

ORIGINAL ARTICLE

Association between endotoxin and mite allergen exposure with asthma and specific sensitization at age 7 in high-risk children

Chris Carlsten¹, Alexander Ferguson², Helen Dimich-Ward¹, Henry Chan¹, Anne DyBuncio¹, Roxanne Rousseau¹, Allan Becker³ & Moira Chan-Yeung¹

¹Occupational and Environmental Lung Disease Unit, Respiratory Division, Department of Medicine; ²Division of Allergy, Department of Pediatrics, University of British Columbia, Vancouver, BC, Canada; ³Section of Allergy and Clinical Immunology, Department of Pediatrics and Child Health, and the Manitoba Institute of Child Health, University of Manitoba, Winnipeg, MB, Canada

To cite this article: Carlsten C, Ferguson A, Dimich-Ward H, Chan H, DyBuncio A, Rousseau R, Becker A, Chan-Yeung M. Association between endotoxin and mite allergen exposure with asthma and specific sensitization at age 7 years in high-risk children. *Pediatr Allergy Immunol* 2011; 10.1111/j.1399-3038.2010.01123.x

Keywords

asthma; allergen sensitization; endotoxin; house dust mite allergen.

Correspondence

Dr. Chris Carlsten, The Lung Centre, 7th Floor, 2775 Laurel Street, Vancouver, BC, Canada V5Z 1M9.

Tel.: 604 875 4122

Fax: 604 875 4695

E-mail: chris.carlsten@vch.ca

Accepted for publication 21 November 2010

DOI:10.1111/j.1399-3038.2010.01123.x

Abstract

The role of endotoxin and house dust mite allergen (HDM) in allergen sensitization and asthma is unclear. The timeframe of exposure and asthma assessment appears critical. We aimed to determine, in children at 7 yr of age, the association between current exposure to endotoxin and HDM and risks of recurrent wheeze, paediatric allergist diagnosed asthma and allergen sensitization. Three hundred and eighty children who had an increased risk of asthma because of family background were assessed at age 7 yr by a questionnaire-standardized interview, allergen skin testing and clinical examination by a paediatric allergist. Dust samples were collected from their homes and analysed for levels of endotoxin and HDM (Der p 1 and Der f 1). Levels of endotoxin in dust samples were associated with protection from paediatric allergist diagnosed asthma with inhaled steroid use (OR 0.69, 95% CI 0.53–0.91) and specific sensitization to dog allergen (OR 0.68, 95% CI 0.51–0.90) at the age of 7 yr; both endotoxin and HDM were associated with decreased risk of sensitization to dog allergen. In high-risk children at age 7, endotoxin levels were associated with decreased sensitization to dog, as well as with decreased asthma.

Endotoxin is the outer lipopolysaccharide component of gram-negative bacteria cell walls (1). It has been well established that occupational exposure to endotoxin in the grain and cotton industries leads to chronic airway diseases (2, 3). Although it has been accepted that high endotoxin exposure is associated with acute exacerbation of disease in patients with asthma (4), the role of endotoxin in the inception of asthma and allergen sensitization is not clear. In some studies, exposure to high levels of endotoxin is associated with increased prevalence of wheezing (5, 6); in others, it is associated with a protective effect (7, 8). Recent literature suggests that endotoxin can affect the airways or sensitization at any time and not necessarily limited to early life (9, 10).

The role of allergen exposure in sensitization is also controversial. While a dose-dependent relationship was found between the levels of exposure and the prevalence of sensitization to house dust mite (HDM) allergen, such a relationship was not found with other aeroallergens such as cat (11). There was no relationship between the levels of exposure to

indoor aeroallergens and the prevalence of asthma in childhood although the risk of asthma is increased with presence of sensitization to these allergens (12–14).

In this report, we examined the relationship between current levels of endotoxin or HDM allergen in homes and the prevalence of asthma diagnosis and specific sensitization to various aeroallergens at 7 yr of age, in children at high-risk of developing asthma and allergic diseases.

Methods

Study population

The children in this study were recruited between 1994 and 1995 for a study on the primary prevention of asthma. Detailed descriptions of this cohort have been published previously (15–17). Briefly, mothers of high-risk children, defined as those with at least one first degree relative with asthma or two first degree relatives with other allergic diseases such as

hay fever or atopic eczema were recruited during the third trimester of pregnancy. In parents and older siblings, the presence of these disorders was self-reported. After assessment of the home environment, the families were randomized into intervention and control groups. The intervention groups received assistance to reduce early exposure to specific allergens and environmental risk factors for asthma whereas the control group received usual care from their physicians (15–17). As noted further below, we were cautious to account for this group randomization in subsequent analyses.

House dust samples were collected at the time of the assessment of the children at 7 yr. The sites sampled were the children's bedroom floor and mattress, the floor of the most commonly used room and the upholstered furniture in that room using a standard protocol (15); the four sites' samples were mixed before extraction.

At the age of 7 yr, the children were interviewed by a research nurse using an asthma questionnaire used in the International Study of Asthma and Allergies in Children (18). In each centre, children were examined by a single paediatric allergist who had no knowledge of the randomization, did not participate in the care of the children or their families and was not aware of the results of the questionnaire, allergen skin tests, or methacholine challenge tests that were done as part of the assessment.

Of the original cohort of 545 families recruited, 380 were examined and assessed by the two paediatric allergists; methacholine challenge was carried out on 348, allergen skin tests on 366 and dust samples collected from the homes of 334 children at 7 yr of age.

Allergen skin tests

Allergen skin tests were performed using the epicutaneous method with a prick lanceter (Bayer Inc., Canada) with the following allergens (Bayer Inc.): *Dermatophagoides pteronyssinus* and *farinae*, cat, dog, and *Alternaria alternata*. In our prior studies, we obtained data on a broader panel of antigens but limited analyses in this study to the allergens above, pre-selected as common aeroallergens in order to limit comparisons. A mean wheal diameter 3 mm or greater than the negative control was considered a positive reaction. A positive histamine (0.1%) control was used each time.

Determination of mite allergen levels

The house dust samples were analysed in duplicate for HDM allergens using ELISA with purified monoclonal antibodies against group 1 mite allergens, Der p 1 and Der f 1 (19). The total HDM allergen (sum of Der p 1 and Der f 1) was averaged and reported as µg/g dust.

Measurement of endotoxin

Dust samples were extracted according to the method described by Park et al. (5) at a concentration of 5 mg/ml (0.05 M potassium phosphate with 0.01% triethylamine, pH 7.5) in a sonic bath for 60 min at room temperature. The

supernatant after centrifugation was then immediately analysed using a Limulus Amebocyte Lysate (LAL), QCL-1000 test kit (Cambrex Bio Science Walkersville Inc., Walkersville, MD, USA). Briefly, 50 µl of sample (in three different serial dilutions), standards (0.1–1.0 EU/ml, EU = endotoxin unit) and a blank were added into the appropriate microplate well. The microplate was pre-equilibrated at 37°C in a heating block. At time zero, 50 µl of LAL was added to each well. One hundred microlitre of substrate (pre-warmed to 37°C) was added to each well at 10 min followed by 100 µl of stop reagent at 16 min. Once the stop reagent was added, the plate was removed from the heating block and the absorbance was read at 405 nm in an ELISA reader. A standard graph was generated (absorbance vs. optical density) and the sample results were read from the graph.

Ethics

This study was carried with the approval of the Clinical Ethics Review Boards of the University of British Columbia and the University of Manitoba.

Data analysis

Altogether, 334 children were examined by a paediatric allergist had allergen skin tests and had house dust samples collected from their homes.

Levels of HDM and endotoxin in dust samples measured at 7 yr were log-normally distributed and analyses were performed after logarithmic transformation. The correlation between exposure variables was determined by Pearson correlation. The following outcomes at 7 yr of age were used in the analyses: recurrent wheeze (defined as 3 or more episodes of wheeze in the past 12 months); asthma (paediatric allergist-diagnosed asthma based on clinical assessment); asthma and use of medications for asthma in the past 12 months (either inhaled steroids or bronchodilator); and positive skin test to HDM allergen (*D. pteronyssinus* and *D. farinae*), cat allergen, dog allergen and *Alternaria*.

First, the linear-by-linear association test was used for relating sensitization to indoor aeroallergens with endotoxin and HDM allergen in dust tertiles. Then, odds ratios (OR) and 95% confidence intervals (CI) were obtained by stepwise multiple logistic regression analyses to examine the adjusted risk of each of the outcomes associated with continuous levels of endotoxin or HDM allergen (separately) with the following independent variables included in the model: sex, race, first born, history of asthma (in mother, in father and/or older siblings), maternal education, city of residence, and intervention versus control group allocation.

Statistical analysis was carried out using STATA (Stata/SE 8.0, College Station, TX, USA) and SPSS/PC Version 10 (SPSS, Chicago, IL, USA.)

Results

The characteristics of children who returned for assessment at age 7 yr were not different from those who did return

Table 1 Endotoxin level and characteristics of families and homes of children at 7 yr of age

	N	Endotoxin EU/mg dust, geom mean (s.d.)	p-value	House dust mite µg/g dust, geom mean (s.d.)	p-value
Winnipeg	175	62.6 (3.45)	0.775	1.98 (3.44)	0.045
Vancouver	159	60.4 (2.66)		2.92 (3.42)	
<i>Mother education</i>					
No post- secondary	73	59.4 (3.48)	0.762	2.75 (3.65)	0.506
Post- secondary	261	62.2 (2.74)		2.36 (3.39)	
<i>No of people in the home</i>					
≤3	50	77.4 (2.46)	0.122	5.86 (4.75)	0.786
>3	284	59.1 (3.16)		2.47 (3.50)	
<i>Pet ownership</i>					
No	210	56.7 (3.41)	0.063	2.55 (3.64)	0.534
Yes	124	70.8 (2.47)		2.26 (3.14)	
<i>Cat ownership</i>					
No	264	60.0 (3.10)	0.438	2.54 (3.14)	0.444
Yes	70	67.6 (2.96)		2.11 (3.45)	
<i>Dog ownership</i>					
No	289	59.7 (3.26)	0.044	2.43 (3.47)	0.931
Yes	45	74.7 (1.74)		2.49 (3.36)	
<i>Air conditioning</i>					
No	185	70.3 (3.08)	0.017	3.03 (3.36)	0.009
Yes	149	52.2 (2.99)		1.83 (3.47)	
<i>Water damage in the home</i>					
No	259	62.7 (3.07)	0.853	2.60 (3.56)	0.210
Yes	75	57.8 (3.05)		1.94 (3.04)	
<i>Visible mould in the home</i>					
No	171	60.7 (3.23)	0.585	1.83 (3.20)	0.003
Yes	163	62.4 (2.91)		3.22 (3.61)	
<i>Group allocation</i>					
Control	152	62.7 (2.93)	0.817	2.98 (3.85)	0.056
Intervention	182	80.6 (3.27)		2.06 (3.09)	

p-value by *t*-tests.

(data not shown) except for a higher proportion of non-whites among those who returned.

Table 1 shows the geometric mean and standard deviation of endotoxin and HDM allergen levels from the homes of the children who participated in this study by their family and home characteristics at 7 yr of age. Dog ownership was associated with a significantly higher level of endotoxin in the dust samples and homes with air conditioning had significantly lower level of endotoxin and HDM allergen compared with homes without air conditioning. Visible moulds in the homes were associated with increased level of HDM allergen levels but not endotoxin levels. A significant correlation was found between HDM allergen level and endotoxin level ($r = 0.12$, $p = 0.03$).

Tertiles of level of endotoxin were negatively related to sensitization to dog allergen; they had no statistical relationship with HDM, *alternaria*, or cat (Fig. 1).

Tertiles of levels of Der p 1/Der f 1 were related positively with the prevalence of sensitization to HDM allergen and negatively with sensitization to dog allergen (Fig. 2); they had no statistical relationship with *alternaria*, or cat.

Levels of endotoxin and Der p 1/Der f 1 in dust samples and respiratory outcomes

The prevalence of recurrent wheeze and paediatric allergist diagnosed asthma at 7 yr of age are shown in Table 2. Neither endotoxin nor Der p 1/Der f 1 were associated with

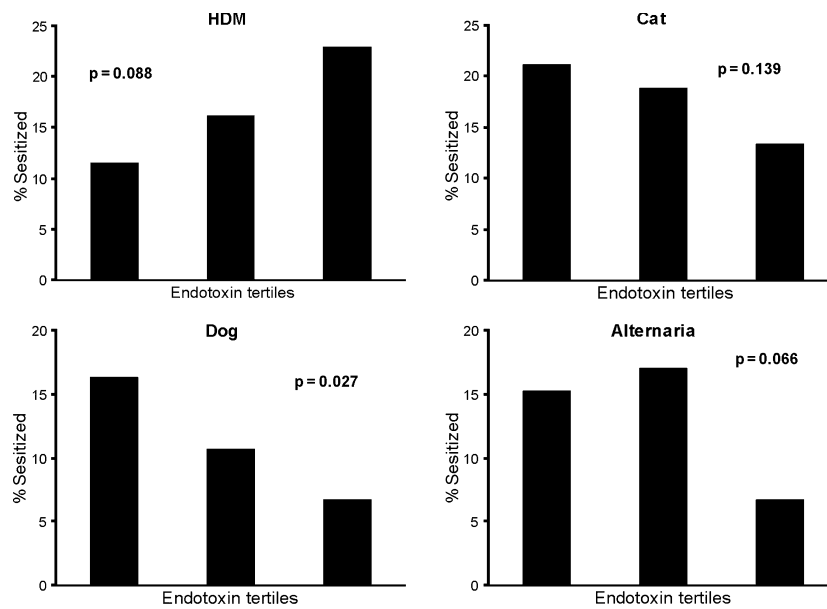


Figure 1 Prevalence of sensitization to aeroallergens by tertiles of endotoxin exposure. First tertile = 0–56.13 EU/g dust; second tertile = 56.13–103.5 EU/g dust; third tertile = >104.5 EU/g dust. p-value was derived from chi-square analysis for trend.

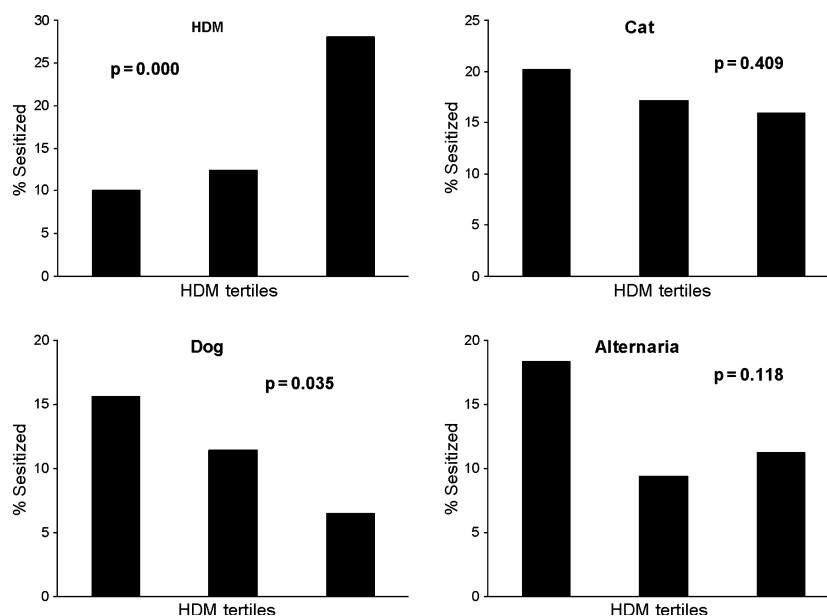


Figure 2 Prevalence of sensitization to aeroallergens by tertiles of house dust mite (HDM) exposure. First tertile = 0–0.34 µg/g dust; second tertile = 0.34–3.394 µg/g dust; third tertile = >3.394 µg/g dust. p-value was derived from chi-square analysis for trend.

Table 2 Association between endotoxin and house dust mite (HDM) allergen levels and risk of recurrent wheeze, asthma diagnosis – results of stepwise multiple logistic regression analysis

Outcome	N	Endotoxin	HDM allergen
		Adjusted odds ratio† (95% CI)	Adjusted odds ratio† (95% CI)
Recurrent wheeze	47 (12.4)	0.81 (0.60–1.08)	0.95 (0.71–1.27)
Paediatrician-diagnosed asthma	71 (18.7)	0.77 (0.60–1.00)*	1.02 (0.80–1.31)
Asthma + use of Broncodilator	49 (14.7)	0.72 (0.55–0.92)*	0.98 (0.76–1.28)
Asthma + use of inhaled steroids	39 (11.7)	0.69 (0.53–0.91)*	1.00 (0.75–1.33)

*p < 0.05; †Adjusted for sex, maternal history of asthma, father and sibling with asthma, race, firstborn, maternal education, city of residence and group allocation. Each odds ratio represents risk associated with each log-normalized unit of respective exposure.

Table 3 Association between endotoxin and house dust mite (HDM) allergen levels and risks of specific sensitization to aeroallergens – results of stepwise multiple logistic regression analyses

Positive skin test to	N (%)	Endotoxin	HDM allergen
		Adjusted odds ratio† (95% CI)	Adjusted odds ratio† (95% CI)
HDM allergen	62 (16.3)	1.33 (0.94, 1.87)	1.56 (1.22–1.99)*
Dog allergen	41 (10.8)	0.68 (0.51–0.90)*	0.65 (0.45–0.94)*
Cat allergen	68 (17.9)	0.82 (0.64–1.06)	0.93 (0.73–1.20)
Alternaria	52 (13.7)	0.77 (0.55–1.07)	0.85 (0.62–1.16)

* $p < 0.05$; †Adjusted for sex, race, firstborn, history of asthma in mother, history of asthma in father/sibling, maternal education, city of residence, group allocation. Each odds ratio represents risk associated with each log-normalized unit of respective exposure.

recurrent wheeze but endotoxin was associated with a significantly lower risk of paediatric allergist diagnosed asthma, asthma diagnosis with use of bronchodilators in the past 12 months and asthma diagnosis with use of inhaled corticosteroids in the past 12 months (Table 2).

Levels of endotoxin and HDM allergen in dust samples and allergen sensitization

Endotoxin in dust samples was associated with a significantly lower risk of sensitization to dog allergen [adjusted OR (per increase in one endotoxin-unit) 0.7, 95% CI 0.5–0.9] but was not significantly associated with risk in sensitization to other aeroallergens (Table 3).

A higher level of HDM allergen in dust samples was associated with a significantly higher risk of sensitization to HDM allergen [adjusted OR (per increase in one mg/ml HDM allergen) 1.56, 95% CI 1.22–1.99] but a lower risk of sensitization to dog allergen (adjusted OR 0.65, 95% CI 0.45–0.94) after adjustment for confounders (Table 3).

Discussion

This is a cross-sectional study of the relationship between levels of endotoxin and allergen in dust samples collected from the homes of children who at birth had a high-risk of developing asthma and allergic disease and the risk of adverse respiratory outcomes and positive skin test reactivity to common allergens at the age of 7 yr. We found that levels of endotoxin in urban homes of children at the age of 7 yr was associated with decreased risk of paediatric-allergist-diagnosed asthma especially when the definition required the use of asthmatic medications. Moreover, level of endotoxin was associated with a significantly reduced risk of positive skin test reactions to dog allergen. Not surprisingly, higher levels of HDM allergen in dust was associated with an increased risk of positive skin test reaction to HDM allergen; unexpectedly, higher levels of HDM allergen in dust was also associated with an decreased risk of positive skin test to dog allergen.

The role of endotoxin in the pathogenesis of asthma and allergic sensitization is complex. It seems that endotoxin can induce wheezing or prevent asthma depending on the dose, timing of exposure, host genetics and presence or absence of pre-existing disease. Endotoxin is known to cause acute exacerbations in those with pre-existing asthma (4). In children under the age of one, exposure to high levels of endotoxin was found to increase the risk of wheezing (5). On the other hand, early life exposure to endotoxin has been reported to be associated with a protective effect on subsequent development of allergy associated disease including asthma in a number of studies (7, 8). This protective effect exhibited a dose-dependent relationship with the number of farm animals and was attributed to exposure to endotoxin by the investigators and is supported by at least one other study (20, 21). Recently, studies have shown that the protective effect probably occurs in early life and even prenatally. Ege et al. (22) reported that maternal exposure to an environment rich in microbial compounds might protect against the development of allergic sensitization. Gerhold et al. (23) demonstrated that prenatal-initiated and post-natal-sustained lipopolysaccharide exposure increased endotoxin susceptibility and prevented later allergen sensitization in offspring through inhibition of T_H2 immune responses in a mouse model (8).

The association between high endotoxin exposure and lower prevalence of atopy has been reported not only in early life but also in later childhood and in adulthood (8, 24–26). Our finding of significantly reduced prevalence of sensitization to dog allergens and the diagnosis of asthma, in those exposed to higher levels of endotoxin, is in accord with these prior findings. We do not know the age at which endotoxin exposure is most influential as we do not have data on endotoxin exposure levels prior to 7 yr of age; although this group of children is a birth cohort and we have collected house dust samples during early life, the dust samples were originally extracted for the purpose of measurement of indoor aeroallergens and previous thawing and re-freezing rendered the samples unsuitable for measurement of endotoxin. However, it has been shown that within-home levels of endotoxin are well-correlated over several years of childhood (21).

The production of specific IgE antibodies has been shown to be influenced by the level of endotoxin exposure in animal models (27, 28). In a mouse model of IgE-specific tolerance to ovalbumin, low dose exposure to aerosolized endotoxin (LPS 40 ng/ml) just before and once during the 2 wk of administration of ovalbumin reversed the development of IgE-specific tolerance leading to higher levels of ovalbumin-specific IgE (27). On the other hand, Tulic et al. (28) exposed rats to 1000 times higher concentration of endotoxin than those used by Wan et al. (27) either one day before or up to 6 days after ovalbumin injection intraperitoneally and prevented the specific IgE response. The levels of endotoxin in dust samples from the homes of our children were high, with a median of 75.2 EU/mg dust, and this may explain the significantly protection from dog allergen sensitization associated with endotoxin in our study. The discrepancy in the effect of endotoxin on sensitization to different allergens is difficult to explain biologically. Study of molecular struc-

tures of various allergens may help us to understand the mechanism.

HDM allergens in dust samples in this study were not related to risks of recurrent wheeze, similar to findings of other investigators (12–14). A significantly reduced risk of sensitization to dog was found to be associated with increasing levels of HDM allergen. This finding cannot be explained by the high levels of endotoxin in dust samples with high HDM allergen levels as there was no correlation between the levels of endotoxin and HDM. Other microbial agents not represented by endotoxin, such as beta-glucan, may also be responsible for the protective effect (29).

In addition to the lack of measurement of endotoxin in dust samples collected in early life, the children in this study were selected to be at high-risk for developing asthma. For this reason, the results of this study are applicable only to high-risk children and may not be applicable to children in the general population. A limitation of our analysis is that we are not able to account for all the environmental factors that may have changed incrementally in the years following birth.

In summary, current exposure to either endotoxin or HDM allergen was not associated with the presence of recurrent wheeze; endotoxin was associated with a significantly lower risk of asthma, by various definitions, in this high-risk cohort. Current exposure to higher levels of endotoxin reduced significantly the risks of sensitization dog allergen. HDM allergen independently and significantly increased the risk of sensitization to HDM allergen and decreased the risk of sensitization to dog. Further studies including measurement of specific IgE and specific IgG antibodies to various allergens and with consideration of timing of exposure are necessary to elucidate the relationship between exposures to endotoxin, aeroallergens and sensitization.

Acknowledgments

We wish to thank the children and their families for participating in this study, the students and the research staff who took part in making the study possible. The Study was supported by the Canadian Institutes of Health Research and the British Columbia Lung Association.

References

- Jagiello PJ, Thorne PS, Watt JL, et al. Grain dust and endotoxin inhalation challenges produce similar inflammatory responses in normal subjects. *Chest* 1996; **110**: 263–70.
- Schwartz DA, Thorne PS, Yagla SJ, et al. The role of endotoxin in grain dust-induced lung disease. *Am Rev Respir Dis* 1995; **152**: 603–8.
- Castellan RM, Olenchock SA, Kinsley KB, Hankinson JL. Inhaled endotoxin and decreased spirometric values: an exposure-response relation for cotton dust. *N Engl J Med* 1987; **317**: 605–10.
- Rizzo MC, Naspitz CK, Fernandez-Caldas E, Lockey RF, Mimica I, Sole D. Endotoxin exposure and symptoms in asthmatic children. *Pediatric Allergy Immunol* 1997; **8**: 121–6.
- Park JH, Gold DR, Spiegelman DL, et al. House dust endotoxin and wheeze in the first year of life. *Am J Respir Crit Care Med* 2001; **163**: 322–8.
- Thorne PS, Kulhankova K, Yin M, et al. Endotoxin exposure is a risk factor for asthma: the national survey of endotoxin in United States housing. *Am J Respir Crit Care Med* 2005; **172**: 1371–7.
- Kilpelainen M, Terho EO, Helenius H, Koskenvuo M. Farm environment in childhood prevents the development of allergies. *Clin Exp Allergy* 2000; **30**: 201–8.
- Braun-Fahrlander C, Riedler J, Herz U, et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. *N Engl J Med* 2002; **347**: 869–77.
- Reed CE, Milton DK. Endotoxin-stimulated innate immunity: a contributing factor for asthma. *J Allergy Clin Immunol* 2001; **108**: 157–66.
- Douwes J, Le Gros G, Gibson P, Pearce N. Can bacterial endotoxin exposure reverse atopy and atopic disease? *J Allergy Clin Immunol* 2004; **114**: 1051–4.
- Sporik R, Squillace SP, Ingram JM, et al. Mite, cat and cockroach exposure, allergen sensitization and asthma in children: a case-control study of three schools. *Thorax* 1999; **54**: 675–80.
- Lau S, Illi S, Sommerfeld C, et al. Early exposure to house dust mite and cat allergens and development of childhood asthma: a cohort study. *Lancet* 2000; **356**: 1392–7.
- Custovic A, Hallam CC, Simpson BM, et al. Decreased prevalence of sensitization to cats with high exposure to cat allergen. *J Allergy Clin Immunol* 2001; **108**: 537–9.
- Lau S, Illi S, Platts-Mills TAE, et al. Longitudinal study in the relationship between cat allergen and endotoxin exposure, sensitization, cat-specific IgG and development of asthma in childhood-report of the German Multicentre Allergy Study (MASn90). *Allergy* 2005; **60**: 766–73.
- Chan-Yeung M, Manfreda J, Dimich-Ward H, et al. A randomized controlled study on the effectiveness of a multifaceted intervention program in the primary prevention of asthma in high-risk infants. *Arch Pediatr Adolesc Med* 2000; **154**: 657–63.
- Becker A, Watson W, Ferguson A, et al. The Canadian asthma primary prevention study: outcomes at 2 years of age. *J Allergy Clin Immunol* 2004; **113**: 650–6.
- Chan-Yeung M, Ferguson A, Dimich-Ward H, et al. The Canadian Primary Prevention of Asthma in Childhood- outcomes at 7 years. *J Allergy Clin Immunol* 2005; **116**: 49–55.
- ISAAC Coordinating Committee. Manual for the International Study of Asthma and Allergies in Childhood (ISAAC). Bochum and Aukland: ISAAC coordinating Committee, 1992.
- Luczynska C, Arruda L, Platts-Mills T, et al. A two-site monoclonal antibody ELISA for the quantification of the major *Dermatophagoides* spp. allergens, Der p 1 and Der f 1. *J Immunol Methods* 1989; **118**: 227–35.
- Peters M, Kauth M, Schwarze J, et al. Inhalation of stable dust extract prevents allergen induced airway inflammation and hyperresponsiveness. *Thorax* 2006; **61**: 134–9.
- Abraham JH, Gold DR, Dockery DW, Ryan L, Park JH, Milton DK. Within-home versus between-home variability of house dust endotoxin in a birth cohort. *Environ Health Perspect* 2006; **113**: 1516–21.
- Ege MJ, Bieli C, Frei R, et al. Prenatal farm exposure is related to the expression of receptors of the innate immunity and to atopic sensitization in school-age children. *J Allergy Clin Immunol* 2006; **117**: 817–23.
- Gerhold K, Avagyan A, Seib C, et al. Prenatal initiation of endotoxin airway exposure prevents subsequent allergen-induced sensitization and airway inflammation in mice. *J Allergy Clin Immunol* 2006; **118**: 666–73.
- Portengen L, Sigsgaard T, Omland O, et al. Low prevalence of atopy of young Danish farmers and farming students born and raised on a farm. *Clin Exp Allergy* 2002; **32**: 247–53.

25. Eduard W, Douwes J, Omenaas E, et al. Do farming exposure cause or prevent asthma? Results from a study among adult Norwegian farmers. *Thorax* 2004; **59**: 381–6.
26. Schram-Bijkerk D, Doekes G, Boeve M, et al. Bacterial and fungal components in house dust of farm children, Rudolf Steiner school children and reference children. *Allergy* 2005; **60**: 611–8.
27. Wan GH, Lin CS, Lin RH. Airborne endotoxin exposure and the development of airway antigen-specific allergen responses. *Clin Exp Allergy* 2000; **30**: 426–32.
28. Tulic MK, Wale JL, Holt PG, Sly PD. Modification of the inflammatory response to allergen challenge after exposure to bacterial liposaccharide. *Am J Respir Cell Mol Biol* 2000; **22**: 604–12.
29. Schram-Bijkerk D, Doekes G, Boeve M, et al. Nonlinear relations between house dust mite allergen levels and mite sensitization in farms and nonfarm children. *Allergy* 2006; **61**: 640–7.