

Original article

Working with male rodents may increase risk of allergy to laboratory animals

Background: Our aim was to study the risk of laboratory animal allergy (LAA) among research staff working in laboratories separate from the animal confinement area. The roles of atopy and exposure intensity in LAA were studied with special regard to exposure to male rodents, who excrete higher levels of urinary allergens than female rodents.

Methods: Eighty rodent-exposed subjects gave blood samples for the analysis of total IgE, Phadiatop, and specific IgE against rat (RUA) and mouse urinary allergens (MUA), and answered questionnaires. Air samples were collected for RUA and MUA aeroallergen measurement in both laboratories and animal confinement facilities.

Results: Twenty percent of the subjects had IgE >0.35 kU/l to RUA and/or MUA, and 32% had experienced animal work-related symptoms, although 90% of aeroallergen samples from the research department laboratories were below the detection limit (<0.26 ng RUA per m³ and <0.8 ng MUA per m³). Atopy (positive Phadiatop), total IgE >100 kU/l, other allergies (especially to other animals), or more than 4 years of exposure significantly increased laboratory animal sensitization and symptoms. Working with mainly male rodents gave odds ratios (95% CI) of 3.8 (0.97–15) for sensitization and 4.4 (1.4–14) for symptoms. Subjects with both exposure to mainly male rodents and atopy or elevated total IgE had a 10-fold higher frequency of sensitization than exposed subjects with neither risk factor.

Conclusions: A majority of subjects with a combination of exposure to mainly male rodents and atopy or elevated total IgE developed sensitization to and symptoms from laboratory animals. Current low exposure seems to maintain the presence of specific IgE. Further measures must be undertaken to provide a safe workplace for laboratory animal workers.

**A. Renström¹, A.-S. Karlsson¹,
P. Malmberg¹, P. H. Larsson¹,
M. van Hage-Hamsten²**

¹Respiratory Health and Climate, National Institute for Working Life, Solna; ²Department of Medicine, Division of Clinical Immunology and Allergy, Karolinska Institutet and Hospital, Stockholm, Sweden

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Anne Renström, PhD
Respiratory Health and Climate
National Institute for Working Life
S-112 79 Stockholm
Sweden

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Previous studies show that 11–62% of rodent-exposed staff in biomedical research and industry have laboratory animal allergy (LAA) symptoms (e.g., rhinitis, asthma, and urticaria) or are sensitized to the animals (1–4). Atopy has consistently been found to be a risk factor in these studies. This has led to suggestions, sometimes put into practice, that staff be preselected according to atopic status. However, the predictive value of a common personal trait such as atopy in the selection of personnel is low (2, 5). In Sweden, as in other countries, efforts have instead been made to improve animal work conditions. Exposure in the laboratory may today be low, particularly if work with the animals is done with ventilated benches or cabinets with displacement ventilation, which have been shown to reduce the

amount of airborne allergen (6). Many workers routinely use gloves, and some use masks or ventilated helmets, which reduce symptoms in subjects with LAA asthma (7).

Hollander et al. found an exposure–response relationship among 118 subjects who were rat exposed for fewer than 4 years, a relationship which was statistically significant among atopics. Moreover, among atopics, the increased prevalence of rat sensitization compared to unexposed workers was significant even in the lowest exposure group (4).

We wished to investigate further what may constitute risk factors or protective factors for rodent sensitization and symptoms among low-exposed laboratory workers. One aim was to assess the relationship between atopy (defined as positive

Phadiatop) or total IgE of >100 kU/l and different types of exposures to laboratory animals for sensitization and symptoms. The risk of working with male as opposed to female rodents was studied, since male rodents have been shown to excrete up to 100-fold higher levels of urinary allergens than female rodents (8–11). Furthermore, Sakaguchi et al. (12) found that male mice produced higher airborne allergen levels in an animal confinement building than female mice. This factor has not been studied previously.

To obtain a sufficient number of low-exposed subjects, in addition to subjects from a prospective study (2), we undertook a cross-sectional study in a university facility (13), resulting in a total of 80 rat- or mouse-exposed research department workers. A control group of 130 non-rodent-exposed laboratory workers was collected during these and other LAA studies (14). To confirm low exposure, we measured aeroallergen concentrations in research department laboratories and compared them with animal confinement measurements in the university facility in which the cross-sectional study was undertaken.

The studies were approved by local ethics committees.

Material and methods

Subjects

Prospective study subjects. Laboratory assistant students ($n=225$) were studied before their first laboratory animal contact and again after 2 years of work as laboratory assistants. Thirty-two subjects fulfilled the criteria given below and were included in this study. The student cohort has been presented in detail elsewhere (2, 15).

Cross-sectional study subjects. Forty-eight research department employees were included from a cross-sectional study undertaken in a university facility (13).

Control sera. Sera from research department personnel ($n=130$) not exposed to rats or mice were collected during these and two other European studies (4, 16).

Criteria. Only subjects who had worked with either rats or mice in research department laboratories for at least 5 months in the 2 years prior to the investigation were included in the study. Pet rodent owners were excluded.

The subjects filled out extensive questionnaires concerning family and personal history of allergy and animal exposure (e.g., years of exposure, hours of animal work, proportion of male animals used, and use of protective devices). All subjects gave blood samples ($n=210$).

Phadiatop, total IgE, and specific IgE analysis

Sera from exposed subjects were assayed by Phadiatop (Pharmacia CAP System Phadiatop FEIA, Pharmacia and Upjohn AB, Uppsala, Sweden), a mixture of common inhalant allergens (no rodent allergens), and total IgE (Pharmacia CAP System IgE FEIA).

IgE to rat or mouse urinary allergen was determined in serum by an enzyme immunoassay, as described previously (14). The cutoff value used for rodent sensitization was 0.35 kU/l specific IgE.

Analysis of airborne rat and mouse urinary allergens

Elution of all filters and the analysis for airborne RUA were done as described previously (17). Briefly, all filters were eluted on the day sampled in PBS, 0.5% Tween 20, and 0.15% Kathon for 1 h during rotation, after which the filter was discarded and the eluates were frozen until analysis. The RUA content was determined in an amplified two-monoclonal sandwich ELISA (standard curve range 20–200 pg/ml), specific for Rat n 1 (17).

MUA in the filter eluates was analyzed by preparing mouse urine standard and rabbit antibodies and using them in an enzyme immunoassay as follows. Urine from postpubertal male NMRI mice was collected in metabolism cages and filtered through 0.8- μ m filters and then 0.45- μ m filters. The urine was concentrated by ultrafiltration through a YM-2 filter (cutoff 2 kDa, Amicon, Danvers, MA, USA), and it was sterile filtered after buffer change in a PD-10 column (Sephadex G25M, Pharmacia). The proteins were size separated by FPLC gel filtration on Superose 12 columns (Pharmacia). Fractions from the protein peak (Mus m 1) were collected and freeze-dried. New Zealand White rabbits were immunized according to Hudson & Hay (18) with 400 μ g Mus m 1 in Freund's complete adjuvant (Sigma) and boosted twice before bleeding. The serum was Na_2SO_4 precipitated for IgG enrichment and then dialyzed, after which the rabbit antibodies were affinity purified with Mus m 1 coupled to CNBr-activated Sepharose 4B (Pharmacia).

In the ELISA against MUA, Maxisorp (Nunc Intermed) microtiter plates were coated with rabbit antibodies against Mus m 1 at 10 μ g/ml overnight. Standard (50–1500 pg/ml) and filter eluates diluted at least twofold in dilution buffer were added to the plates (100 μ l/well) and incubated for 1.5 h at 37°C. Bound antigen was detected with biotinylated rabbit anti-Mus m 1, followed by streptavidin-horseradish peroxidase incubation (both 1 h at 37°C), and visualized with TM-Blue (soluble form, TSI-CDP, Milford, MA, USA). The color reaction was stopped with 1 M H_2SO_4 and read at 450 nm.

Air sampling

Air samples were collected at 2 l/min flow on 25-mm, 0.8- μ m polycarbonate filters (Millipore, Sundbyberg, Sweden) both in the animal department facility and in the research department laboratories with SKC portable samplers (Scantec, Edinburgh, UK) with IOM sampling heads (Scantec). Ambient air (static) samples were collected in the animal rooms, animal laboratories, and corridors at 1–1.5 m in height, and personal samples were collected during normal animal work tasks with sampling heads fastened on the lapel.

A total of 108 air samples were analyzed for RUA content, of which 15 samples were discarded because of technical problems. Median (range) sampling time for ambient air samples ($n=63$) was 63 (45–167) min; for personal air samples ($n=30$), it was 65 (45–155) min. In air-filter eluates with undetectable RUA levels, the mean volume of air sampled was 154 l, giving a mean detection limit of 0.26 ng/m³ for twofold diluted samples.

Fifty-five air samples were analyzed for MUA content. The mean (range) sampling time for ambient air samples ($n=45$) was 74 (20–194) min, and the duration of the personal samples ($n=10$) was 43 (19–60) min. In filter eluates with undetectable MUA levels, the mean detection limit calculated as above was 0.8 ng/m³.

For statistical calculations, all samples below the detection limit were assigned a value of 2/3 of the mean detection limits (19).

Statistics

Patient and exposure data were analyzed with Statview (SAS Institute, Inc., Cary, NC, USA). Significance ($P<0.05$) was determined by the chi-square test (or Fisher's exact test) and the

Table 1. Characteristics of subjects exposed to rodents in research laboratories

	Rodent-exposed subjects (<i>n</i> = 80)	Rat-exposed** (<i>n</i> = 69)	Mouse-exposed** (<i>n</i> = 44)
Age, mean (SD)	33 (±10)	34 (±10)	33 (±10)
Women (%)	54 (68%)	46 (67%)	30 (68%)
Smoking (%)	16 (20%)	15 (22%)	8 (18%)
Atopy (positive Phadiatop), <i>n</i> (%)	21 (27%)*	19 (28%)*	11 (25%)
Total IgE ≥ 100 kU/l, <i>n</i> (%)	20 (25%)*	19 (28%)*	9 (20%)
Atopy or total IgE ≥ 100 kU/l, <i>n</i> (%)	31 (39%)*	29 (43%)*	16 (36%)
Rat work years, median (range)	2 (0–29)	2.4 (0.2–29)	1.5 (0–29)
Mouse work years, median (range)	0.7 (0–29)	0 (0–29)	2 (0.2–29)
Symptoms to rats, <i>n</i> (%)	22 (28%)	22 (32%)	9 (20%)
Symptoms to mice, <i>n</i> (%)	11 (14%)	10 (14%)	9 (20%)
Symptoms to rodents, <i>n</i> (%)	26 (32%)	25 (36%)	12 (27%)
Sensitized to rats, <i>n</i> (%)	14 (18%)*	14 (21%)*	5 (11%)
Sensitized to mice, <i>n</i> (%)	10 (13%)*	9 (13%)*	5 (11%)
Sensitized to rodents, <i>n</i> (%)	16 (20.2%)*	15 (22%)*	7 (16%)

* One blood sample missing.

** Among total rodent exposed.

Mann–Whitney *U*-test. Univariate or multiple logistic regression was used to calculate odds ratios (with 95% confidence intervals) for different risk factors for symptoms and specific IgE against rodents.

Results

Subjects, sensitization, and symptoms from laboratory animals

Rat- and mouse-exposed subjects are presented in Table 1. Among exposed subjects, 14 (17.7%) were sensitized to rat urine (median 2.3, range 0.7–115 kU/l) and 10 (12.7%) to mouse urine (median 2.7, range 0.4–16 kU/l). Among the 130 laboratory workers not working with rodents (mean [$\pm$ SD] age, 33 years [$\pm$ 12], 31% with IgE > 100 kU/l, as defined in Heederik et al. [14]), 10% were sensitized to rat urine and 2.3% to mouse urine ($P < 0.05$ and $P < 0.01$ compared to the exposed subjects).

Furthermore, five subjects exposed only to mice were positive to both RUA and MUA. Subjects who were sensitized to one rodent species had a high risk of being sensitized to the other species; $P < 0.0001$, odds ratio (95% CI) = 42 (7–244). Sensitization to rodents was also strongly associated with expression of animal-work related symptoms ($P < 0.0001$). Rat and mouse exposure, sensitization, and symptoms, respectively, were pooled in the main analyses.

Relationship between sensitization and atopy and exposure

Of the laboratory rodent-exposed group ($n = 80$), significantly more subjects were sensitized to rodents and had experienced symptoms among those who were atopic, had elevated total IgE, or were allergic to other furred animals (Table 2). Those working with mainly

Table 2. Risk factors for sensitization (specific IgE of > 0.35 kU/l to rat and/or mouse) and symptoms when working with laboratory rodents. Univariate logistic regression with 95% confidence intervals

Factor	Sensitized*				Symptoms			
	<i>n</i> and (%) sensitized with factor	<i>n</i> and (%) sensitized without factor	Odds ratio	95% confidence interval	<i>n</i> and (%) symptomatic with factor	<i>n</i> and (%) symptomatic without factor	Odds ratio	95% confidence interval
Smoking	3/16 (19)	13/63 (21)	0.87	0.22–3.6	6/16 (38)	20/64 (31)	1.3	0.42–4.1
Total IgE > 100 kU/l	10/20 (50)	6/59 (10)	8.8	2.6–30	12/20 (60)	13/59 (22)	5.3	1.8–16
Atopy (positive Phadiatop)	9/21 (43)	7/58 (12)	5.5	1.7–18	12/21 (57)	13/58 (22)	4.6	1.6–13
Atopy or total IgE > 100 kU/l	13/31 (42)	3/48 (6.2)	11	2.8–43	17/31 (55)	8/48 (17)	6.1	2.2–17
Allergy in parents/siblings**	10/31 (32)	6/47 (13)	3.3	1.0–10	11/31 (35)	14/48 (29)	1.3	0.51–3.5
Allergy to pollen/dust**	8/18 (44)	8/61 (13)	5.3	1.6–17	9/18 (50)	17/62 (27)	2.6	0.90–7.8
Allergy to other animals**	9/14 (64)	7/65 (11)	15	3.9–57	9/14 (64)	17/66 (26)	5.2	1.5–18
Rodent exposure > 4 years	12/31 (39)	4/48 (8.3)	6.9	2.0–24	16/32 (50)	10/48 (21)	3.8	1.4–10
Exposure ≥ 5 h/week**	8/37 (23)	7/39 (18)	1.3	0.41–3.9	16/38 (42)	9/39 (23)	2.4	0.91–6.5
≥ 60% male rodents**	13/44 (30)	3/30 (10)	3.8	0.97–15	21/45 (47)	5/30 (17)	4.4	1.4–14
Use of gloves**	7/31 (23)	9/43 (21)	1.1	0.36–3.4	10/31 (32)	16/44 (36)	0.83	0.32–2.2
Ventilated bench**	13/49 (27)	3/25 (12)	2.6	0.68–10	18/49 (37)	8/26 (31)	1.3	0.47–3.6

* = One missing blood sample.

** = One or more subjects answered “don’t know” or did not answer question.

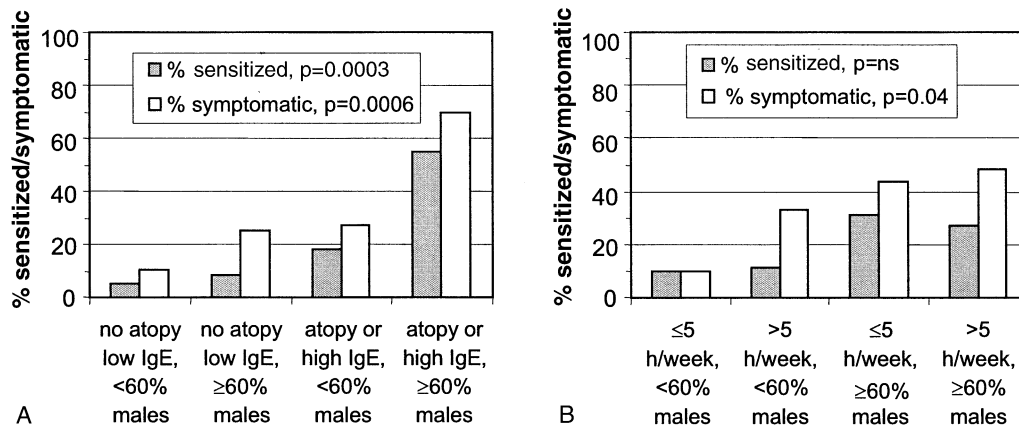


Figure 1. Combinations of risk factors for sensitization and symptoms to laboratory rodents. A) Atopy or total IgE of > 100 kU/l and working with mainly male rodents. B) Exposure more than 5 h per week and working mainly with male rodents.

male rodents ($\geq 60\%$ males) had a threefold higher frequency of both sensitization (NS) and symptoms (significant) compared to those working less with male animals. Those working less or more than 5 h per week did not differ significantly in sensitization nor symptoms. However, working more than 5 h per week correlated with mainly male rodent work ($P < 0.05$). Subjects with more than 4 years of exposure were significantly more often symptomatic and sensitized, and the sensitized subjects had higher specific IgE levels (range 0.8–115 kU/l to RUA [$n = 11$], and 0.4–16 kU/l to MUA [$n = 9$]) than those exposed less than 4 years (0.7–3.9 kU/l to RUA, $n = 3$, and 3.2 kU/l to MUA, $n = 1$). Subjects exposed more than 4 years also worked significantly more with mainly male rodents (75% vs 48%, $P < 0.05$), but not more hours per week with either rats or mice than those exposed less than 4 years.

Subjects with a combination of atopy or total IgE of > 100 kU/l and working with mainly male rodents had a 10-fold increase in sensitization and sixfold increase in symptoms compared to subjects with neither risk factor (Fig. 1A). Combining indicators of exposure intensity ($\geq 60\%$ male rodent work and exposure of > 5 h per week) gave a significant increase in the frequency of symptoms, but not of sensitization (Fig. 1B). All seven subjects with a combination of atopy or total IgE of > 100 kU/l, allergy to other furred animals, and $\geq 60\%$ male rodent exposure were sensitized, whereas only 1/18 with none of these risk factors was sensitized. Use of gloves and/or ventilated benches was not associated with lower sensitization or symptom frequency.

Multiple logistic regression analysis showed that exposure to mainly male animals, with atopy or total IgE of > 100 kU/l, sex, and smoking included in the model, gave an odds ratio (95% CI) of 4.3 (1.3–14) for symptoms and 4.1 (0.92–18) for specific IgE against rodents. The odds ratios for working over > 5 h/week with atopy, total IgE of > 100 kU/l, sex, and smoking in the model were 2.2 (0.76–6.6) and 1.1 (0.31–3.8), respectively.

Aeroallergen measurements

RUA values for 93 samples collected at 63 sampling instances were calculated (mean values for parallel samples). Only two of 26 samples from the research departments were above the detection limit. The RUA levels in personal animal work samples from the animal house (median [range] 1.5 [< 0.26 –5.3] ng/m³, $n = 11$) were significantly higher than the personal animal work samples in the research departments (< 0.26 [< 0.26 –0.51] ng/m³, $n = 7$), $P = 0.01$. The static samples were also significantly higher in the animal house (< 0.26 [< 0.26 –13] ng/m³, $n = 26$) than in the research departments (< 0.26 [< 0.26 –0.32] ng/m³, $n = 19$), $P < 0.001$.

Fifty-five air samples collected on 34 occasions were analyzed for MUA content (mean values calculated for parallel samples). In the animal house, 13/21 of the samples and in the research departments, 11/13 of the samples were below the detection limit. However, the differences between air samples collected in the animal house (median [range] < 0.8 [< 0.8 –26] ng/m³) and in the research departments (< 0.8 [< 0.8 –1.1] ng/m³) did not reach statistical significance.

Discussion

Male rodents excrete up to 100-fold higher amounts of Mus m 1 isoallergens (mouse) (8, 9) and Rat n 1 (α_2 -globulin) (rat) isoallergens (10, 11) in urine than females (20, 21). These lipocalin proteins, which have been shown to function as pheromone carriers, constitute major allergens and are present in animal room dust (22–24). Male mice have also been shown to cause higher airborne allergen levels than female mice (12). Therefore, we hypothesized that working with male rodents might be an overlooked risk factor for development of LAA. Indeed, we found that working with mainly male rodents gave an odds ratio of sensitization to and symptoms from laboratory animals

of about 4, adjusted for atopy or total IgE of >100 kU/l, smoking, and sex. Subjects with both atopy or total IgE of >100 kU/l and male rodent work had a 10-fold higher frequency of rodent sensitization than those with neither risk factor. Working with male rodents appeared to be more important than working many hours per week with rodents. However, both factors correlated ($P<0.05$). Furthermore, it is possible that subjects with symptoms have taken steps to decrease exposure time, but hardly to decrease the use of male rodents. We are not aware of any other study that has taken the sex of the rodents into account.

Air sampling in epidemiologic studies may circumvent this problem, since working with male rodents would give higher allergen levels than working with female rodents. However, in a large cross-sectional study by Hollander et al. (4), including animal handlers, technicians, and scientific staff, among subjects exposed less than 4 years, the time-multiplied exposure level alone had a moderate effect on the prevalence rate ratio of LAA. As in our study, in combination with atopy or total IgE of >100 kU/l, the risk was significantly increased also in the low exposure range. Although the allergen levels in air were very low in the whole-day personal samples, intermittent peak exposure to the male rodent allergens, either through inhalation or perhaps even via the skin, may have contributed to increasing the risk of allergy.

As most air samples from the research laboratories were below detection level (<0.26 ng/m³ for RUA and <0.8 ng/m³ for MUA, respectively), research staff working with special ventilation solutions, such as ventilated benches or cabinets, should be exposed to minimal allergen amounts. Allergen measurements using these methods were not available for the 32 research department staff not from the studied facility. However, we had previously measured allergen levels for this subject group, and found very low allergen levels working with ventilated benches in research facilities compared to other situations (25). However, the use of gloves and/or ventilated benches did not appear to protect from sensitization or symptoms, but conclusions are difficult, since LAA subjects might be more inclined to use such protection.

There are several possible explanations for the high prevalence of symptoms and IgE against rodents among those exposed for over 4 years. The low exposure may have led to a delayed onset of sensitization, instead of the 1–3 years usually found (1). Although these subjects did not work more hours/week with rodents, they worked to a greater degree with male rodents. This group may also have had a higher exposure in the past. The working environment for laboratory animal-exposed personnel had progressively improved over the last decade in the studied facility (main ventilation improvements, gradual installation of ventilated

benches and cabinets, improvements in work routines, and use of personal protective devices). Incidentally, most of the LAA subjects exposed for over 4 years reported having been skin prick test or RAST positive to rodents previously, some for more than a decade prior to our study (data not shown). Instead of healthy worker selection, despite having experienced symptoms and being aware of their sensitization, these subjects remained at work (“stubborn workers”). Thus, the exposure levels were perhaps low enough for subjects to continue working, with manageable symptoms, yet high enough to maintain sensitization. Of course, it is possible that additional staff had left because of LAA.

Besides testing for atopy, we measured total IgE, since we had previously found that total IgE appeared to be a good predictor of LAA (2). This was later confirmed in a cross-sectional study by Hollander et al. (4). Indeed, in the present study, we found that, by combining these measurements, we obtained the highest odds ratios. Absence of either indicator was associated with a low risk of LAA.

In several studies (4, 14, 16), simultaneous exposure to rats and mice has been ignored. Since many laboratory animal workers are actually exposed to both of these species, we hypothesized that all rodent exposure may be of importance for the development of IgE-mediated reactions to a rodent species. Indeed, we observed that subjects who had developed sensitization to one rodent species were highly likely to be also sensitized to the other (odds ratio [95% CI]=42 [7–244], although they were not always exposed to both species. The cause of this immunologic cross-reactivity might be that both the rodent allergens, Mus m 1 and Rat n 1, and the rodent albumins, respectively, are closely related (the lipocalins Rat n 1 and Mus m 1, to which 90% of rat and mouse allergics react, respectively, share 66% homology [26, 27]). Allergy to other animals also greatly increased the odds ratio for LAA sensitization, as also found by Hollander et al. (28) in their cross-sectional study. There are several structurally similar lipocalins among the main allergens from furred animals, e.g., Can f 1 (dog), Bos d 2 (cow), and Equ c 1 (horse) (29), and dog and cat allergens share IgE epitopes (30). Having specific IgE or symptoms against one animal allergen thus appears to increase the risk of developing it against others.

The prevalence of sensitization among a normal population not working with laboratory rodents will not be zero: in our unexposed laboratory worker control group ($n=130$), 10% percent had IgE of >0.35 kU/l against rats and 2.3% against mice. Therefore, a total elimination of sensitization among the exposed subjects is unrealistic.

In conclusion, in low-exposure situations, as in this study, exposure to male rodents may contribute to LAA development. In an improved working environment, Botham et al. showed that first-year symptom incidence

decreased, yet sensitization was high (31, 32). Similarly, we found that the allergen level threshold for sensitization development and maintenance of specific IgE appears to be low indeed. We suggest that to further diminish allergen load and LAA development, female rodents should be used when possible (considering the scientific question at hand). Atopy or total IgE of >100 kU/l, or allergic symptoms, especially reactions to other animals, appear to have the greatest importance for LAA development, followed by exposure intensity. However, neither risk factor predicts LAA usefully enough when considered alone – as we found in our prospective study (2), and as also shown by Fisher et al. (33). Combining atopy or total IgE of >100 kU/l

with exposure intensity strongly increases the risk of LAA (see also Hollander et al. [4]), and hence increases the predictive accuracy considerably (5).

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