

Endotoxin exposure and symptoms in asthmatic children

Rizzo MC, Naspitz CK, Fernández-Caldas E, Lockey RF, Mimiça I, Solé D. Endotoxin exposure and symptoms in asthmatic children. *Pediatr Allergy Immunol* 1997; 8: 121–126. © Munksgaard 1997.

Endotoxins (ET) are pro-inflammatory substances present in house dust which may increase non-specific bronchial reactivity in asthmatic patients. Endotoxins (EU/g) and *Der p* 1 levels were compared in the homes of ten asthmatic and ten control children, aged 6–16 years, living in São Paulo, Brazil. The houses were visited once a month from February 1993 to February 1994 and dust samples were collected from the bedding and floor of each subject's house. No significant differences were observed in ET and *Der p* 1 levels in the homes of asthmatics and controls. The highest ET levels were detected in January and November, whereas the lowest levels were detected in April and August ($p < 0.05$), demonstrating a distinct seasonal distribution. The highest *Der p* 1 levels in bedding were observed in July and the lowest in February ($p < 0.05$), while *Der p* 1 levels in floor did not show significant differences throughout the year. Symptom and medication scores were evaluated monthly in the group of asthmatic children. There was a significant correlation ($p < 0.05$, $r = 0.63$) between clinical symptom scores and ET exposure, however no significant correlation was found for mite exposure ($p > 0.05$, $r = 0.19$). The results suggest that ET exposure exacerbates asthmatic symptoms in mite allergic, asthmatic children.

**M. Cândida Rizzo¹,
Charles K. Naspitz¹,
Enrique Fernández-Caldas²,
Richard F. Lockey², Igor Mimiça¹,
Dirceu Solé¹**

¹Federal University of São Paulo, Paulista School of Medicine, São Paulo, Brazil, ²University of South Florida, Tampa, FL, USA

Charles K. Naspitz, M.D., Rua Sergipe, 634- 13.A
CEP 01243-000 São Paulo, Brazil

Key words: endotoxin; asthma; mites; *Der p* 1; Brazil; seasonal variation

Received 10 August, 1997
Accepted 22 October, 1997

The importance of the indoor environment on respiratory diseases has been increasingly recognized over the past 30 years. The concentration and the duration of exposure to many identified pollutants has increased, and this may be attributed to a tendency for children to spend more time indoors (1).

Microbial organisms present in house dust may induce pulmonary and systemic symptoms upon inhalation. Research focused on endotoxin (ET), a lipopolysaccharide (LPS) present in the outer membrane of gram negative bacteria (GNB), has demonstrated a variety of cellular events in response to these molecules. The lipid portion of LPS (lipid A) is chemically distinct from all other lipids in biological membranes and is responsible for the toxicity of endotoxin. Macrophages exposed to picogram amounts of ET have been shown to produce tumor necrosis factor (TNF α), interleukins 1 and 6 and platelet activating factors (2, 3). These potent mediators then activate other immune effector cells.

It has been demonstrated that inhaled ET influences the cell kinetics of the airways and lung walls of guinea pigs (4). This effect consists of a rapid in-

crease in the number of inflammatory cells, particularly neutrophils, with a return to normal values within a few days. In humans, endotoxin inhalation has been shown to cause an airway response (5–7) and an increase in airway responsiveness (6, 7). Inhaled endotoxin produces a dose-related bronchoconstriction 4 to 6 hours after exposure, which is more accentuated among patients with hyperreactive airways (6, 7). Michel et al. (8) have shown that the inhalation of 20 μ g of LPS in normal subjects did not induce bronchial obstruction, however it produced a systemic inflammatory response.

Dust mites are an important source of allergens in the indoor environment. Mite allergens are able to cause inflammatory allergic responses in sensitized individuals. A major dust mite allergen *Der p* 1 from *Dermatophagoides pteronyssinus*, is mainly present in fecal pellets that accumulate in bedding and other surfaces. The highest levels of this allergen are detected in bedding, mattresses, carpets and upholstered furniture (9, 10). Although there are regional and local differences, domestic mites are thought to be one of the most important perennial allergenic causes of childhood asthma (11). Previous studies in Brazil

have shown that the mites *D. pteronyssinus*, *Blomia tropicalis* and *B. kulaginii* are important causes of sensitization among asthmatic patients (12–13).

The purpose of this prospective study was to determine *Der p 1* and endotoxin levels and correlate them with clinical symptoms of asthma in ten mite allergic, asthmatic children living in São Paulo, Brazil, over a period of one year.

Material and methods

Patient population

A total of 20 children (12 boys, eight girls), aged 6 to 16 years old, were enrolled at the beginning of the study, ten with asthma and ten controls. One patient had mild asthma (patient no. 4), eight patients had moderate asthma (patients 1, 2, 3, 5, 6, 7, 9, 10) and one patient had severe asthma (patient no. 8). The classification of the severity of asthma was based on a report of the British Thoracic Society (14). All the children had positive skin prick tests (mean wheal diameter > 5 mm) to a standardized *D pteronyssinus* extract (Bayer Laboratories, Spokane, Washington) and were selected among patients who attended the Pediatric Allergy Clinic, at the Federal University of São Paulo, Brazil. One of these patients (patient 5) did not complete the study due to poor compliance. Ten control children, with no personal history of allergic disease, negative skin tests to *D pteronyssinus* and no respiratory symptoms were selected from the

Pediatric Emergency Room of the same Institution. The mean Metacholine PC20 values for the asthmatics was ≤ 2.5 mg/ml (range 0.25 to 2.5); the ten control children had Metacholine PC20 values ≥ 10.0 mg/ml (Table 1).

Clinical evaluation

The clinical evaluation was based on a combined quantitative index for symptoms and therapeutic scores, obtained from diary cards. The symptoms and therapeutic scores were grouped forming a global clinical score (symptom + therapeutic score). Asthmatic patients registered 2 daily peak flow meter (PFM) readings (AM and PM) (data not shown). A quantitation index for symptom scores was used as follows: 0 = none; 1 = trivial or doubtful; 2 = mild, clearly present, but causing little or no discomfort; 3 = moderately severe, causing marked discomfort; 4 = severe, some interference with sleep or activities but not incapacitating; and 5 = incapacitating (15). Medical consumption was evaluated according to Table 2 (16).

None of the asthmatic children were using anti-inflammatory inhaled drugs when admitted in the study. During the study, they received inhaled or oral bronchodilators and oral systemic corticosteroids as needed.

IgE antibodies to mites

IgE antibodies to *D pteronyssinus* and *B tropicalis* were measured by radioallergosorbent test (RAST), as previously described (17). Values > 5% of total counts bound were considered positive in this assay.

Analysis of dust samples

Analysis of coded dust samples for ET and *Der p 1* levels was performed, in a blinded fashion at the University of South Florida, by investigators who were not aware of the source of the samples or clinical presentation of the patients. House dust samples were collected monthly from February '93 to February '94, in houses from São Paulo, using methods previously described (18). Briefly, dust samples were collected on a fine mesh cloth filter inserted in a dust "trap filter" between the dusting attachment and the end of the hose of a portable vacuum cleaner (ELECTROLUX, 1300 W). Two dust samples were collected, from the mattress surface and the bedroom floor, in an area of 1 m² for 2 min. Samples were kept in plastic bags at -20°C until extraction to determine ET and *Der p 1* content. Samples were sieved through a 0.3 mm mesh screen to obtain fine dust and 100 mg were extract-

Table 1. Individual characteristics of the asthmatics (1–10) and controls (11–20)

Patient no.	Sex	Age (years)	Degree of asthma severity	Metacholine PC20 (mg/ml)
1	M	9	moderate	0.25
2	F	14	moderate	0.25
3	M	9	moderate	2.50
4	M	12	mild	1.00
5	M	10	moderate	2.50
6	M	10	moderate	1.00
7	M	16	moderate	2.50
8	M	15	severe	1.00
9	M	8	moderate	2.50
10	M	13	moderate	1.00
11	M	6	moderate	>25.00
12	F	12	moderate	>25.00
13	F	14	moderate	>25.00
14	M	7	moderate	10.00
15	F	11	moderate	>25.00
16	F	12	moderate	>25.00
17	M	8	moderate	>25.00
18	F	10	moderate	>25.00
19	F	9	moderate	>25.00
20	F	8	moderate	>25.00

Table 2. Drug equivalency scoring system

Drug	One Unit Equivalent
No drug	0
Antihistamines	0
Oral short acting or intravenous aminophylline	200 mg
Long-acting xanthines	100 mg
Sympathomimetic metered dose inhalers (MDI)	1 puff
Sympathomimetic tablets	
Salbutamol tablets	2.0 mg
Terbutaline tablets	2.5 mg
Ipratropium bromide (MDI drops for nebulization)	20.0 µg
Inhaled corticosteroids (MDI)	25.0 µg
Prednisone (oral)	0.5 mg

ed with 2 ml of borate-buffered saline, pH 8.0. Mite allergens (*Der p 1*) was measured by monoclonal antibody based ELISA, as described elsewhere (19). For ET quantitation purposes, dust samples were extracted in pyrogen free water in a 1: 40 ratio and further diluted to 1: 2000 or higher dilutions. ET was measured by the Lymulus amoebocyte lysate test following the instructions provided by the manufacturer (Bio Whittaker, Inc). This test is based on the characteristics of the lipid A portion of endotoxin to activate the coagulation cascade of the amoebocyte lysate from the horseshoe crab (*Limulus polyphenus*) (20).

Bacteria counts

Samples obtained by imprinting the surfaces of shoes, bedroom floor and mattresses, were collected in each house, monthly. The URIBAC system (PROBAC adapted to bacteria growth, CLED and SIMMONS) was used to imprint the surfaces. After each collection, the URIBAC were incubated at 37°C, for 18–24 hs, and the colony forming units (CFU) were determined. In addition, samples of air inside and outside homes were collected using a Biotest RCS centrifugal air sampler (BIOTEST). Using this system, air samples were collected at a flow rate of 40 l air/minute, and impacted on strips containing appropriate bacterial culture medium (Macconkey-Agar).

Statistical analysis

Statistical analysis was performed by nonparametric methods: Friedman, Mann-Whitney, Wilcoxon and Spearman's rank-correlation tests, and p values < 0.05 were considered as significant.

ET and Der 1 Monthly Median Levels (Floor and Bedding) Collected in Homes of 10 Asthmatics and 10 Controls

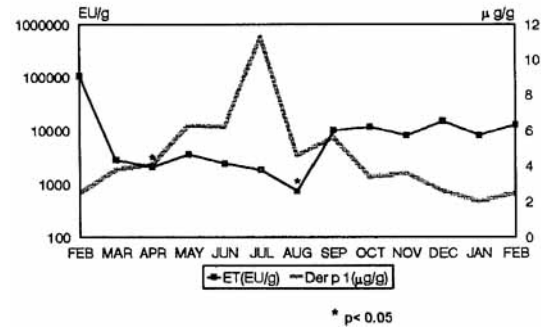


Fig. 1.

Results

Exposure to indoor allergens

Mite allergens were measured monthly from bedding and bedroom floor during the study. The highest levels of *Der p 1* were found in bedding samples ($p < 0.05$) without differences between asthmatics and controls houses. The range of median *Der p 1* levels monthly in bedding were 2.0 µg/g in January to 11.3 µg/g in July. Nine out of nine asthmatic patients and 9/10 control children's dust samples had *Der p 1* levels equal or > 10 µg/g of dust at least in one month of the year. The levels of *Der p 1* showed no significant differences throughout the months of the year (Fig. 1).

IgE response to inhalant allergens

Nine of the ten (90%) and 7/10 (70%) of asthmatics had detectable serum IgE to *D pteronyssinus* and *B tropicalis*, respectively, whereas none of the children in the control group presented IgE to mite allergens.

GNB (cfu) on Shoes' Soles, Bedroom Floor and Bedding 10 asthmatics and 10 Controls Children Feb 93-Feb 94

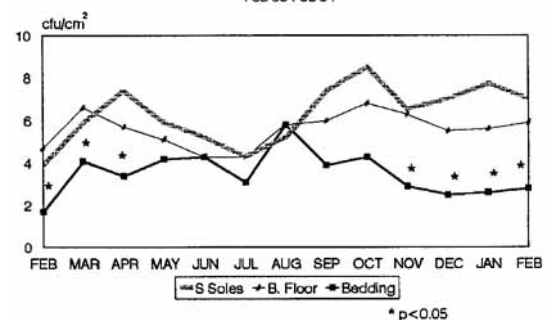


Fig. 2.

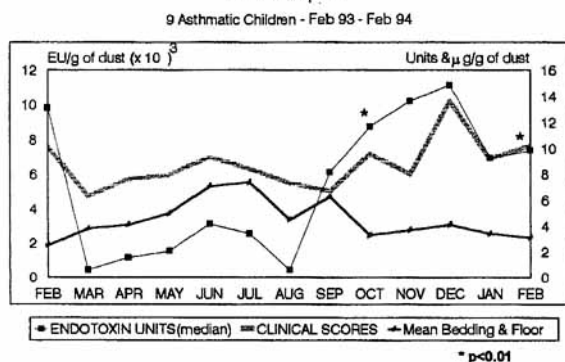
Clinical Scores, Endotoxin Levels and *Der p 1* in Floor and Bedding Dust samples

Fig. 3.

Results of the RAST showed that the median of total counts bound in sera from asthmatics was 14.7% and 13.2% for *D pteronyssinus* and *B tropicalis*, respectively. On the other hand, all sera from control children bound < 5% total counts in this assay.

Endotoxin exposure

The highest levels of ET levels (EU/g of dust) were observed in the summer months (Dec., Jan., Feb.) and the lowest levels were observed in Apr. and Aug. ($p < 0.05$) (Fig. 1).

Endotoxin levels in dust samples from bedding and floor from asthmatics and control houses were statistically similar.

Exposure to bacteria

There were no differences in bacteria CFU between asthmatics and control houses. There were significantly more GNB CFU on soles of shoes and/or floor than in bedding ($p < 0.05$) in the months of Feb., Mar., Apr., Nov., Dec. 1993; and Jan. and Feb. 1994 (Fig. 2). Gram negative bacteria were found predominantly on surfaces, whereas gram positive bacteria were measured in higher concentrations airborne.

Clinical evaluation

There was no correlation between clinical scores and: PFM measurements ($r = 0.15$, $p > 0.05$), gram positive bacteria CFU on surfaces and airborne ($r = 0.30$; $r = 0.17$, $p > 0.05$) and gram negative bacteria on surfaces and airborne ($r = 0.46$; $r = 0.03$, $p > 0.05$).

There was a positive correlation between ET exposure year round and clinical scores ($r = 0.63$,

Table 3. Monthly clinical scores in nine asthmatic patients

	PAT. 1	PAT. 2	PAT. 3	PAT. 4	PAT. 6	PAT. 7	PAT. 8	PAT. 9	PAT. 10
FEB	20	12	11	5	3	12	18	1	10
MAR	9	5	8	2	1	4	20	1	8
APR	4	4	8	2	1	4	20	1	8
MAY	5	5	9	2	3	12	21	7	8
JUN	7	5	3	1	17	4	20	19	8
JUL	8	5	3	2	3	5	21	9	20
AUG	8	5	3	1	3	4	21	9	8
SEP	7	5	3	2	3	4	20	9	8
OCT	10	5	22	3	1	4	20	11	11
NOV	20	5	3	1	3	4	20	7	10
DEC	33	7	5	1	9	7	30	7	24
JAN	20	14	3	4	2	5	21	7	8
FEB	9	5	3	1	2	15	19	28	11

$p < 0.05$), with a tendency for positive correlation in all months, significant in Oct. 1993 ($r = 0.68$, $p < 0.05$) and Feb. 1994 ($r = 0.86$, $p < 0.01$) (Fig. 3).

There was no correlation between clinical scores and *Der p 1* levels in bedding and floor dust samples ($r = 0.21$; $r = 0.52$, $p > 0.05$) (Fig. 3).

Discussion

In this prospective, one year study we have described seasonal variation in *Der p 1* and ET levels in house dust, and their relationship with clinical scores in asthmatic children. The results showed no significant differences between *Der p 1* allergens and ET levels in houses of asthmatic patients and controls. The highest levels of *Der p 1* were measured in bedding samples ($p < 0.05$). These results are in agreement with our previous work in São Paulo, Brazil (12, 21) and with reports from the United States and Europe (22, 23). In the present study, there were no significant differences in *Der p 1* levels throughout the year, probably due to the high temperatures and humidity that can be detected year round in São Paulo. During the period of the present study, the monthly mean range of absolute indoor humidity varied from 9.8 g/Kg in August to 16.2 g/Kg in Feb. 1994, and the lowest levels were found in the winter months (May to August, $p < 0.05$, data not shown). In addition, the mean temperature ($^{\circ}\text{C}$) and relative humidity (%) in all houses visited were 23.5°C (19.8 – 28.3°C) and 67.6% (62.0–73.3%), respectively. It is thought that relative humidity may be a better parameter than absolute humidity to describe the metabolism of house dust mites (24). In keeping with previous studies, there was no monthly correlation between *Der p 1* levels and relative humidity. However, in tropical coast locations, a good correlation between absolute humidity and allergen levels has been obtained (25, 26).

There was no correlation between bacteria counts (cfu) and ET levels, probably because dead bacteria account for the most of the released ET. The prevalence of high GNB counts on soles of shoes and/or floor, as compared to counts in bedding, alert us to the possibility that these microorganisms may be carried from the streets to the houses. The median ET levels in the dust of the homes of nine asthmatic patients during the year, was 10 800 EU/g, ranging from 30 to 1.100.000 EU/g. These levels were similar to the levels reported in a previous study (20).

In keeping with previous studies (20, 27) our results show a positive correlation between total ET exposure and clinical scores, and a positive correlation tendency in all months. This correlation was significant in October '93 ($r=0.68$, $p<0.05$) and Feb. '94 ($r=0.86$, $p<0.01$). We were unable to demonstrate a significant correlation between *Der p 1* levels and clinical scores or between *Der p 1* and ET levels, as previously reported (28). Although the relationship between exposure to dust mites proteins and sensitization to these allergens among asthmatic patients has been clearly demonstrated in multiple studies, the relationship between exposure and severity of symptoms is less clear. It has been shown that the interaction between allergen exposure and other factors, including rhinovirus infections, ozone and endotoxin, can influence the inflammatory responses in the lungs. The enhancing effects of such factors could explain why in some studies it has been difficult to demonstrate a dose-response relationship between allergen exposure and severity of asthma symptoms. For each of our patients, there was a relation between higher levels of ET and higher clinical scores, suggesting endotoxin's ability to potentiate inflammatory responses as an important factor in modulating asthma severity. To establish an ET level of clinical relevance, a large number of patients would be necessary.

In a recent study (29) it was shown that inhaled LPS may activate an inflammatory cascade that may amplify the inflammatory response to inhaled antigens (ragweed) in some asthmatic patients. There are also data showing that the use of prophylactic antibiotics for six winter months, in severely asthmatic children, has caused reductions in upper respiratory tract infections and in severity of asthma (30, 31). At present, the role of bacterial infections in modulating Th1/Th2 selection in response to inhaled allergens is still unclear.

Endotoxin, as a pro-inflammatory substance might increase bronchial hyperreactivity, and may act to potentiate allergic release of histamine and other mediators (6, 32). Therefore lower amounts of mite allergen ($<10 \mu\text{g Der p 1/g}$ of dust) could be sufficient to induce symptoms. Alternatively, we could speculate that the increased inflammation

might facilitate the absorption of mite allergens. It is possible that ET might be an adjuvant to mites in the induction of symptoms, thus explaining the lack of seasonal correlation between mite levels and symptoms. It is also important to consider the role that soles of shoes may play in the introduction of gram negative bacteria in the home environment. It may be advisable to leave the shoes outside the home or outside the patient's bedroom.

We conclude that endotoxin concentrations exhibit seasonal variations, that mite allergen variations are not necessarily associated with seasonal worsening of asthma symptoms and that endotoxin exposure may be responsible for exacerbating mite allergic asthmatic children in São Paulo, Brazil. Although similar results have already been obtained in Europe (20, 27), additional studies are needed in other geographical locations to confirm these observations.

Acknowledgement

We thank L. Karla Arruda, M. D. for helping in the preparation of the manuscript.

References

- DALES RE, BURNETT R, ZWANENBURG H. Adverse health effects among adults exposed to home dampness and molds. *Am Rev Respir Dis* 1991; 143: 505-9.
- LEMAY DR, LEMAY LG, KLUGER MJ, D'ALECY LG. Plasma profiles of IL-6 and TNF with fever-inducing doses of lipopolysaccharide in dogs. *Am J Physiol* 1990; 259: R126-R132.
- RYLANDER R, BEIJER L. Inhalation of endotoxin stimulates alveolar macrophage production of platelet-activating factor. *Am Rev Respir Dis* 1987; 135: 83-6.
- FOGELMARK B, GOTO H, YUASA K, MARCHAT B, RYLANDER R. Acute pulmonary toxicity of inhaled b-1,3-glucan and endotoxin. *Agents-Actions* 1992; 35: 50-6.
- CAVAGNA G, FOA V, VIGLIANI EC. Effects in man and rabbits of inhalation of cotton dust or extracts and purified endotoxin. *Br J Ind Med* 1969; 26: 314-16.
- RYLANDER R, BAKE B, FISCHER JJ, HELANDER IM. Pulmonary function and symptoms after inhalation of endotoxin. *Am Rev Respir Dis* 1989; 140: 981-6.
- MICHEL O, DUCHATEAU J, SERGYSLS R. Effect of inhaled endotoxin on bronchial reactivity in asthmatic and normal subjects. *J Appl Physiol* 1989; 66(3): 1059-64.
- MICHEL O, DUCHATEAU J, PLAT G, CANTINIEAUX B, HOTIMSKY A, GERAIN J, et al. Blood inflammatory response to inhaled endotoxin in normal subjects. *Clin Exp Allergy* 1995; 25: 73-9.
- WHARTON GW. House dust mites. *J Med Entomol* 1976; 12: 577-621.
- TOVEY ER, CHAPMAN MD, WELLS CW, PLATTS-MILLS TAE. The distribution of dust mite allergen in the houses of patients with asthma. *Am Rev Respir Dis* 1981; 124: 630-5.
- Committee on The Health Effects of Indoor Allergens. Agents, Sources, Source Controls. In: *Indoor Allergens*. National Academy Press, Washington, D.C. 1993: 86-130.
- ARRUDA LK, RIZZO MC, CHAPMAN MD, FERNÁNDEZ-CALDAS E, BAGGIO D, PLATTS-MILLS TAE, et al. Exposure

- and sensitization to dust mite allergens among asthmatic children in São Paulo, Brazil. *Clin Exp Allergy* 1991; 21: 433-9.
13. VAN HAGE-HAMSTEN M, MACHADO L, BARROS MT, JOHANSSON SGO. Immune response to *Blomia kulagini* and *Dermatophagoides pteronyssinus* in Sweden and Brazil. *Int Arch Allergy Appl Immunol* 1990; 91: 186-91.
14. BRITISH THORACIC SOCIETY. Guidelines on the management of asthma. *Thorax* 48 (2 suppl): S1-S24, 1993.
15. BUSSE WW, WILSON AF. Report of The American Academy of Allergy and Immunology. Task force on guidelines for clinical investigation of nonbronchodilator anti asthmatic drugs: Assessment of efficacy. *J Allergy Clin Immunol* 1986; 78(3): 525-8.
16. BERNSTEIN IL, HARGREAVE FE, NICKLAS RA, REED CE. Report of the American Academy of Allergy and Immunology task force on guidelines for clinical investigation of non bronchodilator anti-asthmatic drugs. *J Allergy Clin Immunol* 1986; 78(3): 489-546.
17. LLERENA LP, FERNÁNDEZ-CALDAS E, GRACIA LRC, LOCKEY RF. Sensitization to *Blomia tropicalis* and *Lepidoglyphus destructor* in *Dermatophagoides* spp-allergic individuals. *J Allergy Clin Immunol* 1991; 88: 943-50.
18. ARLIAN LG, BERNSTEIN IL, GALLAGHER JS. The prevalence of house dust mites, *Dermatophagoides* spp, associated environmental conditions in homes in Ohio. *J Allergy Clin Immunol* 1982; 69: 527-32.
19. LUCZYNSKA CM, ARRUDA LK, PLATTS-MILLS TAE, MILLER JD, LOPEZ M, CHAPMAN MD. A two site monoclonal antibody ELISA for the quantification of the major *Dermatophagoides* spp. allergens, *Der p 1* and *Der f 1*. *J Immunol Meth* 1989; 118: 227-35.
20. MICHEL O, GINANNI R, DUCHATEAU J, VERTONGEN F, LE BON B, SERGYSSELS R. Domestic endotoxin exposure and clinical severity of asthma. *Clin Exp Allergy* 1991; 21: 441-8.
21. RIZZO MC, ARRUDA LK, CHAPMAN MD, FERNÁNDEZ-CALDAS E, BAGGIO D, PLATTS-MILLS TAE, et al. IgG and IgE antibody responses to dust mite allergens among children with asthma in Brazil. *Ann Allergy* 1993; 71 (2): 152-8.
22. GELBER LE, SELTZER LH, BOUZOUKIS JK, POLLART SM, CHAPMAN MD, PLATTS-MILLS TAE. Sensitization and exposure to indoor allergens as risk factors for asthma among patients presenting to hospital. *Am Rev Respir Dis* 1993; 147: 573-8.
23. LAU S, FALKENHORST G, WEBER A, WERTHMANN I, LIND P, BUETTNER-GOETZ I, et al. High mite-allergen exposure increases the risk of sensitization in atopic children and young adults. *J Allergy Clin Immunol* 1989; 84: 718-25.
24. DORNELAS DE ANDRADE A, BIRNBAUM J, LANTEAUME A, IZARD JL, CORGET P, ARTILLAN MF, et al. Housing and house-dust mites. *Allergy* 1995; 50: 142-6.
25. FERNÁNDEZ-CALDAS E, PUERTA L, MERCADO D, LOCKEY RF, CARABALLO LR. Sequential determinations of *Dermatophagoides* spp. allergens in a tropical city. *J Invest Allergol Clin Immunol* 1996; 24: 98-112.
26. PUERTA L, FERNÁNDEZ-CALDAS E, MERCADO D, LOCKEY RF, CARABALLO LR. Sequential determinations of *Blomia tropicalis* allergens in a tropical city. *J Allergy Clin Immunol* 1996; 97: 689-91.
27. 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.
28. BJÖRNSSON E, NORBÄCK D, JANSON C, WICSTROM J, PALMGREN U, STROM G, et al. Asthmatic symptoms and indoor levels of microorganisms and house dust mites. *Clin Exp Allergy* 1995; 25: 423-31.
29. DUBIN W, MARTIN TR, SWOVELAND PE, et al. Asthma and endotoxin: lipopolysaccharide-binding protein and soluble CD14 in bronchoalveolar compartment. *Lung Cell Mol Physiol* 1996; 14: L736-44.
30. SNYDER RD, STIEFEL FH. Prophylactic antibiotics in asthmatic children. *Ann Allergy* 1970; 28: 307-12.
31. AAS K, BERDAL P, HENRICKSEN SD. "Bacterial allergy" in childhood asthma and the effect of vaccine treatment. *Acta Paediatr Scand* 1963; 52: 338-44.
32. SANDSTROM T, BJERMER L, RYLANDER R. Lipopolysaccharide (LPS) inhalation in healthy subjects increases neutrophils, lymphocytes and fibronectin levels in bronchoalveolar lavage fluid. *Eur Respir J* 1992; 5: 992-6.