BACKGROUND: Acid phosphatase (Api m 3) is a major allergen in honeybee (Apis mellifera) venom, and its availability as a recombinant protein may facilitate the development of improved diagnostic tests and immunotherapies. OBJECTIVE: One objective is the determination of the complete primary structure of Api m 3 and to obtain recombinant Api m 3 on the basis of expression in insect cells. Another objective is the quantitative analysis of patient serum IgE antibody reactive to recombinant Api m 3.

METHODS: The cloning of Api m 3 from venom gland cDNA and its expression as a full-length protein in eukaryotic insect cells is described. The immunoreactivity of serum IgE antibodies of honeybee venom-sensitized patients to recombinant Api m 3 was determined in an enzyme immunoassay.

RESULTS: PCR amplification generated a 1122-bp DNA fragment whose identity as the coding sequence of Api m 3 was verified by several means. Recombinant Api m 3, expressed in Trichoplusia ni cells, showed an expected molecular weight and enzymatic activity at pH 4.5. Analysis of tryptic fragments of purified recombinant Api m 3 by mass spectrometry confirmed its identity. In immunoassays, recombinant Api m 3 is specifically recognized by IgE antibodies of pooled serum in Western blots and by 37% of the individual sera of honeybee venom-sensitized patients in ELISA analysis.

CONCLUSION: The availability of
recombinant Api m 3 provides a tool for both the development of improved diagnostic tests and the design of safer and more effective immunotherapeutic approaches for honeybee venom allergy. CLINICAL IMPLICATIONS: The recombinant venom allergen Api m 3 is a key element in the search for an optimized component-resolved approach to honeybee venom allergy with regard to both the development of superior diagnostic tests and the improvement of allergen immunotherapy.

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