Apostolovic, D.; Sánchez-Vidaurre, S.; Waden, K.; Curin, M.; Grundström, J.; Gafvelin, G.; Cirkovic Velickovic, T.; Grönlund, H.; Thomas, W. R.; Valenta, R.; et al. The Cat Lipocalin Fel d 7 and Its Cross-Reactivity with the Dog Lipocalin Can f 1. *Allergy: European Journal of Allergy and Clinical Immunology* **2016**, *71* (10), 1490–1495. https://doi.org/10.1111/all.12955

Received Date : 24-Mar-2016

Revised Date : 07-Jun-2016

Accepted Date : 09-Jun-2016

Article type : Brief Communication

Title: The cat lipocalin Fel d 7 and its cross-reactivity with the dog lipocalin Can f 1

D. Apostolovic[1,2*], S. Sánchez-Vidaurre[1*], K. Waden[1*], M. Curin[3], J. Grundström[1], G. Gafvelin[4], T. Cirkovic Velickovic[2], H. Grönlund[4], WR. Thomas[5], R. Valenta[3], C. Hamsten[±1,6] & M. van Hage[±1]

[1]Department of Medicine Solna, Immunology and Allergy Unit, Karolinska Institutet, and Karolinska University Hospital, Stockholm, Sweden;

[2]Center of Excellence for Molecular Food Sciences, Faculty of Chemistry, University of Belgrade, Belgrade, Serbia;

[3]Division of Immunopathology, Department of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Austria;

[4]Department of Clinical Neuroscience, Therapeutic Immune Design Unit, Center for Molecular Medicine (CMM), Karolinska Institutet, Stockholm, Sweden;

[5]Center for Child Health Research, Telethon Institute of Child Health Research, University of Western Australia, Perth, Australia;

[6]Center for Inflammatory Diseases, Karolinska Institutet, Stockholm, Sweden.

[*] These authors contributed equally to this work.

[±] These authors contributed equally to this work.

Correspondence
Marianne van Hage, MD, PhD
Karolinska Institutet,
Department of Medicine, Solna
Immunology and Allergy Unit
Karolinska University Hospital Solna L2:04
SE - 171 76 Stockholm, Sweden
Tel +46-8-517 75942, Fax +46-8-33 57 24
E-mail: marianne.van.hage@ki.se

Short title: Characterization of the cross-reactive cat lipocalin Fel d 7

Key words: Can f 1; cat allergy; cross-reactivity; dog allergy; Fel d 7.

Abbreviations

BAT – basophil activation test

Can f – *Canis familiaris*

CD – circular dichroism

CDE – cat dander extract

ELISA – enzyme-linked immunosorbent assay

Fel d – *Felis domesticus*

Abstract

We investigated the prevalence of sensitization to the cat lipocalin Fel d 7 among 140 cat-sensitized Swedish patients and elucidated its allergenicity and cross-reactivity with the dog lipocalin Can f 1. Sixty-five of 140 patients had IgE to rFel d 7 whereof 60 also had IgE to rCan f 1. A moderate correlation between IgE levels to rFel d 7 and rCan f 1 was found. rFel d 7 activated basophils *in vitro* and inhibited IgE-binding to rCan f 1 in 4/13 patients, whereas rCan f 1 inhibited IgE-binding to rFel d 7 in 7/13 patients. Fel d 7 and Can f 1 showed high similarities in protein structure and epitopes in common were found by using cross-reactive antisera. Fel d 7 is a common allergen in a Swedish cat-sensitized population that cross-reacts with Can f 1, and may contribute to symptoms in cat- but also in dog-allergic patients.

Introduction

Allergy to pets like cat (*Felis domesticus*) and dog (*Canis familiaris*) is the second most common indoor allergy in the western world affecting both children and adults (1, 2). Many patients are co-sensitized to cat and dog (3, 4), suggesting allergenic cross-reactivity. Lipocalins constitute the largest mammalian allergen family (5, 6). They are found in body fluids and secretions (7, 8), and despite the highly conserved structure, the sequence identity between lipocalins is generally low (9). The major dog allergen Can f 1 together with Can f 2, Can f 4 and Can f 6 belong to the lipocalin family (10, 11). With respect to cat, the minor cat allergen Fel d 4 and the newly identified Fel d 7 are lipocalins (12). The degree of allergenic cross-reactivity between lipocalins varies (9, 13) and it has been reported that the dog Can f 6 cross-reacts with the cat Fel d 4, as well as the horse Equ c 1 (14, 15). Cross-reactivity between Can f 2 and Fel d 4 has also been reported (11).

Fel d 7 and Can f 1 have sequence identity close to 60%, indicating potential cross-reactivity (12, 16). Sensitization to Fel d 7 has reported to be common (38%) among cat allergic patients in Australia (12). More information regarding the prevalence of IgE recognition of Fel d 7 and its cross-reactivity and allergenicity is needed. We have investigated the prevalence of sensitization to Fel d 7 in a Swedish cat-sensitized population and elucidated the allergenicity of Fel d 7 and its role in cross-reactivity with Can f 1 on an epitope level.

Methods

For details, see the supporting information

Study population

Sera from 140 Swedish patients with an IgE antibody level >2 kU_A/l (median 14.0, range 2.2-190 kU_A/l) to cat dander extract (CDE) and 45 cat IgE negative sera (<0.1 kU_A/l) were collected at the Department of Clinical Immunology at Karolinska University Hospital, Stockholm. In addition, four patients with doctor's diagnosed allergy to cat and IgE-positive to Fel d 7 were included for basophil activation test (BAT). The study was approved by the local ethics committee.

Allergen preparations and characterization

Recombinant (r) Fel d 7 and Can f 1 were produced and characterized as previously described (11, 12). IgE levels to rFel d 7 and rCan f 1 were determined by ELISA (11). Spearman's correlation test was used for comparing rFel d 7- and rCan f 1-specific IgE levels, where $p<0.05$ was considered significant.

Basophil activation test and IgE cross-reactivity between Fel d 7 and Can f 1

Allergenicity was analyzed by BAT (17). The potential cross-reactivity between IgE to Fel d 7 and Can f 1 was investigated by inhibition ELISA.

Identification and molecular modeling of Fel d 7 cross-reactive epitopes

Six overlapping peptides spanning the Can f 1 sequence (Table S1) were synthesized as described (18). Peptide-specific IgG antibodies were obtained by immunizing rabbits with the corresponding Can f 1-peptides. Can f 1-peptide antisera were used to evaluate cross-reactive binding sites between Can f 1 and Fel d 7.

A molecular model comparing Fel d 7 and Can f 1 was created based on the human tear lipocalin/von Ebners gland protein structure (PDB entry 3EYC_A) using SWISS-MODEL (19) and PyMOL (www.pymol.org).

Results

IgE-reactivity and allergenicity of Fel d 7 and Can f 1 in Swedish cat dander-sensitized patients

Sixty-five of 140 (46.4%) Swedish cat-sensitized patients were rFel d 7-positive (median 2.76, range 0.16-37 kU_A/l, Table S2) and almost all of those (92.3%) were rCan f 1-positive (median 8.11, range 0.17–33 kU_A/l). A moderate correlation between IgE levels to rFel d 7 and rCan f 1 was found (rho=0.62, $p<0.0001$, Figure 1A). IgE levels to dog dander extract among the rFel d 7-positive patients (median 14, range 0.12-95 kU_A/l) were similar compared to CDE levels (median 14, range 2.2-190 kU_A/l). The allergenicity of rFel d 7 in relation to rCan f 1 was investigated in a BAT using blood from Fel d 7-sensitized cat-allergic patients

whereof two were also co-sensitized to Can f 1 (Table S3). rFel d 7 induced basophil degranulation in all patients, while rCan f 1 induced a similar response in the two Can f 1 co-sensitized patients (Figure 1B). No activation was seen upon stimulation with controls (data not shown).

IgE cross-reactivity between Fel d 7 and Can f 1

IgE cross-reactivity was evaluated in sera from 13 patients IgE-positive to rFel d 7 and rCan f 1, with five representative patients shown in Figure 1C and the eight remaining patients shown in Figure S1. At the highest concentration (1µg/ml), rFel d 7 inhibited at least 70% of the IgE-binding to rCan f 1 in four patients, whereas 1µg/ml of rCan f 1 inhibited more than 90% of the IgE-binding to rFel d 7 in seven patients. Notably, rFel d 7 and rCan f 1 were equally low in heterologous inhibitions for two patients (# 5 and 6). Homologous inhibition reached at least 65% in four of five patients (Figure 1C). No inhibition of IgE-binding to Can f 1 was observed in Fel d 7 negative sera (data not shown).

Can f 1 peptide antisera identify cross-reactive epitopes on Fel d 7 and Can f 1

The primary structure of Fel d 7 shows high sequence identity (63%) with Can f 1 (Figure S2A) which is manifested by similar secondary and tertiary structures, as observed by CD spectra (Figure S2B). In the far-UV CD spectra there is a difference in molar elipiticity between these two allergens. The maximum at 195nm for rCan f 1 shows higher elipiticity than in rFel d 7. Furthermore, the CD spectrum of rCan f 1 has a clearly visible minimum at 208nm, also representing α-helix content. Using the CONTINILL algorithm with SP37 base to calculate the percentage of secondary structures results in a total difference of 14.2% in sum of α-helix, β-sheets and β-turn structures between Fel d 7 and Can f 1 (Table S4). These differences can be also noticed in the near-UV CD spectra where the dissimilar molar elipticity probably is due to slight differences in local 3D environment of the aromatic chromophores.

When investigating the locations of shared epitopes between Fel d 7 and Can f 1 using Can f 1-peptide antisera, we found that all five antisera bound rFel d 7 and rCan f 1, while none of them bound the unrelated allergen Fel d 1 (Figure S3). Two of the rabbit antisera, covering the N- and C- terminal parts of Can f 1 (Peptide 1 and Peptide 6 in Table S1), displayed the strongest binding to rFel d 7. A 3D model comparing Fel d 7 and Can f 1 was created based on sequence homology to human tear lipocalin/von Ebners gland protein (Figure 2A and B) showing surface exposed cross-reactive epitopes (Figure 2C).

Discussion

This study shows that Fel d 7 is an important allergen in a Swedish cat-sensitized population as nearly half (46.4%) of the included patients had IgE-reactivity to Fel d 7, which was even higher than the prevalence reported among Australian cat-allergic patients (7). We noted that the majority of the rFel d 7 positive patients had correlating rCan f 1 IgE levels, indicating

IgE cross-reactivity between these lipocalins. However, when investigating the allergenicity by BAT, rCan f 1 only stimulated degranulation of basophils from the two patients that were co-sensitized to Can f 1.

A previous report showed IgE cross-reactivity between Fel d 7 and dog dander extract in one out of two patients tested (12), but whether this inhibition could be assigned to Can f 1 was not explored. Overall we observed that the degree of cross-reactivity varied between the patients and did not always correlate with IgE levels to rFel d 7 and rCan f 1, which probably depended on differences in affinity to the respective allergens. Based on the inhibition results, half of the patients seem to be primarily sensitized to Can f 1. However, when also considering BAT, we identified some patients that were exclusively sensitized to Fel d 7. No inhibition of rCan f 1 by rFel d 7 was observed in patients IgE-negative to rFel d 7. These findings indicate that Fel d 7 and Can f 1 have epitopes in common but also have IgE-binding structures specific for each allergen.

When exploring the location of cross-reactive structures on the allergens, we noted that rabbit antisera against peptides covering the N- and the C- terminal part of Can f 1 had a stronger IgG-binding to rFel d 7 compared to the other antisera. The free C- and N-terminals are regions where longer linear epitopes are exposed. The lower degree of binding of the antisera covering the middle part of the protein sequence could be due to the fact that this part harbors conformational epitopes and the antisera were produced against linear peptides. A 3D model was created to visualize where the potential cross-reactive sequences are located. The model revealed that the identical sequences of Fel d 7 and Can f 1 are exposed on the surface of the molecule, including the N- and C- termini, in line with our results from Can f 1 peptide antisera.

In conclusion, we show that Fel d 7 is an important allergen in a Swedish cat-sensitized population that has a similar structure and shares epitopes with the major dog allergen Can f 1. Our findings widen the understanding of allergenic cross-reactivity between cat and dog, where Fel d 7 may contribute to symptoms not only in cat- but also in dog-allergic patients by IgE cross-reactivity with Can f 1. Thus, including Fel d 7 in panels for pet allergy diagnosis has the potential of improving cat allergy diagnosis.

Acknowledgments

We thank N. Bigdeli for excellent technical assistance. The authors are grateful to Dr M. K. Milcic for protein 3D model assistance.

Funding source

The Swedish Research Council, The Stockholm County Council, The Swedish Asthma and Allergy Association's Research Foundation, The King Gustaf V 80th Birthday Foundation, The Swedish Heart-Lung Foundation, The Center for Inflammatory Diseases, The Hesselman

Foundation, The Konsul Th C Bergh Foundation, The Swedish Cancer and Allergy Foundation, The Magnus Bergvall Foundation, the Swedish Association for Allergology, Ministry of Education Science and Technological Development Republic of Serbia (GA No. 172024), FP7 RegPot project FCUB ERA (GA No. 256716), Karolinska Institutet and in part by grant F4605 of the Austrian Science Fund (FWF).

Authors' contributions

The study was conceived by DA, SSV, KW, WRT, RV, CH and MvH. KW, DA, SSV, MC and JG performed the experiments. DA, SSV, KW, MC, JG, RV, CH and MvH wrote the manuscript and all authors contributed to interpretation of the data and provided critical review of the manuscript.

Conflict of interest

None of the authors has any conflict of interest to declare.

Supporting information:

Data S1 - Methods

Table S1 - Characteristics of synthesized Can f 1-derived peptides

Table S2 - Serological characteristics of the 65 rFel d 7-positive patients

Table S3 - Serological characteristics of patients in basophil activation test

Table S4- Percentages of secondary structures of Fel d 7 and Can f 1

Figure S1 - IgE cross-reactivity between rFel d 7 and rCan f 1 in eight additional patients

Figure S2 - Structural similarities between Fel d 7 and Can f 1

Figure S3 - Can f 1 peptide-specific IgG binding to rFel d 7, rCan f 1 and rFel d 1

References

1. Ronmark E, Bjerg A, Perzanowski M, Platts-Mills T, Lundback B. Major increase in allergic sensitization in schoolchildren from 1996 to 2006 in northern Sweden. *J Allergy Clin Immunol* 2009;**124**(2):357-363, 363 e351-315.
2. Schmitz R, Ellert U, Kalcklosch M, Dahm S, Thamm M. Patterns of sensitization to inhalant and food allergens - findings from the German Health Interview and Examination Survey for Children and Adolescents. *Int Arch Allergy Immunol* 2013;**162**(3):263-270.
3. Wegienka G, Johnson CC, Havstad S, Ownby DR, Nicholas C, Zoratti EM. Lifetime dog and cat exposure and dog- and cat-specific sensitization at age 18 years. *Clin Exp Allergy* 2011;**41**(7):979-986.
4. Konradsen JR, Fujisawa T, van Hage M, Hedlin G, Hilger C, Kleine-Tebbe J, et al. Allergy to

furry animals: New insights, diagnostic approaches, and challenges. *J Allergy Clin Immunol* 2015;**135**(3):616-625.
5. Hilger C, Kuehn A, Hentges F. Animal lipocalin allergens. *Curr Allergy Asthma Rep* 2012;**12**(5):438-447.
6. Jensen-Jarolim E, Pacios LF, Bianchini R, Hofstetter G, Roth-Walter F. Structural similarities of human & mammalian lipocalins, and their function in innate immunity and allergy. *Allergy* 2015.
7. Virtanen T, Zeiler T, Mantyjarvi R. Important animal allergens are lipocalin proteins: why are they allergenic? *Int Arch Allergy Immunol* 1999;**120**(4):247-258.
8. Virtanen T, Kinnunen T. Mammalian allergens. *Clin Allergy Immunol* 2008;**21**:201-218.
9. Virtanen T, Kinnunen T, Rytkonen-Nissinen M. Mammalian lipocalin allergens--insights into their enigmatic allergenicity. *Clin Exp Allergy* 2012;**42**(4):494-504.
10. Niemi MH, Rytkonen-Nissinen M, Janis J, Virtanen T, Rouvinen J. Structural aspects of dog allergies: the crystal structure of a dog dander allergen Can f 4. *Mol Immunol* 2014;**61**(1):7-15.
11. Madhurantakam C, Nilsson OB, Uchtenhagen H, Konradsen J, Saarne T, Hogbom E, et al. Crystal Structure of the Dog Lipocalin Allergen Can f 2: Implications for Cross-reactivity to the Cat Allergen Fel d 4. *Journal of Molecular Biology* 2010;**401**(1):68-83.
12. Smith W, O'Neil SE, Hales BJ, Chai TL, Hazell LA, Tanyaratsrisakul S, et al. Two newly identified cat allergens: the von Ebner gland protein Fel d 7 and the latherin-like protein Fel d 8. *Int Arch Allergy Immunol* 2011;**156**(2):159-170.
13. Nilsson OB, van Hage M, Gronlund H. Mammalian-derived respiratory allergens - implications for diagnosis and therapy of individuals allergic to furry animals. *Methods* 2014;**66**(1):86-95.
14. Nilsson OB, Binnmyr J, Zoltowska A, Saarne T, van Hage M, Gronlund H. Characterization of the dog lipocalin allergen Can f 6: the role in cross-reactivity with cat and horse. *Allergy* 2012;**67**(6):751-757.
15. Hilger C, Swiontek K, Arumugam K, Lehners C, Hentges F. Identification of a new major dog allergen highly cross-reactive with Fel d 4 in a population of cat- and dog-sensitized patients. *J Allergy Clin Immunol* 2012;**129**(4):1149-1151.
16. Hales BJ, Chai LY, Hazell L, Elliot CE, Stone S, O'Neil SE, et al. IgE and IgG binding patterns and T-cell recognition of Fel d 1 and non-Fel d 1 cat allergens. *J Allergy Clin Immunol Pract* 2013;**1**(6):656-665 e651-655.
17. Saarne T, Kaiser L, Gronlund H, Rasool O, Gafvelin G, van Hage-Hamsten M. Rational design of hypoallergens applied to the major cat allergen Fel d 1. *Clin Exp Allergy* 2005;**35**(5):657-663.
18. Focke M, Mahler V, Ball T, Kraft D, Valenta R. Nonallergenic peptides from surface-exposed areas or B-cell epitopes of allergens for specific immunotherapy. *Int Arch Allergy Immunol* 2001;**124**(1-3):398-399.
19. Arnold K, Bordoli L, Kopp J, Schwede T. The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. *Bioinformatics* 2006;**22**(2):195-201.

Figure legends

Figure 1. IgE reactivity and allergenicity of rFel d 7 and rCan f 1 and their IgE cross-reactivity

(**A**) Comparison of Fel d 7- and Can f 1-specific IgE levels (kU_A/l) in sera from Fel d 7-positive subjects (n=65), using Spearman's correlation test; (**B**) Allergenicity of rFel d 7 and rCan f 1 as determined by basophil activation in blood from four cat-allergic patients (#I-IV, Table S3) and one non-atopic control. Degranulation is presented as proportion (%) of CD63-positive out of CD203c-positive cells by flow cytometry (y-axes) in response to different

allergen concentrations (x-axes); (**C**) Cross-inhibition and homologous inhibition of rFel d 7 and rCan f 1 shown in five patients (2, 4, 6, 8, 14, Table S2). Percentages of inhibition (y-axes) are plotted against different concentrations of inhibitor allergens (x-axes). IgE-binding to rCan f 1 in sera pre-incubated with rFel d 7–cross-inhibition (grey dots); IgE-binding to rFel d 7 in sera pre-incubated with rCan f 1–cross-inhibition (black squares); IgE-binding to rFel d 7 in sera pre-incubated with rFel d 7–homologous inhibition (dark gray triangles); IgE-binding to rCan f 1 in sera pre-incubated with rCan f 1–homologous inhibition (open triangles).

Figure 2. Fel d 7 and Can f 1 cross-reactive epitopes

(**A**) Ribbon representation and (**B**) surface representation of a 3D model comparing Fel d 7 and Can f 1, based on the human tear lipocalin protein structure (PDB entry 3EYC_A). Pink color corresponds to identical sequences present in all three proteins. Blue color corresponds to identical sequences present only in rFel d 7 and rCan f 1. Grey color corresponds to sequences which are not identical with Fel d 7 and/or Can f 1; (**C**) 3D ribbon (left) and surface representation (right) with highlighted peptides at the N- (Green: Peptide 1 in Table S1) and C-terminal (Red: Peptide 6 in Table S1).