

The relationship between exposure to rats and antibody production in man: IgG antibody levels to rat urinary protein

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Summary

An ELISA procedure has been developed to measure specific IgG antibodies against rat urine. Using this method antibody levels in individuals with differing exposure levels to rats have been measured. Mean antibody titres in groups of workers regularly exposed to rats were higher than those in groups currently or totally non-exposed. However, only in the group of workers exposed and sensitized to rats were IgG levels significantly elevated. Despite this significant difference in mean antibody titres there was considerable overlap between individuals in each group, and there was no direct relationship between exposure to rats and levels of IgG anti-rat urine antibody. Our results suggest a stronger correlation between sensitization to rats and the levels of antigen-specific IgG antibodies.

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Introduction

It has been suggested that although atopic status may be of value in predicting those individuals who are at risk of developing asthma as a symptom of allergy to laboratory animals (ALA), it is of little value in predicting the development of the most common symptoms of the disease such as rhinitis, conjunctivitis and urticaria [1]. However, atopics do appear to develop the common symptoms more quickly than non-atopics, and consequently they may be most susceptible to ALA during their first year of occupational exposure [2]. During subsequent years this susceptibility is not maintained and many atopics do not develop the disease. In this prospective study [2] no other factor was identified as being of value in predicting those individuals at risk of developing ALA. However, it was noted that following the introduction of an education programme and a code of practice for working with animals, designed to increase awareness of the problem and to reduce exposure to laboratory animal allergens, the overall incidence of ALA fell from 37% to between 10% and 12% [2]. However, monitoring of

airborne allergens was not performed and a relationship between reduced exposure and a reduced incidence of symptoms could only be presumed. An attempt has now been made to determine whether it would be possible to address this problem retrospectively by studying the relationship between the levels of IgG antibodies to animal-derived allergens in serum and the development of allergy.

The relationship between exposure to laboratory animal allergens and the development of IgG antibodies against the allergens is unclear. Cockcroft *et al.* [3] failed to detect IgG antibodies to allergens from any of five animal species in exposed workers, whereas Beeson *et al.* [4] reported that 50% of exposed individuals had detectable IgG antibodies to mouse, rat, guinea-pig and rabbit urines; this rose to 80% in exposed workers reporting symptoms of allergy. Similar results to those of Beeson *et al.* [4] have been reported by Lee *et al.* [5]. However, Schumacher *et al.* [6] and Platts-Mills *et al.* [7] have suggested that the levels of IgG antibodies to mice and rat allergens are related more to exposure than to the development of allergy, and consequently they believe that this serological parameter can be used as a measure of exposure to animal-derived allergens.

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We have developed an ELISA procedure to detect IgG antibodies against rat urinary protein. This report describes the relationship between IgG anti-rat urine antibodies, occupational exposure to rats and the development of IgE antibodies against rat urinary protein as a measure of allergic sensitization.

Subjects and methods

Preparation of rat urine allergen

Rat urine allergen was prepared using a modification of the method described by Davies *et al.* [8]. Briefly, rat urine from male rats aged 3–4 months was collected and dialysed against six changes of water. The resulting solution was then freeze-dried. This allergen was used in the assays to estimate levels of both IgE and IgG antibodies against rat urine in human sera.

Estimation of serum IgE antibodies

The modified RAST method of Ceska *et al.* [9] described by Davies *et al.* [10] was used.

Estimation of serum IgG antibodies

Ninety-six well microtitre plates (Immunoplate—Maxisorp, Nunc, Denmark) were coated with the optimal coating density (10 µg/ml (100 µl/well)) of rat urine allergen in bicarbonate buffer (pH 9.6) overnight at 4°C. After washing three times with phosphate-buffered saline (PBS) containing 0.05% Tween 20 (Sigma, Dorset, U.K.), triplicate samples of an optimal dilution of test sera (1/40) in PBS containing 0.05% Tween 20 and 0.1% gelatin were added (100 µl/well) and incubated for 2 hr at room temperature. Plates were then washed with PBS containing 0.05% Tween 20, and peroxidase-labelled goat anti-human IgG (H+L) (Sera Labs, Crawley Down, U.K.) was added at a dilution of 1/3250 in PBS containing 0.05% Tween 20 and 0.1% gelatin (100 µl/well). The plates were then incubated for 2 hr at room temperature. After washing, substrate (160 mg *o*-phenylene diamine in 100 ml citrate-phosphate buffer, pH 5.0, together with 20 mg of urea hydrogen peroxide) was added. After 20 min at room temperature the reaction was stopped with 0.5 M citric acid (50 µl/well) and optical densities (OD) read at 450 nm on a Titertec Multiskan plate reader (Flow Laboratories, Rickmansworth, U.K.).

Absorption of specific antibodies

Samples of each serum (30 µl) were incubated for 3 hr at room temperature and then overnight at 4°C with rat urine allergen coupled to a cellulose disc, as used in the

RAST assay. Two discs were sufficient to remove all specific IgG antibodies. Absorbed sera were then assayed by ELISA. The OD of an absorbed serum sample in the ELISA was subtracted from that of the unabsorbed sample to give a specific antibody level.

Subjects

Subjects were divided into four groups on the basis of exposure and symptoms.

Group 1. Consisted of individuals ($n=17$) employed at a chemical factory (ICI Colours and Fine Chemicals, Huddersfield, U.K.). They had never been exposed to rats occupationally.

Group 2. Consisted of individuals ($n=12$) employed at the ICI Central Toxicology Laboratory, Alderley Park, U.K. This group had not been exposed to rats occupationally for at least 3 yr.

Group 3. Consisted of individuals ($n=17$) employed either at the ICI Central Toxicology Laboratory or ICI Pharmaceuticals, Alderley Park, U.K. This group were currently exposed to rats for at least 3 days per week and had been working with rats for at least 1 yr.

Group 4. Consisted of individuals ($n=16$) employed either at the ICI Central Toxicology Laboratory or ICI Pharmaceuticals, Alderley Park, U.K. This group had the same exposure pattern as Group 3 and were classified as sensitized to rat urinary protein.

The classification of sensitization to rat urine allergen was based on a positive skin test to the allergen of at least 4 mm diameter and a binding of [^{125} I] anti-human IgE in

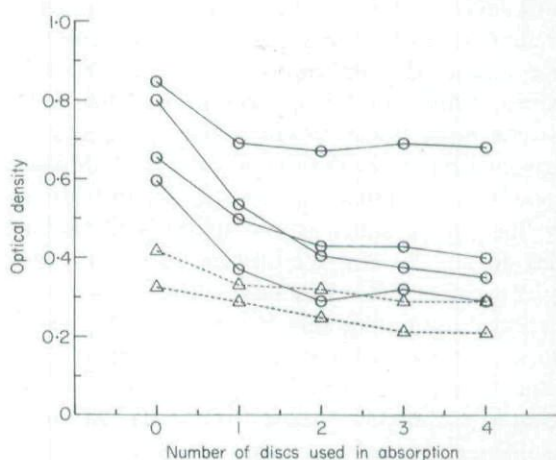


Fig. 1. Representative results of an experiment designed to assess the number of antigen-coated cellulose discs required to remove all specific IgG antibody from a 30-µl serum sample. (○) samples from Group 4 (exposed and sensitized), and (△) samples from Group 1 (unexposed).

the RAST assay of at least 4%. Only one individual in the sensitized group had not reported symptoms to the allergen.

Results

The detection of specific IgG antibodies against rat urinary protein in human sera using a standard direct ELISA assay was compromised by the finding of significant non-specific binding of the human sera to the polystyrene plates (data not shown). This made discrimi-

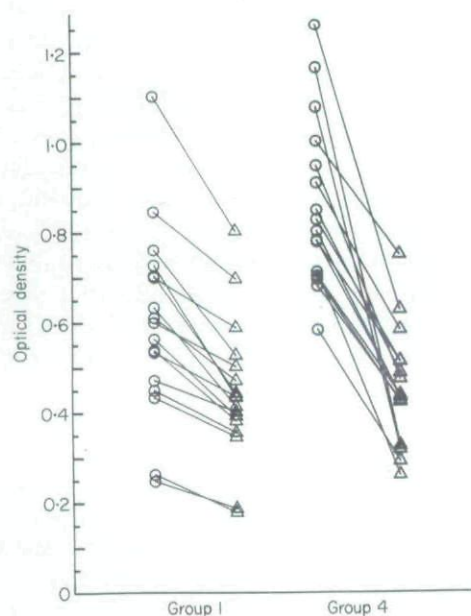


Fig. 2. Optical density reading for each serum sample in Group 1 (unexposed) and Group 4 (exposed and sensitized) before and after absorption with two antigen-coated cellulose discs. (○) unabsorbed sera, and (Δ) absorbed sera.

nation of specific antibody levels against rat urinary protein impossible. To overcome this problem a method was devised involving the incubation of 30 μ l aliquots of human serum with rat urine-coated cellulose discs. Figure 1 shows the result of incubating aliquots of serum with varying numbers of discs for six representative serum samples. Two discs were found to be sufficient to remove specific antibody against rat urinary protein and all subsequent assays followed this method. Specific antibody activity was calculated by subtracting the optical density values obtained using pre-incubated samples from those obtained using un-incubated sera. Incubation of serum samples with cellulose discs coated with an irrelevant antigen (a trimellitic anhydride-human serum albumin conjugate) failed to remove antibody activity in all cases (data not shown).

Figure 2 shows the difference in OD values obtained from absorbed and unabsorbed serum samples from individuals who had never been exposed to rats occupationally (Group 1) and from individuals who have been exposed and sensitized to rats (Group 4). The reduction in OD values of individual serum samples after absorption is greater in Group 4, indicating that there are higher specific antibody titres in this group. The reason for the variability in non-specific binding of antibodies to the coated ELISA plates is unclear. It is possible, however, that it reflects individual variations in the concentration of total serum IgG.

Table 1 shows the levels of specific serum IgG antibody against rat urinary protein in four exposure groups. No significant difference in antibody levels was found between those individuals who had either never (Group 1) or not recently (Group 2) been exposed to rats occupationally and those who were currently exposed but not sensitized (Group 3). However, significant differences were found between the exposed and sensitized group (Group 4) and the other three groups.

Table 1. Mean IgG levels to rat urinary protein (OD units) in four exposure groups

	Group 1, never exposed (not sensitized) (<i>n</i> = 17)	Group 2, currently exposed (not sensitized) (<i>n</i> = 12)	Group 3 (not sensitized) (<i>n</i> = 17)	Group 4 (sensitized) (<i>n</i> = 16)
Mean	0.096	0.094	0.148	0.308
SD	± 0.06	± 0.04	± 0.09	± 0.18

Group 1 vs. Group 4: $P < 0.001$.

Group 2 vs. Group 4: $P < 0.001$.

Group 3 vs. Group 4: $P < 0.01$; Wilcoxon two-sample test.

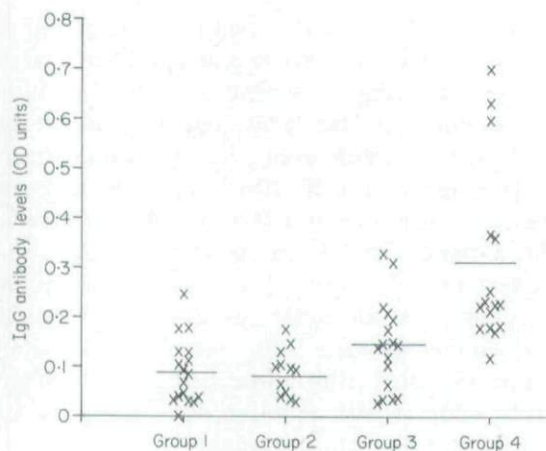


Fig. 3. IgG anti-rat urine levels for each individual within the four exposure groups. Group 1—never exposed to rats ($n=17$); Group 2—currently not exposed to rats ($n=12$); Group 3—exposed to rats but not sensitized to rat urine ($n=17$); and Group 4—exposed to rats and sensitized to rat urine ($n=16$). (—) mean value for each group.

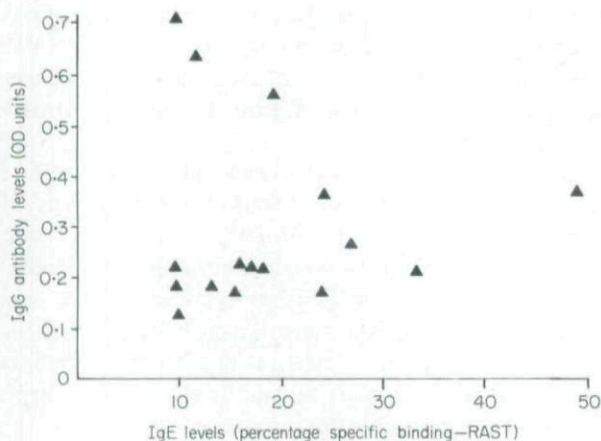


Fig. 4. The correlation between IgG anti-rat urine antibody levels, as measured by ELISA, and IgE anti-rat urine antibody levels, as measured by RAST, in the group of exposed and sensitized individuals (Group 4). Correlation co-efficient = -0.025 .

Figure 3 shows the distribution of individual IgG levels within the different populations. Despite the difference in mean values there is considerable overlap between individual antibody levels when Groups 1, 2 and 3 are compared with the sensitized group (Group 4). This variability in IgG values within the sensitized group does not appear to be related to measurable levels of circulating IgE antibody (Fig. 4; $r = -0.025$).

Discussion

A reliable ELISA procedure for measuring specific serum IgG antibody levels against rat urinary protein has been developed. The method requires the assay of sera both before and after absorption against paper discs coated with rat urinary protein. Viander *et al.* [11] have also shown that absorbing sera from birch pollen and dog-allergic patients with antigen coated cellulose discs could selectively remove antibodies to either dog or birch antigens without affecting unrelated antibodies.

Doubts have been raised about the suitability of using ELISA systems which rely on the direct binding of a mixed protein extract to polystyrene plates [7,11]. It has been argued that differential binding may occur, producing incomplete antibody profiles. To avoid this, either a capture antibody [11,12] or a purified antigen could be used. Rat urine itself is a complex antigen in which two major allergenic components, urinary pre-albumin and $\alpha 2$ -euglobulin (now classified as Rat_nI and Rat_nII [13]) have been identified [14]. Platts-Mills *et al.* [7] reported that these two allergens completely cross-inhibited each other in experiments performed on IgG positive sera and thus it could be argued that the use of either of these two proteins would give an indication of antibody levels to both allergens. However, non-allergenic proteins are also present in a rat urine extract and it is likely that IgG antibodies will also be produced to these proteins. Thus, in our studies, to assess total IgG antibody production a whole urine extract was preferred.

The results of this study show that there is a statistically significant difference between the levels of IgG anti-rat urine antibodies in groups of individuals who are regularly exposed to rats but not sensitized to them (Group 3), and those who are exposed and sensitized (Group 4) ($P < 0.01$, Wilcoxon two-sample test). In Groups 3 and 4 all subjects had been exposed regularly to rats for at least 1 yr. A more significant difference in antibody titres was seen when the unexposed groups (1 and 2) were compared with Group 4 ($P < 0.001$, Wilcoxon two-sample test). However, no significant difference in antibody titres was found between the unexposed groups (1 and 2) and the group of exposed non-sensitized individuals (Group 3).

Although these differences are clear when populations are considered, Fig. 3 shows that there is considerable overlap between individual values in each group. Thus, in contrast to Platts-Mills *et al.* [7] and Schumacher *et al.* [6] we believe that levels of specific IgG antibody against rat urinary protein do not reliably reflect exposure. In agreement with Price and Longbottom [15] we believe that IgG levels are higher only in certain individuals within a population sensitized to rat urine. The reason for the significantly higher IgG antibody titres seen in three

individuals in Group 4 is unclear. There were no differences in the clinical symptoms of these individuals and the higher levels of IgG were not associated with higher levels of IgE. However, it is possible that the pattern of exposure to rats may influence the extent of specific IgG antibody production to rat urine allergen and this is now being addressed in a prospective survey of ALA.

Lee *et al.* [5] have suggested that increased uptake of allergen, made possible by damage to the nasal mucosa in individuals with rhinitis, could account for the elevated production of IgG antibodies. It is also possible that the production of both IgG and IgE antibodies against rat urinary protein may reflect a genetically determined high responder status to the allergen in certain individuals. Consequently, we are currently assessing the development of IgG antibodies against a number of animal allergens from four species (rats, mice, guinea-pigs and rabbits) in a prospective study of ALA, to see whether the development of allergen-specific IgG antibodies in exposed individuals may precede that of IgE antibodies. IgG antibody levels will be measured in individuals at 3-monthly intervals over a 3-year period. This may allow an early warning of sensitization and the possibility of an individual taking additional precautions to minimize or even avoid exposure to one or more laboratory animals.

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