Heterogeneity of Allergen Content in Male Dog Urine and Dander

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Dogs have become an important source of allergens and are responsible for respiratory allergic symptoms including rhinoconjunctivitis and asthma. To date, 7 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 is a member of the MD2-like lipid recognition family [1,2]. Can f 5, which has a high prevalence of sensitization [3], is a 28-kDa prostatic kallikrein found mainly in male dog urine [1,4,5]. In the manufacture of allergen 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, which are present in skin, saliva, dander, and urine, and the differences observed in the presence of allergen between males and females, make it difficult to select raw material for production of allergen extracts with an appropriate allergen composition for diagnosis and treatment. These facts could explain the limited clinical efficacy of dog allergen-specific immunotherapy [2,7,8].

The aim of our study was to investigate the allergen composition of male dog urine and dander, the 2 most relevant allergen sources in dogs, and to define the raw material necessary for the production of an allergen extract that would contain the most appropriate panel of dog allergens.

Eight freeze-dried urine extracts were manufactured from the urine of 8 male beagles aged over 6 years of age (Isoquimen). Protein content was measured following the Bradford technique (Thermo Fisher Scientific). In parallel, a dog dander extract prepared with a mixture of raw material from different breeds (Allergon) was manufactured in line with the principles of Good Manufacturing Practice (Laboratorios LETI). The antigen profile of urine samples and dander extract was compared. One hundred micrograms of each extract underwent SDS-PAGE under reducing and nonreducing conditions before Coomassie Blue staining. The presence of the main allergens Can f 5 and Can f 1 was investigated in both types of extract. Can f 5, which corresponds to a 28-kDa protein band, was cut from the nonreduced gel and analyzed by mass-spectrometry (MS). Can f 1 was identified using Western blot and quantified using a commercial ELISA kit (EL-CF1, Indoor Biotechnologies).

In order to analyze the sensitization profile of dog-allergic patients, 2 sera were purchased from Plasmalab International, which complies with the regulations of the United States Food and Drug Administration. Specific IgE antibody levels to dog dander extract, rCan f 1, and rCan f 5 were determined using ImmunoCAP (Thermo Fisher Scientific). The values for patient 1 were as follows: dog dander extract, 23.2 kU_A/L; Can f 1, 6.4 kU_A/L; Can f 5, 49.7 kU_A/L. The values for patient 2 were as follows: dog dander extract, ≥100 kU_A/L; Can f 1, 38.2 kU_A/L; Can f 5, 5.4 kU_A/L. Proteins from nonreduced 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 antihuman IgE:HRP (1:20 000) (Southern Biotech) and developed by chemiluminescence.

The protein concentration of the different urine extracts was not homogeneous, ranging 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 analysis, while 16 different bands were detected in

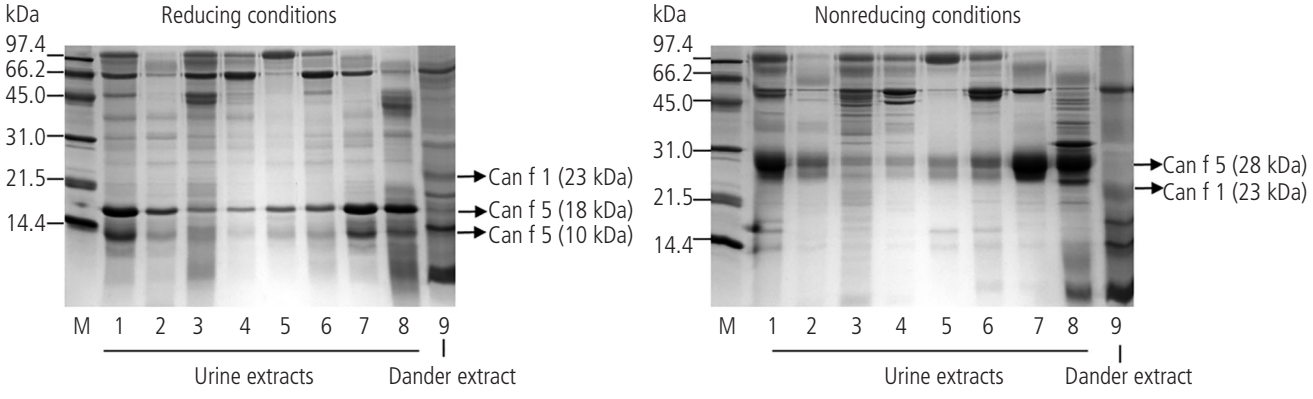

Figure. Protein profile of dander and urine extracts investigated by SDS-PAGE. Extracts were analyzed under reducing and nonreducing conditions. Bands corresponding to the main allergens are highlighted. In the case of reducing conditions, the band of Can f 5 is divided into 2 bands (10 and 18 kDa). Lane M, molecular weight marker proteins; Lanes 1-8, urine extracts; Lane 9, dander extract.

the nonreduced one (Figure). 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 into bands with 10 and 18 kDa [3]. Can f 1 was not detected in urine extracts.

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

As for sensitization profile, patient 1 mainly recognized bands from urine extracts, while patient 2 more intensely recognized bands from dog dander (Supplementary Material, Figure 1). This difference came about because of the different individual allergen sensitization associated with specific IgE levels of Can f 1 and Can f 5 and the differences in the main allergen content in the extracts. Consequently, the allergenic activity of the extracts varied greatly depending on each patient's characteristics, as well as on the allergen components present in the extracts [9,10]. An epidemiologic study including a numerous population that is deeply characterized according to factors affecting exposure to the dog allergen (eg, sex and breed) and clinical manifestations could prove useful when attempting to establish allergenic profiles and correlations with the sensitization profile.

In summary, this study demonstrates the heterogeneity of the raw material used for producing dog allergen extracts. This is the first report of the individual differences in allergenic profile in urine extracts from male dogs of the same breed and a similar age. The variability in dog fur, saliva, and hair extracts has been previously demonstrated, with significant variations for most 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 the individual patients. Therefore, in order to guarantee the clinical efficacy of diagnostics and immunotherapy, dog extracts must be prepared according to the allergenic profile of the individual patient, thus necessitating that the final products contain, at appropriate concentrations, the allergens responsible for allergic sensitization to dog. This fact is especially relevant for well-known allergens, such as Can f 1, but also for recently described allergens, such as Can f 5, for which a specific quantification assay must be developed.

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Conflicts of Interest

Dr. Calzada and Dr. Carnés are employees of Laboratorios LETI. S.L.U.

Dr. Iraola has been an employee of Laboratorios LETI. S.L.U.

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