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ORIGINAL ARTICLE

Development and characterization of an allergoid of cat dander for immunotherapy

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Abstract
Background: Allergy to cats is a frequent cause of sensitization to indoor allergens and currently there are few alternatives to specific immunotherapy with cat native extracts. The objective is to develop and characterize a new allergoid to increase the tools available for use in clinical practice.
Methods: The allergoid cat dander extract (ACD) was developed from a native cat dander extract (NCD) by modification with glutaraldehyde, and the optimal process control was determined by SDS-PAGE, DOT BLOT and determination of free amine groups. The ACD was characterized in protein profile by SDS-PAGE, size exclusion chromatography (SEC) and peptide footprint. The allergenic profile of ACD was determined by immunoblot, IgE CAP inhibition and IgG competition ELISA. The major allergen content in NCD was obtained by the ELISA sandwich protocol and was extrapolated to ACD.
Results: The control process determined the optimal development of the allergoid. The ACD obtained contains 182.28 μg/mg of protein and 11.90 μg/mg of Fel d 1. SDS-PAGE and SEC confirmed the presence of high molecular weight proteins in ACD, and the peptide footprint showed the presence of Fel d 1 and Fel d 7. The high degree of polymerization was evidenced with the determination of the reduction of lysine residues in the allergoid, resulting 91.96%. The ACD showed a significant loss of allergenicity respect to NCD, while the IgG-binding capacity was maintained.
Conclusions: The ACD obtained presents a good safety profile, so would be a good alternative for treatment of cat allergy.

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Introduction

Domestic animals are considered risk factors for the development of allergic rhinitis and asthma in the domestic and occupational environment, constituting an important source of indoor allergens.[1] Sensitization to this kind of allergens is quite ubiquitous and not dependent on geographic or climatic variables.[2] The domestic animals that cause most allergies are dogs, cats and horses. Of these, cat allergy causes more complications, because their allergens can remain volatilized up to six months after the presence of the animal in a place, and is the second most frequent cause of sensitization to indoor allergens, after house dust mites in most European countries.[3] Spain as the lowest percentage of prevalence of sensitization to cat allergens, not exceeding 4%,[4] in contrast with Sweden where it reaches 20% of the population tested by skin prick test (SPT).[3] Globally, the European population evaluated by SPT shows 25% reactivity to house dust mites, while about 10% show allergy to cat allergens.[4] The percentage in the US is 15% prevalence of sensitization to cat allergens.[5]

Currently it is considered that specific immunotherapy (SIT) is the only treatment capable of modifying the natural history of allergic disease,[6] even more so in this specific type of allergy where non-exposure to the animal does not guarantee the absence of an allergic reaction or prevent possible sensitization.[7,8]

The conventional strategy carried out with the treatment with SIT consists of increasing administration of allergen source doses, in order to redirect the immune response of patients, avoiding the production of mediators of immediate hypersensitivity. It has been shown that the use of allergen extracts for hyposensitization therapy is effective when the allergen dose is suitable, but augmentation of the concentration increases the risk of possible adverse reactions. Throughout the history of SIT various strategies have been adopted, such as physical or chemical modifications of the allergen which allow the administration of larger amounts, minimizing the risk of adverse reactions.[9]

Conformational changes of the allergen due to its chemical modification with glutaraldehyde is a well-established SIT strategy; it is possible to alter the capacity of recognition of the allergens by the IgE of the individuals sensitized, substantially reducing the ability of the allergen to induce IgE-mediated reactions.[10] This allergoid, with low allergenicity, maintains its immunogenicity and can induce T cell-mediated responses that are not dependent on conformational epitopes.[11] The effectiveness of allergoids in SIT is widely documented, and their ability to favor non-IgE-dependent immune responses is proven, possibly because they are not presented to T lymphocytes by IgE-dependent mechanisms.[12]

Based on these findings and because the current treatment with SIT for cat allergy is merely vaccines whose active principle is a native protein extract of epithelium and/or dander, the objective of this study was the development and characterization of a polymerized cat dander extract to increase the tools for use in clinical practice. An optimal characterization and standardization of allergenic extracts, is essential to ensure the quality of the extracts used for diagnosis and treatment of allergic diseases. While for the native allergen extracts there are standard methodologies described for their development, characterization and standardization, in the case of allergoids there is little information about the usefulness of these techniques and there are few reference documents available for the standardization of these products, although recently a study on the efficacy and safety of a polymerized cat dander extract has been published which shows a lower interaction with cat-specific IgE by the polymerized extract compared to the native extract, suggesting greater safety.[13]

The guidelines for the development, characterization and standardization of the allergoid cat dander extract were developed on the basis of what is described in both the EMA guides: Guideline on Allergen Products: Production and Quality Issues (2008),[14] and the product allergens monograph European Pharmacopoeia (2010, 1063, Product Allergenic-European Pharmacopeia)[15]; they were also based on the ICH Q6B Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products.[16]

The quality of the new active principle was evaluated through the analysis of the protein, allergenic and immunogenic profiles. Since allergenic activity is essential to establish the safety of the product for use in SIT, is necessary to establish standards of both allergenic activity and immunogenic ability of the allergoid to estimate its potential quality as a hyposensitizing agent.[17]

Methods

Allergoid production

Development of the extract

The allergoid cat dander extract (ACD) was developed from a native cat dander extract (NCD) with raw material from Allergon, manufactured under Good Manufacturing Practices (GMP). The NCD had a major allergen concentration of Fel d 1 of 8.3 μg/mg quantified by Fel d 1 ELISA kit EL-FD1 (6F9/3E4) (Indoor Biotech, VA, USA) and 127.12 μg/mg protein, quantified by elemental nitrogen analyzer (AEN) according to the method described in European pharmacopoeia 7.0 (2.5.33, 7B method) to determine total protein by nitrogen analysis.[18] The NCD was polymerized with glutaraldehyde 10 mM for 4 h at room temperature. The polymerization process was stopped by addition of excess glycine, and the resulting extract was diafiltered against phosphate buffered saline (PBS) in Biomax® 100 kDa membrane (Millipore, Bedford, Mass., USA), in order to remove impurities corresponding to compounds of low molecular weight, such as residues of glycine and non-polymerized protein fractions. The resulting solution was evaluated in protein concentration by AEN for dosing prior to lyophilization.

Process control

The suitability of the production process was assessed at various points by different techniques:

SDS-PAGE: Samples were collected at different times during the course of the polymerization process. These samples were loaded under reducing conditions in 4–20% acrylamide-bisacrylamide gradient gels, with a glycine running buffer

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