Original Paper

International Archives of Allergy and Immunology

Int Arch Allergy Immunol 2010;152:151–158
DOI: 10.1159/000265536

Received: June 2, 2009
Accepted after revision: August 11, 2009
Published online: December 16, 2009

Occupational Endotoxin Exposure Reduces the Risk of Atopic Sensitization but Increases the Risk of Bronchial Hyperresponsiveness

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Key Words

Asthma · Atopy · Bronchial responsiveness · Epidemiology · Farming · Hygiene hypothesis · Occupational exposure

Abstract

Background: Microbial exposures in both childhood and adult life are protective against atopy, allergic rhinitis and atopic asthma. In adults, this protective effect is paralleled by an increased prevalence of non-atopic asthma. This study was undertaken to investigate associations between occupational endotoxin exposure and atopic sensitization and bronchial hyperresponsiveness to methacholine (BHR) in agricultural workers. In addition, the role of atopy in endotoxin-related respiratory effects was studied. ***Methods:*** Data were available for 427 farmers and agricultural industry workers, for whom airborne endotoxin exposure levels were estimated by 249 personal exposure measurements. Atopy was assessed as specific serum IgE to common inhalant allergens, and respiratory symptoms and personal characteristics by standardized questionnaires. BHR was determined in a subset of 113 subjects. Associations were adjusted for age, sex, smoking and living on a farm during childhood. ***Results:*** Endotoxin exposure was positively associated with BHR and wheeze ($p < 0.05$). In contrast, endotoxin exposure was inversely associated with atopy and IgE to grass pollen ($p < 0.001$). The proportions of wheeze and BHR that were attributable to atopy were only 16.6 and 32.8%, respectively. ***Conclusions:*** High endotoxin exposure is a risk factor for BHR and wheeze, which were characterized by a predominantly non-atopic phenotype. At the same time, endotoxin exposure is related to a reduced risk of atopy and IgE to grass pollen in adults. It is unlikely that this is entirely a result of healthy worker selection, as significant inverse associations between endotoxin and IgE to grass pollen were found regardless of reported allergic symptoms.

Introduction

There is increasing evidence that farm exposures throughout life are protective against atopy, allergic rhinitis and atopic asthma [1]. Several studies have observed a strongly decreased prevalence of allergic sensitization [2–4], hay fever [5] and asthma [6] among adults with childhood and current farm exposures. Farmers, and children growing up on farms, are exposed to high levels of microbial agents, such as bacterial endotoxin and fungal β(1→3)-glucans [7, 8]. It has been hypothesized that exposure to such agents may induce a shift from atopic Th2 responses to Th1 responses through stimulation of

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the innate immune system and regulatory T cells [9]. Protective effects of house dust endotoxin on the development of atopy and asthma have been shown in children [10–12], and more recently, studies among adults have shown similar inverse relationships between endotoxin exposure and atopic asthma [7], allergic sensitization [13, 14] and hay fever [15].

Since endotoxin is also a potent pro-inflammatory agent, the downside of increased exposure can be an elevated risk of non-allergic respiratory health effects [16–18]. Only a few studies have explicitly reported the Janus-faced nature of endotoxin – a protective effect on atopic disease, paralleled by an increased risk of non-atopic asthma and nonspecific airway hyperresponsiveness – in the same population [7, 11, 13].

We recently observed this dual nature, specifically associated with adult exposure in farmers and agricultural processing workers. We found a significantly increasing prevalence of wheeze at high endotoxin exposure, but an inverse association between endotoxin exposure and self-reported hay fever [15]. The latter finding is compatible with a presumed down-regulatory effect of endotoxin exposure on atopic immune responses, since hay fever symptoms are usually strongly associated with atopic sensitization [19]. The dose-response relationship between endotoxin and wheeze was independent of self-reported allergy, a surrogate for atopy [15], suggesting that wheezing reported in this population may not be due to atopic airway inflammation. This might explain the paradoxical association with wheezing at high endotoxin exposure, since wheeze is usually associated with atopy [20].

The aim of the present study was to further elucidate this apparent paradox by using objectively measured health data: serum IgE to common allergens and non-specific bronchial hyperresponsiveness (BHR). Specific objectives were to test the hypotheses that (1) occupational endotoxin exposure in agricultural workers is positively associated with airway hyperresponsiveness, but inversely with atopic sensitization, and (2) atopy plays a minor role in endotoxin-related respiratory effects.

Subjects and Methods

Study Population

Figure 1 depicts a flow diagram illustrating study population recruitment. As described earlier [15], questionnaires were sent to employees of 23 companies in 4 agricultural processing sectors (onion trade, flower bulb trade, animal feed industry and vegetable seed industry). We received 376 completed questionnaires (response rate 90%). Farmers were recruited by sending questionnaires to 748 farm owners, and we received 525 completed questionnaires from 418 farms (farm owners' response rate 61%). Selection of the present study population has been described in detail elsewhere [21, 22]. Briefly, a total of 342 agricultural processing workers (91% of 376 eligible) consented to blood sampling. A total of 340 farmers (65% of 525 eligible) indicated that they would not object being contacted for a medical examination. However, for logistical reasons, only approximately 100 farmers could be included. A random sample of 122 farmers was approached by telephone, and 92 farmers participated. Data from 427 of 434 participating subjects were used (338 agricultural processing workers, 89 farmers), as we excluded 4 subjects aged <18 or >65 years, and 3 subjects with incomplete data. Farmers who participated in the medical examination did not differ significantly from farmers in the original sample with respect to endotoxin exposure, potential confounders and wheeze, but hay fever was more prevalent in the farmers who participated (17 vs. 9%; $p = 0.02$, χ^2 test). Selection bias was a potential concern, and therefore, sensitivity analyses were performed to see whether results changed after excluding farmers.

The study protocol was approved by the institutional ethics committee and all participants gave their written informed consent.

Endotoxin Exposure

Endotoxin sampling, quantification and modelling has been described before [15]. Briefly, 249 personal full-shift inhalable dust samples were collected in a sample of participants. Endotoxin was analysed by the quantitative kinetic chromogenic Limulus amebocyte lysate assay. Endotoxin exposure levels for each participant were estimated by work site and job title. Endotoxin exposure levels were expressed as endotoxin units (EU) per m^3.

IgE Serology

Specific IgE to common allergens house dust mite, grass pollen (mix of timothy and perennial ryegrass), cat and dog, as well as total serum IgE were determined by enzyme immunoassays as described previously [13, 23]. Total IgE levels were dichotomized by using 100 IU/ml as cut-off level. Atopy was defined as the presence of specific serum IgE antibodies to 1 or more of the common allergens.

Bronchial Hyperresponsiveness

BHR to methacholine was assessed in subjects from 7 randomly selected agricultural processing companies that were visited on a second occasion, as shown in figure 1. In total, 113 of 119 subjects who were present again on the second occasion consented to BHR (response 95%). Forced expiratory lung function and BHR were assessed according to the European Respiratory Society guidelines [24, 25]. Methacholine was inhaled from 0.019 mg up to a cumulative dose of 2.5 mg, as described before [26]. The test was stopped after the maximum cumulative dose was reached, or when the forced expiratory volume in 1 s (FEV_1) fell by 20% or more.

Data Analysis

BHR was defined as a fall in FEV_1 of at least 20% at a methacholine dose of 2.5 mg or less. Bronchial challenge test results were also expressed by the dose-response slope (DRS), calculated

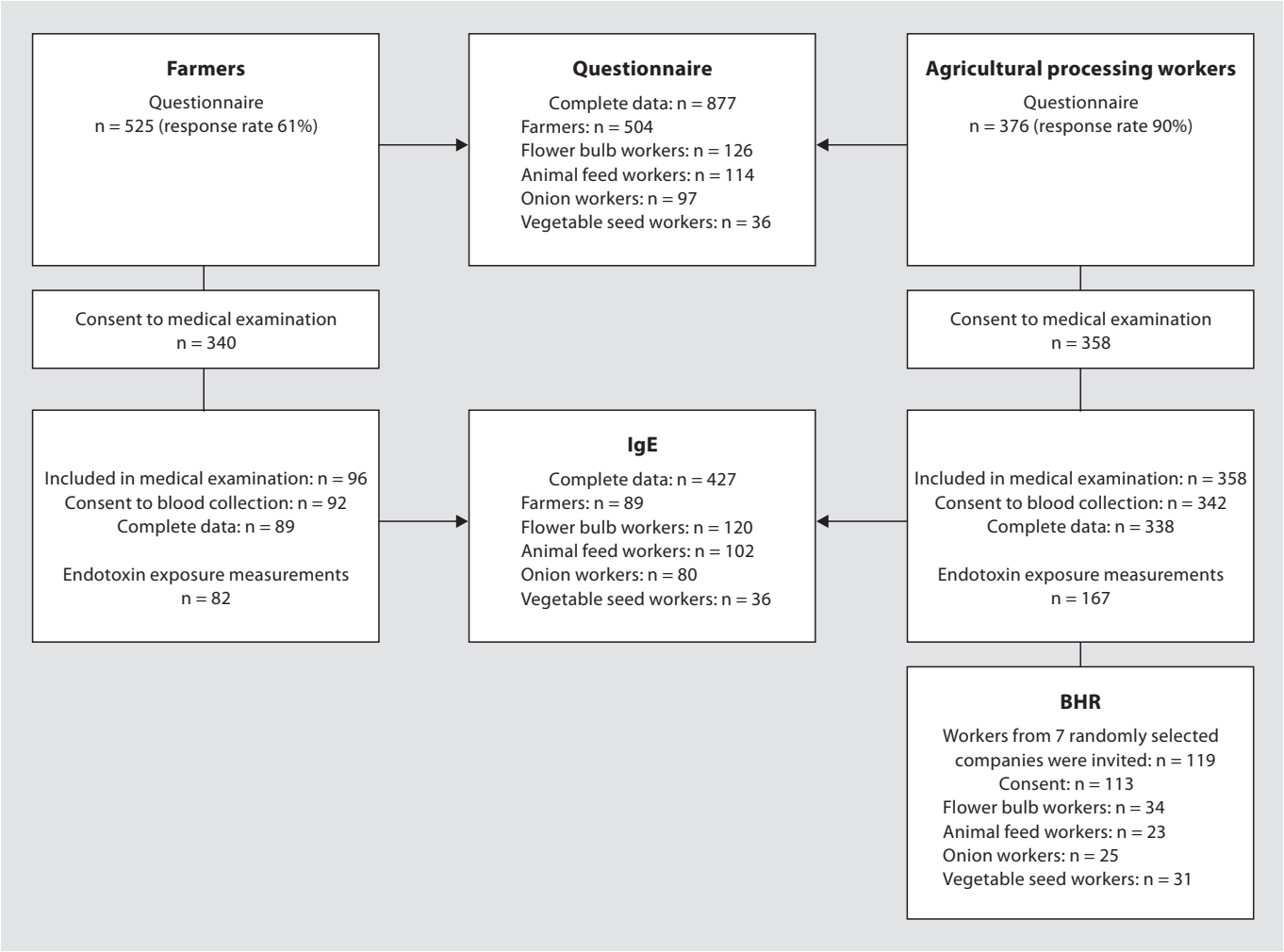

Fig. 1. Flow diagram representing study population recruitment. The questionnaire and endotoxin exposure measurements are described in detail by Smit et al. [15].

as the maximum percentage fall in FEV_1 per mg inhaled methacholine [27]. Natural log-transformed DRS values were used in calculations.

Associations between natural log-transformed endotoxin levels and dichotomous and continuous health outcomes were assessed by multiple logistic and linear regression analysis. Odds ratios (ORs) for the interquartile range increase in endotoxin exposure were calculated and were adjusted for age, sex, smoking habits and farm childhood, unless stated otherwise. Exposure-response relationships between endotoxin exposure and health outcomes were further studied by assessing the shape of the exposure-response relationship by means of generalized additive modelling (smoothing; SAS Proc GAM), using Akaike's information criterion to select the degree of smoothing [13]. The proportion of health outcomes attributable to atopy (i.e. population attributable risk) was calculated as described by Pearce et al. [28].

Results

Characteristics of the study population are presented in table 1. Endotoxin exposure levels ranged over 3 orders of magnitude (approximately 10–10,000 EU/m^3) with a geometric mean endotoxin exposure level of 319 EU/m^3, which was similar in the original sample (265 EU/m^3).

First, the association between endotoxin and wheeze and BHR was explored. Positive and statistically significant relationships between endotoxin exposure and wheezing and BHR (as a dichotomous variable) were found (table 2). The association between endotoxin exposure and wheeze hardly changed when we only considered the processing workers and removed farmers from

Table 1. Characteristics of the study population

Total	427
Male	371 (86.9)
Age, years	41.8 ± 10.5
Smoking habits	
Never	173 (40.5)
Former	134 (31.4)
Current	120 (28.1)
Farm childhood	193 (45.2)
Endotoxin exposure, EU/m^3	
Geometric mean	319
Geometric SD	5.5
Interquartile range	168–720
Range	14–10,645

Figures in parentheses are percentages.

Table 2. Associations between occupational endotoxin exposure and wheezing, BHR, atopic sensitization, elevated total IgE and hay fever in 427 agricultural workers

	n	%	Endotoxin exposure[1]		p value
			OR	95% CI	
Wheezing[2]	55	12.9	1.30	1.01–1.67	0.04
BHR[3]	30	26.6	1.55	1.03–2.35	0.04
Atopy	81	19.0	0.67	0.54–0.84	0.0005
Grass pollen IgE	49	11.5	0.59	0.45–0.79	0.0003
House dust mite IgE	46	10.8	0.84	0.65–1.09	0.18
Total IgE					
(≥100 IU/ml)	104	24.4	0.87	0.72–1.05	0.15
Hay fever[2]	51	11.9	0.57	0.43–0.75	<0.0001

[1] Data are presented as the OR for an interquartile range increase in endotoxin exposure levels with 95% CI. ORs are adjusted for age, smoking habits, sex and farm childhood.

[2] Self-reported [15].

[3] BHR was measured in a subgroup of 113 workers. ORs are adjusted for age, smoking habits, sex, farm childhood and atopy.

Table 3. Association between occupational endotoxin exposure and atopy or grass pollen sensitization, stratified by self-reported allergy

	n/N	%	Endotoxin exposure[1]		p value
			OR	95% CI	
No self-reported allergy[2]					
Atopy	32/325	9.8	0.79	0.58–1.08	0.14
Grass pollen IgE	15/325	4.6	0.59	0.38–0.93	0.02
Self-reported allergy					
Atopy	49/102	48.0	0.61	0.41–0.93	0.02
Grass pollen IgE	34/102	33.3	0.72	0.47–1.09	0.12

n = Atopic subjects in stratum or subjects having a positive test for grass pollen IgE; N = all subjects in stratum.

[1] Data are presented as the OR for an interquartile range increase in endotoxin exposure with 95% CI. ORs are adjusted for age, smoking habits, sex and farm childhood.

[2] Symptomatic subjects are those who gave a positive answer to the question: 'Have you ever had any allergies?'

the analysis (OR 1.39, 95% CI 1.04–1.86). The median DRS (maximum percentage fall in FEV_1 per mg methacholine) was 4.5% (interquartile range 2.5–8.5%). In a linear regression model, the log-transformed DRS showed a positive, borderline statistically significant association with endotoxin exposure ($\beta = 0.14$, 95% CI –0.02 to 0.30; $p = 0.08$).

We then explored associations between endotoxin exposure and atopy. Specific serum IgE to 1 or more common allergens was found in 81 subjects (19%). Very few subjects had a positive IgE test to cat (n = 7, 1.6%) or dog (n = 3, 0.7%). The geometric mean total IgE level was 25.6 IU/ml (geometric SD 7.5). In contrast to wheezing and BHR, endotoxin exposure was inversely associated with atopy, grass pollen sensitization and hay fever, with ORs of 0.67, 0.59 and 0.57, respectively ($p < 0.001$; table 2). The inverse relationships between endotoxin exposure and house dust mite sensitization and increased total IgE were weaker and not statistically significant (table 2). Analysis of total IgE as a continuous variable (log transformed) did not show an association with endotoxin exposure ($p > 0.6$) either. Adjustment had a small influence on the risk estimates (1–20% difference between adjusted and unadjusted ORs). Sensitivity analyses restricted to the 338 agricultural processing workers showed an OR of 0.69 (95% CI 0.55–0.87) for the association between endotoxin and atopy. Adjustment for occupational group (farmer versus agricultural processing worker) yielded similar results.

To examine the possibility that healthy worker selection bias was (partly) responsible for the inverse relationship between occupational endotoxin exposure and atopy, we investigated whether the inverse associations between endotoxin and atopy were also present in workers who reported that they never had any allergies. As shown in table 3, inverse associations between endotoxin exposure and atopy/grass pollen IgE were observed regardless of self-reported allergy (p values between 0.02 and 0.14, p value for interaction >0.3).

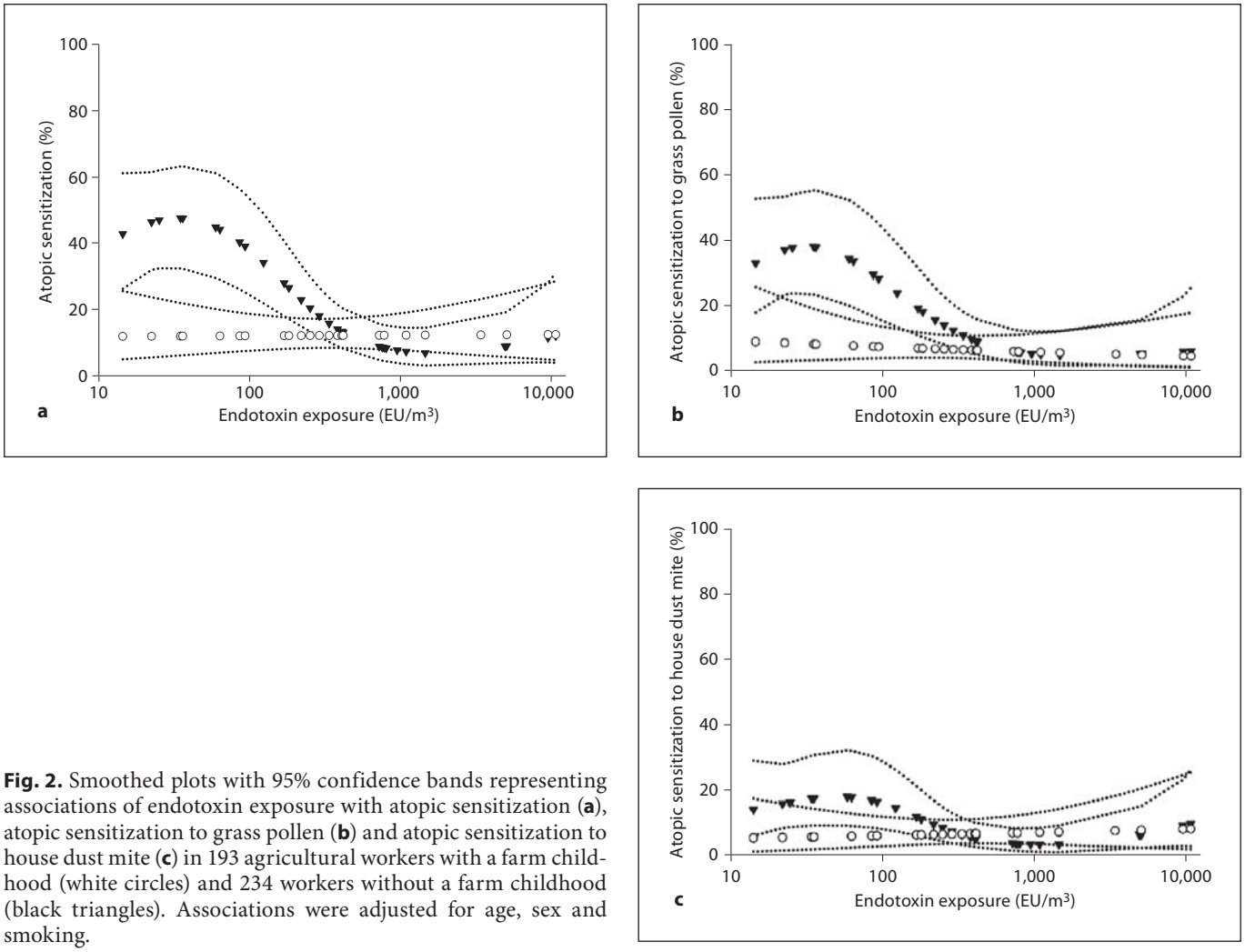

Fig. 2. Smoothed plots with 95% confidence bands representing associations of endotoxin exposure with atopic sensitization (**a**), atopic sensitization to grass pollen (**b**) and atopic sensitization to house dust mite (**c**) in 193 agricultural workers with a farm childhood (white circles) and 234 workers without a farm childhood (black triangles). Associations were adjusted for age, sex and smoking.

Workers who had grown up on a farm had a lower prevalence of atopic sensitization than workers without a farm childhood (atopy 14.5 vs. 22.7%, p = 0.03; grass pollen IgE 7.8 vs. 14.5%, p = 0.03; house dust mite IgE 7.8 vs. 13.3%, p = 0.07). In figure 2, the shape of the exposure-response relationships between occupational endotoxin exposure and atopic sensitization is shown separately for workers with and without a farm childhood. These figures suggested a non-linear relationship between endotoxin exposure and atopy in workers without a farm childhood. At very low endotoxin exposures (<35 EU/m^3), the highest prevalence of atopy was seen, after which a decline in the predicted prevalence was observed up to levels of around 1,000 EU/m^3. At higher endotoxin exposure levels, a constant and very low prevalence of atopic sensitization was seen. For workers with a farm childhood, the prevalence of sensitization was low over the whole range of exposure.

Finally, we explored interrelationships between wheezing, BHR and atopy. As expected, hyper-responsive subjects often reported wheezing (OR 6.4, 95% CI 2.1–19.8). BHR and wheezing were also positively associated with atopy, but the proportions of wheeze and BHR attributable to atopy were only 16.6 and 32.8%, respectively (table 4). Exposure-response relationships between endotoxin exposure and wheeze and BHR were similar in non-atopic and atopic subjects when these associations are described as ORs (table 4). However, the prevalence of wheezing and BHR differs more than a factor 2 in atopics and non-atopics. Hay fever was strongly associated with atopy (table 4) and grass pollen sensitization (OR 18.1, 95% CI 9.0–36.3). Despite the high ORs, the proportion

Table 4. Association between atopy and wheezing, rhinitis and BHR, and association between endotoxin exposure and wheezing, rhinitis and BHR, according to atopic status

		n/N	%	Atopy		Cases attributable to atopy, %	Interquartile range increase in endotoxin exposure[1]		p interaction
				OR	95% CI		OR	95% CI	
Wheezing	No atopy	38/346	11.0	2.15	1.14–4.05	16.6	1.43	1.04–1.97	0.92
	Atopy	17/81	21.0				1.46	0.87–2.45	
BHR	No atopy	17/87	19.5	4.12	1.62–10.5	32.8	1.94	1.11–3.41	0.55
	Atopy	13/26	50.0				2.12	0.71–6.30	
Rhinitis	No atopy	20/346	5.8	10.1	5.35–19.1	54.8	0.73	0.48–1.12	0.29
	Atopy	31/81	38.3				0.52	0.31–0.87	

n = Subjects in stratum reporting wheeze, rhinitis (hay fever), or having a positive test for BHR; N = all subjects in stratum.
[1] ORs for associations between endotoxin exposure and health outcomes are adjusted for age, smoking habits, sex and farm childhood.

of hay fever attributable to atopy was only 54.8%. When hay fever was subdivided by atopic status and analyzed as atopic and non-atopic rhinitis, only 'atopic rhinitis' showed a statistically significant inverse association with endotoxin (table 4).

Discussion

In this study, we showed that high occupational exposure to endotoxins was a risk factor for BHR, whereas endotoxin exposure showed an inverse dose-response relationship with atopic sensitization. Since atopy was positively associated with wheezing and BHR, the opposite effects of endotoxin exposure may seem paradoxical. However, the proportions of wheeze and BHR in our population that were attributable to atopy were only 16.6 and 32.8%, respectively. These apparently low proportions are consistent with data presented by Pearce et al. [28] who demonstrated that the proportion of asthma-related outcomes in population-based studies that is attributable to atopy is on average <40%. In adults exposed to high levels of pro-inflammatory, non-allergenic agents such as endotoxin, one may expect that the attributable risk of atopy is even lower [29]. The proportion of self-reported hay fever that was attributable to atopy was 54.8%. Although this proportion appears to be low, in a review of large population-based surveys it has been shown that the average percentage of self-reported seasonal rhinitis or allergen-related rhinitis attributable to atopy was only 48% [30].

According to the hygiene hypothesis, bacterial and viral infections, and environmental exposures to microbial compounds, may protect from the development of allergic disease by influencing immune responses [31, 32]. It has been argued that the immune system is most susceptible to the influence of microbial exposures during infancy or early childhood and, as a result, it seems plausible that timing of exposure plays a critical role [32]. Several studies have shown that farm exposures during childhood may exert long-lasting protective effects on allergy or allergic asthma [3, 33], and the lower prevalence of atopic disease that is often found among farmers is therefore likely to be partly a result of early microbial exposures [2–6, 34]. In accordance with the hypothesis that effects of early exposures can be long-lasting, we observed a significantly lower prevalence of atopy in agricultural workers with a farm childhood (14.5%) compared with workers without a farm childhood (22.7%). The prevalence of sensitization to common aeroallergens in our study is very low compared with the general Dutch population (32%, subjects aged 20–70 years) [35] and other Dutch occupational populations (36.5%, workers in spray-painting companies; 33%, bakery workers) [26, 36]. The low prevalence of atopy in workers without a farm childhood compared with other Dutch populations can obviously not be attributed to early farm exposures. It has been suggested that immune deviation may take place throughout life [6], and exposure in adulthood to endotoxin and other microbial compounds has indeed been associated with a lower prevalence of allergy or allergic asthma [7, 13–15]. Our study provides evidence for the hypothesis that exposures at adult age may be associated

with a protective effect by showing strong inverse associations between (adult-onset) occupational endotoxin exposure and atopy. In workers without a farm childhood, steep inverse dose-response relationships between endotoxin exposure and atopy were observed for exposure levels >35 EU/m^3. In workers grown up on a farm, the prevalence of atopy and grass pollen IgE was clearly reduced over the whole range of occupational endotoxin exposures. The protective effect of endotoxin exposure was strongest for grass pollen sensitization, which is consistent with other studies in children and adults [13, 14, 37, 38]. Moreover, it has been reported that cigarette smoking, which is also a source of endotoxin, has a similar inverse effect on sensitization to grass pollen but not to house dust mite [39, 40]. Therefore, it could be speculated that endotoxin or other microbial factors may have a strong influence on grass pollen sensitization, whereas levels of allergen exposure or genetic factors may be more important for other allergies and total IgE levels. As we argued before [15], we cannot exclude the possibility that the observed relationships were at least partially due to other microbial agents than endotoxin. Given its potency as an inducer of inflammatory reactions and activator of immunoregulatory pathways, a major role for endotoxin itself seems, however, highly likely.

The present study population was derived from a questionnaire survey in 877 subjects [15], showing significant inverse associations between endotoxin exposure and hay fever. Due to logistical constraints, we could only conduct medical examinations in a relatively small sample of farmers who had completed the questionnaire. On the other hand, a large proportion of agricultural industry workers (>90%) participated in the examination, and results of the questionnaire survey were confirmed in the 427 subjects with IgE data available, also when farmers, where selection effects might have occurred, were excluded from analyses. Confounding by agrochemical exposures is unlikely, as the farmers in this study practiced organic farming and are not allowed to use chemical pesticides and certain toxic disinfectants. An earlier survey showed only small differences in the prevalence of hay fever and asthma symptoms between conventional and organic farmers, which could partly be explained by disinfectant use and farm childhood [5]. Therefore, we assume that the associations between endotoxin exposure, atopy and BHR can very likely be generalized to conventional farmers as well.

Healthy worker effects are likely to be important in agriculture, either because less healthy subjects would prefer not to take over a farm or be employed in a dusty agricultural processing company, or because less healthy workers are more likely to leave high-exposure jobs [41]. In western countries, migration out of rural areas and agricultural occupations has occurred over more than a century. However, the strong and positive associations between endotoxin exposure and wheeze and BHR argue against a major influence of the healthy worker effect. Furthermore, one would not expect strong healthy worker hire effects nor survivor effects to have taken place among workers who reported that they never had any allergies. Thus, the statistically significant inverse exposure-response relationships between endotoxin exposure and grass pollen IgE in the subgroup of non-symptomatic subjects (table 3) supports the hypothesis that microbial exposures in adulthood might be causally related to less atopic sensitization. However, more and especially longitudinal studies are needed to assess causal associations.

In conclusion, occupational endotoxin exposure was a risk factor for BHR and wheeze, which were characterized by a predominantly non-atopic phenotype. In contrast, a significant inverse dose-response relationship between occupational endotoxin exposure and atopy was observed. This study adds to the evidence that exposure to endotoxin later in life may protect against atopic sensitization. However, longitudinal studies are necessary to draw conclusions about causality.

Acknowledgments

This work was supported by a grant from the Netherlands Asthma Foundation (grant No. 3.2.03.70). Jack Spithoven, Siegfried de Wind, Isabella Oosting, Mischa Zengeni and Jos Rooijackers are gratefully acknowledged for fieldwork and laboratory assistance.

References

1 Heederik D, Sigsgaard T: Respiratory allergy in agricultural workers: recent developments. Curr Opin Allergy Clin Immunol 2005;5:129–134.
2 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.
3 Portengen L, Sigsgaard T, Omland O, Hjort C, Heederik D, Doekes G: Low prevalence of atopy in young Danish farmers and farming students born and raised on a farm. Clin Exp Allergy 2002;32:247–253.
4 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.

5 Smit LA, Zuurbier M, Doekes G, Wouters IM, Heederik D, Douwes J: Hay fever and asthma symptoms in conventional and organic farmers in The Netherlands. Occup Environ Med 2007;64:101–107.
6 Douwes J, Travier N, Huang K, Cheng S, McKenzie J, Le Gros G, von Mutius E, Pearce N: Lifelong farm exposure may strongly reduce the risk of asthma in adults. Allergy 2007;62:1158–1165.
7 Eduard W, Douwes J, Omenaas E, Heederik D: Do farming exposures cause or prevent asthma? Results from a study of adult Norwegian farmers. Thorax 2004;59:381–386.
8 Schram D, Doekes G, Boeve M, Douwes J, Riedler J, Ublagger E, von Mutius E, Budde J, Pershagen G, Nyberg F, Alm J, Braun-Fahrländer C, Waser M, Brunekreef B, PARSIFAL Study Group: Bacterial and fungal components in house dust of farm children, Rudolf Steiner school children and reference children – the PARSIFAL Study. Allergy 2005;60:611–618.
9 Romagnani S: Immunologic influences on allergy and the TH1/TH2 balance. J Allergy Clin Immunol 2004;113:395–400.
10 Gereda JE, Leung DY, Thatayatikom A, Streib JE, Price MR, Klinnert MD, Liu AH: Relation between house-dust endotoxin exposure, type 1 T-cell development, and allergen sensitisation in infants at high risk of asthma. Lancet 2000;355:1680–1683.
11 Braun-Fahrlander C, Riedler J, Herz U, Eder W, Waser M, Grize L, Maisch S, Carr D, Gerlach F, Bufe A, Lauener RP, Schierl R, Renz H, Nowak D, von Mutius E: Environmental exposure to endotoxin and its relation to asthma in school-age children. N Engl J Med 2002;347:869–877.
12 Douwes J, van Strien R, Doekes G, Smit J, Kerkhof M, Gerritsen J, Postma D, de Jongste J, Travier N, Brunekreef B: Does early indoor microbial exposure reduce the risk of asthma? The Prevention and Incidence of Asthma and Mite Allergy birth cohort study. J Allergy Clin Immunol 2006;117:1067–1073.
13 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.
14 Gehring U, Bischof W, Schlenvoigt G, Richter K, Fahlbusch B, Wichmann HE, Heinrich J: Exposure to house dust endotoxin and allergic sensitization in adults. Allergy 2004; 59:946–952.
15 Smit LA, Heederik D, Doekes G, Blom C, van Zweden I, Wouters IM: Exposure-response analysis of allergy and respiratory symptoms in endotoxin-exposed adults. Eur Respir J 2008;31:1241–1248.
16 Liu AH: Endotoxin exposure in allergy and asthma: reconciling a paradox. J Allergy Clin Immunol 2002;109:379–392.
17 Park JH, Gold DR, Spiegelman DL, Burge HA, Milton DK: House dust endotoxin and wheeze in the first year of life. Am J Respir Crit Care Med 2001;163:322–328.
18 Thorne PS, Kulhankova K, Yin M, Cohn R, Arbes SJ Jr, Zeldin DC: Endotoxin exposure is a risk factor for asthma: the national survey of endotoxin in United States housing. Am J Respir Crit Care Med 2005;172:1371–1377.
19 Droste JH, Kerhof M, de Monchy JG, Schouten JP, Rijcken B: Association of skin test reactivity, specific IgE, total IgE, and eosinophils with nasal symptoms in a community-based population study. The Dutch ECRHS Group. J Allergy Clin Immunol 1996;97:922–932.
20 Pekkanen J, Sunyer J, Anto JM, Burney P: Operational definitions of asthma in studies on its aetiology. Eur Respir J 2005;26:28–35.
21 Smit LA, Heederik D, Doekes G, Krop EJ, Rijkers GT, Wouters IM: Ex vivo cytokine release reflects sensitivity to occupational endotoxin exposure. Eur Respir J 2009;34:795–802.
22 Smit LA, Heederik D, Doekes G, Wouters IM: Exhaled nitric oxide in endotoxin-exposed adults: effect modification by smoking and atopy. Occup Environ Med 2009;66:251–255.
23 Doekes G, Douwes J, Wouters I, de Wind S, Houba R, Hollander A: Enzyme immunoassays for total and allergen specific IgE in population studies. Occup Environ Med 1996;53:63–70.
24 Sterk PJ, Fabbri LM, Quanjer PH, Cockcroft DW, O'Byrne PM, Anderson SD, Juniper EF, Malo JL: Airway responsiveness. Standardized challenge testing with pharmacological, physical and sensitizing stimuli in adults. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J Suppl 1993;16:53–83.
25 Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC: Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J Suppl 1993;16:5–40.
26 Pronk A, Preller L, Raulf-Heimsoth M, Jonkers IC, Lammers JW, Wouters IM, Doekes G, Wisnewski AV, Heederik D: Respiratory symptoms, sensitization, and exposure response relationships in spray painters exposed to isocyanates. Am J Respir Crit Care Med 2007;176:1090–1097.
27 O'Connor G, Sparrow D, Taylor D, Segal M, Weiss S: Analysis of dose-response curves to methacholine. An approach suitable for population studies. Am Rev Respir Dis 1987;136: 1412–1417.
28 Pearce N, Pekkanen J, Beasley R: How much asthma is really attributable to atopy? Thorax 1999;54:268–272.
29 Douwes J, Gibson P, Pekkanen J, Pearce N: Non-eosinophilic asthma: importance and possible mechanisms. Thorax 2002;57:643–648.
30 Zacharasiewicz A, Douwes J, Pearce N: What proportion of rhinitis symptoms is attributable to atopy? J Clin Epidemiol 2003;56:385–390.
31 Strachan DP: Hay fever, hygiene, and household size. BMJ 1989;299:1259–1260.
32 Schaub B, Lauener R, von Mutius E: The many faces of the hygiene hypothesis. J Allergy Clin Immunol 2006;117:969–977; quiz 978.
33 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;164:1829–1834.
34 Chen Y, Rennie D, Cormier Y, McDuffie H, Pahwa P, Dosman J: Reduced risk of atopic sensitization among farmers: the Humboldt study. Int Arch Allergy Immunol 2007;144: 338–342.
35 Kerkhof M, Droste JH, de Monchy JG, Schouten JP, Rijcken B: Distribution of total serum IgE and specific IgE to common aeroallergens by sex and age, and their relationship to each other in a random sample of the Dutch general population aged 20–70 years. Dutch ECRHS Group, European Community Respiratory Health Study. Allergy 1996; 51:770–776.
36 Jacobs JH, Meijster T, Meijer E, Suarthana E, Heederik D: Wheat allergen exposure and the prevalence of work-related sensitization and allergy in bakery workers. Allergy 2008; 63:1597–1604.
37 Stern DA, Riedler J, Nowak D, Braun-Fahrlander C, Swoboda I, Balic N, Chen KW, Vrtala S, Grönlund H, van Hage M, Valenta R, Spitzauer S, Von Mutius E, Vercelli D: 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.
38 Waser M, Michels KB, Bieli C, Flöistrup H, Pershagen G, von Mutius E, Ege M, Riedler J, Schram-Bijkerk D, Brunekreef B, van Hage M, Lauener R, Braun-Fahrländer C: Inverse association of farm milk consumption with asthma and allergy in rural and suburban populations across Europe. Clin Exp Allergy 2007;37:661–670.
39 Jarvis D, Chinn S, Luczynska C, Burney P: The association of smoking with sensitization to common environmental allergens: results from the European Community Respiratory Health Survey. J Allergy Clin Immunol 1999;104:934–940.
40 Omenaas E, Bakke P, Elsayed S, Hanoa R, Gulsvik A: Total and specific serum IgE levels in adults: relationship to sex, age and environmental factors. Clin Exp Allergy 1994; 24:530–539.
41 Le Moual N, Kauffmann F, Eisen EA, Kennedy SM: The healthy worker effect in asthma: work may cause asthma, but asthma may also influence work. Am J Respir Crit Care Med 2008;177:4–10.