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Animal-derived lipocalin allergens exhibit immunoglobulin E cross-reactivity

S. Saarelainen*, M. Rytkönen-Nissinen*, J. Rouvinen†, A. Taivainen‡, S. Auriola§, A. Kauppinen*, T. Kinnunen* and T. Virtanen*

*Department of Clinical Microbiology, University of Kuopio, Kuopio, Finland, †Department of Chemistry, University of Joensuu, Joensuu, Finland, ‡Department of Pulmonary Diseases, Kuopio University Hospital, Kuopio, Finland and §Department of Pharmaceutical Chemistry, University of Kuopio, Kuopio, Finland

Clinical and
Experimental
Allergy

Summary

Background Although knowledge of the IgE cross-reactivity between allergens is important for understanding the mechanisms of allergy, the regulation of the allergic immune response and the development of efficient modes of allergen immunotherapy, the cross-reactivity of animal allergens is poorly known.

Objective The aim of this study was to characterize IgE cross-reactivities between lipocalin proteins, including five animal-derived lipocalin allergens and one human endogenous lipocalin, tear lipocalin (TL).

Methods The recombinant proteins were validated by chromatography and mass spectrometry. The IgE-binding capacity of the allergens was confirmed by IgE immunoblotting and IgE immunoblot inhibition. IgE ELISA was performed with sera from 42 atopic patients and 21 control subjects. The IgE cross-reactivities between the lipocalin proteins were determined by ELISA inhibition.

Results ELISA inhibition revealed IgE cross-reactivities between Can f 1 and human TL, between Can f 1 and Can f 2, and between Equ c 1 and Mus m 1. Low levels of IgE to human TL were found in the sera of seven dog-allergic patients of whom six were IgE-positive for Can f 1.

Conclusion Several lipocalins exhibited IgE cross-reactivity, probably due to the sequential identity of the proteins and also due to similarities in their three-dimensional structures. The clinical significance of the findings needs to be elucidated. Low-level IgE cross-reactivity can play a role in regulating immune response to lipocalin allergens.

Keywords allergy, cross-reactivity, endogenous lipocalin, lipocalin allergen, three-dimensional structure

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Correspondence:

Soili Saarelainen, Department of Clinical Microbiology, University of Kuopio, Harjulantie 1 C, 70211 Kuopio, Finland.
E-mail: Soili.Saarelainen@uku.fi

Introduction

Knowledge of the IgE cross-reactivity between allergenic molecules is important for several reasons. For example, it makes it possible to predict the risk of developing allergic reactions to other similar allergen sources, to improve the identification of sensitization to specific allergens [1], and to develop new therapies for allergic diseases [2]. Moreover, the cross-reactivity of IgE antibodies can also be implicated in the regulation of the allergic immune response [3].

Most important animal-derived respiratory allergens belong to the lipocalin protein family [4], members of which are present in both humans and animals [5]. Despite the fact that they are important respiratory sensitizers [6], their IgE cross-reactivity is poorly characterized.

Although studies on animal allergen extracts suggest that lipocalin allergens can show IgE cross-reactivity [7, 8], IgE cross-reactivity with natural or recombinant lipocalins have only been reported between Cav p 1 and Cav p 2 (the major allergens of guinea pig) [9], and between Can f 1 and Can f 2 (major and minor allergens of dog, respectively) [10].

Proteins showing a certain degree of amino acid identity tend to have similar (but not identical) three-dimensional (3D) structures [1, 3]. However, on the basis of recent studies with plant allergens, it seems that IgE cross-reactivity may be due to the highly conserved 3D structures rather than due to high similarities at the amino acid level [11, 12]. Interestingly, lipocalin proteins of which sequential identity is often approximately 20% or lower [5] share a similar 3D structure. For instance, the 3D

structure of human tear lipocalin [TL, or von Ebner's gland protein, (VEGP) [13]] has been reported to resemble closely the crystal structure of Bos d 2 [14] although the sequence identity between these two proteins is only 18%. On the other hand, the amino acid identity between lipocalins can be considerable: it is close to 60% between human TL and dog Can f 1 [15]. Because the crystal structure of Can f 1 has not yet been resolved a structural comparison is not possible.

The aim of our study was to characterize the IgE cross-reactivity of five lipocalin allergens (cow Bos d 2, dog Can f 1 and Can f 2, horse Equ c 1, and mouse Mus m 1) and one human endogenous lipocalin, TL. We show here that human IgE can exhibit cross-reactivity between lipocalin proteins, including TL. We speculate that IgE cross-reactivity between endogenous and exogenous proteins can be a factor modulating the allergic disease.

Materials and methods

Subjects

A total of 42 atopic subjects (20 women, mean age 39.9 ± 10.4 SD and 22 men, mean age 42.6 ± 10.2 SD) presenting allergic symptoms upon exposure to dog ($n = 31$), cow ($n = 18$), mouse ($n = 15$) or horse ($n = 21$) were recruited to the study, together with 21 healthy non-atopic individuals as controls (14 women, mean age 38.5 ± 13.7 SD and seven men, mean age 36.1 ± 11.4 SD). With the exception of one mouse-allergic subject who had self-reported symptoms, allergy was diagnosed at the Department of Pulmonary Diseases of the Kuopio University Hospital. A patient was classified as allergic if the result of the UniCAP fluoroenzyme-immunometric assay (Pharmacia, Uppsala, Sweden) was >0.7 kU/L or the result of skin prick test (SPT; epithelial preparations from ALK Abelló, Hørsholm, Denmark) was ≥ 3 mm with the allergen extract from the symptom-causing animal. The SPTs were performed according to European recommendations as described previously [16]. The study was approved by the Ethics Committee of the Kuopio University Hospital.

Allergen preparations and control proteins

The recombinant (r) Can f 1, rCan f 2 and rBos d 2 were cloned, transformed and expressed in *Pichia pastoris*, and then purified as described previously [17, 18]. For TL, a plasmid containing TL cDNA (a kind gift from Dr Bernhard Redl, Institut für Molekularbiologie, Innsbruck, Austria) was used as a template in generating the rTL construct with TL-specific primers based on the published nucleotide sequence [19]. Specific primers for the generation of rEqu c 1 were designed based on the published nucleotide sequence of the allergen [20]. The 3' primers used in each construction included a polyhistidine tag sequence. A

template cDNA for the rEqu c 1 construct was originated by cloning from the sublingual tissue of horse. The created rEqu c 1 and rTL constructs were cloned into the pPIC9 expression vector and their nucleotide sequences were confirmed by DNA sequencing.

P. pastoris yeast was transformed with the constructs pPIC9Equ c 1 and pPIC9TL according to the manufacturer's instructions (Invitrogen, San Diego, CA, USA). The *P. pastoris* expression clone pHIL-D2MUP for rMus m 1 was a kind gift from Dr Elena Ferrari, Istituto di Chimica Biologica, Facoltà di Medicina e Chirurgia, Università di Parma, Parma, Italy. The allergens and rTL were produced according to the instructions of the manufacturer of the *P. pastoris* expression system (Invitrogen). rEqu c 1 and rTL were first purified with the HisTrap Kit (Amersham Pharmacia Biotech AB, Uppsala, Sweden) according to the manufacturer's instructions and then by gel filtration. Recombinant Mus m 1 and natural (n) Mus m 1 from mouse urine were purified by ion exchange chromatography basically as described by Ferrari et al. [21]. Recombinant psoriasin (with a polyhistidine tag) was used as a control protein, produced and purified as described previously [22]. Protein concentrations were determined by absorbance spectroscopy.

Immunoblotting and immunoblot inhibition

Patients' IgE reactivity against the commercial horse SPT preparation (8 µg/lane), rEqu c 1, nMus m 1, and rMus m 1 (40 ng/lane each) was detected by SDS-PAGE and immunoblotting, as described previously [17], with minor modifications. In brief, membrane strips were incubated overnight with patient and control sera (1 : 10) followed by incubation for 2 h with monoclonal horseradish peroxidase-labelled mouse anti-human IgE (Southern Biotechnology Associates Inc., Birmingham, AL, USA) at 1 : 2000. The reactive bands were visualized with ECL detection reagents (Amersham International, Buckinghamshire, UK).

The inhibition of IgE immunoblotting was performed essentially as described previously [17]. Serum pools reactive to Equ c 1 or Mus m 1 were mixed with the respective recombinant allergens or recombinant psoriasin as a control protein so that the final serum dilution was 1 : 30 and protein concentration was 100 µg/mL. After an incubation of 2 h at $+37^\circ\text{C}$, IgE reactivity against the horse epithelial preparation or nMus m 1 was visualized as described above.

Enzyme-linked immunosorbent assay and enzyme-linked immunosorbent assay inhibition

IgE antibodies to recombinant Can f 1, Can f 2, Bos d 2, Equ c 1, Mus m 1, and TL were measured with indirect ELISAs as described previously [17], with a few modifications. In brief, the plates were coated with 5 µg/mL of rCan

Table 1. Summary of data from the patients whose sera were included in the serum pools

Patient no.	Sex	Age (Y)	Total IgE (kU/L)	Serum pool	Skin prick test (mm)				Specific IgE (kU/L)			
					Dog	Horse	Cow	Mouse	Dog	Horse	Cow	Mouse
2	F	42	1143	d, e	4	8	7	0	7.6	9.9	48.2	<0.7
4	F	39	124	a, b	11	ND	ND	ND	13.4	1.2	<0.7	<0.7
5	M	38	345	d, f	5	7.5	2	3	5.8	8.2	<0.7	2.6
6	M	32	1816	f	5	0	0	5	41	<0.7	<0.7	3.2
7	F	40	2010	a, c	6	2	0	0	15.5	1.4	<0.7	<0.7
8	F	48	388	a	5	0	0	3	73	4.0	<0.7	<0.7
11	M	33	382	c	4.5	8	0	0	28.1	7.4	<0.7	<0.7
13	M	26	832	f	3	5	8.5	5	99	2.8	2.0	4.9
18	F	46	306	b	9.5	14.5	0	2	15.8	1.8	<0.7	<0.7
19	M	32	930	a, b, c, d	18	26	2	0	>100	12.4	<0.7	<0.7
20	M	45	464	c	5	10	0	2	12.1	1.1	<0.7	1.2
22	M	52	172	a	7	0	0	0	36.8	2.6	<0.7	<0.7
27	F	46	146	e	3	5	6.5	ND	<0.7	ND	14.87	ND
28	F	58	361	e	3	0	7	ND	ND	ND	98.24	ND
30	M	39	294	d, e	5	9	4.75	ND	ND	3.2	74.7	ND
31	F	44	397	d	3	3.5	13	ND	ND	ND	50.37	ND
32	M	47	537	e	5	8	7	ND	ND	11.7	>100	ND
38	M	34	1211	f	ND	ND	ND	ND	ND	ND	ND	28.8
42	M	34	104	f	ND	ND	ND	ND	ND	ND	ND	25.3

Serum pools used in inhibition assays: (a) Can f 1, (b) Can f 2, (c) TL, (d) Equ c 1, (e) Bos d 2, (f) Mus m 1; ND, not determined; M, male; F, female.

f 1 and rCan f 2 or with 10 µg/mL of rEqu c 1, rBos d 2, rMus m 1 and rTL. Sera diluted 1 : 10 were incubated for 1 h at +37 °C. The bound IgE was detected by biotinylated rabbit anti-human IgE (1 : 1000; Southern Biotechnology Associates Inc.) combined with AMDEX™ streptavidin-HRP (1 : 10 000; Amersham International). The colour reaction was developed by the TMB single solution reagent (Zymed Laboratories Inc., South San Francisco, CA, USA). The results were expressed as optical density (OD) values at 450 nm. The cut-off for a positive reaction was defined as the mean OD of the control subjects +3 SD.

Pooled sera from three to five patients with the highest titres of specific IgE to lipocalin allergens were used in ELISA inhibitions. The summary of data from the patients whose sera were included in the serum pools is in Table 1. The ELISA inhibitions were performed essentially in the same way as the indirect ELISAs but with an additional preincubation (1 h at +37 °C) of the serum pools with the inhibitor proteins (rCan f 1, rCan f 2, rEqu c 1, rBos d 2, rMus m 1, rTL and the control proteins human transferrin (Sigma, Steinheim, Germany) and recombinant psoriasin). In the preincubation, the final serum dilution was 1 : 10 to 1 : 50 and the lipocalin concentrations ranged from 0.6 to 200 µg/mL. The results were expressed as inhibition percentages.

Statistical analyses

Statistical differences between groups were determined with the Kruskal–Wallis test and *post hoc* comparisons

with the Mann–Whitney *U*-test. For determining correlations, the Spearman rank correlation test was used.

Results

Validation of the recombinant proteins

After affinity chromatography and gel filtration, rEqu c 1 was eluted as a dimer and rMus m 1 as a monomer. rTL was eluted at two approximate molecular sizes (20 and 40 kDa), compatible with being monomeric and dimeric forms of the protein. SDS-PAGE revealed rEqu c 1, rMus m 1, and rTL as single bands with molecular masses of 26, 18 and 22 kDa, respectively. The sequences of the tryptic digests of the recombinant proteins obtained by HPLC-electrospray ionization mass spectrometry (data not shown) also matched those reported for rEqu c 1 [20], rMus m 1 [21], and rTL [19]. As TL has been reported to be a dimer in humans [23, 24], the dimeric form was chosen for further studies.

The IgE-binding capacities of rEqu c 1 and rMus m 1 were verified with immunoblotting. As shown in Fig. 1, the sera of horse and mouse-allergic patients reactive to the proteins corresponding to natural Equ c 1 and Mus m 1 by the molecular size (23 and 20 kDa, respectively) also recognized the recombinant allergens. Figure 1a, right panel, shows that when a serum pool with IgE reactivity to nEqu c 1 was pre-incubated with rEqu c 1, it resulted in the elimination of IgE binding to the 23 kDa component in the extract. In an experiment with rMus m 1, no IgE

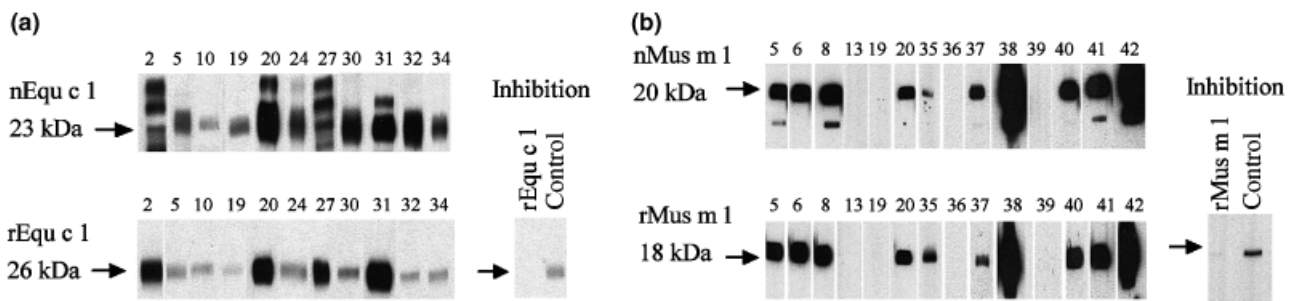


Fig. 1. IgE reactivity of individual horse-(a) and mouse-(b) allergic patients to natural and recombinant Equ c 1 and Mus m 1, respectively, in SDS-PAGE immunoblotting. The allergen-specific serum pool was incubated with a recombinant allergen (or a control protein) before immunoblotting against the natural allergen extract of horse (a) or natural Mus m 1 (b), right panels.

binding was observed to the natural allergen after prior incubation with rMus m 1 (Fig. 1b, right panel). Control sera did not show IgE binding to the recombinant allergens (data not shown). The biophysical characteristics and the IgE-binding capacities of rBos d 2, rCan f 1, and rCan f 2 have been demonstrated previously [14, 16, 17, 25]. SPTs with recombinant Bos d 2 [14], Can f 1 and 2 [17], and Equ c 1 (data not shown) further confirmed that the allergens were immunologically functional.

Immunoglobulin E reactivity to lipocalin allergens and to human tear lipocalin

The IgE reactivity of dog, horse, cow and mouse-allergic patients to dog rCan f 1 and 2, cow rBos d 2, horse rEqu c 1, and mouse rMus m 1, respectively, was measured by indirect ELISA (Fig. 2). The prevalence of dog-allergic patients sensitized to Can f 1 and Can f 2 was 42% and 16%, respectively. Eighty-three percent of cow-allergic patients were sensitized to Bos d 2, whereas 76% of horse-allergic patients were sensitized to Equ c 1. Sixty-six percent of mouse-allergic patients had specific IgE to Mus m 1. The IgE levels between control subjects and allergic patients differed statistically significantly (Fig. 2), the exception being the level of Can f 2-specific IgE ($P > 0.05$). The IgE levels of dog-allergic patients to Can f 1 and Can f 2 showed significant correlation ($r = 0.514$, $P = 0.003$). As has been found previously, all Can f 2 IgE-positive patients were also Can f 1 IgE positive [17].

The measurement of specific IgE levels of all subjects to rTL revealed that seven allergic patients out of 42 had specific IgE to the protein (Fig. 3). Importantly, all the seven rTL IgE-positive patients were dog allergic and six of them were Can f 1 IgE positive (Fig. 3). The rTL-specific IgE level of Can f 1-positive patients differed significantly ($P = 0.014$) from that of Can f 1-negative patients. Moreover, a high correlation ($r = 0.81$, $P = 0.007$) was seen between the rCan f 1 and rTL-specific IgE levels of Can f 1-allergic patients.

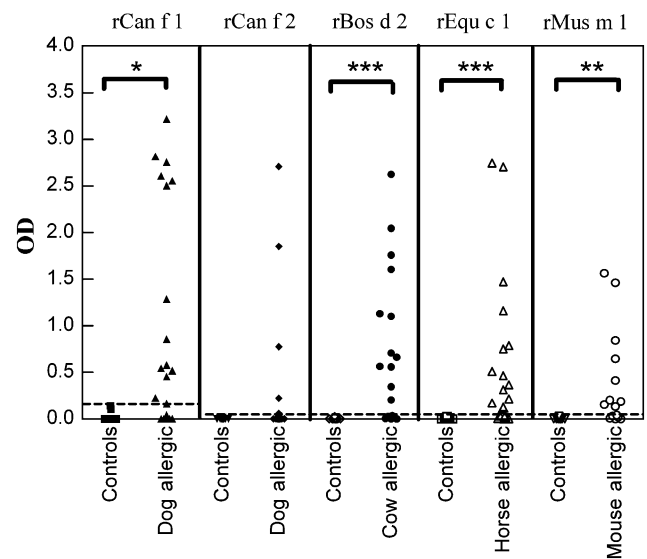


Fig. 2. IgE reactivity of dog-, cow-, horse- and mouse-allergic patients to recombinant mammalian lipocalin allergens measured by ELISA. The background values ($OD < 0.15$) have been subtracted. The IgE levels between the control and allergic patients differed statistically significantly, the exception being the Can f 2 IgE level. The cut-offs are indicated by a dash. * $P = 0.02$, ** $P < 0.002$, *** $P < 0.001$. OD, Optical density.

Immunoglobulin E cross-reactivity between lipocalin proteins

To examine IgE cross-reactivity between lipocalins, three to five sera with a high level of specific IgE to a lipocalin allergen or to rTL were pooled and incubated with the allergens, rTL or the control proteins before adding to the plate coated with a lipocalin. The results of the ELISA inhibition experiments are shown in Fig. 4.

Figure 4a shows that rTL inhibited dose dependently the IgE binding of the rCan f 1-specific serum pool to rCan f 1. The concentration of rTL needed for the 50% inhibition was about 400-fold higher than that of rCan f 1. Recombinant Can f 1 was reciprocally able to inhibit the IgE binding of rTL-positive serum pool to rTL (Fig. 4b).

Interestingly, rCan f 1 was about 100-fold more efficient than rTL in inducing the 50% inhibition.

The pre-incubation of the rCan f 2-specific serum pool with rCan f 1 inhibited IgE binding to rCan f 2 very weakly (Fig. 4c). However, as indicated in Fig. 4a, rCan f 2 could not prevent the binding of specific IgE to rCan f 1. The situation was analogous with rEqu c 1 and rMus m 1, as the pre-incubation of rMus m 1-specific serum pool with rEqu c 1 inhibited the binding of IgE to rMus m 1 (Fig. 4d) while the preincubation of rEqu c 1-specific serum pool with rMus m 1 could not inhibit the binding of IgE to rEqu c 1 (data not shown). No IgE cross-reactivities were seen between Bos d 2 and other lipocalin proteins (data not shown).

Modelling of immunoglobulin E cross-reactive sites

To assess the potential sites in the molecules for the IgE cross-reactivities, the amino acid sequences of the proteins were compared. The highest amino acid identities

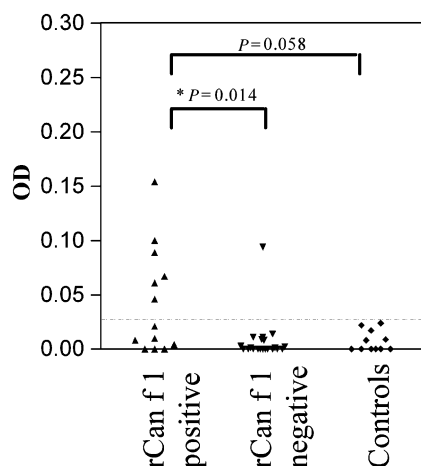


Fig. 3. ELISA results of tear lipocalin-specific IgE levels of patients with sensitization to Can f 1 or to other mammalian lipocalin allergens. The background values (OD <0.15 in all tests) have been subtracted. The dash line indicates the cut-off. OD, Optical density.

were observed between Can f 1 and TL (61%) and between Mus m 1 and Equ c 1 (47%; the SIB BLAST network service at the Swiss Institute of Bioinformatics, 5 April, 2007). The sequence alignments of the lipocalins with IgE cross-reactivity show that the identical residues form short continuous segments (Fig. 5). Surprisingly, the residues in the secondary structure elements (α -helices and β -strands) are not more conserved than the residues in the loops. For example, there are residues in the loops between strands B and C, D and E, F and G, G and H as well as in the C-terminus, which can be found in both TL and Can f 1. Thus, there are several potential areas for IgE cross-reactivity.

Crystallographic 3D structures are available only for TL and one IgE cross-reacting pair, Equ c 1 and Mus m 1 (Protein Data Bank codes 1XKI, 1EW3, and 1MUP, respectively, <http://www.rcsb.org/pdb/>). The mapping of identical residues on the surfaces of Equ c 1 and Mus m 1 (Fig. 6) again shows quite even distribution. The interface between the two monomers of Equ c 1 contains residues mainly from strands F, G, and H which are not very similar compared with those of Mus m 1. The largest similar area (and putative epitope) on the surface can be found around the twofold axis containing the N-terminus of strand C and the C-terminus of strand D of the monomers. It is noteworthy that according to the amino acid sequences, these parts in strands C and D are clearly similar between the IgE cross-reacting pair TL-Can f 1, and there are also some similarities in the Can f 2-Can f 1 pair.

Discussion

IgE cross-reactivities of animal allergens are poorly known except for the IgE cross-reactivities of serum albumins [26, 27]. As the major mammal-derived respiratory sensitizers are lipocalins [4], a protein family also comprising human proteins, there are both clinical and immunological reasons for establishing whether they exhibit IgE cross-reactivity. Interestingly, some other allergens are known to be IgE cross-reactive with their

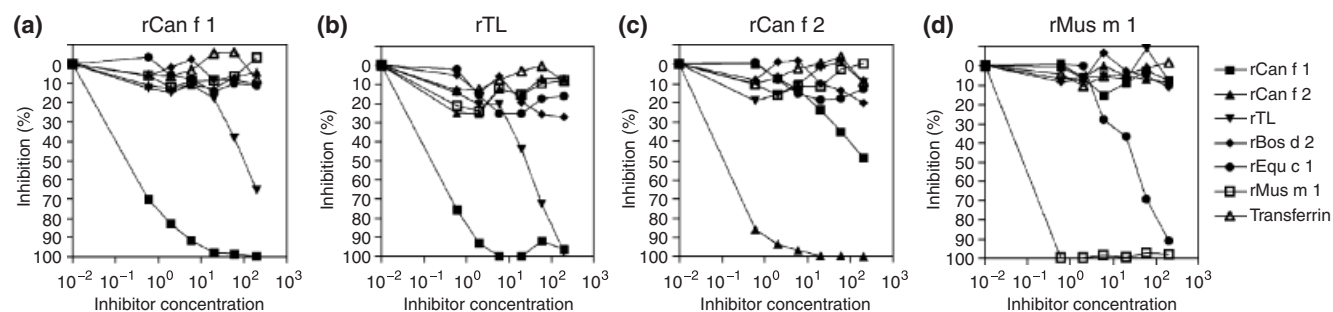


Fig. 4. ELISA inhibition of IgE binding to rCan f 1, rTL, rCan f 2 or rMus m 1 by lipocalin proteins. Pooled sera with IgE reactivity to the recombinant proteins were pre-incubated with recombinant lipocalin allergens, rTL or control proteins at different concentrations. The results are representative of three to four independent experiments. TL, tear lipocalin.

[illegible]

Fig. 5. Sequence alignments between the pairs of lipocalins showing cross-reactivity. Identical amino acid residues are indicated with grey boxes, α -helices by double underlining (=), and β -strands by arrows ($\rightarrow$). The peptide segments of the putative IgE-binding epitope are indicated with XXX.

human counterparts [27–29]. The aim of our study was to characterize the IgE cross-reactivity of five lipocalin allergens (recombinant Bos d 2, Can f 1 and 2, Equ c 1 and Mus m 1) and one human lipocalin, TL (or VEGP).

The biophysical characteristics and the IgE-binding capacities of the recombinant proteins used in this study were found to be correct (Fig. 1, [14, 16–18, 25]). SPTs further confirmed the immunological functionality of the allergens ([14, 17], and data not shown). The prevalences of IgE reactivity to the recombinant allergens were at the same level as reported previously with natural or recombinant allergens [17, 30–32].

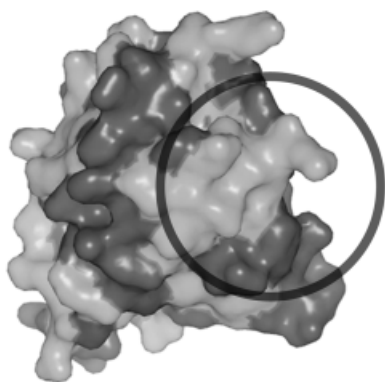
Four out of six lipocalins showed IgE cross-reactivity. As the 3D structure of a protein is dependent on the primary sequence, the sequence similarity between two proteins is related to cross reactivity [3]. This was also the case in our study, because human TL and Can f 1, which have the highest sequential identity (61%, Fig. 5) between the proteins examined, showed the strongest IgE cross-reactivity (Fig. 4). However, cross-reactivity can also occur with lower identities as was observed between Mus m 1 and Equ c 1 (an identity of 46%) and between Can f 1 and Can f 2 (23%). The sequence alignments (Fig. 5) and

3D modelling (Fig. 6) suggest that multiple sites on the surface of the proteins can participate in the IgE cross reactivities. In addition, the comparison of the surfaces of Mus m 1 and Equ c 1 suggested that one large similar area (Figs 5 and 6) could be the cross-reactive epitope.

Cross-reactivity can be asymmetric in which case the allergens contain both common and unique IgE-binding determinants [12, 26, 33]. This is probably the case with Mus m 1 and Equ c 1 and with Can f 1 and Can f 2, because cross-reactivity was not reciprocal between the pairs (Fig. 4). Although the presence of common and unique epitopes can explain the phenomenon, there are other possible explanations for the finding. For example, a dimeric protein (here Equ c 1) may have a higher allergenic potential than a monomer because dimerization may create additional new IgE epitopes [34].

Glycosylation has been observed to be involved in the IgE cross-reactivity of plant and insect-derived glycoproteins [35]. Because TL and Mus m 1 are not glycosylated [20, 23] the cross-reactivities between the pairs Can f 1-TL and Equ c 1-Mus m 1 cannot be explained by glycosylation but by sequential identity and/or similar structures. In contrast, the dog allergens contain putative

Mus m 1



Equ c 1

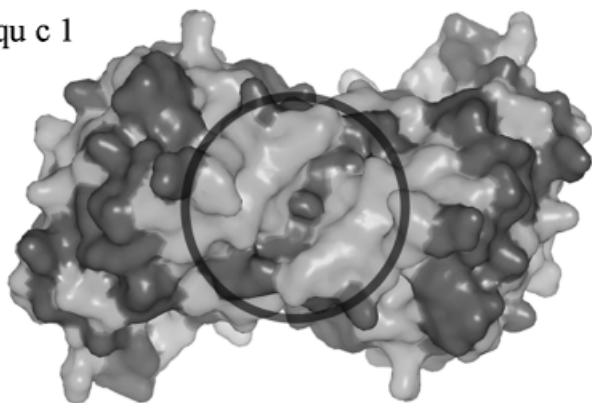


Fig. 6. Three-dimensional models of dimeric Equ c 1 and monomeric Mus m 1. The similar areas are indicated by light grey. The largest putative IgE cross-reacting area is circled.

glycosylation sites [15], and Can f 2 has been confirmed to be glycosylated [17]. However, it is unlikely that carbohydrates could account for the observed IgE cross-reactivity between rCan f 1 and 2 since it has also been observed with the *Escherichia coli*-produced allergens [10].

Despite the increasing knowledge of structural and immunological similarities between environmental and human proteins, such as calcium-binding proteins [28], animal serum albumins [27] and cyclophilins [29], the significance of IgE cross-reactivity between endogenous proteins and exogenous allergens is not clear. In our study, almost a half of Can f 1-allergic patients had IgE against human TL. Furthermore, Can f 1 and TL showed IgE cross-reactivity. Because some autoreactive IgE antibodies are cross-reactive with an exogenous allergen, it might be that autoreactive IgE production could be induced, at least in some instances, by an exogenous allergen [28, 36]. In our study, this was probably the case with anti-TL IgE, because Can f 1 inhibited the IgE binding to TL substantially more strongly than TL itself (Fig. 4b). On the other hand, it can be speculated that endogenous proteins that are IgE cross-reactive with exogenous allergens could regulate or tolerize an allergic immune response to some degree [3]. This regulation might be mediated through, for example, the FcεRI-mediated

induction of indoleamine 2,3-dioxygenase in antigen-presenting cells (APC) [37]. It has recently been reported that FcεRI is highly expressed on the surface of dendritic cells of atopic patients [38, 39] and also on the surface of oral Langerhans cells of non-atopic patients [38]. Moreover, it has been suggested that different FcεRI-bearing APC subsets in the skin of patients with atopic dermatitis might regulate inflammatory processes through the production of T-helper type 1 (Th1)/Th2-polarizing signals and other factors involved in the induction of tolerance [40].

We have shown here directly that several lipocalins can exhibit IgE cross-reactivity even though the sequence similarities were lower than 50%. The most important finding is that the major dog allergen Can f 1 showed IgE cross-reactivity with human TL. Although high-affinity IgE antibodies are considered to play a central role in the clinical manifestation of cross-reactivity [41], it can be speculated that IgE cross-reactivity between endogenous lipocalins and exogenous lipocalin allergens is implicated in the regulation of allergy. It is clear, however, that further studies are needed to elucidate whether our findings have clinical significance. Knowledge of the cross-reactivities between allergens is important for understanding the mechanisms of allergy, the regulation of allergic immune response and the development of efficient modes of allergen immunotherapy.

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