Cloning, Expression and Patient IgE Reactivity of Recombinant Pru du 6, an 11S Globulin from Almond.

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Abstract:
Background: IgE-reactive proteins have been identified in almond; however, few have been cloned and tested for specific patient IgE reactivity. Here, we clone and express prunin 1 and prunin 2, isoforms of the major almond protein prunin, an 11S globulin, and assay each for IgE reactivity. Methods: Prunin isoforms were PCR-amplified from an almond cDNA library, sequenced, cloned and expressed in Escherichia coli . Reactivity to the recombinant (r) allergens, Pru du 6.01 and Pru du 6.02, was screened by dot blot and immunoblot assays using sera from almond-allergic patients and murine monoclonal antibodies (mAbs). Sequential IgE-binding epitopes were identified by solid-phase overlapping peptide analysis. Epitope stability was assessed by assaying denatured recombinant proteins by immunoblot. Results: IgE reactivity to rPru du 6.01 and rPru du 6.02 was found in 9 of 18 (50%) and 5 of 18 patients (28%), respectively. Four patients (22%) demonstrated reactivity to both isoforms. Murine anti-almond IgG mAbs also showed greater reactivity to rPru du 6.01 than to rPru du 6.02. Both stable and labile epitopes were detected. Six IgE-binding sequential epitope-bearing peptide segments on Pru du 6.01 and 8 on Pru du 6.02 were detected using pooled almond-allergic sera. Conclusions: rPru du 6.01 is more widely recognized than rPru du 6.02 in our patient population. The identification of multiple sequential epitopes and the observation that treatment with denaturing agents had little effect on IgE-binding intensity in some patients suggests an important role for sequential epitopes on prunins.