

Respiratory Infection, Exposure to Mouse Allergen and Breastfeeding: Role in Recurrent Wheezing in Early Life

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Key Words

Asthma • Endotoxin • Mouse allergen • Recurrent wheezing • Respiratory infection

Abstract

Background: Environmental factors may influence the development of allergen sensitization and asthma. The aim of this study was to evaluate the role of endotoxin and allergen exposure in early life as a risk factor for recurrent wheezing. **Methods:** One hundred and four infants from low-income families, at high risk of asthma, were enrolled at birth. Dust samples were collected from the bedding and bedroom floor within 6 months after birth. Recurrent wheezing was defined as 3 or more wheezing episodes in the past year. Endotoxin was determined by Limulus amoebocyte lysate assay, and major indoor allergens were quantitated by ELISA in dust extracts. IgE antibodies were measured by ImmunoCAP at 30 months of age. **Results:** At 30 months, 51 of the 99 infants who completed the study (51.5%) had recurrent wheezing. Respiratory infection was strongly associated with recurrent wheezing (OR 6.67, 95% CI 1.96–22.72), whereas exclusive breastfeeding for at least 1 month was a protective factor (OR 0.09, 95% CI 0.01–0.51). Exposure to high levels of

mouse allergen was more frequent among non-recurrent wheezers, approaching significance (OR 0.12, 95% CI 0.01–1.13; $p = 0.064$). None of the children were sensitized to mouse. Sensitization to mite was found in 26/90 (28.8%) children, with no association with recurrent wheezing. **Conclusion:** Respiratory infection was strongly associated with recurrent wheezing in the first 30 months of life, in children at high risk of asthma, living in a socially deprived community in Brazil.

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Introduction

Asthma induction seems to result from complex interactions between environmental exposures and genes during crucial periods in the natural history of the disease [1]. Sensitization to aero-allergens from indoor and outdoor environments, particularly those derived from dust mites, pets and cockroaches, has been consistently linked to the development of allergic symptoms in the upper and/or lower respiratory tract [2–4]. In addition, infections with respiratory viruses including respiratory syncytial virus and rhinovirus have been recognized as

major triggers of wheezing in young children [5, 6] and may be associated with persistence of symptoms and development of asthma later in life, particularly in children who present early (≤ 2 years of age) sensitization [7]. Current data highlight the potential synergistic effect of sensitization, allergen exposure and concomitant viral infection in augmenting inflammatory responses in the airways, contributing to increased severity of symptoms and subsequent development of persistent asthma [8].

Quantitative assessments of exposure to major allergens and endotoxin in the home early in life have revealed different and even paradoxical effects on sensitization and development of asthma. Exposure to cats and dogs may protect against subsequent development of asthma [9–11]. A modified Th2 response, characterized by decreased allergen sensitization and pronounced allergen-specific IgG production, has been described among children exposed to very high levels of cat allergen Fel d 1 [12]. Recent reports have highlighted the importance of mouse allergen as a cause of sensitization among children with asthma living in inner-city and suburban environments in US cities, such as Baltimore and Boston [13–17], and have demonstrated that the combination of sensitization to mouse and high-level exposure to mouse allergen ($>0.5 \mu\text{g Mus m 1/g}$ of dust) in the home was associated with increased morbidity due to asthma in 2- to 6-year-old children belonging to low-income families, predominantly of African-American origin [17].

Indoor exposure to high levels of endotoxin has been associated with exacerbation of pre-existing asthma in both children and adults [18, 19], as well as with increased risk of repeated wheeze in the first year of life [20]. However, other studies have shown that early life exposure to high levels of endotoxin in both farming and non-farming environments might prevent the development of asthma and atopy [21, 22]. The impact of endotoxin may be genetically determined [23]; in a birth cohort study, increasing endotoxin exposure was associated with a reduced risk of allergic sensitization and eczema, and with increased risk of non-atopic wheeze, only in children with the CC genotype at -159 of the CD14 gene [23].

The aim of this prospective birth cohort study was to examine the influence of environmental factors including exposure to indoor allergens and to endotoxin, infections and breastfeeding on the development of recurrent wheezing in young children from very low-income families at high genetic risk of asthma and allergies in Brazil.

Subjects and Methods

Subjects

In this cohort study, 104 newborn infants were enrolled at birth. All the children belonged to low-income families (yearly income $<\text{USD } 5,000$, with 3–12 inhabitants per household) and were at high risk of asthma. Mothers were recruited from those attending a social program for pregnant women at the Albert Einstein Hospital, São Paulo, targeting low-income families living in a nearby community. Of the 200 women participating in the program, 178 were interviewed; 104 were selected based on the presence of at least 1 parent with a history of allergies and/or asthma, and all of them agreed to participate. They were enrolled in the study between January and March 2004. Ninety-nine children and their families remained in the study after 30 months of follow-up. Five children were lost to follow-up or excluded from the study due to the following reasons: moving to another city (3 children); death from bacterial meningitis (1 child); recurrent infections after the mother was diagnosed with AIDS (1 child). The study was approved by the Ethics Committees of the Paulista School of Medicine Hospital and the Albert Einstein Hospital. All parents or caregivers gave written informed consent to participate in the study.

Study Design

Within the first 6 months following birth, dust samples were collected by the research team from the bedding and floor in the infant's bedroom. Samples were analyzed for allergen and endotoxin contents, as previously described [5]. In 2 households where infants slept with their mothers, samples were collected from the mother's bedroom. Floor dust samples were obtained by vacuuming an area of 1 m^2 for 2 min, using an Electrolux vacuum cleaner with an airflow rate of 45 l/min. Fine dust was obtained by sieving samples through a mesh screen, and dust extracts were prepared [5].

The following information was provided by the parents by means of phone interviews based on a structured questionnaire: number of wheezing episodes and general health, respiratory infection episodes requiring antibiotics, during the preceding 3 months, from 3 to 30 months of age. Outcome measures at 30 months are described for 99 children. Serum IgE antibodies to inhalant allergens were measured in 90 children, at 30 ± 3 months of age.

Quantification of Allergens and Endotoxin in House Dust Samples

Major allergens from *Dermatophagoides* sp. mites (Der p 1, Der f 1), cockroach species *Blattella germanica* and *Periplaneta americana* (Bla g 1 and Per a 1, respectively), cat (Fel d 1), dog (Can f 1), mouse (Mus m 1) and rat (Rat n 1) were quantitated in dust extracts by ELISA (Indoor Biotechnologies Inc., Charlottesville, Va., USA). High-level exposure to mites and cockroach was defined as group 1 mite allergens $\geq 2 \mu\text{g/g}$ of dust, and cockroach allergens as Bla g 1 and/or Per a 1 $\geq 2 \text{ U/g}$ of dust [24, 25]. High-level exposure to cat, dog and mouse was characterized as Fel d 1 $\geq 8 \mu\text{g/g}$, Can f 1 $\geq 10 \mu\text{g/g}$ [25] and Mus m 1 $\geq 1.6 \mu\text{g/g}$ of dust [14], respectively.

Separate dust samples were collected for endotoxin analysis and were kept in pyrogen-free glassware. Extracts were prepared by shaking 10 mg of dust in 400 ml of pyrogen-free distilled water

Table 1. Univariate analysis of factors associated with recurrent wheezing in 30-month-old children at high risk for allergy

Risk factor	Total (n = 99)	Recurrent wheezers (n = 51)	Non-recurrent wheezers (n = 48)	OR
Endotoxin level >100 EU/mg				
Bedding and/or floor	14	4	10	0.32 (0.09–1.11)
Bedding	10	2	8	0.20 (0.04–1.01)*
Floor	7	2	5	0.35 (0.06–1.90)
High-level allergen exposure				
Mite group 1 >2 µg/g bedding	78	39	39	0.75 (0.28–1.98)
Mite group 1 >10 µg/g bedding	29	18	11	1.83 (0.76–4.44)
Mus m 1 >1.6 µg/g	7	1	6	0.14 (0.02–1.21)
Bla g 1 >2 U/g	4	1	3	–
Per a 1 >2 µg/g	3	1	2	–
Fel d 1 >8 µg/g	0	0	0	–
Can f 1 >10 µg/g	0	0	0	–
Report of respiratory infection(s)				
Infection(s) requiring antibiotics	69	44	25	5.78 (2.17–15.38)*
Gender				
Male	56	29	27	1.02 (0.46–2.27)
Breastfeeding				
Exclusive breastfeeding for at least 1 month	80	35	45	0.14 (0.04–0.54)*
Sensitization at 30 months of age ¹				
IgE antibodies to at least 1 inhalant allergen	26	13	13	1.00 (0.40–2.49)
Other parameters				
Use of inhalant corticosteroids ²	11	10	1	11.46 (1.41–93.41)*
Gastroesophageal reflux disease	6	5	1	5.11 (0.57–45.42)
Physician-diagnosed eczema	24	15	9	1.80 (0.70–4.63)
Asthmatic parent(s)	13	9	4	2.36 (0.67–8.24)
Cigarette smoking in the house	28	15	13	1.12 (0.47–2.69)
Pet ownership	29	14	15	0.83 (0.35–1.98)

Figures in parentheses are 95% CIs.

¹ Sensitization data obtained from 90 children.

² Use of inhaled corticosteroids for 2 or more months over the past year.

* $p < 0.05$.

for 5 min, followed by a 1-hour resting period. After centrifugation, supernatants were removed and assayed without freezing. Endotoxin quantitation was carried out by the Limulus amoebocyte lysate assay (Pyrogen-5000, Cambrex). A replicate of each sample was spiked with a standard preparation of endotoxin. If spike recovery was <45%, the suspension was concentrated; if recovery was >200%, the suspension was diluted. Assays were run in duplicate, and results are expressed as endotoxin units per milligram (EU/mg) of dust. High-level exposure to endotoxin was considered as >100 EU/mg of dust, in accordance with Park et al. [20].

IgE Antibodies

Serum IgE antibodies to *D. pteronyssinus*, *D. farinae*, cat, dog, *B. germanica*, mouse epithelium, grass pollen and mold mixtures were quantitated using the Phadia ImmunoCAP system, and lev-

els of IgE >0.7 kUA/l (class 2 or greater) were considered positive to minimize inclusion of nonspecific results due to high total IgE levels, in accordance with previous studies carried out in Brazil [5, 26].

Outcome Definitions

Recurrent wheezing was defined as 3 or more wheezing episodes requiring treatment with an inhaled β_2 -agonist in the past year [5, 26]. A report of respiratory infection was defined as infection of the upper and/or lower respiratory tract requiring treatment with antibiotics prescribed by a physician. Eczema was considered as eczema diagnosed by a physician.

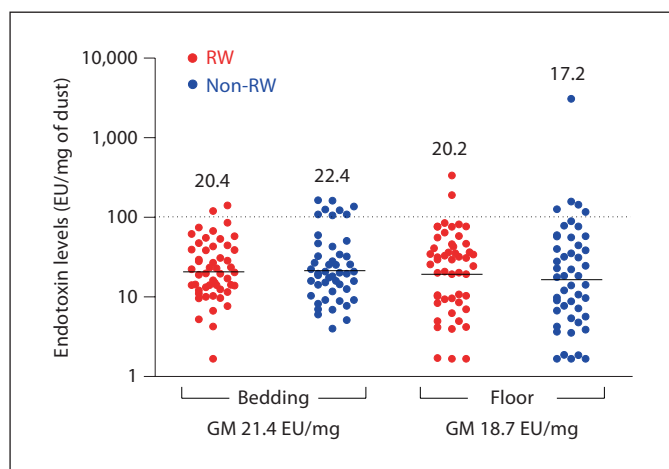


Fig. 1. Endotoxin levels in dust samples from the bedding and bedroom floor collected from the homes of children identified as recurrent wheezers (RW) and non-recurrent wheezers (non-RW). The dotted line indicates a level of 100 EU/mg of dust, defined as high exposure [20]. Children were followed from birth to 30 months of age, and the endotoxin content was determined in dust samples collected within the first 6 months of life. GM = Geometric mean.

Statistical Analysis

Comparisons among recurrent and non-recurrent wheezers were performed using the χ^2 test for proportions. The Fisher exact test was used when the observed frequency of any variable was <5 . Univariate and multivariate analyses were carried out by logistic regression, using STATA version 6.0. $p < 0.05$ was considered as significant.

Results

At 30 months, 51/99 (51.5%) children presented recurrent wheezing. Univariate analysis revealed that a report of respiratory infection was more frequent in recurrent wheezers ($p < 0.001$). On the other hand, high levels of endotoxin in bedding (>100 EU/mg of dust) and exclusive breastfeeding for at least 1 month were more frequent in non-recurrent wheezers ($p = 0.047$ and 0.002 , respectively). Likewise, the comparison between the groups in terms of exposure to high levels of mouse allergen in the home approached statistical significance ($p = 0.055$), with non-recurrent wheezers being more exposed (table 1). Exposure to high levels of allergens derived from dust mites or cockroach showed no effect on recurrence of wheezing (table 1).

Endotoxin from the bedding and bedroom floor ranged from 1.6 to 158 EU/mg and from 1.6 to 2,955 EU/

mg of dust, with geometric means of 21.4 EU/mg and 18.7 EU/mg of dust, respectively (fig. 1). Mouse allergen Mus m 1 was detectable in the homes of 34/99 children, with levels ranging from 0.03 to 26.9 $\mu\text{g/g}$ of dust. Very low levels of rat allergen Rat n 1 were detectable in the homes of 5/99 children, ranging from 0.05 to 0.2 $\mu\text{g/g}$ of dust (data not shown). Mite allergen Der p 1 from the bedding and bedroom floor ranged from 0.02 to 27.2 $\mu\text{g/g}$ and from 0.02 to 21.7 $\mu\text{g/g}$, with geometric means of 5.4 and 1.5 $\mu\text{g/g}$, respectively. Mite allergen Der f 1 from the bedding and bedroom floor ranged from 0.02 to 21.1 $\mu\text{g/g}$ and from 0.02 to 1.3 $\mu\text{g/g}$, with geometric means of 0.4 and 0.2 $\mu\text{g/g}$, respectively. Overall, the levels of allergens from cockroach, cat and dog were very low. Cockroach allergens Bla g 1 and Per a 1 ranged from 0.2 to 59.2 U/g and from 0.1 to 3.5 $\mu\text{g/g}$, with geometric means of 0.7 U/g and 0.5 $\mu\text{g/g}$, respectively. Levels of cat allergens Fel d 1 ranged from 0.006 to 2.8 $\mu\text{g/g}$, with a geometric mean of 0.03 $\mu\text{g/g}$, and levels of dog allergen Can f 1 ranged from 0.1 to 20 $\mu\text{g/g}$ of dust, with a geometric mean of 0.5 $\mu\text{g/g}$. Analysis using allergen and endotoxin levels as continuous variables showed no significant correlation with recurrent wheezing (data not shown). In addition, no significant correlation was observed between endotoxin levels and mite group 1 or Mus m 1 allergen levels in dust samples (data not shown).

Exclusive breastfeeding for at least 1 month showed a protective effect, with an OR of 0.14 (95% CI 0.04–0.54; $p < 0.05$) (table 1). This effect was not observed for longer durations (3, 4 or 6 months) of exclusive breastfeeding. Sensitization to mites (*D. pteronyssinus* and/or *D. farinae* CAP score ≥ 2) at the age of 30 months was found in 26/90 (28.8%) children. Of those, 13/45 were recurrent wheezers and 13/45 were non-recurrent wheezers. Four children were sensitized to *B. germanica*, 1 to cat, 1 to grass pollen mixture, and none to dog or to mold mixture. None of the children were sensitized to mouse at the age of 30 months (even considering a lower cut-off value for IgE antibodies of 0.35 kUA/l), including those exposed to high levels of mouse allergen in their homes early in life. No significant association of allergen sensitization and recurrent wheezing was observed in these young children after 30 months of follow-up.

Additional factors including male gender, presence of eczema, exposure to tobacco smoke and pets in the house, and having an asthmatic parent showed no effect on recurrence of wheezing in these children, whereas use of inhaled corticosteroids was more commonly reported among recurrent wheezers due to obvious bias of indication for treatment (table 1).

Table 2. Multivariate analysis of risk factors for recurrent wheezing in 30-month-old children at high risk of allergy

	OR	P
Respiratory infection	6.67 (1.96–22.72)	0.002
High exposure to mouse allergen ¹	0.12 (0.01–1.13)	0.064
High exposure to endotoxin ²	0.55 (0.14–2.21)	0.407
Exclusive breastfeeding for at least 1 month	0.09 (0.01–0.51)	0.006
Sensitization to at least 1 inhalant allergen ³	1.09 (0.36–3.24)	0.871
Gender, male	0.71 (0.26–1.93)	0.511

Figures in parentheses are 95% CIs.

¹ Mus m 1 >1.6 µg/g in bedding and/or bedroom floor dust samples.

² Endotoxin >100 EU/mg in bedding and/or bedroom floor dust samples.

³ CAP score ≥2 to at least 1 inhalant allergen.

Multivariate analysis revealed that the report of respiratory infection was strongly associated with recurrent wheezing, whereas exclusive breastfeeding for at least 1 month showed a protective effect (table 2). Exposure to high levels of mouse allergen (Mus m 1 >1.6 µg/g of dust) approached statistical significance as a protective factor for recurrent wheezing. Male gender, high-level exposure to endotoxin and sensitization to at least 1 inhalant allergen at 30 months of age had no effect.

Discussion

In the present study, 51.5% of the children at high risk of development of allergies and asthma, living under very low socioeconomic conditions in the city of São Paulo, Brazil, had recurrent wheezing at 30 months of age. A report of respiratory tract infection was strongly associated with recurrent wheezing at 30 months. Our results are in agreement with those reported by a previous study carried out in metropolitan Boston, involving infants with a parental history of asthma or allergies. In that study, the report of at least 1 lower respiratory tract illness was associated with wheezing in the first year of life [27]. In the present study, respiratory infection was usually coincident with wheeze. Previous data from Brazil revealed that infection with respiratory viruses was a major risk factor for acute wheezing among children <2 years of age [5].

One limitation of the present study is that the specific pathogen(s) responsible for each episode of acute respiratory infection was not identified. We used the criterion of reports of respiratory infections requiring antibiotics prescribed by a physician to increase the accuracy of the

information provided and to select for more severe illness. To investigate this issue in detail, Kusel et al. [7] conducted a community-based study involving 198 children, assessing postnasal aspirates for viral identification in all episodes of acute respiratory illnesses in the first year of life. Viruses were detected in 69% of the 815 episodes, most commonly rhinovirus (48.3%) and respiratory syncytial virus (10.9%). The results showed that acute severe lower respiratory tract infections caused by rhinovirus or respiratory syncytial virus in the first year of life were important contributors to current asthma and persistent wheezing at age 5; this serves to highlight the major role of respiratory viruses in early life wheezing.

We found that exclusive breastfeeding for at least 1 month had a protective effect against recurrent wheezing. However, this effect was lost when exclusive breastfeeding for 3, 4 or 6 months was analyzed. Similar results were obtained in a previous study carried out in Brazil, to evaluate risk factors for persistence of wheezing among children 2–4 years of age [26]. This leads to the question of whether breastfeeding per se does indeed have a protective role against wheezing. However, it has been shown that human colostrum and early milk (<15 days) are enriched with anti-inflammatory cytokines, particularly transforming growth factor (TGF)-β₁ and IL-10, and that the levels of these cytokines fall substantially in mature milk (>40 days) [28–30]. TGF-β₁ promotes secretory IgA production, which might increase defense against viruses in the respiratory mucosa and decrease food sensitization in the gut. In experimental models, TGF-β₁ regulates signaling mechanisms of proliferation and differentiation of lung cells, enhancing lung development [28]. We speculate that bioactive factors abundant in colostrum and ear-

ly milk may have an important role in establishing an anti-inflammatory milieu and increasing defense mechanisms in the respiratory mucosa. This may be the reason why longer breastfeeding does not seem to confer additional beneficial effects.

Our results suggest that exposure to high levels of mouse allergen early in life may protect against recurrent wheezing, raising the possibility of tolerance induction. In keeping with this, a study with preschool, inner-city, predominantly African-American children has shown that the relationship of exposure to mouse allergen and sensitization to mouse seems to follow a linear pattern with low or moderate levels of exposure; however, at very high exposure (Mus m 1 >29.9 µg/g) an attenuated prevalence rate of mouse sensitization was observed [31]. The rate of mouse allergen detected in the present study (34%) was comparable with that reported in other areas, including inner-city homes in Poland (46%) [32] and metropolitan Boston (42%) [27]; however, it was lower than the rates observed in inner-city and suburban homes in Baltimore (100%) [13, 16] and in other inner-city homes in the US (70%; overall rate 95%) [13]. Exposure range was also not as high as that observed in inner-city US homes [13]. In the present study, high-level exposure was defined as mouse allergen >1.6 µg/g, which has been associated with a greater risk of sensitization to mouse among school-age children participating in the National Cooperative Inner-City Asthma Study [14]. Only 7/99 homes presented levels above this threshold. Interestingly, it has also been shown that a report of exposure to mouse in the household was associated with increased risk of wheezing in the first year of life, in a group of high-risk infants followed prospectively [27]. To interpret these seemingly paradoxical effects, it is important to consider that both airborne and settled dust mouse allergen levels vary substantially over time within homes and may be affected by acquisition of a cat, low indoor humidity and season [33]. Repeated exposure may be more suitable to assess the strength of the association between mouse exposure and wheeze, as suggested by Matsui et al. [33]. In the present study, repeated allergen measurements were not considered in the study planning.

An alternative hypothesis is that high-level exposure to mouse allergen may represent a surrogate marker for exposures to microbial products such as endotoxin and other components, which may be abundant in homes with poor hygiene conditions and might confer protection for the subsequent development of allergies and asthma. In the present study, we observed a negative association between exposure to high levels of endotoxin in

bedding and recurrent wheezing on univariate analysis, but this association lost significance on multivariate analysis. A prospective study involving 226 participants, carried out in a non-farming environment, has shown decreasing rates of wheezing among children exposed to higher levels (>81.3 EU/mg) of house dust endotoxin, as compared with those exposed to lower levels, at age 5–9 years [34]. On the other hand, Perzanowski et al. [35] found that domestic endotoxin exposure, analyzed as quartiles (up to 35.6 EU/mg, 35.6–77.6 EU/mg, 77.6–155 EU/mg, and >155 EU/mg) was inversely associated with eczema at 1 year of age, but positively associated with wheeze at 2 years, in inner-city New York. It has become evident that not only do the effects of endotoxin exposure depend on dosing and timing of exposure but they also depend on individual genetic background [23].

Some limitations of the present study include the relatively small sample size, which might have decreased the power to detect significant differences, and acquisition of clinical data by parental report. The conclusion that exposure to high levels of mouse allergen in early life may be a protective factor for recurrent wheezing was based on results of 7/99 homes, and a larger sample size could contribute to defining clinical relevance. However, this study with children living in socioeconomically deprived areas is the first prospective birth cohort study carried out in South America. As reported for other areas of the world, infections of the respiratory tract were strongly and independently associated with recurrent wheezing at the age of 30 months. In addition, exclusive breastfeeding for at least 1 month was protective. Investigating the role of viral respiratory infections in asthma inception, in the context of early sensitization, environmental exposure to allergens and endotoxin and other risk factors, as well as establishing the genetic and immunologic basis of protective effects of environmental exposures, will be useful for the definition of early intervention strategies.

Acknowledgements

The authors wish to thank Lucila Camargo, MD, Cintia S.K. La Scala, MD, Fabrício L. D'Octaviano, MD, Daniele K. Arari, MD, M. Socorro Damasceno, RN, and Jade Cury, MD, for their assistance with the selection of patients, home visits and collection of dust samples. L.K. Arruda, MD, and Dirceu Solé, MD, are recipients of CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) scholarships. This work was supported by grants No. 03/01745-8 and 03/01746-4 from FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) and by the Institute of Investigation in Immunology, CNPq, Brazil.

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