

Occupational asthma and rhinitis related to laboratory rats: Serum IgG and IgE antibodies to the rat urinary allergen

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Allergic reactions to rat urinary proteins are an important cause of occupational asthma and rhinitis among laboratory workers. We have measured IgG and IgE antibodies to a purified rat urinary allergen in sera from 179 laboratory workers of whom 30 reported symptoms on exposure to rats. There was a very good correlation between IgE antibodies and positive skin tests. In addition, there was a close correlation between reported asthmatic reactions and serum IgE antibody to rat allergen: IgE ab was present in 12/18 of workers with asthmatic reactions but in only 2/135 of workers without symptoms ($p < 0.001$). Serum IgG antibodies to rat allergen were present in all sera with IgE antibody but were also present in 30% of asymptomatic individuals. The incidence and quantity of IgG antibody correlated with the degree of exposure to animals (i.e., hours per day) but not with the length of exposure in years. Our results on rat allergy confirm that there is an increased incidence of asthma among individuals who were atopic as judged by positive skin tests to other allergens. However, this relationship did not apply to individuals with rhinitis alone, and excluding atopic individuals from employment would have been a very inefficient method of reducing asthma or rhinitis in this group. Our results confirm that IgE antibody responses to rat urinary allergen are an important cause of occupational disease. The results for IgG antibody suggest that their prevalence represents a marker for the degree of exposure to rat proteins. (J ALLERGY CLIN IMMUNOL 1987;79:505-15.)

Acute respiratory symptoms are very common among laboratory workers who work with or look after small mammals.¹⁻⁷ A large proportion of these patients develop positive skin tests, serum IgE antibodies, and positive in vitro histamine release to extracts of serum, dander, or urine from these animals. In most studies rats have been found to be an important or the most important cause of symptoms, including acute and sometimes severe attacks of asthma.^{1, 2, 5, 6} Although positive skin tests have been observed to rat serum and rat dander, there is good evidence that the main cause of reactions to rats is a protein or proteins present in high concentration (i.e., >1 mg/ml) in rat

Abbreviations used

Rat allergen: The α_2 -euglobulin form of rat urinary allergen
SAS: Saturated ammonium sulfate
CIE: Crossed immunoelectrophoresis

urine.^{1, 5, 8, 9} The fact that the source of rat allergen is a protein solution makes it very different from the particulate allergens, e.g., pollen grains, fungal spores, or mite fecal pellets. Recent studies have demonstrated that the quantities of guinea pig, rat, mouse, or rabbit allergens in litter (up to 4 gm per cage) and that become airborne (~ 100 $\mu\text{g}/\text{m}^3$) can be very high.¹⁰⁻¹² Furthermore, it appears that the airborne allergen for rat urinary allergen is carried on relatively small particles (~ 7 μm in diameter) that remain airborne longer than the particles that carry dust mite allergens (>10 μm in diameter).^{10, 11} Studies on the immune response to inhalant allergens have found that most nonallergic individuals make no antibody response and can be considered as nonresponders.¹³⁻¹⁶ However, it is well established that beekeepers make

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0.1ml serum dilution (or SAS fraction of serum)
 + 7ng ^{125} I labelled rat allergen
 carrier protein (IgG or IgE myeloma protein)
 4 hours room temp.
 + goat anti-IgG or anti-IgE
 Precipitate overnight 4°C
 Wash 3x and count

FIG. 1. Radioimmunoassay for antibodies to rat allergen.

IgG antibody to venom whether they are allergic or not. It has been reported that a large proportion of asymptomatic laboratory workers develop IgG ab to animal allergens.^{17, 18}

In a previous epidemiologic survey, Cockcroft et al.¹ reported results for skin tests and symptoms on 179 people working with laboratory animals. In that study no specific IgG antibodies could be measured with either gel diffusion or the ELISA technique.¹ With the use of radiolabeled rat urinary protein (α_2 -euglobulin) and a radioimmunoprecipitation technique for measuring IgG and IgE antibodies, we have assayed sera from the same 179 individuals.^{1, 10, 14, 16} The results demonstrate that specific antibodies to rat urinary allergen can be measured in sera from a large proportion of the symptomatic individuals, particularly individuals with positive skin tests to rat urine. In addition, a considerable number of asymptomatic individuals had detectable IgG antibodies to rat allergen. Although there was no overall tendency for the most heavily exposed individuals (animal handlers) to develop more allergy,¹ there was a strong correlation between increasing exposure and the development of serum IgG antibodies.

METHODS

Study population

The survey was carried out in three Medical Research Council institutes in the United Kingdom, and the results of questionnaire and skin tests were reported previously.¹ Overall, 213 individuals were studied, including 54 animal handlers, 125 individuals who used animals during their work (users), and 34 clerical or administrative workers who were only inadvertently exposed to laboratory animals. The questionnaire asked about past history of allergic diseases and symptoms on exposure to specific animals, including rhinitis and asthma. In addition, participants were asked about the number of years they had worked with animals and with which species they had worked. Animal users were separated into light and heavy according to whether they

were normally exposed for less than 2 hours or more than 2 hours per day.

Sera were also available from 54 pregnant women who were bled as part of their routine prenatal study and who were questioned about previous hay fever, asthma, and exposure to small animals. The pregnant women were not skin tested.

Skin testing

Skin testing was carried out as described previously with 26-gauge needles and the prick technique. Common inhalant skin test reagents were obtained from Bencard Ltd. (Betchworth, U. K.). *Aspergillus* and small-animal extracts were obtained as described previously.^{1, 19} Rat urine obtained from Wistar-Firth rats with a metabolic cage was dialyzed (molecular weight cutoff, 3000) overnight against water, heated at 56°C for 30 minutes to kill viruses, and filtered through a Millipore (Millipore Corp., Bedford, Mass.) filter, 0.22 μ m. The dialyzed urine was then diluted with physiologic saline to elicit a final solids concentration, as measured by dry weight, of 5 mg/ml.

Skin test responses both to animal extracts and common inhalants were read at 15 minutes and were regarded as positive if they demonstrated erythema and a wheal diameter of 3 mm more than control subjects or 5 mm more than control subjects if there was no erythema. Modifying the criteria used for skin tests to 5 mm more than control subjects with erythema and 8 mm more than control subjects without erythema would have reduced the number of positive skin tests to rat urine from 22 to 18, and the incidence of associated antibodies to rat allergen would have changed from 20/22 to 18/18 for IgG ab and from 14/22 to 14/18 for IgE ab. With a lesser criteria of wheal size, 2 mm more than control subjects, five additional individuals would have been judged to have positive skin tests, none of whom reported symptoms with rats and only one of whom had IgG ab.

Radioimmunoprecipitation assays for serum IgG and IgE antibodies to rat urinary allergen

Rat allergens were purified from the urine of Sprague-Dawley rats as described previously.^{5, 9} Two purified fractions were obtained, an α_2 -euglobulin and a prealbumin of molecular weight 20,000 and 16,000 daltons, respectively.⁹ The two purified proteins were $\geq 95\%$ pure as judged by CIE. The α_2 -globulin is referred to as euglobulin because it precipitates after prolonged dialysis against distilled water. This is the same protein that was first identified in 1965²⁰ and was designated in 1966 as α_{2u} -globulin, where the u denotes that it is urinary.²¹ The α_2 -euglobulin and the prealbumin have previously been demonstrated to cross-react by use of CIE and were found to have very similar skin reactivity.²² For each of those proteins, 20 μ g were radio-labeled with 1 mCi of ^{125} I with the chloramine-T technique. The labeled proteins that were separated on a Sephadex G-75 column had a specific activity of 50,000 to 70,000 cpm/ng.

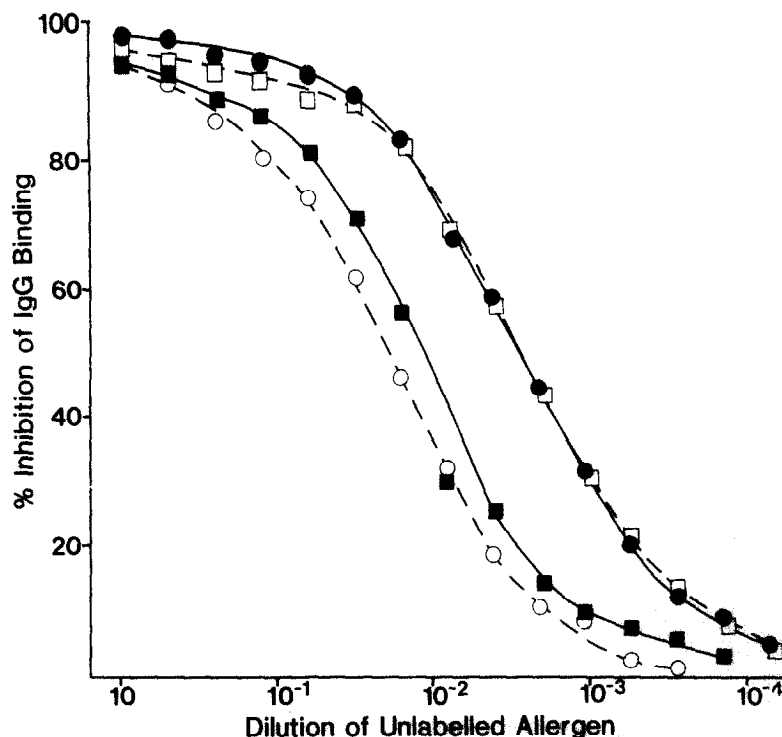


FIG. 2. Cross-inhibition between two allergens purified from rat urine. The binding of human IgG antibody for each allergen radiolabeled with ^{125}I was inhibited by the same or the other allergen. Radiolabeled α_2 -euglobulin inhibited by unlabeled α_2 -euglobulin ($\bullet$ — $\bullet$) or prealbumin ($\circ$ — $\circ$); radiolabeled prealbumin inhibited by unlabeled prealbumin ($\blacksquare$ — $\blacksquare$) or α_2 -euglobulin ($\square$ — $\square$).

The protocol for the antigen-binding assay was similar to protocols used previously for grass-pollen, dust mite, and mouse urinary allergens.^{14, 16, 18} Briefly, dilutions of sera were incubated with ~ 7 ng of ^{125}I -labeled rat allergen for 4 hours, carrier IgG or IgE protein, and then goat anti-IgG or goat anti-IgE was added to precipitate all the immunoglobulin of that isotype. Myeloma serum containing ~ 20 mg of the relevant isotype was used diluted 1:100 as a source of carrier protein. After overnight incubation in the cold, the precipitates were washed three times and counted in a four-channel gamma counter (Micromedic 4/200, Micromedic Systems, Inc., Harsham, Pa.) (Fig. 1). In preliminary assays, sera from 14 rat-allergic patients and three control subjects were assayed at two dilutions against both rat allergens. One serum (M. H.) that demonstrated good IgG and IgE binding for both proteins was chosen as a control serum and arbitrarily allotted 2500 units of IgG antibody to rat-allergen α_2 -euglobulin fraction. For quantitation serial, two-fold dilutions of that serum were assayed for IgG antibody in parallel with two dilutions of each test serum and results were interpolated from the control curve.¹⁶ Repeated assays on 17 sera demonstrated between-assay variability of $\pm 17\%$ for IgG antibody. Both IgG and IgE antibody results were interpolated from the same IgG antibody control curve, and serial dilutions of a serum demonstrating high levels of IgE antibody elicited a curve parallel to that for IgG antibody. In the present studies, two techniques were used to minimize

the interference of IgG antibody with quantitation of IgE antibody. First, sera were diluted to reduce the quantity of IgG antibody. Assaying antibodies in diluted sera was made possible by reducing the nonspecific radioactivity to a minimum by use of high levels of protein in the diluent buffer (1.0% bovine serum albumin) and by transferring washed precipitates to clean tubes.¹⁶ Second, sera were fractionated with SAS, and IgE antibody was assayed in the IgE-rich 33% to 50% SAS fraction.¹⁶ In order to ensure that results for IgE antibody were not influenced by the inhibitory effects of IgG antibody, either sera or SAS fractions were diluted so that $\leq 25\%$ of total precipitable radiolabeled was bound by the two classes.¹⁶ Overall, between-assay variability for IgE antibody measurements, including assays on SAS fractions, was $\pm 21\%$. Values were regarded as positive when they elicited counts > 2 SDs above the mean for six negative sera run in parallel with each assay.

For inhibition studies, the IgG binding of serum J. H. against both allergens was inhibited by serial dilutions of each allergen unlabeled. In each case, a 1:12.5 dilution of the serum was incubated with the inhibitor for 2 hours before adding radiolabeled allergen for 4 hours. Finally, goat anti-IgG Fc was added, and precipitates were allowed to form overnight and washed as described above. Each unlabeled antigen was diluted to approximately 5 $\mu\text{g}/\text{ml}$ before starting serial dilutions.

Antisera used for the assays were prepared as described

TABLE I. Antibodies to rat urine in symptomatic and asymptomatic laboratory workers

Symptoms*	Apparent cause	Positive skin tests (to rat urine) (%)		IgG Ab		Mean fort positives (U/ml)	IgE Ab		Mean fort positives (U/ml)
				Incidence	(%)		Incidence	(%)	
Asthma and rhinitis	Rats‡	13/18	(72)	15/18	(83)	2076	12/18	(66)	165
	Any animal	15/21	(71)	16/21	(76)	—	12/21	(57)	—
Rhinitis alone	Rats	6/12	(50)	8/12	(66)	7568	3/12	(25)	42
	Any animal	6/23	(26)	11/23	(48)	—	3/23	(13)	—
No symptoms									
Exposed§	—	1/135	(0.7)	41/135	(30)	271	2/135¶	(1.5)	98
Unexposed§	—	0/34	(0)	6/34	(17)	72	0/34	(0)	—

*Symptoms reported by individual workers and their assessment of the animals causing symptoms.

†Geometric mean of specific antibodies to rat urinary allergen values in units per milliliter. IgG values for asymptomatic individuals were significantly lower than for rhinitis and asthma groups ($p < 0.001$).

‡Symptoms were classified under rat if rats were the only animals reported or rats were included with others; any animal includes all individuals reporting symptoms on exposure to laboratory animals.

§Exposed includes handlers as well as light and heavy users; unexposed includes technical, secretarial, and administrative workers with minimal or no exposure to animals i.e., less than 2 hours per year.

||Incidence significantly different from rat asthma group ($p < 0.01$) and rat rhinitis group ($p < 0.05$).

¶Incidence significantly different from rat asthma group ($p < 0.001$) but not significantly different from rat rhinitis group.

previously.^{14,16} Goats were immunized repeatedly with either the Fc fragment of human IgG or the Fc fragment of human myeloma IgE. IgE myeloma serum was kindly provided by Dr. K. Ishizaka, Johns Hopkins University. In each case, goat antisera were absorbed and tested by immunodiffusion against purified proteins. Antisera were judged to be mono-specific by the presence of a single line against the immunizing antigen and no lines against the opposite antigen, purified IgM, or purified IgA.

Statistics

The incidence of serum antibodies and skin tests in groups of individuals studied was compared with the chi-squared test with Yates correction for small numbers. The quantities of IgG antibody in positive sera from different groups were compared by Student's unpaired *t* test.

RESULTS

Radioimmunoassay for IgG and IgE ab to rat urinary allergen

By use of a radioimmunoprecipitation technique and radiolabeled rat urinary proteins, we assayed sera from 14 rat-allergic patients and three control subjects and found closely comparable IgG and IgE binding activity for both the α_2 -euglobulin and the prealbumin. The similarity of binding of the two proteins suggested that they must cross-react extensively.²² In order to compare the reactivity of human IgG antibody for these two proteins, we carried out cross-inhibition experiments. The results demonstrate that the two allergens completely cross-inhibited each other and that the inhibition curves for autologous and heterologous protein were closely parallel (Fig. 2). In all further experiments, ¹²⁵I-labeled α_2 -euglobulin fraction was

used to measure serum antibodies and is referred to as rat allergen (Fig. 1).

Relationship between skin tests, serum antibodies to rat urinary proteins, and symptoms on exposure to rats

There was a highly significant correlation between positive skin tests to rat urine and reported symptoms (asthma and/or rhinitis) on exposure to rats. Thus, 19 of 30 symptomatic individuals were positive, whereas only two of 135 asymptomatic individuals had positive skin tests ($p < 0.001$) (Table I). Comparable figures for serum IgE ab to rat urinary protein were 16 of 30 and two of 135 for symptomatic and asymptomatic individuals, respectively ($p < 0.001$) (Table I). There was also a close relationship between symptoms and IgG ab in that 20/20 skin test positive symptomatic individuals had serum IgG ab to rat urinary protein (Fig. 3). Many asymptomatic individuals (30%) had serum IgG ab to rat allergen (Table I and Fig. 3), and the levels in sera from some of these nonallergic subjects were as high as in those with symptoms and positive skin tests (Fig. 3).

Relationship between intensity of exposure and length of exposure to laboratory animals and serum antibodies to rat urinary allergen

The study included 125 workers who used laboratory animals experimentally (users) and 54 individuals whose primary job was looking after laboratory animals (handlers). The incidence of skin tests and symptoms was only slightly higher among the han-

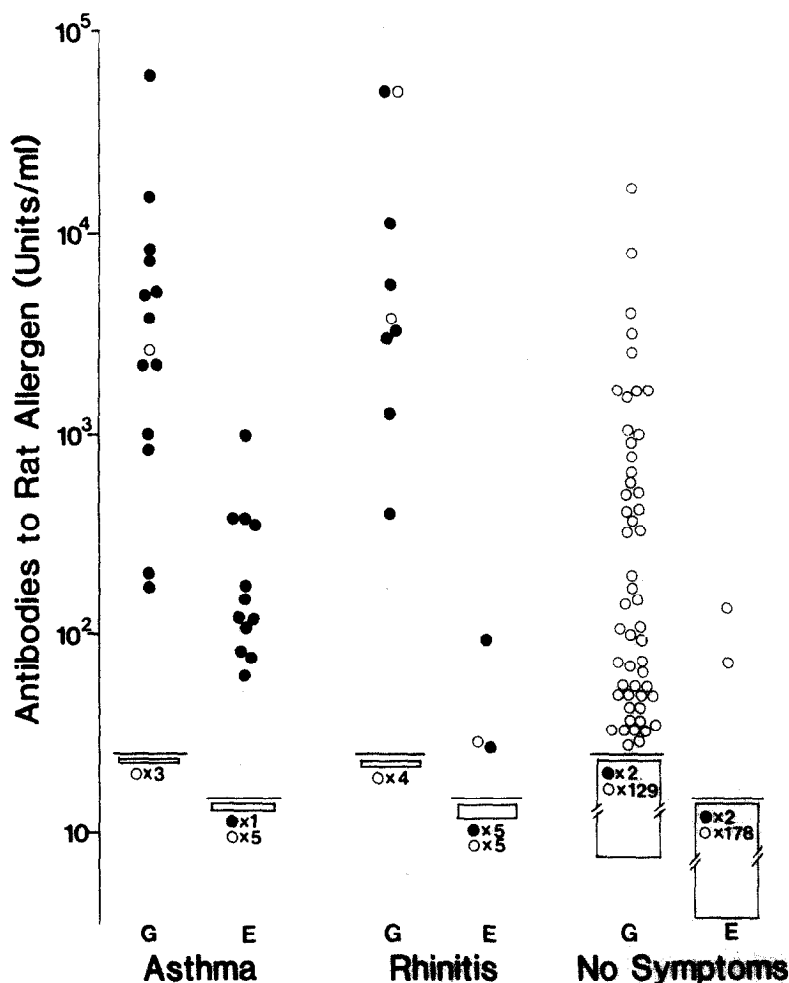


FIG. 3. Isotype-specific antibodies to rat allergen in sera from individuals reporting asthma, rhinitis, or no symptoms. IgG antibodies (G) and IgE antibodies (E) were measured by radioimmunoprecipitation, and values are presented in arbitrary units. The asymptomatic group includes the 54 sera from unexposed individuals. Individuals who had positive skin tests to rat urine are indicated by solid circles (●). The number of sera demonstrating no detectable antibodies is illustrated below the line for the lower limit of the assay.

dlers (Table II).¹ However, both the incidence and the mean quantity of IgG ab was directly related to the intensity of exposure. The incidence of IgG ab was 58% among handlers (Table II). Surprisingly, even among the "unexposed" workers, we identified six individuals with serum IgG ab to rat allergen. However, only three sera from 54 pregnant women had detectable IgG antibody, and the levels found were only twofold above the background for the assay (Table II). Dividing the laboratory workers into light users (less than 2 hours per day) and heavy users revealed further evidence that both the incidence and quantity of IgG ab to rat allergen increased progressively with the extent of exposure (Fig. 4). The incidence of serum IgE ab increased from 0% among unexposed or occasionally exposed individuals to 20% among handlers, but there was no correlation between

the degree of exposure and the quantity of IgE ab (Fig. 4).

The length of employment in a laboratory varied from 1 month to 32 years. As reported previously, the mean time before symptoms developed was approximately 2½ years. Overall, the handlers developed symptoms slightly faster than the users. Relating IgG ab to length of employment revealed a wide range of results (Fig. 5). Many individuals including handlers had worked with rats for years without developing serum IgG or IgE antibodies to rat allergen. There was a significant tendency for workers who had high levels of antibody and had worked for some years to have symptoms. Among individuals who had worked more than 3 years and had >2000 units of IgG ab, 11/12 reported symptoms and had positive skin tests, whereas among individuals who had

TABLE II. Exposure to laboratory animals and antibodies to rat urinary protein

	Zero* (%)	Unexposed† (%)	Users‡ (%)	Handlers (%)
Skin tests	ND	0/34	15/125 (12)	7/54 (13)
IgG Ab				
Incidence	3/54 (5.5)	6/34 (18)	37/125 (30)	31/54 (57.5)
Mean for positives§	42	71	332	1389
IgE Ab				
Incidence	0/54	0/34 (0)	8/125 (5.5)	9/54 (19)
Mean for positives§	—	—	181	206

*Pregnant women who had no history of laboratory work and were not skin tested.

†Includes clerical and administrative workers who had only inadvertent exposure.

‡Includes light and heavy users.

§Geometric means units per milliliter. IgG values for handlers were significantly different from unexposed ($p < 0.001$) and from users ($p < 0.002$). IgG values for users were significantly different from unexposed individuals ($p < 0.02$).

||Incidence significantly different from users ($p < 0.01$) and unexposed ($p < 0.01$) groups.

TABLE III. Prevalence of atopy among laboratory workers with or without symptoms on exposure to rats

	Rhinitis and asthma (%)	Rhinitis alone (%)	No symptoms (%)	Total (%)
Atopic by history* and skin test	8/18 (44)†	3/13 (23)‡	30/148 (20)‡	41/179 (22)
Atopic by skin test§	12/18 (66)†	5/13 (38)‡	54/148 (36)‡	71/179 (40)

*Individuals who had a history of asthma or seasonal hay fever and a positive skin test to one or more common inhalant allergen.

†Incidence significantly higher than the asymptomatic group ($p < 0.05$).

‡Not significantly different from the total group.

§Individuals who had a positive skin test to one or more common inhalant allergen with or without a history of allergic disease.

worked less than 3 years and had <2000 units of IgG ab, only 2/13 reported symptoms, and only one had positive skin tests ($p < 0.05$ for symptoms and <0.01 for skin tests). In addition, most of the animal workers who had high levels of IgG ab without symptoms had worked for less than 4 years. Of the 13 individuals who had developed ≥ 400 units of IgG antibody to rat allergen within the first 2 years of working with animals, 10 were animal handlers.

Serum antibodies to rat allergen in atopic and nonatopic individuals

The study involved both a questionnaire and skin testing with six common inhalant allergens, i.e., cat, house dust, dust mite *Dermatophagoides pteronyssinus*, grass pollen, mixed trees, and *Aspergillus fumigatus*. From these results, 22% of the laboratory workers were judged to be atopic because they had both a history of inhalant allergy and at least one positive skin test. When a positive skin test alone was taken as evidence of atopy, then 40% of workers were judged to be atopic (Table III). As reported previously, only the laboratory workers who reported asthmatic

reactions to laboratory animals had an increased incidence of atopy.¹ This was equally true of the rat-allergic individuals and also whether atopy was judged by skin tests or by history and skin tests (Table III). The quantities of IgG and IgE antibodies in atopic and nonatopic individuals who reported symptoms were very similar (Fig. 6). Equally, both the incidence and the quantity of IgG antibody in sera from asymptomatic individuals were not influenced by whether or not they were atopic (Fig. 6).

DISCUSSION

The correlation between IgE ab to rat allergen and positive skin tests to rat urine strongly supports the view that this is the major allergen of rat urine. Furthermore, the correlation with histories suggests that this is the main allergen produced by rats. Our results also suggest that the two forms of the allergen must be closely related proteins, since they cross-react almost completely. We have demonstrated here that the incidence of IgG antibodies to this protein correlates with exposure to animals. Thus, handlers (approximately 8 hours per day) have a greater incidence than

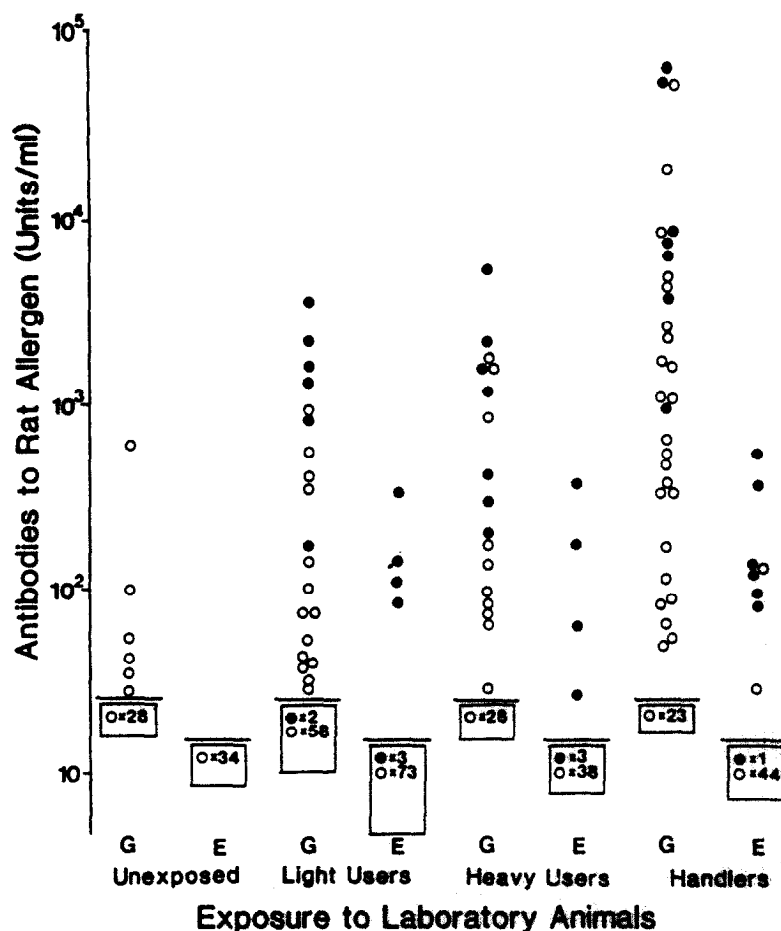


FIG. 4. IgG (G) and IgE (E) antibodies to rat allergen in sera from groups with different degrees of exposure to laboratory animals. Individuals with a positive skin test to rat urine are indicated by solid symbols (●). Exposure grades are as in Table I except that uses are divided into heavy (more than 2 hours per day) and light (less than 2 hours per day) categories.

heavy users (approximately 2 hours per day), and light users have a lower incidence still. On rather limited data, it appears that long-term exposure is not so important as the intensity of exposure. Presumably, the inhaled allergen has to reach a critical concentration on an antigen-presenting cell in the lymph nodes draining the nose.²³ In the previous study concerning these sera, it was reported there was no difference in IgG antibodies between the groups.¹ However, those results were obtained by immunodiffusion and an ELISA that required dilution of the sera 1:1000 to avoid nonspecific background.¹ As discussed elsewhere, antigen-binding technique with mouse urinary allergen elicited results very similar to those reported here,¹⁸ whereas solid-phase techniques for IgG antibody (presumably because they are not sufficiently sensitive) have failed to demonstrate significant differences between exposed and unexposed individuals.^{1, 24} The present serum antibody results are in keep-

ing with previous results on animal handlers but strikingly different from results reported for pollen or dust mite allergens.¹³⁻¹⁶ It is unusual with pollens to find nonallergic or skin test negative individuals who have any detectable IgG antibody. This has been taken as evidence that the genetic control over inhalant allergy controls both IgG and IgE ab and that the nonallergic individuals are "nonresponders."^{15, 25} In the animal workers reported here, all the IgE antibody responders were also IgG antibody responders. In addition, 30% of the individuals who reported no symptoms on exposure to rats also had IgG antibody in their serum.

There are several possible reasons why animal handlers or users should produce IgG ab to rat urinary protein, although they are not allergic. It is possible that the rat allergen is different in some way from other allergens so that it tends to induce IgG ab. However, rodent urinary allergens do not appear to have special physicochemical features.^{9, 18, 20, 21} Alterna-

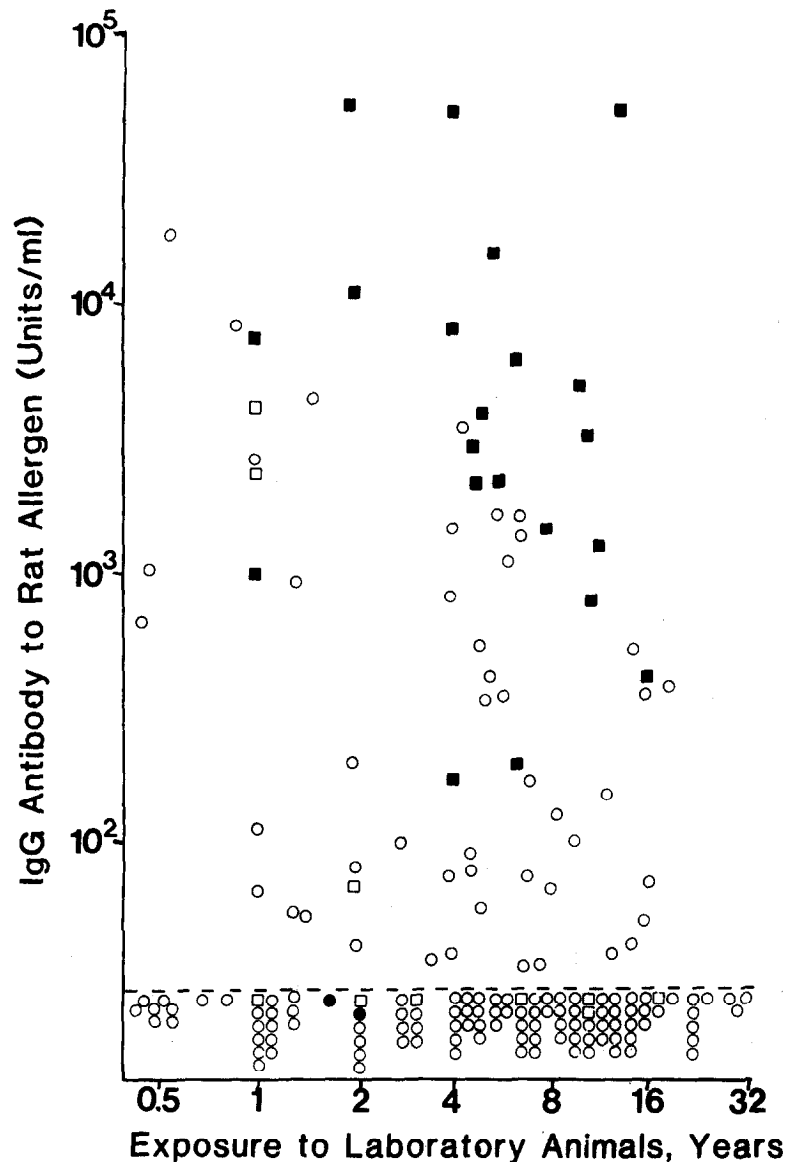


FIG. 5. Relationship between serum IgG antibodies to rat allergen and the number of years working with laboratory animals. Individuals who had reported symptoms of rhinitis and/or asthma precipitated by rats are indicated by *squares*, and asymptomatic individuals are indicated by *circles*. *Solid symbols* indicate individuals with a positive skin test to rat urine (■ and ●). Sera with <25 units of IgG antibody per milliliter are indicated below the dotted line.

tively, it is possible that exposure to rat urine or animal products in general may act as an adjuvant for the production of IgG antibody. The most likely explanation, however, is that exposure to rat urinary allergen is quantitatively greater than exposure to pollen or dust mite allergens. Indeed, recent studies on allergens in animal houses suggest that exposure is higher because the allergens are continuously present in the air; the maximum quantities in the air can rise as high as $100 \mu\text{g}/\text{m}^3$; the quantities of allergen in litter, i.e., grams, are very high and may be handled.¹⁰⁻¹² Particle size measurements suggest that

rat allergen is predominantly airborne on particles of $\sim 7 \mu\text{m}$ in diameter, which may explain both the fact that the allergen remains airborne and the fact that rat-allergic individuals often develop acute symptoms within minutes of entering a rat room. At the present, it appears most likely that the production of IgG in asymptomatic individuals represents a response to relatively "high dose" exposure in individuals who would probably fail to respond to a lower dose. It is possible that some of the individuals who had developed IgG ab at the time of this study will subsequently develop IgE ab. The results presented here suggest that the

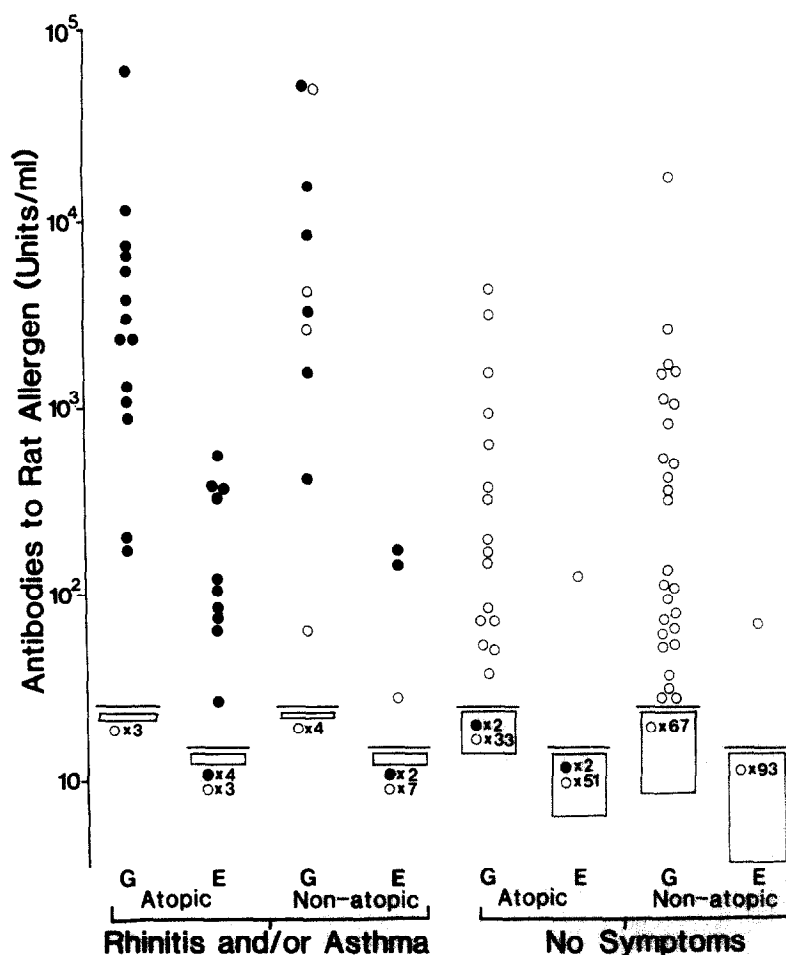


FIG. 6. Serum IgG (G) and IgE (E) antibodies to rat allergen in laboratory workers who were atopic or nonatopic. Atopy was judged by the presence of a positive skin test to at least one of five common inhalant allergens. Solid circles (●) indicate individuals with a positive skin test to rat urine.

prevalence of serum IgG ab could be used as an indirect method of assessing the exposure of groups of laboratory workers to rat allergens.

The initial result of this survey demonstrated that preexisting "atopy" had a significant impact on the development of asthma but little or no effect on other symptoms. Our present results confirm that view. Interestingly, the quantities of IgG and IgE ab found in the sera of atopic individuals were not significantly different from those found in nonatopic individuals. This suggests that atopic status may influence the initial response to an inhalant allergen but has little effect on the quantity of the antibody response. In addition, atopy did not influence the production of IgG ab in asymptomatic individuals. These observations would be in keeping with the view that atopy represents an increased tendency to recognize and respond to low doses of inhalant proteins and that the isotype of the response reflects both the low dose and prolonged

immunization.²⁵ From the initial survey it was concluded that screening applicants for laboratory work would be a very ineffective method of reducing the incidence of allergy to laboratory animals.¹ The present data on the rat-allergic individuals certainly support that view. In order to exclude 66% of workers who developed asthma on exposure to rats, it would have been necessary to reject 71 (of 179) individuals who had positive skin tests to inhalants. Rejecting the 41 individuals with positive history of inhalant allergy and positive skin tests to inhalants would only have avoided 50% of the cases of asthma and only 33% of the cases of rhinitis associated with exposure to rats. Thus, a large proportion of rat-allergic individuals were not previously "atopic" and most atopic individuals did not develop allergic symptoms, positive skin tests, or serum IgE antibodies when they worked with laboratory animals. At the present, it appears that attempts to reduce the problem of laboratory animal

allergy should be focused on the design of cages and animal houses.^{3, 6, 10-12}

In the present study, three separate observations can be correlated. First, workers were asked whether they were aware of symptoms on exposure to laboratory animals. Second, they were skin tested with animal extracts. Third, their serum was assayed for antibodies to a purified rat allergen. The most striking correlation found was between a history of asthma on exposure to rats, positive skin tests to rat urine, and serum IgE ab to rat allergen. Thus, 12 of the 18 people who reported asthma on exposure to rats had detectable IgE ab, and for those with positive skin tests as well, the figure was 12/13. By contrast, among 135 asymptomatic laboratory workers, only two had positive skin tests, and none had both positive skin tests and IgE ab. The obvious implication of these results is that IgE ab, but not IgG ab, is causally related to the development of asthmatic reactions in these individuals. In most cases asthma or rhinitis caused by rats develops within a few minutes of exposure. The timing of the asthmatic response is therefore comparable to the results of direct provocation studies with other allergens. In the present study, only three individuals had abnormal lung function at the time of the study, and bronchial reactivity was not tested. In a previous study, several patients who reported asthmatic reactions on exposure to rats did *not* have increased bronchial reactivity to methacholine.²⁶ It is well established that patients with ragweed hay fever will develop bronchospasm when they are provoked with nebulized ragweed extract, even though they do not have bronchial reactivity and have never had asthma.^{27, 28} Thus, the response of rat-allergic individuals on entering a laboratory may be comparable to the response that all or most ragweed-allergic patients have to experimental bronchial provocation.

In conclusion, our results on serum antibodies to rat allergen confirm a close relationship between immediate hypersensitivity to this allergen and asthmatic reactions in animal facilities housing rats. In addition, there was a progressive increase in the prevalence of IgG antibodies in relation to increasing intensity (i.e., hours per day) of exposure to laboratory animals. It appears likely that the high incidence of asthmatic reactions to rat allergen and of antibody response to this allergen reflects both the quantities and the form in which rat urine becomes airborne.

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Elevated urinary histamine in the hyperimmunoglobulin E and recurrent infection (Job's) syndrome: Association with eczematoid dermatitis and not with infection

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*To test the hypothesis that IgE-mediated release of histamine may be, in part, responsible for the abnormal inflammatory response observed in the hyperimmunoglobulin E (HIE) and recurrent infection syndrome, urine and plasma histamine levels were measured. Twenty-four-hour urinary histamine concentrations from 15 patients with HIE ($23.7 \pm 6.9 \mu\text{g}/24 \text{ hr}$) were significantly elevated ($p < 0.001$) compared to a large historical control population ($10.5 \pm 0.7 \mu\text{g}/24 \text{ hr}$; $n = 97$). However, urinary histamine levels in HIE were much less abnormal ($p < 0.01$) than in five patients with biopsy-proven systemic mastocytosis ($159 \pm 62 \mu\text{g}/24 \text{ hr}$) and were not significantly elevated when levels were compared to 13 concurrently studied normal subjects ($10.1 \pm 1.7 \mu\text{g}/24 \text{ hr}$) and nine patients with chronic granulomatous disease ($8.1 \pm 1.2 \mu\text{g}/24 \text{ hr}$). Overall, there was no clear relationship between urine histamine values and the presence of infection as well as no significant correlation between urine histamine and total IgE or anti-Staphylococcus aureus IgE. However, urine histamine levels in a subgroup of six patients with HIE with chronic eczematoid dermatitis ($42.4 \pm 12.5 \mu\text{g}/24 \text{ hr}$) were elevated compared with values from the historical control subjects ($p < 0.001$), the concurrent control subjects ($p < 0.01$), the patients with chronic granulomatous disease ($p < 0.01$), and five patients with HIE who did not have skin manifestations ($4.6 \pm 1.1 \mu\text{g}/24 \text{ hr}$; $p < 0.05$). In related studies, plasma histamine levels were normal ($<500 \text{ pg/ml}$) in 19 samples from eight patients with HIE. Thus, histamine is moderately increased in the urine of patients with HIE and is not increased in the plasma. Elevated urine histamine excretion in HIE appears to be related to the presence of eczematoid dermatitis and not to the presence of infection. Therefore, it appears unlikely that abnormal histamine release plays an important role in the pathophysiology of recurrent infections in patients with HIE. (*J ALLERGY CLIN IMMUNOL* 1987;79:515-22.)*

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The hyperimmunoglobulin E (HIE, Job's) and recurrent infection syndrome is a complex disorder characterized by onset early in life, markedly elevated serum IgE, eczematoid dermatitis, and serious recurrent bacterial infections of the skin and sinopulmonary tract.^{1, 2} The presence of circulating IgE directed against *S. aureus* has been demonstrated^{3, 4} and has