

Importance of albumin in cross-reactivity among cat, dog and horse allergens

R. Cabañas¹, M.C. López-Serrano¹, J. Carreira², P. Ventas², F. Polo², M.T. Caballero¹, J. Contreras¹, P. Barranco¹ and A. Moreno-Ancillo¹

¹Allergy Service, Hospital La Paz, Madrid, and ²Research Department, ALK-Abelló, Madrid, Spain

SUMMARY

Different allergenic proteins have been involved in cross-reactivity among animals. Albumins seem to be cross-sensitizing allergenic components. The aim of this study was to assess the importance of albumin as a cross-reactive allergen in patients sensitized to cat, dog and horse. One hundred and seventeen patients sensitized to cat were tested for IgE reactivity using skin prick tests and RAST assays with cat, dog and horse hair/dander extracts and their purified albumin extracts. RAST-inhibition studies were carried out to assess cross-reactivity among cat, dog and horse and among their purified albumins. It was found that 22% of patients exhibited specific IgE to cat albumin; 41% of patients sensitized to cat were also sensitized to dog and horse. Out of these patients, 21% had IgE to three albumins and 17% to two. Reciprocal inhibitions were observed among cat, dog and horse albumins and also among cat, dog and horse hair/dander extracts, using in the latter experiment sera from patients not sensitized to albumins. IgE binding to horse extract was inhibited 30% by its homologous albumin and IgE binding to cat and dog extracts in almost 15% by their respective albumins. It was concluded that albumins from these three animals share some epitopes that account for the cross-reactivity observed in around one-third of patients sensitized to cat, dog and horse. Nevertheless, more than 50% of specific IgE that cross-reacts among these three animals is directed to allergens other than albumin.

Key words: Cat, dog, horse sensitization - Albumin - IgE-cross-reactivity - RAST-inhibition

INTRODUCTION

Cat sensitization is a well known and increasing cause of rhinoconjunctivitis and asthma. Cat allergy is

often found in combination with dog and horse allergies. The question may be raised whether this is due to the fact that atopic individuals are highly predisposed to becoming sensitized to the aeroallergens they are exposed to, or whether this phenomenon is due to the existence of cross-reactivity among allergens from these animals.

Different authors have reported the existence of cross-reactivity among animals. Different allergenic proteins, such as immunoglobulins (1-3) α_1 -antitrypsin (3) and albumin (1-6), have been proposed as being responsible for this cross-reactivity. The allergenicity of serum albumin has received some attention and cross-reactivity among albumins from different animals has been demonstrated by Spitzauer *et al.* (5, 7) and Goubran Botros *et al.* (6). Studies by Spitzauer *et al.* (8) indicate the existence of common epitopes between major cat and dog allergens. This then led to the question as to whether there are any other common allergens that could contribute to the cross-reactivity observed among animals.

Our first objective was to determine how often cat sensitization appears in combination with dog and/or horse sensitization. We then studied the frequency of albumin sensitization in the group of patients with sensitization to two or three of these animals in order to determine the percentage of patients in whom albumin could account for the cross-reactivity.

Secondly, we aimed to confirm the existence of cross-reactivity among cat, dog and horse hair/dander extracts and among albumins from these three animals. Finally, we looked for the proportion of specific IgE to cat, dog and horse that was directed against albumin in the patients sensitized to these animals in order to de-

termine to what extent IgE to albumin could be responsible for the cross-reactivity observed among hair/dander extracts.

MATERIALS AND METHODS

Characterization of allergic patients

One hundred and seventeen patients with rhinoconjunctivitis and/or asthma and sensitized to cat according to duplicate positive skin prick test were selected. All patients were tested for IgE reactivity by skin prick tests and RAST assays with cat, dog and horse hair/dander extract, and with cat, dog and horse albumin. We considered that a patient was sensitized to any of these aeroallergens if they presented a positive skin prick test and/or RAST.

Among the patients with positive sera to cat, dog and horse hair/dander extracts, seven sera from patients with the highest IgE titers to albumins from cat, dog and horse and another seven patients without IgE to these albumins were chosen for RAST-inhibition studies.

Natural allergen extracts

Cat and dog hair/dander extracts were from ALK-Abelló (Horsholm, Denmark). Powdered hair/dander from horse was purchased from Allergon (Allergon, Engelholm, Sweden). Hair/dander was mixed with phosphate buffered saline (PBS) at 10% weight/volume and stirred for 1 h at room temperature. After centrifugation at 27,000 g for 20 min at 4 °C, the supernatant was dialyzed, filtered and it was lyophilized for later use. The protein concentration was measured by Lowry's method (9).

Purified cat, dog and horse albumins were purchased from Sigma (Sigma, St. Louis, MO, USA).

Skin prick testing and RAST experiments

Skin prick tests were carried out in duplicate with extracts of cat (100 BU/ml; Fel d1: 40 µg/ml), dog (100 BU/ml) and horse (5% wt/vol) and purified albumins from these three animals prepared at 10 mg/ml in glycerol. The different vials for the tests were from the same manufacturing lot. Histamine at 10 mg/ml and serum saline were used as positive and negative controls, respectively. Criteria for positivity used were as recommended by the European Academy of Allergology and Clinical Immunology (10). A skin prick test was considered as positive if a wheal (≥ 3 mm in diameter) was present with flare.

Total serum IgE level was measured by microparticle enzyme immunoassay (MEIA) testing using the IMx system (Abbott Diagnostics, Chicago, IL, USA). The results were expressed in kilounits per liter (KU/L).

In vitro specific IgE to cat, dog and horse hair/dander extracts and to cat, dog and horse albumin were detected in duplicate by radioallergosorbent test. The different extracts at 1 mg/ml were placed onto cyanogen bromide activated cellulose discs according to the method described by Ceska (11).

To detect specific IgE against the different extracts, 50 µl of each undiluted sera was incubated with antigen-coated discs for 3 h at 37 °C. After washing three times with PBS-T the discs were incubated with 50 µl of ^{125} I-antihuman IgE (12) (100,000 c.p.m.) overnight at room temperature. Then the discs were washed four times and the radioactivity bound was measured in a Packard Multiprias gamma-scintillation counter (Packard Instruments, Downers Grove, USA). The control employed was HSA (commercial human serum albumin) (Sigma, St. Louis, MO, USA).

The results were expressed in RAST class according to the reference curve of the Phadebas RAST system (Pharmacia Diagnostics, Uppsala, Sweden), graded from class 0 (level of allergen absent or undetectable) to class 4 (very high). A RAST class higher or equal to 1 was considered positive.

RAST-inhibition experiments

We performed three different RAST-inhibition experiments.

Experiment A

Firstly, we used cat, dog and horse hair/dander extract as solid phases and as inhibitors and seven individual sera from patients with IgE to these extracts but without IgE to their respective albumins.

Experiment B

Secondly, by using seven individual sera from patients with IgE reactivity to the three animals and also to their respective albumins, we performed a RAST-inhibition assay using in this case cat, dog and horse albumin as solid phases and as inhibitors.

Experiment C

Finally, we performed RAST-inhibition experiments using cat, dog and horse hair/dander extract as solid phases and the respective albumin as inhibitor and a pool of sera from four patients with IgE to the extracts and to their albumins.

The RAST-inhibition experiments were performed by adding 50 µl of each undiluted sera or diluted 1:2

(depending on the RAST class), to 50 µl of inhibitor extract at 10 mg/ml or HSA as a control, and incubating for 30 min at 37 °C. Coated discs were then added and incubated for 3 h at 37 °C. After washing three times, the discs were incubated with 50 µl of ¹²⁵I-anti-human IgE (100,000 c.p.m.) overnight at room temperature. Discs were washed another three times with PBS-T and radioactivity was measured with a gamma-counter.

The percentage of inhibition of IgE-binding to the solid phase for each inhibitor extract was calculated according to the following formula:

$$\% \text{ Inhib} = 100 \times 1 - \frac{(\text{c.p.m. after inhib with each extract}) - (100\% \text{ inhib})}{(\text{c.p.m. after inhib with HSA}) - (100\% \text{ inhib})}$$

RESULTS

Patients sensitized to cat, dog and/or horse

Forty-eight patients (41.0%) showed a positive skin prick test and/or RAST to cat, dog and horse; 21 (17.9%) only to cat and dog; 13 (11.1%) to cat and horse; and 35 (29.9%) did not show any IgE reactivity to either dog or horse. This means that 70% of patients sensitized to cat were also sensitized to at least one of the other animals we tested for.

Patients sensitized to albumin

Twenty-six patients (22.2%) had specific IgE to cat albumin, 16 (13.7%) were sensitized to dog albumin and 24 (20.5%) to horse albumin. Of the 48 patients sensitized to cat, dog and horse, only 10 (20.8%) had IgE reactivity to the albumins of these three animals and eight (16.6%) against two of them. In the group of patients sensitized to the three epithelia, 21 out of 48 (43.8%) had IgE reactivity against cat albumin. No patients were sensitized only to two epithelia presented IgE against two albumins.

RAST-inhibition experiments

Experiment A

The results of the experiment of inhibition of hair/dander extracts with hair/dander extracts are shown in Figure 1. Cat and dog hair/dander extract inhibited each other to practically the same extent, with some individual variations, ranging from 30-75%; there was one serum for which the cat extract did not inhibit the dog extract.

Horse hair/dander extract was inhibited by dog and cat hair/dander extract at a high percentage (above 70%) in all sera; however, horse extract only inhibited cat extract by around 30%, and for dog extract, except for one serum at 48% and another at 15%, in the remaining sera the inhibition was not significant.

Experiment B

The results of the inhibition of albumin extracts with albumin extracts are shown in Figure 2.

Cat albumin extract inhibited horse albumin at a very high percentage. Dog albumin was inhibited by cat albumin but with wide individual variation (in four sera 71-100%, in two sera 20-35% and in one sera there was no inhibition).

Strong inhibition of IgE binding to cat and horse albumin was observed on preincubation of all sera with dog albumin, but with a lower percentage for cat albumin (49-99%) than for horse albumin (70-100% except one serum 24%). Dog albumin inhibits cat albumin more than vice versa.

Horse albumin was clearly the least inhibitory. Using dog albumin as solid phase, the inhibition reached by horse albumin was not significant in four sera; in another serum it was 18% and in two 50%. Inhibition of IgE binding to cat albumin was slightly higher. In two sera it was high (87-97%) and in five it was low (15-30%).

Experiment C

We inhibited the binding of specific IgE to cat, dog and horse hair/dander extract by preincubating the pool of four sera with each homologous albumin. The inhibitions observed were 15% by dog albumin, 16% by cat albumin and 30% by horse albumin.

DISCUSSION

Our first objective was to confirm the existence of cross-reactivity among cat, dog and horse allergens reported by other authors and to assess the role of albumin in this cross-reactivity.

The results obtained by skin prick tests and RAST with cat, dog and horse hair/dander extracts indicate that there is a strong association between sensitization to cat and dog and less so to horse, which is not a chance finding.

We observed that only 30% of patients sensitized to cat were not sensitized to either dog or horse and 41% were sensitized to cat, dog and horse. Twenty-one percent of the patients sensitized to these three epithelia exhibited IgE to the three albumins and 17% against two of them. We believe that albumin may only play a

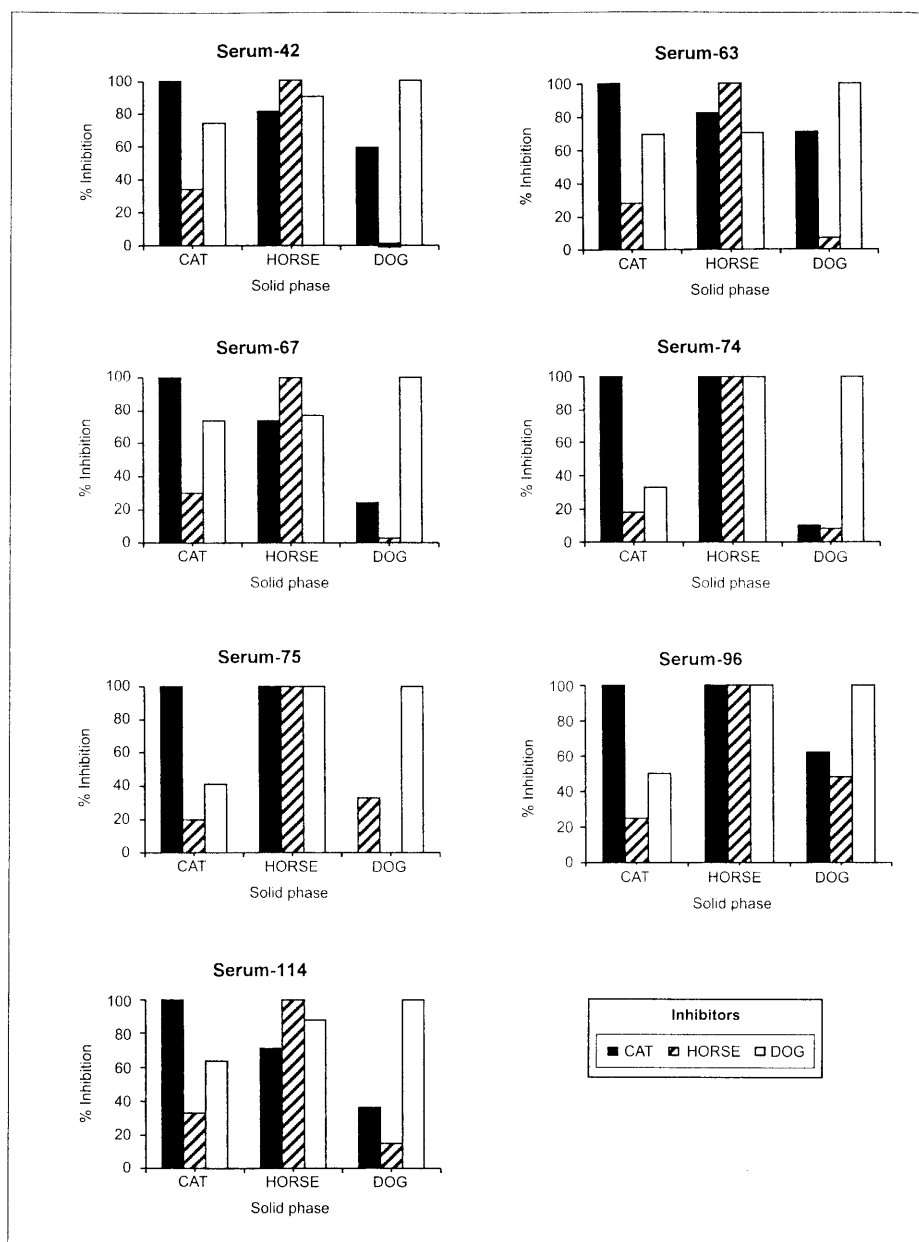


Fig 1. RAST-inhibition assays using cat, dog and horse hair/dander extracts as solid phases and as inhibitors and seven individual sera from patients with IgE to these extracts but not to their albumins.

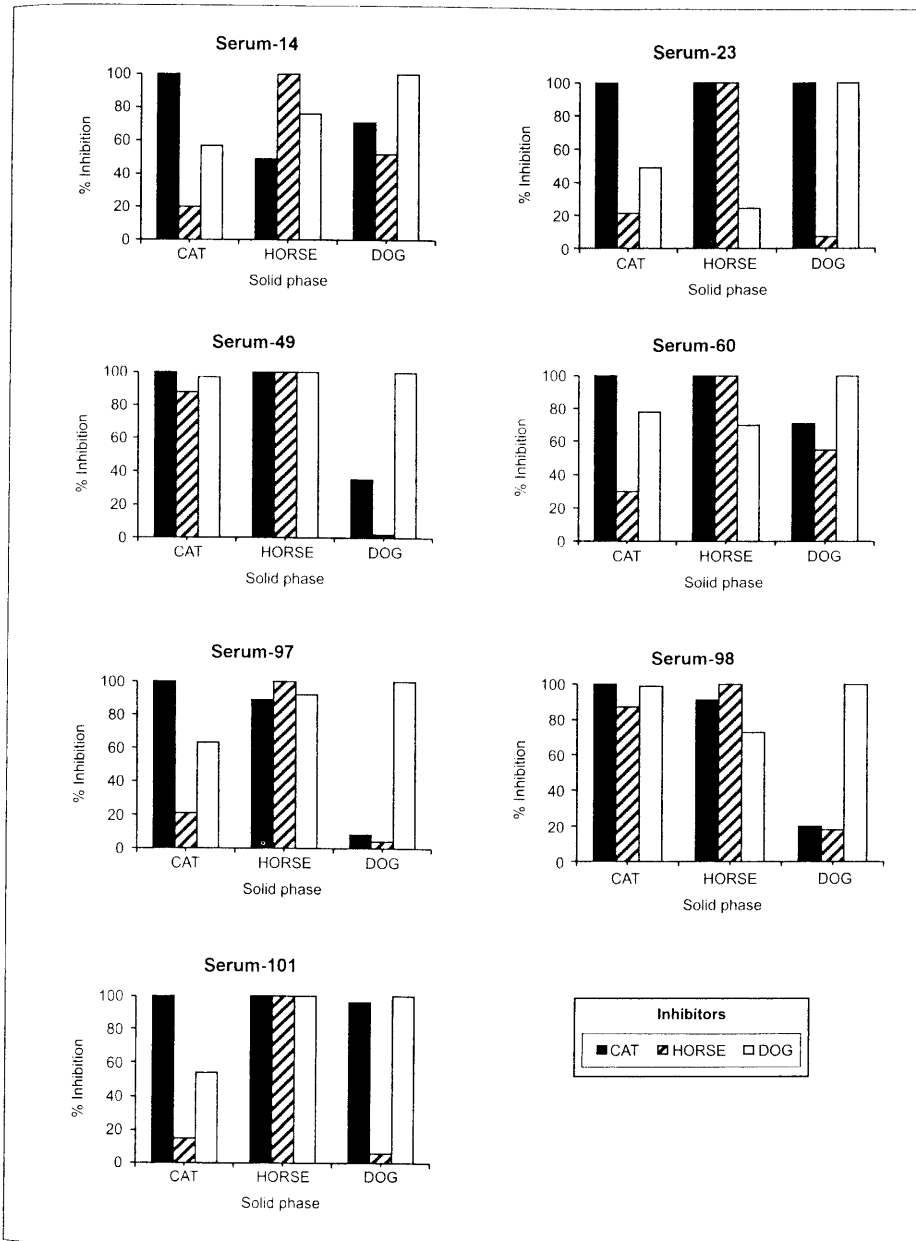


Fig 2. RAST-inhibition assays using cat, dog and horse albumin extracts as solid phases and as inhibitors, and seven individual sera from patients with IgE to cat, dog and horse hair/dander extracts and also to their respective albumins.

role in cross-reactivity in patients exhibiting IgE to two or three albumins, meaning from 17-38% of patients sensitized to cat, dog and horse. In the 62-83% of the cases remaining, cross-reactivity among other allergens could be involved or they could be independent sensitizations.

There was another interesting finding: of the patients sensitized to three epithelia, 43.8% exhibited IgE reactivity to cat albumin, which is double the prevalence found in the whole group of patients sensitized to cat (22.2%). Therefore, we think that sensitization to cat serum albumin should be considered a useful marker of possible sensitization to other animals.

The 22% frequency of sensitization to cat serum albumin found in our patients is similar to that found by Hilger *et al.* (13), which lies between 14% and 23%. We confirmed that cat albumin is a minor allergen in cat sensitization according to actual allergen nomenclature (14), as has been suggested by Duffort *et al.* (15) and Lowenstein *et al.* (16).

Through RAST-inhibition experiments we confirmed the existence of cross-reactivity among albumins, since a reciprocal inhibition was observed among them. This phenomenon has been pointed out by different authors; our results are in agreement with these studies in some respects and differ in others.

In the inhibitions between cat and dog albumin, we observed that dog albumin always inhibits cat albumin at a high percentage; nevertheless, as an inhibitor cat albumin shows great individual variations. This indicates that cat and dog albumin have some antigenic determinants that are common between them and others that are specific to them, with dog albumin having more specific determinants. Our findings differ from those expressed by Goubran Botros *et al.* (6), which showed that the allergenicity and antigenicity of both dog and cat albumin were almost completely identical.

As for horse albumin, this was highly inhibited by dog and cat albumin (70-100%); nevertheless, horse albumin only slightly inhibited cat and dog albumin. This indicates that cat and dog albumin share some common epitopes with horse albumin, but they also have specific epitopes. From our results, the most specific of the albumins tested seems to be dog, followed by cat then horse.

Goubran Botros *et al.* (6) performed ELISA-inhibition assays and found very similar results to ours using horse albumin as solid phase (inhibition near 100%). Nevertheless, using horse albumin as inhibitor and dog albumin as solid phase, the inhibition observed was 54-65%, which are much higher values than those we obtained.

Spitzauer *et al.* (7) performed ELISA-inhibition assays and found a strong inhibition of IgE binding to all albumins on preincubation with either dog or cat albumin, which is similar to our results. However, there was a difference in the behavior of horse albumin between the experiments by Spitzauer *et al.* (7), Goubran Botros *et al.* (6) and our experiment. In those by Spitzauer *et al.*

et al., the inhibition that cat and dog albumin produced on IgE binding to horse albumin was lower than the inhibition that horse albumin produced on dog and cat albumin, which in five sera was close to 100% and the opposite to what we observed.

Our results and those from other authors should be interpreted cautiously due to the small number of sera used [Goubran Botros *et al.* used five (6), Spitzauer *et al.* six (5) and eight (7) and we used seven]. This limitation is a reflection of the difficulty in finding patients with high IgE titers to the different albumins and indicates that the prevalence of concomitant sensitization to some albumins is rather low, which then limits the extrapolation of these results to determine clinical importance. Besides the reduced number of sera used, we think that the differences in the results could be explained by the different criteria used for sera selection. Goubran Botros *et al.* (6) selected patients allergic to horses, Spitzauer *et al.* (5) those allergic to dogs or animals in general (7), and we selected patients sensitized to cats.

Goubran Botros *et al.* (6) have suggested that IgE against albumins from different animals is primarily induced by dog serum albumin. They reached this conclusion due to the behavior of albumin in ELISA-inhibition experiments and the fact that in the horse-allergic patients selected, the specific IgE titer to dog albumin was the highest, followed by the IgE titer to cat albumin and then to horse albumin. Our results and those of Spitzauer *et al.* (7) are not in agreement with Goubran Botros *et al.* (6) on this point. In fact, the IgE titers to cat and dog albumin that we detected were very similar (RAST class 3 and 4) and slightly lower for horse albumin (RAST class 2 and 3). From the behavior of albumin in the RAST-inhibition assay alone we could hypothesize on the primary sensitization for each patient.

Our RAST-inhibition experiments using cat, dog and horse hair/dander extract as inhibitors and as solid phases showed that there is cross-reactivity among these extracts and that, in this case, since we used sera from patients without IgE to albumin, the albumin was not involved. This indicates that there are other common allergens among cats, dogs and horses besides albumin. Major cat and dog allergens could be responsible in part for this cross-reactivity as demonstrated by Spitzauer *et al.* (8).

Spitzauer *et al.* (8) performed CAP-inhibition with sera from five patients allergic to both dog and cat but with no IgE to albumin by preadsorbing with cat or dog hair/dander extract. Dog extract inhibited IgE binding to cat extract by an average 86.6%, whereas cat extract was able to inhibit dog extract by 57%. These results are higher than those obtained in our study (86.6% vs. 58% for dog and 57% vs. 42% for cat). We think that the differences can be explained again by the small number of sera used (five by Spitzauer *et al.* and seven by us) with great individual variations, and with clear repercussions on mean inhibition value. On the other

hand, the techniques used were different (CAP and RAST). The results obtained in the RAST-inhibition assays should be interpreted cautiously, since the apparent deficiency of specific allergens in one animal species could be due to the primary sensitization of the subject.

Spitzauer *et al.* (5) found that in patients sensitized to dog albumin, 70-100% of dog-specific IgE was against albumin. Our results are quite lower, at 15%. This variation again can probably be explained by the difference in the method used, since they performed immunoblotting and preincubation of six individual sera with dog albumin, whereas we carried out a RAST-inhibition assay preincubating a pool of sera with dog albumin.

The percentage of cat-specific IgE against cat albumin detected in our study (16%) is in agreement with the literature: Lowenstein *et al.* (16) indicate 25% and Schou (17) 10-25%. From these results we conclude that albumin could only be partially responsible for the reciprocal inhibitions observed among cat, dog and horse hair/dander extract (less than 30% from our results); the rest of inhibition observed should be attributed to other allergens.

ACKNOWLEDGEMENTS

This study was carried out with the support of a grant from the Spanish Society of Allergy and Clinical Immunology Foundation.

RESUMEN

Se ha implicado a diferentes proteínas alergénicas en las reacciones cruzadas entre animales. La albúmina parece tener componentes alergénicos de sensibilización cruzada. El objetivo del estudio fue determinar la importancia de la albúmina como alérgeno de reacción cruzada en pacientes sensibilizados al perro, gato y caballo. En 117 pacientes sensibilizados al gato, se determinó la presencia de IgE mediante skin prick test y RAST con extractos de epitelio de gato, perro y caballo, y sus extractos purificados de albúmina. Se llevó a cabo RAST-inhibición para determinar la reactividad cruzada entre gato, perro y caballo y entre sus albúminas purificadas. Un 22% de los pacientes mostraron IgE específica frente a la albúmina de gato; un 41% de los pacientes sensibilizados al gato lo fueron también al perro y al caballo. De estos pacientes, el 21% tuvieron IgE frente a tres albúminas y el 17% a dos de ellas. Se observó una inhibición recíproca entre la albúmina de gato, perro y caballo y también entre extractos de epitelios, utilizando en este último experimento suero de pacientes no sensibilizados a la albúmina. La unión de la IgE al extracto de caballo fue inhibida en un 30% la albúmina homóloga y la unión de IgE al extracto de gato y perro en un porcentaje cercano al 15% por sus respectivas albúminas. Se concluye que las albúminas de estos tres animales comparten algunos epítomos que justifican la reactividad cruzada observada en alrededor de un tercio de los pacientes sensibilizados al gato, perro y caballo. Sin embargo, más del 50% de la IgE específica que reacciona cruzadamente entre estos tres animales está dirigida a alérgenos diferentes a la albúmina.

Palabras clave: Sensibilización al gato, perro, caballo - Albúmina - IgE reactividad cruzada - RAST-inhibición

REFERENCES

- Ohman, J.L., Bloch, K.J., Kendall, S., Lowell, F.C. Allergens of mammalian origin. IV. Evidence for common allergens in cat and dog serum. *J Allergy Clin Immunol* 1976, 57: 560-8.
- Lowenstein, H., Markussen, B., Weeke, B. Identification of allergens in extract of horse hair and dandruff by mean of crossed radioimmuno-electrophoresis. *Int Arch Allergy Appl Immunol* 1976, 51: 38-47.
- Blands, J., Lowenstein, H., Weeke, B. Characterisation of extract of dog hair and dandruff from six different dog breeds by quantitative immunoelectrophoresis. Identification of allergens by crossed radioimmuno-electrophoresis (CRIE). *Acta Allergologica* 1977, 32: 147-69.
- Boutin, Y., Hébert, H., Vrancken, E.R., Mourad, W. Allergenicity and cross-reactivity of cat and dog allergenic extracts. *Clin Allergy* 1988, 18 (3): 287-93.
- Spitzauer, S., Schweiger, C., Sperr, W.R., Pandjaitan, B., Valent, P., Muhl, S., Ebner, C., Scheiner, O., Kraft, D., Rumpold, H., Valenta, R. Molecular characterisation of dog albumin as a cross-reactive allergen. *J Allergy Clin Immunol* 1994, 93: 614-27.
- Goubrian Botros, H., Gregoire, C., Rabillon, J., David, B., Dandeu, J.P. Cross-antigenicity of horse serum albumin with dog and cat albumins: Study of three short peptides with significant inhibitory activity towards specific human IgE and IgG antibodies. *Immunology* 1996, 88: 340-7.
- Spitzauer, S., Pandjaitan, B., Söregi, G., Muhl, S., Ebner, C., Kraft, D., Valenta, R., Rumpold, H. IgE cross-reactivities against albumins in patients allergic to animals. *J Allergy Clin Immunol* 1995, 96: 951-9.
- Spitzauer, S., Pandjaitan, B., Muhl, S., Ebner, C., Kraft, D., Valenta, R., Rumpold, H. Major cat and dog allergens share IgE epitopes. *J Allergy Clin Immunol* 1997, 99: 100-6.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951, 193: 265-75.
- Mailing, H.J. Methods of skin testing. Position paper. Allergen standardisation and skin tests. *Allergy* 1993, 48 (4): 55-56.
- Ceska, M., Eriksson, R., Varga, J.M. Radioimmunosorbent assay of allergens. *J Allergy Clin Immunol* 1972, 49: 1-9.
- Sánchez-Madrid, F., Morago, G., Corbi, A., Carreira, J. Monoclonal antibodies, three distinct epitopes on human IgE: Their use for determination of allergen-specific IgE. *J Immunol Methods* 1984, 73: 367-78.
- Hilger, C., Kohnen, M., Grigioni, F., Lehnert, C., Herntges, F. Allergic cross-reactions between cat and pig serum albumin. Study at the protein and DNA levels. *Allergy* 1997, 52 (2): 179-87.
- King, T.P., Hoffman, D., Lowenstein, H., Marsh, D.G., Platts-Mills, T.A.E., Thomas, W. Allergen nomenclature. *J Allergy Clin Immunol* 1995, 96: 5-14.
- Duffort, O., Carreira, J., Lombardero, M. Characterisation of the main IgE-binding components of cat dander. *Int Archs Allergy Appl Immunol* 1987, 339-44.
- Lowenstein, H., Lind, P., Weeke, B. Identification and clinical significance of allergenic molecules of cat origin. *Allergy* 1985, 40: 430-41.
- Schou, C. Defining allergens of mammalian origin. *Clin Exp Allergy* 1993, 23: 7-14.

Correspondence: Rosario Cabañas, Avda. Europa 4, P.2, 1-C, 28224 Madrid, Spain.