

Heterogeneity of the Allergen Content in Male Dog Urine and Dander

Calzada D, Iraola V , Carnés J

R&D Allergy & Immunology Unit. Laboratorios LETI S.L.U Madrid, Spain

Corresponding author:

Jerónimo Carnés
Laboratorios LETI S.L
Tres Cantos, Madrid, SPAIN
E-mail: jcarnes@leti.com

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Dogs have become an important allergenic source, responsible for respiratory allergic symptoms including rhino-conjunctivitis and asthma. To date, seven allergens have been identified. Can f 1, 2, 4 and 6 are members of the lipocalin family, Can f 3 is an albumin and Can f 7 was reported as a member of the MD2-like lipidic recognition family [1,2]. Finally, and related with its high prevalence of sensitization, recent studies have shown the importance of Can f 5 [3], a 28 kDa prostatic kallikrein presented mainly in male dog urine [1,4,5]. For the manufacturing of allergenic extracts, control methods and acceptance criteria relating to the handling of the source material are well established by the European Pharmacopoeia [6]. However, the wide distribution of dog allergens, present in skin, saliva, epithelia and urine, and the differences observed in allergen presence between males and females, make difficult the raw material selection for producing allergen extracts with an appropriate allergen composition for diagnosis and treatment. These facts could be behind the limited clinical efficacy of dog allergen-specific immunotherapy [2,7,8].

The aim of the study was to investigate the allergen composition of male dog urine and dander, the two most relevant allergenic sources of dogs, and to define the raw material, for producing an allergenic extract which contain most appropriate panel of dog allergens.

Eight freeze-dried urine extracts were manufactured from urines from 8 male beagledogs, over 6 years of age, that were provided by Isoquimen (Barcelona, Spain). Protein content was measured by Bradford (Thermo Fisher-Scientific) following manufacturer's instructions. In parallel, a dog dander extract, prepared with a mixture of raw material from different breeds (Allergon, Ängelholm, Sweden), was manufactured in compliance with GMP principles (Laboratorios LETI, Madrid, Spain). The antigen profile of urine samples and dander extract was compared. A hundred μg of each extract were run in a SDS-PAGE, under reducing and non-reducing conditions and stained with Coomassie. The presence of the main allergens Can f 5 and Can f 1 was investigated in both type of extracts. Can f 5, corresponding to a 28 kDa protein band, was cut from the non-reduced gel and analyzed by mass-spectrometry (MS). Can f 1 was identified by western blot and quantified by ELISA, using a commercial kit (EL-CF1) (Indoor biotechnologies, VA, USA) and a secondary antibody anti-rabbit IgG-HRP (1/300.000) (Bethyl, TX, USA).

To analyze the sensitization profile of dog allergic patients, two different sera were purchased from Plasmalab International (WA, USA), which operates in compliance with U.S. FDA regulations. Specific IgE antibodies levels to dog dander extract, rCan f 1 and rCan f 5 were determined by ImmunoCAP (Thermo Fisher-Scientific). Patient 1 (Dog dander extract = 23.2 kUA/l; Can f 1 = 6.4 kUA/l; Can f 5 = 49.7 kUA/l) and Patient 2 (Dog dander extract ≥ 100 kUA/l; Can f 1 = 38.2 kUA/l; Can f 5 = 5.4 kUA/l). Proteins from non-reduced and reduced SDS-PAGE gels were electrotransferred. Membranes were blocked and incubated overnight with the serum (1/10). After washing, membranes were incubated with mouse anti-human IgE:HRP (1:20,000) (Southern Biotech, AL, USA) and developed by chemoluminescence.

The protein concentration was not homogeneous in different urine extracts, varying from 28.3 µg/mg to 104.1 µg/mg. Regarding the protein profile, a total of 11 different bands (from 10 kDa to 100 kDa) were detected in the reduced SDS-PAGE while 16 different bands were detected in the non-reduced one (Figure 1). ImageQuant analysis revealed that a band at 28 kDa, identified by MS as Can f 5 (sequence coverage 58.46%), was present in all urine extracts. Urines from dogs 1, 7 and 8 showed the highest intensity, whereas urines 3 and 4 showed the lowest. Similar results were obtained in reduced gels where Can f 5 was divided in bands with 10 and 18 kDa [3]. Can f 1 was not detected in urine extracts.

Concerning dog dander extract, 13 different bands were detected in the reduced gel and 7 in the non-reduced. The presence of Can f 5 and Can f 1 was confirmed by MS, with sequence coverages of 30% and 60%, respectively. The concentration of Can f 1 was 7.39 ± 0.47 µg/mg dander lyophilized product. The presence of Can f 2, 3, 4, 6 and 7 were also detected by MS.

Regarding sensitization profile, patient 1 mainly recognized bands from urine extracts while patient 2 recognized more intensely bands from dog dander (Supplementary Material, Figure 1). This difference comes about for two reasons, the different individual allergen sensitization showed by Can f 1 and Can f 5 specific IgE levels and the differences in the main allergens content in the extracts. This means that the allergenic activity of the extracts varied greatly depending on each patient's characteristics as well as the allergen components present in the extracts [9,10]. An epidemiologic study including a numerous population and deeply characterized according to the dog allergen exposure (males or females, breed...) and clinical manifestations could be useful to establish different allergenic profiles and correlate with the sensitization profile.

In summary, this study demonstrates the heterogeneity of the raw material used for producing dog extract. In case of urine, this is the first article showing the individual differences of the allergenic profile in male-dog urine extracts of the same breed and similar age. On the other hand, the variability of dog fur, saliva and hair extracts has been previously demonstrated, revealing significant variations on most of dog allergens regardless of breed [2,11]. Additionally, and from a clinical point of view, our results also confirmed the variability of the allergenic profile of individuals. According to that, and in order to guaranty the clinical efficacy of diagnostics and immunotherapy, it seems clear that dog extracts must be prepared according to the allergenic profiles of patients. This means that the final products must contain, in an appropriate concentration, the responsible allergens of dog allergic sensitization. This fact is especially relevant for known allergens, as Can f 1, but also for recently described allergens, as Can f 5, that needs the development of a specific quantification assay.

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Conflicts of interests

Dr. Calzada reports he is an employee of LETIPharma.

Dr. Iraola reports he has been an employee of LETIPharma.

Dr. Carnés reports and Jerónimo Carnés is an employee of LETIPharma.

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References

1. González-de-Olano D, Gandolfo-Cano M, de-Calzada-Bustingorri MP, González-Mancebo E, de-Andrés-Martín A, Cuesta-Herranz J, et al. Prevalence of allergy to human seminal fluid among women with allergy to male dog and sensitization to Can f 5. *Clin Exp Allergy*. 2018;48(10):1368-70.
2. Wintersand A, Asplund K, Binnmyr J, Holmgren E, Nilsson OB, Gafvelin G, et al. Allergens in dog extracts: Implication for diagnosis and treatment. *Allergy*. 2019;74(8):1472-9.
3. Mattsson L, Lundgren T, Everberg H, Larsson H, Lidholm J. Prostatic kallikrein: a new major dog allergen. *J Allergy Clin Immunol*. 2009;123(2):362-8.
4. Liccardi G, Calzetta L, Salzillo A, Apicella G, Piccolo A, Di Maro E, et al. Dog allergy: can a prevalent or exclusive sensitization to Can f 5 be considered a lucky or negative event in real life? *Eur Ann Allergy Clin Immunol*. 2018;50(6):283-5.
5. Dávila I, Domínguez-Ortega J, Navarro-Pulido A, Alonso A, Antolín-Amerigo D, González-Mancebo E, et al. Consensus document on dog and cat allergy. *Allergy*. 2018;00:1-17
6. Council of Europe. European Pharmacopoeia 9.0. Animal epithelia and outgrowths for allergen products. 01/2017:2621. 1736-37.
7. Liccardi G, Calzetta L, Salzillo A, Apicella G, Di Maro E, Rogliani P. What Could the Role of Can f 5 Allergen Be in Dog-Sensitized Patients in "Real Life"? *J Invest Allergol Clin Immunol*. 2017;27(6):397-8.
8. Liccardi G, Calzetta L, Milanese M, Lombardi C, Savi E, Passalacqua G, et al. Critical aspects in dog allergen immunotherapy (DAI). May Component

- Resolved Diagnosis (CRD) play a role in predicting the efficacy? *Hum Vaccin Immunother.* 2018;3;14(6):1438-41.
9. Riccio AM, De Ferrari L, Chiappori A, Ledda S, Passalacqua G, Melioli G, et al. Molecular diagnosis and precision medicine in allergy management. *Clin Chem Lab Med.* 2016;54(11):1705-14.
10. Fernández-Caldas E, Cases B, El-Qutob D, Cantillo JF. Mammalian raw materials used to produce allergen extracts. *Ann Allergy Asthma Immunol.* 2017;119(1):1-8.
11. Vredegoor DW, Willemse T, Chapman MD, Heederik DJ, Krop EJ. Can f 1 levels in hair and homes of different dog breeds: lack of evidence to describe any dog breed as hypoallergenic. *J Allergy Clin Immunol.* 2012;130(4):904-9.

Figure 1. Protein profile of dander and urine extracts investigated by SDS-PAGE. Extracts were analyzed under reducing and non-reducing conditions. Bands corresponding to main allergens are highlighted: blue box: Can f 1; red box: Can f 5. In case of reducing conditions the band of Can f 5 is divided in two bands (10 and 18 kDa). Lane M: MW marker proteins; Lanes 1-8: urine extracts; Lane 9: dander extract.

