

Prospective study of laboratory-animal allergy: factors predisposing to sensitization and development of allergic symptoms

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In a prospective study of laboratory technicians, selected indicators of allergy and atopy were studied in an attempt to determine predictors of laboratory-animal allergy (LAA). Laboratory technicians underwent spirometry, methacholine provocation tests, and blood sampling, and responded to a questionnaire during training and after 2 years' work. Among 38 laboratory-animal-exposed subjects, total IgE before exposure gave the best correlation ($P < 0.01$; Mann-Whitney U-test) to reported symptoms caused by laboratory animals ($n = 8$) at follow-up. The prevalence of atopy and allergic symptoms had increased in exposed technicians at follow-up, but this was also found among unexposed matched referents ($n = 36$ pairs). One subject in the exposed group reported asthma before exposure, compared with seven at follow-up ($P < 0.05$; Fisher's exact test). However, the prevalence of asthma had increased from two to six (not significant) also among unexposed technicians. There were no significant differences between the groups in any measured variable at follow-up. Among 43 subjects who later worked with laboratory animals, 21% had a positive skin prick test for common allergens, as compared with 37% among 112 without animal exposure ($P = 0.06$; χ^2 test), suggesting selection for laboratory animal work.

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Laboratory-animal allergy (LAA) is common among workers and researchers in biology and medicine. Of those exposed to laboratory animals, 10–30% become sensitized or report symptoms caused by the animals (3, 9, 10, 19). The allergens are potent: low levels of inhaled allergen, perhaps a few micrograms per year, may cause allergies, and nanogram amounts may cause symptoms in sensitized persons (8, 12, 14). Most of those who develop symptoms do so within 2–3 years of exposure (3, 17).

A prospective study was designed in which laboratory technicians were examined for lung function and immunology during their education. Those who later worked as laboratory technicians and were exposed to laboratory animals were reexamined 2 years after starting work together with matched unexposed referents. The first aim was to assess factors predisposing to development of sensitization (development of specific IgE antibodies) and allergic symptoms caused by laboratory animals. The second aim was to compare exposed and a matched group of unex-

posed laboratory technicians for change in lung function, allergic symptoms, and atopic status. A third aim was to assess possible selection among laboratory technicians for work that included animal exposure.

Material and methods

Laboratory technicians

Students from five laboratory technician training schools in the first year of their 2-year course were invited to participate in the study of 1986–7. About 60% participated, and 225 students were examined. According to a postal follow-up questionnaire, 2 years after graduating, 168 (77%) had completed their course and worked at least 6 months as laboratory technicians (Table 1). The 168 did not differ in allergy, atopy, lung function, etc. from those who had not worked as technicians. Forty-three technicians had worked with laboratory animals (rat, mouse, rabbit, hamster, horse, pig, chicken, and

Table 1. Prospective laboratory technician study

1986-7:	225 (about 60% of available) students investigated
1990:	219 (97%) answered questionnaire
	168 (77%) worked >6 months as technicians
	43 were exposed to laboratory animals
	112 were unexposed
	13 were sporadically or home exposed and were excluded
1990-1:	38 (of 43) exposed technicians investigated
	36 unexposed referents investigated

vole) more than 5 months. Thirteen subjects were excluded: they had been exposed at work only sporadically or had one of these animals at home. Exposure to dog or cat was disregarded, as most subjects had been exposed to one or the other at home, often for a long time before the investigation. For study of selection, the 1986-7 values from the 43 laboratory-animal-exposed subjects were compared with those of the 112 unexposed subjects.

In the follow-up study of 1990-1, laboratory animal workers and matched referent technicians were invited to undergo the same medical tests with the same methods and equipment as in the 1986-7 investigation. One was excluded, as she was exposed to an animal (vole) for which there were no available tests. Matching was based on 1) 1986-7 atopy (skin prick test or Phadiatope® positivity), 2) smoking/nonsmoking, 3) age, and 4) sex. In a few cases with difficulty in finding matches, an age difference of up to 12 years was accepted, and the fourth criterion was abandoned. Some of the matched referents had owned rabbits or other animals as pets as children, as had the exposed subjects. None of those who had owned rats or mice as pets as children were chosen as unexposed referents. The project was approved by the Karolinska Hospital ethics committee, and it had the informed consent of each participant.

Questionnaires

For both the 1986-7 and 1990-1 studies, laboratory technicians completed extensive questionnaires on allergic heredity, personal allergic and medical history, exposure to a number of irritants, smoking habits, and animal exposure. A postal follow-up questionnaire asked subjects whether they had graduated, worked as technicians, etc.

Lung physiology

Spirometry was done with a wedge spirometer (Vitalograph®, Buckingham, UK) at least 20 min before the bronchial provocation. The highest value of three forced expirations was chosen as FEV₁ (forced expiratory volume during 1 s), and the highest of three forced and three slow maximal expirations was taken as VC (vital capacity) for each patient.

In bronchial provocation tests, methacholine in isotonic saline was inspired in increasing concentrations (0, 0.5, 2, 8, and a maximum of 32 mg/ml). Details of the methacholine provocation tests are described elsewhere (11). The cumulative inhaled dose of methacholine which caused a 20% decrease in FEV₁ was calculated by interpolation on a log cumulative dose scale (PD₂₀ FEV₁). If FEV₁ did not decrease by ≥20% at the highest concentration, PD₂₀ was assigned a value of 10 mg. The slope of the relationship between percent change in FEV₁ and the cumulative methacholine dose (linear scale) was calculated by linear regression (7, 13).

Skin prick tests

Extracts from eight common aeroallergens were tested on the volar aspects of both forearms at 100 000 BU/ml (Pharmacia, Uppsala, Sweden): birch, timothy, mugwort, dog, cat, two types of mold (*Alternaria* and *Cladosporium*), and house-dust mite (*Dermatophagoides pteronyssinus*). Histamine chloride, 10 mg/ml, was used as a positive reference, and dilution solution as negative control. Reactions ≥2 mm in diameter were considered to be positive. A wheal size equal to the histamine wheal was defined as +++ (1). A person with at least one +++ reaction or two ++ (wheal area = half of histamine wheal area) reactions was defined as atopic. Sensitivity to laboratory animals was determined by skin prick tests with hair extracts from guinea pig, mouse, rat, rabbit, and hamster (1:20 w/v, ALK, Copenhagen, Denmark).

Phadiatope® and total IgE

Sera from 1986-7 and 1990-1 were stored at -70°C until analyzed. Atopy was also tested with Phadiatope® (Pharmacia). Total IgE was analyzed with ELISA (enzyme-linked immunosorbent assay) in which human IgE was detected with alkaline phosphatase-conjugated antibody.

Specific IgE against laboratory animals

Sera of 1990-1 from exposed laboratory technicians were tested for IgE against rat and mouse urinary proteins, respectively, with ELISA. A mouse and rat positive serum (6.3 and 2.4 PRU, respectively) was used in both tests as a standard curve reference. Microtiter wells (Maxisorp, Nunc, Roskilde, Denmark) were coated at 10 µg/ml with purified mouse urinary allergen protein, MA-1, and YM-2 (Amicon, Beverly, MA, USA) concentrated and dialyzed rat urine, respectively. Undiluted and 1:3 diluted sera were added after washing, as was standard curve serum, diluted threefold from 1:1 to 1:81.

Biotinylated rabbit anti-IgE was added, and bound antibody was detected with streptavidin horseradish peroxidase and the substrate TMBblue (TSI/CDP, Milford, MA, USA). The reaction was terminated with 1 M H₂SO₄, and absorbance was read at 450 nm in an ELISA reader Vmax (Molecular Devices, Menlo Park, CA, USA). Sera from laboratory technicians exposed to other animals were tested with RAST for specific IgE (Pharmacia).

Statistics

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

Significance was tested with the Statview® Student's *t*-test, the chi-square test, Fisher's exact test, the Wilcoxon signed rank test, or the Mann-Whitney U-test, as appropriate. A *P* value of <0.05 was considered to be significant. Values in text and tables are shown with median values and interquartile range.

Results

Factors predisposing to sensitization and allergic symptoms

Thirty-eight of the 43 laboratory-animal-exposed subjects participated in the follow-up. Seven subjects had developed a positive SPT (skin prick test) for one or more of the animal hair extracts from animals encountered at work. Six of these and another two subjects reported allergic symptoms related to laboratory-animal exposure. Several potential risk indicators from the investigation before exposure were compared between the seven subsequently sensitized and the 31 nonsensitized (Table 2). No significant differences were detected, although there was a tendency of association between some indicators and later sensitization; for instance, total IgE (*P* = 0.09; Mann-Whitney U-test). Three subjects had atopy according to SPT before exposure to animals, of whom one later became sensitized.

Total IgE was significantly higher before exposure among the eight who developed symptoms from the laboratory animals than among the 30 nonsymptomatic subjects; 150 (114–202) and 29 (17–101) kU/l, respectively (*P* < 0.01; Mann-Whitney U-test) (Fig. 1).

Of those who reported symptoms caused by laboratory animals, 7/8 had a total IgE > 100 kU/l for 1986–7. Among nonsymptomatic subjects, the number was 8/30 (*P* < 0.01; Fisher's exact test).

The positive predictive value (2, 18) was 0.44 for total IgE > 100 kU/l for development of symptoms.

Table 2. Preexposure data from subjects who later worked with laboratory animals. No statistical differences were found between values from sensitized and nonsensitized groups. Median (interquartile range) values for total IgE and lung function

	Sensitized (n=7)	Nonsensitized (n=31)
Positive SPT*	2 (29%)	6 (19%)
Atopy, SPT	1 (14%)	2 (6%)
Atopy, Phadiatope test	1 (14%)	5 (16%)
Symptoms, nasal/eye	2 (29%)	2 (6%)**
Asthma symptoms	0 (0%)	1 (3%)
Allergy, family	3 (43%)	15 (48%)
Smoking (≥ 1 cig/d)	2 (29%)	7 (23%)
Furred animals at home	7 (100%)	24 (77%)
Total IgE, kU/l	120 (50.5–187)	36 (17–130)***
PD ₂₀ , mg methacholine	3.49 (1.07–7.10)	1.74 (0.64–> 10 ⁺)
Slope, %/mg	–10.2 (–18.7 to –2.2)	–4.2 (–19.7 to –2.2)

* Any reaction ≥ 2 mm; ** *P* = 0.15; Fisher's exact test; *** *P* = 0.09; Mann-Whitney U-test; + not determined because of censored data.

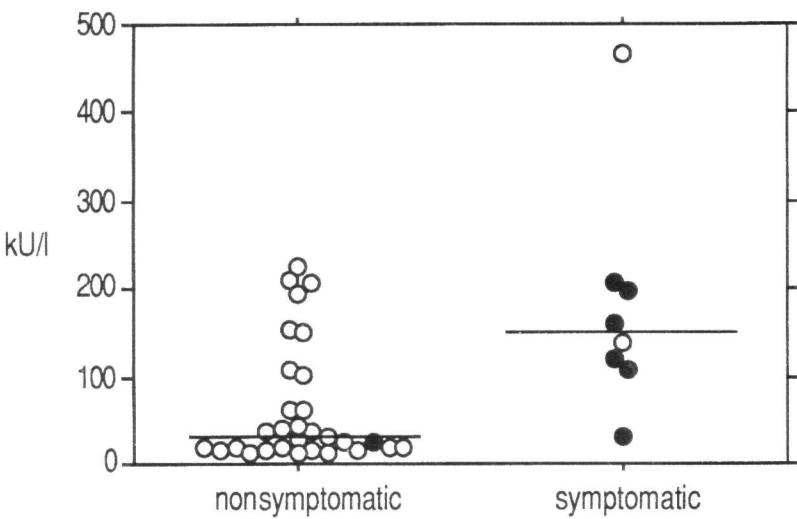


Fig. 1. Preexposure total IgE values (kU/l) for subjects who at follow-up reported no symptoms from laboratory animals (median = 29) and symptomatic subjects (median = 150) (*P* < 0.01; Mann-Whitney U-test). Filled circles represent laboratory-animal-sensitized subjects.

For development of sensitization, the predictive values were 0.33 for total IgE and 0.40 for previous rhinitis/conjunctivitis. The predictive value of family allergy was 0.17.

Comparison between exposed and matched unexposed subjects

Thirty-six pairs were matched for preexposure Phadiatope and SPT positivity, smoking (≥ 1 cigarette/d), age (within 10 years), and sex, with six exceptions (one pair was unmatched for smoking and three for sex, and two had an age difference of > 10 years). There were no significant differences between the groups in age (median age difference = 2 years), sex (four men in the exposed group; three in the unexposed group), and total IgE or lung function values at preexposure measurements.

At follow-up, the exposed and unexposed groups

had not developed significant differences for total IgE or lung function variables (Table 3).

The prevalence of atopy and allergic symptoms was increased in both the exposed and unexposed groups at follow-up. The increase in reported asthma symptoms in the exposed group was statistically significant ($P = 0.03$; Fisher's exact test). However, the differences between the groups were not significant (Table 4).

Lung function values did not differ significantly between the matched exposed and unexposed subjects. At follow-up, both FEV₁ and VC had decreased significantly by an average 28 and 12 ml/year, respectively ($P < 0.01$; paired t -test, combined groups). In the methacholine provocation tests, there were no differences between exposed and unexposed subjects.

Selection

The 155 (139 women) laboratory technicians had a median age of 21 (range 18–51) years in 1986–7. The prevalence of different indicators of allergic disposition among those who subsequently worked with laboratory animals ($n = 43$) and those who did not ($n = 112$) is shown in Table 5. No significant differences were found. However, there was a tendency to lower prevalence of positive SPT for common allergens among subsequently exposed subjects, as compared with unexposed subjects ($P = 0.06$).

Discussion

Many laboratory technician students are exposed to laboratory animals during their training course. To obtain never-exposed subjects, we had to do the preinvestigation at an early stage of their course. As it was impossible to predict who would later work with animals (about 25%), a fairly large sample of students was needed. Students from five out of six training schools in Sweden were studied, of whom about 75% completed their course and worked as technicians.

The design allowed a study of selection mechanisms for laboratory animal work. Allergic diagno-

Table 4. Changes in atopy, symptoms, and lung function from preexposure to post-exposure. For total IgE and lung function variables, median values are given with interquartile ranges in parentheses. No differences between exposed and unexposed subjects were statistically significant

	Exposed ($n=36$)	Unexposed ($n=36$)
Atopy, SPT	+2 (3→5)	+1 (4→5)
Atopy, Phadiatope test	+2 (6→8)	+1 (5→6)
Symptoms, nasal/eye	+6 (4→10)	+4 (4→8)
Asthma symptoms	+6 (1→7)	+4 (2→6)
Total IgE change, kU/l	-0.5 (-47.5-9)	+1.5 (-12.5-5.5)
FEV ₁ change, l/s	-0.06 (-0.17-0.02)	-0.11 (-0.24-0.04)
VC change, l	-0.02 (-0.16-0.06)	-0.14 (-0.22-0.04)
Slope change, %/mg	-0.1 (-9.9-5.6)	-0.1 (-9.0-4.9)

Table 5. Selection: atopy and allergy in laboratory technicians before work. No differences were statistically significant

	Exposed ($n=43$)	Unexposed ($n=112$)
Positive SPT*	9 (20.9%)	41 (36.6%)**
Atopy, SPT***	5 (11.6%)	22 (19.6%)
SPT laboratory-animal positive	2 (4.7%)	15 (13.4%)
Allergic diagnosis/medication	6 (13.9%)	18 (16.1%)
Atopy, Phadiatope	8 (19.0%; $n=42$)	24 (21.4%; $n=110$)
Total IgE kU/l, median (i-q range)	43 (19-151)	42 (20-120)

* Any reaction ≥ 2 mm; ** $P=0.06$; chi-square test; *** At least one +++ or two ++ reactions in SPT to common aeroallergens.

sis and SPT positivity were known to the subjects (the latter as a result of participating in our preexposure study), but they were unaware of their Phadiatope and total IgE status before starting work. Interestingly, there was a roughly doubled prevalence of preexposure SPT-positive and atopic subjects among those who did not work with animals (not significant). Other indicators were similar among later exposed and unexposed subjects.

A second consequence of the design was that it allowed comparison with a well-matched referent group. To our knowledge, this has not been reported before. There was a clear tendency to increase in the prevalence of allergic symptoms in the exposed group (reported asthma symptoms were significantly increased). However, the cause of this is difficult to interpret, since unexposed referents showed similar increases in allergic symptoms. Possibly, the increase in symptoms reported by both groups at follow-up was partly a result of increased awareness (recall bias). Environmental exposures other than that to laboratory animals may also have contributed to the increase. Thus, when interpreting the results of longitudinal studies, these confounders must be seriously considered.

Atopy is a well-documented risk factor for sensitization. In several other papers on laboratory-animal allergy, a significant relationship was shown between preemployment allergic symptoms and later development of LAA (4, 9, 15, 18). In this study, 2/7

Table 3. Postexposure data from matched laboratory-animal-exposed and unexposed subjects. Median (interquartile range) values; no differences were significant

	Exposed ($n=36$)	Unexposed ($n=36$)
Total IgE, kU/l	38 (21.5-104)	34.5 (21.5-72)
FEV ₁ , l/s	3.50 (3.16-3.80)	3.48 (3.15-3.86)
VC, l	4.16 (3.66-4.59)	4.13 (3.70-4.43)
PD ₂₀ , mg methacholine	1.51 (0.74- $\geq 10^*$)	1.88 (0.70-5.58)
Slope, %/mg	-12.8 (-20.5 to -2.0)	-9.9 (-23.0 to -2.6)

* Not determined because of censored data.

sensitized and 2/31 nonsensitized subjects had previous rhinitis/conjunctivitis and only one nonsensitized subject had asthma symptoms.

Skin prick tests are widely used to define atopy. The SPT criteria used in this study (atopy: $\geq$ one histamine equivalent wheal reaction (+ + +) or $\geq$ two half area (+ +) reactions (1)) are stringent. In other reports (18, 20), a reaction of 2–3 mm to any of three allergens defines atopy. In this study, before exposure, only 3/38 subjects were SPT atopic, of whom one later became sensitized to laboratory animals, resulting in a nonsignificant increased risk for atopic subjects of sensitization. In several other reports with larger proportions and numbers of atopic subjects (5, 6, 18, 20), the relation is significant, especially for the development of chest symptoms. By a looser definition of atopy (any positive reaction $\geq$ 2 mm), two of the seven sensitized subjects were previously atopic. The Phadiatope test includes a mixture of about 10 allergens and seems to define more subjects as atopic: six, as compared with three by SPT in this study.

Yet, preventing atopic subjects (SPT or Phadiatope definition) from animal work would only have reduced the nine sensitized and/or symptomatic subjects to seven. In the present study, as in those above, a relatively high proportion of laboratory-animal-sensitized and/or symptomatic subjects were previously nonatopic; conversely, many atopic subjects do not develop laboratory-animal allergy.

From our results, it does not seem likely that refusing to employ atopic subjects in animal work will prevent the development of LAA.

In this study, the best predictor for symptoms and sensitization was not atopy, but elevated total-IgE level (predictive value 0.44; $P < 0.01$ (Mann-Whitney U-test) for development of symptoms). Predictive values are generally low for the variables studied from before exposure. Results were similar in earlier reports of predictive values for LAA which compared several criteria of atopy (16, 18).

In the present study, preventing subjects with total IgE levels > 100 kU/l from working with animals would have reduced the number of subjects developing LAA to two instead of nine. On the other hand, eight nonreactive subjects (after this study) would also have been excluded from such work.

The indicators of allergic disposition are not sufficiently good predictors of LAA to be used as pre-employment selection instruments. The possible selection value of total IgE deserves further investigation.

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