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Allergic sensitization is associated with increased bronchial responsiveness: a prospective study of allergy to laboratory animals

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Allergic sensitization is associated with increased bronchial responsiveness: a prospective study of allergy to laboratory animals. A. Renström, P. Malmberg, K. Larsson, P.H. Larsson, B-M. Sundblad. ©ERS Journals Ltd 1995.

ABSTRACT: The purpose of this prospective study was to investigate the extent of change in bronchial responsiveness and the prognostic value of methacholine provocation in early sensitization to laboratory animals.

Thirty eight laboratory technicians were studied during training (before first exposure) and after having been exposed to laboratory animals for a median 18 (range 5–33) months. On both occasions they were subjected to spirometry, bronchial methacholine challenge, skin-prick tests and blood sampling, and responded to questionnaires.

Nine (24%) developed laboratory animal allergy (LAA), defined as animal work-related symptoms (n=8), or specific immunoglobulin E (IgE) (n=7) or both. In the LAA group, bronchial responsiveness was normal before employment, but had increased significantly at follow-up compared to technicians who had not developed LAA. Six of the nine LAA subjects had a more than threefold increase in bronchial responsiveness, and three of these reported chest symptoms. Spirometric values were not different between the groups prior to exposure or at follow-up, and had no prognostic value. However, a pre-employment level of total IgE >100 kU·L^{-1} predicted the development of LAA (relative risk 2.8).

Thus, early LAA was associated with increased bronchial responsiveness in most subjects. In contrast to total IgE, the level of pre-employment bronchial responsiveness or lung function did not influence the magnitude of change in responsiveness, nor predict sensitization.

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Laboratory animal allergy (LAA) is a well-known occupational hazard for workers in biological and medical education, research and industry. The prevalence of LAA is often reported to be 10–30% of exposed subjects. The majority of subjects with LAA develop symptoms during the first 3 yrs of exposure. Atopics are overrepresented among LAA subjects [1–5]. The most commonly reported symptom caused by the animals is rhinitis, often in combination with conjunctivitis. About 25–50% of the subjects with rhinitis develop asthma, with a time lag of months or several years, with a yearly incidence of about 2% of exposed subjects [4, 6], and a workplace prevalence of about 10% [2, 7, 8].

In recent years, increased awareness of the risk of developing LAA has prompted efforts to reduce exposure. We have also noted that laboratory technicians with knowledge of atopy according to skin-prick test, tend to avoid positions that involve exposure to laboratory animals. In spite of this, we have found a high percentage of sensitization to laboratory animals among exposed technicians during the first few years of employment, chiefly among those with high levels of total immunoglobulin E (IgE) [9]. In this report, the extent of change in bronchial responsiveness associated with development of LAA and the prognostic value of pre-exposure airway responsiveness and lung function are described.

Subjects and methods

Laboratory technicians

Thirty eight students (four men) were examined during the first year of their 2 year laboratory technician education, and were re-examined after having worked for a median 24 months (range 13–40 months; 25th–75th percentile 24–25 months). At entry into the study, their median age was 21 (range 18–43) yrs. At follow-up, they had worked with animals 18 months (range 5–33 months).

Laboratory animal exposure included rat (n=31), mouse (n=23), rabbit (n=7), hamster (n=2), horse (n=1), pig (n=1), and chicken (n=1). Nineteen of the 38 subjects worked with two or more species. Laboratory exposure to dog and cat were not included, as the majority of all technicians

had been exposed to these animals in private, prior to and during the study.

On both occasions, the 38 subjects underwent several tests using identical conditions and equipment. They also responded to extensive questionnaires on animal exposure, family history of allergy, personal allergic and medical history, *etc*.

Respiratory physiology

Spirometry was performed with a wedge spirometer (Vitalograph®, Buckingham, UK) at least 20 min prior to the bronchial provocation. The highest value of three forced expirations was chosen as the forced expiratory volume in one second (FEV_1) and the highest of three forced and three slow maximal expirations was chosen as the vital capacity (VC) for each patient. In the bronchial provocation tests, after inhalation of the diluent, methacholine in isotonic saline was inhaled by tidal breathing (15 breaths·min^{-1}) over 1 min in subsequent concentrations of 0.5, 2, 8 and 32 mg·mL^{-1}.

The nebulizer output was 0.4 mL·min^{-1} and the nebulisate passed through a drying device before inhalation, regulating inspiratory flow to 0.4 L·s^{-1} and drying the nebulisate to achieve maximum deposition in the lungs [10]. Each dose-step cycle lasted 6 min and FEV_1 was measured five min after start of the inhalation. The test was stopped at an FEV_1 decrease of ≥20% compared to the value measured after inhalation of the diluent, or after inhalation of the highest concentration.

The cumulative inhaled dose was calculated from nebulizer output and inhalation time. The cumulative methacholine dose which provoked a 20% decrease in FEV_1 was calculated by interpolation on a log cumulative dose scale (PD_{20} FEV_1). The methacholine test was designed so that PD_{20} FEV_1 could be measured in most healthy subjects [10, 11]. In cases where FEV_1 did not decrease by >20% at the highest concentration, PD_{20} FEV_1 was assigned a value of >10 mg. The average percentage decrease in FEV_1 per mg of inhaled methacholine (cumulative dose, linear scale; "slope") was calculated by linear regression [12, 13]. Change in slope was calculated as the ratio of the slope at follow-up divided by pre-exposure slope. In table 1 this ratio is presented after logarithmic transformation, due to skewed distribution.

Skin-prick test

Subjects were skin-prick tested on the volar aspects of both forearms using hair extracts from the most commonly used laboratory mammals: mouse, rat, guinea-pig, rabbit and hamster (1:20 w/v; ALK, Copenhagen, Denmark). Histamine dihydrochloride, 10 mg·mL^{-1}, was used as a positive reference and dilution solution as negative control.

Skin-prick tests were also performed with extracts from eight common aeroallergens (birch, timothy, mugwort, dog, cat, two types of moulds (Alternaria and Cladosporium), and house dust mite (*Dermatophagoides pteronyssinus*) at 100,000 biological units (BU)·mL^{-1} (Pharmacia, Uppsala, Sweden)). Reactions ≥2 mm in diameter were considered positive. A weal size equal to the histamine weal was defined as +++ [2].

Table 1. – Methacholine provocation data at follow-up and change from pre-exposure to follow-up among sensitized and nonsensitized subjects

	Sensitized n=7	Nonsensitized n=30	p-value
$PD_{20}FEV_1$ mg	0.30 (0.22–2.94)	1.97 (0.89–>10)	<0.05
Slope %·mg^{-1}	-43.2 (-57.5–-6.8)	-9.5 (-17.1–-1.7)	<0.05
log slope$\Delta^{\dagger}$	0.52 (0.32–0.82)	-0.08 (-0.29–0.53)	<0.05

Slope represents percentage FEV_1 change per milligram methacholine (cumulative dose). Δ: change. †: change calculated as ratio of slope at follow-up divided by pre-exposure slope. Values are presented as median, and 25th–75th percentile in parenthesis. Statistical analysis performed by means of Mann-Whitney U-test. FEV_1: forced expiratory volume in one second; PD_{20}: dose provoking a 20% decrease in FEV_1.

Specific IgE against laboratory animals in sera

Pre-employment and follow-up sera were stored at -70°C until analysed. Follow-up sera were tested for IgE against rat and mouse urinary proteins, respectively, using enzyme linked immunosorbent assay (ELISA) as described previously [9]. In subjects exposed to other species, sera were tested with Phadebas radioallergosorbent test (RAST)® (Pharmacia) for specific IgE against these animals (rabbit, hamster, horse, pig and chicken) by Pharmacia, Uppsala, Sweden.

Total-IgE and Phadiatope®

Pre-employment and follow-up serum total-IgE were analysed with ELISA. Microtitre wells (Maxisorp, Nunc, Roskilde, Denmark) were coated with 10 µg·mL^{-1} rabbit anti-human IgE antibodies. Dilutions of IgE (0.5–100 kU·L^{-1}, calibrated with paper radioimmunosorbent test (PRIST)® standard extracts [14]) and patient sera diluted 1/10 were added. Alkaline phosphatase conjugated rabbit anti-IgE was added and after reaction with the substrate p-nitrophenyl phosphate (pNPP) (Sigma, St Louis, Mo, USA), bound IgE was detected at 405 nm in a Vmax ELISA reader (Molecular Devices, Menlo Park, CA, USA). Phadiatope® analysis (Pharmacia) was performed at the Department of Clinical Immunology, Karolinska Hospital, Stockholm.

Definitions

Laboratory animal sensitized. An individual was considered sensitized to a laboratory animal if he/she had a positive skin-prick test or specific IgE in serum to the allergen.

Laboratory animal allergy. An individual was considered to be an LAA subject if he/she was sensitized and/or

had experienced symptoms (rhinitis, conjunctivitis, wheezing, tightness of the chest, coughing, or urticaria) clearly related to laboratory animal work.

Atopy. A person with at least one +++ reaction or two ++ (weal area=half of histamine weal area) reactions against any of eight common aeroallergens in skin-prick tests or a positive Phadiatope® test, was defined as atopic.

Statistics

Statistical analyses were performed by means of Chi^2-test, Wilcoxon signed rank test or Mann-Whitney U-test, as appropriate. Results are presented as median (25th–75th percentile) values. A p-value of less than 0.05 is considered significant.

All calculations and statistics were performed using Microsoft® Excel (Microsoft Corp, Redmond, WA, USA), or Statview® (Abacus Concepts, Berkeley, CA, USA) software on Macintosh microcomputers (Apple Computers, Cupertino, CA, USA).

Results

Reactions against laboratory animals

None of the 38 technicians exposed to laboratory animals had positive skin-prick tests (SPT) against the animal hair extracts in the pre-employment tests. At follow-up, seven had at least one positive SPT. Six of the SPT positive and two SPT negative (in total 8 out of 38 exposed) reported animal work-related symptoms at follow-up (table 2). Four of the SPT positive subjects were also positive in the ELISA test against rat or mouse urine. All RAST tests against other animals were negative. There was a highly significant correlation between reported symptoms and demonstrated sensitization (6 of the 7 sensitized had symptoms compared to 2 of the 31 nonsensitized; $p<0.001$). Both of the SPT negative subjects who reported symptoms had stopped working with the animals more than a year prior to follow-up, one expressly because of these symptoms. The prevalence of sensitization and/or symptoms against laboratory animals ("LAA") was thus 9 out of 38. Symptoms, SPT and specific IgE test results for these nine, laboratory animal allergic subjects, are shown in table 2. Among the 31 technicians exposed to rats, six were SPT positive against rat (19%), and among the 23 exposed to mice, two were SPT positive against mouse. One SPT positive subject (no. 7) had rhinitis but was uncertain whether or not the symptoms were connected to work exposure to laboratory animals.

Of the three who reported light or moderate chest symptoms (wheezing, tightness of the chest, or coughing) during animal work, one had physician diagnosed asthma. The other two had not sought medical help. The subject with the most hyperresponsive airways had experienced chest symptoms, but reported only urticaria at laboratory animal contact. She kept a laboratory rat at home as a pet and had high levels of rat specific serum IgE. She subsequently needed asthma medication.

Comparison between laboratory animal exposed technicians with and without LAA

Lung function and bronchial responsiveness

FEV_1 and VC values did not differ between the nine LAA subjects and the 29 non-LAA subjects before first exposure or at follow-up. Neither was there any difference between the groups regarding change in FEV_1 or VC from before first exposure to follow-up (tables 3 and 4).

No differences between those who developed LAA and non-LAA subjects with regard to bronchial responsiveness was observed before first exposure. At follow-up, LAA subjects had significantly more reactive airways than non-LAA subjects ($p<0.05$) (table 3). All LAA subjects had lower PD_{20} FEV_1 values ($p<0.01$) and a steeper slope ($p<0.01$) at follow-up than before first exposure (table 2 and figure 1a). Bronchial responsiveness among non-LAA subjects was on average unchanged (figure 1b), although there was a trend in the opposite direction (eight subjects had a higher slope value at follow-up, 1 was unchanged, and 19 had a lower value). Between the LAA and the non-LAA group, the change in slope from before first exposure to follow up is significantly different ($p<0.01$) (table 4).

Table 2. – Subjects symptomatic and/or test positive against laboratory animals (LAA group)

Subj	Exposure				Skin-prick tests		Symptoms *vs* lab animals				Specific IgE ng·mL^{-1}		PD_{20} FEV_1		Slope %·mg^{-1}	
No.	Rat	Mouse	Other	m	Rat	Mouse	N	E	C	S	Rat	Mouse	Before	After	Before	After
1		+		18	2+	4+ *	+	+			0.4	2	>10	4.15	-2.6	-2.8
2	+	+		16	4+	2+	+	+	+	+	18	0	2.31	0.30	-10.2	-43.2
3	+			25	2+	2+				+	350	0	4.05	0.13	-14.6	-121.4
4	+			16	2+	0	+	+	+		2	0	8.11	1.52	-2.0	-15.2
5	+	+		25	1-2+	0	+	+			0	0	0.58	0.27	-23.6	-43.4
6	+	+		25	1+	0				+	0	0	3.49	3.41	-1.2	-4.0
7	+	+	+	30	1-2+	0	(+)				0	0	0.66	0.20	-20.1	-62.2
8	+			14	0	0	+	+			0	0	4.55	0.96	-3.8	-16.7
9	+	+	+	5	0	0	+		+	+	0	0	1.79	0.56	-4.6	-31.9

*: also guinea-pig 2+; Slope: represents percentage FEV_1 change per milligram methacholine (cumulative dose). LAA: laboratory animal allergy; Subj: subject; lab: laboratory; IgE; immunoglobulin E; m: months; N: nasal; E: eye; C: chest; S: skin. For further abbreviations see legend to table 1.

Table 3. – Follow-up data on LAA subjects (sensitized and/or symptomatic) and non-LAA subjects

	LAA subjects (n=9)	Non-LAA subjects (n=29)	p-value
Age at follow-up yr	25 (24–28)	25 (23–28)	NS
Exposure months	18 (15.5–25)	18 (13.8–24)	NS
Exposure h·month^{-1}	80 (20.5–160)	18 (3–60)	<0.05
Total IgE kU·L^{-1}	109 (59–158)	35 (19–64)	<0.01
FEV_1 L·s^{-1}	3.38 (3.24–3.63)	3.59 (3.10–3.88)	NS
VC L	3.95 (3.66–4.38)	4.15 (3.63–4.59)	NS
PD_{20} FEV_1 mg	0.56 (0.25–1.99)	3.16 (0.89–>10)	<0.05
Slope %·mg^{-1}	-31.9 (-48–-12.4)	-7.5 (-16.4–-1.6)	<0.05

Slope represents percentage FEV_1 change per milligram methacholine (cumulative dose). Values are presented as median, and 25th–75th percentile in parenthesis. Statistical analysis by means of Mann-Whitney U-test. VC: vital capacity; NS: nonsignificant.

Table 4. – Change in lung function from pre-exposure to follow-up among LAA subjects (sensitized and/or symptomatic) and non-LAA subjects

	LAA subjects (n=9)	Non-LAA subjects (n=29)	p-value
ΔFEV_1 L·s^{-1}	-0.15 (-0.24–0.03)	-0.05 (-0.15–0.04)	NS
ΔVC L	-0.01 (-0.17–0.07)	0.00 (-0.14–0.07)	NS
log slope Δ	0.63 (0.43–0.85)	-0.09 (-0.35–0.18)#	<0.01

#: n=28. Slope represents percentage change in FEV_1 per milligram methacholine. Values are presented as median, and 25th–75th percentile in parenthesis. Statistical analysis by means of Mann-Whitney U-test. For further abbreviations see legends to tables 1–3.

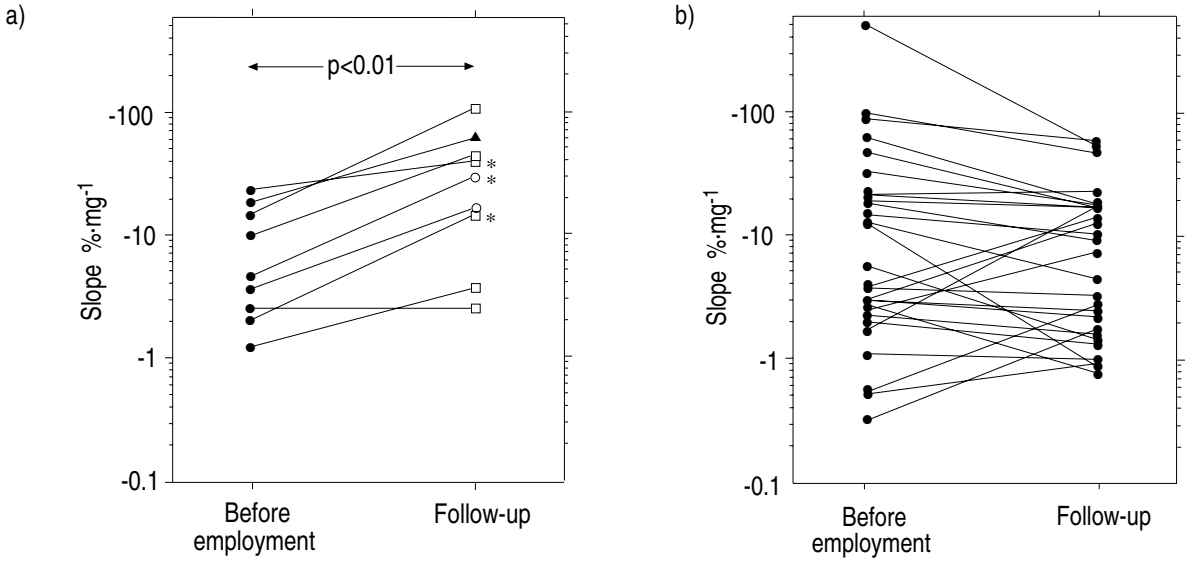

Fig. 1. – a) Slope (percentage change in FEV1 per milligram methacholine (cumulative dose) for LAA subjects (sensitized and/or symptomatic)) before employment and at follow-up. The change in slope is significant, p<0.01 (Wilcoxon signed rank test). *: subject reported chest symptoms related to animal work. ● : SPT-, symptom-; ○ : SPT-, symptom+; ▲ : SPT+, symptom-; □ : SPT+, symptom+. b) Slope for non-LAA subjects. The change is not significant. FEV1: forced expiratory volume in one second; LAA: laboratory animal allergy.

The same results were found, when comparing those who were sensitized against laboratory animals according to SPT or specific IgE (n=7) with the remaining subjects (n=31). At follow-up, the $PD_{20}FEV_1$ values were significantly (p<0.05) different between the groups (table 1). Slope values at follow-up, and change in slope from before exposure to follow-up were also significantly different between sensitized and non-sensitized (table 1).

There was no difference in numbers of months of exposure to animals between LAA subjects and those who did not develop LAA (table 3). Sensitized or LAA subjects worked with anmlas significantly more hours per month than non-LAA subjects. Among nonsensitized subjects, exposure (h·month^{-1}) was not correlated to follow-up bronchial hyperresponsiveness or change in bronchial responsiveness. There was no difference in slope change between those who were currently continuously laboratory animal exposed (n=23) and those who had not been exposed to laboratory animals during the last month prior to the follow-up examination (n=14), either in the LAA-group or the non-LAA group.

Atopy and total IgE

Prior to animal work, two of the nine LAA subjects and four of the 29 non-LAA subjects were atopic according to SPT and/or Phadiatope®. At follow-up, 3 of the 9 LAA subjects and 5 of the 29 non-LAA subjects were atopic (not significant, Chi^2-test).

Total IgE serum levels before exposure were 138 (89.5–199) $kU \cdot L^{-1}$ in the LAA subjects and 31 (17–102.5) $kU \cdot L^{-1}$ in the non-LAA subjects ($p<0.05$). At follow-up, the difference between the groups remained significantly different ($p<0.01$) (table 3). Prior to exposure, 8 out of 9 LAA and 8 of 29 non-LAA subjects had total IgE >100 $kU \cdot L^{-1}$ (relative risk 2.8; 95% confidence interval 1.3–6.0).

Discussion

Most technicians who became sensitized to laboratory animals also developed increased bronchial responsiveness. $PD_{20}FEV_1$ decreased in all nine subjects with LAA. In six subjects, a more than threefold decrease in $PD_{20}FEV_1$ was observed. In exposed nonsensitized technicians, $PD_{20}FEV_1$ tended to increase rather than decrease, although this difference did not reach statistical significance.

Three of the technicians reported chest symptoms related to exposure to laboratory animals. A fourth subject (with the most hyperresponsive airways) may have underreported chest symptoms, since she kept a pet rat at home. However, at least two subjects with a marked change in $PD_{20}FEV_1$ did not report chest symptoms related to animal exposure. At the time of investigation, after a median 18 months of exposure, symptoms were generally mild and had not been identified or treated as asthma, with the exception of one subject. Most cases in the LAA group had low levels of specific IgE antibodies as determined by the size of the skin-prick test, ELISA or RAST and in two subjects animal specific IgE was not demonstrated. Thus, the changes in airway responsiveness occurred in a population with mild reactions to laboratory animal allergens after a rather short period of work exposure.

We are not aware of other studies where bronchial responsiveness has been measured prior to the first exposure and compared with values after a short period of exposure to laboratory animal allergens. This prospective design allowed a more detailed description of changes induced by the animal allergens than would have been possible in a cross-sectional study. NEWILL *et al.* [15] reported an association between LAA and bronchial hyperreactivity, in a study in which subjects who had reported chest symptoms at animal contact had been excluded beforehand. A significantly higher percentage of symptomatic subjects was defined as having bronchial hyperreactivity compared to nonsymptomatic animal exposed. In the present study, it is confirmed that increased bronchial responsiveness can also be demonstrated in subjects who do not report chest symptoms. However, the finding that most subjects with LAA develop increased bronchial responsiveness, has not been demonstrated previously.

Bronchial hyperresponsiveness to methacholine or histamine does not seem to be strongly associated with reported chest symptoms in the population [16]. These findings are in accordance with those of the present study.

A conclusion of the present study is that neither pre-exposure lung function nor bronchial hyperresponsiveness predicted sensitization or the development of chest symptoms against laboratory animals. Our results do, however, indicate that a change in bronchial responsiveness is a more sensitive indicator of lower airway involvement than a change in spirometric values.

The increase in bronchial responsiveness was associated with sensitization to animal allergens rather than to exposure to toxic dusts associated with the allergens. Most technicians worked in modern research laboratories, not animal housing facilities, and levels of such dusts is probably low. The change in bronchial hyperresponsiveness was, thus, seen in the group of sensitized subjects and correlated with intensity of exposure. In nonsensitized subjects exposed to the same types of environment, there was no change in average bronchial responsiveness, and no correlation with exposure intensity. The design of the present study, however, does not allow firm conclusions as to whether or not sensitization *per se* or continued allergen exposure in sensitized subjects caused the bronchial responsiveness. In the murine model described by SALOGA *et al.* [17], local allergen challenge was necessary in sensitized mice to provoke increased airways responsiveness. In the present study, two sensitized subjects who had increased bronchial responsiveness at follow-up, had not been exposed to laboratory animals for at least a month prior to follow-up. Two more subjects, with increased bronchial responsiveness, had left exposure partly because of work-related respiratory symptoms more than a year before examination. These subjects did not have measurable specific IgE at follow-up. It cannot be determined whether they had signs of specific IgE after LAA symptom development, or if events unrelated to laboratory animal exposure may have contributed to the increase in bronchial responsiveness in these subjects. Thus, although this study is based on a rather small cohort, in contrast to SALOGA *et al.* [17], there were no differences in slope change between those who were currently laboratory animal exposed and those who had not been exposed for at least a month prior to follow-up, either among LAA or non-LAA subjects. Thus, the LAA group had a greater increase in bronchial reactivity, irrespective of recent exposure.

The degree of change in bronchial responsiveness associated with sensitization was moderate in comparison with the interindividual variability in $PD_{20}FEV_1$ values observed before exposure. The magnitude of change among LAA subjects from pre-exposure to follow-up was similar in those with more hyperresponsive airways compared to those with less responsive airways. Thus, the LAA subjects with the most responsive airways before exposure also had the most responsive airways after exposure. Whether or not asthma symptoms relate to the magnitude of change in responsiveness or to the absolute level of bronchial responsiveness cannot, however, be determined from the present study.

If allergic reactions to animal allergens is a characteristic of those who develop LAA, one might have expected that these subjects had asymptomatic allergic inflammation due to exposure to commonly present dog or cat allergens. However, the subjects who developed LAA did not appear to have more hyperresponsive airways prior to first exposure than other subjects.

The diagnosis of sensitization was based both on skin-prick tests against hair allergens from the commonly used laboratory animals, supplemented by measuring specific IgE in serum against other species or allergens, for instance against the major rat and mouse urinary proteins [1, 18–20]. Several studies show, however, that specific IgE cannot be demonstrated with the methods available in many subjects who experience symptoms [7, 8, 15, 21]. This was the rationale for including the two subjects with symptoms related to laboratory animal exposure but lacking evidence of specific IgE in the LAA group. Omission of these subjects does not change the conclusions drawn. High pre-exposure total-IgE levels predicted development of LAA: an elevated total-IgE level prior to work exposure was shown to have an approximately three times higher relative risk of sensitization and/or symptom development against the laboratory animals. This was indicated similarly in both LAA asthma and test positive rhinitis groups compared to nonsymptomatics in another study [21].

In conclusion, the present study shows that sensitization to laboratory animals was associated with increase in bronchial responsiveness in the majority of laboratory technicians. The magnitude of the change rather than the level of bronchial responsiveness correlated with sensitization. The duration of exposure was short and the majority of the sensitized subjects were nonatopics by SPT or Phadiatope® prior to exposure. Increased total IgE in serum prior to exposure predicted the development of LAA, but the level of lung function or bronchial responsiveness did not.

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