

Analysis of the allergenic composition of rat dust

S. GORDON, R. D. TEE and A. J. NEWMAN TAYLOR

Department of Occupational and Environmental Medicine, National Heart and Lung Institute, Imperial College, London, UK

Summary

Background Allergy to rats is an important occupational health problem. The allergens of rat urine have been well defined but those in rat room dust, a potentially important source of inhalant exposure, have not.

Objective To describe the allergens present in rat room dust and to identify a suitable marker protein which may be used to quantify airborne rat allergen.

Methods Dust collected from the air-conditioning system (bulk dust, 'bd') and with an air sampler (airborne dust, 'ad') were analysed by radioallergosorbent test (RAST) inhibition, immunoblotting and immunoblot inhibition techniques and comparisons made with hair and urine extracts prepared from adult male Wistar rats.

Results Extensive crossreactivity was found between the extracts by RAST inhibition under different experimental conditions. Dust was more potent as an inhibitor than other extracts. The immunoblotting patterns of both dusts were similar although 'ad' contained an allergen at 29 kDa not found in 'bd'. Forty-two sera from rat allergic subjects were used to identify 18 allergens in 'bd'. Three 'major' allergens were found; 100% of subjects had immunoglobulin (Ig)E to a 44 kDa allergen and 74% and 88% of subjects had IgE with bound to the 20.5 and 17 kDa allergens respectively. Immunoblot inhibition experiments identified the 17 kDa dust allergen as α_{2u} -globulin (Rat nI).

Conclusions Rat dust is a complex allergenic source. The 17 kDa dust allergen has immunological identity with Rat nI and is a suitable marker protein for the quantitation of airborne rat allergen.

Keywords: rat dust allergens, laboratory animal allergy

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Introduction

Allergy to rats is an important occupational health problem which may affect up to one-third of those working directly with rats [1]. Although urine is recognized as an important source of the allergens involved [2,3] and is commonly used for diagnostic purposes, the airborne dust present in rat housing rooms is a potential source of allergenic material. The low levels of dust in rat rooms may originate directly from the animals (e.g. skin scales and hairs [4]) or be generated via animal hus-

bandry procedures or animal activity (e.g. litter particles contaminated with urine). The study of rat room dust allergens is therefore important to determine the relationship of the dust allergens to those from other sources (such as urine and pelt). We have recently reported the measurement of occupational exposure to rats using a rat urinary allergen standard [5]. The analysis of the allergens present in rat dust will assist the development of improved assays to quantify airborne rat allergen.

There have been two studies examining the antigenic and allergenic composition of rat dust which have used crossed-radioimmunoelectrophoresis as the main analytical technique [6,7]. Three main dust constituents were described by Walls and Longbottom which had electrophoretic properties similar to that of albumin,

Correspondence: Dr S. Gordon, Department of Occupational and Environmental Medicine, National Heart and Lung Institute, Imperial College, Manresa Road, London SW3 6LR, UK.

prealbumin and α_{2u} -globulin [6]. An additional study by Longbottom and Austwick identified two main dust components, one with immunological similarity to prealbumin and one of very high molecular weight when assessed by gel filtration chromatography [7]. Both studies have analysed dust allergens using sera from a relatively small number of rat allergic subjects.

The aims of the current investigation were to assess the immunological relationship between dust collected from the air conditioning system of a rat room with that collected with air samplers, use immunoblotting to describe the allergens present in rat dust using sera from large numbers of rat allergic individuals and identify a suitable marker protein which may be used to quantify airborne rat allergen.

Materials and methods

Antigen preparation

Dust was collected in bulk from the exit grille of the ventilation system of a room housing only rats. The rat room was at positive pressure to the corridor. The grille was cleaned monthly. Airborne dust was additionally collected onto polytetrafluoroethylene filters using a high volume air sampler (Air Sentinel, Quan-Tec-Air Inc., Rochester, Minnesota, USA; 150 L/min) in the same room. Soluble antigen was extracted from the filters overnight at 4°C in 0.1 M ammonium bicarbonate (pH 7.0), and the supernatant filtered (0.22 µm; Sartorius Instruments Ltd, Epsom, Surrey, UK), dialysed against distilled water and lyophilized. Urine collected with a metabolism cage was prepared from adult male Wistar rats as described previously [3]. Hair extract was prepared from the unwashed pelts of 1-year-old male Wistar rats. The pelts were placed on a metal tray over a freezing mixture of solid CO₂ (BOC London, UK) and methanol

(BDH Laboratory Supplies, Merck Ltd, Lutterworth, UK) according to the method of Siraganian and Sandberg [8]. Long hairs were removed with scissors close to the skin and extracted overnight at 4°C in 0.1 M ammonium bicarbonate with vigorous shaking. The extract was filtered, dialysed and lyophilized as described above.

The protein concentration of all the extracts was determined by the dye binding method of Bradford [9] (protein assay kit; Bio-Rad Laboratories Ltd, Hemel Hempstead, UK) by comparison with an albumin standard. The mean protein content of each extract was: urine 32.9%, hair 22.9%, bulk dust 13.1% and airborne dust 1.2%. The protein content of the α_{2u} -globulin (Rat nI purified from male rat urine, donated by Dr J. L. Longbottom) was assumed to be 100%.

Sera

Blood was obtained by venepuncture from rat allergic subjects attending occupational allergy clinics and the serum separated after centrifugation, and stored at -20°C in sealed containers until used. All sera were screened by radioallergosorbent test (RAST) for specific IgE antibodies to the rat extracts but not to other animals or environmental allergens. Five pools of sera were established which differed in the relative amount of specific IgE to rat extracts (Table 1).

RAST and RAST inhibition

Both the RAST and RAST inhibition procedures have been described in detail elsewhere [3,5]. Briefly, cyanogen bromide activated paper discs were coupled with rat extracts or, for house dust mite only, were purchased from Pharmacia Ltd (Milton Keynes, UK). Serum (50 µL) was incubated for 16 h with each disc. Bound IgE was detected by further overnight incubation with ¹²⁵I

Table 1. Summary of sera used for serum pools. All measurements were made on undiluted serum. All serum included in each pool contained specific IgE greater or equal to that of the RAST class shown. The GM of the per cent RAST isotope binding is also shown in parentheses.

Pool	'Specificity'	n	RAST class			
			Urine	Hair	'bd' dust	HDM
I	All	8	≥ 2 (29.5)	≥ 2 (29.7)	≥ 2 (30.8)	2* (13.9)*
II	All	5	4 (42.1)	≥ 3 (38.1)	4 (37.2)	2* (7.7)*
III	Urine/dust	4	≥ 2 (21.0)	≥ 1 (7.8)	≥ 2 (14.3)	2* (11.0)*
IV	Hair/dust	5	≥ 2 (11.8)	≥ 2 (21.7)	≥ 2 (19.6)	2* (12.8)*
V	HDM	4	0* (1.8)*	1* (3.1)*	0* (1.8)*	≥ 3 (30.0)

n. Number of serum combined in equal volumes to make pool. *Measurement made on pooled serum.

labelled anti-human IgE (antiserum raised in rabbits and purified by immunosorption techniques, Pharmacia Ltd). Phadebas reference reagents (Pharmacia Ltd) were run in parallel to determine RAST class.

The RAST inhibition procedure was similar to that described above except that the serum pool (50 μ L) was preincubated with 50 μ L of the crossreacting antigens (10-fold dilution series). All dilutions of serum and antigens were made in PBS diluent (0.1 M phosphate buffered saline [PBS] containing 0.3% w/v human serum albumin and 0.1% w/v sodium azide).

Statistical analysis

Where comparisons were to be made between the RAST inhibition curves formed by the antigen dilution series, the % inhibition data for each curves was logit transformed and plotted against $\log_e$ concentration to form a straight line. The regression line in each case was constructed from at least six points. Significant differences in the slopes of the lines were then tested for by analysis of covariance (ANCOVA). The significance of the test was then obtained from *F*-tables.

Immunoblotting

As the technique has been fully described [3], the immunoblotting procedure will be described in brief. The proteins were separated by sodium dodecyl-polyacrylamide gel electrophoresis (SDS-PAGE) in 15% acrylamide gels under non-reduced conditions using the buffer system of Laemmli [10]. Marker proteins of low molecular weight (Pharmacia Biotechnology) were electrophoresed in parallel. The proteins were transferred electrophoretically to nitrocellulose membranes and the immunoblots blocked with TRIS washing buffer containing 0.1% v/v Tween 20 before sequential overnight incubations with serum and ^{125} I-anti-IgE (Pharmacia Ltd) with thorough washing between each step. The washed and dried immunoblots were exposed to x-ray film at -70°C for 140, 60 and 24 h.

The molecular weight of the radiostaining bands (allergens) were determined manually as described [3] and the relative radiostaining was graded as follows; band visible after 140 h = 1, band visible after 60 h = 2, band visible after 24 h = 3, medium staining after 24 h = 4 and strong staining after 24 h = 5. For the purposes of this study the allergens were classified in accordance with the criteria of Lind and Lowenstein [11]; 'major' allergens were those recognized by more than 50% of subjects of whom 25% or more had a strong reaction ($\geq$ grade 3), 'intermediate' allergens were classified as those to whom

more than 10% of subjects had strong staining ($\geq$ grade 3) and all other allergens were classified as 'minor' allergens.

In the immunoblot inhibition experiments, bulk dust was electrophoresed and immunoblotted as before but any remaining sites on the nitrocellulose were blocked with TRIS washing buffer containing 5% w/v MarvelTM prior to incubation with the inhibited serum. Serum pool I (1 mL, diluted 1:4) was inhibited with increasing concentrations of allergen (1 mL) for 1 h prior to overnight incubation with the dust strip. The strips were then washed, labelled and exposed to x-ray film as before.

Results

RAST inhibition

RAST inhibition was used to assess initially the general crossreactivity of the rat derived extracts. Pooled sera containing IgE antibodies against rat hair, urine and dust was inhibited with urine and both dust extracts ('ad' and 'bd') and the non-absorbed antibodies measured by rat urine RAST (Fig. 1a). Both sources of dust shared common or crossreacting epitopes with urine as shown by the parallel inhibition curves (ANCOVA, $P = 0.10$). When expressed by protein concentration, both dust extracts were fivefold more potent than urine at inhibiting the urine RAST. As both 'ad' and 'bd' appeared to have similar inhibitory activity, only 'bd' was used in subsequent RAST inhibition experiments.

Further experiments were conducted to evaluate the apparent crossreactivity of dust and urine with the rat hair antigen. Bulk dust, urine and hair caused dose-dependent inhibition of both the urine and hair RAST systems (Fig. 1b and c) and no significant differences in the slopes were observed (ANCOVA, $P = 0.81$ and 0.60 respectively). Dust was more potent than either of the 'self' inhibitors; fivefold more potent than urine in the urine RAST system and threefold more potent than hair in the hair RAST system. The difference in potency between each 'self' inhibitor and either the urine or the hair was 2.4-fold.

Rat urine RAST inhibition experiments with serum pool III (selected to contain IgE predominantly to urine) and hair RAST inhibition experiments with serum pool IV (selected to contain IgE predominantly to hair) did not reveal any significant differences in the slopes of the inhibition curves obtained between urine, hair and bulk dust extracts (data not shown).

Immunoblotting analysis

Effect of mode of dust collection on allergen composition
Immunoblotting was used to compare further the

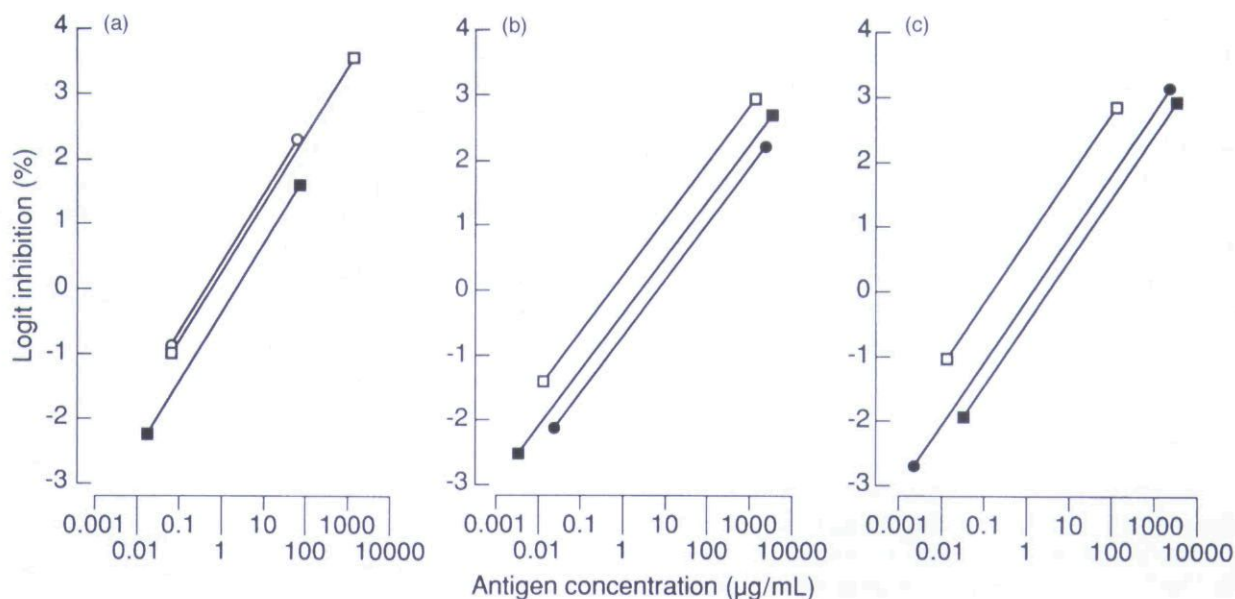


Fig. 1. Analysis of the crossreactivity of rat extracts by RAST inhibition. The data has been logit transformed and only two of the data points plotted for clarity. (a) Urine RAST, serum pool I diluted 1:4, uninhibited RAST isotope binding = 12.0%. (b) Urine RAST, serum pool II diluted 1:16, uninhibited RAST isotope binding = 6.3%. (c) Hair RAST, serum pool II diluted 1:32, uninhibited RAST isotope binding = 9.1%. ○. Airborne dust. □. Bulk dust. ●. Hair. ■. Urine.

allergens in rat room dust with those in urine and hair. Biasing the IgE specificity of the serum pool did not significantly affect the allergens detected in the dust (Fig. 2, tracks 'ad' and 'bd', pools II–IV) and urine (Fig. 2, tracks 'u', pools II–IV) extracts; similar patterns of bands were found although the intensity of radio-

staining varied. The serum pools with more 'hair' specific IgE antibodies (pools II and IV) detected allergens with molecular weight <14.4 kDa in both dust extracts. The dust extracts ('ad' and 'bd') contained the most allergens with discrete areas of radiostaining identified at 17, 19, 21, and 44 kDa. The 44 kDa allergen was not found in

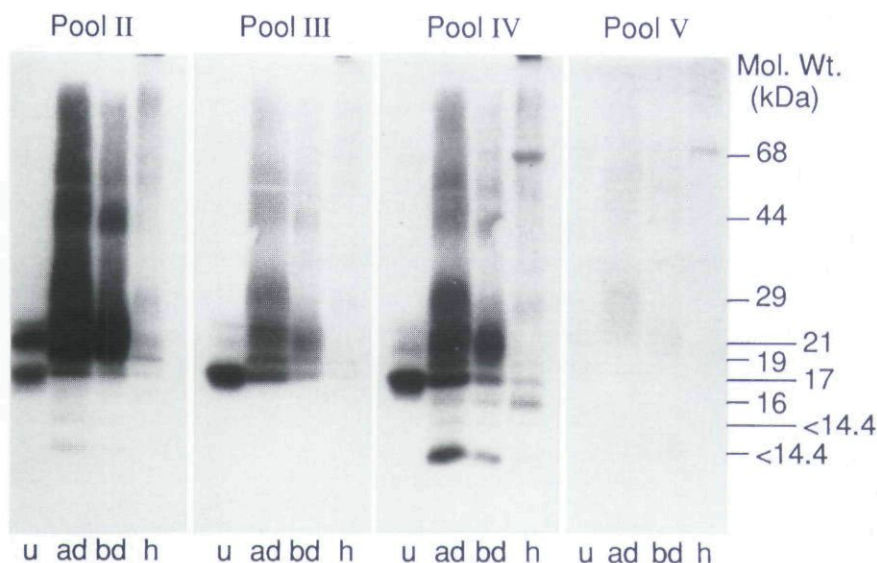


Fig. 2. Immunoblot analysis of the allergens present in rat dust, urine and hair after 60 h exposure. The protein loaded per track was; urine (u) = 13 µg, airborne dust (ad) = 9 µg, bulk dust (bd) = 5.2 µg, hair (h) = 9.2 µg. The serum pools contained IgE predominantly to hair and urine (pool II diluted 1:32. RAST isotope binding; urine = 6.4% hair 9.1%, HDM = 0.7%), urine (pool III diluted 1:4. RAST isotope binding; urine = 6.4%, hair = 5.5%, HDM = 2.3%), hair (pool IV diluted 1:10. RAST isotope binding; urine = 4.1%, hair = 9.5% HDM = 2.8%) and *D. pteronyssinus* (pool V diluted 1:16. RAST isotope binding; urine = 0.8, hair = 0.8, HDM = 11.6%). The molecular weight on the right indicate the position of some of the allergens discussed.

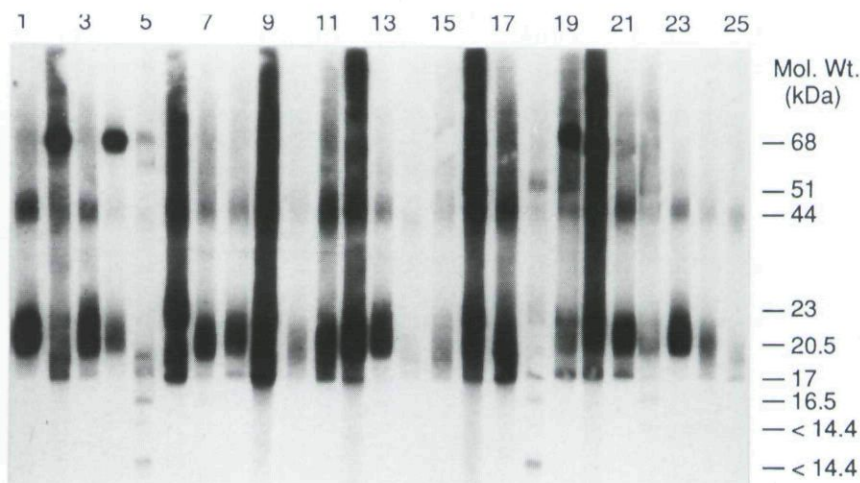


Fig. 3. Autoradiographs of bulk dust immunoblots after 60 h exposure. Tracks 1 through 25 represent individual allergic sera. The molecular weight shown on the right indicate the position of some of the allergens discussed.

the other extracts tested. In addition, a diffuse band at 29 kDa was only seen in the 'ad' extract. The strength of radiostaining of the bands in the 'ad' extract was always greater than that observed in the 'bd' extract which may be a reflection of the higher protein load of the former. Only three allergens were detected in urine (17, 21 and 23 kDa), the 17 kDa of which was the heaviest staining and which was present in all four extracts.

The allergens detected in the hair extract (Fig. 2, tracks 'h') by the different serum pools did vary. A very high molecular weight allergen at the 'origin' of the gel and allergens at 19 and 17 kDa were recognized by all the serum pools used, but only the immunoblot probed with pool IV developed radiostaining at 68, 16 and 2 faint bands at <14.4 kDa. An IgE binding component with a molecular weight of 19 kDa was found in the hair and both dust extracts but not the urine extract.

Faint radiostaining was found with pool V between 25 and 21 kDa in both dusts and at 65–44 kDa in the airborne dust extract suggesting that house dust mite allergens are not present in rat room dust in significant amounts.

Screen of individuals Forty-two sera were used to describe the allergens present in bulk dust. Thirty-two sera were diluted before use (dilution range 1:2–1:80) and the geometric mean (GM) of the RAST isotope binding to bulk dust of all sera used was 14.2% (range 5.0–21.6, GSD 1.3). Figure 3 shows the rat dust ('bd') immunoblots of 25 of the sera tested. Some sera had generalized radiostaining over part of the dust immunoblot (Fig. 3, tracks 6, 9, 12, 16 and 20). The resolution of the radiostaining was improved by decreasing the exposure time.

Fifteen allergens were found in dust and the molecular weights, detection frequencies and grade of radiostaining

for the 'major' and 'intermediate' allergens are shown in Table 2. Other 'minor' allergens were seen at 78, 58, 26.5 diffuse radiostaining (d), 18 (d), 16 and <14.4 ($n = 2$) kDa.

All the subjects tested had some degree of diffuse radiostaining at 44 kDa. A number of distinct patterns of IgE reactivity to dust allergens were apparent. Most sera contained IgE which bound to several of the low molecular weight dust allergens (<25 kDa). IgE binding to the 20.5 kDa dust allergen in the absence of binding to

Table 2. Distribution of the dust allergens recognized by 42 positive sera after 140 h ($\geq$ grade 1) and 24 h ($\geq$ grade 3) of exposure. Only 'major' allergens (in bold) and 'intermediate' allergens are shown

Molecular weight/ kDa	Positive (Grade ≥ 1)		Strong staining (Grade ≥ 3)	
	<i>n</i>	% [†]	<i>n</i>	% [‡]
68	10	23.8	9	90.0
51	15	35.7	9	60.0
44 d	42	100.0	24	58.5
25	13	31.0	7	53.8
23.5	13	31.0	6	46.2
20.5 d	31	73.8	27	87.1
19	12	28.6	7	58.3
17	37	88.1	23	62.2

n. Number of sera.

%[†]. Percentage of total number positive to rat dust.

%[‡]. Percentage of number positive to that molecular weight allergen.

(d). Diffuse staining.

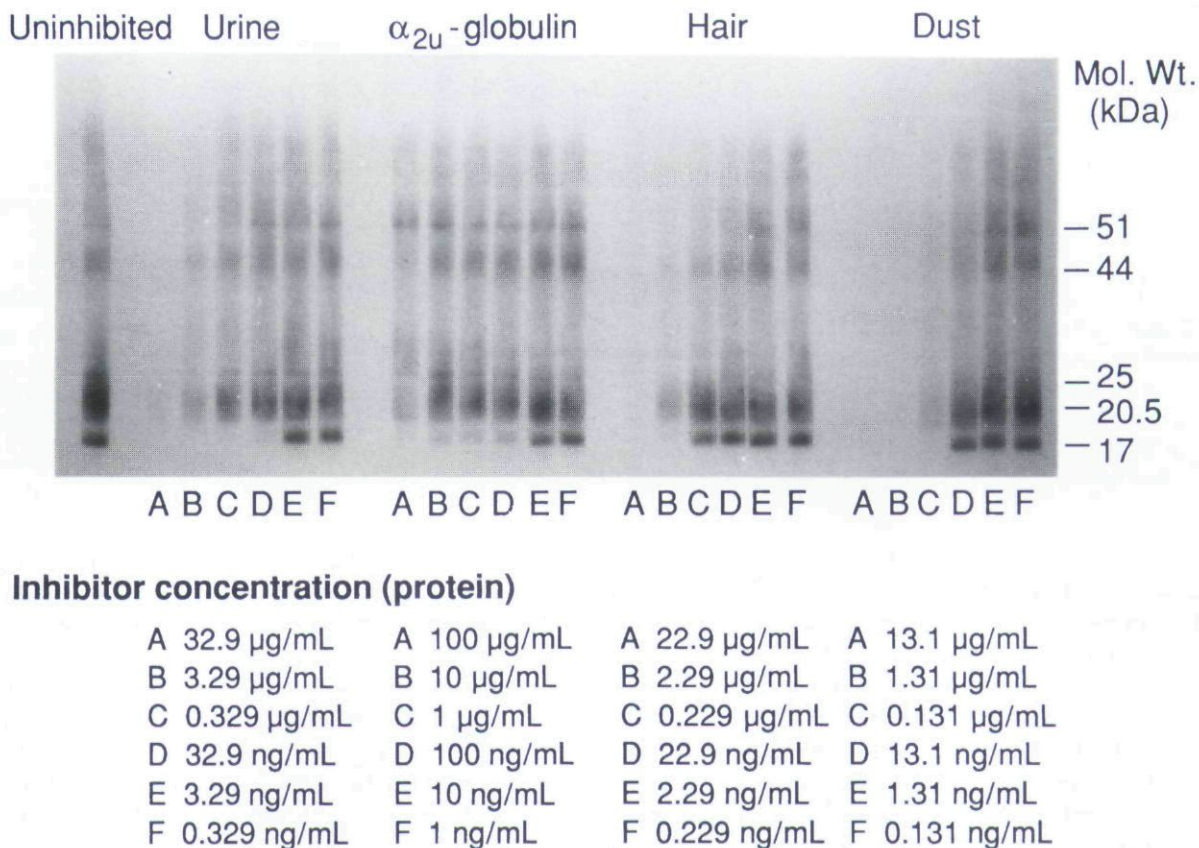


Fig. 4. Immunoblot inhibition analysis of the allergens present in rat dust. The molecular weight shown on the right indicate the position of some of the allergens discussed. Serum pool I was used at a dilution of 1:4, dust RAST isotope binding = 28.8%.

the 17 kDa allergen was uncommon (12.9%; e.g. Fig. 3, tracks 4, 13, 23) but 23% of sera had IgE to the 17 kDa allergen alone (e.g. Fig. 3, track 5). All subjects with IgE to the 23.5 kDa band in dust (31.0%) also had IgE to the 25 kDa band (e.g. Fig. 3, tracks 15 & 18). Although relatively few sera had IgE specific for the 68 kDa allergen, the majority (90%, Table 2) had strong radiostaining. Weakly radiostaining bands at 16.5 and <14.4 kDa ($n = 2$) were seen in 7–14% of subjects (e.g. Fig. 3, tracks 5 & 18).

Immunoblot inhibition experiments

The immunological crossreactivity of rat allergens was analysed by inhibiting serum pool I (diluted 1:4) with rat urine, purified Rat nI (α_{2u} -globulin), hair or dust before performing the dust immunoblot analyses. Five areas of radiostaining were identified by the uninhibited pool in the dust solid phase and which were equivalent to the 'major' dust allergens at 44, 20.5 and 17 kDa and the 'intermediate' dust allergens at 51 and 25 kDa identified before (Fig. 4).

Pre-incubation with high concentrations of urine (32.9 μ g/mL) caused almost complete inhibition of IgE binding to all the dust components (Fig. 4, urine, track A). Partial inhibition of the 17 kDa band occurred at 32.9 ng/mL (track D) and no inhibition of this band was observed below 3.29 ng/mL (track E). Inhibition with 3.29 μ g/mL of rat urine caused partial inhibition of IgE binding to all the other dust components (molecular weight ≥ 20.5 kDa). There was little inhibition of the high molecular weight components (≥ 20.5 kDa) at rat urine inhibitor concentrations ≤ 0.329 μ g/mL (track C).

Pre-incubation with Rat nI (α_{2u} -globulin) had little effect on the density of radiostaining of the high molecular weight components (≥ 20.5 kDa) at the highest concentration used (α_{2u} -globulin, track A; 100 μ g/mL) but completely inhibited IgE binding to the 17 kDa band. Partial specific inhibition of binding to the 17 kDa component was observed between 10 and 0.1 μ g/mL (tracks B–D).

The influence of hair and dust as inhibitors of the binding of serum pool I IgE antibodies to the dust immunoblot were very similar although the dust extract

was approximately 10-fold more potent. Both effected complete inhibition of all bands at the highest concentrations used; 22.9 $\mu\text{g/mL}$ for hair (Fig. 4, hair, track A) and $\geq 1.31 \mu\text{g/mL}$ for dust (dust, tracks A & B). Hair and dust were more effective than the urine extract at inhibiting the binding of IgE to the higher molecular weight components of dust ($\geq 44 \text{ kDa}$); partial inhibition occurred at 0.1–0.2 $\mu\text{g/mL}$ compared with 3.29 $\mu\text{g/mL}$ for urine.

Discussion

Several immunological techniques have been used to examine the allergens in dust collected from a room housing rats. Such studies are complex due to the heterogeneous nature of the allergen extracts and sera used, and this paper is therefore a descriptive study aimed at identifying a suitable marker protein for the improved quantitation of exposure to rat allergen.

Although care was taken in the collection and preparation of the allergenic extracts, minimal cross-contamination between the source materials cannot be excluded. Whilst isolated hairs were observed in the urine collected with a metabolism cage, comparison with urine collected directly by bladder massage has shown any contamination to be minor. Cross-contamination of pelt with urine is more difficult to assess, partly because the major urinary component which might have been used as a marker of urine contamination in male rats, α_{2u} -globulin, has now been shown to be present in modified sebaceous glands [12] as well as the saliva [13]. The contribution of salivary proteins to the apparent 'hair' allergens has not been studied. Contamination of the dust with allergen from other laboratory animals is unlikely but the dust may contain some fungi or bacterial allergens.

The sera used in this study were selected primarily for their high rat specific IgE levels and the volume of sera available; all were uncharacterized with respect to other environmental allergens including other laboratory animals. The selection of 'monospecific' sera was not possible as few subjects have IgE to single rat urinary allergens and those with high levels of specific IgE antibodies invariable had complex immunoblot profiles to all the rat extracts.

RAST inhibition experiments showed that both dust sources were equally potent at inhibiting a rat urine RAST system and were considerably more potent than the urine 'self' inhibitor when expressed on the basis of protein concentration. A possible explanation for this apparent preferential concentration of rat dust allergens may be that a significant amount of allergen in rat room dust is of a non-protein nature. Alternatively, the

allergenic proteins may be more likely to become airborne than the non-allergenic proteins (a hypothesis which is consistent with the major urinary constituents of rats and mice being pheromones [14] as well as allergens) or that just the IgE-binding epitopes of the proteins are becoming airborne.

Immunoblotting experiments confirmed the similarity of the dusts; a 29 kDa allergen was the only allergen present in dust collected with an air sampler that was absent from the exit grille dust and may represent a less stable allergenic dust component present in airborne dust only. Sera from a large number of rat allergic individuals ($n = 42$) were used to further analyse the allergens present in the exit grille dust. Fifteen allergens were detected with 'major' allergens (according to the criteria of Lind and Lowenstein [11]) at 44, 20.5 and 17 kDa. The definition of a 'major' allergen is unclear [15,16] and whilst those described here are clearly important (100% of subjects tested had some degree of radiostaining to the 44 kDa allergen, 74% had IgE to the 20.5 kDa component and 88% had IgE to the 17 kDa component), the contribution of each to the overall allergenic activity of the extract has not been assessed. Their description as 'major' allergens is therefore based solely on the number of clinically sensitive subjects who had IgE specific for that component and a semi-quantitative assessment of the degree of radiostaining observed.

All the sera used in the immunoblotting analysis of dust allergens had previously been used to analyse rat urine allergens [3]. In contrast to urine, many of the 'ad' and 'bd' components separated into diffuse areas of radiostaining rather than discrete bands. Dust contained many more IgE-reactive components of molecular weights of 25 kDa or above than urine [3]. Similarities were noted in subjects' pattern of IgE reactivity to the low molecular weight components of dust and urine [3]; 88% of subjects had IgE-binding to the 17 kDa band in dust compared with 89% to the 17 kDa urinary allergen.

Although the crossreactivity of different sources of rat allergen has been observed before [6,17], the identification of the cross-reacting components has been complicated by the analytical procedures employed. The immunoelectrophoretic studies of Walls and Longbottom found that not only do the mobilities of the main allergens, α_{2u} -globulin and prealbumin, vary slightly depending upon the source material [6], but also that these allergens exist in isoallergenic forms [18]. Our RAST inhibition experiments have confirmed that extensive crossreactivity exists between rat urine, hair and dust under a number of different experimental conditions. The similarity in the immunoblotting patterns of reactivity between the low molecular weight components of dust and urine implicate these as the probable cause of crossreactivity.

Immunoblot inhibition experiments have confirmed that the dust collected from the air-conditioning system of a rat room is not significantly contaminated with allergens from a non-rat source as low concentrations of both rat urine and rat hair (33 and 23 $\mu\text{g}/\text{mL}$ respectively) caused complete inhibition of IgE binding to all the dust components. The specific inhibition of the 17 kDa band in dust by Rat nI (α_{2u} -globulin) confirms that dust contains a protein with immunological identity to Rat nIB at this molecular weight. The 17 kDa dust allergen was also inhibited by the hair extract showing that the distribution of Rat nI in extracts prepared from different source materials is wide. By comparison of the minimum concentration of extracts causing inhibition of the 17 kDa dust band, the relative concentrations of this crossreacting constituent in each extract can be determined. Threefold more urine (0.329 $\mu\text{g}/\text{mL}$) and 17-fold more hair (2.29 $\mu\text{g}/\text{mL}$) were required to cause the same inhibition as 0.131 $\mu\text{g}/\text{mL}$ of dust. Such extensive cross-reactivity explains why similar occupational rat aeroallergen exposure measurements have been obtained with urine, hair or epithelial derived standards. The apparently wide distribution of proteins with Rat nI reactivity indicate that the 17 kDa protein would be a good marker protein for the development of monoclonal antibodies for the measurement of aeroallergen. It should, however, be noted that in the immunoblot inhibition experiments purified Rat nI was less potent at inhibiting the IgE binding to the 17 kDa constituent of dust than urine when compared by protein concentration; complete eradication of radiostaining was achieved at 32.9 ng/mL protein concentration for urine and 100 $\mu\text{g}/\text{mL}$ for purified Rat nIB. It is therefore possible that some purification procedures may affect the allergenicity of the Rat nI.

The origin of the high molecular weight allergens in dust is less clear but the immunoblot inhibition experiments show that hair (and its salivary contaminants) is a potential source. The exact origin of the 44 kDa dust allergen is not known. Immunoblotting studies suggest that 35% of rat allergic subjects have IgE specific for an 'intermediate' allergen present in hair with molecular weight of 45 kDa (unpublished observations). Whilst saliva can not be excluded as an additional source of this allergen, the extensive radiostaining seen at this molecular weight in the dust immunoblots cannot be explained by non-specific binding of the IgE label or the presence of significant house dust mite contamination. The source and crossreactivity of minor dust components cannot be commented upon due to the inability of this method to detect them.

Our study corroborates observations made by others [6,7], although we used many more sera and different

immunological methods. A contrasting observation is the relative importance of prealbumin and α_{2u} -globulin in rat room dust. We have found both a 20.5 kDa allergen (possibly prealbumin) and α_{2u} -globulin (at 17 kDa) in rat room dust whereas Longbottom and Austwick found prealbumin but not α_{2u} -globulin in dust [7]. This difference may reflect the different methodologies used (some problems of which were discussed above) or the heterogeneous nature of dust.

This study is the most comprehensive and detailed of rat room dust allergens yet described. Dust collected in large quantities from a regularly cleaned air-conditioning system has been found to be immunologically representative of the material inhaled in the occupational environment. Rat dust contains many allergens, the predominant source of which is the urine and hair of rats. We have shown that the 17 kDa allergen (Rat nI) is present in all the rat extracts studied and is a suitable marker protein for the development of monoclonal antibodies for the improved quantitation of exposure to rat allergens.

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