

Identification of a *Hevea brasiliensis* Latex Manganese Superoxide Dismutase (Hev b 10) as a Cross-Reactive Allergen

Stefan Wagner^a Slawomir Sowka^a Christina Mayer^b Reto Cramerⁱ
Margit Focke^a Viswanath P. Kurup^{c,d} Otto Scheiner^a Heimo Breiteneder^a

^aDepartment of Pathophysiology, University of Vienna, Vienna, Austria; ^bSwiss Institute of Allergy and Asthma Research, Davos, Switzerland; ^cAllergy Immunology Division, Medical College of Wisconsin, Milwaukee, Wisc., ^dResearch Service, V.A. Medical Center, Milwaukee, Wisc., USA

Key Words

Hevea brasiliensis · Latex allergy · Manganese superoxide dismutase · Pan-allergen · Recombinant Hev b 10 · Mold allergy

Abstract

Background: Cross-reactive allergens play an increasingly important role in latex allergy in complicating both the diagnosis and time course of allergic symptoms. Manganese superoxide dismutase (MnSOD), a ubiquitous protein of prokaryotic and eukaryotic organisms, was described as a cross-reactive allergen in *Aspergillus fumigatus*. Little information is available on the importance of this pan-allergen in *Hevea brasiliensis* latex. The aim of this study was to clone and express MnSOD from *H. brasiliensis* latex, and to obtain the soluble and immunologically active recombinant allergen for diagnosis of latex allergy and to investigate possible cross-reactivities with the structurally related *A. fumigatus* and human MnSODs. **Methods:** A complementary DNA coding for *Hevea* latex MnSOD was amplified by PCR. The recombinant protein was produced in *Escherichia coli* with an N-terminal hexahistidyl tag. Enzymatic activity of the recombinant protein was determined using an enzyme

assay for SODs. IgE immunoblotting and IgE inhibition assays were performed to characterize the recombinant allergen and its cross-reactivity. **Results:** A *Hevea* latex MnSOD consisting of 206 amino acid residues was cloned and expressed in *E. coli*. The allergen was designated Hev b 10. The recombinant protein was enzymatically active, indicating the correct folding of the protein. In immunoblots, latex- as well as *A. fumigatus*-allergic patients revealed IgE binding to recombinant (r)Hev b 10. Cross-reactivity to Asp f 6, the MnSOD from *A. fumigatus*, and human MnSOD was determined by inhibition of IgE binding to these MnSODs by rHev b 10. **Conclusions:** Hev b 10 is a new cross-reactive allergen of *H. brasiliensis* which belongs to the 'latex-mold' group of latex allergens. Furthermore, it is a candidate for primary sensitization in patients allergic to the pan-allergen MnSOD.

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Introduction

Allergy to natural rubber products is recognized as a serious medical problem, especially among health care workers and pediatric patients with congenital abnormalities. Of special interest are proteins from natural rubber

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Correspondence to: Dr. Heimo Breiteneder
Department of Pathophysiology, AKH-EBO 3Q
Währinger Gürtel 18–20
A–1090 Vienna (Austria)
Tel. +43 1 40400 5102, Fax +43 1 40400 5199, E-Mail Heimo.Breiteneder@akh-wien.ac.at

latex (NRL) that can act as cross-reactive allergens. Recent studies have shown that allergy to NRL is significantly associated with hypersensitivity to plant foods, and a 'latex-fruit syndrome' has been postulated [1]. In addition, cross-reactivities between allergens from NRL and *Ficus benjamina*, sweet pepper, grass and weed pollen have also been described [2–4]. Structurally similar proteins in the respective plants are responsible for such extensive cross-reactivity. However, only little information is available about the proteins that are involved in the cross-reactivities.

Some proteins may play a special role in cross-reactivity because of their highly conserved sequence and structure. These ubiquitous families of proteins can act as cross-reactive allergens in distantly related organisms and are called pan-allergens. The first allergen that was named a pan-allergen was profilin [5], and even in *Hevea brasiliensis*, profilin is an allergen [6]. Manganese superoxide dismutase (MnSOD) from *Aspergillus fumigatus* shows a high degree of homology with phylogenetically distant MnSODs. Furthermore, this enzyme has been demonstrated to be a cross-reactive allergen with MnSODs from several prokaryotic and eukaryotic organisms, thus representing a pan-allergen [7].

Interestingly, phylogenetically highly conserved proteins also show cross-reactivity with their human homologues. This IgE autoreactivity was first described for the pollen allergen profilin [8]. Humoral and cell-mediated autoimmunity was also described in individuals sensitized to *A. fumigatus* [9, 10]. Individuals sensitized to MnSOD and the acidic ribosomal P₂ protein from *A. fumigatus* also displayed IgE antibodies recognizing the human counterparts, and the human and fungal proteins elicited specific immediate-type allergic skin reactions [9, 10].

First evidence that MnSOD is an allergen from *Hevea* latex was obtained by immunoprobings two-dimensional *Hevea* latex protein blots with sera from latex-allergic patients [11]. One IgE-reactive spot showed high sequence identity with the N-terminus of the MnSOD from *H. brasiliensis* described by Miao and Gaynor [12]. MnSOD is a highly conserved enzyme involved in defense mechanisms against highly reactive oxygen species by catalyzing the conversion of superoxide anions (O₂⁻) to oxygen and hydrogen peroxide [13]. MnSOD was described as an allergen in *A. fumigatus* (Asp f 6) with cross-reactivity with the human MnSOD [10], and also showed cross-reactivity with MnSODs from yeast, *Drosophila melanogaster* and *Escherichia coli* [7].

Here we report the cloning and expression of the recombinant MnSOD from *H. brasiliensis*, a new cross-reactive latex allergen designated Hev b 10. The results obtained by IgE immunoblots and IgE ELISAs with MnSODs from *Hevea* latex, *A. fumigatus* and humans illustrate a new area of cross-reactivity of this pan-allergen.

Materials and Methods

Isolation of Total RNA from Latex

Fresh latex was collected from regularly tapped Malaysian rubber trees (*H. brasiliensis*, clone RRIM 600). Latex exuding from the tapped trees was collected while continuously mixing it with an equal volume of RNA extraction buffer [0.1 M Tris-HCl, 0.3 M LiCl, 1 mM ethylenediamine tetra-acetic acid (EDTA), 10% sodium dodecylsulfate (SDS), pH 9.5] at ambient temperature. This mixture was centrifuged at 100,000 g for 30 min at 15 °C. The rubber plug was removed, the aqueous phase extracted with chloroform-phenol and total RNA was precipitated with LiCl.

RT-PCR Amplification and Construction of the Expression Plasmid

First-strand complementary (c)DNA synthesis was performed by reverse transcribing 0.5 µg of total latex RNA using a modified oligo-dT primer, T₂₅NN [5'-GGAGAAGGA(T)₂₅(A/G/C)N-3']. For PCR, 10 µl of the first-strand cDNA synthesis was used to amplify the coding sequence for the mature Hev b 10 protein. The primers used in the PCR were SOD1 (5'-ATAGATCTCAGACCTTCTCTCTCCCGATC-3'; *Bgl*II site underlined) and SOD2 (5'-AAGA-TCTTCTACTAAGAAGAAGGGCATTCTTTGGC-3'; *Bgl*II site underlined). The PCR product was ligated into the pGEM®-T easy vector (pGEM®-T Easy Vector System, Promega, Wisc., USA) and propagated in XL1-Blue *E. coli* cells according to the manufacturer's instructions. The PCR products were sequenced using the Thermo Sequenase fluorescent labeled primer cycle sequencing kit (Amersham Life Science, Little Chalfont, England) with the fluorescent labeled primers T7 forward and M13 reverse flanking the vector's multiple cloning site.

The resulting 636-bp-long amplification product was digested with *Bgl*II and ligated into the pQE-40 expression vector (Qiagen, Hilden, Germany). The pQE-40 expression vector provides a sequence coding for a hexahistidyl affinity tag at the 5' end of the Hev b 10 coding sequence. The expression construct pQE-40/Hev b 10 was sequenced using vector-derived primers. The expression construct was then transformed into competent M15[pREP4] *E. coli* cells.

Expression of Recombinant Hev b 10 in *E. coli*

For the expression of recombinant (r)Hev b 10, 1 liter of super broth culture medium containing both ampicillin and kanamycin was inoculated with 10 ml of an overnight culture and grown with vigorous shaking at 37 °C to an OD₆₀₀ of 0.8. Expression of rHev b 10 was induced by adding isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 2 mM. After growing for an additional 16 h at 37 °C, cells were harvested and cell pellets were frozen at -20 °C.

Purification of rHev b 10 under Nondenaturing Conditions

Cell pellets were thawed for 15 min on ice and resuspended in 30 ml of lysis buffer (50 mM NaH₂PO₄, 300 mM NaCl, pH 8.0). Lysozyme (Merck, Darmstadt, Germany) was added to a concentration of 1 mg/ml and the mixture was incubated on ice for 1 h. The highly viscous lysate was treated with DNaseI at a concentration of 200 µg/ml on ice for 1 h. To pellet cellular debris, the lysate was centrifuged for 20 min at 4°C at 13,000 rpm. The supernatant was loaded onto a packed column of 3 ml of 50% w/v nickel-nitrilo-triacetic acid (Ni-NTA) agarose (Qiagen). The column was washed with 20 ml of lysis buffer and 10 ml of wash buffer (same composition as lysis buffer plus 20 mM imidazole). Purified rHev b 10 was eluted with lysis buffer containing 100 mM imidazole. The course of the purification was monitored by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on 12% gels under reducing conditions. The separated proteins were visualized by staining with Coomassie brilliant blue R-250 (Bio Rad Laboratories, Richmond, Calif., USA).

Enzymatic Activity of Hev b 10

For an 8-tube assay, the following reagents were combined and mixed to form an SOD assay reagent: 8 ml of 0.3 mM xanthine solution, 4 ml of 0.6 mM EDTA, 4 ml of 150 mM nitroblue tetrazolium solution, 2.4 ml of 400 mM Na₂CO₃ and 1.2 ml of 1 g/l bovine serum albumin (BSA). For the xanthine oxidase solution, 2.5 µl of xanthine oxidase (Boehringer Mannheim, Germany) was diluted in 250 µl of ice-cold 2 M ammonium sulfate, freshly prepared. The final concentration of xanthine oxidase was then 50 U/ml.

The enzymatic assay was performed by adding 0.5 ml of MnSOD (0–10 µg) to 2.45 ml of SOD assay reagent. The tubes were then placed into a water bath at 25°C. Then, 50 µl of xanthine oxidase solution was added to each tube at 30-second intervals, and each tube was incubated for 20 min. The reaction was then terminated by adding 1 ml of 0.8 mM CuCl₂ solution per tube every 30 s, and the absorbance of each sample was determined at 560 nm.

Under these conditions, the absorbance at 560 nm of the blank tube is about 0.25. The percentage of inhibition is calculated as below:

$$\% \text{ inhibition} = \frac{(A_{\text{blank}} - A_{\text{sample}})}{A_{\text{blank}}} \times 100\%$$

One unit of SOD is defined as the amount of protein that inhibits the rate of nitroblue tetrazolium reduction by 50%.

Cloning, Expression and Purification of rAsp f 6 and Human MnSOD

The cDNA encoding Asp f 6 was obtained from filamentous phages isolated by biopanning of an *A. fumigatus* phage surface display cDNA library. The cDNA encoding human MnSOD was obtained from a commercial human lymphoma U937 lung cDNA library (Stratagene, La Jolla, Calif., USA). Hexahistidyl-tagged recombinant proteins were produced in *E. coli* M15 as inclusion body proteins, purified by Ni²⁺-chelate chromatography under denaturing conditions and refolded by dialysis against a physiologic buffer (PBS, pH 7.4) [10].

Selection of Sera

From among 107 sera of individuals allergic to latex proteins according to case histories, skin test reactivity and RAST, 15 sera were selected that showed IgE binding to *Hevea* latex proteins in the molecular weight range that could be expected to include the

Table 1. Clinical data of patients allergic to latex (patients 1–14), latex and *A. fumigatus* (patient 15) or *A. fumigatus* alone (patients 16–21)

Patient No.	Sex	Total IgE kU/l	Latex CAP kU/l	<i>Aspergillus</i> CAP kU/l	IgE to Hev b 10
1	F	79	12.5	0	–
2	F	115	32.3	0	–
3	F	316	5.7	0	–
4	F	1,401	16.1	0	–
5	F	358	11.9	0	–
6	F	423	4.8	0	–
7	F	138	45.6	0	–
8	F	33	89.9	0	–
9	F	982	2.1	0	–
10	F	2,000	62.6	0	+
11	F	572	3.9	0	–
12	M	282	41.8	0	–
13	F	2,000	22.9	0	+
14	F	2,000	29.3	0	+
15	M	800	82.3	12.3	+
16	F	1,458	0	33.1	–
17	F	8,965	0	>100	–
18	M	1,199	0	43.7	+
19	M	832	0	>100	+
20	M	6,050	0	66.9	–
21	M	1,792	0	37.2	+

MnSOD (table 1, patients 1–15). In addition, 6 sera from *A. fumigatus*-allergic patients were included (table 1, patients 16–21). Total serum IgE and specific serum IgE against latex and *A. fumigatus* extracts were measured with a Pharmacia UniCAP device (Pharmacia, Uppsala, Sweden).

Immunoblot Analysis

Two micrograms of rHev b 10, rAsp f 6 and human MnSOD per lane were separated on 12% SDS-PAGE gels, blotted onto nitrocellulose membranes and treated with blocking buffer (40 mM Na₂HPO₄, 7 mM NaH₂PO₄, 0.5% BSA, 0.05% w/v sodium azide, 0.5% w/v Tween-20, pH 7.5). Membrane strips were then incubated with patients' sera diluted in blocking buffer (1/5) overnight at 4°C. After a washing step, the blots were incubated with ¹²⁵I-labeled anti-human IgE (IBL, Hamburg, Germany) overnight. Binding of patients' IgE to the allergens was visualized on Biomay™ MS film (Kodak, New York, N.Y., USA).

IgE Inhibition ELISA

For cross-inhibition experiments, the individual Hev b 10-, Asp f 6- and human MnSOD-specific sera were diluted 1/6 in Tris-buffered saline (TBS), 0.05% Tween-20 and 0.5% BSA and preincubated (overnight at 4°C) with 1, 10, 50 and 100 µg/ml rHev b 10 and also rHev b 3 [14]. Microtiter plates (Nunc-Immuno Plate, Nalge Nunc International, Denmark) were coated with 4 µg/ml recombinant allergens overnight at 4°C. After blocking with TBS, 0.05% Tween-20 and 1% BSA, preincubated sera were applied onto the coated

	1				50
Hev b 10	QTFSLPDLPY	DYGALEPAIS	GEIMQLHHQK	HHQTYITNYN	KALEQLNDAI
human	-KH-----	-----H-N	A-----S-	--AA-----	VTE-KYQ--L
Asp f 6	-Q-T--P---	P-D--Q-Y--	Q---E---K-	-----G-	A---AQKK-A
	-----	*-----*	*-----*	*-----*	*-----*
	51				99
Hev b 10	EKGDSAAVVK	LQSAIKFNGG	GHVNHHSIFWK	NLAPVREGGG	ELPHGSLGW.
human	A---T-Q-A	--P-L-----	-----T	--S...N--	GE-K-E--E.
Asp f 6	-AT--PKL-S	V-Q-----	-----L--	---EKS---	KIDQAPV-KA
	-----*	*-----*	*-----*	*-----*	-----*
	100				149
Hev b 10	AIDADFGSLE	KLIQLMNAEG	VALQGSGWVW	LALDKELKKL	VVETTANQDP
human	--KR-----	----K-T-AS	-----	-G-N--R-H-	Q-AACP----
Asp f 6	---QR-----	----A--TTL	L-----	-VT-GPK---	D-T--HD---
	**-----*	*-----*	*-----*	*-----*	*-----*
	150				199
Hev b 10	LVTKGPTLVP	LLGIDVWEHA	YYLQYKNVRP	DYLKNIWKVM	NWKYASEVYA
human	-QG.T-----	-----	-----	---A-----	--ENVT---
Asp f 6	V....-AA-	V---M----	-----L-DKA	S-A-G-----	--AE-EN--I
	-----*	*-----*	*-----*	*-----*	*-----*
	200				
Hev b 10	KECPSS....	..			
human	MA--K.....	..			
Asp f 6	AGD-GGHPFM	KL			
	-----*	-----			

Fig. 1. Amino acid sequence alignments of Hev b 10, human MnSOD and Asp f 6. Hev b 10 shows 58.3% identity to the human MnSOD and 48.5% identity to the *A. fumigatus* MnSOD (Asp f 6). Identical amino acid residues are indicated by dashes. The positions of the metal binding ligands are shaded in grey. The sequence differs from the reported sequence of latex MnSOD [12] at position 120, where Val instead of Ala was found in all clones sequenced.

plates and incubated overnight at 4°C. After washing five times with TBS and 0.05% Tween-20, the plates were incubated with alkaline phosphatase-conjugated mouse anti-human IgE antibody (Pharmingen, San Diego, Calif., USA) for 1 h at 37°C, and for another hour at 4°C. Color development was performed using 0.1% disodium *p*-nitrophenyl phosphate substrate (Sigma-Aldrich, Steinheim, Germany) and the optical density was measured at 405 nm (550 nm as reference wavelength) after 30 min using an ELISA reader (Dynatech MR 7000, Alexandria, Va., USA).

Results

Cloning and Sequence Analysis of Hev b 10

Primers designed according to the N- and C-terminus of the mature protein of the MnSOD from *H. brasiliensis* (EMBL: L11707) were used to amplify cDNA spanning

the mature enzyme. Several cDNA clones were sequenced, resulting in the deduced amino acid sequence reported in figure 1. The Hev b 10 cDNA encodes a protein spanning 206 amino acid residues with a predicted molecular weight of 22.9 kD and a calculated pI of 6.3 (fig. 1).

The deduced amino acid sequence of Hev b 10 was compared to sequences in the SWISS-PROT database. Hev b 10 displayed an overall identity of 48.5% to the MnSOD from *A. fumigatus* (Asp f 6), and 58.3% to the human MnSOD (fig. 1). In addition, the putative Mn(III) ligand-binding sites (His-27, His-75, Asp-164, His-168 relative to MnSOD of *Hevea* [12]) were well conserved in all three amino acid sequences (fig. 1). The designation Hev b 10 for the latex MnSOD was approved by the IUIS allergen nomenclature committee.

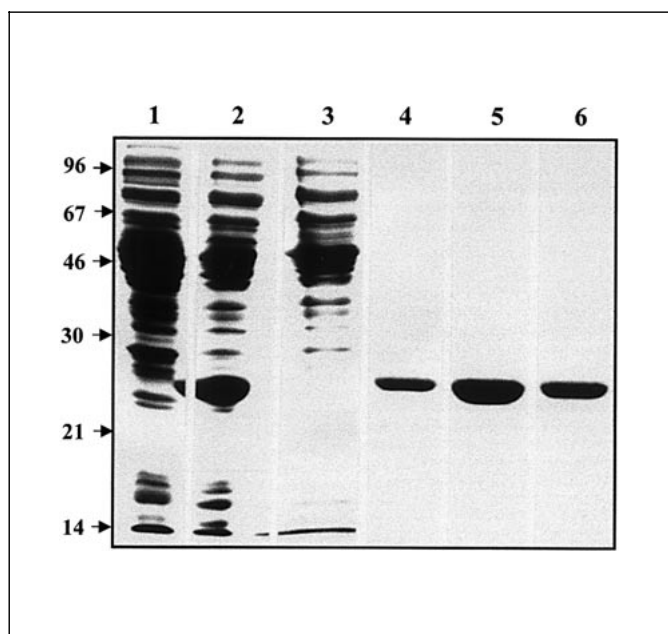


Fig. 2. Expression and purification of recombinant Hev b 10. Protein fractions at different stages of purification were loaded onto a 12% SDS-PAGE and stained with Coomassie brilliant blue R-250. Molecular weight markers are indicated in kD. Lane 1: total protein extracted from *E. coli* harboring pQE-40 without an insert. Lane 2: total protein extract from *E. coli* harboring pQE-40 with the rHev b 10 coding sequence inserted. Lane 3: wash step. Lanes 4–6: elution fractions from the Ni-NTA agarose column.

Expression and Purification of rHev b 10

The coding region for the mature protein of Hev b 10 was ligated into the expression vector pQE-40, and rHev b 10 was produced with a hexahistidyl tag. After induction of transformed M15 *E. coli* with 2 mM IPTG for 16–20 h, cells were harvested and lysed by the addition of 1 mg/ml lysozyme, and rHev b 10 was detected by SDS-PAGE. The recombinant protein was purified by Ni-NTA affinity chromatography and purification was analyzed by SDS-PAGE (fig. 2). Lane 5 in figure 2 shows 5 µg of purified rHev b 10. Since 0.1 µg of protein is detectable by SDS-PAGE stained with Coomassie brilliant blue, the purity of the rHev b 10 is >98%. The yield of purified, soluble rHev b 10 was 70 mg per liter of culture.

MnSOD Activity

Purified rHev b 10 was tested for its enzymatic activity in an assay for SOD. In this assay, xanthine-xanthine oxidase is used as a superoxide generator, which reduces nitroblue tetrazolium. This reduction is inhibited by SOD present in the solution. Four different concentrations of

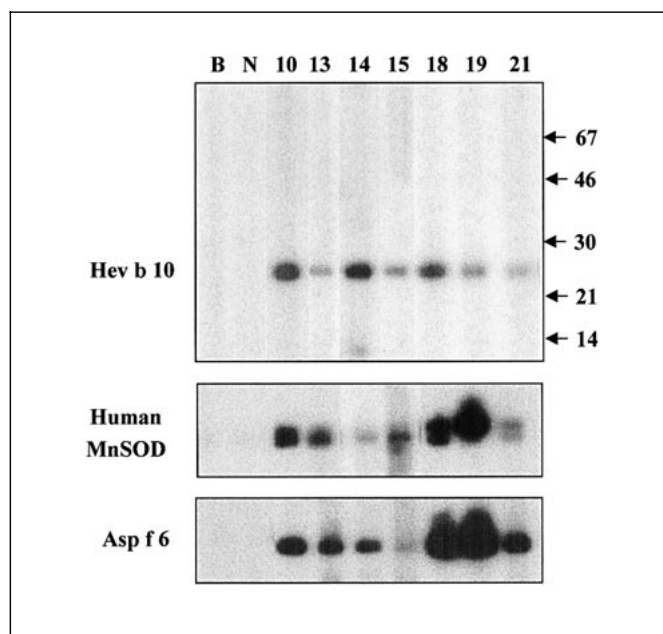


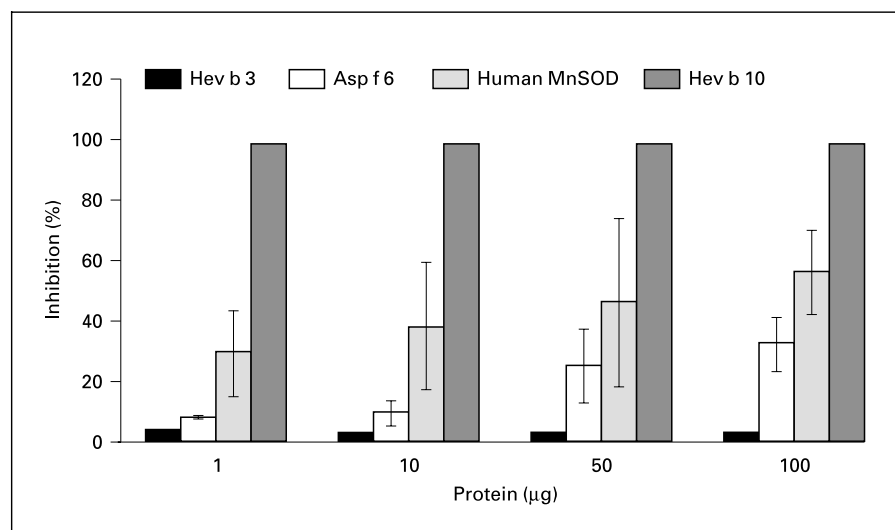
Fig. 3. IgE immunoblots with rHev b 10, recombinant human MnSOD and rAsp f 6. Blotted recombinant proteins were probed with sera from single patients, with a normal human serum pool (lane N) or a buffer control without addition of serum (lane B). Lane numbers correspond to the patient numbers in table 1. Patients 10, 13 and 14 are latex allergic, patient number 15 is latex as well as mold allergic and patients 18, 19 and 21 are mold allergic. Molecular weight markers are indicated in kD.

rHev b 10 were used in the assay, leading to an average calculation estimate of the enzymatic activity of 330 units per milligram of protein.

IgE-Binding Properties of rHev b 10, rAsp f 6 and Human MnSOD

Sera of 21 patients were tested by IgE immunoblot for their IgE binding to recombinant MnSODs from *H. brasiliensis*, *A. fumigatus* and humans. The recombinant allergen preparations were free of IgE-binding contaminants, and IgE against *E. coli* proteins was not detectable in the patients' sera. According to case histories, 14 of these patients were allergic to latex, one of the patients was additionally allergic to *A. fumigatus* and 6 patients were allergic to *A. fumigatus* alone (table 1). IgE binding to the three recombinant proteins could be detected in 7 patients: patients 10, 13 and 14 of the latex-allergic patient group, patient 15, who was allergic to latex and mold, and patients 18, 19 and 21 of the mold-allergic patient group (fig. 3). No IgE binding could be detected when using a serum pool from 6 nonallergic individuals or

Fig. 4. IgE inhibition ELISA. rHev b 10, recombinant human MnSOD and rAsp f 6 were coated at 4 µg/ml. Inhibitions were determined with Hev b 10-specific sera after preincubation with different concentrations of rHev b 10 or rHev b 3. Bars indicate the median.



a buffer control (fig. 3, lanes N and B). Hev b 10 displayed a different IgE-binding capacity in comparison to Asp f 6 and the human MnSOD for all Hev b 10-positive sera, whereas the IgE-binding capacities of Asp f 6 and the human MnSOD were comparable for 4 of the 7 sera (patients 10, 13, 18 and 19 in fig. 3).

ELISA Inhibition Studies with rHev b 10, rAsp f 6 and Human MnSOD

For cross-inhibition studies, the Hev b 10-positive sera were used in ELISA experiments. Therefore, the three recombinant MnSODs were coated (4 µg/µl) and tested with aliquots of the sera after preincubation with increasing amounts of rHev b 10 and rHev b 3 as a negative control. rHev b 10 was able to abrogate 93% of IgE binding to rHev b 10 at a concentration of 1 µg/ml, and almost 100% of binding at concentrations greater than 50 µg/ml. Cross-inhibition was observed with rHev b 10 in the case of Asp f 6 up to an average of 32%, and in the case of the human MnSOD up to an average of 58% (fig. 4). Preincubation of the sera with equivalent amounts of purified rHev b 3 did not influence IgE binding to rHev b 10 (fig. 4).

Discussion

Cross-reactivities of proteins involved in latex allergy are an important part of this severe and so far untreatable disorder. Here we present a novel cross-reactive latex allergen which is an MnSOD, designated Hev b 10. We produced Hev b 10 as a 206-amino acid recombinant pro-

tein. The recombinant protein was able to bind IgE from latex- as well as mold-allergic patients. Furthermore, cross-reactivity to *A. fumigatus* and human MnSODs could be shown using IgE inhibition ELISA.

In our IgE-binding studies we could show that patients sensitized to Hev b 10 also recognized Asp f 6 and the human MnSOD. Furthermore, Asp f 6-sensitized patients showed IgE binding to Hev b 10. IgE-binding intensities were quite similar for Asp f 6 and human MnSOD, but differed for Hev b 10 when tested in IgE immunoblots (fig. 3). The cross-inhibition studies revealed that Hev b 10 inhibited IgE binding to Asp f 6 and the human MnSOD by 32 and 56%, respectively (fig. 4). These data correlate with the higher identity between the human MnSOD and Hev b 10. Complete inhibition of IgE binding with Hev b 10 as described for IgE binding to human MnSOD of sera preincubated with Asp f 6 [10] could not be observed. These results indicate that some IgE-binding epitopes are shared between Hev b 10 and the other MnSODs.

rHev b 10 showed enzymatic activity in an MnSOD activity assay, indicating that the recombinant protein was correctly folded. Sequence comparison of Hev b 10 with *A. fumigatus* and human MnSODs revealed sequence identities of 48.5 and 58.3%, respectively. Beside this, the sequence comparison between the three proteins revealed that the putative Mn(III) ligand-binding sites are well conserved (fig. 1), suggesting similar protein structures. This could be an explanation for the cross-reactivity of Hev b 10 with MnSODs from unrelated organisms.

MnSODs from *A. fumigatus*, humans, *D. melanogaster*, *Saccharomyces cerevisiae* and *E. coli* have been produced as recombinant proteins [7]. Specific IgE raised against all MnSODs could be detected with sera from patients sensitized to the *A. fumigatus* enzyme. In addition, IgE binding to all MnSODs could be inhibited by preincubation of the sera with *A. fumigatus* MnSOD [7]. These results indicated that MnSODs are pan-allergens that are recognized by individuals sensitized to the fungal MnSOD.

Two functional categories of allergens are present in *A. fumigatus*: secreted and cytoplasmic proteins. MnSOD belongs to the nonsecreted allergens and is exclusively recognized by serum IgE of allergic bronchopulmonary aspergillosis patients [15]. Therefore, it represents a highly specific marker for allergic bronchopulmonary aspergillosis. Allergens from *Hevea* latex can also be classified into several groups, such as 'latex only', 'latex-fruit syndrome', 'spina bifida' and now possibly a 'latex-mold' group [16].

Hev b 10 can be placed among the 'latex-mold' group because of its cross-reactivity to Asp f 6. Another latex allergen, an enolase (Hev b 9), shared high sequence identity to enolases from molds [11]. These enolases have been identified as important allergens of the molds *Cladosporium herbarum* and *Alternaria alternata* [17, 18]. Hev b 9 and Hev b 10 are housekeeping enzymes, and represent a new group of cross-reactive allergens. Over the past years, several cross-reactive allergens in plants have been identified. The housekeeping enzymes Hev b 9 and Hev b 10 form a new group of cross-reactive allergens of *H. brasiliensis* possibly connected with a 'latex-mold' syndrome.

Most of the cross-reactive allergens described so far are homologous to pathogenesis-related proteins and represent plant defense-related proteins. β -1,3-glucanase (Hev b 2) and *Hevea* chitinase (Hev b 11) are involved in defense mechanisms against fungi [19, 20]. Hev b 6.02, the latex hevein, and its precursor prohevein, Hev b 6.01, can also be classified as defense-related proteins of a rubber tree [21–23]. Another cross-reactive allergen of *H. brasiliensis* is Hev b 8, the latex profilin [6]. Profilins are cytoskeletal actin-binding proteins and act as pan-allergens in many organisms. Beside MnSOD, profilin is another ubiquitous protein that induced an autoreactivity response in allergic patients [8].

Hev b 10 is a minor allergen of *H. brasiliensis* according to the relatively low incidence of IgE-positive sera (table 1). However, its importance lies in its ability to act as a pan-allergen, thus linking latex and mold allergy in terms of possible IgE-mediated symptoms caused by similar molecular structures. In addition, structural information opens new ways to generate and select hypoallergenic variants through consequent applications of advanced techniques based on molecular breeding [24].

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