

Prevalence of symptoms, sensitization to rats, and airborne exposure to major rat allergen (Rat n 1) and to endotoxin in rat-exposed workers: a cross-sectional study

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Summary

Objective To analyse the relation between airborne exposure to major rat allergen and to endotoxins in exclusively rat-exposed workers and the prevalence of rat-related symptoms and sensitization.

Methods A total of 113 workers answered a standardized questionnaire on their atopy status, occupational exposure to rats, and possible work-related symptoms. Specific IgE against rat urinary proteins (RUP) was measured for 73 subjects. Individual airborne exposure to Rat n 1 and endotoxin were determined with static ($n = 256$) samplings. Rat n 1 was measured with enzyme-linked immunosorbent assay (ELISA) and endotoxin by the *Limulus* method.

Results Forty-four of 113 subjects (38.9%) reported at least one rat-related symptom: asthma (4.4%), rhinitis (34%) and conjunctivitis (16%). Twelve per cent were sensitized to RUP (specific IgE > 0.35 KU/L). But only 30.8% of all symptomatic subjects were sensitized to rat allergens. Airborne Rat n 1 levels were not related to symptoms in workers. Symptomatic patients not sensitized to rats were exposed to higher endotoxin levels, but airborne exposure to endotoxins did not significantly protect against or increase sensitization to RUP or rat-related symptoms.

Conclusion Most symptomatic workers were not sensitized to rat allergen; but no significant relation between rat-related symptoms and endotoxin levels was found. This suggests that more studies are needed to determine causes other than rat allergens or endotoxins that may be responsible for symptoms in rat-exposed workers.

Keywords airborne measurement, endotoxin, occupational asthma, rat allergen, rat quarters

Submitted 15 August 2001; revised 7 June 2002; accepted 8 July 2002

Introduction

The prevalence of symptoms among persons occupationally exposed to rats is reported to vary from 12 to 31% [1–3]. Asthma symptoms are reported in 20–30% of symptomatic workers. In the UK, exposure to laboratory animals is the fifth leading cause of occupational asthma [4]. Only one study, however, has assessed the frequency of sensitization to rats among symptomatic workers [5]; it found that 37% of them were not sensitized to rat allergens. This finding suggests that other agents present in rat quarters may play a role in these symptoms.

It has been suggested that endotoxins influence the pathogenesis of bronchial symptoms [6–8]. Airborne endotoxins have been measured in various occupational environments [9]. Two studies reported low levels of endotoxins in some laboratory animal facilities (rats/mice), based on static sample measurements [10, 11], but individual exposure to endotoxins has not been evaluated over a full work-shift.

Our aims were twofold: first, to determine the prevalence of bronchial, nasal and ocular symptoms, and of sensitization against rat urinary proteins among workers exposed to laboratory rats; and then to analyse the relation between individual doses of airborne major rat allergen (Rat n 1) and of endotoxins and the prevalence of symptoms and sensitization among rat-exposed workers.

Materials and methods

Study design

We enrolled all staff and students occupationally exposed to laboratory rats (animal technicians, laboratory technicians, scientists and students; $n = 113$) in 12 laboratories of public research establishments (medical school and hospital laboratories) in Strasbourg. They completed a standardized questionnaire between September 1997 and July 1998. Specific IgE against rat urinary proteins (RUP) was obtained from 73 volunteers. Individual exposure to airborne rat allergen and endotoxins was assessed with static ($n = 256$) and individual ($n = 227$) air samples during the same period.

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Questionnaire

All 113 subjects completed the questionnaire, a modified version of the ISAAC questionnaire [12], which contained 170 items. It asked about their smoking habits and history of allergic symptoms. Atopy was defined as allergic rhinitis and/or asthma previously confirmed by a medical diagnosis and by the memory of a positive skin test to at least one common aeroallergen. Rat-related symptoms were defined as the presence of self-reported symptoms (cough, wheezing, chest tightness, asthma, runny nose and sneezing, and running and itching eyes) during or after occupational contact with rats. Detailed information about exposure to rats was requested: duration of exposure and tasks performed. This questionnaire was approved by the CNIL (National Committee on Computers and Privacy).

Specific IgE to rat urinary proteins

After approval by the local institutional ethics committee (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale) and informed consent by the subjects, 73 volunteers provided blood samples. The serum was stored at -20°C . Specific IgE antibodies against RUP were measured by RAST (radioallergosorbent test; e74, CAP-System®, Pharmacia, Uppsala, Sweden), following the manufacturer's recommendations. The threshold for clinically relevant positive findings was 0.35 KU/L.

Exposure assessment

Airborne Rat n 1 and endotoxin measurements Exposure assessment included 483 airborne Rat n 1 and endotoxin samples taken from the 12 sites: 227 individual samples (113 for Rat n 1 and 114 for endotoxin, successively) and 256 static samples (128 for Rat n 1 and 128 for endotoxin, simultaneously). All airborne allergen measurements were performed according to French standards [13]. Details of samplings and measurements have been previously reported [14]. Briefly, individual airborne samples were taken by 38 workers randomly selected from each site (nine animal technicians, nine laboratory technicians, nine scientists and 11 students) to determine levels of exposure to Rat n 1 and endotoxin. On six occasions, each subject worked his or her entire shift with a individual air sampler (air flow: 2 L/min). Static samples were taken from each site in rat rooms, both during cage cleaning and rat feeding ($n = 34$) and without any disturbance ($n = 31$), and in experimental rooms, both during rat handling ($n = 31$) and in the absence of rats ($n = 32$). Each sampling lasted 60 min at 20 L/min. All sampler heads contained a fibreglass filter. Rat n 1 and endotoxin were eluted from the filters. Rat n 1 was assayed with an enzyme-linked immunosorbent assay (ELISA) sandwich assay with monoclonal antibodies [15]; the detection limit was 0.31 ng/mL, corresponding to 0.25 ng/m³. The mean intra-assay coefficient of variation (CV) was 11% ($n = 12$) and the mean interassay CV was 9.5% ($n = 20$). Endotoxin was measured with the *Limulus* method [16]. The detection limit was 0.025 UI/mL, corresponding to 0.02 ng/m³. The mean intra-assay CV was less than 7% ($n = 12$) and the mean interassay CV less than 9% ($n = 20$).

Individual daily inhaled doses of Rat n 1 and endotoxin Individual and static samples from the 12 sites, together with the job exposure description for each subject from the questionnaire, enabled us to determine the doses of Rat n 1 and of endotoxin that each worker inhaled over an 8-h shift. Individual and static air samples were available and well correlated (i.e. $r = 0.69$; $P < 0.001$ for Rat n 1 and $r = 0.88$; $P < 0.0001$ for endotoxin) for 38 workers, but we had only static samples for the other 75 subjects. Accordingly, we choose to take only the static values into account in calculating D, the mean inhaled dose during 8 h (ng):

$$D = (C' \times T' + C'' \times T'' + \dots) \times V,$$

with C' , C'' mean airborne concentration in a given room, during a given task ($n = 3$); T' , T'' mean time spent in each room from which static samples were taken, for a cumulative duration of 480 min; and V mean ventilation rate (10 L/min).

Statistical analysis

Different subgroups (symptomatic vs. non-symptomatic subjects, those who had an IgE determination vs. those who did not give a blood sample) were compared with univariate analysis of variance or chi-square test, as appropriate. Symptom prevalence among the four occupational categories was compared with the chi-square test. The rate of blood samples among asymptomatic and symptomatic workers was compared with a chi-square test. All the quantitative values (individual daily inhaled doses of Rat n 1 and endotoxin) were log-transformed on the model ' $\log_{10}(1 + x)$ '. The correlation between individual and static samples was tested with correlation coefficient of Pearson for Rat n 1 and endotoxin exposures. Logistic regression was used to identify risk factors (from the following variables: individual daily inhaled doses of Rat n 1 and of endotoxin, number of years of occupational rat exposure, number of hours of rat exposure per week, atopy, specific IgE against RUP, occupational exposure to disinfectant, smoking habits, sex) for rat related-symptoms and sensitization. Endotoxin airborne exposures among symptomatic sensitized and nonsensitized to RUP and non-symptomatic subjects were compared with analysis of variance. Statistical significance was defined as $P < 0.05$.

Results

Subjects' characteristics

The study included 113 persons, 61 women and 52 men, with a mean age of 38 years (Table 1). They worked at 12 different sites and all had occupational contact with laboratory rats. There were 13 animal technicians, 19 laboratory technicians, 46 researchers and 35 students.

Rat-related symptoms according to the questionnaire

Symptomatic and non-symptomatic subjects did not differ significantly for sex ratio, age, or smoking. Overall, 38.9% ($n = 44$) reported at least one rat-related symptom (Table 1). Rhinitis was reported most frequently (33.6%), followed by conjunctivitis (15.9%) and asthma (4.4%). More than one-third (36.4%),

16/44) of the symptomatic subjects reported at least two rat-related symptoms. Atopy affected 47% of the population. Of subjects with work-related symptoms, 64% were atopic. Symptom prevalence did not differ significantly among the four occupational categories: 38.5% among animal technicians, 31.5% among laboratory technicians, 40% among students and 41.3% among researchers.

Rat sensitization according to specific IgE to RUP Specific IgE was measured in 73 of the 113 workers who completed the questionnaire (19 refused to give blood samples and 21 had left Strasbourg). Consent to giving blood did not differ significantly by professional category (i.e. 69% of the animal technicians, 68% of the laboratory technicians, 71% of the students and 57% of the researchers; $P = 0.32$; chi-square test). Similarly, subjects for whom we were able to determine IgE did not differ significantly from subjects for whom we had no blood sample in terms of age, sex, smoking habits, sensitization to common aeroallergens, or rat-related symptoms including asthma, rhinitis and conjunctivitis. Subjects in whom rat-specific IgE was not determined did have a higher prevalence of one or more rat-related

symptoms and rat-related rhinitis than those in whom this test was performed (i.e. 45% vs. 35% for at least one symptom and 40% vs. 30% for rhinitis, respectively; $P = 0.06$). Twelve per cent (9/73) had specific IgE against RUP. These sensitized subjects included no animal technicians. Blood samples were taken from 26 of the 44 symptomatic subjects, and only 30.8% had specific IgE against RUP (Table 1). The availability of blood samples did not differ significantly between asymptomatic (31.9%) and symptomatic workers (40.9%; $P = 0.32$).

Atopy and sensitization to RUP as risk factors for rat-related symptoms Atopy was a risk factor for rat allergy [odds ratio (OR) 9.24], and especially for rat-related asthma (OR 21.8; Table 2). Presence of specific IgE against RUP was a risk factor for rat-related symptoms (OR 26.0), especially for rat-related rhinitis and conjunctivitis (OR 34.7 and 9.44, respectively).

Rat n 1 and endotoxin exposure assessment

Job characteristics according to the questionnaire The mean duration of rat exposure was 25 h/week (range 5–40 h) for animal technicians; 7.5 h/week (range < 30 min–20 h) for

Table 1. Subjects' characteristics, rat-related symptoms ($n = 113$) and specific IgE to rat urinary proteins (RUP; $n = 73^*$)

	Asymptomatic subjects $n = 69$	At least one rat-related symptom $n = 44$	Symptomatic subjects		
			Asthma rat-related $n = 5$	Rhinitis rat-related $n = 38$	Conjunctivitis rat-related $n = 18$
Mean age	38	37.7	41	37	40
% men	43.5	50	40	47	72
% smoker	20.3	27	40	26	27.8
% atopic	36.2	64	100	60	78
Prevalence†	61.1%	38.9%	4.4%	33.6%	15.9%
Early symptoms (< 30 min)			70%	70%	71%
Specific IgE to RUP ⁽¹⁾	1	8‡	1‡	8§	4¶
Positive	1	8‡	1§	8¶	4**
Negative	46	18	1	14	6

*Of 113 subjects, blood samples were available from only 73. †The percentage values do not add up to 100, because some symptomatic workers had two or three symptoms (asthma and/or rhinitis and/or conjunctivitis). ‡Subjects with at least one rat-related symptom were rat-sensitized more often (presence of specific IgE to RUP) as compared to asymptomatic workers ($P = 0.0005$; Fisher's exact test). §Specific IgE to RUP does not differ significantly between asthmatic and asymptomatic subjects, but the populations are very small ($P = 0.08$). ¶Subjects with rat-related rhinitis were rat-sensitized more often than asymptomatic subjects ($P = 0.0003$). **Subjects with rat-related conjunctivitis were sensitized to rat more often than asymptomatic subjects ($P = 0.0024$).

Table 2. Risk factors in symptoms and allergy to rat: exposure to Rat n 1, to endotoxin, exposure to rats, contact with disinfectant, smoking and presence of specific IgE to rat urinary proteins (RUP) for 113 workers exposed to laboratory rats

	At least one rat-related symptom		Asthma rat-related		Rhinitis rat-related		Conjunctivitis rat-related		Allergy to rats*	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Exposure to Rat n 1 (ng/8 h)	0.997	0.99–1.002	0.99	0.98–1.00	0.996	0.991–1.001	0.99	0.993–1.005	1.001	0.84–1.09
Exposure to endotoxin (ng/8 h)	1.00	1.00–1.001	1.00	0.99–1.00	1.00	1.00–1.001	1.00	1.00–1.001	0.997	0.99–1.002
Years of rat exposure	1.02	0.98–1.06	1.08	0.95–1.23	1.01	0.97–1.05	1.03	0.98–1.09	0.93	0.89–1.08
Hours per week of rat exposure	1.01	0.96–1.06	1.09	0.96–1.24	1.006	0.95–1.06	1.02	0.96–1.08	0.92	0.66–1.23
Contact with disinfectants	0.83	0.28–2.45	0.73	0.72–1.00	0.85	0.27–2.70	0.90	0.20–3.95	0.97	0.08–10.7
Smoking	1.85	0.66–5.19	5.07	0.50–51.1	2.13	0.73–6.18	1.02	0.27–3.87	1.81	0.27–12.0
Atopy	2.096	0.75–5.86	21.8†	2.2–208	1.47	0.50–4.29	2.3	0.65–8.13	9.24†	2.46–34.7
Presence of specific IgE to RUP	26.0‡	3.0–225	7.87	0.44–138	34.7‡	3.95–304	9.44‡	1.90–46.8		

*According to specific IgE to rat urinary proteins (RUP; $n = 73$). † $P = 0.01$; ‡ $P = 0.006$. OR, odds ratio; 95% CI, 95% confidence interval.

laboratory technicians; 6 h/week (range < 30 min–50 h) for scientists and 8 h/week (range < 30 min–30 h) for students. Nearly 60% of the scientists spent fewer than 2 h/week in direct contact with rats. Animal technicians' duties included cleaning rat rooms and cages and feeding rats. Laboratory technicians and students carried out experiments regularly, handling rats for treatments, performing surgery or euthanasia. Student contact had a duration of fewer than 4 years. Researchers had less direct contact with rats; but their offices were often near the rat quarters.

Individual daily inhaled doses of Rat n 1 and endotoxin

Previous results [14] enabled us to determine the mean dose of Rat n 1 and endotoxin that each worker inhaled during an 8-h shift. Each occupational group was assigned to a category of exposure intensity according to their mean doses of either Rat n 1 or endotoxin:

For Rat n 1 exposure: animal technicians were most highly exposed to Rat n 1 (Fig. 1; $P=0.004$); cleaning cages and feeding rats were the tasks that induced the highest exposure to Rat n 1 ($P=0.03$). Researchers (scientists and students) and laboratory technicians were all subjected to medium exposure levels: handling rats, surgery and euthanasia induced a significantly lower exposure than the tasks performed by animal technicians. Among the scientists, those who were rarely in contact with rats were the least exposed ($P=0.005$).

For endotoxin exposure: animal technicians were also most exposed to endotoxins (Fig. 2; $P=0.01$): cleaning cages and feeding rats were again the tasks inducing the highest exposure ($P=0.001$). Exposure was lower for researchers (scientists and students) and laboratory technicians ($P=0.01$): regular activities involving handling rats, surgery and euthanasia did not induce significantly higher endotoxin exposure than those with occasional or rare contact with rats.

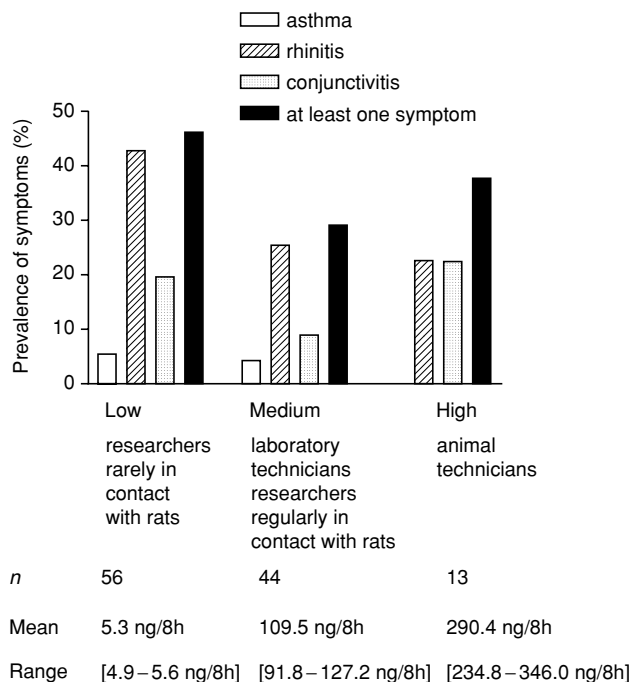


Fig. 1. Prevalence of rat-related symptoms according to category of exposure to Rat n 1.

Exposures to Rat n 1 and endotoxin as risk factors for rat-related symptoms and sensitization

No relation was observed between symptoms or sensitization to rat allergen and airborne exposure to Rat n 1 (Table 2, Fig. 1). Exposure to endotoxin did not protect against or increase sensitization to RUP or rat-related symptoms (Table 2, Fig. 2). Symptomatic subjects who were not allergic to rats were exposed to more endotoxins ($n=18$; mean of 1.64 ng/8 h) than were either symptomatic subjects allergic to rats ($n=8$; mean of 0.96 ng/8 h) or asymptomatic subjects ($n=47$; mean of 0.81 ng/8 h; $P=0.057$). The analysis did not find that number of years of occupational rat exposure, number of hours of rat exposure per week, or occupational exposure to disinfectant were significant risk factors for rat-related symptoms (asthma, rhinitis or conjunctivitis) or rat allergy (Table 2).

Discussion

This cross-sectional study allowed us to analyse the relations between airborne levels of rat major allergen (Rat n 1) and endotoxin, and sensitization to RUP and occupational rat-related conjunctivitis, rhinitis and asthma.

Our questionnaire, a modified French version of the ISAAC questionnaire [12], produced results similar to those in other studies: of 113 subjects working with rats, 38.9% reported at least one rat-related symptom. The prevalence of asthma, rhinitis and conjunctivitis in our population was 4.4%, 33.6% and 15.9%, respectively. In previous publications [1–3], asthma was also the least frequent symptom, with a prevalence ranging from

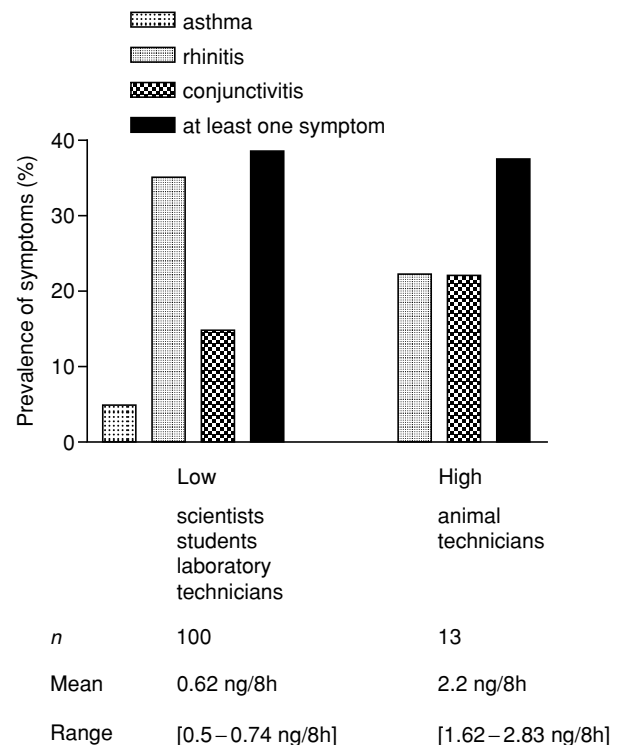


Fig. 2. Prevalence of rat-related symptoms according to category of exposure to endotoxins.

6 to 10%, while rhinitis was the most common (i.e. prevalence of 39.7%) [17]. Two other studies [5, 18] have also reported that the prevalence of IgE specific to RUP among exposed workers was less than 20%. We found, as previous studies have [3, 5, 19–21], that workers sensitized to RUP were more likely to develop rat-related symptoms: the presence of specific IgE to RUP was a risk factor for rat-related rhinitis and conjunctivitis.

In our study, only 30.8% of the symptomatic workers tested were sensitized to RUP. Such a result was suggested by Platts-Mills et al. who found that 63% of symptomatic patients had specific IgE to rat urinary allergens [5]. Our results might indicate that there is no evident relation between symptoms and sensitization to RUP. Moreover, we found no relation between symptoms and airborne Rat n 1 levels; this contradicts the findings by Cullinan et al. [21]. Various hypotheses may account for these discrepancies:

The allergenic extracts of RUP we used might not have detected all the sensitized subjects. Our results raise the issue of the quality of the extracts used for specific IgE measurements: two other studies found a sensitization ratio twice as high as ours when using purified urinary proteins to detect specific IgE [5, 22].

The prevalence of at least one rat-related symptom and rat-related rhinitis were higher among subjects in whom rat-specific IgE had not been determined than among those in whom it was ($P=0.06$). This may in part explain the low prevalence of sensitization to rat among symptomatic workers.

Among symptomatic subjects not sensitized to RUP, 50% were atopic, with a highly significant OR for rat-related asthma (OR 21.8). They may be sensitized to another aeroallergen present in rat quarters, such as soybean-based rat food or woodchip bedding.

We performed a cross-sectional study, which may involve a 'healthy worker effect', especially due to the mean age of the workers. Only a prospective study can minimize this bias. Cullinan and colleagues performed a prospective study over a 4-year period and did not find a significant dose-response relation between rat allergen concentrations and chest, eye and nose symptoms [21]; only new work-related skin symptoms were related to high rat allergen exposures. The lack of sensitivity in our measurements (eight patients of 44 with symptoms described symptoms with non-detectable Rat n 1 levels) may also explain the lack of relation between symptoms and rat allergen exposure.

Other non-allergenic pollutants present in rat quarters, such as endotoxins, might be responsible for symptoms among non-sensitized atopic and non-atopic subjects. For this reason we measured airborne endotoxins in rat quarters. At all 12 sites, endotoxin levels were 100–1000 times lower than the levels found in occupational settings in which endotoxins have been considered to be responsible for respiratory symptoms [9]. However, one can suppose that subclinical inflammation may occur, as Borm et al. have demonstrated [11]: at low levels of airborne endotoxin, equivalent to those we found, the peripheral blood of animal facility workers shows a cellular response to lipopolysaccharide (LPS) with a significant increase of BPI (bactericidal permeability increasing protein) and of sTNF-R75 (75-kDa soluble tumor necrosis factor receptor), two LPS-response mediating proteins. The prevalence of symptoms among workers who were and were

not sensitized to rats was not related to endotoxin levels, even when we assessed exposure as the cumulative dose of 8 h at work, as Vandenplas et al. [23] recommended. Symptomatic subjects not sensitized to rat were, however, exposed at higher levels of endotoxin than symptomatic rat-allergic subjects or asymptomatic subjects. This difference did not reach statistical significance, perhaps because of the small number of subjects. It could be interesting to perform an experiment involving repeated inhalation of low doses of endotoxin, corresponding to levels found in rat quarters, among symptomatic subjects not sensitized to rat, as has been done with cat allergen [24].

In conclusion, the prevalence of symptoms and of sensitization to rat allergen was very similar to that in other studies. We have found that most symptomatic workers were not sensitized to rat allergen. No significant relation between rat-related symptoms, and airborne rat allergen or endotoxin levels was found. Endotoxin did not seem to be an important co-factor for sensitization to RUP and for rat-related symptoms. More studies are needed to determine causes other than rat allergens or endotoxins that may be responsible for symptoms in rat-exposed workers.

Acknowledgements

The authors thank Dr Laurence Guilloux (Unité d'Allergologie, Institut Mérieux, Lyon, France) for the measurement of specific IgE to rat urinary proteins. This research was funded by the Comité Départemental du Bas-Rhin contre les Maladies Respiratoires et la Tuberculose (Lower-Rhine Departmental Committee against Respiratory Diseases and Tuberculosis) and by the Scientific Council of the University ULP (Université Louis Pasteur, Strasbourg, France).

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