

# Does early indoor microbial exposure reduce the risk of asthma? The Prevention and Incidence of Asthma and Mite Allergy birth cohort study

Jeroen Douwes, PhD,<sup>a,b</sup> Rob van Strien, PhD,<sup>a,c</sup> Gert Doekes, PhD,<sup>a</sup> Jet Smit, PhD,<sup>d</sup> Marjan Kerkhof, PhD,<sup>e,f</sup> Jorrit Gerritsen, PhD,<sup>e</sup> Dirkje Postma, PhD,<sup>g</sup> Johan de Jongste, PhD,<sup>h</sup> Noemie Travier, MSc,<sup>b</sup> and Bert Brunekreef, PhD<sup>a,i</sup> Utrecht, Amsterdam, Bilthoven, Groningen, and Rotterdam, The Netherlands, and Wellington, New Zealand

**Background:** Exposure to microbial agents might inhibit the development of atopy and asthma.

**Objective:** We measured the association between microbial exposure assessed at 3 months and the development of atopic sensitization and doctor-diagnosed (DD) asthma and wheeze in the first 4 years in a birth cohort study of children with atopic mothers.

**Methods:** Endotoxin, fungal (1→3)-β-D-glucans, extracellular polysaccharides from the genera *Penicillium* and *Aspergillus* (EPS-Pen/Asp), and dust on living room floors were measured at 3 months of age. Serum IgE levels against common allergens were determined at 1 and 4 years, and questionnaire information about respiratory morbidity was collected yearly.

**Results:** Microbial levels in mattresses were low and not associated with serum IgE levels, DD asthma, and wheeze.

**Floor levels of biocontaminants and dust, on the other hand, were inversely associated with DD asthma, being most**

pronounced for endotoxin (odds ratio [OR], 0.40; 95% CI, 0.21-0.77) and EPS-Pen/Asp (OR, 0.42; 95% CI, 0.18-0.99). Mutual adjustment for other exposures did not significantly alter the results for endotoxin and only moderately affected the results for EPS-Pen/Asp. Persistent wheeze was also consistently less common in the high-exposure group, being significant only for EPS-Pen/Asp (OR, 0.37; 95% CI, 0.15-0.96). Transient wheeze and wheeze in the past 12 months were also reduced, but effects were smaller and not significant. Relationships with serum-specific IgE levels, which could only be assessed in 41% at age 4 years, were less pronounced and statistically significant only for EPS-Pen/Asp.

**Conclusions:** Early exposure to common microbial contaminants, including fungal agents, might protect against asthma.

**Clinical implications:** Microbial exposure in early life might protect against asthma and might constitute a novel target for prevention. (J Allergy Clin Immunol 2006;117:1067-73.)

**Key words:** Asthma, allergy, endotoxin, (1→3)-β-D-glucan, hygiene hypothesis, infant cohort study

From <sup>a</sup>the Institute for Risk Assessment Sciences (IRAS), Utrecht University; <sup>b</sup>the Centre for Public Health Research, Massey University, Wellington; <sup>c</sup>the Department of Environmental Medicine, Municipal Health Service Amsterdam; <sup>d</sup>RIVM-National Institute of Public Health and Environment, Bilthoven; <sup>e</sup>Beatrix Children's Hospital, Groningen University; the Departments of <sup>f</sup>Epidemiology and <sup>g</sup>Pulmonary Medicine, University Medical Center Groningen; <sup>h</sup>the Department of Pediatrics, Sophia Children's Hospital, Erasmus University, Rotterdam; and <sup>i</sup>the Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht.

The Prevention and Incidence of Asthma and Mite Allergy study is supported by the Netherlands Asthma Fund, Zorgonderzoek Nederland, The Ministry of the Environment, and the National Institute of Public Health and the Environment. Analyses for microbial agents in house dust were supported by The Centre for Indoor Air Research (CIAR). Jeroen Douwes has been supported by a research fellowship from the Netherlands Organization for Scientific Research (NWO) and is currently supported by a Sir Charles Hercus Research Fellowship from the Health Research Council (HRC) of New Zealand.

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

Received for publication July 5, 2005; revised January 29, 2006; accepted for publication February 1, 2006.

Available online March 31, 2006.

Reprint requests: Bert Brunekreef, PhD, Institute for Risk Assessment Sciences, Utrecht University, PO Box 80178, 3508 TD, Utrecht, The Netherlands. E-mail: B.Brunekreef@iras.uu.nl.

0091-6749/\$32.00

© 2006 American Academy of Allergy, Asthma and Immunology

doi:10.1016/j.jaci.2006.02.002

It has been suggested that early-life exposures to microbial agents might prevent the development of atopy and asthma.<sup>1</sup> Various studies have reported a reduced risk of atopy, hay fever, and asthma in farmer's children and adolescents with suspected high bacterial endotoxin exposures.<sup>2,3</sup> Other studies in both rural and nonrural environments reported a significant inverse association between indoor endotoxin levels and atopic sensitization,<sup>4,5</sup> hay fever, and atopic asthma.<sup>6</sup> It has also been demonstrated that atopy is associated with a genetic polymorphism for CD14, the endotoxin receptor on monocytes and other inflammatory cells.<sup>7,8</sup> The latter, however, was not confirmed in other recent studies in different populations.<sup>9</sup> Finally, animal studies have demonstrated that endotoxin might protect against atopy and asthma.<sup>10</sup> The mechanisms underlying these protective effects are not clear but might be related to an upregulation of T<sub>H</sub>1 and a downregulation of T<sub>H</sub>2 lymphocyte immunity.<sup>11</sup> Alternatively, microbial exposure might enhance the activity of T regulatory cells, resulting in a downregulation of both T<sub>H</sub>2 and T<sub>H</sub>1 immunity.<sup>11</sup>

*Abbreviations used*

DD:	Doctor diagnosed
DL:	Detection limit
EPS-Pen/Asp:	Extracellular polysaccharides from the genera <i>Penicillium</i> and <i>Aspergillus</i>
EU:	Endotoxin units
OR:	Odds ratio
PIAMA:	Prevention and Incidence of Asthma and Mite Allergy

In contrast to the protective effects, it is well known from occupational and experimental challenge studies that endotoxin exposure might also induce (nonallergic) asthma.<sup>12</sup> There is also evidence that indoor endotoxin exposure might exacerbate pre-existing asthma in both children and adults.<sup>13</sup> Finally, 2 birth cohort studies showed that indoor endotoxin exposure was associated with an increased risk of wheeze during the first year of life.<sup>14,15</sup> Thus the role of endotoxin in the development of allergies and asthma is currently not clear.

Bacterial CpG-containing DNA motifs and peptidoglycans have also been suggested to reduce the risk of allergies and asthma.<sup>16,17</sup> The role of fungal agents has not been studied to date, whereas some fungal agents, such as (1 → 3)-β-D-glucans, cell-wall components of most fungi, have strong immunomodulating characteristics. (1 → 3)-β-D-glucans have previously been associated with respiratory morbidity in occupational populations and increased peak expiratory flow variability in children.<sup>18</sup> Fungal extracellular polysaccharides from the genera *Penicillium* and *Aspergillus* (EPS-Pen/Asp) are nonpathogenic general markers for indoor fungal exposure that have previously been associated with respiratory symptoms in children.<sup>19</sup>

In this article we assessed the role of indoor endotoxin, (1 → 3)-β-D-glucan, and EPS-Pen/Asp on the development of atopy and asthma symptoms in the first 4 years of life of children with atopic mothers in the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort study. We also assessed the same associations for crude house dust levels.

## METHODS

### Study population

The PIAMA study is a prospective birth cohort study conducted in 3 centers in The Netherlands and involved both an intervention and a natural history portion.<sup>20</sup> In this article we only report on the intervention study because no microbial exposure data were collected in the other part of the study. The cohort was recruited during the second trimester of pregnancy in 1996-1997. For the intervention study, 2062 mothers with self-reported allergies, asthma, or both<sup>21</sup> were invited to participate, of whom 855 (41.5%) agreed. Intervention measures were successfully applied in 810 participants: one group was supplied (randomly) with mite-impermeable mattress and pillow covers (AcB; n = 416), and the other (placebo group) was supplied with placebo cotton covers (n = 394) for use both on the infants' and their parents' beds.<sup>20</sup> Both groups were included in the current

study, but we were able to collect dust samples for only 696 subjects; of these, 51% were included in the intervention group, and 49% were included in the placebo group. The study protocol was approved by institutional review boards of each participating institute, and informed consent was obtained from all parents.

### Exposure assessment

Dust from the living room floor and infants' mattresses was sampled at 3 months by using a Rowenta Dymbo vacuum cleaner (Rowenta, Erbach, Germany), with ALK filter holders containing paper filters (Schleicher & Schuell 589; Schleicher & Schuell, Dassel, Germany). Dust was collected from the whole mattress after removing duvets and blankets but not the sheets. Living room samples were taken from 1 m<sup>2</sup>. Bed and floors were sampled for 2 minutes. Dust was weighed and extracted for endotoxin, EPS-Pen/Asp, and (1 → 3)-β-D-glucans, as described previously.<sup>22,23</sup> Extracts were stored frozen at -20°C until analysis.

Endotoxin was analyzed by using a kinetic chromogenic Limulus amoebocyte lysate test (Limulus amoebocyte lysate lot no. 622661, LPS standard lot no 5L4100; BioWhittaker, Walkersville, Md).<sup>22</sup> (1 → 3)-β-D-glucans and EPS-Pen/Asp were analyzed by using an inhibition<sup>23</sup> and a sandwich enzyme immunoassay, respectively.<sup>19</sup> The latter assay was developed to quantify EPS from *Penicillium* and *Aspergillus* species (EPS-Pen/Asp), 2 common fungal genera in house dust.<sup>19</sup> Concentrations were expressed per square meter, and samples with concentrations of less than the detection limit (DL) were given a value of two thirds of the lowest measurable concentration of the samples with concentration greater than the DL. We also measured Der p 1 and Der f 1 allergens in mattress dust samples.<sup>24</sup> Results for these analyses have been reported previously<sup>24</sup> and will not be discussed in this article. However, we will explore whether the associations with microbial agents and atopy and symptoms were confounded by Der p 1 and Der f 1 allergen exposure.

### Questionnaire data

Questionnaires were based on those used in the International Study of Asthma and Allergies in Childhood study<sup>25</sup> and were completed by the parents at 1, 2, 3, and 4 years after birth. Doctor-diagnosed (DD) asthma was defined as a reported diagnosis confirmed by a doctor at any time in the past 4 years. On the basis of the longitudinal questionnaire data on wheeze symptoms, children were divided into 4 wheezing phenotypes: (1) never wheeze, (2) early transient wheeze (at least 1 episode in the first 3 years of life), (3) late-onset wheeze (at least 1 episode in the fourth year of life), and (4) persistent wheeze (at least 1 episode in the first 3 years of life and at least 1 episode in the fourth year of life). Because of the low number of late-onset wheezers (n = 14), we did not analyze this group separately.

### Atopy

Venous blood was collected at 1 and 4 years and analyzed for serum IgE to inhalant allergens (dust mite [*Dermatophagoides pteronyssinus*], cat, dog, grass [*Dactylis glomerata*], birch [*Betula verrucosa*], and mold [*Alternaria alternata*]) and food allergens (egg and milk) by using RASTs. Atopy was defined as an IgE concentration of greater than 0.35 IU/mL for at least 1 allergen. We collected serum in 287 subjects at 4 years (compared with 506 at 1 year) because consent and cooperation were more difficult to obtain at age 4 years. Of these, 248 subjects had also been tested at age 1 year.

### Statistical analyses

Logistic regression analyses were conducted to measure the association between microbial levels measured at 3 months and health outcomes collected in the first 4 years of life (SAS for

**TABLE I.** Endotoxin, (1→3)-β-D-glucan, EPS-*Pen/Asp*, and dust levels per square meter on living room floors and mattresses

Sampling location	Microbial component	n miss*	N	(N < DL)†	P10‡	Median	P90‡
Smooth floor	Endotoxin (EU/m <sup>2</sup> )	24	525	0	13	217	3585
	EPS (EPSU/m <sup>2</sup> )	35	514	401	365	365	2199
	Glucan (μg/m <sup>2</sup> )	41	508	444	90	90	180
	Dust (mg/m <sup>2</sup> )	2	547	196	7	21	161
Carpet	Endotoxin (EU/m <sup>2</sup> )	5	140	0	2,293	9,503	49,053
	EPS (EPSU/m <sup>2</sup> )	6	139	4	4,004	16,751	54,785
	Glucan (μg/m <sup>2</sup> )	13	132	16	90	686	2,080
	Dust (mg/m <sup>2</sup> )	0	145	2	162	373	1,025
Rug	Endotoxin (EU/m <sup>2</sup> )	4	176	0	3,650	27,481	135,660
	EPS (EPSU/m <sup>2</sup> )	9	171	3	6,367	32,045	13,9944
	Glucan (μg/m <sup>2</sup> )	3	177	13	240	1,005	2,875
	Dust (mg/m <sup>2</sup> )	1	179	0	217	638	1,452
Mattress	Endotoxin (EU/m <sup>2</sup> )	60	636	0	190	856	3,616
	EPS (EPSU/m <sup>2</sup> )	62	634	384	365	365	6,518
	Glucan (μg/m <sup>2</sup> )	87	609	463	90	90	549
	Dust (mg/m <sup>2</sup> )	42	654	125	7	66	188

EPSU, Extracellular polysaccharides units.

\*Number missing.

†N &lt; DL = number of samples with concentrations less than the DL.

‡P10 and P90 = 10th and 90th percentiles, respectively.

Windows version 8.0; SAS Institute, Cary, NC). We did the same for crude dust levels. We first explored dose-response associations by using exposure categories, and we subsequently studied the shape of the dose-response association in more detail by using continuous exposure levels and nonparametric smoothing techniques (see below). For each of the microbial agents and dust, 3 exposure levels were defined (low, medium, and high) by using tertiles. For glucan and EPS-*Pen/Asp*, tertiles could not be used because too many samples had undetectable levels (Table I). In those cases the detectable results were grouped into the medium and high groups on the basis of the median level of all detectable results, and the nondetectable results were placed in the low-exposure group. Cutoff levels for endotoxin were 142.2 or less endotoxin units (EU)/m<sup>2</sup> (low group), greater than 142.2 and less than 1657.2 EU/m<sup>2</sup> (medium group), and 1657.2 or greater EU/m<sup>2</sup> (high group). The medium and high cutoffs for EPS-*Pen/Asp*, (1→3)-β-D-glucan, and dust were 365.1 and 8802.3 extracellular polysaccharides units/m<sup>2</sup>, 90.0 and 505.0 μg/m<sup>2</sup>, and 14.5 and 88.8 mg/m<sup>2</sup>, respectively. In case of smooth floors with a rug, we included the concentration of the floor in our analyses and did not use rug concentrations. Analyses were adjusted for the following potential confounders: sex, region, parental education, indoor tobacco smoke, and number of other children in the household. We explored the shape of the dose-response relationship between continuous exposures and symptoms by using generalized additive modeling (smoothing) with PROC GAM (SAS version 8).<sup>26</sup> For this purpose, we log transformed the exposure data because these data were best described by using a log-normal distribution. A logistic model was used, and smoothed curves were computed by using a logit-link function and transformed to prevalences by applying the inverse of the logit function.

## RESULTS

Characteristics of the study population are described in Table II. Most population characteristics were not different among the 3 exposure groups, except for “home dampness during the first year,” which was recorded

more often in the high endotoxin and high dust exposure groups ( $P < .05$ ). The high dust exposure group also had significantly higher numbers of “other children in the household at 4 years” ( $P < .05$ ).

Endotoxin levels were detectable in all samples, whereas (1→3)-β-D-glucan and EPS-*Pen/Asp* concentrations were undetectable in a significant proportion of the samples, particularly on smooth floors (Table I). Endotoxin levels in mattresses were low compared with those in previously published studies in school-age children.<sup>6</sup>

At 1 year, 29% of the infants were sensitized, mostly against milk (25%) and egg (7%), and only a few had positive IgE test responses against inhalant allergens. Forty-four percent had a positive specific IgE test response at 4 years, with a substantially higher proportion (almost 8-fold) of positive test responses against inhalant allergens (Table III). As expected in this high-risk population, DD asthma and wheeze were common (Table III).

Microbial and dust levels in mattresses were not associated with atopy, DD asthma, and wheeze (with odds ratios [ORs] close to 1, data not shown). Living room concentrations, on the other hand, were inversely and significantly associated with DD asthma, with ORs close to 0.5 for children with high levels of biocontaminants and dust on the living room floor (Table IV). Adjustment for confounders did not alter the results. “Persistent wheeze” was also inversely associated with ORs slightly higher than for DD asthma and statistically significant only for EPS-*Pen/Asp* (Table IV). Wheeze in the past 12 months and “early transient wheeze” were somewhat reduced in the high-exposure groups, but this was not statistically significant (Table IV). When we analyzed atopic and nonatopic DD asthma separately, we found no substantial differences in the effect estimates (data not shown).

**TABLE II.** Characteristics of the study population for whom we had exposure data available

	n/N*	%
Girls	334/690	49
Region		
North	191/696	27
Middle	219/696	32
West	286/696	41
Educational level of the mother		
Low	137/628	22
Intermediate	256/628	41
High	235/628	37
Educational level of the father		
Low	151/617	25
Intermediate	215/617	35
High	251/617	41
Cat(s) in the home in the first 12 mo (y/n)	185/636	29
Cat(s) in the home in the first 4 y (y/n)	205/537	38
Dog(s) in the home in the first 12 mo (y/n)	102/633	16
Dog(s) in the home in the first 4 y (y/n)	123/533	23
Tobacco smoking exposure at home in the first year (y/n)	195/640	31
Tobacco smoking exposure at home in the first 4 y (y/n)	234/565	41
Other children in the household at 3 mo of age		
No	341/667	51
1 or 2	312/667	47
≥3	14/667	2
Other children in the household at 4 y of age		
No	74/623	12
1 or 2	426/623	68
≥ or more	23/623	4
Type of floor		
Smooth floor only	369/694	53
Smooth floor and rug	180/694	26
Wall-to-wall carpet	145/694	21
Antibiotic use during the first 4 y of life (y/n)	372/550	68
Home dampness at 1 y of age (y/n)	191/617	31
Gas cooking at 3 mo of age (y/n)	539/659	18

\*Number of children (n) versus total study population (N).

Furthermore, no significant changes were observed after further adjustments for the presence of cats, dogs, or both in the home; antibiotic use; dampness; gas cooking; and mite allergens (Der p 1 and Der f 1) in mattress dust (data not shown). Adjustment or stratification by intervention group (ie, mite-impermeable mattress covers) also did not alter the results. Finally, using nonparametric smoothing techniques, we assessed the shape of the dose-response curves, indicating a near-linear inverse association between living room biocontaminant levels and DD asthma (Fig E1 available in the Online Repository at [www.jacionline.org](http://www.jacionline.org)).

DD asthma was inversely associated with all tested exposures, including dust. As shown in Table V, mutual adjustment for one or more other exposures did not significantly affect the effect estimates for endotoxin. In contrast, the estimates for glucan and dust became highly unstable. The association between EPS-Pen/Asp and DD asthma was only moderately affected when further

**TABLE III.** IgE sensitization at 1 and 4 years and DD asthma and wheeze at 4 years or in the past 4 years

	IgE sensitization			
	1 y		4 y	
	N§	%	N§	%
House dust mite	506	1	287	15
Cat	499	2	285	7
Dog	482	2	283	5
Egg	479	7	271	10
Milk	479	25	274	27
<i>Alternaria</i> species	—	—	263	2
Birch	—	—	276	2
Grass ( <i>Dactylis glomerata</i> )	—	—	277	7
Atopy*	479	29	273	44
Atopy against food allergens†	479	28	272	46
Atopy against inhalant allergens‡	482	3	272	24
				<b>Symptoms</b>
DD asthma in past 4 y			547	18
Wheeze in past 12 mo			531	17
Wheeze in past 4 y				
Never			506	54
Early transient			506	28
Late onset			506	3
Persistent			506	15

\*IgE positive for 1 or more allergens.

†IgE positive for food allergens.

‡IgE positive for environmental allergens.

§Total number of subjects for whom IgE measurements or questionnaire data were available.

adjustments were made for (1 → 3)-β-D-glucan and dust but became less pronounced when adjusted for endotoxin.

Relationships with atopy at 4 years, which could only be assessed in 41%, were less pronounced and statistically significant only for EPS-Pen/Asp (OR, 0.40; 95% CI, 0.18-0.91). No clear association with atopy at 1 year was found (data not shown).

## DISCUSSION

In this prospective birth cohort study, we found that a doctor's diagnosis of asthma at any time in the first 4 years was less common in children exposed to high levels of dust and microbial agents on living room floors. This protective effect was most pronounced and consistent for endotoxin and EPS-Pen/Asp. A similar association was demonstrated for persistent wheeze, but this was only statistically significant for EPS-Pen/Asp. The (inverse) associations with atopy were weak and significant only for EPS-Pen/Asp. No associations were found with microbial concentrations in mattresses.

Our results are in agreement with those of previous studies demonstrating an inverse association between endotoxin exposure and indicators of asthma,<sup>6</sup> although results have not always been consistent.<sup>14,15</sup> Few of these earlier studies were prospective, and those that were have

**TABLE IV.** Microbial contaminant and dust levels, doctor diagnosis of asthma, and respiratory symptoms at age 4 years

	DD asthma		Wheeze					
			In past 12 mo		Early transient in past 4 y		Persistent in past 4 y	
	cOR (95% CI)	aOR (95% CI)	cOR (95% CI)	aOR (95% CI)	cOR (95% CI)	aOR (95% CI)	cOR (95% CI)	aOR (95% CI)
Endotoxin	N = 525	N = 477	N = 509	N = 474	N = 398	N = 382	N = 333	N = 316
Medium	0.54 (0.32-0.92)*	0.47 (0.26-0.86)*	1.13 (0.66-1.93)	1.28 (0.73-2.27)	0.80 (0.49-1.33)	0.70 (0.41-1.19)	0.85 (0.46-1.56)	0.96 (0.49-1.87)
High	0.43 (0.24-0.77)†	0.40 (0.21-0.77)†	0.74 (0.41-1.33)	0.79 (0.42-1.49)	0.82 (0.49-1.36)	0.77 (0.45-1.31)	0.61 (0.32-1.19)	0.67 (0.33-1.37)
Glucan	N = 508	N = 461	N = 493	N = 458	N = 386	N = 370	N = 319	N = 302
Medium	0.71 (0.35-1.45)	0.63 (0.27-1.48)	1.36 (0.72-2.58)	1.50 (0.77-2.94)	0.92 (0.49-1.70)	0.89 (0.46-1.71)	1.12 (0.53-2.36)	1.16 (0.52-2.62)
High	0.61 (0.28-1.33)	0.70 (0.30-1.60)	0.69 (0.31-1.52)	0.76 (0.34-1.72)	0.61 (0.31-1.20)	0.57 (0.28-1.16)	0.47 (0.17-1.25)	0.43 (0.15-1.21)
EPS	N = 515	N = 469	N = 499	N = 466	N = 391	N = 376	N = 327	N = 311
Medium	0.73 (0.40-1.33)	0.78 (0.40-1.55)	1.21 (0.69-2.13)	1.28 (0.70-2.32)	1.08 (0.63-1.85)	0.99 (0.56-1.76)	1.03 (0.53-2.00)	1.07 (0.53-2.16)
High	0.39 (0.18-0.84)*	0.42 (0.18-0.99)*	0.59 (0.29-1.22)	0.63 (0.30-1.32)	0.66 (0.37-1.19)	0.67 (0.36-1.23)	0.37 (0.15-0.92)*	0.37 (0.15-0.96)*
Dust	N = 544	N = 496	N = 528	N = 493	N = 415	N = 399	N = 348	N = 331
Medium	0.79 (0.47-1.30)	0.79 (0.44-1.40)	1.14 (0.67-1.94)	1.19 (0.67-2.11)	1.63 (0.99-2.66)	1.35 (0.81-2.25)	0.98 (0.54-1.79)	1.04 (0.54-2.03)
High	0.45 (0.25-0.80)†	0.54 (0.28-1.03)	0.80 (0.45-1.42)	0.98 (0.54-1.80)	0.95 (0.56-1.61)	0.91 (0.53-1.56)	0.66 (0.34-1.25)	0.78 (0.39-1.54)

Crude (*cOR*) and adjusted (*aOR*) ORs with 95% confidence intervals are shown. ORs are adjusted for sex, region, parental education level, exposure to indoor tobacco smoke in the past 4 years, and other children in the household at 4 years of age.

\**P* < .05.

†*P* < .01.

**TABLE V.** Microbial contaminant and dust levels and DD asthma with (models 2 and 3) and without (model 1) further adjustments for other exposures

	Model 2†				
	Confounding exposure variable				
	Model 1* aOR (95% CI)	Endotoxin aOR (95% CI)	Glucan aOR (95% CI)	EPS aOR (95% CI)	Dust aOR (95% CI)
Endotoxin	N = 477		N = 461	N = 469	N = 475
Medium	0.47 (0.26-0.86)§		0.46 (0.25-0.86)§	0.43 (0.22-0.83)§	0.42 (0.21-0.84)§
High	0.40 (0.21-0.77)		0.36 (0.15-0.87)§	0.43 (0.17-1.11)	0.28 (0.08-0.95)§
Glucan	N = 461	N = 461		N = 454	N = 459
Medium	0.63 (0.27-1.48)	0.79 (0.32-1.96)		0.85 (0.34-2.12)	0.85 (0.33-2.22)
High	0.70 (0.30-1.60)	1.27 (0.42-3.82)		1.86 (0.57-6.05)	1.21 (0.36-4.02)
EPS	N = 469	N = 469	N = 454		N = 467
Medium	0.78 (0.40-1.55)	1.24 (0.54-2.82)	0.73 (0.35-1.54)		0.92 (0.43-2.00)
High	0.42 (0.18-0.99)§	0.69 (0.22-2.15)	0.25 (0.07-0.89)§		0.53 (0.17-1.69)
Dust	N = 496	N = 475	N = 459	N = 467	
Medium	0.79 (0.44-1.40)	1.21 (0.61-2.38)	0.81 (0.44-1.48)	0.82 (0.44-1.52)	
High	0.54 (0.28-1.03)	1.57 (0.47-5.23)	0.49 (0.18-1.31)	0.71 (0.28-1.83)	

Adjusted ORs (*aOR*) with 95% confidence intervals are shown.

\*Adjustment for sex, region, parental education level, exposure to indoor tobacco smoke in the past 4 years, and other children in the household at 4 years of age (same as Table IV).

†Same as model 1, with a further adjustment for 1 other exposure variable at the time.

‡Same as model 1, with a further adjustment for all other exposure variables.

§*P* < .05.

||*P* < .01.



only followed the children until the age of 1 or 2 years.<sup>5,14,15</sup> Despite our population being older, there are limitations with regard to the validity of the reported asthma diagnosis. We therefore also evaluated associations with wheeze, a major symptom of asthma. Because the persistent wheezing phenotype is less associated with viral infections (and more with asthma),<sup>27</sup> we analyzed early transient and persistent wheezers separately. These analyses showed a consistent inverse association for persistent wheeze, statistically significant only for EPS-*Pen/Asp* (Table IV). The associations with early transient wheeze and current wheeze were weaker. DD asthma combined with current wheeze (vs no DD asthma and no current wheeze) resulted in similar but less significant ORs as for DD asthma alone (data not shown). Interestingly, the associations with DD asthma were stronger than for persistent wheeze. In a previous publication<sup>28</sup> we demonstrated that increased levels of exhaled nitric oxide in 4-year-old children of the PIAMA study were also most strongly associated with DD asthma (compared with persistent wheeze), suggesting that in our study DD asthma at this age might be a more valid predictor of asthma.

Previous studies proposed that endotoxin, or microbial exposure in general, might exert its protective effect on asthma by inhibiting the atopic immune response (by reducing T<sub>H</sub>2 immunity). If true, then the strongest protective effect would be expected for atopy. However, in our study atopy was only weakly associated with exposure. This might be due to the relatively low response rate for blood collection (41% at age 4 years), which might have biased the results. In fact, children who had a blood sample taken reported significantly ( $P < .05$ ) more symptoms (data not shown), suggesting that some bias might have occurred. Another reason might be that serum IgE measurements were not sufficiently sensitive to evaluate atopy; use of a skin prick test could have possibly resulted in stronger associations. In any case, even if bias has occurred, it would not affect our main conclusion regarding DD asthma and persistent wheeze because these did not involve serum IgE measurements. The causative mechanism for these observed protective effects remain unclear and require further study, but innate immune responses involving endotoxin-microbial binding receptors, such as Toll-like receptor 4, Toll-like receptor 2, and CD14, might play a key role.<sup>29</sup>

Endotoxin, EPS-*Pen/Asp*, (1→3)-β-D-glucan, and dust were highly correlated, with Pearson correlations ranging from 0.63 to 0.84 ( $P < .01$ ) and κ statistics (a measure of agreement between exposure categories) ranging from 0.22 to 0.59 ( $P < .05$ ). Therefore firm conclusions as to which specific component or components contributed to the observed effects cannot be drawn. Nonetheless, mutual adjustments indicated that the effects were most consistent for endotoxin and, to a lesser extent, EPS-*Pen/Asp*. The effects of (1→3)-β-D-glucan and dust, on the other hand, largely disappeared after controlling for other exposures (Table V). The reason for this might be because a large proportion of the samples had levels less than the DL for (1→3)-β-D-glucan and EPS-

*Pen/Asp*, which might have resulted in some degree of exposure misclassification. Alternatively, endotoxin might be causally related, as suggested by results from animal experiments<sup>10</sup> and recent epidemiologic studies (see above), and the association with EPS-*Pen/Asp* and (1→3)-β-D-glucan could be due to the strong correlation with endotoxin. In any case, on the basis of our results, a role for other microbial agents (including components of fungal origin) cannot be excluded.

The prospective nature of our study, which allowed us to assess exposures before the development of the main health outcomes, is a major strength. Because infants were born of mothers with allergy, asthma, or both, selection effects on the basis of asthma status of the mother are expected to be minimal. Analyses were adjusted for several potential confounders, and these did not alter the results. Finally, we explored dose-response relationships by using smoothing (cubic spline) techniques, confirming that the protective effects on DD asthma were not dependent on the cutoff points chosen to define categoric exposures. These plots (Fig E1) also suggested that the inverse association was present throughout the entire exposure range.

Relatively high temporal variation in airborne concentrations of microbial agents prevents short-term airborne sampling as an accurate measure of chronic exposure, and hence floor dust was sampled, which has been suggested to be a better proxy of long-term exposure.<sup>30</sup> We only found associations with exposure when expressed per square meter, whereas no association was found with exposures expressed per gram of dust (data not shown). This is in agreement with a recent report by the Institute of Medicine<sup>30</sup> that suggested that “for exposure-assessment purposes, it may be more accurate to express exposure as floor-dust concentration per square meter sampled than as concentration per gram of sampled dust.”<sup>30</sup> Despite the fact that infants and children generally spend more time in bed than in the living room, we found no associations with mattress exposures. This might be due to the very low levels measured compared with those in previous studies (Table I), which is most likely explained by the fact that most of the mattresses were purchased new and had only been used for 3 months or less.

There is no standard procedure on how to deal with biocontaminant levels in rugs (on top of smooth floors). Although the concentrations in rugs are high (Table I), the floor area they cover is generally small, and they therefore most likely represent only a minor source of exposure. Therefore in our analyses we have chosen to use the levels measured on the smooth floor instead. Analyses with rug concentrations essentially showed the same results; however, the associations for atopy were somewhat stronger, whereas the associations with DD asthma and symptoms were somewhat weaker (data not shown). Adjusting for type of floor and excluding all subjects with a rug on the floor did not significantly alter the results. This suggests that our findings are robust and do not hinge on our decision to disregard the rug as a potential source of exposure.

In conclusion, although our findings need confirmation at older ages, they provide evidence from a prospective

cohort study that noninfectious microbial exposure at a very young age might protect against asthma. The underlying mechanisms are not clear and require further study.

We thank all the children and their parents for their participation. We also thank Marieke Oldenwening and Isabella Oosting for conducting the field work, Ada Vos for the data management, and Jack Spithoven and Siegfried de Wind for all laboratory analyses.

## REFERENCES

- Gereda JE, Leung DY, Thatayatikom A, Streib JE, Price MR, Klinnert MD, et al. Relation between house-dust endotoxin exposure, type 1 T-cell development, and allergen sensitisation in infants at high risk of asthma. *Lancet* 2000;355:1680-3.
- von Mutius E, Braun-Fahrlander C, Schierl R, Riedler J, Ehlermann S, Maisch S, et al. Exposure to endotoxin or other bacterial components might protect against the development of atopy. *Clin Exp Allergy* 2000;30:1230-4.
- 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.
- Gehring U, Bischof W, Fahlbusch B, Wichmann HE, Heinrich J. House dust endotoxin and allergic sensitization in children. *Am J Respir Crit Care Med* 2002;166:939-44.
- Bottcher MF, Björkstén B, Gustafson S, Voor T, Jenmalm MC. Endotoxin levels in Estonian and Swedish house dust and atopy in infancy. *Clin Exp Allergy* 2003;33:295-300.
- 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;347:869-77.
- Woo JG, Assa'ad A, Heizer AB, Bernstein JA, Hershey GK. The -159 C→T polymorphism of CD14 is associated with nonatopic asthma and food allergy. *J Allergy Clin Immunol* 2003;112:438-44.
- Zambelli-Weiner A, Ehrlich E, Stockton ML, Grant AV, Zhang S, Levett PN, et al. Evaluation of the CD14/260 polymorphism and house dust endotoxin exposure in the Barbados Asthma Genetics Study. *J Allergy Clin Immunol* 2005;115:1203-9.
- Kabesch M, Hasemann K, Schickinger V, Tzotcheva I, Bohnert A, Carr D, et al. A promoter polymorphism in the CD14 gene is associated with elevated levels of soluble CD14 but not with IgE or atopic diseases. *Allergy* 2004;59:520-5.
- Watanabe J, Miyazaki Y, Zimmerman GA, Albertine KH, McIntyre TM. Endotoxin contamination of ovalbumin suppresses murine immunologic responses and development of airway hyper-reactivity. *J Biol Chem* 2003;278:42361-8.
- Romagnani S. The increased prevalence of allergy and the hygiene hypothesis: missing immune deviation, reduced immune suppression, or both? *Immunology* 2004;112:352-63.
- Douwes J, Pearce N, Heederik D. Does environmental endotoxin exposure prevent asthma? *Thorax* 2002;57:86-90.
- Michel O, Kips J, Duchateau J, Vertongen F, Robert L, Collet H, et al. Severity of asthma is related to endotoxin in house dust. *Am J Respir Crit Care Med* 1996;154:1641-6.
- 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;163:322-8.
- Gehring U, Bolte G, Borte M, Bischof W, Fahlbusch B, Wichmann HE, et al. Exposure to endotoxin decreases the risk of atopic eczema in infancy: a cohort study. *J Allergy Clin Immunol* 2001;108:847-54.
- van Strien RT, Engel R, Holst O, Bufe A, Eder W, Waser M, et al. Microbial exposure of rural school children, as assessed by levels of N-acetyl-muramic acid in mattress dust, and its association with respiratory health. *J Allergy Clin Immunol* 2004;113:860-7.
- Jain VV, Kline JN. CpG DNA: immunomodulation and remodelling of the asthmatic airway. *Expert Opin Biol Ther* 2004;4:1533-40.
- Douwes J, Zuidhof A, Doekes G, van der Zee SC, Wouters I, Boezen MH, et al. (1→3)-beta-D-glucan and endotoxin in house dust and peak flow variability in children. *Am J Respir Crit Care Med* 2000;162:1348-54.
- Douwes J, van der Sluis B, Doekes G, van Leusden F, Wijnands L, van Strien R, et al. Fungal extracellular polysaccharides in house dust as a marker for exposure to fungi: relations with culturable fungi, reported home dampness, and respiratory symptoms. *J Allergy Clin Immunol* 1999;103:494-500.
- Brunekreef B, Smit J, de Jongste J, Neijens H, Gerritsen J, Postma D, et al. The prevention and incidence of asthma and mite allergy (PIAMA) birth cohort study: design and first results. *Pediatr Allergy Immunol* 2002;13(suppl 15):55-60.
- Lakwijk N, Van Strien RT, Doekes G, Brunekreef B, Gerritsen J. Validation of a screening questionnaire for atopy with serum IgE tests in a population of pregnant Dutch women. *Clin Exp Allergy* 1998;28:454-8.
- Douwes J, Versloot P, Hollander A, Heederik D, Doekes G. Influence of various dust sampling and extraction methods on the measurement of airborne endotoxin. *Appl Environ Microbiol* 1995;61:1763-9.
- Douwes J, Doekes G, Montijn R, Heederik D, Brunekreef B. Measurement of beta(1→3)-glucans in occupational and home environments with an inhibition enzyme immunoassay. *Appl Environ Microbiol* 1996;62:3176-82.
- Brussee JE, Smit HA, van Strien RT, Corver K, Kerkhof M, Wijga AH, et al. Allergen exposure in infancy and the development of sensitization, wheeze, and asthma at 4 years. *J Allergy Clin Immunol* 2005;115:946-52.
- Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J* 1995;8:483-91.
- Hastie TJ, Tibshirani RJ. Generalised additive models. New York: Chapman and Hall; 1990.
- Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. *N Engl J Med* 1995;332:133-8.
- Brussee JE, Smit HA, Kerkhof M, Koopman LP, Wijga AH, Postma DS, et al. Exhaled nitric oxide in 4-year-old children: relationship with asthma and atopy. *Eur Respir J* 2005;25:455-61.
- Singh J, Schwartz DA. Endotoxin and the lung: Insight into the host-environment interaction. *J Allergy Clin Immunol* 2005;115:330-3.
- Institute of Medicine. Damp indoor spaces and health. Washington (DC): The National Academies Press; 2004.