

January 2000, part 2 • Volume 105 • Number 1

978 Calcium-Binding Allergens: Cross-Reactivity Between Molecules With Two (rAln g 4) and Four (rJun o 2) EF-Hand Domains*Raffaella Tinghino***Brigitte Hayek†**Bianca Barletta***Eleonora Puggioni***Patrizia Iacovacci***Cinzia Butteroni***Claudia Afferni***Gabriella Di Felice***Carlo Pini***Rudolf Valenta†**Adriano Mari****Department of Immunology, Istituto Superiore di Sanità, Rome, Italy †Institute of General and Experimental Pathology, University of Vienna, Austria*

Calcium-binding proteins (CBP) contains a variable number of calcium-binding motifs, termed EF-hands. CBP are presently classified into 32 distinct subfamilies that contain from two to eight EF-hand domains. Several pollen-derived CBP have been described as allergens, and all seem to act as panallergens. Until now no crossreactivity has been described among the two- (e.g. Aln g 4 from alder), three- (e.g. Bet v 3 from birch) and four- (e.g. Jun o 2 from juniper) EF-hand pollen allergens.

We sought to define a possible cross-reactivity between rAln g 4 and rJun o 2 by ELISA- and immunoblotting-inhibition experiments, employing sera from subjects with multiple pollen sensitization (MPS). 176 patients with MPS were screened in ELISA with the two recombinant allergens. Forty subjects (22%) were positive to at least one of the two CBP; 27 (15%) sera had IgE to both allergens, whereas 13 (7%) were found positive for rAln g 4 only. The inhibition assays, performed with sera selected among the double-positive subjects, showed a high degree of cross-reactivity between the two recombinant allergens when rJun o 2 was used as antigen. A complete inhibition was reached in both ELISA (0.06 µg protein/ml) and immunoblotting (10 µg protein/ml). When rAln g 4 was used as antigen in the immunoblotting assay, rJun o 2 gave only a partial IgE-binding inhibition. This result was confirmed by ELISA inhibition experiments performed with sera

from a panel of 11 allergic patients. The sera gave inhibition values ranging from 10 to 60% at the highest inhibitor concentration used (30 µg protein/ml). These results indicate that unique epitope/s are present on rAln g 4 together with epitopes shared with rJun o 2.

To study the presence of these “unique” and “common” allergenic epitopes on a panel of 16 pollen extracts from taxonomically unrelated tree, weed, and grass families, ELISA inhibition experiments using total pollen extracts as inhibitors were performed with a rAln g 4-positive rJun o 2-negative sera on rAln g 4 as antigen, and a double-positive sera on rJun o 2 as antigen. All the extracts were capable to inhibit at different concentrations the IgE binding to rAln g 4 or rJun o 2.

We conclude that: 1) About one out of five patients with MPS have IgE specific to a CBP; 2) rAln g 4 was recognized by most of the CBP sera; it has either unique or common IgE epitopes when compared with a four-EF-hand allergen; both types of epitopes are present in all the allergenic sources tested; 3) IgE epitopes present on rJun o 2 are also present in pollen extracts from taxonomically unrelated plant families; these epitopes are common with rAln g 4.