

Characterization of allergens from deer: cross-reactivity with allergens from cow dander

S. SPITZAUER*, R. VALENTA†, S. MÜHL*, H. RUMPOLD*, H. EBNER‡
and C. EBNER†

*Institute of Medical and Chemical Laboratory Diagnostics, AKH, University of Vienna,

†Institute of General and Experimental Pathology, AKH, University of Vienna, and

‡Allergy-Clinic Reumannplatz, Vienna, Austria.

Summary

Background Animal hair/dander proteins frequently cause Type I hypersensitivities. Species-specific and broadly cross-reacting allergens have been characterized in the past.

Methods Sera from eight individuals suffering from symptoms due to exposure to deer and deer-derived products were investigated by immunoblotting. Extracts from deer, dog, cat, horse, rabbit and cow, respectively, were tested for IgE-binding. To reveal cross-reactivities patients' sera were preadsorbed with these extracts prior to testing with deer extract.

Results Deer allergens with the molecular mass of 22 and 25 kD (major allergens), as well as 60 kD were identified. The 22 and 25 kD allergens are cross-reactive with the corresponding cow allergens.

Conclusions Deer allergy is a rare sensitization mainly affecting persons exposed to deer, who displayed an atopic disposition. From our results it can be assumed that this hypersensitivity is partly associated with allergy to cow dander.

Keywords: Type I allergy, IgE cross-reactivity, deer allergens, cow allergens

Clinical and Experimental Allergy, Vol 27, pp. 196–200. Submitted 3 May 1996; revised 18 June 1996; accepted 1 August 1996.

Introduction

Type I allergy to animal dander is frequently observed in individuals with atopic disposition [1–2]. However, due to the infrequent exposure, allergy to products associated with game and particularly to deer, is rare. It affects hunters, foresters and persons involved in the processing of venison (meat). Until now two case reports of allergy to deer have been described [3–4]. RAST-inhibitions indicated shared IgE-epitopes between deer and horse allergens [4].

In this study we characterized the allergens of deer using sera from eight deer-allergic individuals. In addition, IgE-immunoblot inhibition studies were performed to further investigate the presence of common IgE-epitopes among deer and other mammalian allergens.

Materials and methods

Characterization of allergic patients

Sera from eight patients (five male and three female), aged 30–65 yr with a positive case history, positive skin prick test and positive radio-allergo-sorbent-test (RAST) to deer allergens (Pharmacia, Uppsala, Sweden), were investigated in this study. General features and serological data of the individuals participating in this study are summarized in Table 1.

Pooled sera from 22 healthy individuals (negative case history concerning Type I allergy, negative SPT and RAST) were used as negative controls.

Protein extracts

Deer hair extract: pelt from deer was homogenized under liquid nitrogen and boiled in Laemmli sample buffer [5].

Correspondence: Susanne Spitzauer, Institute of Medical and Chemical Laboratory Diagnostics, AKH, University of Vienna, Währinger Gürtel 18–20, A-1090 Vienna, Austria.

Table 1. Characterization of patients allergic to deer dander

Patient	Age (yr)	Sex	Occupation	Deer-related clinical symptoms	Other sensitizations	RAST (kU(A)/l)	Total IgE (kU/l)
1	37	M	Hunter	co, rh, cu	Grass and tree pollen, dog, house dust mite	e1: 2, e3: 0, e4: 2, e5: 2, e82: 0, Re216: 2	794
2	65	F	Hunters wife	as, cu	Rye and wheat flour, cat, cow, pig, house dust mite	e1: 0, e3: 0, e4: 3, e5: 1, e82: 0, Re216: 3	353
3	61	M	Farmer	cu	Grass and tree pollen,	e1: 0, e3: 2, e4: 2, e5: 0, e82: 0, Re216: 2	560
4	51	F	Hunters wife	rh, as, cu	Grass and tree pollen, cat, dog, cow, horse, pig, guinea pig, house dust mite	e1: 2, e3: 3, e4: 3, e5: 3, e82: 0, Re216: 3	969
5	31	M	Hunter	co, rh, cu	Cow, pig, bee and wasp venom	e1: 0, e3: 0, e4: 3, e5: 0, e82: 0, Re216: 3	162
6	56	F	Hunters wife	as, cu	Dog, cow	e1: 0, e3: 0, e4: 3, e5: 0, e82: 0, Re216: 3	200
7	57	M	Hunter	rh, as	Dog, horse, cow, guinea pig, house dust mite	e1: ND, e3: 3, e4: 4, e5: 4, e82: ND, Re216: 4	1000
8	30	M	Hunter	co, as, cu	Grass and tree pollen, cat, dog, horse, cow guinea pig, rabbit, house dust mite, moulds	e1: 2, e3: 2, e4: 3, e5: 0, e82: 4, Re216: 4	516

co = conjunctivitis; rh = rhinitis; cu = contact urticaria; as = asthma bronchiale; ND = not done; e1 = cat dander; e3 = horse dander; e4 = cow dander; e5 = dog dander; e82 = rabbit dander; re216 = deer dander; kU(A)/L = kilo units (antigen) per litre.

Deer serum and urine: serum and urine were diluted in Laemmli sample buffer and boiled for 10 min.

Cat and dog hair/dander extracts: powdered cat and dog hair/dander were obtained from Allergon, AB (Engelholm, Sweden) and extracted as previously described [6].

Cow, horse and rabbit extracts: cow, horse and rabbit extracts were obtained from ALK (ALK-SQ, Horsholm, Denmark).

The total protein concentration of all extracts was determined [7] and adjusted to a final concentration of approximately 1.5 mg/ml.

SDS-PAGE and immunoblotting

SDS-PAGE was performed on a vertical slab gel apparatus (Hoefer, San Francisco, California, USA) according to the method of Laemmli [3]; 12% homogenous gels with 5% stacking gels were used. Approximately 200 µg/cm gel of each extract was applied. Separated proteins were

transferred to nitrocellulose as described by Towbin [8]. Molecular weights were estimated using protein standards (14.3–200 kD; Amersham, Buckinghamshire, UK). Immunoblotting was performed as described [6,9]. Bound IgE was detected by iodine 125-labelled anti-human IgE antibody (Pharmacia, Uppsala, Sweden). Autoradiography was performed at –70°C for 48–96 h, with Lanex R FAST intensifying screens (Kodak, Heidelberg, Germany) and Kodak Ortho G X-ray films (Kodak, Heidelberg, Germany).

Inhibition experiments

Sera from all patients ($n = 8$) were diluted 1:10 in buffer (50 mM Naphosphate pH 7.5, 0.5% v/v Tween 20) and preincubated overnight with approximately 30 µg of cat, dog, horse, bovine extract or human transferrin (control), at 4°C. Thereafter, preadsorbed sera were used to detect nitrocellulose blotted deer allergens as described above.

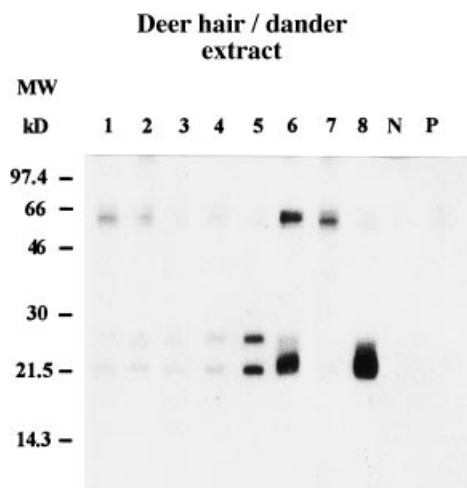


Fig. 1. Deer hair/dander proteins were separated by SDS-PAGE and blotted to nitrocellulose. IgE-binding components were detected with serum IgE from eight deer-allergic patients (lanes 1–8). N = serum pool from non-allergic individuals; P = buffer control.

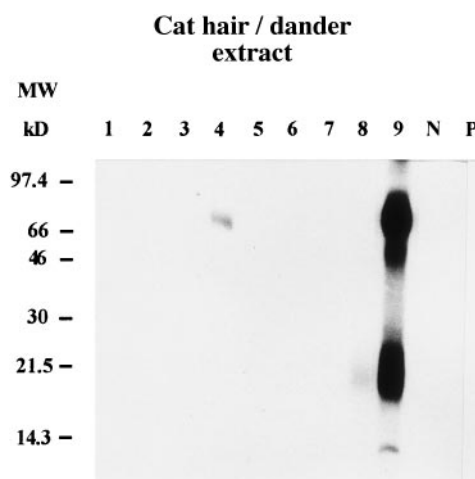


Fig. 2. Cat hair/dander proteins were separated by SDS-PAGE and blotted to nitrocellulose. Lanes 1–8 show the IgE-binding patterns of eight deer-allergic patients to cat allergens compared with the IgE-binding pattern of a cat-allergic patient (lane 9). N = serum pool from non-allergic individuals; P = buffer control.

Results

IgE-reactivity to deer allergens

Seven of eight patients exhibited IgE-binding to proteins with a molecular mass of 22 and 25 kD in the deer pelt extract (with the exception of patient no. 7). In addition, a 60 kD protein was recognized by four patients (#1,2,6,7). The results are shown in Figure 1.

No IgE-reactivity to extracts prepared from deer serum or urine was observed (data not shown).

IgE-reactivity to cat allergens

Patient 4 exhibited IgE-binding to a 69 kD protein, presumably cat albumin. Patient 8 recognized a component of 18 kD, probably the major cat allergen Fel d 1 (Fig. 2).

IgE-reactivity to dog allergens

Patient 4 exhibited IgE-binding to a 69 kD protein, presumably dog albumin. Patients 7 and 8 reacted weakly with a component of 19 kD, patient 7 displayed IgE-binding to a 30 kD protein (Fig. 3).

IgE-reactivity to horse allergens

Patient 4 displayed IgE-binding to a 19 and a 21 kD protein,

patient 7 recognized proteins of 19, 21, 25 and 46 kD, respectively (Fig. 4).

IgE-reactivity to rabbit allergens

IgE-antibodies from patients 7 and 8 bound to a 19 kD and a 15 kD protein (Fig. 5).

IgE-reactivity to cow allergens

Seven out of eight patients exhibited IgE-binding to bands in the molecular weight range of 22 kD, 25 kD and to a 28 kD component (Fig. 6).

Immunoblot-inhibition

Preincubation of patients' sera with cat, dog, horse and rabbit extracts did not inhibit IgE-binding to deer allergens in any serum tested ($n = 8$). A significant reduction of IgE-binding to deer allergens of 22 and 25 kD was observed in seven of eight patients tested after preincubation of patients' sera with the same amount of cow proteins as shown for a representative patient in Figure 7.

Discussion

In comparison with subjects allergic to 'normal' animals, some clinical peculiarities can be observed in patients

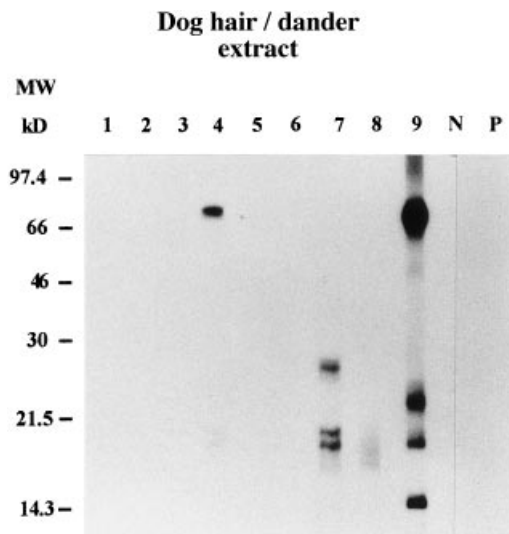


Fig. 3. Dog hair/dander proteins were separated by SDS-PAGE and blotted to nitrocellulose. Lanes 1–8 show the IgE-binding patterns of 8 deer-allergic patients to dog allergens compared with the IgE-binding pattern of a dog-allergic patient (lane 9). N = serum pool from non-allergic individuals; P = buffer control.

reacting to deer. The contact with the allergen is short but very intense. It takes place immediately after the shooting, when the trophies are taken. The symptoms — contact urticaria, conjunctivitis and respiratory problems — are caused by pelt and blood contact. Moreover, the exposure during the transport of the deer can result in severe airway obstruction.

In this study we investigated eight patients with clinical symptoms related to deer products. All male patients ($n = 5$) were exposed to deer allergens during hunting, the women ($n = 3$) with deer allergies all had husbands who were hunters. They experienced allergic symptoms not only after direct processing of the hunted material, but also when cleaning clothes or washing textiles used during the hunt. No food allergy after ingestion of the meat could be explored. All subjects displayed an atopic predisposition, i.e. elevated total IgE levels and additional sensitizations to other common allergens.

We prepared protein extracts from pelt, urine and serum of freshly shot deer and performed IgE-immunoblotting [6]. Using this technique we could detect IgE-binding to allergens in the pelt extract. Seven of eight patients recognized allergens in the MW range of 22 and 25 kD, which therefore can be designated major allergens. Moreover, IgE-binding to a protein of 60 kD was observed. As some of our patients were sensitized to other animals as well, we investigated whether this deer allergy could be caused by cross-reactivity between common animal-derived allergens [2,9]. Sera were

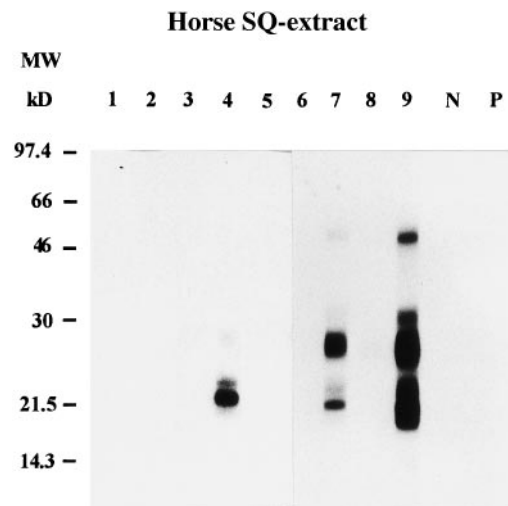


Fig. 4. Horse proteins were separated by SDS-PAGE and blotted to nitrocellulose. Lanes 1–8 show the IgE-binding patterns of eight deer-allergic patients to horse allergens compared with the IgE-binding pattern of a horse-allergic patient (lane 9). N = serum pool from non-allergic individuals; P = buffer control.

tested with extracts from cat, dog, horse, rabbit and cow, respectively. In accordance with personal case-histories additional IgE-reactivities to other animal hair/dander extracts (cat, dog, horse and rabbit) were observed.

Seven of eight patients showed IgE-binding to cow dander allergens. Moreover, in inhibition experiments,

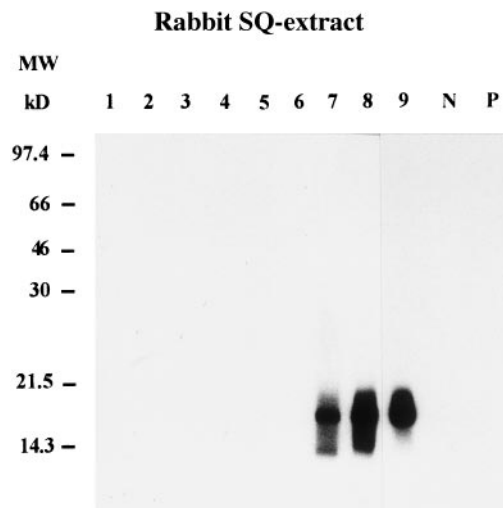


Fig. 5. Rabbit proteins were separated by SDS-PAGE and blotted to nitrocellulose. Lanes 1–8 show the IgE-binding patterns of eight deer-allergic patients to rabbit allergens compared with the IgE-binding pattern of a rabbit-allergic patient (lane 9). N = serum pool from non-allergic individuals; P = buffer control.

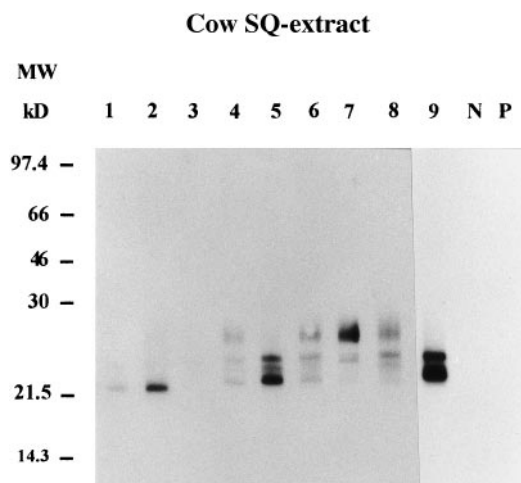


Fig. 6. Cow proteins were separated by SDS-PAGE and blotted to nitrocellulose. Lanes 1–8 show the IgE-binding patterns of eight deer-allergic patients to cow allergens compared with the IgE-binding pattern of a cow-allergic patient (lane 9). N = serum pool from non-allergic individuals; P = buffer control.

only preincubation with cow proteins led to significant blocking of IgE-binding to deer allergens. This observation fits the close taxonomical relationship between deer (*cervidae*) and cow (*bovidae*), which both belong to the ruminants of the order *Artiodactyla* (cloven-hoofed animals). Indeed, the majority of our patients reported that they had experienced allergic symptoms to cows prior to their first symptoms of deer allergy.

In conclusion, deer allergens can occasionally lead to allergic symptoms in predisposed (atopic) individuals, and this sensitization seems to be associated with allergy to cow proteins. After the confirmation of their experience by laboratory tests, all patients were able (with some limitations) to continue with their hobby or work, sometimes with the help of symptomatic medication.

References

- 1 Haahtela T, Jaakonmaki I. Relationship of allergen-specific IgE antibodies skin prick tests and allergic disorders in unselected adolescents. *Allergy* 1981; 36:251–6.

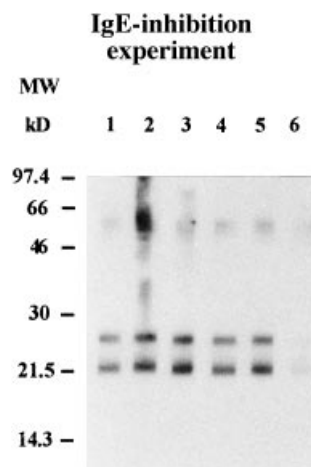


Fig. 7. IgE-immunoblot inhibition experiment. Deer hair/dander proteins were separated by SDS-PAGE and blotted to nitrocellulose. Serum from a representative deer-allergic patient (patient 8) was preadsorbed with transferrin (lane 1), cat extract (lane 2), dog extract (lane 3), rabbit extract (lane 4), horse extract (lane 5) and cow extract (lane 6).

- 2 Schou C. Defining allergens of mammalian origin. *Clin Exp Allergy* 1993; 23:7–14.
- 3 Munro-Ashman D. Sensitivity to deer scurf. *Acta Allergol* 1963; 18:168–9.
- 4 Huwyler T, Wüthrich B. A case of fallow deer allergy. Cross-reactivity between fallow deer and horse allergy. *Allergy* 1992; 47:574–5.
- 5 Laemmli UK. Cleavage of structural proteins during the assembly of the head of the bacteriophage T₄. *Nature* 1970; 227:680–5.
- 6 Spitzauer S, Schweiger C, Anrather J et al. Characterization of dog allergens by means of immunoblotting. *Int Arch Allergy Immunol* 1993; 100:60–7.
- 7 Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; 72:248–54.
- 8 Towbin H, Staehlin T, Gordon J. Electrophoretic transfers of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Nat Acad Sci* 1979; 76:4350–4.
- 9 Spitzauer S, Panjaitan B, Söregi G et al. IgE cross-reactivities against albumins in patients allergic to animals. *J Allergy Clin Immunol* 1995; 96:951–9.