

Prevalence and diagnosis of laboratory animal allergy

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Summary

A survey of the prevalence of laboratory animal allergy to rats, mice, guinea pigs and rabbits among sixty-nine animal workers and 308 other subjects on a pharmaceutical research site revealed a 22% prevalence of laboratory animal allergy among the animal workers. The overall prevalence of atopy was 67% in persons with allergy to laboratory animals. This was significantly greater than the 31% prevalence in other animal workers. Skin-prick tests and specific IgG and IgE assays to urinary protein extracts strongly correlated with the occurrence of laboratory-animal allergy and would appear to have diagnostic value. However, a number of clinically diagnosed laboratory-animal-allergy subjects gave no evidence of immunological response to the urinary allergens and wider diagnoses may have to be applied in these cases.

Introduction

Through the last decade, attention has been increasingly focused on allergy amongst laboratory-animal workers. Several prevalence surveys have been published (Lincoln, Bolton & Garrett, 1974; Lutsky & Neuman, 1975; Taylor *et al.*, 1976; Gross, 1980; Cockcroft *et al.*, 1981; Davies & McArdle, 1981; Slovak & Hill, 1981) and a better appreciation is being gained of the allergens involved. In earlier studies, diagnostic skin-test reagents were prepared from animal epithelial tissue extracts, hair or fur, and from serum (Sorrell & Gottesman, 1957; Rajka, 1961; Neuman & Lutsky, 1976; Gross, 1980; Wahn & Siraganian, 1980; Siraganian & Hook, 1981). More recently, proteins excreted in the urine of rats and mice have been identified as playing a significant role in laboratory-animal allergy (LAA) (Newman Taylor, Longbottom & Pepys, 1977; Schumacher, 1980) and have been used in skin-test diagnostic aids (Newman Taylor *et al.*, 1977; Cockcroft *et al.*, 1981).

The present study was set up to determine the prevalence of LAA at a pharmaceutical research site and to assess the value of immunological tests in the diagnosis of this condition. Urinary protein extracts were used as skin-test diagnostic

reagents and as allergens for use in the radioallergosorbent test (RAST) for IgE antibody and in a radioimmunoassay for specific IgG antibodies. The sensitizing species studied were the mouse, rat, guinea pig and rabbit.

Materials and methods

Prevalence survey

A questionnaire based on that used in a previous study (Davies & McArdle, 1981) was sent to all of the 390 staff working on a pharmaceutical research site. The main questions related to the extent and nature of animal work undertaken by the individual; whether the individual experienced allergy-related symptoms and questions directed at definition of the atopic constitution of the individual. Symptoms considered included asthma, bronchitis, sneezing and rhinitis, conjunctivitis, eczema, urticaria and other skin conditions. No question was included on the association between symptoms and animal exposure.

Skin testing, and medical interviews to clarify and extend the information derived from the questionnaire, were carried out with 312 of the staff. Blood samples were taken from 273 of these individuals.

Skin-test reagents

Commercial skin-test reagents were supplied by Bencard, Brentford, U.K., for the following allergens: house dust mite, mixed grass pollens, cat fur, mouse fur, rat fur, guinea-pig fur and rabbit fur. Experimental skin-test reagents were prepared from bulk materials supplied by Dr J. L. Longbottom of the Department of Clinical Immunology, Cardiothoracic Institute, Brompton, London, for the following allergens: serum and urinary protein extracts from mice, rats, guinea pigs and rabbits. Serum-derived reagents were used at a final concentration of 1 mg ml⁻¹. Fur extracts were prepared as 6% extracts from mouse, rat and guinea-pig fur and as a 150% extract (by vacuum concentration) from rabbit fur.

Similar experimental skin-test reagents were prepared from urinary protein extracts from the four species of experimental animal. These extracts had been prepared by the authors by dialysis and freeze-drying.

Skin testing

The allergen solutions, derived as described above, were used for prick testing. This was done on the flexor aspect of both forearms. Reactions were read after 15 min and recorded as mean weal diameters. Vehicle-treated sites were included on each arm. Weal diameters in excess of 3 mm (or in excess of the largest vehicle reaction if this exceeded 3 mm) were considered to be positive.

Radioallergosorbent tests (RAST)

Serum was stored at -20°C prior to assay by RAST for specific IgE antibodies to each of the four animal species' urinary proteins. Urinary protein extracts, obtained by the authors as described above, were coupled to paper discs and used as detailed by Edwards *et al.* (1983).

Sera were tested in these assays at a dilution of 1 in 5. Results are expressed as the ratio of the counts bound in the presence of the test serum to those bound in the presence of a standard negative control serum (RAST index).

Assays for specific IgG

The sera were also assayed for the presence of specific IgG antibody to the urinary proteins derived from the four animal species. The serum dilution was 1 in 25 and the method was that of Moran, Dupe and Gauntlett (1978), as modified by Lee *et al.* (1982).

Statistical methods

Data have been variously analysed using a chi-squared test with Yate's correction applied, Fisher's exact test or Student's *t*-test.

Results

Prevalence survey

Compliance in the survey was very satisfactory: 97% of staff returned completed questionnaires; 83% of these staff were willing to be interviewed and skin tested; 88% of those interviewed gave blood for antibody assays.

There were no significant differences in the compliance rates between animal workers and the general site population.

Sixty-nine of the 377 staff on-site who returned questionnaires, worked with animals in some capacity. Of these sixty-nine, 44% reported that at the time of the survey they were experiencing one or more of the symptoms identified in the questionnaire. Of the remaining 308, 25% reported symptoms. The difference between the two groups is significant at the 1% level ($\chi^2 = 9.186$).

Of the sixty-two animal workers interviewed, fifteen reported evidence of allergy to a laboratory-animal species under study. Three of these subjects claimed to be free of symptoms at the time of the survey. None of the remaining seven animal workers who could not be interviewed reported relevant symptoms and were, therefore, judged not to represent any animal allergic subjects. The prevalence of LAA in the animal workers was thus 22%.

In only three subjects were symptoms of asthma present. In none was asthma the only symptom and, as shown in Table 1, asthma was always associated with rhinitis or conjunctivitis.

Table 1. Combinations of animal-related symptoms reported

Symptoms	Asthma	Rhinitis/conjunctivitis	Skin
Asthma	0	—	—
Rhinitis/conjunctivitis	3	6	—
Skin	0	4	2
Asthma + skin + rhinitis/conjunctivitis	0		

The figures refer to the numbers of LAA subjects reporting the symptom combinations shown.

Hours of exposure

The twelve subjects experiencing animal allergy at the time of the survey worked an average of 8.3 h week⁻¹ in the presence of animals. The remaining animal workers worked 13.3 h week⁻¹ with animals. This difference is not statistically significant. Likewise, no statistical difference between the two groups of workers is revealed if the numbers of subjects working 10 h week⁻¹, or less with animals, are compared ($\chi^2=0.958$).

Animal-handling procedures used

Consideration was also given to the animal-handling procedures used by current LAA sufferers, as compared with the other animal workers. No significant differences were found.

Association between LAA and atopy

Atopic predisposition was judged by prick-test response to three standard allergens: house dust mite, mixed grass pollen and cat fur. The skin-test data are shown in Table 2. The overall prevalence of atopy in LAA sufferers was 67%. This figure includes all three of the asthmatic subjects. The prevalence of atopy in other animal workers was significantly lower (31%) and comparable with that in non-animal workers (34%).

Atopic disposition was also judged from information on family and personal history of allergy. The association of LAA with an atopic constitution remained significant.

Association between LAA and allergy to common allergens

The data in Table 2 reveal that LAA subjects showed significantly greater prick-test reactivity to house dust mite (HDM) than did other animal workers. A similar relationship between LAA and cat fur may also be indicated by the data. This does not hold true for pollen sensitivity. Animal workers not allergic to animals did not differ from non-animal workers with regard to reactivity to any of the three common allergens.

Table 2. Skin-test responses to standard allergens

Subject group	Group code	Group size	% with positive reaction to:			% Atopic
			HDM	Grass	Cat fur	
LAA—asthma	A	3	100	67	100	100
—others	B	12	33	33	25	58
Other animal workers	C	48	15	21	15	31
Non-animal workers	D	249	20	26	18	34

Statistical analyses: % atopic: A *vs.* B: NS ($P=0.527$; Fisher's exact test); C *vs.* D: NS ($\chi^2=0.028$); (A + B) *vs.* (C + D): $P<0.05$ ($\chi^2=5.590$). Percent responding to standard allergens: (A + B) *vs.* C for HDM: $P<0.05$ ($\chi^2=5.076$); (A + B) *vs.* C for cat fur: $P=0.078$ ($\chi^2=3.090$); (A + B) *vs.* C for grass: NS ($\chi^2=1.320$). C *vs.* D for HDM: NS ($\chi^2=0.390$); C *vs.* D for cat fur: NS ($\chi^2=0.096$); C *vs.* D for pollen: NS ($\chi^2=0.175$)

Immunoassays

Skin-prick tests. There were no significant differences between either the incidence or size of prick-test reactions provoked by the two sets of urinary protein reagents used. Only results obtained using the extracts made by the authors have been used in the data analyses which follow.

A summary of prick-test results obtained with the urinary proteins is contained in Table 3. There was a significant correlation between prick-test reactions and the species of laboratory animal which the subject associated with the provocation of symptoms.

The comparative efficacy of skin-test reagents prepared from urine, fur and serum is indicated in Table 4. The urinary proteins were no better for diagnosis than were the other reagents at the concentrations used. However, for the mouse and the rat the weal diameters were almost invariably greater with the urinary protein extract than with either fur or serum reagents.

Specific IgE measurements. Table 5 reveals that there were significant differences between the total number of positive RAST results identified in the various subject groups. A significant association was found between positive tests and LAA.

Assay for IgG antibodies. Results of the Staph. A Sepharose antigen binding assay to measure IgG antibody specific for urinary proteins have been discussed in detail elsewhere (Lee *et al.*, in press).

Correlation of immunoassay results. Table 6 summarizes the relationships between the results obtained in the three immunoassays. Table 6 can also be used to derive the

Table 3. Analysis of specific urinary protein prick-test results across subject groups.

Subject group	Group code	Group size	Total no. tests	% positive
LAA—asthma	A	3	12	67
—others	B	12	48	23
Other animal workers	C	48	192	10
Non-animal workers	D	249	996	2

Statistical analysis: A *vs.* B: $P < 0.02$ ($\chi^2 = 6.590$); C *vs.* D: $P < 0.001$ ($\chi^2 = 35.803$); (A + B) *vs.* (C + D): $P < 0.001$ ($\chi^2 = 99.766$)

Table 4. Comparison of positive prick-test responses to allergens from fur, serum and urine, for the LAA subjects ($n = 15$)

Source species	No. of subjects with positive prick test to:		
	Urine	Fur	Serum
Mouse	3	2	3
Rat	7	6	4
Guinea-pig	5	4	4
Rabbit	5	7	2

Table 5. Analysis of specific IgE assay results across subject groups.

Subject group	Group code	Group size	Total No. assays	% positive
LAA—asthma	A	3	12	83
—others	B	12	48	25
Other animal workers	C	41	164	2
Non-animal workers	D	217	868	1

Statistical analysis: A *vs.* B: $P < 0.001$ (two-sided Fisher's exact test); C *vs.* D: NS ($\chi^2 = 0.627$); (A + B) *vs.* (C + D): $P < 0.001$ ($\chi^2 = 242$)

Table 6. Summary of positive responses to specific diagnostic tests across subject groups

Immunological assay responses			Subjects with present or past animal exposure		Subjects without animal exposure
Skin test	Specific IgE	Specific IgG	LAA*	No LAA	
+	+	+	7	3	0
+	+	—	0	1	1
+	—	+	1	1	1
—	+	+	0	0	0
—	+	—	1	2	1
+	—	—	0	7	7
—	—	+	4	22	9
—	—	—	2	45	158
Group size			15	81	177

* Results cited for LAA sufferers are for urinary allergen from the species implicated in symptom provocation.

number of individual subjects giving one or more positive reactions in any test system. Thus, positive prick tests occurred in 53% (8/15) of subjects with LAA, but in only 15% (12/81) of individuals with no evidence of clinical LAA but with some form of occupational exposure to the species under consideration. Positive prick tests to the urinary allergens occurred predominantly in the LAA group, including all three asthmatics.

Similarly, 53% (8/15) of the LAA subjects gave one or more positive RAST results. This compares with an incidence of 7% in subjects with documented animal exposure but no identified LAA, and 1% in non-animal workers.

The incidence of specific IgG in the exposed population without LAA (32%; 26/81) was higher than the corresponding incidence of positive prick tests or presence of specific IgE.

Table 7. Skin test and immunoassay results for LAA subjects

Mouse			Rat			Guinea-pig			Rabbit		
Skin test (mm)	IgE ratio	IgG units	Skin test (mm)	IgE ratio	IgG units	Skin test (mm)	IgE ratio	IgG units	Skin test (mm)	IgE ratio	IgG units
0*	<u>11.8</u>	<10	4	<u>16.4</u>	<u>11</u>	<u>5</u>	<u>45</u>	<u>275</u>	4	<u>110</u>	<u>410</u>
<u>3*</u>	<u>3.8</u>	<u>24</u>	<u>2</u>	<u>63</u>	<u>515</u>	0	1.2	<u>11.5</u>	0	1.1	<10
0*	<u>9.2</u>	<10	7	<u>32</u>	<u>51</u>	3	<u>3.4</u>	<u>14.5</u>	1	<u>6.9</u>	<u>26</u>
0	1.1	<10	0	1.4	<u>82</u>	0	0.9	<u>32</u>	0	1.5	<u>18</u>
0	0.9	<10	0	0.9	<10	0	1.2	<u>74</u>	0	1.0	<10
0	1.2	<10	0	0.9	<10	0	1.0	<10	0	0.9	<10
0	0.9	<10	0	1.1	<10	<u>2</u>	1.8	<u>20</u>	0	1.1	<10
0	1.0	<10	0	0.9	<10	0	0.9	<u>138</u>	0	0.9	<10
<u>3</u>	<u>120</u>	<u>240</u>	<u>3</u>	<u>128</u>	<u>820</u>	<u>2</u>	<u>2.0</u>	<u>227</u>	<u>1</u>	1.3	<u>18</u>
0	1.0	<10	<u>1</u>	<u>2.7</u>	<u>12</u>	0	1.1	<10	0	1.0	<10
0	0.9	<10	0	1.1	<10	0	1.0	<10	0	1.1	<10
0	1.1	<u>59</u>	<u>2</u>	<u>2.4</u>	<u>825</u>	0	0.9	<u>11.5</u>	0	0.9	<10
0	<u>4.5</u>	<10	0	<u>20</u>	<10	0	<u>7.0</u>	<10	0	<u>3.2</u>	<10
0	1.2	<10	0	1.2	<10	0	1.6	<u>70</u>	0	1.1	<10
<u>5</u>	<u>13.4</u>	<10	<u>6</u>	<u>9.1</u>	<u>39</u>	<u>3</u>	<u>11.3</u>	<u>27</u>	<u>1</u>	1.1	<10

* Asthmatic subjects. Underlined values are considered to be positive.

Table 7 shows the detailed results for the fifteen LAA subjects. Seven of these subjects had positive results in all three assays relating to one animal species (47%). The corresponding figure for the other subjects with some animal exposure but no LAA was 4% (Table 6). There were no occurrences in the non-animal workers.

Discussion

The prevalence of laboratory animal allergy, as judged by medical interview and response to questionnaire, was 22% in this survey, a figure in accord with published findings (Lincoln *et al.*, 1974; Lutsky & Neuman, 1975; Taylor *et al.*, 1976; Gross, 1980; Cockcroft *et al.*, 1981; Davies & McArdle, 1981; Slovak & Hill, 1981).

One aim of this study has been to measure the immunological response of animal workers and others to animal-derived allergens. Three assays have been used; the skin-prick test, the RAST and a measure of specific IgG antibody. Details of the methods used and of their specificity are published elsewhere.

The value of both skin tests and RASTs in support of clinical diagnosis is apparent from the data reported here. Positive prick tests were obtained in 53% of clinically diagnosed subjects, and in all three of the asthmatic individuals in the group. The use of urinary proteins in addition to fur and serum as prick-test materials did not add significantly to diagnostic capability (Table 4) but the value of each reagent used cannot be judged adequately from our data. No dose-response studies were performed with the skin-test reagents and the optimum concentration for each is not known. Moreover, it is certain that common allergens are to be found amongst the three

allergen sources used (urinary proteins, furs and sera) and without detailed immunochemical definition of the allergen specificity of the reactions, we do not know whether each is identifying common allergens or if allergens unique to a source are involved.

Our survey involved immunological tests on a large number of individuals not exposed occupationally to animals. Only 5% of such individuals responded to prick test with urinary protein allergens. However, 15% of individuals who have some contact with animals but who have no symptoms of LAA also responded (Table 6). It cannot be established at the present time whether such individuals would, given a sufficient level of exposure, develop symptoms. In terms of occupational health strategy it is clearly important to determine the prognostic implications, if any, of this skin-test reactivity.

The RAST, using urinary proteins, was positive in 53% of subjects with clinically diagnosed LAA including all three asthmatic patients. This figure is to be compared with only 1% in the unexposed population and with 7% in the non-symptomatic persons with some documented animal exposure. These figures are essentially the same as those we have obtained using prick tests and the same considerations apply.

The binding assay used for the measurement of IgG antibody specific for urinary proteins has given results of interest. Eighty percent of individuals with clinical symptoms had significant titres of IgG antibody, compared with only 6% of the non-exposed population. Of interest, however, was the finding that as many as 50% of non-symptomatic animal workers had IgG antibody. The conclusion to be drawn is that the presence of antibody of this class is an indicator primarily of exposure although the higher incidence of such antibody in the symptomatic group needs explanation (Lee *et al.*, 1982).

The main question addressed in this study was the value of these immunological tests in the diagnosis of laboratory animal allergy. At first sight it would seem that tests, both prick tests and RASTs, which are positive in only half the clinically diagnosed population, are not promising as diagnostic tools. We do not take this view. It is clear from the data given, and summarized in Table 6, that not all individuals diagnosed as animal allergic on clinical grounds have evidence of a specific immune response to the allergens used.

Thus, of the fifteen individuals originally deemed to be laboratory-animal allergic, two had no detectable immune response in either the IgE class of antibody or the IgG class. Thus there is no evidence even of a significant level of exposure to the relevant allergens. In a further four, the only indication of immunological reactivity to appropriate animal allergens was an IgG response, we believe a consequence only of exposure. Thus in six of the fifteen, no supportive evidence can be obtained that the initial diagnosis was accurate.

Further study is needed to establish the diagnosis of their condition. It is possible that they represent individuals allergic to other allergens, either animal-derived or animal-associated. We believe that it is more likely that, while their condition is related to animal handling, it is not allergic in origin but mediated through, for example, an unusual susceptibility to the irritant character of some animal furs or to histamine excreted in rodent urines.

If we are correct in our assessment of these individuals then both prick tests and radioimmunoassays become tools of some importance to discriminate between those who develop IgE-mediated syndromes and those who share similar symptoms but whose syndrome is of different aetiology.

The relationship between atopy and the development and manifestation of LAA has been addressed in other publications (Neuman & Lutsky, 1976; Gross, 1980; Cockcroft *et al.*, 1981; Davies & McArdle, 1981; Dewdney, 1981; Slovak & Hill, 1981).

Our data are in general agreement with most published work in that, judged either immunologically or on the basis of genetic predisposition, the percentage of atopic individuals was found to be significantly higher in the laboratory-animal allergics than in the other groups (Table 2). The association between these two conditions is particularly high for those who develop asthma to animal allergens; 100% on our data, and this is again in line with published work. As discussed above, until we can be certain of the initial clinical diagnosis of LAA it is not possible to determine what the true percentage figures are for the non-asthmatic, but symptomatic group. It is important that this question is answered by further study as it has clear implications in relation to employment procedures and career selection.

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