

IgE response to fur animal allergens and domestic animal allergens in fur farmers and fur garment workers

J. SAVOLAINEN, J. UITTI*, L. HALMEPURO† and H. NORDMAN†

Departments of Medical Microbiology, Paediatrics and Clinical Allergology, University of Turku, Turku, *Tampere Regional Institute of Occupational Health, Tampere and †Finnish Institute of Occupational Health, Helsinki, Finland

Summary

Objective The aim of this study was to compare the IgE response to the most commonly farmed fur animals with that to domestic animals.

Methods IgE-immunoblotting and RAST-inhibition analyses were performed using RAST-positive sera from fur workers sensitized to fur allergens and sera from patients sensitized to domestic animal allergens.

Results The urine extracts of mink, blue fox, silver fox, racoon dog and fitchew contained more protein bands than the fur extracts did. Allergens with the same molecular weight were found in all of the fur and urine extracts. The most prominent allergenic bands had molecular weights of 62–67 kDa, 23–25 kDa and 18–19 kDa. With crossreacting sera the reciprocal RAST inhibition with all five animal extracts indicated common IgE-binding epitopes, probably common allergens (especially the 62–67 kDa bands). Urine and fur contain common allergens, since urine allergens strongly inhibited the IgE-binding to fur allergens. The IgE binding to allergenic bands of fur animal extracts was also observed in immunoblotting when dog and cat RAST-positive sera were used, but not for cow RAST-positive sera. RAST inhibition of dog-positive sera with fur animal extracts and fur-positive sera with dog extract confirmed the crossreactivity of these IgE antibodies. No such inhibition was seen with cow extract.

Conclusion The results of the RAST inhibition and immunoblotting suggest that fur animals have IgE binding epitopes or allergens in common with cat and dog – possibly albumin – but not with cow.

Keywords: crossreactivity, domestic animal allergens, fur, animal allergens, IgE response, fur industry

Clinical and Experimental Allergy, Vol. 27, pp. 501–509. Submitted 27 October 1995; revised 29 September 1996; accepted 20 November 1996.

Introduction

In the mid-1980s more than 20 000 Finns were involved in fur animal farming. Because of allergic manifestations among these workers a cross-sectional study was carried out to assess the prevalence of allergies to fur allergens and respiratory symptoms among exposed fur farmers and fur garment workers ($n=363$), and unexposed referents ($n=299$) [1]. The prevalence of positive skin tests to fur animal extracts (fur or urine) did not differ between the fur

animal workers and the referents. In addition, positive skin tests to domestic animal extracts showed a close correlation with positive skin tests to fur animal allergens. This finding suggested that fur animals and domestic animals have common allergens.

Studies using immunoelectrophoresis have demonstrated partial identity of antigens from several mammalian species [2,3]. IgE antibodies to allergens of cat and dog are known to crossreact, for both serum proteins and dander antigens [2,4–9]. Allergens of fur animals have not been studied.

This investigation was undertaken to compare the IgE response to the most commonly farmed fur animals with that to domestic animals.

Correspondence: Dr J. Uitti, Tampere Regional Institute of Occupational Health, Clinic of Occupational Medicine (Tampere University Hospital), PO Box 486, FIN-33101 Tampere, Finland.

Table 1. Distribution of positive skin-prick test and specific IgE to fur animal extracts in studied sera ($n = 20$). Result of skin-prick/specific IgE to each fur animal extract in all sera is expressed as + or – (fg = fur garment worker, ff = fur farmer)

Sera	Mink fur	Mink urine +	Mink urine –	Silver fox fur	Silver fox urine	Blue fox fur	Blue fox urine	Fitchew fur	Fitchew urine	Raccoon fur	Raccoon urine
1 (fg)	+/+	+/+	–/–	+/+	+/+	+/+	+/+	–/–	–/–	+/+	+/+
2 (ff)	+/-	+/+	–/–	+/+	+/+	+/+	+/+	–/–	+/+	+/+	+/+
3 (ff)	–/–	+/+	+/-	–/–	–/–	–/–	–/–	–/–	+/+	+/+	–/–
4 (fg)	–/–	+/+	–/–	–/–	+/-	–/–	–/–	–/–	–/–	–/–	+/+
5 (ff)	+/-	+/-	+/-	+/+	+/+	+/-	+/+	–/–	–/–	+/+	+/+
6 (ff)	–/–	+/-	+/-	–/–	+/+	–/–	+/-	–/–	–/–	–/–	–/–
7 (ff)	–/–	+/+	+/+	–/–	+/+	–/–	+/-	–/–	+/-	–/–	–/–
8 (ff)	+/-	+/-	+/-	–/–	–/–	–/–	–/–	+/-	–/–	+/-	–/–
9 (fg)	–/–	–/–	–/–	–/–	–/–	+/-	+/-	–/–	–/–	–/–	–/–
10 (fg)	–/–	–/–	+/-	+/-	–/–	–/–	–/–	–/–	–/–	–/–	–/–
11 (fg)	–/–	–/–	–/–	+/-	+/-	+/-	–/–	–/–	–/–	–/–	+/-
12 (fg)	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	+/-	–/–
13 (ff)	–/–	–/–	–/–	–/–	–/–	+/-	+/-	–/–	–/–	–/–	–/–
14 (ff)	–/–	–/–	–/–	–/–	–/–	–/–	+/-	–/–	–/–	–/–	+/-
15 (ff)	–/–	–/–	–/–	+/-	–/–	+/-	–/–	–/–	–/–	–/–	–/–
16 (ff)	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	+/-	–/–
17 (ff)	–/–	–/–	–/–	+/-	–/–	–/–	–/–	–/–	–/–	–/–	+/-
18 (ff)	–/–	–/–	–/–	+/-	–/–	–/–	–/–	–/–	–/–	–/–	–/–
19 (ff)	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	+/-	–/–
20 (ff)	–/–	–/–	–/–	+/-	–/–	–/–	–/–	–/–	–/–	–/–	+/-
	4/1	8/5	6/1	9/3	7/5	7/2	8/3	1/–	3/2	8/5	8/4

Material and methods

Sera

Twenty serum specimens were collected from the fur farmers and garment workers who had displayed the strongest reactions to fur animal extracts in a skin-prick test. In skin tests the greatest diameter and the diameter perpendicular to it were measured for each weal. The mean of the diameters was the test result. A test was considered positive if the mean of the diameters in the test minus the mean of the diameters of the negative controls was equally large or larger than 3 mm [10,11]. A person was considered atopic, if she/he had at least one positive skin-test result to common environmental allergens. Most of these 20 workers had positive reactions to more than one fur animal extract in a skin-prick test (Table 1). Sixteen of the 20 workers were atopic, 15 had positive skin-test results to at least one animal allergen, mostly cat or dog. Seven of the 20 specimens proved to be RAST positive to fur animal allergens; three of them were confirmed to be borderline positive in a later analysis. Atopic sera with positive RAST tests (classes 2–4) to dog, cat, cow and horse were used to determine possible allergenic crossreactivity. Atopic sera were from town inhabitants who never had worked in the fur industry.

Sera with a negative skin-prick test to fur or domestic animals and pooled non-atopic sera were used as controls.

Allergen extracts

The fur and urine extracts of mink (*Mustela vison*), silver fox (*Vulpes vulpes*), blue fox (*Alopex lagopus*), racoon dog (*Nyctereutes procyonoides*) and fitchew (*Mustela putorius*) were prepared as follows: 150–500 mL of urine from each animal was collected through a funnel leading to plastic bottles under the cages. The iron-wired mesh bottom of the cages allowed the urine to be collected free of faeces and other contaminants. Urine specimens were also obtained from mink suffering from plasmacytosis (aleuthian disease), a widespread viral disease causing marked proteinuria among minks. More than 50% of the mink stock was infected. The urine samples were stored at -20°C , and the antigen preparation was performed at $+4^{\circ}\text{C}$. The urine samples were first filtered through Whatman 1 filter paper to eliminate insoluble material. Urine from animals with plasmacytosis contained sedimented insoluble proteins. After rough filtration the urine specimens were filtered sequentially through Millipore filters having pore sizes of 1.2 μm , 0.8 μm , 0.45 μm and 0.22 μm . After the last

filtration, the urine was sterile. The sterile urine was concentrated in an Amicon chamber with threefold volume of sterilized water. The dialysed (cut-off 1000 Da), concentrated urine was freeze-dried and stored at -20°C . The specimens for the fur extracts were collected by cutting and brushing hairs near the skin without damaging the skin. The specimens were immersed in Coca solution (1:20) and centrifuged overnight. The work-up of the centrifuged pellet was the same as with the urine extracts.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis and immunoblotting

The urine and fur extracts and the molecular weight markers (LMW Calibration Kit, Pharmacia Fine Chemicals, Uppsala, Sweden) were separated with sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) in 5–17.5% gradient gels according to Laemmli [12] and transferred to nitrocellulose sheets (Millipore 0.22 μm , Millipore Co, Bedford, MA, USA) in a Transphor apparatus (LKB, Bromma, Sweden) according to Towbin *et al.* [13].

IgE antibody detection

The specific IgE binding to allergens on nitrocellulose was detected according to Bengtsson *et al.* [14]. The same 20 sera from the fur farmers and fur garment workers were used for the IgE immunoblotting. The dilution of individual patient sera, as well as the dilution of the anti-IgE antibodies labelled with beta-galactosidase (Phadezym-RAST, Pharmacia Diagnostics, Uppsala, Sweden), was 1:4. The beta-galactosidase-labelled bands were detected as previously described by Bengtsson *et al.* [14].

RAST inhibition

Each extract was fixed on CNBr (cyanogen-bromide) activated paper discs (Munktell-OOH filtration paper) at a concentration of 1 mg/mL (dry weight), 50 μL /disc [15]. The RAST determination was done according to the instructions of the manufacturer (Pharmacia, Sweden). The result was read from reference curves and considered positive when it was 0.35 kU/L or greater [16].

Two sera showing the strongest RAST positivity to silver fox fur and mink urine were used for the RAST inhibition tests. Serum (30 μL) was incubated with inhibiting extracts (20 μL , dilution series 1:1, 1:5, 1:25, 1:625, 1:3125, stock 10 mg/mL dry weight) or with 0.9% NaCl for 3 h. An allergen-coated disc was added, and an ordinary RAST assay was performed. The discs were coated with solutions with 1–3 mg protein/mL (Fig. 1). The results were expressed as the percentage of the absorbance obtained

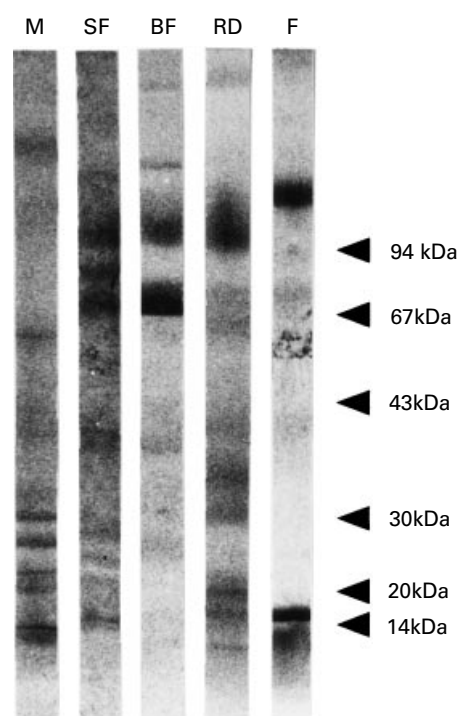


Fig. 1. Urine extracts after SDS-PAGE and stained for proteins with Coomassie Brilliant Blue. The amounts of extract corresponding to one nitrocellulose strip are 1 mg for mink (plasmocytosis positive), 1.5 mg for silver fox, blue fox and racoon dog and 1.7 mg for fitchew. The molecular weight markers (Pharmacia) are shown on the right. M, mink; SF, silver fox; BF, blue fox; RD, racoon dog; F, fitchew.

with a disc inhibited by NaCl at 420 nm [17]. The parallelism of the inhibition curves was analysed according to Schröder and Yman [18].

Results

The urine extracts run in SDS-PAGE-gel and Coomassie-stained for proteins of the five animals studied are shown in Fig. 1. The urine extracts of all the animals clearly contained more protein bands than the fur extracts (SDS-PAGE profiles not shown), which mainly contained traces of the most prominent proteins of the urine extracts (62–67 kDa, 23–25 kDa and 18–19 kDa).

The IgE immunoblotting of the extracts with seven IgE-positive sera is presented in Fig. 2. For different fur animal extracts IgE antibodies bound simultaneously to protein bands with similar molecular weights. The binding occurred predominantly to bands having molecular weights of 62–67 kDa, 23–25 kDa and 18–19 kDa. The strongest simultaneous binding was seen for the 62–67 kDa bands. Occasional IgE binding to other bands also occurred. No

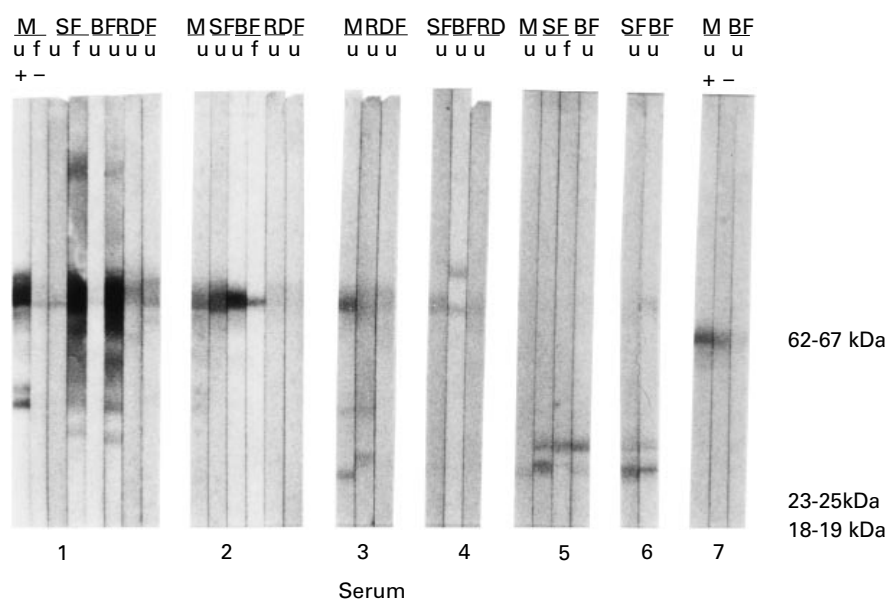


Fig. 2. Urine and fur extracts immunoblotted after SDS-PAGE with seven study sera that showed a positive skin-prick test to fur animal extracts. A selection of sera with crossreactivity is presented. The IgE detection was performed with anti-IgE labelled with beta-galactosidase after incubation with patient serum. u, urine; f, fur; M, mink; SF, silver fox; BF, blue fox; RD, racoon dog; F, fitchew. +, plasmacytosis-positive; –, plasmacytosis-negative.

binding was observed with the control sera. Table 2 summarizes the results from the immunoblotting experiments.

For serum 2 (Fig. 3a), the inhibition obtained with blue fox urine and fur antigen extracts was similar whether the urine or fur was used as the solid phase. Similar results were observed for serum 1 when inhibited with fitchew, mink or silver fox extracts (Figs 3b–3d). These findings indicate the presence of common allergens in the urine and fur extracts. The urine obtained from the minks with plasmacytosis was more potent than normal urine at inhibiting IgE binding to

both fur and urine discs (Fig. 3c). With the same sera as in Fig. 3, allergens of all five fur animals clearly inhibited the binding of IgE antibodies to silver fox urine allergens and mink urine allergens (Fig. 4). Combined with the immunoblotting data from Fig. 1, these results suggest crossreacting IgE antibodies against the 62–67 kDa protein of all of these animals. The immunoblotting experiments with dog and cat RAST-positive sera gave IgE binding to the 62–67 kDa, 23–25 kDa and 18–19 kDa bands (Fig. 5). The immunoblotting with cow RAST-positive sera revealed no bands.

Table 2. Frequency of IgE binding to fur animal protein bands

Molecular weight band	Mink	Silver fox	Blue fox	Racoon dog	Fitchew
150 kDa	–	1(14%)	–	1(13%)	–
120 kDa	–	–	–	2(25%)	–
110 kDa	–	1(14%)	–	–	–
72 kDa	–	–	1(17%)	–	–
62–67 kDa	4(67%)	5(71%)	5(83%)	5(63%)	3(100%)
55 kDa	2(33%)	–	–	–	–
47–50 kDa	3(50%)	2(29%)	2(33%)	–	–
40 kDa	–	–	1(17%)	–	–
34–35 kDa	2(33%)	1(14%)	2(33%)	–	–
30 kDa	3(50%)	1(14%)	2(33%)	–	–
23–25 kDa	–	3(43%)	3(50%)	1(13%)	–
18–19 kDa	2(17%)	2(29%)	2(33%)	1(13%)	–
13 kDa	–	–	–	1(13%)	–
No of IgE positive sera to each animal	6	7	6	8	3

Percentage of all IgE positives for each fur animal in parentheses.

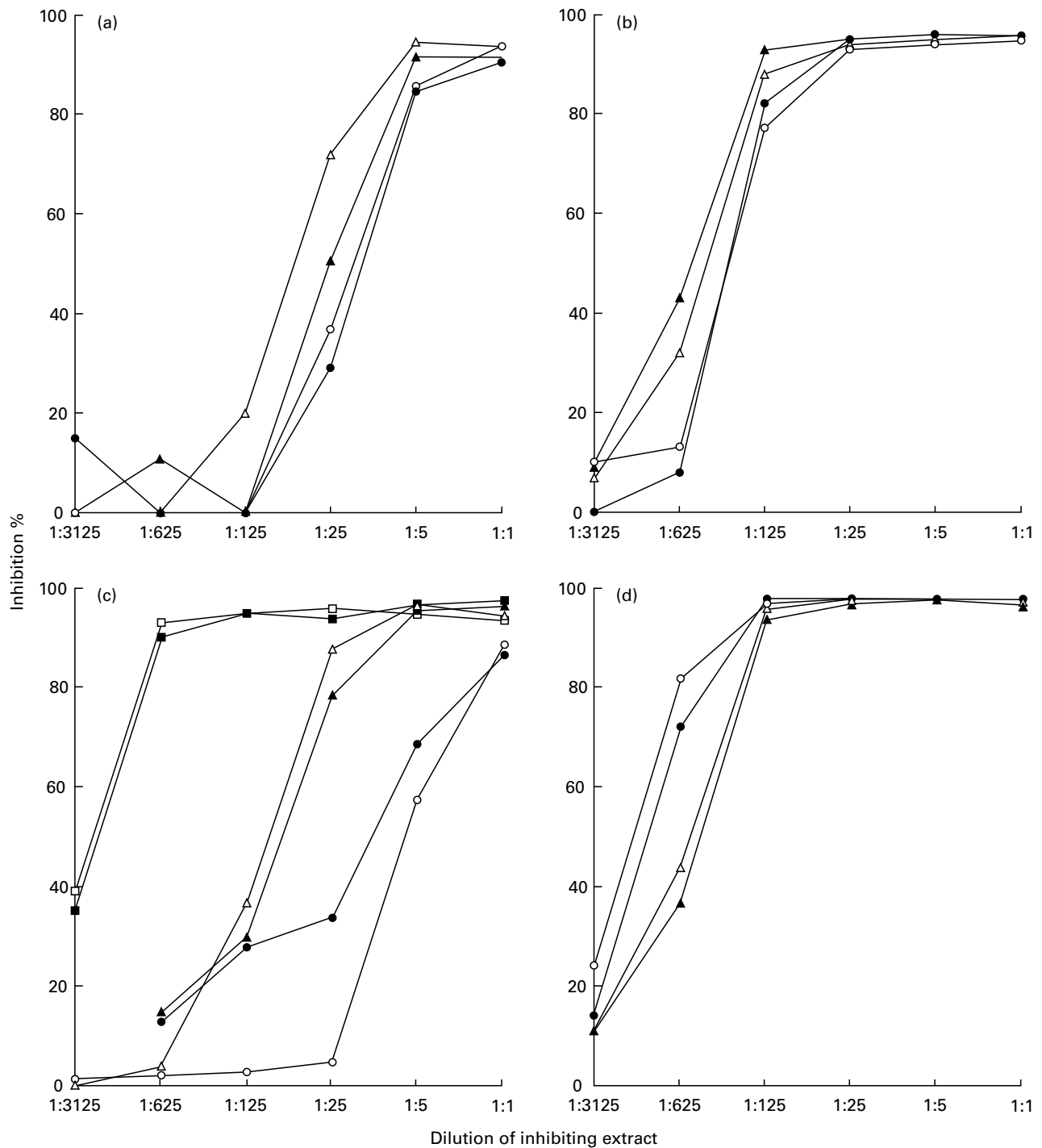


Fig. 3. RAST inhibition performed with fur and urine extracts from four fur animals and using two sera, numbered as in Fig. 2. ▲, △, inhibiting fur extract; ●, ○, inhibiting urine extract; ■, □, plasmacytosis-positive mink urine extract, the closed symbols representing urine extract discs and open symbols representing fur extract discs. (a) serum 2-Blue fox, (b) serum 1-fitchew; (c) serum 1-mink; (d) serum 1-silver fox.

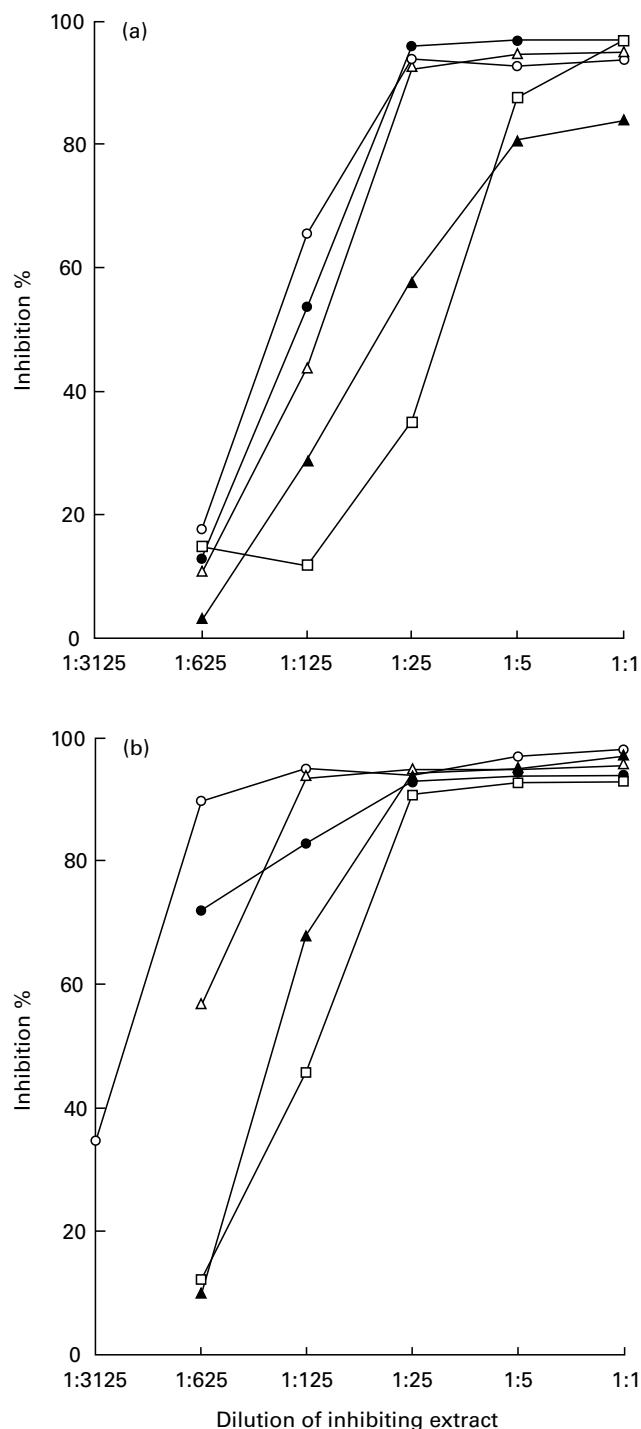


Fig. 4. RAST inhibition with urine extracts from the five fur animals. With serum 2 the discs were coated with urine extract from silver fox, and with serum 1, discs coated with urine from plasmacytosis-positive mink were used (inhibitor symbols: ○ mink, △ silver fox, □ blue fox, ● racoon dog, ▲ fitchew). (a) serum 2-disc silver fox; (b) serum 1-disc mink.

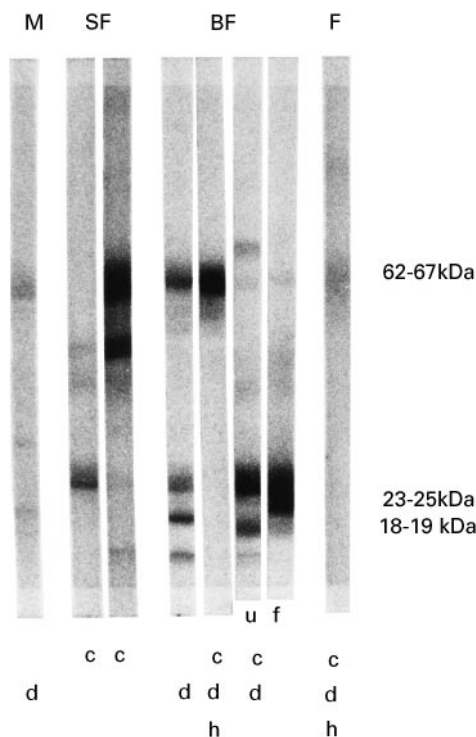


Fig. 5. Urine extracts from the fur animals and fur extract (f) from blue fox, as immunoblotted after SDS-PAGE analysis with seven sera positive for different household animals in the RAST: c = cat, d = dog and h = horse. The IgE detection was performed with anti-IgE labelled beta-galactosidase after incubation with serum from the patient. No IgE binding was seen with cow RAST-positive sera. M, mink; SF, silver fox; BF, blue fox; F, fitchew; u, urine; f, fur.

The RAST inhibition tests confirmed that this observation was due to crossreacting IgE antibodies (Fig. 6). Clear inhibition was shown for different fur animal extracts with dog extract but not with cow extract (Fig. 7).

Discussion

This study showed that IgE antibodies from sensitized fur workers bind to fur animal allergens. Crossreactivity was found between antibodies to the fur and urine allergens of the studied fur animals. Similar crossreactivity has been observed in the IgE response to other animal allergens [19]. Common allergens were found in the fur and urine extracts. The likely explanation is that the fur was contaminated by saliva, urine and other forms of excretion. Contamination is hard to avoid, but from the point of view of our objective (i.e. to study the prevalence of animal allergy), contamination was not considered a problem.

The fur extracts showed stronger inhibition in the RAST tests than the urine extracts despite their lower protein contents. One explanation may be that the allergen content of fur extracts was proportionally greater than that of the

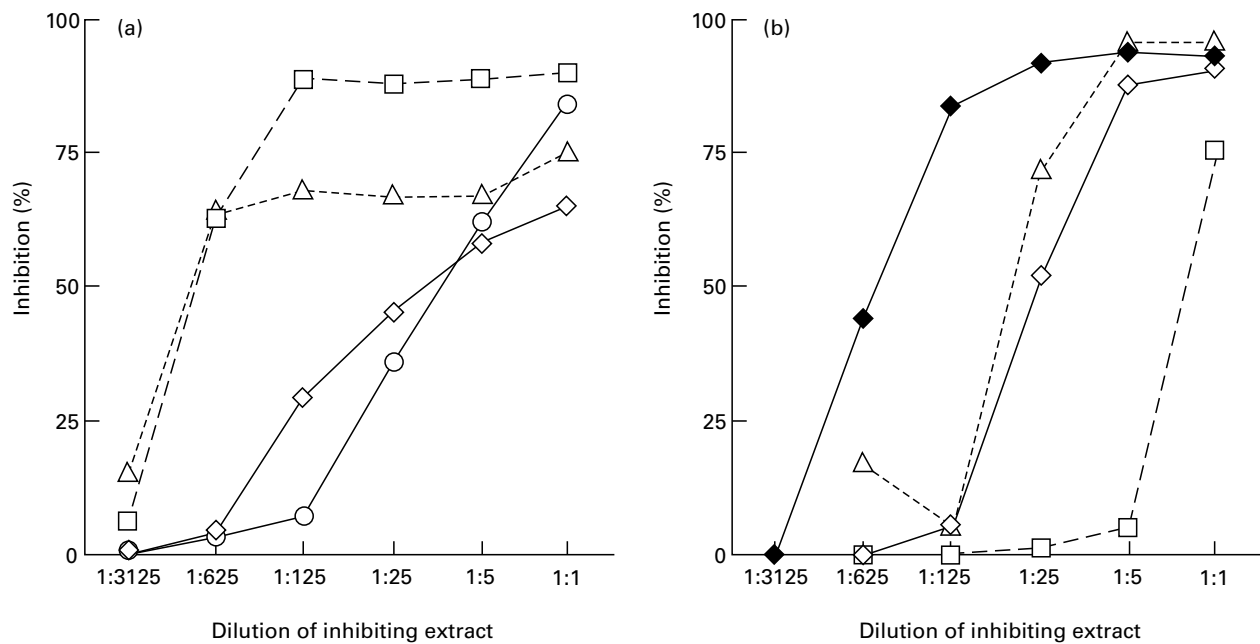


Fig. 6. (a) RAST inhibition tests using dog-RAST positive serum (—) and fur-RAST positive serum (···no 1) indicating crossreactivity between dog and fur animal extracts. □ dog disc inhibited using silver fox extract, △ dog disc inhibited using mink extract, ◇ dog disc inhibited using racoon dog extract, ○ dog disc inhibited using dog extract. (b) RAST inhibition tests using two fur-RAST positive sera (···serum 1, - - serum 2) and dog-RAST positive sera (—) indicating crossreactivity between dog and fur animal extracts. ◆. Racoon dog disc inhibited using racoon dog extract. △. Mink disc inhibited using dog extract. ◇. Racoon dog inhibited using dog extract. □. Silver fox disc inhibited using dog extract.

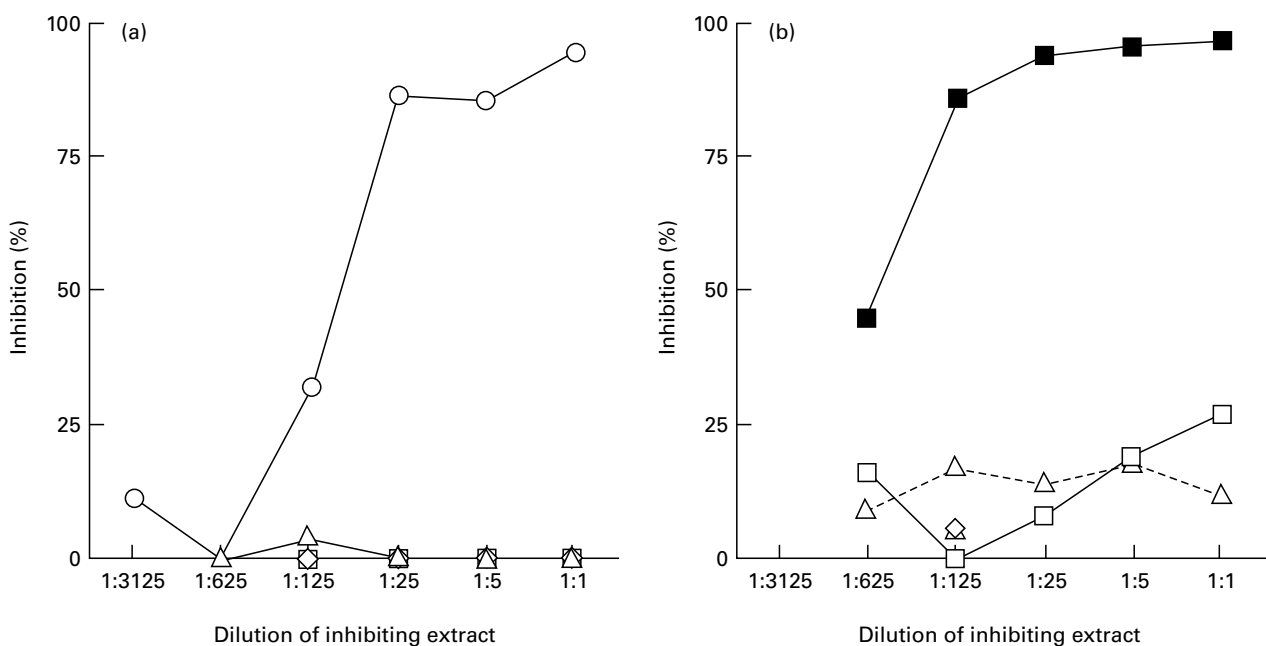


Fig. 7. (a) RAST inhibition tests using cow-RAST positive serum (—) and fur-RAST positive serum (···no 1) indicating crossreactivity between cow and fur animal extracts. ○, cow disc inhibited using cow extract; □, cow disc inhibited using silver fox extract; △, cow disc inhibited using mink extract; ◇, cow disc inhibited using racoon dog extract. The low degree of inhibition of cow by racoon dog and silver fox is technically difficult to represent correctly in the figure. (b) RAST inhibition tests using fur-RAST positive sera (···serum 1) and cow-RAST positive sera (—) indicating crossreactivity between cow and fur animal extracts. ■. Silver fox disc inhibited using silver fox extract. □. Silver fox disc inhibited using cow extract. △. Mink disc inhibited using cow extract.

urine extracts. The urine extracts contained many other proteins in addition to allergenic proteins. However, in this first study characterizing fur animal allergens the allergen extracts were not standardized. Conclusions as regards reasons and order of potency can therefore not be drawn. Plasmacytosis urine contains much albumin, and this phenomenon explains its stronger inhibiting capacity.

Fur farmers are exposed to both fur and urine allergens in their working environment, whereas fur garment workers are seldom, if ever, exposed to urine allergens since they exclusively handle dressed furs. Nevertheless, some of the fur garment workers reacted to both fur and urine extracts in the skin tests and nasal provocation, some even without a positive reaction to domestic animal allergens which could explain such a reaction [1]. This result suggests that urine and fur may contain antigens/allergens that probably correspond to serum albumin. In addition they proved to have species-specific allergens.

The crossreactivity of the IgE antibodies to different fur animals was clearly shown in the RAST inhibition tests. In the immunoblotting the band representing the molecular weight of 62–67 kDa displayed the most prominent binding of the IgE antibodies. It is likely that the shared epitopes are in this protein fraction. The crossreactivity between antibodies to fur and domestic animals was confirmed by both the immunoblotting and the RAST inhibition. Such cross-reactivity between foxes and minks on one hand, and dog and cat on the other, has been proposed by Løwenstein [3], on the basis of the phylogenetic relations between these animal species. Moreover, the results also indicated the existence of crossreacting IgE antibodies against 62–67 kDa protein in all the fur and domestic animals studied. The molecular weight of this protein refers to albumin, which has been earlier found to be an important allergen in dog [2,7,20].

IgE antibodies to serum albumin (69 kDa) have been observed more frequently among British than among Finnish subjects sensitized to dog [21]. Specific dog allergens with a molecular weight of 22–24 kDa [4,15] or 25 kDa have also been identified [22]. The antibodies to 69 kDa protein of dog and cat have been shown to crossreact [4,5]. In IgE immunoblot inhibition studies subjects who react to dog albumin exhibit IgE reactivity with purified albumins from cat, mouse, chicken, and rat [20]. It is possible that the albumin of domestic and fur animals share common epitopes within the different species. The RAST inhibition and immunoblotting studies with sera containing IgE antibodies to fur animal extracts have shown clear crossreactivity with IgE antibodies to dog and cat extracts but not with IgE antibodies to cow extract. The major cow allergen has a molecular weight of 20 kDa [23], and therefore our result was to be expected.

From the occupational health point of view, the most conspicuous result of this study was the crossreactivity of antibodies to extracts from dog, cat and farmed fur animals. It appears possible that fur workers who have been sensitized to domestic animal allergens (i.e. cat or dog) would more likely develop symptoms when exposed to fur animals. This possibility should be kept in mind in vocational guidance and occupational health care activities in the fur industry.

Acknowledgements

We would like to thank Mrs Kaarina Jokela for her proficient technical assistance and Mrs Birgitta Kaprali for her assistance with the figures. The study has been supported by the Finnish Work Environment Fund and the Medical Research Foundation of the Tampere University Hospital.

References

- 1 Uitti J. Turkisompelijoiden ja turkistarhaajien allergiat ja yliherkkyyssreaktiot [Allergies and hypersensitivity reactions among fur farmers and fur garment workers]. Työ jaihiminen 1992; suppl. 2. (Academic dissertation, summary in English).
- 2 Blands JL, Løwenstein H, Weeke B. Characterization of extract of dog hair and dandruff from six different dog breeds by quantitative immunoelectrophoresis. Identification of allergens by crossed radioimmunoelectrophoresis (CRIE). *Acta Allergol* 1977; 32:147–69.
- 3 Løwenstein H. Immunological partial identity between animal allergens. *Allergy* 1985; 40(suppl 3):64–6.
- 4 Boutin Y, Hebert J, Vrancken ER, Mourad W. Allergenicity and cross-reactivity of cat and dog allergenic extracts. *Clin Allergy* 1988; 18:287–93.
- 5 Boutin Y, Hebert J, Vrancken ER, Mourad W. Mapping of cat albumin using monoclonal antibodies: identification of determinants common to cat and dog. *Clin Exp Immunol* 1989; 77:440–4.
- 6 Brandt R, Yman L. Dog Dander allergens. Specificity studies based on the radioallergosorbent technique. *Int Arch Allergy Appl Immunol* 1980; 61:361–70.
- 7 Ohman JL Jr, Bloch KJ, Kendall S, Lowell FC. Allergens of mammalian origin. IV. Evidence for common allergens in cat and dog serum. *J Allergy Clin Immunol* 1976; 57: 560–8.
- 8 Uhlin T, Reuterby J, Einarsson R. Antigenic/allergenic composition of poodle/alsatian dandruff extract. *Allergy* 1984; 39:125–33.
- 9 Viander M, Valovirta E, Vanto T, Koivikko A. Crossreactivity of cat and dog allergen extracts. RAST inhibition studies with special reference to the allergenic activity in saliva and urine. *Int Arch Allergy Appl Immunol* 1983; 71:252–60.
- 10 Cockcroft A, McCarthy P, Edwards J, Andersson N. Allergy in laboratory animal workers. *Lancet* 1981; ii:827–30.
- 11 Venables KM, Tee RD, Hawkins ER et al. Laboratory animal allergy in a pharmaceutical company. *Br J Ind Med* 1988; 45:660–6.
- 12 Laemmli UK. Cleavage of structural proteins during the

- assembly of the head of bacteriophage T4. *Nature* 1970; 227:680–5.
- 13 Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc Natl Acad Sci USA* 1979; 76:4350–4.
- 14 Bengtsson A, Rolfsen W, Einarsson R. Characterization of allergens and patient sera by a Nitrocellulose Immunoprint Technique. *Int Arch Allergy Appl Immunol* 1985; 78:139–44.
- 15 Ceska M, Lundkvist U. A new and simple radioimmunoassay method for the determination of IgE. *Immunochemistry* 1972; 9:1021–30.
- 16 Björkstén F, Haahtela T, Hannuksela M. Assay of allergen preparations using direct RAST titration and skin tests. *Allergy* 1980; 35:671–7.
- 17 Yman L, Ponterius G, Brandt R. RAST-based allergen assay methods. *Dev Biol Stand* 1975; 29:151–65.
- 18 Schröder H, Yman L. Standardization of the RAST inhibition assay. *Allergy* 1980; 35:234–6.
- 19 Berrens L, van Dijk AG, Bollenbakker-Baars A, Huizinga M. RAST with animal dander, urine, saliva and serum. *Ann Allergy* 1983; 51:543–6.
- 20 Spitzauer S, Schweiger C, Sperr W R et al. Molecular characterization of dog albumin as a cross-reactive allergen. *J Allergy Clin Immunol* 1994; 93:614–27.
- 21 Ford AW, Alterman L, Kemeny DM. The allergens of dog. I. Identification using crossed radio-immunoelectrophoresis. *Clin Exp Allergy* 1989; 19:183–90.
- 22 Schou C, Svendsen UG, Løwenstein H. Purification and characterization of the major dog allergen. *Can f I. Clin Exp Allergy* 1991; 21:321–8.
- 23 Ylönen J, Nuutinen J, Rautiainen M, Ruoppi P, Mäntyjärvi R, Virtanen T. Comparative analysis of bovine extracts by immunoblotting and ELISA-inhibition. *Allergy* 1990; 45:30–9.