

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

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SUMMARY

Horse serum albumin is present in the near vicinity of the animal, while dog and cat serum albumins are very common allergens present in house dust. Human patients clinically defined as allergic to horse could react with horse serum albumin by means of IgE or IgG antibodies. Studies regarding the specificities of these antibodies by inhibition enzyme-linked immunosorbent assay (ELISA) and depletion experiments have demonstrated that they are directed against dog serum albumin and cross-react not only with horse serum albumin but with other serum albumins from different origins. To investigate these observations further, we isolated and characterized three tryptic peptides (P1, P2 and P3) from horse serum albumin. The peptide P1 contains loops 1 and 2 of the first domain, P2 is derived from loop 4 of the second domain, and P3 contains the disulphide loop 9 of the third domain. These were able to inhibit the binding of the patients' IgE and IgG antibodies to horse albumin as well as to dog and cat serum albumins. This indicates that these peptides are involved in the observed cross-reactions. They also shared common epitopes, as revealed by human IgE antibodies. After reduction and alkylation, they totally lost their inhibitory capacity, suggesting that the intra-chain disulphide bridges, essential for the preservation of the loop structure, probably maintain their allergenic/antigenic reactivity.

INTRODUCTION

Allergenicity of serum albumin from different animal species has received some attention and several studies have reported on its importance in humans at different levels of efficiency.^{1–4} Purified horse serum albumin was shown to be an allergen as early as 1950.⁵ In 1967 Chan & Porter⁶ demonstrated the presence of antibodies (assigned to the IgE class) that bind horse serum albumin in the sera of patients sensitive to horse. Histamine release from human blood basophils, after triggering the cells with the allergen, was also used as an *in vitro* assay to study IgE-mediated reactions; the allergen induces bridging between two IgE antibodies that are fixed on the target cells (mast cells or basophils) via high-affinity receptors, and causes a release of vasoactive mediators such as histamine and serotonin. Wahn *et al.*⁷ demonstrated the capacity of purified horse serum albumin to induce around 30% of histamine release from human blood basophils in three out of 22 children allergic to horses. By means of Western blot analysis, it was demonstrated that horse serum albumin showed IgE-binding activity in a sera pool of eight horse-allergic patients.⁸

In addition to the horse serum albumin, the molecule subject of this report, different studies have evaluated the capacity of other serum albumins from different animal species to bind human IgE antibodies. Dog, rat, mouse and chicken serum albumins were capable of inducing histamine release from blood basophils of a patient allergic to dog.⁹ The histamine release observed was more pronounced with dog albumin (40%) than with rat, mouse and chicken albumin (15–25%). The ability of rat and mouse serum albumin to provoke histamine release from the basophils of patients allergic to rats was compared.¹⁰ Twenty-four per cent of these patients had basophils releasing at least 10% and up to 100% of their histamine when triggered with rat serum albumin. The dose–response curve with mouse serum albumin was similar. The basophils from bovine serum albumin (BSA)-sensitive patients as well as from BSA-immunized rabbits triggered by intact BSA or large BSA fragments, BSA-P_{1–306}, BSA-P_{307–582} and BSA-T_{377–582}, released around 50–60% of the total histamine, but failed to release histamine with the fragment BSA-P_{505–582}.¹¹ The authors suggested the presence of at least four IgE-reactive antigenic sites on BSA.

In our study we investigated the reactivity of IgE and IgG antibodies from humans naturally sensitized to horse allergens towards various albumins, mainly from dog and cat, the most common antigens present in the human environment. We kept horse serum albumin as a reference. The study was performed by means of inhibition enzyme-linked immunosorbent assay (ELISA)

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and antibody depletion of the human patients' sera on immobilized albumins. We also analysed the allergenic and antigenic activity of peptides generated by tryptic hydrolysis of a highly purified horse serum albumin, without previous rupture of the disulphide bonds. These peptides showed significant immunochemical inhibitory activity towards specific human IgE and IgG antibodies.

MATERIALS AND METHODS

Albumins

Albumins from different animal species were purchased from Sigma Chemicals (St Louis, MO): human serum albumin (HSA), bovine (cow) (BSA), chicken (CkSA), pigeon (PnSA), pig (PSA), rat (RSA), goat (GSA), rabbit (RbSA), donkey (DkSA), equine (horse) (ESA), cat (CSA) and dog (DSA). Zebra serum albumin (ZSA), not commercially available, was purified from zebra serum on immobilized metal ion affinity chromatography (IMAC)¹² using copper as the ligand and based on a previous report where cat albumin was purified.¹³ ESA (Sigma) was further purified by the same method.

Chemicals

Alkaline phosphatase-conjugated goat anti-human IgE (ϵ -chain specific), peroxidase-conjugated goat anti-human IgG (γ -chain specific), p-nitrophenyl-phosphate (p-NPP), o-phenylenediamine dihydrochloride, L-1-tosylamide-2-phenylethylchloromethyl ketone (TPCK)-treated trypsin, dithiothreitol and iodoacetamide were obtained from Sigma. Acetonitrile and trifluoroacetic acid were from Merck (Darmstadt, Germany).

Patients' allergic sera

Human sera were obtained from volunteers (at Bichat Hospital, Paris, France), allergic to different allergens (such as mites, pollen or animals).

Human sera IgE and IgG antibody depletion

To deplete patients' sera of their IgE or IgG antibodies specific to different albumins, we coupled these albumins to CNBr-activated Sepharose 4B (Pharmacia, Uppsala, Sweden), according to the manufacturer's instructions, then diluted sera were incubated for 2 hr at 37° with the immobilized albumin. The collected unadsorbed fractions (the depleted sera) were tested in ELISA to determine the specificity of the remaining IgE or IgG antibody-binding activity after complete adsorption with the homologous immobilized albumin.

Direct ELISA

Human IgE and IgG antibody concentrations to different albumins were evaluated by ELISA.¹⁴ Concerning inhibition ELISA, the patients' sera were previously mixed with an equal volume of serially diluted antigens. The percentage of inhibition was calculated according to the following formula: $[1 - (\text{OD in presence of inhibitor} / \text{OD in absence of inhibitor})] \times 100$.

The statistical significance of the observed results was tested by using the chi-squared test.

Tryptic digestion of ESA

Purified horse serum albumin was digested with TPCK-treated trypsin in 0.1 M ammonium bicarbonate buffer, pH 8.2, at 37°, at an enzyme/substrate ratio of 1:50, for 15 min at 37°.

Isolation and purification of ESA peptides

Peptides were separated by reversed-phase high-performance liquid chromatography (RP-HPLC) on a LiChrosphere 100 RP-18, 25 × 0.4 cm column (Merck). Elution was performed using a linear gradient from 10% to 60% acetonitrile-trifluoroacetic acid at a flow rate of 0.5 ml/min; the eluate was monitored at 214 nm.

Identification of ESA fragments

The ESA peptides were identified by N-terminal amino acid sequencing of different amino acid residues using Edman degradation on a 473 protein sequencer (Applied Biosystems, Foster City, CA). Phenylthiohydantoin (PTH) derivatives of amino acids were separated and identified by on-line RP-HPLC with a RP-18 column (Brownlee Labs, Applied Biosystems). Biobrene (Applied Biosystems) was added to prevent wash-out and to improve the initial yields.¹⁵

Reduction and carboxymethylation of proteins

Horse serum albumin and purified ESA peptides were reduced with dithiothreitol and alkylated with iodoacetamide.¹⁶

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot

PAGE (18% gel) was performed in the presence of SDS using Laemmli's discontinuous buffer system¹⁷ in a Novex (San Diego, CA) electrophoresis unit. The separated proteins were transferred to a nitrocellulose membrane (Schleicher & Schuell, Dassel, Germany). Unoccupied sites were blocked by milk. The membranes were then incubated with patient serum, and IgE-binding antigens were detected with ¹²⁵I-labelled rabbit anti-human IgE antibody (Pharmacia), followed by autoradiography.

Rocket immunoelectrophoresis (RIE)

The antigenic properties of the ESA peptides were investigated by RIE¹⁸ using rabbit serum raised against native ESA. The antibody containing agarose gel was poured in two parts; the upper part contained 0.5 $\mu\text{l}/\text{cm}^2$ and the lower part 0.1 $\mu\text{l}/\text{cm}^2$. Different concentrations (0.1 and 1.0 mg/ml) of ESA and ESA peptides were added to the wells.

RESULTS

Human sensitization towards albumins from different animal origins

Results were essentially obtained using five patients' sera selected after performing the following experiments. Sera of 43 patients clinically defined as allergic to horse were tested with a highly purified horse major allergen Equ c1,¹⁹ in order to confirm the diagnosis. These sera were also examined by direct ELISA for the presence of IgE antibodies to 13 albumins from different animal species. We found that 39% of the patients' sera contained IgE antibodies to DSA and CSA, 18% to ESA, 15% to DkSA and ZSA, 12% to GSA, PSA and CkSA, 9% to RbSA and RSA, 2% to PSA and PgSA and 0% to HSA. Most of these sera essentially displayed IgE antibody-binding activity to DSA, CSA and ESA. Thus we selected the five patients' sera displaying the highest IgE antibody titre towards those three albumins. Figure 1 shows the degree of binding activity between IgE and IgG antibodies of the five selected sera against the different albumin species (average data from all five patients with error bars). IgE antibodies were reactive

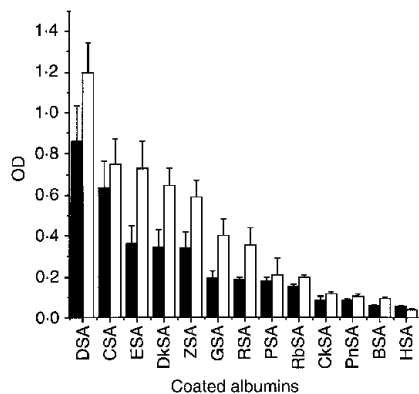


Figure 1. Comparison between IgE and IgG antibodies binding against 13 different albumin species. The assay plate was coated with 13 albumins from different animal species (100 μ l of each antigen, 10 μ g/ml) in 0.1 M carbonate-bicarbonate buffer, pH 9.6, overnight at 4°. Unoccupied sites in the wells were saturated by 1 hr incubation at 37° with 100 μ l 0.5% gelatin in PBS. All wells were washed five times with PBS containing 0.1% Tween-20 between each incubation step. Diluted allergic sera (100 μ l, dilution 1:10 for IgE antibody evaluation and 1:25 for IgG) were added and incubated at 37° for 2 hr. After washing, secondary antibodies (alkaline phosphatase-conjugated goat anti-human IgE antibody or peroxidase-conjugated goat anti-human IgG antibody) were added at 100 μ l/well for 1 hr at 37°. After additional washes the amount of alkaline phosphatase bound to the wells was measured at 405 nm for IgE determination (■) and the amount of peroxidase bound to the wells was measured at 490 nm for IgG determination (□). Negative control was performed using non-allergic serum where the optical density (OD) obtained was <0.05 and subtracted from all the other values.

against eight different albumin species, while IgG antibodies displayed binding activity against 12. From a clinical point of view we could have expected that the higher the sensitivity to horse was, the higher the IgE or IgG antibody level should have been towards both major allergen Equ c1 and horse serum albumin; in fact the five selected sera exhibited the strongest IgE or IgG antibody reactivity to DSA followed by CSA and ESA.

The reactivity of these five sera against other dog proteins was not considered as only antibodies towards albumin were tested. Nevertheless, this reactivity was tested against a crude dog dander extract and we observed a good reactivity. The latter was significantly inhibited by DSA when we tested the patients' sera against the crude dog dander extract (Fig. 2). These results suggested that DSA was the main molecule involved in the observed cross-reactions in our study.

We used the inhibition ELISA to characterize the specificities of the IgE and IgG antibodies against ESA, CSA and DSA. We preincubated the five sera with different concentrations (100–0.001 μ g/ml) of ESA, as well as DSA and CSA, against which these sera showed high IgE and IgG antibody-binding activity. Then we determined the residual IgE and IgG antibody activity against ESA and DSA. Incubation of the five patients' sera with ESA, CSA and DSA, respectively, totally eliminated IgE and IgG antibody binding to coated ESA, i.e. 99% inhibition. When the remaining binding activity towards DSA was tested after preincubation with ESA, we observed between 54% and 65% inhibition for IgE antibodies and between 61% and 70% inhibition for IgG antibodies. Because the results of the inhibition experiments for the five patients' sera were similar, only the

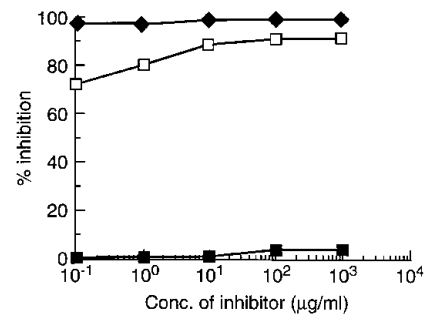


Figure 2. Inhibition of human IgE antibodies binding to dog dander extract. Patients' sera (100 μ l, diluted 1:10) were mixed with different concentrations (1000–0.01 μ g/ml) of dog dander extract (◆), DSA (□) and HSA (■) and were preincubated for 2 hr at 37°. The samples were added to the ELISA plate coated with dog dander extract. Incubation was performed at 37° for 2 hr and alkaline phosphatase-conjugated goat anti-human IgE antibody was added. The amount of alkaline phosphatase bound to the wells was measured at 405 nm. The percentage inhibition was determined, taking the optical density in the absence of inhibitor as 100%. Negative controls were performed using non-allergic serum as well as HSA.

percentage inhibition of IgE antibody for one serum is presented in Fig. 3.

Depletion of the patients' sera on specific immunoadsorbents

In order to characterize further the specificities of the IgE and IgG antibodies as defined above against the tested albumins, we adsorbed, in a preparative manner, three samples of each of the five selected patients' sera on immobilized ESA, CSA and DSA, respectively. Proteins were linked to CNBr-activated Sepharose 4B. After incubation for 2 hr at 37°, we tested for residual IgE and IgG antibody-binding activity against coated homologous and heterologous albumins in ELISA. Patients' sera adsorbed on the immobilized ESA lost their IgE and IgG reactivity to the coated homologous albumin, but retained significant IgE reactivity, 55% (mean of the five selected sera) of the total activity before adsorption to DSA and CSA. The residual IgG reactivity was 38.5%. On the other hand, when the sera were adsorbed on the immobilized DSA or CSA as well, they completely lost their IgE

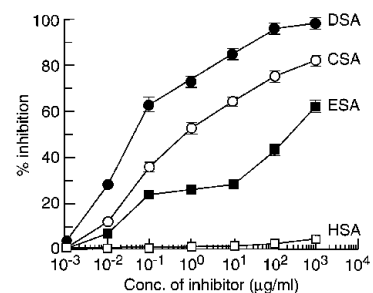


Figure 3. Inhibition of human IgE antibodies binding to dog serum albumin by DSA, CSA, ESA and HSA. Patients' sera (100 μ l, diluted 1:10) were mixed with different concentrations (100–0.001 μ g/ml) of free native DSA (●), CSA (○), ESA (■) and HSA (□), and were preincubated for 2 hr at 37°. These samples were added to the ELISA plate coated with DSA. The remaining steps of the ELISA assay were performed as described in the legend to Fig. 2.

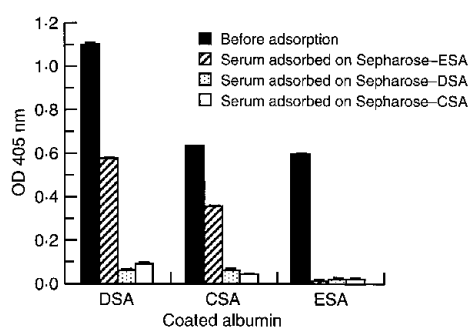


Figure 4. Determination of remaining IgE antibodies after adsorption of the patients' sera on immobilized albumins. The patients' sera were adsorbed on immobilized dog, cat and horse serum albumins linked to CNBr-activated Sepharose 4B. The unadsorbed fractions were tested for the remaining IgE antibody-binding activity against coated DSA, CSA and ESA. The ELISA assay was performed as described in the legend to Fig. 1.

and IgG reactivities against these three serum albumin species. As the results of the five sera were similar, only the residual IgE antibody-binding activity for one serum (Dec) is presented in Fig. 4. These results are in agreement with those described above.

ESA peptides

Commercial horse serum albumin was further purified on immobilized metal ion affinity chromatography before digestion by trypsin without previous reduction. The experimental conditions, based on previous kinetic studies, were an enzyme/substrate ratio of 1:50 at 37° for 15 min. Using RP-HPLC, we separated the generated digests into fractions that were analysed by SDS-PAGE showing protein bands in a molecular weight (MW) range between 50 000 and 3000 (Fig. 5). Using Western blot analysis, we identified the fraction containing the smallest peptides able to bind human IgE antibodies from sera of horse-sensitive patients (Fig. 6).

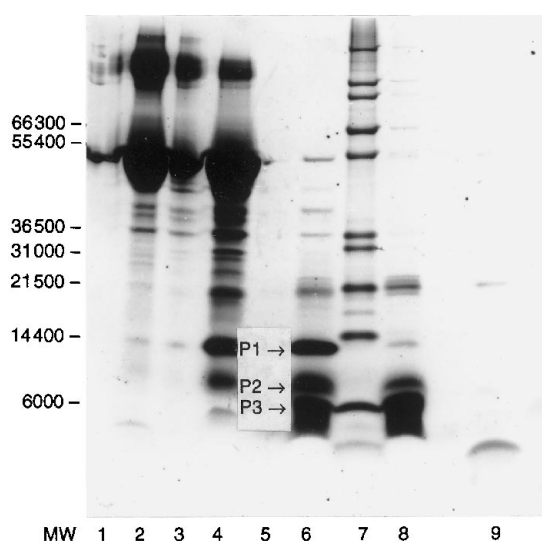


Figure 5. SDS-PAGE of ESA tryptic digests. Homogenous 18% gel was used. Lanes 1–6, 8 and 9 are the different fractions obtained after the first RP-HPLC of the ESA tryptic fragments generated. Lane 7, protein standards.

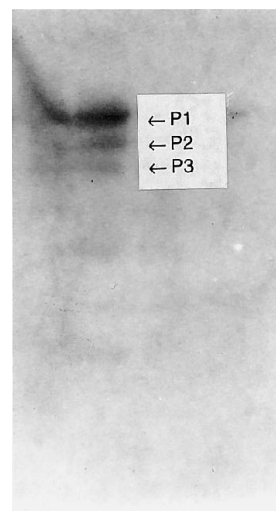


Figure 6. Western blot analysis. After separation of the ESA tryptic digest by SDS-PAGE (18% gel), the peptide bands were transferred to nitrocellulose membrane. The latter was then incubated with patient serum and IgE-binding antigens were detected with ^{125} I-labelled rabbit anti-human IgE antibody.

Complete resolution of these peptides (P1, P2 and P3) was obtained by rechromatography of the partially resolved peaks of the first chromatographic separation. Their MW, established by SDS-PAGE, was around 7400 for P3, 11 400 for P2 and 12 000 for P1 (Fig. 7). To characterize these peptides further, the N-terminal amino acid sequence of each peptide was analysed, which allowed us to localize them within the complete amino acid sequence of the equine serum albumin, as defined by Ho *et al.*²⁰ The different N-terminal amino-acid sequences were: KGLVLVAF for P1, ILLSS for P2 and KAETFTFHA for P3.

The five patients' sera exhibited IgE and IgG antibody-binding activity to the three ESA peptides, as shown by direct ELISA (Fig. 8). We tested the ability of these peptides to inhibit the binding of IgE and IgG antibodies to native ESA and DSA. For this we arbitrarily chose two sera (Mar and Thi) among the five previously selected ones. As the results of both patients' sera were similar, only the results obtained with one of them (Mar) are presented in Fig. 9. They show the increasing inhibition of the patient's IgE antibody binding to native ESA (Fig. 9a) and native DSA (Fig. 9b) by various concentrations (10–0.001 μ M) of each of the ESA peptides. The inhibition obtained reached a plateau at 1.0 μ M/ml and did not exceed 50% for each of the three peptides. Comparable results and similar curves were also obtained when testing the ability of these peptides to inhibit the binding of IgG antibodies to both ESA and DSA. In this case the maximum percentage inhibition obtained at a plateau was between 40% and 50%. Reduced and alkylated ESA, P1, P2 and P3 completely lost their inhibitory capacity towards native ESA.

Based on the previous inhibition experiments, we examined the possible existence of common epitopes on the ESA peptides. We preincubated the patient serum (Mar) with different concentrations (10–0.001 μ g/ml) of P1, P2 and P3, followed by testing the residual IgE antibody binding activity against coated homologous and heterologous peptides. The percentage inhibition obtained at a plateau was between 85% and 90% when the homologous inhibitor was used, and between 65% and 82% when a heterologous inhibitor was used (Fig. 10).

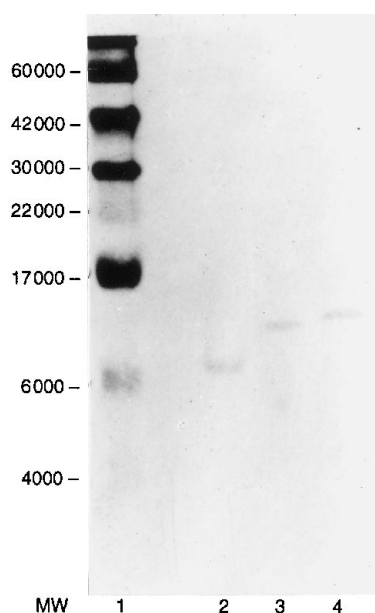


Figure 7. SDS-PAGE of the three purified ESA peptides. Homogenous 18% gel was used. Lane 1, protein standards from 200 to 6000 MW; lane 2, P3; lane 3, P2; lane 4, P1.

DISCUSSION

Allergy to animal proteins is a subject of interest. Several studies concerning animal dander allergens have indicated the relative importance of serum albumin as an allergen. They have shown the presence of human IgE antibodies to serum albumin from different animal species such as dog, cat, horse, etc., in sera of patients shown to be hypersensitive to these animals.²¹ In this study, we

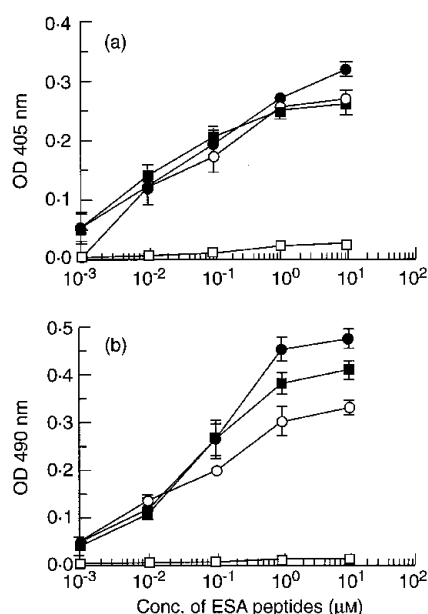


Figure 8. Reactivity of human IgE and IgG antibodies to ESA peptides. Patients' sera (100 μ l, diluted 1:10) were added to the ELISA plate coated with different concentrations of P1 (○), P2 (●) and P3 (■). Lysozyme (□) was used as a negative control. Bound IgE (a) and IgG (b) antibodies were determined as described in the legend to Fig. 1.

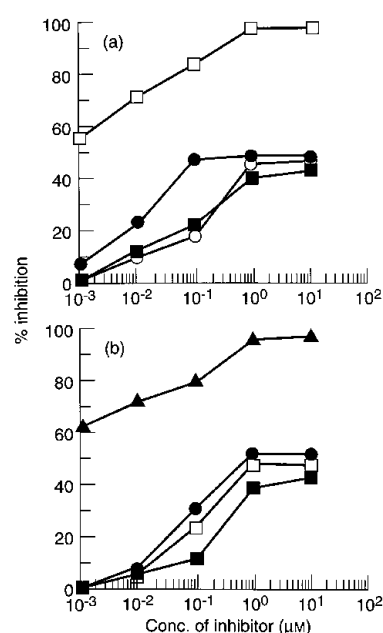


Figure 9. Inhibition of human IgE antibodies binding to horse and dog serum albumins by ESA peptides P1, P2 and P3. Patients' sera (100 μ l, diluted 1:10) were mixed with different concentrations (10–0.001 μ M/ml) of free native ESA (□), free native DSA (▲), P1 (○), P2 (●) and P3 (■), and were preincubated for 2 hr at 37°. These samples were added to the ELISA plate coated with ESA (a) and DSA (b). Incubation was performed at 37° for 2 hr. Alkaline phosphatase-conjugated goat anti-human IgE antibody was added. The amount of alkaline phosphatase bound to the wells was measured at 405 nm. The percentage inhibition was determined taking the optical density in absence of inhibitor as 100%. Negative controls were performed using non-allergic serum as well as unrelated peptide at 10 μ M/ml.

investigated the properties of IgE antibodies present in horse-sensitized patients' sera that contained IgE and IgG antibodies against different allergens present in horse hair and dander extract. They exhibited a high reactivity towards the horse major allergen Equ c1 and towards the horse serum albumin on which we especially focused. The antigenic structure of the ESA, as well as its cross-antigenicity relationship with other albumins, was examined by the bias of peptides obtained after tryptic hydrolysis. Cross-reactions have been observed previously between dog and cat albumins,²² mouse and rat serum albumin,¹⁰ and cat, mouse, chicken and rat serum albumins.⁹ We observed cross-antigenicity between horse, dog and cat serum albumins when they reacted with IgE antibodies from sera of horse-sensitive patients. In these cases the positive reactions observed could be owing either to parallel sensitization or to cross-reactivity of the antibodies with the different albumins. To discriminate between these two possibilities, we further investigated the cross-antigenicity by inhibition ELISA and depletion experiments.

These sera were tested against 13 mammalian albumins from different animal species by ELISA, with special interest focused on ESA. Each albumin displayed a different level of reactivity with the patients' sera tested, probably corresponding to different IgE or IgG antibody concentrations as well as to their different affinities for the antigenic sites involved. If we compare results obtained for IgG and IgE antibodies, we can observe that IgE antibodies reacted with a more limited number of albumins than IgG antibodies. This

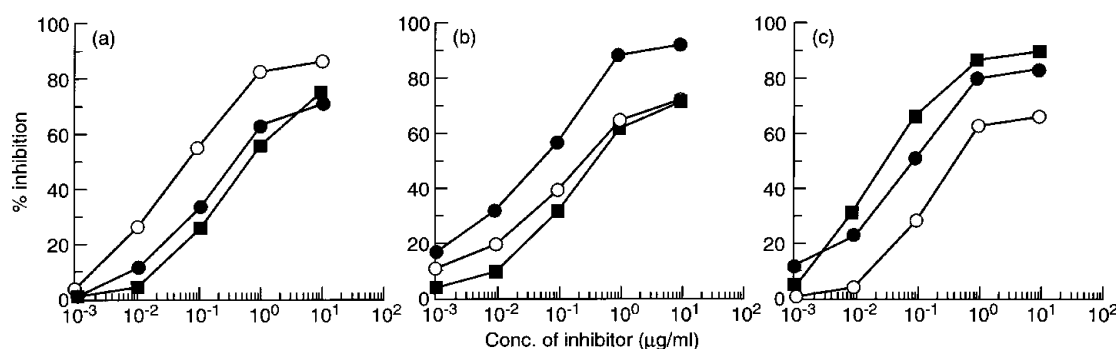


Figure 10. Determination of cross-antigenicity between ESA peptides P1, P2 and P3. We preincubated 100 μ l (diluted 1 : 10) of the patient serum (Mar) with different concentrations (10–0.001 μ g/ml) of P1 (○), P2 (●) and P3 (■) for 2 hr at 37°. These samples were added to the ELISA plate coated with (a) P1, (b) P2 and (c) P3. Alkaline phosphatase-conjugated goat anti-human IgE antibody was added. The amount of alkaline phosphatase bound to the wells was measured at 405 nm. The percentage inhibition was determined taking the optical density in absence of inhibitor at 100%. Negative control was performed using unrelated peptide at 10 μ M/ml.

suggests that IgE antibodies recognize a smaller number of epitopes than IgG antibodies.

Several studies have already shown the presence of IgE antibodies to the albumin of the animal species to which the patient is reportedly allergic.^{2,3,10,23,24} As the present patients were reportedly allergic to horse, it seemed relevant to find the highest IgE antibody reactivity towards horse serum albumin. Unexpectedly the highest titre was found against dog serum albumin, immediately followed by cat serum albumin, while the IgE antibody titre towards ESA was significantly lower. The specificity of these antibodies was investigated using inhibition ELISA. Inhibition was total by DSA, and only partial by CSA and ESA.

Adsorption on immobilized DSA or CSA led to a complete loss of IgE and IgG reactivity towards ESA. We could assume that human IgE and IgG antibodies against albumins from different animal origins are primarily induced by dog serum albumin, the most common albumins along with cat albumin, present in the human environment. Dog serum albumin probably contains all the common determinants that are shared by horse and cat plus its own specific determinants. The similarity in inhibition patterns of the specific human IgE antibodies binding to ESA by DSA and CSA suggests an almost complete allergenic/antigenic identity between both dog and cat albumins.

The sequence homology and the conservation of either the primary or the tertiary structure of the entire albumin could explain the phenomenon of cross-reactivity. Serum albumin is a single-chain protein organized into a series of nine double loops (by 17 disulphide bridges) grouped into three structurally similar domains of three loops each.²⁵ The existence of high levels of conserved sequences in different albumin species,²⁰ and of internal homology, suggests that albumin could have evolved from a single domain, a common precursor of about 190 amino acids.²⁵ Alignment of the ESA²⁰ and CSA amino acid sequence¹³ shows 83% homology.

We further investigated the antigenic structure of ESA by tryptic digestion carried out on a highly purified molecule. The antigenicity of the RP-HPLC-purified peptides isolated and characterized in this study was examined by ELISA. Among the peptides obtained by tryptic hydrolysis and shown to bind human IgE and IgG antibodies, we chose the three smallest peptides P1, P2 and P3, with MW of 12 000, 11 400 and 7400, respectively.

Considering the three-dimensional conformation model of BSA,²⁵ the primary structure of which shows a very high level of homology with ESA (Fig. 11), we attributed a similar conformational structure to ESA. Knowing the N-terminal amino acid sequence of the ESA peptides P1, P2 and P3 and their apparent molecular weight, we were able to localize them within the complete structure of ESA. Peptide P1 could include amino acid residues 21–113, and contains loops 1 and 2 of the first domain; P2 (188–275) probably includes the disulphide loop 4 of the second domain; and P3 (503–560) could be derived from loop 9 of the third domain (Fig. 12).

ESA peptides P1, P2 and P3 were shown to induce 50% inhibition of the IgE and IgG antibodies binding from sera of horse-sensitive patients, not only to ESA but also to DSA, showing that they were involved in the observed cross-reactions between horse, dog and cat serum albumins. They did not form any precipitate line in the RIE experiment, suggesting that the number of epitopes they bear is too small to induce the formation of a precipitating network with specific rabbit IgG antibodies.

In our attempt to investigate the possible existence of cross-antigenicity between ESA peptides P1, P2 and P3 themselves, cross-inhibition experiments were performed, using the serum of a horse-sensitive patient, where each peptide was alternatively used as antigen or as inhibitor. Our results showed that P1, P2 and P3 share common epitopes. This is relevant to the structural homologies between the different domains of the ESA molecule to which these peptides belong. Similar observations have been made by other authors for HSA²⁶ and BSA.²⁷

Finally, to emphasize the possible role of the loop structure of the whole ESA molecule and of the three ESA peptides P1, P2 and P3 in their reactivity with IgE and IgG antibodies, we studied the effect of reducing the disulphide bonds responsible for the stabilization of the spatial configuration of the proteins. After reduction and alkylation of the resulting thiol groups, the whole ESA as well as ESA peptides P1, P2 and P3 completely lost their inhibitory capacity. Therefore we can assume that the intra-chain disulphide bridges bring the sequentially distant amino acids involved in IgE binding into spatial proximity, and this leads us to the conclusion that the allergenic sites on ESA and ESA peptides could include discontinuous epitopes.

Whatever the clinical importance of serum albumin as an allergen, it appears to be an interesting model to study the structure

DTHKSEI AHRFNDLGEKHF KGLVLVAFSQYLQQCPFEDHVKLVNEVTEFAKKCAA	55
K E I DE L T V	
DESAENCDSKSLHTLFGDKLCTVATLRATY GELADCCCKQEPERNECFLTHKDDHP	110
HAG E E K S E DM EQ S S	
NLPKL- KPEPDAQCAAFQEDPDKFLGKYLYEVARRHPYFYGPPELLFHAE EYKADF	164
D D NTL DG KA GK W I A Y NK NGV	
TECCPADDKLACLIPKLDALKERILLSSAKERLKCSSFQNFGERAVKAWSVARLS	219
Q Q E G L IETMR KV T RQ R A I K L	
QKFPKADF AEVSKI VTDLT KVHKECCHGDLLECADDRADLAKYICEHQDSISGKL	274
E V T L DN T S	
KACCDKPLLQKSHCI AEVKEDDLPSDLPALAADFAEDKEICKHYKDAKDVFLGT F	329
E E EK AI E P T DV N QE A S	
LYEYSRRHPDYSVSLLLRI AKTYEATLEKCCAEADPPACYRTVFDQFTPLVEEPK	384
E A V L E E KD H TS KLKH D Q	
SLVKKNCDLFEVGEYDFQNALIVRYTKKAPQVSTPTLVEIGRTLKGKVGSRCKL	439
N I Q Q KL G R V VS S T TK	
PESERLPCSENHLALALNRLCVLHEKTPVSEKITKCCTDSLAEERRPCFSALELDE	494
M T DY S I V VN TP	
GYVPKEFKAETFTFHADI CTLPEDKQIKKQSALAEVLVKKPKATKEQLKTVLGN	549
T A DEKL DT T V L E ME	
FSAFVAKCCGREDKEACFAEEGPKLVASSQLALA-	583
V D AAD V V T T	

Figure 11. Sequence alignment of ESA and BSA. The amino acid sequences of ESA,²⁰ the first line, and BSA,²⁵ the second line, were compared. ESA exhibited 73.9% sequence homology with BSA. The percentage homology of the ESA peptides with the corresponding BSA regions was 76%, 79% and 72% for P1, P2 and P3, respectively. Concerning the BSA sequence, only amino acids which differed from those of ESA are indicated.

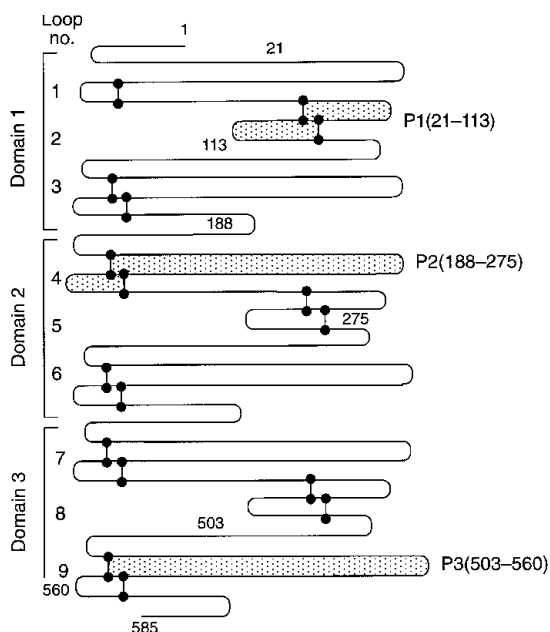


Figure 12. Localization of P1, P2 and P3. Based on the primary structure of ESA²⁰ and the three-dimensional conformational model of BSA, where the albumin molecule is organized into nine double loops [by 17 disulphide bridges (● - - ●)] grouped into three domains of three loops each,²⁵ we attempt to predict the structure of ESA peptides. P1 contains loops 1 and 2 of the first domain, P2 derives from loop 4 of the second domain and P3 contains the disulphide loop 9 of the third domain. The loop areas included in the peptides are shaded.

involved in the allergenicity of a protein. Otherwise, from our results, the assumption may be made that an apparent sensitization to a given allergen could result in fact from a priming of the immune system by another protein carrying common epitopes.

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