TP1657 - Development of a new *Dermatophagoides farinae* extract for its specific use in allergic dogs

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Background

Canine atopic dermatitis (CAD) is a pruritic allergic skin disease, being *Dermatophagoides farinae* one of the main responsible agents. Different profiles of sensitization to *D. farinae* have been described between human and dogs. High Molecular Weight (MW) proteins, Der f 15 and Der f 18, are major allergens in dogs, while groups 1 and 2 are considered major allergens for humans. Despite these differences, allergic dogs have traditionally been treated using extracts intended for human immunotherapy.

The objective of this study was to develop a specific allergen extract for veterinary practice enriched in the major allergens for dogs and to demonstrate its *in vitro* efficacy.

Methods

Canine samples

Serum samples were collected from 16 dogs clinically diagnosed with CAD and presenting specific IgE (sIgE) against *D. farinae*. Peripheral Blood Mononuclear Cells (PBMCs) were obtained from 4 atopic animals and 3 healthy controls (Isoquimen S.L., Spain).

Veterinary mite extract manufacturing

The veterinary mite extract (VME) was manufactured from a *D. farinae* culture (Laboratorios LETI) following in-house manufacturing procedures, looking for an increase in high MW proteins. A *D. farinae* extract used for human immunotherapy (HME) was used as control.

Protein and allergenic profile

Protein profile was investigated by SDS-PAGE and Size Exclusion Chromatography (SEC). Allergenic profile was studied by immunoblot using a pool of sera from allergic dogs

Major allergens relative quantification

Relative quantification of Der f 15 and Der f 18 was estimated by scanning densitometry using the ImageQuant TL 2005 software (GE Healthcare).

Der f 15 was also relatively quantified by mass-spectrometry. Searches were performed against Uniprot *D. farinae* database. Three peptides (Table 1) from Der f 15 were selected for quantitative and statistical analysis.

ELISA assays

The sIgE of individual serum samples against VME or HME was analyzed by direct ELISA. The pool of sera was used to analyze the biological potency of VME respect to HME by ELISA inhibition assays.

Cellular studies

PBMCs were stimulated with VME or HME (40 µg protein/ml) and the production of IL-10 and IFN-γ cytokines determined in supernatants by ELISA-based Milliplex® Mag Dog kit (Merck Millipore). Concanavalin A and LPS were used as positive controls.

Results

Veterinary mite extract characterization

VME showed a higher content of proteins >40 kDa respect to HME (Figure 1A), recognized with a higher intensity by the pool of sera (Figure 1B). The fold-increase of Der f 15 and Der f 18 by densitometry analysis in VME respect to HME was 2.3 and 2.5, respectively (Figure 1C).

VME presented in SEC analysis (Figure 2), contrary to HME, >50% of the chromatographic area corresponding to proteins between >1500 kDa-40 kDa.

Relative quantification analysis by mass-spectrometry revealed a fold-increase of Der f 15 in VME respect to HME of 2.97 (95% CI:2.28 to 3.86; $p<0.001$) at protein level.

In vitro efficacy

All serum samples presented significantly higher specific IgE levels against VME (Figure 3A). The 50% of inhibition of the pool of sera was reached with 0.132 µg of HME, which represents a 2.13 fold-increase respect to VME (0.062 µg) (Figure 3B).

Both extracts induced significantly higher levels of IL-10 than negative control (1170 pg/ml for HME and 1748 pg/ml for VME). IFN-γ was also significantly higher than negative control in atopic dogs cells treated with HME (52.1 pg/ml) and VME (50.4 pg/ml) (Figure 4).

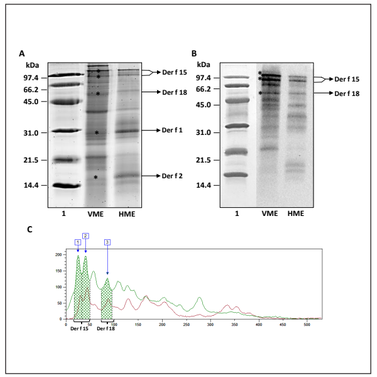

Figure 1. Protein profile (A) and immunoblot (B) of VME (10 µg protein) and HME (10 µg protein). 1: Low Range Molecular Weight Standard. C) Scanning densitometry comparing VME (green line) and HME (red line).

m/z	Star time (min.)	End time (min.)	Sequence
835.38	41	47	FDGLDLDWEYPGSR
776.38	41	47	VDPYTIEDIDPFK
813.89	37	43	IWVGYDDLASISCK

Table 1. List of m/z acquired for quantification of Der f 15 (Uniprot Q9U6R7).

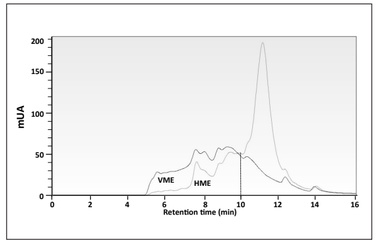

Figure 2. SEC of VME (black line) and HME (gray line) extracts detected at 215 nm.

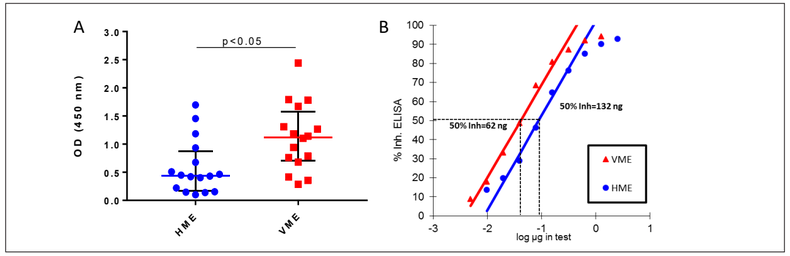

Figure 3. A) OD of sIgE levels of individual serum samples against HME and VME extracts. B) ELISA inhibition assay for biological potency determination. 50% Inh= 50% inhibition.

Conclusions

> A veterinary *D. farinae* extract with a higher content of dog major allergens Der f 15 and Der f 18 has been developed and its *in vitro* efficacy demonstrated.

> This extract represents an optimal candidate for allergen specific immunotherapy in atopic dogs allergic to mites.

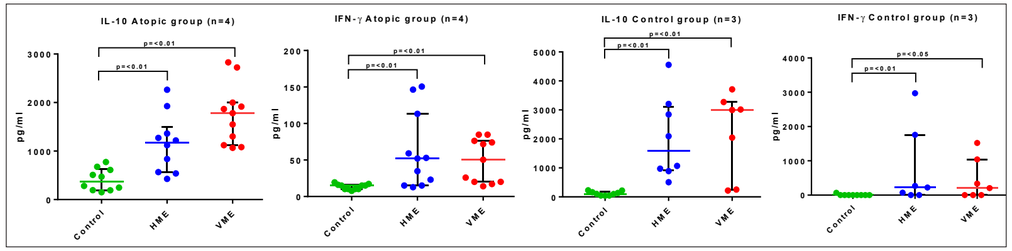

Figure 4. IL-10 and IFN-γ produced by PBMCs from atopic and control group after 24 (IL-10) or 48 (IFN-γ) hours of incubation with negative control, HME or VME extracts.

Conflict of interest: In relation to this presentation, I declare the following, real or perceived conflicts of interest: R. Moya, L. Ramió-Lluch, N. Parody, P. Brazis and J. Carnés are employees of Laboratorios LETI.