Clinical and Translational Allergy

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Analysis of the IgE epitope profile of soybean allergen Gly m 4

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Background

Individuals with birch pollinosis may show allergic reactions after consumption of soybean-containing food. This is caused by cross-reaction of IgE directed against the major birch pollen allergen Bet v 1 with its homologue Gly m 4 from soybean. Birch pollen allergic subjects without soybean allergy often present IgE binding to Gly m 4.

Objective

To identify IgE epitopes of Gly m 4 in birch pollen-allergic subjects with and without allergy to soybean.

Methods

Sera from 33 birch pollen-allergic patients with and without clinical reactivity to soy as determined by oral food challenge or convincing history of soy allergy were included in the study. Specific IgE against Bet v 1 and Gly m 4 was determined by ImmunoCAP. IgE binding of the sera to rGly m 4 and soy extract was tested in western blot analysis. To predict putative IgE epitopes, 20 IgE-binding phage-displayed peptide mimics and heptamers representing the total Gly m 4 amino acid sequence were bioinformatically mapped onto the molecular surface of Gly m 4. To verify predicted epitopes, Gly m 4 variants with substituted epitope residues were expressed in and purified from *E. coli*. Secondary and tertiary structures were assessed by CD and NMR spectroscopy. IgE binding of variants was analyzed by competitive immunoblotting and inhibition ELISA.

Results

Gly m 4- and Bet v 1-specific IgE levels ranged from 0 to >100 kU_A/L in sera of both patient groups. CAP values were above 3.5 kU_A/L in 18 sera (86%) of patients with clinical soy allergy and in 7 sera (58%) of patients sensitized, but without clinical reactivity to soy, respectively. Intensity of IgE binding in western blot corresponded to ImmunoCAP analysis. Bioinformatic mapping resulted in four distinct epitopes on Gly m 4 surface. Several amino acid residues were chosen for generation of substitutional variants. Purified rGly m 4 variants showed a typical Bet v 1-like structure according to CD spectra and 1D-^{1}H-NMR spectroscopy. IgE binding to several substitutional variants was reduced, indicating a multiple number of epitopes on Gly m 4 surface.

Conclusion

No significant differences in Gly m 4-specific IgE binding could be observed between birch pollen-allergic patients with and without clinical soy allergy in ImmunoCAP and immunoblot analysis. However residual IgE reactivity persists in substitutional rGly m 4 variants, indicating the presence of further IgE epitopes not yet identified.

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