

Dander is the best epithelial source for dog allergenic extract preparations

Martínez A, Martínez J, Sanz ML, Bartolomé B, Palacios R. Dander is the best epithelial source for dog allergenic extract preparations. Allergy 1994; 49: 664–667. © Munksgaard 1994.

Four different commercially available raw materials of dog epithelia (dander, hair and skin scraping, skin scraping, and whole skin) were compared by means of biochemical methods such as SDS–PAGE and chromatography with the SMART™ System, immunochemical methods such as RAST and SDS–PAGE immunoblotting, and cutaneous tests. Dander extract clearly exhibited the highest IgE binding and *in vivo* allergenic activity. The dog major allergens *Can f*I and the 19-kDa protein were adequately detected only in dander extract. Thus, it is concluded that dander is the most suitable epithelial source for preparing dog allergenic extracts.

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Key words: dog allergens; dog epithelia; SMART™ System.

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Accepted for publication 12 January 1994

Precise diagnostic and effective immunotherapy treatment of dog allergy depends on the use of the appropriate allergenic extracts. False negative diagnoses are commonly found by *in vivo* and *in vitro* methods when dog extracts are tested (9). Differences that are due to origin (epithelia, serum, saliva, and urine) and breed are already substantiated (1, 3, 13, 14).

During the last few years, dog extracts have drawn the attention of several research groups, leading to the establishment of the World Health Organization (WHO) International Standard (8) and the characterization and definition of a major allergen: *Can f*I (3, 11, 13), thus allowing a better comparison of different dog extracts.

Since epithelial origin is considered to be the most suitable one, the aim of the present study was to analyze the different commercially available epithelial materials. Four different sources of dog epithelia were tested by *in vitro* and *in vivo* methods. The following raw materials were compared: whole skin (from now on referred to as DWS), dander (DD), hair plus skin scraping (DHS), and skin scraping (DS).

Material and methods

Ten patients with a clear history of dog allergy (six patients with asthma, three with rhinitis, and one

with asthma-urticaria) were selected at random for cutaneous prick tests and as serum donors for *in vitro* assays. All patients showed sensitization to other allergens (three patients to grass pollen, seven to *Dermatophagoides*, seven to molds, and two to foods). Control nonatopic subjects and negative sera, from healthy donors, were used to ascertain the specificity of responses.

The four dog epithelial sources were purchased from Allergon, AB (Sweden). All of them were from Alsatian dogs. The process followed for protein extraction with partial purification was exactly the same for the four raw materials. The procedure involved lipid removal with acetone, mechanical stirring in saline solution (10% w/v), and tangential ultrafiltration with a 5-kDa cutoff.

SDS–PAGE was done by the method of Laemmli (7), in 12.5% polyacrylamide gels. Separated protein bands were electrophoretically transferred to Immobilon-P membranes. Immunochemical staining was done as described previously (4), with peroxidase-conjugated rabbit immunoglobulins against human ϵ -chains (DAKO, Glostrup, Denmark).

High-resolution chromatography of the dog extracts was achieved with the new SMART™ System (Pharmacia LKB, Sweden). A gel filtration column, Superdex 75 PC 3.2/30, was used. Samples were applied at a concentration of 2 mg/ml in 0.05 M phosphate buffer, 0.15 M NaCl pH 7.0 (PBS), and

injected with a loop of 20 μ l. A flow rate of 40 μ l/min was maintained during the whole chromatographic process. The column was calibrated with a kit of proteins of known mol. mass. Chromatography was done at 8°C.

Solid-phase antigen for the RAST method was obtained by coupling the extract solution (10 mg/ml) to the 6-mm diameter CNBr-activated paper disks, according to the method of Ceska & Lundqvist (2). RAST was done with Phadezym RAST reagents (Pharmacia Diagnostics, AB), according to the manufacturer's instructions.

Results

The yield obtained after the process of extraction and partial purification of the four extracts, expressed as a percentage which refers to the mass of freeze-dried extract obtained from the mass of raw material, was greatest for dander (2.6%), which also gave rise to a lyophilizate containing 43.0% of protein. Yields of 0.78, 0.69, and 0.65% were obtained for DS, DWS, and DHS, exhibiting protein contents of 44.7, 37.8, and 30.1, respectively.

By SDS-PAGE analysis, DD and DHS presented a similar band pattern, while that of DS resembled that of DWS to a large extent. A main band at about 63.8 kDa consistent with dog albumin was present in the four extracts, but in a much higher ratio in DS and DWS. There was also another band at 14.8 kDa, common to all the extracts, but exhibiting a more diffuse shape in DD and DHS extracts.

SDS-PAGE immunoblotting with a pool serum from the 10 sensitized patients indicated the high degree of IgE binding in dander extract (Fig. 1) with strong but diffuse binding at mol. mass higher than 30 kDa and two sharp and intense bands at about 23.2 (the predominant one) and 18.6 kDa. These two bands were not detected in DWS and DS, while

the DHS extract faintly exhibited the band at 23.2, but not the one at 18.6 kDa. The four extracts showed IgE binding on the albumin band (63.8 kDa). As in SDS-PAGE, DWS produced a band pattern highly similar to that of DS; however, by SDS-PAGE immunoblotting, clear differences between DHS and DD were detected (Fig. 1).

Biochemical comparison of the four extracts at the level of native components was achieved by the high resolution of SMART chromatography on a gel filtration column. Fig. 2 shows the recorded chromatograms after ASCII format exportation to a graphic software, with a certain loss of resolution. The range of protein separation was between 0.8 and 2.5 ml. The mol. masses indicated were determined according to the calibration of the column with eight known proteins with mol. masses ranging within the 102–2.85-kDa interval, where the column produced a linear separation. Comparison of the chromatograms showed that from this native point of view DS and DWS also have a fairly similar composition, while DD and DHS show quite distinct features. Only the albumin peak, detected at 66.1 kDa, is common to the four elution profiles, but with different relative ratios. The main peak produced by DD extract at 1.63 elution volume, corresponding probably to a polypeptide peak (5.4 kDa), is not present in DS and DWS. Some peaks caused by other UV-absorbing molecules at elution volumes from 1.8 to 2.4 ml appear in the four chromatograms.

The allergenic activity of the dog epithelial extracts was comparatively studied by RAST and cutaneous prick tests. Paper disks with coupled dander extract showed the highest degree of IgE binding from the 10 different sera with a large difference from the other three extracts, which appear to have, on average, a similar IgE-binding capacity. For corroboration of these results by *in vivo* assays, the allergenic activity of the four extracts was determined

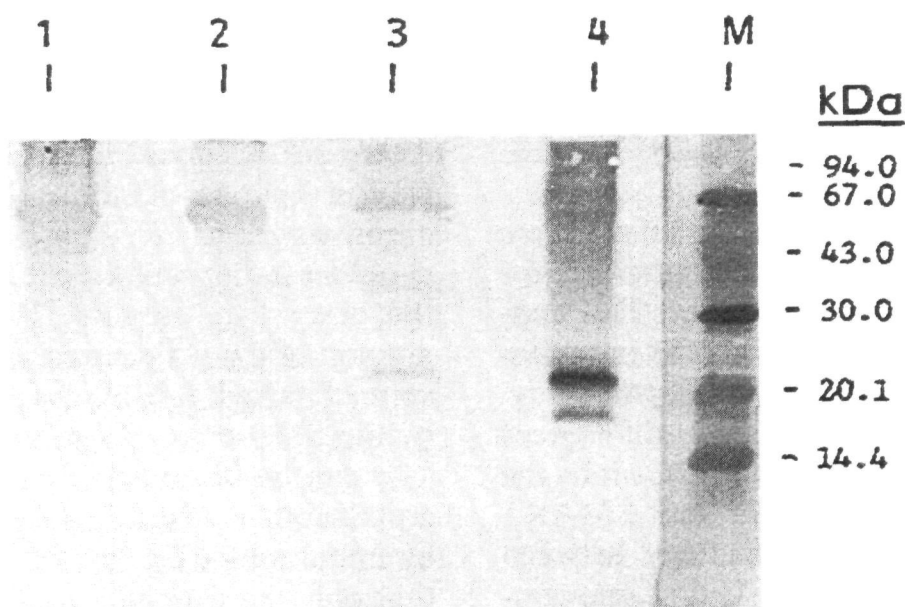


Fig. 1. SDS-PAGE immunoblotting of epithelial extracts: 1) DWS, 2) DS, 3) DHS, 4) DD. M: mol. mass markers.

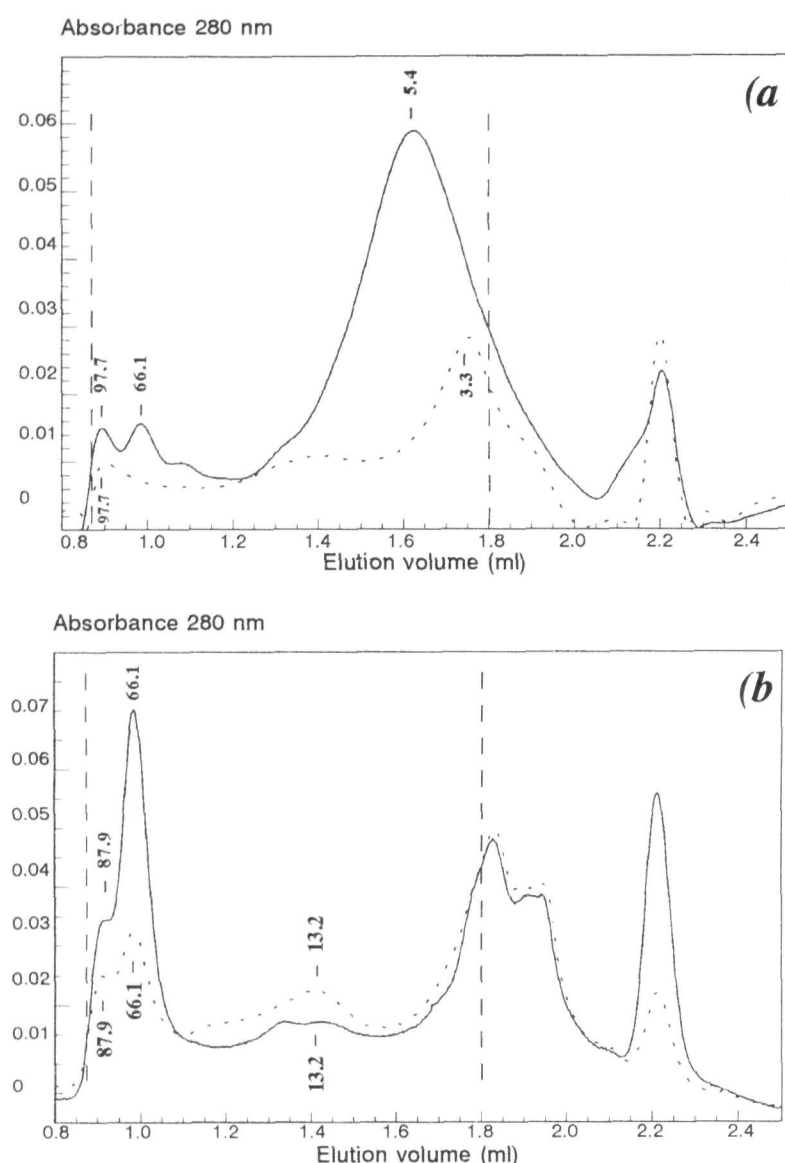


Fig. 2. SMART chromatograms of dog extracts. Elution profiles correspond to: a) DD (continuous line), DHS (dotted line); b) DS (continuous line), DWS (dotted line). Figures on or under peaks indicate mol. mass (kDa) estimated from column calibration. Chromatography limits detected with blue dextran (BD) and potassium chromate (PC) are indicated as vertical dashed lines.

by the cutaneous response elicited in prick test. DD caused the highest wheal in 8/10 dog-allergic patients, and DHS in the other two. Again, there was no homogeneity in the preponderance of the rest of the extracts. None of the dog extracts produced positive signals in either RAST or cutaneous tests when applied to nonatopic control subjects.

Discussion

Dander is certainly the most suitable epithelial source for preparing dog allergenic extracts intended for diagnostic and immunotherapy purposes. This conclusion is based on the results of all the immunochemical procedures completed in the present study.

Important qualitative biochemical differences among the four extracts were found, as shown by the patterns obtained in SDS-PAGE and SMART chromatography, with a close homology between DWS and DS extracts, although the first one contained a greater amount of albumin. DD and DHS

also exhibited an evident degree of similarity, as deduced especially from the SDS-PAGE analysis, although the higher-resolution SMART chromatography revealed significant differences. It is worth noting that SDS-PAGE and chromatographic patterns were largely concordant, because main bands of the extracts corresponded in mol. mass to main peaks of the elution profiles. Therefore, it can be deduced that most of the dog epithelial proteins were monomeric.

While these analytic methods revealed the differences in composition of the four extracts, the assays of biologic and IgE-binding activity indicated clearly the convenience of using dog dander as raw material because DD yielded the highest signal in SDS-PAGE immunoblotting (Fig. 1) and RAST, and also produced the biggest wheals in most of the patients tested. The finding that two patients showed higher cutaneous response for DHS, even though the RAST signal was lower than for DD, lies within the 18% discordance reported between RAST and skin test (6) and indicates that there are allergens recognized by some patients (these being responsible for a proportion of the skin activity of the extracts) which are not revealed in RAST, as a result either of its lack of linkage to the solid-phase support, or of its chemical instability under coupling conditions, or even of interference with specific IgG antibodies in both sera (10).

The major dog allergen *CanfI* was described by De Groot et al. (5) as a protein showing a band at 25 kDa by SDS-PAGE, and by Schou et al. (11) as a double band with major and minor components at 25 and 18 kDa, respectively. They postulated tentatively that the latter one was a breakdown derivative of the 25-kDa protein. Recently, Spitzauer et al. (13) have shown that the minor band, with redefined mol. mass of 19 kDa, represents another immunologically independent major allergen. This allergen, according to SDS-PAGE immunoblotting (Fig. 1), was detectable only in dander extracts.

CanfI has been characterized in its native mol. mass by high-performance liquid size-exclusion chromatography to be a protein of 22 kDa (12). Therefore, it should have appeared as a peak at an elution volume of about 1.27 ml in the SMART chromatogram (Fig. 2); in fact, it appeared as a slight shoulder in the profiles of DD and DHS, and not in the other two extracts. This was due to the low amount of *CanfI* present in the complete extract (in spite of its high IgE-binding capacity), as also shown by the SDS-PAGE pattern.

It can be observed from Fig. 1 that both *CanfI* and the other, 19-kDa allergen could not be detected by immunoblotting in DWS and DS extracts, and that only the first one could be detected (faintly) in DHS, although *CanfI* is preferentially expressed in

hair (and saliva) and the 19-kDa protein in skin (and saliva), according to Spitzauer et al. (13). This fact reinforces the unlikelihood of achieving a precise diagnosis and effective treatment of dog allergy by using both kinds of extracts.

Acknowledgments

This work was supported in part by funds from the Spanish Ministerio de Industria y Energía, Plan Nacional de I + D, Farma II (Ref. 952/91).

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