

428 Identification of the Russian Hamster Allergens (*Phodopus sungorus*)

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RATIONALE: The aim of our study was to identify the Russian hamster allergens from different sources.

METHODS: Four patients were recruited. One of them had asthma and another had experienced an anaphylactic reaction due to hamster bite. A third patient had asthma and was sensitized to Russian hamster and Golden hamster (*Cricetulus gender*). The fourth patient was sensitized only to Golden hamster. Skin prick tests and IgE determinations to common allergens and animal epithelia were performed. Extracts of epithelium, urine, salivary glands and serum of Russian hamster were prepared for skin tests, specific bronchial challenges and immunoblotting.

RESULTS: Skin prick tests with Russian hamster extracts were positive with epithelium, urine and salivary glands, and negative with serum, on the three patients sensitized to Russian hamster. Specific bronchial challenge with Russian hamster epithelium extract on the two asthmatic patients elicited an early asthmatic reaction in one patient and a dual asthmatic reaction in another, along with significant variations in the post-provocation PC20 methacholine. Immunoblotting showed common IgE-binding bands at 18, 21 and 23 kDa on the epithelium, urine and salivary glands extracts. These bands were similar in the two patients sensitized to Russian hamster and in the patient sensitized to both hamster species, who also recognized a 66 kDa band. All tests performed with Russian hamster extracts were negative in the patient sensitized to Golden hamster.

CONCLUSIONS: Several allergens between 18-23 kDa from different sources were identified in Russian hamster. Moreover, another 66 kDa allergen was detected on the patient sensitized to both hamster species.

429 Assessment of the Potential Cross-Allergenicity between Hen's Egg Lysozyme and Recombinant Human Lysozyme

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RATIONALE: Human lysozyme (rhu lys) extracted from genetically modified rice is available and regulatory strategies are currently under evaluation. Since the sequence of human lysozyme shows 60% homology with hen's egg lysozyme, and because egg lysozyme is recognized by 35% of sera from egg allergic patients, searching for a possible cross-allergenicity between human and egg lysozyme is needed. This is in keeping with the recommendations from the WHO/FAO Joint Expert Committee and the Codex Alimentarius Commission.

METHODS: Forty-one sera from egg allergic patients, with specific IgE to egg lysozyme were screened by ELISA inhibition using egg lysozyme and rhu lys. Basophil activation tests (BAT) were performed in 28 egg lysozyme-sensitized patients using 3 preparations of rhu lys with a purity of 85%, 95% and 99%, human milk lysozyme, and rice proteins. Sera from 5 patients not allergic to egg were used as controls.

RESULTS: In the tested sera, ELISA inhibition showed no cross-reactivity between human and hen egg lysozyme. Positive BAT to rhu lys in patients was associated to positive BAT to rice extract. The percentage of basophil activation decreased with the increase of the purity of rhu lys, consistent with the notion that contaminating rice proteins are responsible for the BAT positive responses. No positive BAT test was observed with human milk lysozyme.

CONCLUSIONS: Our data support the conclusion that rhu lys does not cross react with human IgE directed against hen egg lysozyme.

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430 Specific IgE levels to Der p 1 and Der p 2 in patients sensitized to *Dermatophagoides* spp

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RATIONALE: Der p 1 and Der p 2 are important mite allergens. However, the relative importance of sensitisation to these individual allergens remains unclear. The aim of this study was to determine specific IgE levels against Der p 1 and Der p 2 in a mite allergic population of Spain (Galicia).

METHODS: 27 sera from allergic patients suffering from rhinoconjunctivitis and/or asthma and sensitized to *D. pteronyssinus* were analysed. Specific IgE was determined by ELISA using purified allergens.

RESULTS: All patients had specific IgE against Der p 2 and 23 to Der p 1. Mean value specific IgE levels to Der p 1 was 14.89 ± 17.43 (0.59 to 59.19 IU/ml), and to Der p 2 20.45 ± 26.07 (0.56 to 107.5). The correlation coefficient between Der p 1 and Der p 2 levels was 0.54. In eight patients the ratio between Der p 1 and Der p 2 was >1 (mean ratio 2.6) and in 19 was <1 (mean ratio 0.36). Important individual differences between specific IgE levels to Der p 1 and Der p 2 were detected. However, the mean overall value of the ratio was 1.03, demonstrating the importance of both allergens in a region, where *D. pteronyssinus* is highly prevalent.

CONCLUSIONS: This study demonstrates that mite allergic patients can show significant differences in specific IgE levels against Der p 1 and Der p 2. These differences may have clinical implications in the correct diagnosis and treatment of mite allergic individuals.

431 Production of Purified Native Alt a 1 at an Industrial Scale

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RATIONALE: The increasing interest in component-resolved diagnostics and development of novel allergy immunotherapy strategies based on the use of single allergenic components has induced a need for well characterised and standardised allergen molecules. In this study a method for producing large amounts of purified native Alt a 1 has been developed.

METHODS: The culture conditions of an industrial allergen source material strain of *Alternaria alternata* were optimised for a high production of Alt a 1. A scaleable purification process for the isolation of Alt a 1 was developed by means of liquid chromatography.

RESULTS: The developed purification process is cost effective with few production steps. The process yields highly purified Alt a 1 in gram quantities. Immunological characterisation showed that the antigenicity of Alt a 1 was maintained after purification.

CONCLUSIONS: A method for producing large amounts of purified native Alt a 1 has been developed. The starting material can be obtained in large amounts through cultivation of *A. alternata* at optimal conditions. The study illustrates the feasibility of using natural sources for the generation of pure single allergens.

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