

Domestic endotoxin exposure and clinical severity of asthma

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Summary

Endotoxins are potent pro-inflammatory substances present in several natural environments and in commercial house dust extracts. To investigate the possible effect of chronic endotoxin exposure on asthma, 28 patients with perennial chronic asthma (20 allergic to house dust mite and eight intrinsic asthmatics) were evaluated during a 4-month period (lung function, clinical and immunological criteria). At the same time, two house dust samples were collected from each patient's home to determine total house dust weight (mg/m²), endotoxin concentration and house dust mite antigen content (evaluated indirectly by guanine content with HPLC method). The mean ($\pm$ s.d.) endotoxin concentration, as measured by quantitative Limulus assay was 2.59 ($\pm$ 3.41) ng/mg house dust, ranging from 0.12 to 20 ng/mg. The mean guanine content was 0.13 ($\pm$ 0.16) mg/100 mg house dust. There was no correlation between endotoxin and house dust mite concentrations. Patients were compared according to the low or high grade exposure to dust, endotoxins and guanine. Compared with patients with low grade ($\leq$ 5.6 ng/ml) exposure, subjects exposed to high endotoxin concentrations ($>$ 5.6 ng/ml) showed a significant increase in dyspnea (median 2.6 *vs* 3.3; $P < 0.05$) and treatment (median 14 *vs* 44.3; $P < 0.01$) scores, oral corticosteroid (median 0.0 *vs* 13.5 mg/24 hr; $P < 0.01$) and β_2 -mimetics (median four *vs* eight puffs/day; $P < 0.01$) intake, and a significant decrease in FEV₁/FVC (median 84.5 *vs* 67% of predicted value; $P < 0.01$). In contrast, no differences were found between the two groups exposed to low ($<$ 0.07 mg/100 mg house dust) and high ($\geq$ 0.07 mg/100 mg house dust) concentrations of guanine, respectively. We conclude that endotoxins are present in normal domestic environment and could have a deleterious effect on the chronic asthmatic disease.

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Introduction

House dust mite (HDM) is probably the most important perennial allergen. Indeed dust mite is demonstrable in 99% of homes from allergic patients [1] and it is the most frequent allergen giving a positive skin test in asthmatic subjects [2]. Moreover, new environmental circumstances leading to mite growth have been associated with an outbreak of asthma limited to that part of population having the specific ability to produce an IgE antibody response [3]. The threshold concentration of antigen P1 (*Der p* 1, from *Dermatophagoides pteronyssinus*, Dpt) equivalent to 10 μ g/g house dust, seems to be a major risk

factor for occurrence of asthma in HDM-sensitized patients [4]. However, in these latter, acute symptoms are not correlated with the highest levels of HDM seasonal variation [4]. Although some mite avoidance studies ($>$ 2 months) have shown that a significant decrease of mite allergen was associated with an improvement of symptoms [5], other studies have not confirmed these results [6]. Thus, it seems that mite allergen in itself does not explain all the symptoms related to house dust asthma and that it is but one of several factors leading to asthma.

Ozone, NO₂, SO₂, tobacco smoke and infectious agents are inhalable substances with a potential to cause inflammation that could participate to the inflammatory bronchial process. Endotoxins, a part of the outer membrane of Gram-negative bacteria, are potent pro-inflammatory

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substances [7], present in several domestic [8,9] or occupational environments [10,11], that could play an important role in the severity of asthma. This is suggested by a recent study, in which we showed that acute inhalation of lipopolysaccharides (LPS), the major constituents of endotoxins, induced in asthmatics, but not in normal subjects, a bronchial obstruction associated with an increase in histamine bronchial responsiveness [12].

The purpose of the present study was to assess whether LPS is present in house dust and whether this correlates with the clinical severity of asthmatics patients.

Subjects and methods

Patients

In our patients asthma was assessed (as recommended by the American Thoracic Society [13]) by the presence of a typical clinical picture, associated with either an increase of 20% or more of forced expiratory volume in 1 sec (FEV₁) after inhalation of two puffs of 100 µg each of salbutamol (Ventolin, Glaxo), or a decrease of at least 20% of FEV₁ after inhalation of a histamine cumulative dose < 480 µg. All patients had to present either Dpt allergy (based on clinical history, RAST and skin-prick test) or non-allergic asthma with perennial clinical presentation. To assume the criteria of chronicity, the patients had to be followed in our clinic for at least 1 year, with a frequency of at least four visits a year, prior to inclusion in the study. Patients with seasonal asthma, positive skin test or RAST related to pollenic allergy were excluded. Following the above criteria 28 patients (14 women, 14 men, mean age of 42.3 ± 11.1 years) were included. Twenty patients were allergic to HDM and eight were intrinsic asthmatics. The average duration of disease was 12.0 (± 9.5) years. Three patients were smokers and 25 non-smokers.

General study design

The study was performed between March and September, 1988. All the patients underwent a complete clinical evaluation on day 1, 60 and 120 and an immunological and functional evaluation on day 1 and 120. At the same time, on day 40 and 100, trained nurses working for the FARES (Fondation contre les Affection Respiratoires et pour l'Éducation à la Santé) visited patients' homes. Each time, they recorded the clinical score, the value of peak expiratory flow (PEF) and the objective drug intake and collected samples of house dust. The first sample was obtained by mean on 12 May (± 39 days) and the second on 24 July (± 49 days). During the first visit, the nurses described the house and its hygienic characteristics, filling a pre-established card. A global hygienic score of the house was calculated (0–300 points corresponding of high

to low hygienic degree). This arbitrary score included general home characteristics and description of each room (presence of animals, carpets, sofa, wool-bedding and mattress, presence of humidity traces, amount of house dust, mode of heating and frequency of vacuum cleaning). Each patient was evaluated, time by time, by the same physician and the same nurse.

Clinical evaluation

A dyspneic score was estimated at five different times, by the patient's answers about the frequency of respiratory discomfort: never, 0; 1 episode/week, 1; > 1 episode/week, 2; > 1 episode/day, 3; > 5 episodes/day, 4; and > 10 episodes/day, 5. For each patient, the mean dyspneic score was calculated on the base of three responses given to the physician (three visits) and two responses given to the nurse (two visits). During home visits, the nurse also evaluated patient's drug intake, to determine it as accurately as possible. Compared to the physician recommendations, the amount of drugs really consumed by patients, was the same for oral corticosteroids ($r = 1.00$) but not for topical corticosteroids ($r = 0.77$), xanthines ($r = 0.80$) and β_2 mimetics ($r = 0.58$). A mean therapeutic score was calculated as reported elsewhere [14].

Lung functional evaluation

FEV₁, FEV₁/FVC and RAW (airway resistance in cm H₂O/L/s) were all measured on a constant volume computerized Bodyscreen II plethysmograph (Jaeger) [12] and expressed as percentage of the predicted value (CECA); the mean of measurements obtained at visit 1 and 3 was then calculated. Peak expiratory flow (PEF, measured on a mini-Wright peak flow meter, Clement Clarke) was assessed considering the best of three successive values obtained during each of the home visits and expressed in l/min.

Allergological evaluation

Total serum IgE in IU/ml (Prist Test, Pharmacia; normal value < 200 IU/ml), specific serum IgE to Dpt and house dust in IU/ml (RAST, Pharmacia), plasma C3d in mg/dl, obtained as described elsewhere [15] and blood absolute eosinophilia (normal value < 500/mm³) were measured on visit 1 and 3 and their means calculated. Skin-prick tests for Dpt and house dust (Bencard, ref. 2801 and 3201)

were performed at visit 1 and 3 on the patient's back. Flare reactions were read after 15 min and estimated as a score 0 to ++++ (as recommended by Bencard).

House dust samples

Samples of house dust were collected by means of a hand-held vacuum cleaner (Black & Decker Model no. 936 B 780340-02) in which a paper filter was incorporated to cover the plastic filter. In the bedroom, two samples of dust were collected from mattress surface and floor, vacuum cleaning an area of 1 m² for 2 min. The samples were pooled in a sterile flask and kept at -20°C until extraction.

Samples ($n=56$) were sieved through a 0.3-mm mesh screen to obtain fine dust. Samples < 10 mg fine dust were excluded ($n=2$). Samples of 5 mg house dust/ml PBS (phosphate buffered saline, pH 7.2, from Bio Mérieux) were extracted by shaking (vortex 1 min) followed by a continuous rotation for 3 hr at 4°C. After centrifugation (5 min at 2000 g) supernatant was stored frozen at -20°C as well as the remainder of the 'base' fine house dust.

Endotoxin content of house dust extract was measured by a method based on the characteristic of the lipid A portion of endotoxin to activate the coagulation cascade of the amoebocyte lysate from the horseshoe crab (*Limulus polyphenus*). After activation by endotoxin of a pro-enzyme present in the *Limulus* lysate, paranitroaniline is cleaved from the chromogenic substrate S-2423 (Coatest-R Endotoxin, Kabivitrum Diagnostica, Amsterdam, The Netherlands) leading to a colorimetric reaction [16]. Standard endotoxin curves were established with *Escherichia coli* O111:B4 (Kabivitrum Diagnostica). Albumin 0.1% was added to counteract the risk of losses of endotoxin to test tube surfaces. The average LPS content for each patient's samples was calculated and expressed in ng/ml house dust extract (i.e. ng/5 mg house dust). The sensibility of this method is very high (detection limit being 5 ng LPS/l [16]). The specificity was tested by comparison of the slope, after several dilution, of the standard curve and the house dust extract response; the curves obtained (2 standards and 5 house dust extracts) were parallel, assuming the specificity of the technique. The intrasample reproducibility (different extractions on one sample) was satisfactory ($n=10$, coefficient of variability=21%).

Sample *Der p* I content was evaluated by a quantitative dosage of guanine, which has been shown to correlate with both *Der p* I concentration in house dust [17,18] and skin response to mite antigen [19]. Guanine was extracted by treatment with methanol-NaOH and chromatographed using reversed-phase high-performance liquid

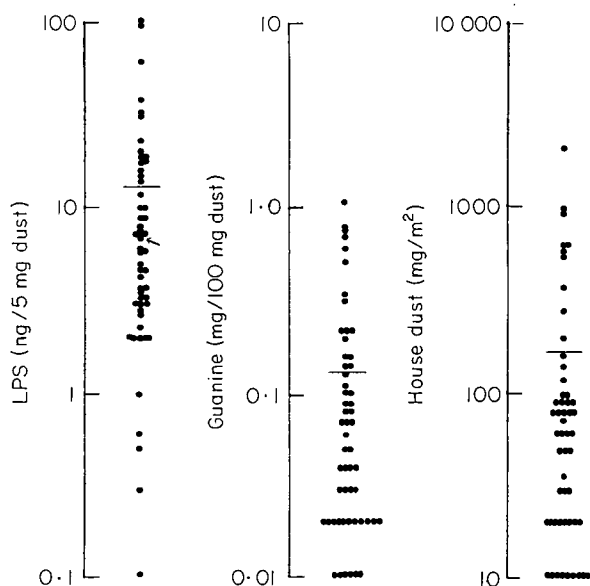


Fig. 1. Distribution of lipopolysaccharides (LPS) and guanine concentration in house dust extract and distribution of total house dust weight/m².

chromatography (Ultrapac, particle size 5 µm, isocyanic solvent system, phosphate buffer 40 mM/methanol 98:2%, flow rate 1 ml/min, u.v. detection 245 nm). The intrasample reproducibility was very good for guanine HPLC determination ($n=10$, coefficient of variability=5%).

Statistical analysis

Results are expressed as mean $\pm$ 1 s.d. or as median with its range. The non-parametric Mann-Whitney *U*-test was used for comparison of variables and the Kendall's rank correlation coefficient was calculated to summarize the relation between two variables [20]. Results expressed as frequency were tested by the χ^2 test with Yates' correction for small numbers [20]. $P < 0.05$ was considered significant.

Results

Average fine house dust total weight was 171.6 (± 296.2) mg/m² and average LPS and guanine concentrations were 2.59 (± 3.41) ng/ml house dust and 0.13 (± 0.16) mg/100 mg house dust, respectively (Fig. 1). In view of the results of Bischoff and Schirmacker [17], this last result corresponds approximately to *Der p* I concentration of 5.4 µg/g house dust. As shown in Fig. 2, no significant correlation was found between LPS and guanine contents. No significant differences were found in both LPS and

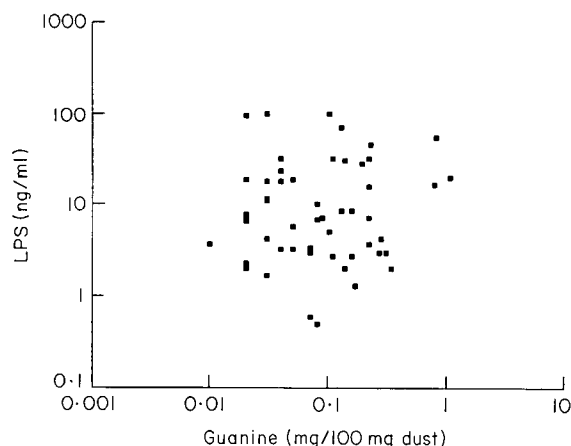


Fig. 2. Relation between lipopolysaccharides (LPS) and guanine ($n=54$) concentration in house dust (Kendall test, $r=-0.01$; P not significant).

guanine exposure (medians 5.25 vs 14.1 ng/ml and 0.05 vs 0.09 mg/100 mg house dust, respectively) between allergic and non-allergic subjects. The two groups of patients were then pooled for the subsequent analysis.

In view of the abnormal distribution of LPS content, the median value (5.6 ng/ml) was chosen to divide the population into two groups: 14 patients exposed to low (≤ 5.6 ng/ml) and 14 exposed to high (> 5.6 ng/ml) LPS concentrations. These two groups were compared and the results are shown in Table 1. Compared with patients exposed to low LPS concentration, patients with high-grade exposure showed a significantly greater bronchodilator and oral corticosteroid intake ($P<0.01$ for both), significantly greater dyspnea and treatment scores ($P<0.05$ and $P<0.01$, respectively) and a significant reduction in FEV_1/FVC ($P<0.01$). Individual values of corticosteroid intake and FEV_1/FVC are shown in Fig. 3, according to a low or an intense exposure to LPS or HDM. High LPS level exposure was also associated with higher doses of xanthines and topical corticosteroids, higher RAW and lower FEV_1 values, but differences were not statistically significant. Average patients' age, duration of disease and allergological parameters were similar in the two groups. No difference was found in term of HDM exposure (median of guanine 0.070 vs 0.055 mg/100 mg house dust).

The analysis of home environment showed no association between low or high LPS content in house dust, on the one hand and presence of wool mattresses or bedding, non synthetic pillows, carpets, mode of heating, frequency of vacuum cleaning, presence of humidifiers or wall humidity traces, presence of pets or global house hygienic score, on the other hand.

The median guanine content was 0.07 mg/100 mg

house dust. No significant differences in terms of treatment and clinical, functional and allergological parameters were found between the two groups of patients exposed to high (≥ 0.07 mg/100 mg house dust) and low (< 0.07 mg/100 mg house dust) concentrations of guanine, respectively. The same results were obtained considering only the patients who were allergic to dust mites ($n=20$). Only six patients were exposed to *Der p 1* higher than 10 $\mu\text{g/g}$ house dust (corresponding to a guanine level of 0.17 mg/100 mg house dust [15]), considered as a risk to develop attacks of asthma [4]. Four of them were allergic to Dpt (two were severe corticoid dependent and two were slight asthmatics). Furthermore, we failed to find any significant difference in environmental home parameters between the two groups of patients with low or high grade exposure to guanine, except for the specific bedroom score (including the presence of carpets, non-synthetic mattresses and blankets and the absence of heating) and the total house dust/ m^2 which were, associated with a higher guanine level (Table 2).

The median of total fine house dust was 65 mg/m^2 . No significant difference in terms of treatment and clinical, allergological and functional parameters could be observed between patients exposed to low (≤ 65 mg/m^2) or high (> 65 mg/m^2) house dust level. Furthermore, we failed to find any significant relationship between total house dust weight and LPS concentration ($r=0.02$; $P=\text{NS}$).

Discussion

Results of this study show a significant relation between natural endotoxin exposure and the clinical and functional status of asthmatic patients. In contrast, we failed to find any significant correlation between either total house dust or mite level exposure and clinical and functional status.

Clinical evaluation of asthma severity remains a difficult problem. Some parameters like pulmonary function tests assess patient clinical status at a given moment of the nyctemia; other parameters like dyspnea scores and drug scoring systems have the advantage to regard a longer period (days to months). We used a very simple dyspneic score to avoid interferences in the patient answers and we tried to increase their objectivity by using two different examiners in different places and at different moments. The drug intake is a useful index for the assessment of patient clinical status [14], because it is dependent on the clinical and functional condition of the patient, and it is a long-time retrospective criterion. Nevertheless, drug intake is a valid index provided that patient compliance is documented by repeated visits and that the threshold dose (i.e. the dose below which a

Table 1. Comparison of patients exposed to low or high lipopolysaccharide (LPS) level

	Low LPS (≤ 5.6 ng/ml)	High LPS (> 5.6 ng/ml)	P
Clinical details			
Age (years)	46.0 (19–56)	40.5 (30–61)	NS
Female/male	11/3	3/11	< 0.05
Asthma onset (years)	13.5 (2–35)	6.5 (2–26)	NS
Dyspneic score	2.6 (0–3.5)	3.3 (1.5–8)	< 0.05
Treatment			
Methylprednisolone (mg/day)	0.0 (0–15)	13.5 (0–40)	< 0.01
β_2 mimetics (puffs/day)*	4.0 (1–10)	8.0 (1–10)	< 0.01
Beclomethasone (puffs/day)	1.75 (0–6.5)	2.75 (0–8)	NS
Xanthines (mg/day)	425 (0–1000)	637 (0–1000)	NS
Treatment score	14.0 (2–61.5)	44.3 (11.5–133.5)	< 0.01
Lung function			
FEV ₁ (%)	64.0 (33–92)	55.5 (32–77)	NS
FEV ₁ /FVC (%)	84.5 (47–99)	67.0 (52–87)	< 0.01
RAW (%)	150 (61–292)	213 (67–497)	NS
PEF (l/min)	280 (150–420)	295 (125–610)	NS
Allergology†			
IgE (IU/ml)	337 (83–1168)	345 (54–912)	NS
RAST Dpt (IU/ml)	10.5 (0–17.5)	3.2 (0–15.3)	NS
RAST house dust (IU/ml)	1.2 (4–4.5)	0.5 (0–5.1)	NS
C3d (mg/dl)	0.4 (0.2–0.8)	0.3 (0.2–0.5)	NS
Eosinophilia (cells/mm ³)	384 (135–840)	239 (40–948)	NS
Dpt skin test	4.0 (3.5–4.0)	4.0 (2.0–4.0)	NS
House dust skin test	3.0 (3.0–4.0)	2.5 (0–4.0)	NS

* Fenoterol or salbutamol.

† In the 20 allergic subjects only.

All the results are expressed as median, with ranges in parentheses.

Dpt, *Dermatophagoides pteronyssinus*; NS, not significant.

clinical and functional worsening is noted) for each drug is determined by an accurate selection of patients already followed in the clinic for 1 year or more.

We used the house dust collecting method recommended for mite evaluation by Platts-Mills *et al.* [21]; a more complex method like airborne sampling leads to no particular advantage because disturbance in the room is requested to obtain suspended allergen-associated airborne particles [22]. In the same way, the content of airborne endotoxin from indoor environments seems also related to personal activity, leading to difficulties in standardizing the creation of air movements [23]. Indirect estimation of HDM level by guanine quantitative measurement is not a mite species specific method but is correlated with the P1 antigen content [8]. Considering the results of Bischoff and Schirmacker [17], a guanine

concentration of 0.07% is approximately equivalent to 1.24 μg *Der p* I/g house dust. This result, obtained from samples collected in the first half of the year, is consistent with that of Wood *et al.* [1] who reported for the same year period a median total dust mite antigen content of 1.12 μg *Der p* I/g house dust (range 0.03–30.17).

The quantitative *Limulus* test is a very sensitive one showing a detection limit for LPS in blood samples of 5 ng/l [16]. Furthermore, it is also specific because interferences of substances other than endotoxins seem to be minimal [24]. Nevertheless, peptidoglycans, from the membrane of Gram-negative and Gram-positive bacteria, cause gelation of the *Limulus* test but at concentrations many orders higher than for endotoxin (400 μg of *Streptococcus* group A peptidoglycan/ml compared with only 0.0005 μg of purified endotoxin/ml) [25]. In view of

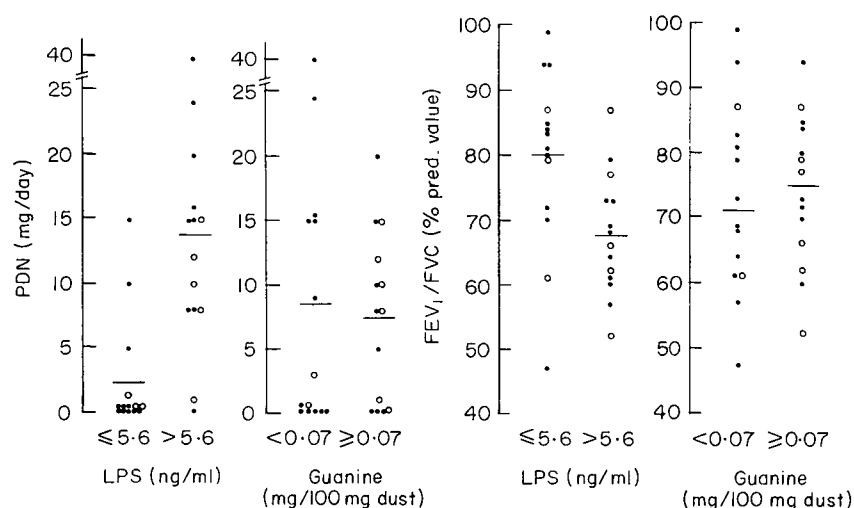


Fig. 3. Comparison of daily prednisolone (PDN) intake and FEV₁/FVC in allergic patients (●) and patients with intrinsic asthma (○) exposed to low or high lipopolysaccharides (LPS) concentration and to low or high guanine concentration.

the significant correlation ($r=0.73$) between glucan and endotoxin associated with their similar range of concentration in airborne house dust [23], the interference of glucan in the present results is probably unimportant. Moreover, the glucan coagulation pathway of the *Limulus* test is independent of the LPS-mediated gel formation [26]; thus in presence of significant amounts of glucan, the slope of the response after several dilutions of the house dust extracts should have been different from the slope of the standard curve. The LPS concentration found in our

patients' house dust samples (median 1.1 ng/mg house dust; range 0.1–20) corresponds to that reported in several studies concerning occupational exposure to airborne agricultural dusts [10,11] or cotton dust [27]. In silage dust, all size particles (ranging from $<0.43\ \mu\text{m}$ to $>10\ \mu\text{m}$) are contaminated with LPS and can reach every level of the respiratory tract; in particulates of $4.7\text{--}9.0\ \mu\text{m}$, the range of endotoxin concentration is 0.59–41.04 ng/mg dust [11]. Endotoxin levels in poultry farms ranges from 13.8 to 47.4 ng/mg dust [10].

Table 2. Domestic house characteristics in relation to high or low guanine level exposure

	Guanine in house dust (%)		P
	<0.07	≥0.07	
Wool bedding	4/14	7/14	NS
Wool mattress and/or pillow	3/14	9/14	NS
Carpet (bedroom)	6/14	6/14	NS
Heating (bedroom)	7/14	5/14	NS
Humidifier or plants	3/14	3/14	NS
Humidity traces (bedroom)	2/14	2/14	NS
Cat or dog	2/14	2/14	NS
Vacuum cleaner (< once/week)	4/14	4/14	NS
Total house scoring*	66.0 (39–124)	77.0 (57–112)	NS
Bedroom scoring†	1.0 (0–4)	2.5 (0.0–4.0)	<0.05
Total house dust (mg/m ²)*	52.5 (10–195)	97.5 (10–1365)	<0.05
LPS (ng/ml)*	5.6 (0.1–57.5)	7.45 (1.6–65.9)	NS

* Expressed as median and range of values.

† Absence of heating, 1; carpet, 1; wool mattress, 1; wool bedding, 1.

We failed to find any significant relationship between the low or high grade exposure to LPS and mean age, duration of disease or smoking habits. Furthermore, there were no significant differences in terms of allergological parameters between the two groups exposed to different endotoxin groups. This could be due to a similar antigen exposure [28] in view of the comparable guanine content in the two groups' house dust samples. However, clinical and functional characteristics significantly differed in the two groups of patients. Indeed, we found in subjects exposed to high LPS levels a significant increase in methylprednisolone and β_2 -mimetic intake and in therapeutical and dyspneic scores, while FEV₁/FVC values were significantly decreased. Differences, though not statistically significant, were found also in topical corticosteroid and xanthine intake and in FEV₁ and RAW values. All these parameters are consistent with a more severe asthma in patients exposed to high endotoxin concentrations, independently of HDM exposure.

No association between clinical asthma and HDM exposure (expressed by guanine) was demonstrable in our patients. Nevertheless, in our study, most of the patients are exposed to a level of *Der p* I below the threshold value ($< 10 \mu\text{g/g}$ house dust) considered as a risk of asthma crisis [4]; this could explain the poor association between their clinical status and mite antigenic exposure. The absence of correlations between LPS and guanine level in house dust and between this latter and patients' clinical status, are ulterior arguments for the influence of LPS on the severity of asthma independently of the presence of HDM. This could also explain the weak correlation between decrease in antigen concentration and improvement of symptoms found in some Dpt eviction long-term studies [6] and the discrepancies found between house dust levels and patients' clinical status [1,4]. Indeed, studies demonstrating a significant clinical improvement after eviction measures are those where patients were moved away from their homes for a long time [5]; the results could thus be due to variation of environmental characteristics (may be LPS content but also other pollution atmospheric factors) other than modification in *Dermatophagoides* concentration.

Determination of domestic sources of Dpt and LPS are important to define future eviction measures. Our results agree with those of others [4,29] in indicating wool mattresses and/or bedding, carpets and absence of bedroom heating as factors increasing the risk of higher mite density. In the present study, however, no domestic characteristic was associated with a high LPS level and thus eviction of LPS remains a problem. Theoretically, all factors enhancing the growth of gram negative bacteria at 20°C could lead to high LPS level in house dust.

Mean LPS concentration was about 12.9 ng/5 mg

house dust with a mean total weight of about 171.6 mg/m² and thus a mean total LPS weight of 446 ng/m². If one supposes a bedroom of 10 m² surface (25 m³) and an exposure of one night (10 hr), in a subject ventilating a total of 5 m³, only 1% of total house dust should be airborne and inhalable to obtain a total LPS intra-bronchial level of 9 ng. This inhalable LPS dose is similar to that present in some cotton extracts shown to induce a significant FEV₁ decrease in cotton workers [27] and pre-selected normal subjects [30]. In these studies FEV₁ decrease was correlated with the LPS concentration in cotton dust and not with total cotton dust weight. Our results are also consistent with a recent report by Rylander *et al.* [23] showing airborne LPS concentration, in sick buildings, up to 18.1 ng/m³ and a significant association between LPS exposure and clinical complaints. Moreover, in an animal model (rat), the LPS induced bronchial hyper-responsiveness associated with an infiltration of inflammatory cells in the airways, depends on the selected rat strain [31]. Thus, a genetic predisposition could determine the potential bronchial response to LPS and this could be an explanation for the onset of asthma in some 'intrinsic' asthmatics.

This study suggests that the clinical manifestations of asthma are modulable by non-specific proinflammatory substances like endotoxins. Up to now it is impossible to say if LPS is a substance acting in synergy with specific allergens or if it has its own activity. To try to give an answer, studies including skin and bronchial challenge tests with solutions of endotoxins and allergens are in progress.

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