

Cloning and Molecular Characterization of Hev b 3, a Spina-bifida-Associated Allergen from *Hevea Brasiliensis* Latex

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Key Words

Allergy · cDNA · *Hevea brasiliensis* · Latex · Recombinant allergen · Spina bifida · IgE

Background

Type I allergy (urticaria, rhinoconjunctivitis, bronchospasm, anaphylaxis) to proteins of natural rubber latex, the milky sap from the tropical rubber tree *Hevea brasiliensis*, occurs predominantly in risk groups, e.g. health care workers or children with spina bifida (SB) [1, 2]. In SB patients, the rate of latex sensitization is 29–72% [3–6]. It still remains to be clarified whether repeated early exposure or possibly a genetic predisposition represents the major factor for sensitization [6].

Two *Hevea* latex allergens, Hev b 1 and Hev b 3, were found to be associated with SB. Hev b 1, the rubber elongation factor [7], was cloned and sequenced [8]. The second SB-associated latex allergen, Hev b 3, has been reported with different molecular weights: a 27-kD SB allergen [9, 10], a 23-kD SB allergen [11] and finally a 24-kD protein [12]. Hev b 3 had a tendency to fragment into several polypeptides of 24 to about 5 kD even when stored at –20°C. Sequence comparisons of these three allergens [10–12] showed a good consensus in all fragments studied [13]. Mass spectrometry of Hev b 3 gave several species of

molecular weights differing by multitudes of 266 D and starting at 22,258 D. Without doubt, patients afflicted with SB would profit from more selective diagnostic measures with Hev b 1 and Hev b 3. Moreover, availability of recombinant latex allergens will also make specific immunotherapy of the disease conceivable.

Results and Discussion

In this study we describe cloning and expression of recombinant Hev b 3 (rHev b 3) in *Escherichia coli* and its characterization by immunoblots, IgE inhibition experiments, T-cell proliferation assays and establishment of Hev-b-3-specific T cell clones. Hev-b-3-encoding cDNA was prepared by RT-PCR using primers designed according to tryptic peptides [11]; the sequences at the 5' end was completed by RACE. The complete cDNA consisted of 922 bp (open reading frame: base 105–719) encoding 204 amino acids. By calculation, the protein had an M_r of 22.3 kD and pI of 4.6. The Hev b 3 sequence showed 47% amino acid sequence identity to Hev b 1 and 54% identity to PvSRP, a stress-related protein from kidney bean.

Hev b 3 carrying a hexahistidyl tag was expressed in *E. coli* (expression vector pQE-40); rHev b 3 was refolded and purified by Ni affinity chromatography. rHev b 3 remained soluble after purification and storage at –20°C.

In immunoblot experiments, 29/35 (83%) of SB patients with positive skin prick test to latex allergens (glove extract) and 4/15 (27%) with negative SPT reacted with purified rHev b 3. Preincubation of selected patient sera with rHev b 3 significantly reduced or abolished IgE binding to allergens from natural rubber latex extract corresponding to Hev b 3 as well as to Hev b 1.

PBMC from an SB patient with IgE antibodies to Hev b 3 showed a weak but significant proliferative response to rHev b 3 (stimulation index >2, Δ cpm = 7,000). However, 5 independent Hev-b-3-specific T-cell clones established from this patient vigorously reacted with Hev b 3 (stimula-

tion index >100, Δ cpm >100,000), but only moderately with latex C serum as a source of Hev b 3 (stimulation index 10–100). The data clearly show that purified rHev b 3 can be expected to represent a valuable tool for diagnosis, but possibly also for specific immunotherapy of latex allergy, particularly in SB patients.

Acknowledgements

Supported by grants P12838-GEN and S06707-MED from the 'Fonds zur Förderung der Wissenschaftlichen Forschung', Vienna, Austria.

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