

Involvement of Can f 5 in a Case of Human Seminal Plasma Allergy

Maria Basagaña^a Borja Bartolome^b Carlos Pastor-Vargas^c Lars Mattsson^e
Jonas Lidholm^e Moises Labrador-Horrillo^d

^aAllergy Department, Hospital Universitari Germans Trias i Pujol, Universitat Autònoma de Barcelona, Badalona,

^bBial-Aristegui, R&D, Bilbao, ^cDepartment of Biochemistry and Molecular Biology, Universidad Complutense, Madrid, and ^dAllergy Department, Hospital Universitari de la Vall d'Hebron, Universitat Autònoma de Barcelona, Barcelona, Spain; ^ePhadia AB, Uppsala, Sweden

Key Words

Allergens • Cross-reactivity • Cross-reactive allergen • Dog

Abstract

Background: The existence of IgE binding to dog dander extract without IgE antibodies against the described dog allergens (Can f 1, 2, 3 and 4) implies the presence of other dog allergens yet to be identified. Recently, an IgE-binding protein was isolated from dog urine and identified as prostatic kallikrein; it has been named Can f 5. Cross-reactivity between a dog dander allergen and human prostate-specific antigen (PSA) has been described. The aim of this study was to identify the dog dander allergen that presents cross-reactivity with PSA and demonstrate its clinical relevance in our patient with human seminal plasma allergy. **Methods:** SDS-PAGE immunoblotting and inhibition tests were performed. Mass spectrometry was carried out to identify the protein involved in the allergy reactions. **Results:** SDS-PAGE immunoblotting-inhibition with an IgE-binding protein from dog prostatic secretion showed total IgE binding inhibition to a 28-kDa IgE-reactive band identified as PSA. The electroeluted protein from dog prostatic secretion was identified by mass spectrometry as Can f 5. IgE immunoblotting of human seminal

plasma incubated with the serum of the patient revealed two IgE-binding bands (28 and 32.7 kDa). Both SDS-PAGE immunoblotting inhibition assays, with human seminal plasma or purified PSA in solid phase, showed complete IgE binding inhibition when the serum of the anaphylactic patient was pre-incubated with dog dander extract or recombinant Can f 5. **Conclusions:** The dog dander allergen that shows cross-reactivity with human PSA has been characterized and turns out to be the recently described Can f 5. We demonstrated the clinical relevance of this cross-reactivity in a patient.

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Introduction

Human seminal plasma allergy (HSPA) is a rare phenomenon, but the exact prevalence of this condition is unknown. Several seminal plasma allergens have been described, ranging in molecular mass between 12 and 75 kDa [1].

In two separate studies of HSPA, prostate-specific antigen (PSA) has been identified as the causative allergen [2, 3]. In one of these studies, cross-reactivity between human PSA and dog dander proteins was demonstrated [2].

In a recently concluded epidemiological study carried out in Spain [4], an increased prevalence of sensitization to animal epithelia was observed. These epithelia represent the third most common cause of sensitization in rhinitis and asthma.

Dog dander is an important cause of allergy respiratory symptoms, including rhinoconjunctivitis, asthma and bronchial inflammation. Among the described dog allergens, Can f 1, Can f 2 and Can f 4 are all members of the lipocalin protein family and have been purified and expressed as recombinant proteins [5, 6]. Can f 3, dog serum albumin, is a highly conserved protein, and antibodies directed to Can f 3 cross-react extensively with albumin of other animal species [7]. Approximately 50% of dog-allergic patients present IgE antibodies against Can f 1, 20% against Can f 2 and 15–40% against Can f 3 and Can f 4 [6].

Recently, a new IgE-binding protein was isolated from dog urine, identified as prostatic kallikrein and named Can f 5. The protein was shown to be present also in dog dander and bound IgE antibodies from 70% of sera of dog-allergic patients were tested in that study, so it was found to be a major dog allergen [8].

Methods

Here we present the case of a 38-year-old nulligravida woman who experienced anaphylactic reactions after sexual intercourse since her first coitus, as previously described by our group [2, 9]. The patient was monosensitized to dog dander since she was 18 years old, and she suffers from bronchial asthma due to this sensitization.

The patient reported the presence of a dog at home since she was a teenager and now she has frequent contact with dogs. The patient also followed a specific immunotherapy to dog epithelium for 1 year after the beginning of HSPA symptoms; however, the treatment was discontinued because of the low response to this desensitization treatment. The patient provided written informed consent for the current study, and the study was approved by the hospital ethical committee.

Allergen Source

Seminal fluid was collected by condom from a healthy donor. It was used to perform all the in vitro assays.

Samples of dog prostatic secretions were collected at the Small Animals Reproduction Service of the Veterinary Faculty at the Universitat Autònoma de Barcelona. Prostatic secretions were collected by manual manipulation of the dog's penis. Untreated samples were used to perform all the in vitro assays.

Skin Prick Test

Skin prick tests with dog epithelium (3 mg/ml) and dander (1.3 mg/ml) extracts were performed according to a standard

procedure [10]. Histamine phosphate (10 mg/ml) and sterile 0.9% saline were used as positive and negative controls, respectively. A mean wheal area of 7 mm² or greater, 15 min after puncture, was considered a positive response.

Specific IgE Determination

Determination of serum-specific IgE against dog dander, dog epithelium and human seminal plasma was performed using the ImmunoCAP regular test from Phadia. Test results were expressed as kilounits per liter, according to the manufacturer's instructions. ImmunoCAP results higher than 0.35 kU/l were considered positive.

Enzyme Allergosorbent Test Inhibition

Enzyme allergosorbent test inhibition was performed according to the methods reported by Yman et al. [11]. HSP was used as the solid phase, and the patient's serum was preincubated with dog dander extract (1.8 mg/ml) and Can f 1 and Can f 2 recombinant molecules at 120 µg/ml (Department of Clinical Microbiology, University of Kuopio, Kuopio, Finland).

SDS-PAGE Immunoblotting and Inhibition Tests

To perform the in vitro assays, seminal fluid from a healthy human donor, prostatic secretions collected from two different dogs, purified PSA from human semen (Sigma-Aldrich) and purified recombinant Can f 5 were used.

SDS-PAGE was carried out according to the method of Laemmli [12]. When reducing conditions were used, samples were dissolved in 62 mM Tris-HCl, pH 6.8, and were dissociated with 2% SDS and 5% β-mercaptoethanol at 100°C for 5 min; in non-reducing conditions, 2-mercaptoethanol was not added. When Western inhibition assays were performed, patient serum was preincubated with different inhibitor phases (Can f 5 at 10 µg/ml) overnight at 4°C; then membranes were incubated with the patient serum sample obtained.

Mass Spectrometry

Bands were extracted from the gel and digested with trypsin, and the proteins were identified by mass spectrometry as previously described [13].

Results

The patient showed positive skin prick tests to dog dander and dog epithelium. The serum IgE titer was 5 kU/l to complete human seminal plasma, 7.5 kU/l to dog epithelium, 8.9 kU/l to dog dander and 95 kU/l to dog prostatic secretion.

Enzyme allergosorbent test inhibition assays were performed with the serum from the patient with HSPA to assess whether HSP proteins cross-reacted with Can f 1 and Can f 2, the 2 major dog epithelium allergens. The results showed that Can f 1 and Can f 2 recombinant allergens as inhibitors (120 µg/ml) were not able to reduce the IgE antibody binding to seminal fluid, whereas in the same assay, dog dander extract (1.8 mg/ml) produced a

total inhibition (data not shown). This result is consistent with the absence of known homologues of Can f 1 and Can f 2 in HSP.

An immunoblotting assay in reducing conditions with patient serum using 2 samples of dog prostatic secretions in solid phase revealed two IgE-binding bands of 31 and 17–18 kDa (fig. 1, panel 1).

The IgE-binding bands in dog prostate secretions were identified by mass spectrometry and turned out to match with the recently described Can f 5 (arginine esterase peptidase S1 family, kallikrein subfamily) [8]. In order to examine the relationship with human PSA, the 31-kDa band was electroeluted and used as inhibitor in IgE immunoblotting inhibition experiments. Preincubation of the anaphylactic patient's serum with this protein, at a concentration of 50 µg/ml, caused complete inhibition of IgE binding to PSA in an HSP immunoblotting inhibition assay (results not shown) as well as to the 17- to 18-kDa band in the dog dander immunoblotting inhibition assay in reducing conditions (fig. 1, panel 2). PSA inhibition is explained by the sequence homology described between dog prostate arginine esterase (31-kDa band) and human PSA, as both proteins belong to the kallikrein subfamily, and the inhibition on the 17- to 18-kDa dog dander protein by the aforementioned Can f 5 present in dog dander.

IgE immunoblotting in nonreducing electrophoretic conditions of human seminal plasma, incubated with serum of the patient with HSPA, revealed an IgE-binding band of 28 kDa and a faint one of 32.7 kDa (the 28-kDa protein was identified as PSA as we previously described [2]; fig. 1, panel 3). Preincubation of this serum with either dog dander extract (1 mg/ml) or purified recombinant Can f 5 (10 µg/ml) caused complete inhibition of IgE binding to both bands (fig. 1, panel 3). Furthermore, an SDS-PAGE immunoblotting inhibition assay using purified PSA from human semen (Sigma-Aldrich) in solid phase and dog dander extract (1 mg/ml) and recombinant Can f 5 (100 µg/ml) as inhibitors showed the capacity of both samples to produce total IgE binding inhibition on the purified PSA protein (online suppl. fig. 1, www.karger.com/doi/10.1159/000336388).

Discussion

In this case of a dog dander-sensitized patient displaying HSPA, Can f 5 was clearly identified as a dog allergen cross-reactive with PSA from human seminal plasma.

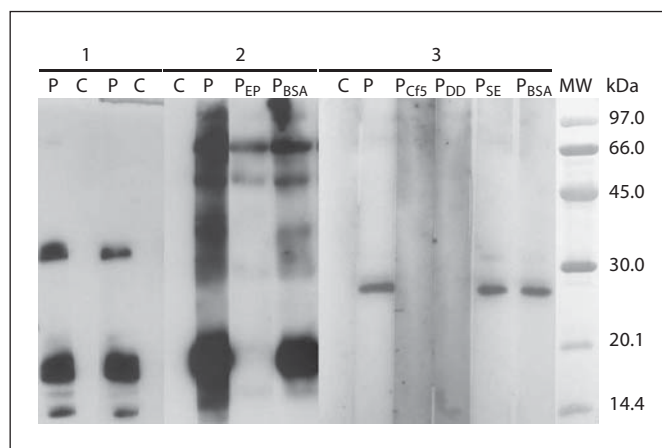


Fig. 1. IgE immunoblotting results. Panels 1 and 2: reducing conditions; panel 3: nonreducing conditions. Panel 1 shows two samples of dog prostatic secretions incubated with serum from our patient with HSPA (lane P) and with a pool of sera from nonatopic patients (lane C). Panel 2 shows dog dander incubated with serum of our patient with HSPA under the following conditions: not preincubated (lane P), preincubated with the 31-kDa electroeluted protein at 50 µg/ml (lane P_{EP}) or preincubated with BSA at 50 µg/ml (lane P_{BSA}). Panel 3 shows human seminal plasma from a healthy donor incubated with serum from our patient with HSPA under the following conditions: not preincubated (lane P), preincubated with Can f 5 at 10 µg/ml (lane P_{Cf5}), preincubated with dog dander extract at 1 mg/ml (lane P_{DD}), preincubated with shrimp extract at 1 mg/ml (lane P_{SE}) or preincubated with BSA at 10 µg/ml (lane P_{BSA}). Lane C: solid phase incubated with a pool of sera from nonatopic patients. Lane MW: molecular weight standard.

We have noted with several serum determinations that high concentrations of IgE antibodies against Can f 5 are accompanied by weak positive responses to HSP ImmunoCAP. Unlike the HSPA case presented here, it appears that these low IgE levels against HSP typically do not elicit allergy symptoms (unpublished observations).

Mattsson et al. [8] described the molecular weight of Can f 5 to be about 28 kDa in nonreducing conditions and two bands at about 18 and 10 kDa under reducing conditions in urine and dander. They found that Can f 5 expressed in *Pichia pastoris* gives two bands, one of about 31 kDa and another of about 18 kDa [8], as we describe in the present study. The differences may be due to more complete processing of the proteins purified from natural sources (urine, dander). The 31-kDa protein, purified from the original source (prostatic secretions), represents Can f 5 in an unprocessed form.

In conclusion, studying a patient with anaphylactic HSPA, we have demonstrated cross-reactivity between

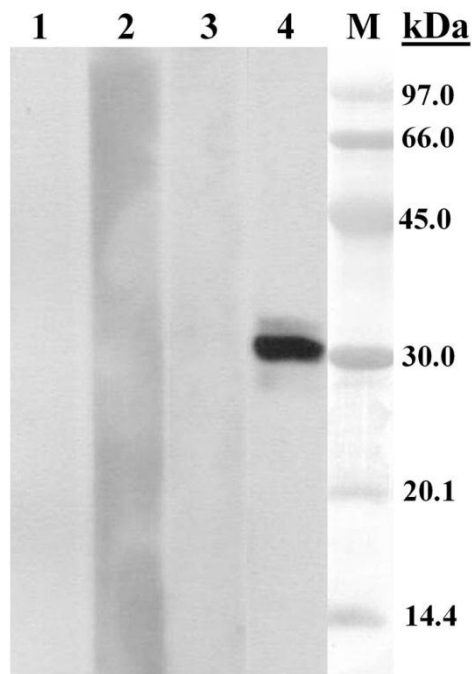
the allergenic protein from HSPA, PSA, and the recently characterized dog allergen Can f 5, suggesting that dog protein sensitization may in some cases be relevant to HSPA, perhaps as a predisposing factor or as an environmental booster of the immune response against PSA.

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References

- Halpern BN, Kyt RB: Clinical and immunological study of an exceptional case of reaginic type sensitization to human seminal fluid. *Immunology* 1967;12:247–259.
- Basagaña M, Bartolome B, Pastor C, Torres F, Alonso R, Vivanco F, Cisteró-Bahima A: Allergy to human seminal fluid: cross-reactivity with dog dander. *J Allergy Clin Immunol* 2008;121:233–239.
- Weidinger S, Mayerhofer A, Raemsch R, Ring J, Kohn FM: Prostate-specific antigen as allergen in human seminal plasma allergy. *J Allergy Clin Immunol* 2006;117:213–215.
- SEIAC: Alergológica 2005: factores epidemiológicos, clínicos y socioeconómicos de las enfermedades alérgicas en España en 2005, ed 1. Madrid, Luzan.
- Saarelaïnen S, Taivainen A, Rytönen-Nissinen M, Auriola S, Immonen A, Mäntyjärvi R, Rautiainen J, Kinnunen T, Virtanen T: Assessment of recombinant dog allergens Can f 1 and Can f 2 for the diagnosis of dog allergy. *Clin Exp Allergy* 2004;34:1576–1582.
- Mattsson L, Lundgren T, Olsson P, Sundberg M, Lidholm J: Molecular and immunological characterization of Can f 4: a dog dander allergen cross-reactive with a 23 kDa odorant-binding protein in cow dander. *Clin Exp Allergy* 2010;40:1276–1287.
- Cabañas R, López-Serrano MC, Carreira J, Ventas P, Polo F, Caballero MT, Contreras J, Barranco P, Moreno-Ancillo A: Importance of albumin in cross-reactivity among cat, dog and horse allergens. *J Invest Allergol Clin Immunol* 2000;10:71–77.
- Mattsson L, Lundgren T, Everberg H, Larsson H, Lidholm J: Prostatic kallikrein: a new major dog allergen. *J Allergy Clin Immunol* 2009;123:362–368.
- Ferré-Ybarz L, Basagaña M, Coroleu B, Bartolomé B, Cisteró-Bahima A: Human seminal plasma allergy and successful pregnancy. *J Invest Allergol Clin Immunol* 2006;16:314–316.
- Malling HJ: Allergen standardisation and skin tests. *Methods of skin testing. Allergy* 1993;48(suppl 14):55–56.
- Yman L, Ponterius G, Brandt R: RAST-based allergen assay methods. *Dev Biol Stand* 1975;29:151–165.
- Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T. *Nature* 1970;227:680–685.
- Pastor C, Cuesta-Herranz J, Cases B, Pérez-Gordo M, Figueredo E, de las Heras M, Vivanco F: Identification of major allergens in watermelon. *Int Arch Allergy Immunol* 2009;149:291–298.



Online Supplementary Fig. 1. IgE- Immunoblotting results (in non-reducing conditions). Purified PSA in solid phase. Lane 1: control serum (pool of sera from non atopic subjects); lanes 2, 3, 4: patient's serum previously incubated with dog dander extract (1mg/ml), or purified rCan f 5 (100 μ l/ml), or BSA (100 μ l/ml), respectively. Lane M: Molecular weight standard.