

Short communication

Analysis of allergens in rat fur and saliva

Background: The fur is an important source of allergens in many mammal species, but this source has not been extensively studied in rats. Rat room dust contains high-molecular-weight allergens that have been found to cross-react with fur and its presumed salivary contaminants. The role of rat fur and saliva as a source of respiratory allergens merits further investigation. The objective was to describe the allergens present in the fur and saliva of male rats.

Methods: Allergen extracts were prepared from the saliva and fur of the pelts from adult male rats. Immunoblotting was used to describe the allergens present in rat fur and saliva with serum from 76 and 25 individuals, respectively.

Results: There was considerable variation between the individuals in the binding of IgE to the separated fur and saliva allergens. Immunoblot analysis identified 23 allergens in rat fur. “Major” allergens were found at the “origin”, and at 55, 51, 19, and 17 kDa, and “intermediate” allergens at 74, 67 (probably albumin), and 21.5 (diffuse) kDa. Seventeen salivary allergens were described with “major” allergens at 21.5, 19.5, 19, 18, and 17.5 kDa. Many subjects had IgE to the 67 kDa (56%) and 43 kDa (64%) allergens but the density of staining was weak.

Conclusions: Rat fur and saliva are the sources of many allergens. Fur contained five “major” allergens and was a complex source of allergens of relatively high molecular weight (> 22 kDa). The most important salivary allergens have molecular weights of less than 22 kDa. Fur is the most probable source of the high-molecular-weight allergens found in rat room dust.

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Laboratory animals, particularly rats and mice, are an important cause of occupational allergic disease; data from the SWORD (Surveillance of Work and Occupationally-related Respiratory Disease) study consistently cite laboratory animals among the top three causes of occupational asthma in the UK (1). In 1998, laboratory animal allergy (LAA) was officially recognized as an occupational hazard in the USA (2). Epidemiologic studies have demonstrated that the pathogenesis of LAA is common worldwide, and that typically one-third of exposed people will experience allergic symptoms (3). It is probable that, as with sensitisation to domestic animal species, exposure to low levels of allergen is sufficient to cause the disease in some people (4). It therefore remains important to understand as fully as possible the factors that contribute to the pathogenesis of LAA.

Identification of both the sources of exposure and the allergens is important. The allergens produced by rats are particularly well characterized, and it is currently believed that the urine of adult male rats is the most important source of allergen. Three major allergens with molecular weights of 17, 21, and 23 kDa, respectively, are present in the urine of sexually mature rats (5). The amino-acid sequence of two of

these, Rat n 1.02 (17 kDa) and Rat n 1.01 (21 kDa), have been determined and identified as members of the lipocalin family of proteins (6). In contrast, dust collected from a rat holding room was shown to contain 18 allergens with “major” allergens at 44, 20.5, and 17 kDa (7). Inhibition studies demonstrated the low-molecular-weight, but not the high-molecular-weight, dust allergens to be of urinary origin (7). This suggests that other potential sources of respiratory allergens, such as the fur or saliva, may also be important in allergy to rats. Fur and saliva are important sources of allergen in other laboratory animal species, particularly the rabbit (8). However, there have been relatively few studies of these sources for rat (9, 10). We therefore present a descriptive study of the allergens present in the fur and saliva of rats identified by immunoblotting.

Material and methods

Antigen preparation

The unwashed pelts were removed from adult Wistar rats (male, 1 year old) and frozen on a metal tray over a mixture of solid CO₂ and methanol (11). Long hair was removed with scissors from areas uncontaminated with urine or blood and extracted in 0.1 M

NH_4HCO_3 (pH 7.0) overnight at 4°C. The supernatant was filtered (0.22 µm, Sartorius Instruments Ltd, Epsom, Surrey, UK) before dialysis and lyophilization. Conditions for the collection of saliva were optimized to ensure that no contamination with blood or lachrymal fluid occurred. Saliva from Wistar rats (male, 4 months old) was collected with cotton buds after subcutaneous injection of pilocarpine nitrate (10 mg, subcutaneous). The saliva was eluted twice in 5 ml phosphate-buffered saline (10 mM phosphate, 0.15 M sodium chloride, pH 7.6) and centrifuged (10°C at 2000 g for 2 × 10 min), and the supernatant was dialysed and lyophilized.

Serum

Serum was obtained from 76 individuals attending occupational allergy clinics. All the subjects had specific IgE to rat urinary proteins, as measured by RAST of class 1 or more (≥ 0.35 kU/ml).

SDS-PAGE and immunoblotting

The extracts were separated by SDS-PAGE under dissociating (10% SDS) but nonreducing conditions by the method of Laemmli (12). Low-molecular-weight standards (Pharmacia Biotech Ltd, St Albans, UK) were run in parallel. The proteins were then electrophoretically transferred onto nitrocellulose (13) and blocked with 0.1% v/v Tween 20. Nitrocellulose strips (0.5 cm wide) were incubated initially with the subjects' serum, washed, and then incubated with ^{125}I -labelled anti-IgE label (Pharmacia Biotech Ltd). After further washing, the strips were exposed to radiographic film at -70°C for 140, 60, and 24 h. The molecular weight of the areas of radiostaining was determined manually, and the relative density graded as follows: band visible after 140 h: 1; after 60 h: 2; 24 h: 3; medium staining after 24 h: 4; and strong staining after 24 h: 5 (5, 7). The classification system of Lind & Löwenstein (14) was used. Allergens were termed "major" if more than 50% of subjects had IgE which bound to that allergen (and of whom 25% had radiostaining of grade 3 or higher), and they were termed "intermediate" if more than 10% of subjects had strong staining (grade 3 or higher).

Results

Rat fur

The immunoblotting study of allergens of rat fur employed serum from 76 rat-allergic individuals. Fifty-seven sera were diluted before use (range 1:2–1:50). A complex immunoblot pattern was seen after visualization of the bound serum IgE (Fig. 1A). There was considerable variation between different individuals in the numbers and molecular weights of the allergens identified. Several sera ($n=13$) showed generalized diffuse radiostaining of the high-molecular-weight material (Fig. 1A, lanes 5, 13, and 18). These sera were not included in the calculation of the prevalence of IgE to the high-molecular-weight allergens. Very high-molecular-weight material, which did not migrate far from the cathode, was described as at the "origin". Twenty-three allergens were identified, including five "major" allergens and three "intermediate" allergens (Table 1). Three allergen "pairs" were detected at 55/51, 26/23, and 19/17 kDa. The 55/51 and 19/17 kDa pairs were only minor protein constituents of the fur extract (data not shown). Most of the sera (95%) with IgE binding to either the 55 or 51 kDa allergens

(Fig. 1A, lanes 14 and 15) and 75% of sera with IgE binding to either the 19 or 17 kDa allergens had a positive response to both constituents of the pair. However, the density of radiostaining of each individual band of the pair varied. In addition, 81% of subjects with IgE binding at 23 kDa also had IgE to the 26 kDa allergen.

Rat saliva

Sera from 25 of the subjects were also used in the immunoblotting studies of rat saliva, all of which were diluted before use (range 1:2–1:40). As for fur, there was considerable variation in the immunoblot profiles. The allergens binding IgE were predominantly of low molecular weight (Fig. 1B and Table 2). Seventeen allergens were found, of which one was detected as a diffuse area of radiostaining. Five "major" allergens were observed that all had a molecular weight of less than 22 kDa. Although these allergens varied by as little as 500 Da, the clear separation of these proteins made identification possible. One "intermediate" allergen was observed at 17 kDa. Important allergens with molecular weights of 66 and 43 kDa were identified, and 56% and 64% of subjects respectively had IgE that bound to these allergens. However, they could not be classified as "major" allergens due to the relatively weak blackening of the film.

Discussion

For many animal species, the pelt is the most significant source of allergens causing respiratory symptoms. Apart from the work of Walls & Longbottom (9), there have been few studies of the allergens present in rat fur and rat saliva. To our knowledge, the present study represents the largest report of the allergens present in rat fur and rat saliva.

Twenty-three allergens were identified in the extract of rat fur, many of which were of high molecular weight. "Major" allergens were found at the "origin" and at 55, 51, 19, and 17 kDa. These allergens may have originated from the hair shaft, skin secretions, or saliva, but they were probably not of serum origin because of the method of preparation of this extract. The complex pattern observed here is in sharp contrast to that made for other animal species. In immunoblotting studies of rabbit pelt and dog pelt, fewer than 10 IgE-binding bands are usually seen and, apart from albumin, the allergens are of low molecular weight (8, 15). The significance of this observation is hard to interpret, as other published reports use antigens from commercial sources. In addition, detailed information about the electrophoresis conditions employed is not always given; the conditions used can significantly influence the protein separation and will be discussed in more detail below.

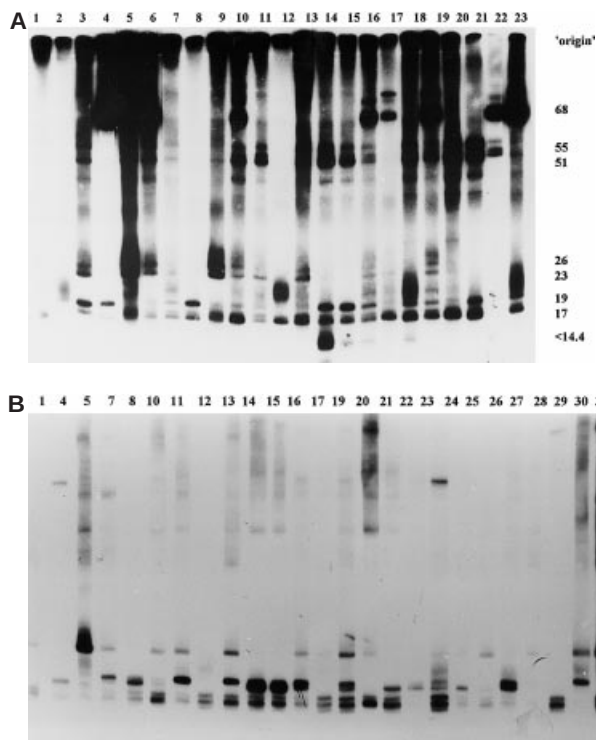


Figure 1. Autoradiograph of immunoblots after 60-h exposure. A) Fur, lanes 1–23 individual sera. B) Saliva, 1–23 individual sera as used in (A). Lanes 24–31 are additional individual sera.

In contrast, immunoblot analysis of saliva revealed allergens of predominantly low molecular weight with five “major” allergens of 21.5 kDa or less. Although more than half the subjects studied had IgE specific for the 66 and 43 kDa salivary allergens, the staining intensity to these allergens was weak, precluding their classification as “major” or “intermediate” allergens. No salivary allergens were described with molecular weights of 55 or 51 kDa or at the “origin”, suggesting that saliva is unlikely to be a source of the high-molecular-weight allergens found in the fur and dust extracts. Electrophoretic analysis of saliva from several animal species has been reported previously as part of studies to investigate the physiologic mechanism of exocrine gland secretion (10, 16). Salivary proteins from animals may be prone to aggregation when separated at low concentrations of SDS. Rat parotid saliva is complex and is rich in amylase and proline-rich proteins. Williams et al. (10) have tentatively identified the low-molecular-weight proteins (<20 kDa) in rat saliva as common salivary proteins, and formal identification of these proteins as the allergens described here is now required.

The electrophoresis experiments in this study have been performed under dissociating (10% SDS) but nonreducing conditions. The use of agents to reduce protein disulphide bonds in the immunoblotting investigation of allergens is controversial; employing

Table 1. Distribution of IgE-binding proteins of male rat fur recognized by 76 positive sera. Data based on visual evaluation of immunoblots exposed for 140 h (grade 1 or higher) and 24 h (grade 3 or higher). “Major” allergens are shown in bold; “intermediate” allergens in italics

Molecular weight kDa	Positive (grade 1 or higher)		Strong staining (grade 3 or higher)	
	<i>n</i>	%*	<i>n</i>	%**
Origin	69	90.8	58	84.1
> 94	5	6.6	3	60.0
77	8	10.5	6	75.0
74	17	22.4	8	47.1
67	25	32.9	21	84.0
57	1	1.3	0	0
55	38	50	11	28.9
53	16	21.1	3	18.8
51	38	50	17	44.7
45	27	35.5	2	7.4
40	9	11.8	0	0
36 (diffuse)	22	28.9	1	4.5
27 (diffuse)	14	18.4	3	21.4
26	28	36.8	4	14.3
23	32	42.1	5	15.6
21.5 (diffuse)	29	38.2	10	34.5
21.5	5	6.6	1	20.0
19	51	67.1	13	25.5
18	9	11.8	1	11.1
17	60	78.9	30	50.0
16.5	9	11.8	4	44.4
< 14.4	13	17.1	2	15.4
< 14.4	6	7.9	0	0

* Percentage of total number positive to rat fur.

** Percentage of number positive to that molecular weight allergen.

Table 2. Distribution of IgE-binding proteins of male rat saliva recognized by 25 positive sera. Data based on visual evaluation of immunoblots after exposure for 140 h (grade 1 or higher) and 24 h (grade 3 or higher). “Major” allergens are shown in bold, “intermediate” allergens in italics

Molecular weight kDa	Positive (grade 1 or higher)		Strong staining (grade 3 or higher)	
	<i>n</i>	%*	<i>n</i>	%**
> 94	2	8.0	0	0
> 94	6	24.0	2	33.3
> 94	7	28.0	1	14.3
80	6	24.0	0	0
74	7	28.0	0	0
66	14	56.0	0	0
43	16	64.0	2	12.5
36	8	32.0	0	0
34	11	44.0	0	0
29	3	12.0	0	0
21.5	20	80.0	8	40.0
20 (diffuse)	10	40	0	0
19.5	15	60.0	0	66.7
19	16	64.0	11	68.8
18	22	88.0	13	59.1
17.5	25	100	14	56.0
17	13	52.0	3	23.1

* Percentage of total number positive to rat saliva.

** Percentage of number positive to that molecular weight allergen.

such conditions may enhance the detection of continuous epitopes but may reduce the ability to detect conformational epitopes whose structures are determined by the presence of intact disulphide bonds (17). We have used nonreducing conditions to give the proteins their greatest chance to assume their native conformation after blotting (18). However, under these conditions, it is possible that high-molecular weight allergens may consist of aggregates or polymers of smaller allergens.

Comparison of our results with the CRIE studies of Walls & Longbottom (9) is difficult, as the preparation of extracts and the electrophoretic methods used are so different. We have found more allergens in both fur (23 compared to 14) and saliva (17 compared to 12) than Walls & Longbottom, as well as differences in the relative importance of the main allergens. However, both studies demonstrate that fur and saliva are undoubtedly important sources of rat allergens.

The extensive cross-reactivity of different sources of rat allergen, particularly urine and fur, has been well documented. While cross-reactivity studies were not performed as part of this study, such inhibition experiments using Rat n 1.02 (α_{2u} -globulin, a lipocalin [7, 19]) and individual serum and salivary proteins would clearly help to elucidate the origin of the allergens described here. As part of our extensive immunoblotting studies of rat allergens, we have compared the immunoblot profiles of individuals to the different rat extracts tested (data not shown).

Subjects with IgE to just one urinary component (i.e., seemingly monospecific serum) frequently had IgE to several of the low-molecular-weight allergens in the fur, dust, and saliva extracts. There is therefore no clear relationship of IgE reactivity between the low-molecular-weight allergens in the rat extracts. This suggests either that isoforms of the allergens may exist at different molecular weights (as has been shown for urinary proteins [20]) or that the relative concentrations of the allergens in the extracts studied may vary, or both. It has recently been reported that a 23 kDa major dog allergen is preferentially expressed in fur and saliva, whereas another major allergen (19 kDa) was found only in saliva and skin (15). It is therefore possible that allergenically similar proteins can be found among animal species in more than one source material. Without the amino-acid sequence data of these proteins, the complex relationship among the many allergens from urine, fur, and saliva is unlikely to be fully understood.

In conclusion, we have proven that the fur and saliva of rats are important sources of allergens. In the occupational environment, people will predominantly be exposed to these sources when handling the animals. We have described five "major" allergens in fur, which is also a rich source of allergens of relatively high molecular weight. Like urine, the most important salivary allergens have molecular weights of less than 22 kDa. Fur is the most likely source of the high-molecular-weight allergens identified in rat room dust.

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