

Allergy to mice. I. Identification of two major mouse allergens (Ag 1 and Ag 3) and investigation of their possible origin

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Summary

An extract of dust from the outlet filters of a mouse isolator was used as a basis for determining the source of inhalant allergens for subjects sensitive to this species. The antigenic components, identified by crossed immunoelectrophoresis (XIE), were compared to those found in extracts of other mouse-derived source materials, i.e. urine, fur, dander and saliva. Of the eight dust components, one (Ag 1) was identified as antigenically identical to the major urinary pre-albumin whilst the others were detected in fur, and to a lesser extent dander and saliva. None of the dust antigens was detected as a component of food or bedding.

Crossed radio-immunoelectrophoresis (XRIE), performed using sera from a group of fifteen mouse-allergic subjects (positive by RAST to mouse extracts), identified seven of the dust antigens as IgE-binding components. Antigens 1 and 3 were reactive with all the sera tested and have, therefore, been termed the 'major' allergens. Varied responses were obtained to the other 'minor' antigens.

Ag 1 (urinary pre-albumin) and Ag 3 were detected in all samples of mouse dust studied. RAST and RAST inhibition also indicated the presence of urinary pre-albumin. These findings suggest that the major mouse inhalant allergens may be derived predominantly from urine and secretions originating in the skin and present on the fur.

Introduction

Allergy to laboratory mice has in recent years become largely attributed to allergens isolated from urine, in particular, to urinary pre-albumin [1,2]. This component, also known as the mouse urinary protein (MUP) complex [3], constitutes >90% of the crude mouse urine [4] and has been shown to be reactive in skin-prick test, bronchial challenge, RAST and histamine-release tests. It has a molecular weight of 17–22 Kd with pIs in the range 4.2–4.9, and its presence in dust and pelt extracts has been

demonstrated immunochemically. Extensive cross-reactivity exists between the urinary pre-albumins derived from various mouse strains.

Other allergens present in extracts of pelt, dander and serum have also been implicated, although not studied in such detail. A 67 Kd component of mouse pelt identified as mouse serum albumin (MSA) was shown by histamine-release studies to be allergenic for a high proportion of mouse allergic subjects [5], although another study indicated it to be important in only the most highly sensitive [6], and by skin test it was much less reactive than the urinary allergen [2].

However, even though mouse-allergic subjects, in particular those with asthma, have been identified by skin test and RAST using the mouse urinary allergens, some patients with lesser symptoms, i.e. rhinitis/urticaria, have not reacted [7,8]. This has therefore cast doubt as to whether the reactivity in such subjects is IgE-mediated or whether other allergenic components may be responsible. It was hoped that immunochemical studies of mouse dust, which could be considered more representative of the material to which workers are exposed, may help resolve this problem, and that comparisons of its antigenic content to those of other mouse-derived extracts would yield information as to possible sources. Furthermore, by using the sera of allergic patients in crossed radio-immunoelectrophoresis tests, the full spectrum of allergenic components would be identified; this is important not only for diagnostic tests but also if desensitization is to be considered, since with identification of all the allergens involved there is a greater likelihood of both successful diagnosis and treatment.

The aim of this study was to identify the allergenic components present in extracts of mouse dust and to investigate their sources.

Materials and methods

Collection and preparation of extracts

Dust was collected from the extract filters of several mouse isolators (CBA, DBA, BALB/c and C57/BL) and the air-vent filter(s) of a mixed mouse housing area(s) (strains as above). Fur was shaved from several strains of mouse (i.e. CBA, DBA, BALB/c) and used to make a mixed extract. Dander (epithelial scales) was scraped from the shaved pelts with a razor blade after first freezing the pelts on a metal surface placed on dry ice. Urine was collected daily in metabolic cages and stored at -20°C until sufficient volumes were obtained. Saliva was obtained by stimulation with pilocarpine nitrate (0.1 ml of a 20 mg/ml solution), injected subcutaneously. Unused samples of food and bedding were obtained from all establishments where dust was collected.

Extraction for all solid materials was in Coca's solution (pH 7.2) for 48 hr. These extracts, and liquid source materials, were filtered (0.45 μm pore size), dialysed extensively against distilled water, and freeze-dried.

Antisera

New Zealand White/Dutch Lop cross-bred rabbits were immunized with whole mouse dust, urine and saliva extracts. All extracts (2 mg/ml in normal saline) were emulsified in Freund's incomplete adjuvant (Sigma Chemical Company, Poole, U.K.) and injected in two intramuscular and two subcutaneous sites in the rabbit. This was

repeated after 4 weeks, followed by intramuscular injections in saline every 3–4 weeks until a satisfactory response (as determined by XIE) was obtained. Anti-mouse serum raised in rabbits was obtained commercially (Dakopatts, High Wycombe, U.K.)

Crossed immunoelectrophoresis (XIE)

XIE was performed as described previously [9,10]. Briefly, 1% agarose gels (BDH, Poole, U.K.) were poured on 9 × 9 cm glass plates (Photoscience, Canvey Island, U.K.) and the freeze-dried extracts (10 mg/ml in 0.9% NaCl/0.05% NaN₃) were electrophoresed in the first dimension at 4 V/cm for 70 min. The second dimension electrophoresis was performed at 1 V/cm overnight through an intermediate gel (0.8 × 5 cm) containing anti-mouse serum (and in some cases a second intermediate gel of similar size incorporating another antiserum) into a main gel area (3 × 5 cm). These antiserum-containing intermediate gels were used to identify antigenically related components within the different extracts, e.g. the anti-mouse serum to identify serum components present in the extracts.

Crossed radio-immunoelectrophoresis (XRIE)

XRIE was performed, as described previously [11], using as a template a dried mouse dust XIE profile incorporating a second intermediate gel containing anti-mouse urine. Two autoradiographs were produced for each serum tested and radioactive uptake graded (independently by two observers) for each antigen peak for the individual serum, as *strong* (dense radiostaining after 2 days), *moderate* (weak radiostaining after 2 days, and *weak* (radiostaining visible only after a 7-day exposure).

Human sera

Fifteen serum samples were obtained from allergic individuals who associated their symptoms (asthma and/or rhinitis) with exposure to mice. Sera from non-atopic subjects were employed as controls.

Radio-allergo-sorbent test (RAST)

RAST for the determination of IgE levels specific for mouse dust and mouse urine was carried out as described elsewhere [12]. The extracts (10 mg) were coupled to cyanogen bromide activated Sepharose 4B (1 g) according to manufacturer's instructions (Pharmacia, Uppsala, Sweden). The resultant antigen particle complex (APC) was stored at a stock concentration of 25% in PBS/0.3% HSA/0.02% NaN₃, at 4°C.

For the assay, 100 µl of 2.5% APC were incubated with 50 µl of serum (in duplicate) and shaken at room temperature overnight. Each tube was washed with 3 ml of PBS/0.5% Tween 20 three times with centrifuging (500 g for 10 min). Fifty µl of ¹²⁵I-anti-IgE (Pharmacia, Uppsala, Sweden) were added to each tube, again shaken overnight, washed (six times) as previously and then counted for 2 min on a gamma counter. Results were expressed as percentage binding of the ¹²⁵I-anti-IgE added.

RAST inhibition

Fifty microlitre aliquots of pooled sera from allergic subjects, or non-atopic control individuals, were added to 100 µl of inhibiting extract in increasing concentrations in PBS (in duplicate) and incubated on a shaker at room temperature for 6 hr. One-hundred microlitres of APC were added to each tube and then the RAST procedure performed as above.

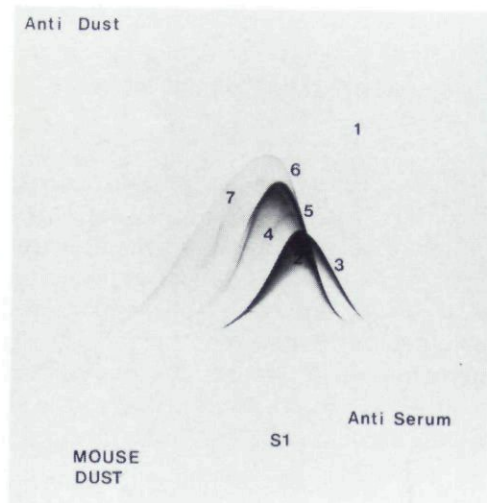


Fig. 1. XIE reference profile for mouse dust (30 μg), with anti-mouse dust in the main gel (20 $\mu\text{l}/\text{cm}^2$), and anti-mouse serum in the intermediate gel (12.5 $\mu\text{l}/\text{cm}^2$). The immunoprecipitin peaks are arbitrarily numbered from anode.

The percentage inhibition was calculated as:

$$\frac{(A - C) - (a - c)}{(A - C)} \times 100$$

where A and C were the counts per min obtained for allergic and control sera, respectively, without the addition of inhibiting extract, and a and c were the counts per minute for the allergic and control sera with the inhibiting extract added.

Results

Antigenic characterization of dust

A reference XIE precipitin pattern (Fig. 1) was established for the extract of dust collected from the extract filter of a mixed mouse isolator. Of the eight precipitin peaks (numbered arbitrarily from the anode), seven were identified as specific dust components; however, one peak, S1, was identified as a serum component by its position in the intermediate anti-mouse serum gel (Fig. 1). Further tests (not shown) have confirmed this component as serum albumin.

The presence of the specific urinary pre-albumin component in mouse urine was demonstrated by XIE using an anti-mouse urine (Fig. 2a). By incorporation of this antiserum into a second intermediate gel in the mouse-dust XIE profile, complete absorption of the peak indicated (i.e. Ag 1) resulted (Fig. 2c); thus this component was identified antigenically as urinary pre-albumin. Similarly, the many antigenic components specific to mouse saliva were identified in XIE using its homologous antisera (Fig. 2b), and by incorporation of this antiserum into the dust profile peak 2 was completely, and peaks 3, 5 and 6 partially, absorbed (Fig. 2d). There appeared to be no effect on the urinary pre-albumin peak, indicating that this component was absent in

saliva, whereas the weak partial absorption of peaks 3, 5 and 6 indicated their presence, but only in low amounts. Many of the slower migrating components of saliva appeared to be absent from the dust.

XRIE analysis of the dust extract

Autoradiographs for sera from three mouse-allergic subjects are illustrated, together with the protein-stained XIE template (Fig. 3a–d). The XIE pattern chosen was that incorporating a second intermediate gel with anti-mouse urine to absorb Ag 1, in order to avoid the possibility of co-precipitation of this antigen within the immunoprecipitates of the other components. This could occur if the reference XIE dust pattern (Fig. 1) had been used for these tests.

The XRIE results (as determined from the degree of radiostaining seen for each serum after both 2- and 7-day exposure to X-ray film—see 'Materials and methods') for a group of fifteen sera are shown (Fig. 4). All sera reacted with antigens 1 and 3, and

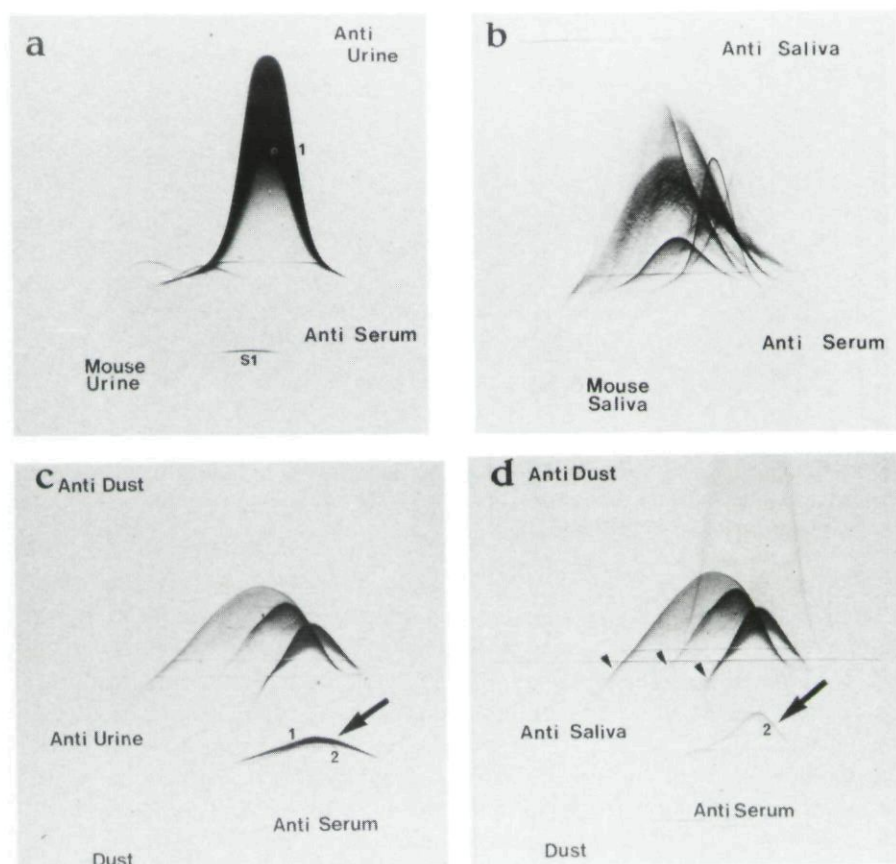


Fig. 2. (a) XIE of mouse urine (20 μg) against anti-mouse urine (20 $\mu\text{l}/\text{cm}^2$) with an intermediate gel of anti-mouse serum. (b) XIE of mouse saliva (34 μg) against anti-mouse saliva (20 $\mu\text{l}/\text{cm}^2$) with intermediate gel of anti-mouse serum. (c) XIE of mouse dust/anti-mouse dust, as in Fig. 1, but with a second intermediate gel incorporating the anti-mouse urine (12.5 $\mu\text{l}/\text{cm}^2$). The absorbed Ag 1 peak is indicated. (d) XIE of mouse dust/anti-mouse dust, as in Fig. 1, but with a second intermediate gel incorporating the anti-mouse saliva. The absorbed Ag 2 and partially absorbed Ags 3, 5 and 6 are indicated.

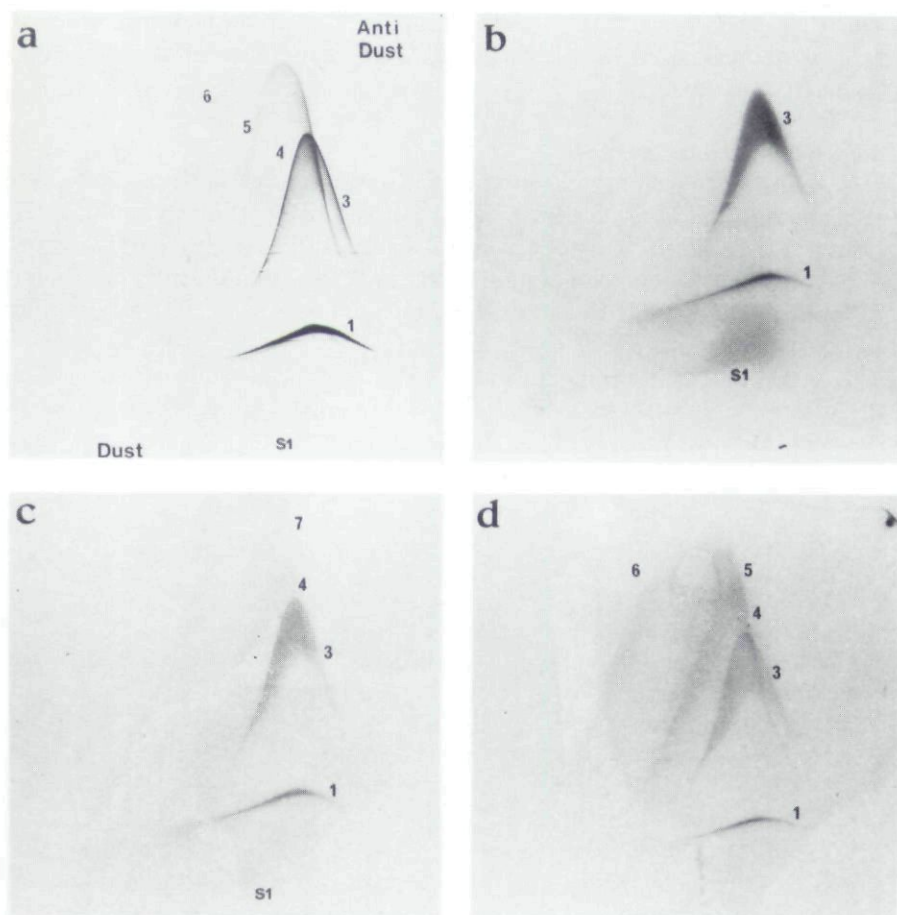


Fig. 3. (a) XIE precipitin pattern for mouse dust/anti-mouse dust with intermediate gels containing anti-mouse serum and anti-mouse urine (cf. Fig. 2c). (b–d) XRIE autoradiographs for three mouse allergic patients (antigen peaks numbered as in Fig. 1).

for each antigen the majority of the sera exhibited strong/moderate binding. No radiostaining was observed on the autoradiographs of control sera.

Significant correlation ($P < 0.01$) was seen between the dust RAST and the number and intensity of radiostaining of the dust components in XRIE, as determined by Spearman's coefficient of rank correlation (r_s) [13]. There was not, however, such a good correlation between the urine RAST and the XRIE assessment ($P > 0.02$), although there was a significant correlation between the dust RAST and urine RAST ($P < 0.01$), as determined by linear regression analysis.

RAST inhibition

The inhibition by dust and urine of a dust RAST is shown in Fig. 5a and of a urine RAST in Fig. 5b. Although at concentrations of $100 \mu\text{g/ml}$ both urine and dust were able to inhibit completely the urine RAST, only dust and not urine was able to inhibit

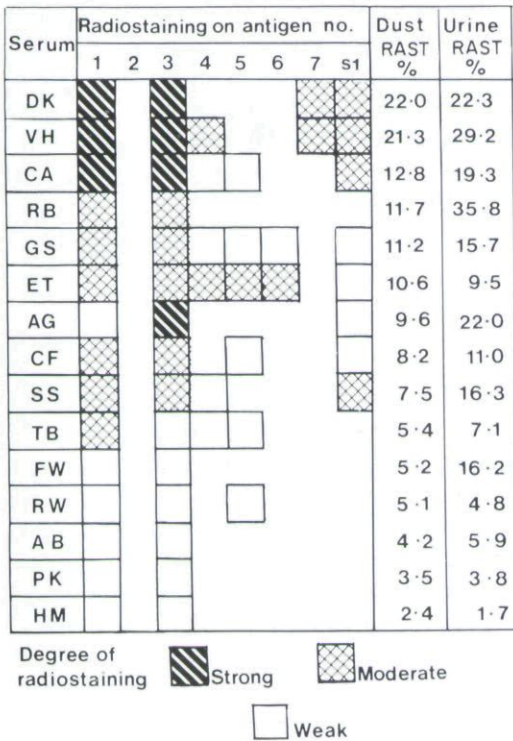


Fig. 4. XRIE radiostaining of mouse dust antigens with sera from 15 mouse allergic patients. Radioactive uptake for each antigen was graded as strong, moderate and weak, as described in the 'Materials and methods'. Also included are the RAST results (percentage binding) for specific IgE to mouse dust and mouse urine.

completely the dust RAST. These results indicate not only high levels of urinary pre-albumin, but also the presence of non-urinary allergenic components in the dust extract.

Presence of antigens 1 and 3 in other mouse extracts

Figure 6 shows the XIE profiles of dusts collected from various sources—all contained both Ag 1 and Ag 3. Figure 6a is very similar to the reference dust profile, whilst Fig. 6b indicates a lower level of Ag 1. Both of these were prepared from the extract filters of mouse isolators. The profile of mouse dust collected from a mixed mouse housing area (CBA, DBA, BALB/c and C57/BL), Fig. 6c, has very high levels of antigen 3, whereas that obtained from a nude mouse isolator, Fig. 6d, has rather lower levels of both major allergens. Trace amounts of Ag S1 are detectable in the dusts in Fig. 6a and c.

XIE of mouse dander (Fig. 7a) and mouse fur (Fig. 7b), using the mouse dust antiserum, showed the presence of Ag 3 in both extracts, and furthermore revealed the strong resemblance of the fur profile to the dust reference pattern.

Discussion

An extract of dust obtained from a filter in a mouse isolator (rather than a single source material) was used as a reference to reflect more closely the inhalant antigen exposure of an individual.

Seven out of the eight dust antigens were identified as allergenic by XRIE, and although a variety of responses was obtained, two of the antigens (Ag 1 and Ag 3), due to their binding of specific IgE in all sera studied, were recognized as being major allergens. (A 'major' allergen in this system is defined as one for which 50% or more of the subjects studied demonstrate IgE and for which at least 25% show strong specific IgE binding [14,15].) An appropriate XIE pattern was chosen for these tests as the position of Ag 1 in the reference XIE dust profile may have resulted in its co-precipitation within the other immunoprecipitates, thus causing non-specific uptake of the tracer antiserum [16]. The absence of radiostaining on the control autoradiographs indicated the specificity of these results. The dust RAST scores correlated with both the number of antigens recognized by specific IgE and the degree of radiostaining seen ($P < 0.01$).

The mouse serum-derived component Ag S1 (serum albumin) showed binding by 50% of the sera but, due to the position of the component on the XIE plate, there is some doubt as to the specificity of this binding, which could partially be due to allergen co-precipitation (see above). Most of the radiostaining seen on this antigen was classed as 'weak', and consequently this component was regarded only as a minor allergen, although greater specificity may have been achieved if an extract containing higher amounts had been used. These findings are in accordance with the earlier reported studies [5,6] of the allergenicity of mouse serum albumin.

Antigens 4, 5, 6 and 7, with their weak/moderate radiostaining for a minority of sera, can also be classed as minor allergens.

All the mouse dust antigens investigated appeared to be derived from the mice themselves as they were all identified in mouse source materials, and extracts of food and bedding did not contain components recognized by the anti-dust. The possibility of mite contamination of the dust was considered, however, no mite bodies could be

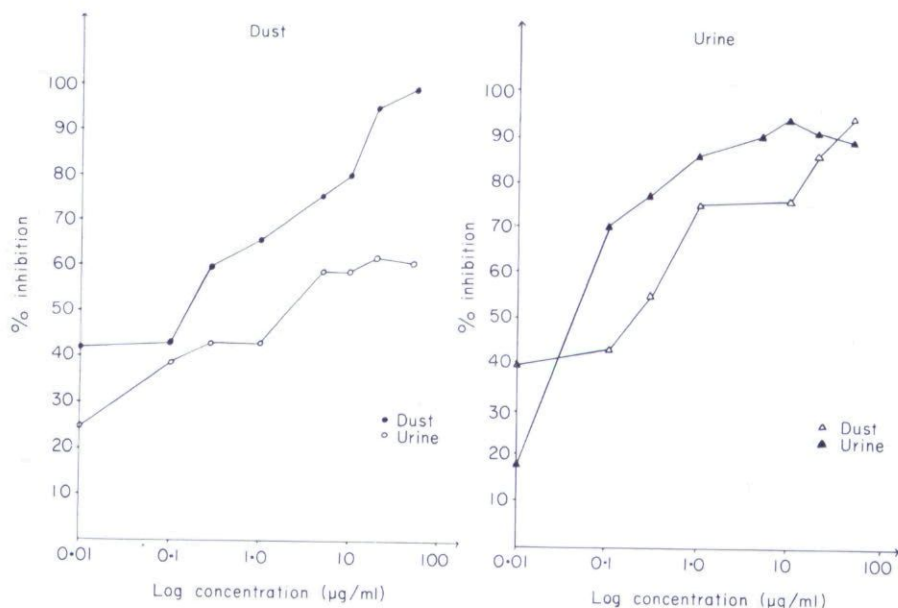


Fig. 5. Inhibition of IgE RAST for mouse dust (a) and for mouse urine (b) with their homologous and heterologous extracts.

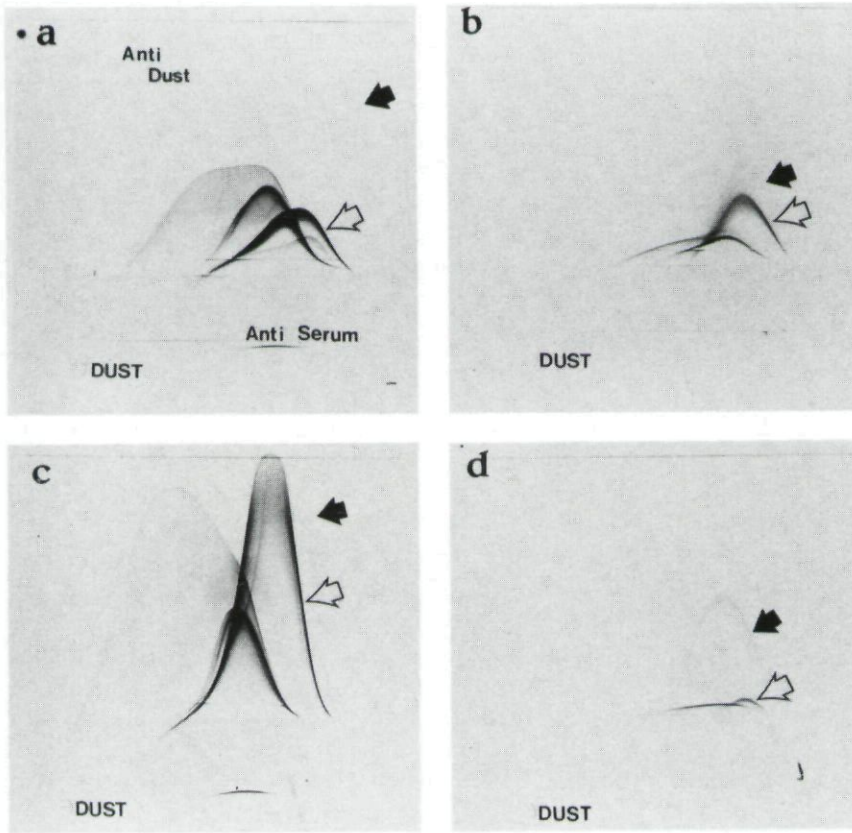


Fig. 6. XIE profiles of four mouse dust extracts ($30 \mu\text{g}$) against anti-mouse dust ($20 \mu\text{l}/\text{cm}^2$) with intermediate gel of anti-mouse serum ($125 \mu\text{l}/\text{cm}^2$). (a) Dust from second filter from same mouse isolator as reference dust. (b) Dust from similar type mouse isolator. (c) Dust collected from mixed mouse housing area. (d) Dust from nude mouse isolator. Ag 1 indicated ($\blacktriangle$); Ag 3 indicated ($\triangle$).

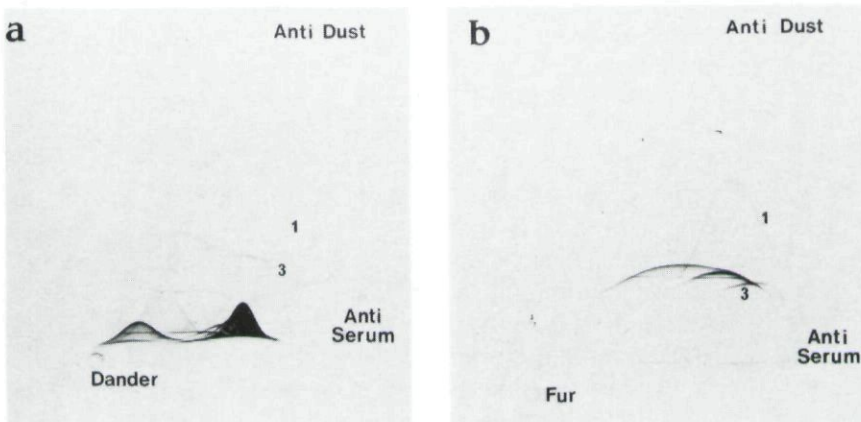


Fig. 7. XIE of mouse dander ($34 \mu\text{g}$) (a) and mouse fur ($30 \mu\text{g}$) (b) against anti-mouse dust ($20 \mu\text{l}/\text{cm}^2$ and $5 \mu\text{l}/\text{cm}^3$, respectively) with intermediate gel of anti-mouse serum.

identified by microscopic investigation.

Antigen 1, from its absorption by the mouse urine antiserum, was identified as antigenically identical to the urinary pre-albumin. This component, which constitutes the major urinary protein (MUP) complex [3], has previously been shown to be highly allergenic for mouse allergic subjects [1,2,4]. Its presence, immunochemically, in dust and pelt extracts has previously been reported [2,17], and dust extracts were also shown to inhibit a mouse urine RAST although one hundred-fold less effectively than the urinary protein [2]. Thus, it was concluded that the presence of the urinary pre-albumin component in dust was probably due to dispersal of urinary proteins from litter in mouse cages, and that such components from mouse pelt and dander would only contribute to its presence. However, the close similarity between the XIE profiles of dust and fur found in our studies suggests that although urine is an important source of this major allergen, its presence in airborne dust samples may involve the fur becoming contaminated with urine (and skin secretions) rather than originating directly from the litter.

The source(s) of the other antigenic components of the dust, and in particular Ag 3, were more difficult to determine. They were detected in extracts of fur, dander and saliva, although in the latter only in trace amounts. Furthermore, the presence in saliva of many other components that were not identified in the dust suggests that this is not the main source of these antigens, although the presence of saliva on the fur, due to grooming, is probably adding to their levels in the fur extracts. Dander, i.e. epithelial scrapings of the shaved pelt, although demonstrating some of the antigens, did not quantitatively reflect the dust levels as did the fur, suggesting that the antigens might be related to the hairs themselves. Further immunohistochemical studies have confirmed the presence of these antigens in close relationship to the secretions present on the hairs and stratum corneum (J. A. Price and J. L. Longbottom, manuscript in preparation).

Small amounts of Ag 3 were detected by XIE as present in the mouse urine, i.e. the anti-mouse urine did contain a very low titre of antibodies to this component (results not shown). However, investigations of urine obtained directly from the bladder have indicated that, with the collection of urine in metabolic cages over quite long periods, contamination with 'dust' from the fur of the animals occurred, and Ag 3 is therefore not present in the urine itself.

The two major allergens Ag 1 and Ag 3 were detected, although varying quantitatively, in all dust samples studied. Animal husbandry appeared to play an important role in the quantitative differences observed between the extracts, as lower levels of the allergens were demonstrated in those samples taken from areas where more stringent standards of cleanliness were enforced. The lowest levels of both components were seen in the extracts of dust from nude mouse isolators, implying either that these mice actually produce less of the antigens, or that the absence of fur decreases the level of antigen becoming airborne.

Although in XRIE all patients' sera reacted with both Ag 1 and Ag 3, there was some variation in the degree of radiostaining and, therefore, the current trend to use mouse urinary proteins, i.e. almost exclusively Ag 1, for diagnostic tests may result in some mouse allergic individuals being overlooked. These studies suggest that a more relevant source of mouse allergens to include both the recognized major allergens, Ag 1 and Ag 3, for extracts used for diagnosis (i.e. skin test and RAST) and also for desensitization, would be the mouse fur, but that dust from isolators (available in greater amounts) would be equally suitable.

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