Endotoxin exposure, wheezing, and rash in infancy in a New Zealand birth cohort

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Background: Wheezing in infancy is common and is associated with small lungs, viral respiratory tract infection, and environmental tobacco smoke exposure. Recently, increased levels of endotoxin in the domestic environment have also been associated with infant wheezing, particularly among infants with a family history of atopic disease.

Objective: To explore associations between exposure to endotoxin at 3 months of age and reported symptoms of wheezing, rhinitis, itchy scaly rash, and atopy at 15 months in a birth cohort of 881 New Zealand children.

Methods: Using standardized methods, a 1-m^2 site from the bedroom floors of the 3-month-old infants was sampled and analyzed for endotoxin.

Results: Wheezing was significantly associated with higher endotoxin levels (odds ratio [OR], 1.54; 95% CI, 1.03-2.30), particularly among infants with a parental history of allergic disease (OR, 1.67; 95% CI, 1.07-2.60). Higher endotoxin concentrations were also strongly associated with recurrent itchy rashes (OR, 1.87; 95% CI, 1.14-3.05), particularly among infants who were atopic (OR, 4.64; 95% CI, 1.56-13.77) or had a parental history of allergic disease (OR, 2.10; 95% CI, 1.22-3.61).

Conclusion: Domestic endotoxin was associated with reported airway and skin symptoms in this large group of New Zealand infants. The role of endotoxin in the development of respiratory and skin disease in infancy deserves further study.

Clinical implications: Reducing domestic endotoxin exposure might reduce infant wheezing and atopic dermatitis, but the long-term benefits of this remain unclear. (J Allergy Clin Immunol 2006;118:1265-70.)

Key words: *Endotoxin, atopy, wheezing, infants, rash, asthma, birth cohort, allergic disease*

Wheezing occurs in more than a third of infants in the first 3 years of life with 3 partial and overlapping phenotypes described: transient early wheezing, nonatopic wheezing, and atopic wheezing.[1] Transient wheezing is associated with reduced lung function from birth that tracks over time with symptoms and generally remits by 5 years.[2] Nonatopic wheezing is associated with viral infections. This type of wheezing tends to remit by 10 to 11 years of age and is not associated with persistent lung function impairment. Wheezing associated with the development of atopy tends to be more severe and persistent, often continuing into adulthood.[1]

Recently, a number of longitudinal studies have shown a positive association between early wheezing and domestic endotoxin exposure.[3,4] Endotoxin exposure in infancy may also be associated with a decreased risk of acquiring asthma by 4 years.[5] Farm residence in the first year of life, particularly when associated with close contact with farm animals, is associated with reduced atopy and atopic disease in childhood.[6,7]

Endotoxin is a biologically active lipopolysaccharide that makes up part of the cell wall of Gram-negative bacteria. It was first described in house dust in the mid-1960s, when it was found in high concentrations in house dust preparations used to desensitize dust-sensitive children with asthma.[8] Endotoxin is ubiquitous in the environment and is found in very high concentrations in occupational settings containing textiles, grains, and livestock, where it has been associated with respiratory and systemic symptoms.[9] Acute inhalation of endotoxin causes both systemic and airway responses including myalgias, fever, and wheezing in subjects with and without asthma.[10] Subjects with asthma and atopic subjects respond to lower doses of endotoxin than normal subjects. This response manifests as increased airway responsiveness and neutrophil recruitment into the airway.[11,12]

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Abbreviations used
EU: Endotoxin unit
OR: Odds ratio

New Zealand has high rates of asthma and allergic disease in children and adults. The role of endotoxin in the early presentation of asthma and allergic disease has not been investigated. We have prospectively examined the associations between exposure to bedroom floor endotoxin at 3 months of age and respiratory and allergic disease symptoms and atopy among a birth cohort at 15 months of age.

METHODS

Cohort assembly

Midwives, who provide almost all maternity care in New Zealand, were randomly selected from all midwives currently practicing in the 2 regions. They were asked to enroll all mothers in their care and were trained and supported by trial coordinators. Expectant mothers were approached by their midwives to give written informed consent to be enrolled in the study before delivery. Midwives were asked to keep records of all mothers who were approached and mothers who declined to participate.

Ethical approval was gained from Wellington and Christchurch Regional Ethics Committees.

Home visits and exposure measurements

Questionnaires were interviewer-administered at 3 and 15 months of age. The 3-month questionnaire collected detailed exposure information about the infants.

A 1-m^2 section of the 3-month-old infant's bedroom floor was sampled for dust using a 1100-W vacuum cleaner with the dust collected in a 25-μm nylon mesh sock inserted into the furniture attachment of the vacuum.[13] A 1-minute sampling time was used for rug and carpet coverings, and a 2-minute sampling time was used for bare floors.

After collection, dust was sifted through a 425-μm mesh sieve, and then the fine dust was weighed and divided into aliquots for various analyses. Endotoxin was only analyzed if there was sufficient dust after indoor allergen assays had been performed. Dust samples were extracted by placing 0.2 g sifted dust in endotoxin-free glass tubes with 5 mL extraction fluid, which consisted of pyrogen-free water containing 0.05% Tween-20.

The endotoxin activity of the house dust samples was determined by a kinetic-QCL assay (Bio Whittaker, Walkersville, Md). Aliquots (100 μL) of endotoxin standards and dust extracts were pipetted into pyrogen-free polystyrene microplates and assayed via the addition of the limulus amebocyte lysate reagent and substrate. Four assay reagent blanks were included to serve as reference and as a control for the reagent water, centrifuge tubes, pipette tips, and microplates. To minimize differences between analytical batches, the same lot number was used for all the analyses. The lot number was 2L7230. One sample from each of the 23 plates used in the analysis was repeated on a single plate. The geometric mean of the 23 study samples was 470 endotoxin units [EU]/g (95% confidence interval [CI], 214-1028) and of the duplicate samples was 471 EU/g (95% CI, 218-1012). Our correlation coefficient ($r = 0.968$) confirms good between-plate repeatability.

Outcome definitions and measurement

At 15 months, the Quintest system (Bayer Corp, West Haven, Conn) and Bayer allergens were used to determine skin prick test positivity to *Dermatophagoides pteronyssinus*, cat pelt, cockroach, rye grass, cow's milk, egg white, peanut, *Aspergillus fumigatus*, a positive histamine control (10 mg/mL), and a negative control. Sensitivity to dog hair was tested separately using a Bayer prick lancet. Wheal diameters were recorded as the mean of the 2 perpendicular diameters after 15 minutes. A reaction was considered positive if there was an allergen to histamine wheal ratio >0.5 after subtraction of the negative control mean wheal size. Atopy was defined as a positive reaction to any allergen. Infants who did not show a reaction to histamine have been excluded from the analysis.

All questionnaire outcome data were collected at 15 months of age. The mother or primary caregiver answered symptom questions including a history of wheeze, history of rash defined as an itchy/scaly rash coming and going for at least 6 months, and rhinitis defined as a runny or blocked nose when the infant did not have a cold or flu. The rash question was adapted from the United Kingdom Working Party diagnostic criteria for atopic dermatitis.[14] Parental history of allergic disease was defined as a history of allergic disease, asthma, eczema, or hay fever at any time.

Data analysis

The analysis was undertaken using SAS statistical software, version 8 (SAS Institute, Cary, NC,). Endotoxin levels were expressed as EU per gram of dust (EU/g). Because endotoxin was log-normally distributed, analysis was based on log-transformed data, and the significance of differences between those with and without disease was compared using *t* tests. Odds ratios (ORs) and 95% CIs were calculated for quartiles of endotoxin with the lowest quartile used as the reference category before and after adjustment for potential confounders. These were the total number of people and the total number of rooms in the house; owning a pet; having a damp, musty smell, dampness, or mold in the bedroom; having an open fireplace; maternal smoking; type of flooring in the bedroom; and the New Zealand Deprivation Index 2001 as a small area marker of socioeconomic status.[15] The model with atopy as the dependent variable was also adjusted for the 2 study locations. Statistical significance was assessed using the Mantel-Haenszel method. Interaction terms were added to the models to determine whether the effects of endotoxin on outcome variables varied significantly ($P \leq .05$) by family history, atopy, and sex.

RESULTS

A total of 1105 infants were recruited to the study at birth, of whom 1064 (96%) remained in the study at 3 months of age and 1011 at 15 months. Most analyses were restricted to the 881 (80% of those recruited) infants with sufficient dust collected at 3 months for endotoxin analysis and questionnaire outcome data available at 15 months.

We undertook an analysis of those not included in our present analysis (n = 224; Fig 1). There was a similar prevalence of having a parental history of allergic disease (82%) at 3 months, and the prevalence of any reported wheeze (35%), rhinitis (22%), and recurrent itchy scaly rash (21%) were also similar at 15 months to those included in the analysis. The prevalence of atopy was slightly higher (34%). None of these differences was significant.

The study population was composed of 434 females (49%) and 447 males (51%) from both study regions,

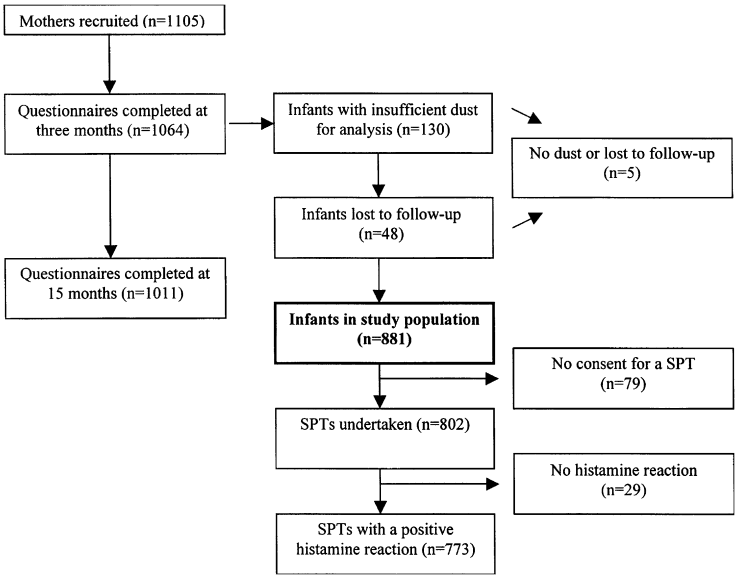

FIG 1. Flow diagram of cohort progress to 15 months. *SPT,* Skin prick test.

Christchurch (n = 421; 48%) and Wellington (n = 460; 52%). At 15 months of age, 39% of the infants had reported wheezing, 25% rhinitis, 21% a recurrent itchy scaly rash, and 27% atopy. Parental history of allergic disease was reported for 735 (84%) infants.

Measured endotoxin concentrations ranged from 0.41 to 609,910 EU/g with a geometric mean of 9244 (95% CI, 8071-10,588). Geometric mean endotoxin concentrations were higher among symptomatic infants but lower among those with atopy, although these differences were not significant (Table I).

The relationship between endotoxin from bedroom floor dust at 3 months of age and the development of allergic symptoms by 15 months was further explored using quartiles of endotoxin after adjusting for potential confounders (Table II). There was a dose-response effect of wheezing with increasing endotoxin concentrations, which was significant at the highest quartile. A dose response association was seen for rash, which was significant for the third and fourth quartiles. No associations were found for rhinitis and atopy.

We tested the significance of a possible interaction effect of family history, sex, and atopy on the relationships between endotoxin and symptoms. There were no significant interactions of sex with any outcome; thus, a stratified analysis was not performed. For wheezing, there was a significant interaction for family history at the third quartile only ($P = .03$), but none for atopy. For rash, there were significant interactions at the third quartile for parental history ($P = .05$) and for the third and fourth quartiles for atopy ($P = .02$ for both). There were no significant interactions for rhinitis.

Associations between endotoxin and allergic symptoms were examined, with infants stratified by parental history of allergic disease and the infant's atopic status (Table III). For wheezing, the associations with endotoxin quartiles were confined to those with a parental history. Infants with a parental history of disease exposed to the highest floor levels of endotoxin had 67% more wheezing compared with infants in the lowest quartile. When infants were stratified by atopic status, both atopic and nonatopic infants showed a nonsignificant trend for more wheezing with exposure to higher endotoxin levels. Rhinitis showed no obvious consistent association with endotoxin levels for infants with or without a parental history. An itchy rash was associated with exposure to higher endotoxin levels among infants with a parental history of allergic disease and showed stronger associations among infants who were atopic.

TABLE I. Prevalence of reported symptoms and atopy and mean bedroom floor endotoxin concentrations with 95% CIs (n = 881)

Outcome	Prevalence (%)	EU/g	(95% CI)	*P*
Wheezing*				
Yes, n = 342	38.9	10,856	(8678-13,581)	.065
No, n = 538		8351	(7041-9905)	
Rhinitis*				
Yes, n = 217	24.9	10,728	(8919-12,904)	.11
No, n = 656		8736	(7358-10,372)	
Rash*				
Yes, n = 186	21.2	11,350	(8171-15,766)	.16
No, n = 692		8757	(7548-101,589)	
Atopy*				
Yes, n = 209	27.0	7044	(4984-9955)	.066
No, n = 564		10,054	(8599-11,755)	

*Numbers do not add to total n because of missing data.

DISCUSSION

In this birth cohort study, we measured endotoxin levels in dust samples collected from the bedroom floors of

TABLE II. Adjusted ORs* and 95% CIs showing associations between respiratory and allergic symptoms, and atopy at 15 months of age by quartiles of bedroom floor endotoxin concentrations at 3 months of age

Outcome	Endotoxin (EU/g)			
	1st Quartile <4621	2nd Quartile 4621-10,749	3rd Quartile 10,750-23,672	4th Quartile >23,672
Wheezing	1.00	1.08 (0.72-1.61) $P = .72$	1.23 (0.82-1.84) $P = .31$	1.54 (1.03-2.30) $P = .04$
Rhinitis	1.00	0.97 (0.62-1.51) $P = .86$	0.98 (0.63-1.52) $P = .92$	0.94 (0.60-1.46) $P = .77$
Rash	1.00	1.48 (0.90-2.43) $P = .12$	1.82 (1.12-2.96) $P = .02$	1.87 (1.14-3.05) $P = .01$
Atopy†	1.00	1.01 (0.64-1.60) $P = .96$	1.25 (0.79-1.97) $P = .34$	0.73 (0.45-1.19) $P = .21$

*ORs are adjusted for total number of people in the house; total number of rooms in the house; owning a pet; having a damp, musty smell; dampness or mold in the bedroom; having an open fireplace; maternal smoking; type of flooring in the bedroom; and New Zealand Deprivation Index.
†Atopy is additionally adjusted for location (Christchurch vs Wellington).

3-month-old infants as a marker of infant exposure to endotoxin. Our main finding was that endotoxin exposure at 3 months of age was associated with reported wheezing and an itchy scaly rash at 15 months but was not associated with rhinitis or atopy.

For wheezing, the effect was confined to infants with a parental history of allergic disease and was not modified by the infant's atopic status. The effects of endotoxin on infant wheezing were similar to those found in previous birth cohorts.[3,4] As in our study, the influence of a family history in these birth cohort studies suggests that susceptibility to endotoxin in infancy is at least partly inherited. The lack of modification by atopic status in our study suggests that the mechanism underlying the association is not related to enhanced IgE-mediated airway inflammation. In studies by Park et al[4] and Bolte et al,[3] the associations were found for endotoxin in either bedding dust or family room dust. In this study, infant bedroom floor dust was used because we had insufficient bed dust available. Endotoxin levels in infant bedroom floor dust have been shown to correlate with those in bed dust[16] but are nevertheless likely to have revealed less marked associations than for bedding or living room dust exposure.

Experimental airway challenge with endotoxin is associated with airflow obstruction, increased airway hyperresponsiveness, macrophage and neutrophil recruitment to the airway, and increased inflammatory mediators[16] and occurs in subjects with and without asthma.

Uniquely, we found a positive association between endotoxin and a history of recurrent itchy rash. Like the association with wheezing, this was confined to infants with a family history of allergic disease. In contrast with the association with wheezing, the effect of endotoxin on rash was enhanced among infants with atopy, suggesting that the mechanism is likely to be IgE-mediated. Studies of allergen sensitization in a mouse model have shown a significant enhancement of the response when sensitization occurs in the presence of endotoxin.[4]

It is possible that endotoxin could be topically proinflammatory on the skin, with a greater effect among atopic infants. Keratinocytes in the skin produce a wide variety of cytokines[17] and express functional CD14 and Toll-like receptor 4, which recognize endotoxin and can induce inflammatory responses.[18] Presumably this effect is most likely to be topical, although aeroallergen challenge can exacerbate eczema, suggesting that airway exposure may affect skin responses.[19] Inhalation of endotoxin experimentally can lead to a transient skin rash associated with increased circulating monocyte $CD4^+$ and CD4 $CD25^+$ lymphocytes.[20]

Domestic endotoxin levels have been associated with increased eczematous skin rashes.[21] This study of German households suggests that prolonged storage of organic waste indoors was associated with a 3-fold to 7-fold increase in dust endotoxin and fungal extracellular polysaccharides. Among the households storing waste for more than 2 days compared with households storing waste for less than 2 days, there was a 3-fold increase in recurrent itchy skin rashes, with a 6-fold increase among atopic family members.[21]

Our question on rash, modified from the International Study of Asthma and Allergies in Childhood (ISAAC) study question and based on the United Kingdom Working Party definitions of atopic dermatitis, was designed to capture recurrent and prolonged itchy scaly rashes. We believe that it will have captured significant atopic dermatitis, but the question has not been specifically validated in New Zealand infants. Parental reports of eczema, at least in older children, have been shown to have poor sensitivity (22%) compared with itchy rash (78%).[22]

Our cohort assembly was designed to produce a random sample of the New Zealand population, although we do not have information on response rates or subjects who refused to participate. However, ethnicity (14.7% identify as Maori) is similar to the 2001 New Zealand Census of Population and Dwellings, in which 15.2% identify as Maori.[23] A family history of allergic disease is high (84%), raising the possibility of selective participation by allergic families. The average age of mothers in our

TABLE III. Adjusted ORs* and 95% CIs showing associations between stratified parental history and atopy and quartiles of bedroom floor endotoxin concentrations at 3 months of age and respiratory and allergic symptoms at 15 months of age

	Endotoxin (EU/g)			
Symptom	**1st Quartile <4621**	**2nd Quartile 4621-10,749**	**3rd Quartile 10,750-23,672**	**4th Quartile >23,672**
Wheezing among infants with parental history of allergic disease (n/N)	1.00 58/177	1.08 (0.68-1.69) *P* = .75 64/180	1.46 (0.94-2.28) *P* = .09 81/187	1.67 (1.07-2.60) *P* = .02 88/191
Wheezing among infants without parental history of allergic disease (n/N)	1.00 15/41	1.37 (0.51-3.69) *P* = .54 14/35	0.47 (0.15-1.50) *P* = .20 6/30	1.32 (0.47-3.76) *P* = .60 11/29
Wheezing among infants with atopy (n/N)	1.00 21/52	1.11 (0.49-2.53) *P* = .80 25/54	1.35 (0.62-2.96) *P* = .45 32/63	1.94 (0.80-4.72) *P* = .14 20/40
Wheezing among infants without atopy (n/N)	1.00 46/141	1.04 (0.62-1.75) *P* = .87 49/140	1.17 (0.70-1.96) *P* = .56 50/133	1.42 (0.86-2.34) *P* = .17 65/150
Rhinitis among infants with parental history of allergic disease (n/N)	1.0 43/177	1.06 (0.65-1.73) *P* = .81 45/180	1.12 (0.69-1.81) *P* = .66 49/183	1.12 (0.69-1.82) *P* = .65 52/189
Rhinitis among infants without parental history of allergic disease (n/N)	1.00 11/41	0.73 (0.24-2.23) *P* = .58 7/35	0.78 (0.22-2.77) *P* = .70 5/29	0.31 (0.08-1.30) *P* = .11 3/29
Rhinitis among infants with atopy (n/N)	1.00 12/52	1.14 (0.45-2.86) *P* = .79 14/54	3.31 (1.37-7.96) *P* = .008 26/63	1.08 (0.39-3.02) *P* = .88 9/40
Rhinitis among infants without atopy (n/N)	1.00 35/141	0.91 (0.52-1.58) *P* = .73 33/140	0.58 (0.32-1.06) *P* = .08 22/128	0.98 (0.57-1.69) *P* = .94 40/149
Rash among infants with parental history of allergic disease (n/N)	1.00 26/176	1.69 (0.97-2.92) *P* = .06 41/180	2.12 (1.24-3.61) *P* = .006 50/187	2.10 (1.22-3.61) *P* = .007 49/190
Rash among infants without parental history of allergic disease (n/N)	1.00 5/41	0.49 (0.12-1.96) *P* = .31 4/35	0.36 (0.07-1.78) *P* = .21 2/30	0.54 (0.12-2.44) *P* = .42 4/29
Rash among infants with atopy (n/N)	1.00 7/52	3.20 (1.12-9.20) *P* = .03 17/54	4.07 (1.47-11.28) *P* = .007 23/63	4.64 (1.56-13.77) *P* = .006 16/40
Rash among infants without atopy (n/N)	1.00 20/141	1.38 (0.72-2.62) *P* = .33 27/140	1.65 (0.86-3.14) *P* = .13 27/133	1.91 (1.02-3.57) *P* = .04 33/150

*ORs are adjusted for the total number of people in the house; total number of rooms in the house; owning a pet; having a damp, musty smell; dampness or mold in the bedroom; having an open fireplace; maternal smoking; type of flooring in the bedroom; and New Zealand Deprivation Index.

cohort at entry was 31 years. We therefore explored this in the Dunedin Multidisciplinary cohort study[24] at the 32-year survey. The response rate at 32 years was 96%. Seventy percent of female cohort members reported a history of 1 or more allergic diseases, asthma, allergic rhinitis, or eczema, and 64% of males (Dr R Hancox, Deputy Director, personal communication, March 2006), showing that 89% of random pairs of the Dunedin cohort would have a history of allergic disease. This suggests that there has been no appreciable self-selection of allergic families into the cohort, but rather it reflects the high prevalence of these conditions in New Zealand. The smaller number of infants with no family history reduces our power to observe an effect of endotoxin in this group and is reflected in the wider CIs associated with the estimates.

We used the Quintest after difficulties using a standard method of skin prick testing in an earlier study of 15-month old infants.[25] It became clear that the Quintest application was producing smaller wheals than would have been expected. We subsequently confirmed this and published the results.[26] For this reason and

additionally to correct for any operator or allergen batch effects, we used a ratio method (comparing the ratio of allergen wheals to histamine wheals) to assess atopic status. This method has been recommended for younger children in previous studies.[27]

It remains to be determined whether any of these early associations between endotoxin and respiratory and skin symptoms have any influence on the later development of allergic disease either positively or negatively. Our findings suggest that endotoxin exposure in infancy deserves further investigation and may be an important determinant of early allergic or nonallergic disease manifestations, in particular infant wheezing and atopic dermatitis among those at increased risk.

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