811 Structural and immunological characterisation of a broad-spectrum grass allergoid vaccine

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RATIONALE: Immunological and structural characterisation of a complex broad-spectrum grass allergoid vaccine was sought. Mapping IgG epitopes and their functional capacity to induce blocking antibodies for one of the major grass allergens (Lol p1) allows further insights into the mechanism of action for allergoid SIT and provides steps toward standardising allergoid vaccine formulations.

METHODS: Reduced allergenicity and maintained immunogenicity potential of a grass allergoid vaccine was determined using an ELISA inhibition platform. Specificity of immunogenic determinants including Lol p1 IgG-binding epitopes were identified via ELISA inhibition experiments with Lol p1 specific synthetic peptides. The blocking capacity of Lol p1 induced IgG was assessed via IgE ELISA specificity and SDS-PAGE/Western blotting.

RESULTS: Attenuation of IgE immunoreactivity and maintenance of IgG immunoreactivity following glutaraldehyde modification of the mixed-grass native extract was confirmed. Retention of six Lol p1 IgG-binding epitopes on a solvent exposed area of the N-terminal domain of Lol p1 homology model was demonstrated. A novel IgG epitope was identified, not previously characterised, and was classified as immune-dominant. Lol p1 specific IgG antibodies exhibited functional capacity to block 50% of IgE binding sites from the native grass extract.

CONCLUSIONS: Structural and immunological characterisation of the mixed-grass allergoid vaccine formulation demonstrated a high degree of preservation of Lol p1 IgG binding epitopes. It supports the concept of using an allergoid vaccine for treatment of grass allergies and provides further insights of its immunogenicity potential. The blocking function of IgG antibodies reaffirms the protective function of immunotherapy induced antibodies.

812 Isoallergen Distribution of Der p 1 in Mite Extracts and in the Highly Purified Allergen

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RATIONALE: Highly purified, well-characterized allergens can be used for molecular allergy diagnostics and as reference materials. Reference materials are important for standardization of allergen extracts, determination of potency of allergy vaccines and validation of molecular diagnostics. The objective of this study was to analyze purity and isoallergen distribution in affinity-purified dust mite allergen Der p 1.

METHODS: Isoform distribution of Der p 1 in mite culture, during bioprocessing and in preparations of purified Der p 1 was compared by LC-MS/MS. Data from digests were analyzed against individual Der p 1 isoform sequences. Der p 1 was lyophilized using different buffer conditions. Real time stability data were collected from frozen liquid allergens and lyophilized allergens.

RESULTS: Patterns of Der p 1 isoforms were identical in mite culture and purified allergens. Based on diagnostic peptides, five isoforms of Der p 1 (0101, 0102, 0106, 0108, and 0124) were identified and 17 Der p 1 isoforms could be excluded. Purified Der p 1 was free of contaminants. Real time stability tests of frozen liquid allergens and of frozen lyophilized allergens showed comparable potency in allergen-specific ELISA and no signs of degradation on SDS-PAGE.

CONCLUSIONS: Mass Spectrometry is a valuable tool to assess purity and isoform composition of purified allergens. Affinity-purification of natural Der p 1 does not affect the original isoform distribution found in mite culture. Bioprocessing pathways have been established to yield high purity mite allergens with homogenous isoform profiles. Purified natural Der p 1 can be used as molecular reference material for allergen standardization.

813 Allergen VLPs

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RATIONALE: The use of antigenic VLPs has recently impacted the vaccine industry by allowing the development of efficacious, safe and low cost recombinant vaccines. We are currently investigating the potency of allergens presented in the form of recombinant VLPs for immunotherapy.

METHODS: Transient expression of a fusion protein made of a natural non-immunogenic carrier peptide fused to an allergen component was used to produce VLP particles harboring spikes of either homotrimers or homotetramers of a major dust mite allergen. These VLPs were purified and characterized in preparation for a head-to-head mouse efficacy study in comparison with the same allergen in a soluble form and whole dust mite commercial extracts.

RESULTS: VLPs were readily formed with both trimeric and tetrameric forms of the the antigen. Their purification was performed with a succession of simple filtration and ion-exchange chromatography steps. The particles were between 130 and 180 nm in diameter, harboring an average of 900 spikes of the homopolymers on their surface. Their membrane was made of lipids typical of membrane rafts. Their production showed high reproducibility both in yield and quality. They are currently under efficacy trial in mice. This combination of a new manufacturing technology and a new 3D antigen display technology is GMP compliant, low cost and has unlimited capacity. Its functionality has now been tested with some of the major allergens.

CONCLUSIONS: This new technology has the power to bridge the gap between the rapidly increasing knowledge of the immunological basis of allergy and the manufacturing of therapeutic allergens.

814 Do Human Mite Allergen Extracts Contain the Relevant Allergens for Treating Canine Atopic Dermatitis?

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RATIONALE: Der f 15 and Der f 18 are major allergens in dogs, while the human major allergens, Der f 1 and Der f 2, are considered minor allergens. However, canine atopic dermatitis is being treated with the same allergen extracts used for humans. This study aims to demonstrate and quantify the presence of relevant *Dermatophagoides farinae* allergens for dogs in conventional immunotherapy used in veterinary practice.

METHODS: A native allergen extract of *D. farinae* was manufactured from a mites culture (body content >80%). The protein profile was analyzed by SDS-PAGE. Concentration of each allergen was calculated by scanning densitometry, according to the whole protein content. The presence of relevant allergens was identified by mass spectrometry (amino-acid sequencing). Allergenic profile was studied by immunoblot using individual serum samples from 18 Spanish-residing dogs, suffering from atopic dermatitis.

RESULTS: The extract showed a protein concentration of 220 mcg/mg. Der f 15 (119 and 96 kDa), Der f 18 (57 kDa), Der f 14/Der f 11 (43 kDa), Der f 3/Der f 14 (34 kDa), Der f 1 (30 kDa) and Der f 2 (15 kDa) were identified. The allergenic profile showed that dogs were mainly sensitized to Der f 15, Der f 18, Der f 1 and Der f 2. The concentration of these allergens was 18, 9.5, 17 and 21 mcg/mg freeze-dried material respectively.

CONCLUSIONS: The commercial *D. farinae* extract (Laboratorios LETI, Madrid, Spain) contains the major allergens for dogs, being a good candidate for treating allergen-dependent canine atopic dermatitis.