

514 Comparison of Microarray-Based IgE Profiling with Established Diagnostic Methods in Patients with Latex Allergy

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RATIONALE: Immediate reactions to natural rubber latex (NRL) still have to be considered a clinically relevant phenomenon with up to 17% of exposed health care workers. Routine *in vitro* and *in vivo* tests for latex allergy employ non-standardized allergen extracts. Therefore sensitivity and specificity are subjects to fluctuation. However, several latex proteins have been characterized and synthesized as recombinant allergens and are available for a component-resolved diagnosis in patients with latex allergy.

METHODS: We studied 63 adult patients suffering from clinically significant symptoms as defined by latex allergy and in whom type-I-sensitization to NRL had priorly been confirmed by an established fluorescence enzyme immunoassay using allergen extracts. We applied a new microarray-based method in which a computer-assisted qualitative and quantitative analysis of interacting human IgE antibodies with an array of recombinant allergens was performed incubating only 20 μ l of patient serum on a solid-phase chip. In addition, we detected specific IgE against latex with an established fluorescence immunoassay (UniCAP, Pharmacia) by using recombinant latex allergens, too, and compared the results of the two methods.

RESULTS: Statistical analysis revealed a moderate degree of correlation between chip-analysis and the established enzyme immunoassay results in the case of rHev b 5 ($r=0,64$) and rHev b 6 ($r=0,73$) whereas other latex-components showed less correlation.

CONCLUSIONS: Microarray-based detection of latex-specific allergen components is feasible using only minute amounts of patient serum. As this novel technique shows correlation with established diagnostic tools, it may be used to improve the currently difficult diagnosis of latex allergy in the near future.

515 The Role of Glycosylation of nHev b 2, The β -1,3-Glucanase from Hevea Brasiliensis Latex, in IgE Recognition

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RATIONALE: The aim of this study was to determine the contribution of the carbohydrate moiety of nHev b 2 for IgE binding. nHev b 2 is glycosylated with the MMXF structure, a common sugar-motif of plant glycoproteins.

METHODS: nHev b 2 was purified from fresh Hevea latex. A portion was treated with periodate to destroy carbohydrate epitopes. CD-spectroscopy and a glucanase activity assay were performed for both samples. Out of 107 latex sensitized patients, 47 (44%) sensitized to nHev b 2 were tested by IgE-ELISA experiments on nHev b 2 and periodate treated nHev b 2.

RESULTS: No significant differences in the secondary structure between native and periodate treated nHev b 2 were observed. 55% (26/47) of patients with IgE to nHev b 2 showed latex allergic symptoms, 45% (20/46) did not. 39% (10/26) of latex allergic patients did not react to nHev b 2 treated with periodate. However, 61% (16/26) of sera contained IgE reactive only to protein epitopes. From patients without symptoms, 67% (13/21) reacted only with the carbohydrate chain, whereas 33% (7/21) of the sera showed a positive reaction to nHev b 2 and periodate-treated nHev b 2.

CONCLUSIONS: In general, IgE of individual patients is directed either to protein or carbohydrate epitopes. Protein epitopes are more frequently recognized by allergic subjects' IgE (61%) than by sensitized subjects (33%). The clinical relevance of the cross-reactive carbohydrate structure of nHev b 2 remains to be investigated.

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516 Allergen Concentration in Natural Rubber Latex

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RATIONALE: The serum from *Hevea* latex is commonly used as the *in vivo* and *in vitro* reference antigen for latex allergy diagnosis since it contains the entire complement of latex allergens. This study determines the concentrations of the significant allergens in latex serum and examines its suitability as an antigen in latex allergy diagnostics and immunotherapy.

METHODS: The serum phase was extracted from centrifuged latex that was repeatedly freeze-thawed or glycerinated. Quantitation of latex allergens was performed by two-site immunoenzymetric assays. Abundance of RNA transcripts of the latex allergens was estimated from the number of their clones in an Expressed Sequence Tags library.

RESULTS: The latex allergens, Hevb 1, 2, 3, 4, 5, 6, 7 and 13, were detected in freeze-thawed and glycerinated latex serum at levels ranging from 75 (Hev b 6) to 0.06 nmol/mg total proteins (Hevb 4). Hevb 6 content in the latex was tens to a thousand times higher than those of the other seven latex allergens, depending on source and/or preparation procedure. Allergen concentration was reflected in the abundance of mRNA transcripts.

CONCLUSIONS: Latex allergy diagnostics and immunotherapy that use whole latex serum as antigen may not be optimal because of the marked imbalance of its constituent allergens. When used as the antigen, dilute latex serum may bias the diagnostic outcome towards sensitization to Hevb 6. Tests that make use of dilute latex serum may fail to detect latex-specific IgE reactivity in subjects who are sensitized only to allergens that are present at very low concentrations.

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517 The Association between Alcohol Consumption and Atopy in Danish Adults

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RATIONALE: There is evidence that alcohol increases serum total IgE and has an influence on the immune system. It is less clear whether alcohol is associated with increased risk of specific IgE sensitisation. The aim was to investigate the association between alcohol consumption and atopy.

METHODS: The analyses included a total of 3317 subjects aged 30-60 years who participated in a population-based cross-sectional study in 1982-84 in Copenhagen, Denmark. Alcohol consumption (drinks per week on average last 12 months) was assessed by questionnaire. Atopy was defined as detectable serum levels of specific IgE against a panel of common inhalant allergens as reflected by a positive ADVIA Centaur® Allergy Screen Assay test. Risk estimates were adjusted for potential confounders by logistic regression analyses.