALEX*		PARSIFAL†		GABRIEL phase I		GABRIEL phase II‖	
	Farm children‡	Nonfarm children	Farm children§	Nonfarm children	Farm children§	Nonfarm children	Farm children§	Nonfarm children
Asthma diagnosis ever	5.4%	11.8%	6.3%	9.1%	6.5%	10.1%	8.3%	12.1%
Hay fever diagnosis ever	5.9%	15.9%	1.3%	4.4%	3.0%	9.5%	3.9%	10.6%
Atopic dermatitis diagnosis ever	—	—	7.1%	9.9%	10.6%	14.5%	12.8%	17.8%
Atopic sensitization¶	17.9%	32.9%	22.7%	34.7%	—	—	24.7%	40.8%

*Riedler et al.[E8]

†Alfven et al.[E9]

‡Farm children were defined as children with contact with farm milk or stables ever.

§Farm children were defined as children currently living on a farm run by the child's family.

‖Weighted prevalences (weighted to GABRIEL phase I).

¶ALEX: IgE ≥3.5 kU/L for house dust/storage mites, cat, grass, birch, and cow; PARSIFAL: IgE ≥0.35 kU/L in Phadiatop or mix of common food allergens (fx5); GABRIEL: IgE ≥0.70 kU/L for house dust mite, cat, and birch or IgE ≥0.35 kU/L for grass mix.

SCIENCE AND SOCIETY

Farm living: effects on childhood asthma and allergy

Erika von Mutius and Donata Vercelli

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

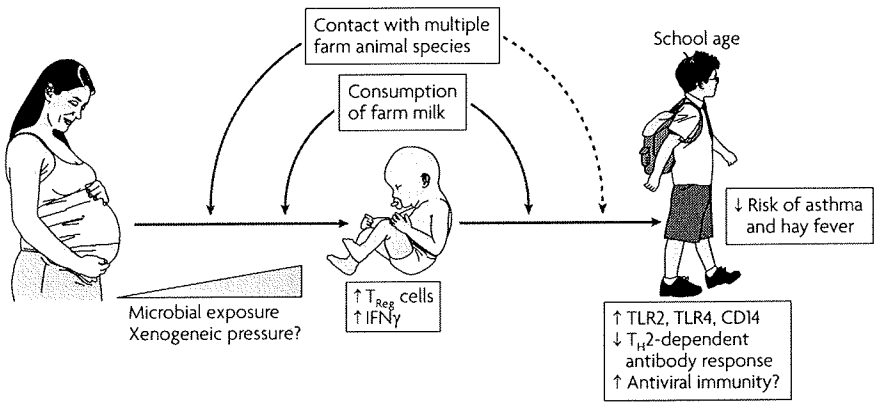

Figure 1 | **A working model of the immunobiology of farm exposure.** Contact with multiple animal species, combined with consumption of farm milk, results in strong microbial exposure of, and possibly xenogeneic pressure on, women who carry out farming duties during pregnancy. These combined exposures,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

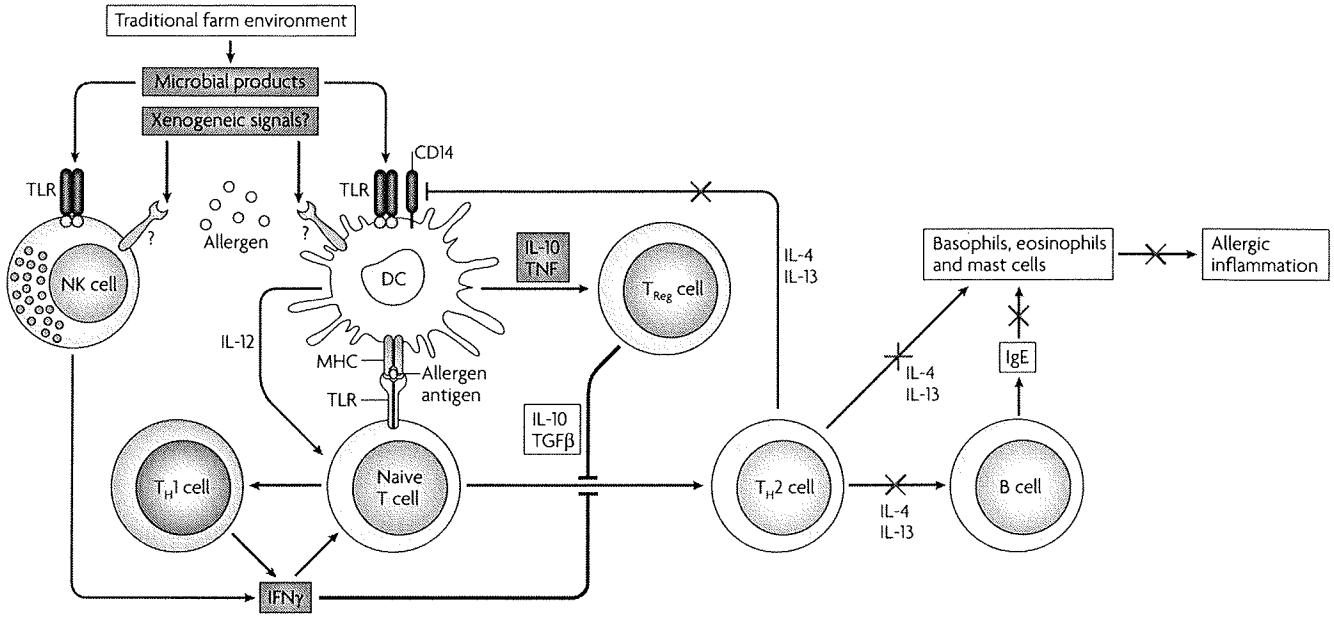

Figure 2 | **Mechanisms potentially underlying the impact of farm exposure on the human immune system.** In this model, the biological diversity of a traditional farm environment (in particular, high numbers of farm animal species) results in intense microbial pressure on the innate immune system. This 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.

Erika von Mutius is at the Dr. von Haunersche Kinderklinik Ludwig Maximilians Universität, 80337 Munich, Germany.

Donata Vercelli is at the Arizona Respiratory Center, Arizona Center for the Biology of Complex Diseases and Department of Cell Biology, University of Arizona, Tucson, Arizona 85719, USA.

e-mails: erika.von.mutius@med.lmu.de; donata@arc.arizona.edu

doi:10.1038/nri2871
Published online 9 November 2010

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
See online article: S1 (table)

ALL LINKS ARE ACTIVE IN THE ONLINE PDF

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].