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## Respiratory allergy induced by exclusive polysensitization to serum albumins of furry animals

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### KEY WORDS

*Allergic rhinitis, allergic sensitization, animal allergy, bronchial asthma, cat, dog, hypersensitivity, respiratory allergy, pet, serum albumin*

### SUMMARY

*In this report we describe an unusual case of exclusive allergic sensitization to furry animals, as a possible study model to speculate about different modalities of sensitization to allergens of common and less common mammalian species. A 27-year-old woman referred in our Allergological Centre for the occurrence of conjunctival and severe respiratory symptoms after contact with several animals such as cats, dogs, rabbits, horses, cows etc. Patient underwent clinical and anamnestic evaluation including a detailed information on the modality of exposure to different furry animals. Skin-prick-test (SPT) was performed with our routine panel of commercial standardized extracts (Lofarma Laboratories, Milan, Italy). Some animal allergenic extracts (rabbit, horse, rat, mouse, cavia, cow and hamster) have been tested by SPT one week after the routine SPT. A blood sample was taken for measurement of total IgE and specific IgE (CAP System, Phadia, Uppsala, Sweden) as well as Immunoblotting procedures. The results of *in vivo* and *in vitro* procedures revealed allergic sensitization only to animal – derived allergens. Total IgE were 59,3 kU/L. Immunoblotting showed a specific IgE-mediated sensitization of the patient to cow's, rabbit's and horse's serum albumins (SA). In conclusion, our case report confirms the role of SA as cross-reacting agent in allergic sensitization to furry animals. This finding suggests to perform SPTs to several furry animal allergens in all individuals with high level of allergic sensitization to common pets (cats and / or dogs) in order to identify allergy to other animals and consequently to avoid future exposures at risk.*

### Introduction

Exposure to furry animal – derived materials is a well recognized cause of occupational sensitisation for people who are in contact with animals in labs and other settings such as pet shops, farms, etc. (1).

It is well known that a large percentage of cat or dog-sensitized individuals has never kept these animals in their domestic environments (2). As a consequence, these pets' allergens are now considered ubiquitous being transferred in pet-free environments (private homes, schools, public

transport vehicles etc) through different transfers such as clothes and hair (3, 4).

Another mechanism of allergic sensitization to animal allergens without previous contact includes a cross-reaction through the lipocalins (5) or serum albumin (6).

To the best of our knowledge, no previous report on multiple allergic sensitization to different animal allergens has been published.

We describe an unusual case of exclusive allergic sensitization to several furry animals through different modalities of sensitization (direct/indirect contact and cross-reaction).

## Case report

A 27-year-old woman was referred in our Allergological Centre due to the occurrence of conjunctival and severe respiratory symptoms (rhinitis, cough, wheezing and dyspnea) mainly after contact with common pets such as cats and dogs, but also after occasional exposure to other animals (e.g. rabbits, horses, cows etc.).

Patient reported that no animal was kept steadily in her domestic environment.

Respiratory allergic symptoms of slight/moderate intensity were persistent all year round. Asthmatic symptoms were not controlled by usual long-term anti-asthma therapy (Fluticasone propionat: 500 mcg + Salmeterol xinafoate: 50 mcg – bid/die) plus Montelukast (10 mg/die). The patient denied any cutaneous symptom after contact with animals and/or after the ingestion of meats/milk.

## Methods

### *Personal data*

Detailed information about exposure to different furry animals was sought.

A familiar history of atopy was reported but patient did not have a personal history of previous cutaneous and/or respiratory symptoms.

### *Skin-prick-tests (SPT)*

SPT were performed with commercial extracts (Lofarma Laboratories, Milan, Italy) of house dust mites, *Parietaria* species, grasses, cat and dog dander, olive, birch, *Alternaria alternata*, *Cladosporium herbarum* and mugwort. Further, rabbit, horse, rat, mouse, cavia, cow and hamster were tested as well. Skin tests were performed and read according to accepted guidelines (7).

### *Evaluation of specific IgE antibodies*

Specific IgE to animal allergens were measured by ImmunoCAP (Phadia, Uppsala, Sweden).

### *PolyAcrylamide Gel Electrophoresis (PAGE) and Immunoblotting*

Protein separation was performed by electrophoresis using gels with the following composition:

*Running gel:* 15% acrylamide; 0.14% bis-acrylamide; 0.36 M TRIS-HCl buffer pH 8.8; 35% glycerol; 0.02% ammonium persulfate; and 0.15% N,N,N',N'-tetramethylethylenediamine (TEMED).

*Stacking gel:* 3.5% acrylamide; 0.09% bis-acrylamide; 0.125 M TRIS-HCl buffer pH 6.8; 0.02% ammonium persulfate; and 0.15% TEMED.

*Running buffer:* 25mM TRIS, 0.19M glycine, pH 8,8.

### *Immunoblotting*

After PAGE, proteins were transferred to PVDF membrane (Millipore) by western blotting in a Trans-blot Electrophoretic Transfer Cell (Bio-Rad). The membranes were blocked with 1% gelatin and washed three times with 0.25% gelatin solution (in 150 mM NaCl, 5mM EDTA, 50 mM Tris, 0.05% Triton-X) to prevent non-specific adsorption of the immunological reagents. The membrane was then immersed in 10 mL of 0.25% gelatin solution containing 300 µL of serum from allergic subject. Antigen-IgE complexes were detected using 10 µL of goat anti-human IgE antibodies labeled with alkaline phosphatase (Sigma, Milan, Italy). The developing solution contained 15% bromochloroindolyl phosphate (BCIP) and 30% nitro blue tetrazolium (NBT) (Sigma, Milan, Italy) in alkaline phosphatase buffer (100 mM Tris-HCl, pH 9.5, 100 mM NaCl, 5 mM MgCl<sub>2</sub>).

Quantitative evaluation of the immunoreactive bands was performed by a gel scanner (Sharp JX-330, Pharmacia Biothec) and the Image Master™ 1D Software. It allows the quantification of proteins by calculating the average density of pixels across the band length and integrating over the band width.

Classes of positive reactions were defined on the basis of an arbitrary scale of densitometric values and 6 classes of reactivity were identified.

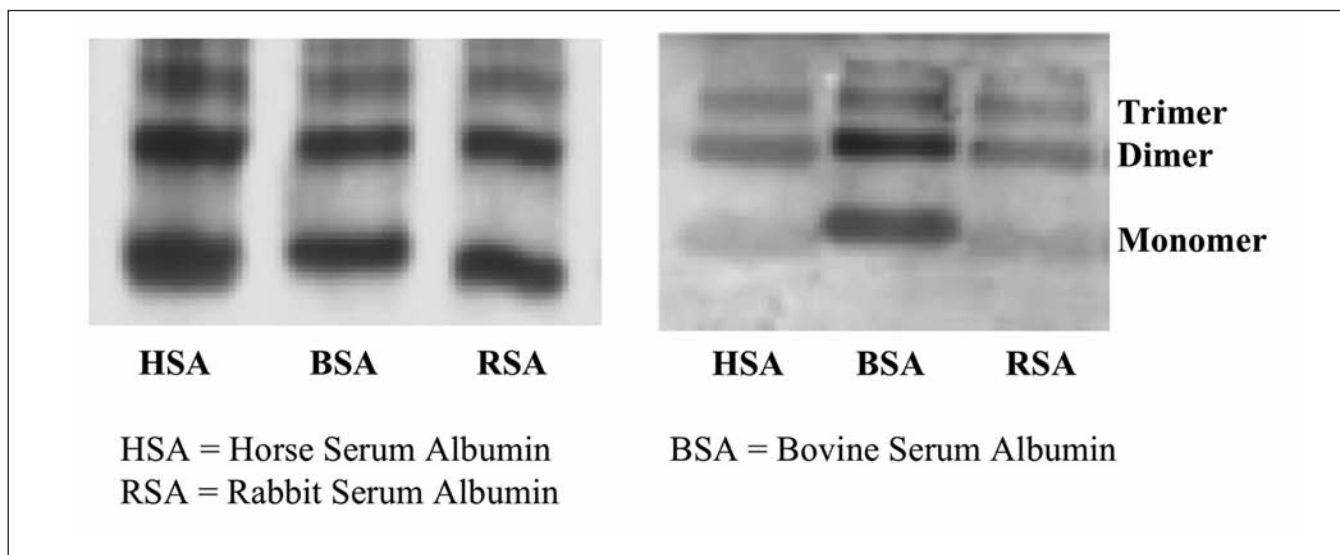
## Results

SPT revealed positivity only to animal – derived allergens (dog, cat, rabbit, horse, rat, mouse, cavia, cow and hamster).

Specific IgE were detected for all tested animal allergens (Tab. 1). Immunoblot analysis revealed IgE-reactivity to cow's, rabbit's and horse's serum albumins, with a good correlation between severity of response in SPT (wheal diameter) and the densitometric class of reaction in immunoblotting. No IgE reactivity against other proteins has been found (Fig. 1).

The strict avoidance of animal contact outdoors along with an intensive cleaning of indoor environments resulted in a progressive significant reduction of respiratory symptoms over the next two months.

**Figure 1** - SDS-PAGE (left) and Immunoblotting (right) of mammal's serum albumin. These procedures revealed IgE-reactivity to cow's, rabbit's and horse's serum albumins



**Table 1** - Cutaneous, laboratory and exposure data of a patient sensitized only to allergens of furry animals

Allergen	SPTs (Wheal diameter)	IgE (KU/L)	IgE (Class)	Animal exposure
Dog epithelia	9 x 9 mm	19.7	4	Direct (outside her home)
Cat dander	10 x 11 mm	24.8	4	Direct (outside her home)
Horse epithelia	7 x 6 mm	1.1	2	No
Rabbit epithelia	9 x 10 mm	1.7	2	Indirect
Hamster epithelia	8 x 9 mm	4.0	3	No
Cow epithelia	10 x 12 mm	0.5	1	No
Mouse epithelia	11 x 11 mm	0.4	1	No
Rat epithelia	7 x 7 mm	2.0	2	No
Guinea pig epithelia	6 x 6 mm	8.8	3	No
Rabbit serum	n.a	4.5	3	Indirect
Rabbit urine	n.a.	2.9	2	Indirect
Horse serum prot	n.a.	2.3	2	No
Rabbit meat (*)	4 x 4 mm	1.7	2	n.a.
Cow milk (*)	5 x 4 mm	0.5	1	n.a.

n.a. = not applicable

(\*) No symptoms after ingestion

## Discussion

To the best of our knowledge this is the first report of a multiple sensitization to furry animal allergens in a subject without professional exposure.

As shown in table 1, allergic sensitization to common pets

can be easily explained by the reported direct and frequent contact with cats/dogs although outside patient's home and the transport of cat/dog allergens indoors through her clothing.

Allergic sensitization to rabbit epithelia as well as rabbit serum and urine is likely to be induced by an indirect

mechanism of exposure because the frequent presence, at patient's home, of a couple of friends owners of four rabbits as pets.

Sensitization to other animal allergens cannot be explained neither by exposure direct (denied by patient) nor indirect considering their low ownership in our geographical area.

A likely explanation of high number of animal sensitization in our patient is the cross-reactivity between serum albumins of different mammals.

Serum albumin (SA), a thermolabile protein of approximately 68 kDa, constitutes an important panallergen involved in milk, meat, and epithelia allergy (8-10). Some important allergens of cat such as Fel d 2 (11) and dog such as Can f 3 (12) are SA and it may explain the high frequency of allergic sensitization to both pets. It has been shown that SA constitutes the cross-reacting allergen between epithelia of cat, dog, horse and pig (13, 14).

Although the primary modality of allergic sensitization to SA is the ingestion of different meats such as beef, pork etc., in some cases an inhalation route has been demonstrated (15).

Recently, it has been shown that first contact with SA was through cow's milk and that sensitization to SA may occur even without direct contact with animals (6). This mechanism could explain why this patient became allergic to uncommon mammalian allergens even in the absence of any contact with such animals.

Our patient showed cutaneous and serological sensitization to cow milk, rabbit meat and relative serum albumins, but the ingestion of these foods did not induce cutaneous/respiratory symptoms. The lack of reactions is likely connected to the heat lability of serum albumin with consequent modification of sensitization mechanisms (16).

In conclusion, our case report confirms the role of SA as cross-reacting agent in allergic sensitization to furry animals. This finding suggests to perform SPTs to several furry animal allergens in all individuals with high level of allergic sensitization to common pets (cats and/or dogs) in order to identify allergy to other animals and consequently to avoid future exposures at risk.

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