

Relevance of the recombinant lipid transfer protein of *Hevea brasiliensis*: IgE-binding reactivity in fruit-allergic adults

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Background: Lipid transfer proteins (LTPs) are relevant allergens in certain plants. The role of the LTP of *Hevea brasiliensis* in the latex-fruit syndrome is widely unknown.

Objective: To study IgE reactivity with recombinant *Hevea* LTP in sera of fruit-allergic adults with and without natural rubber latex (NRL) allergy.

Methods: An LTP-specific complementary DNA of *H brasiliensis* leaves was amplified, subcloned into the pMAL expression system, and analyzed. The recombinant protein was coupled to ImmunoCAP, and the IgE-binding properties were studied in sera of 10 NRL-allergic patients without symptoms to fruit and 48 atopic patients with fruit allergy. Eleven of these 48 patients were also allergic to NRL, 14 displayed sensitization to NRL without symptoms on NRL exposure so far, and 23 had neither symptoms nor IgE antibodies to NRL.

Results: After expression in *Escherichia coli*, a soluble maltose-binding protein-rHev b 12 fusion protein was isolated and coupled to ImmunoCAP to determine rHev b 12 specific IgE reactivity. rHev b 12 specific IgE binding was found in 3 fruit-allergic patients with NRL sensitization (0.68, 0.88, and 0.96 kU/L) and in 3 fruit-allergic patients without NRL sensitization (1.58, 2.25, and 2.27 kU/L). The remaining 52 serum samples and all maltose-binding protein control test results were negative (<0.35 kU/L).

Conclusions: In these patients, rHev b 12 specific IgE reactivity seems to result from common cross-reactive epitopes with some of the fruit LTPs tested and underscores only an involvement in co-recognition. No clinical relevance of IgE binding to the LTP of *H brasiliensis* in association with NRL allergy was detected.

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INTRODUCTION

Nonspecific lipid transfer proteins (LTPs) belong to the PR-14 family of pathogenesis-related proteins.¹ Plant nonspecific LTPs are small proteins with antifungal and antibacterial properties that facilitate the transfer of phospholipids and other lipids across membranes.^{2,3} Studies⁴ have shown that LTPs are important allergens, especially of the Prunoideae fruits, including peach, apricot, plum, and cherry.

Recently, Beezhold and coworkers⁵ demonstrated by means of immunoblot experiments the presence of specific IgE to a recombinant LTP of *Hevea brasiliensis* in 9 (24%) of 37 natural rubber latex (NRL)-allergic individuals tested, whereby 2 had clinical reactivity to Rosaceae fruits. For the remaining 7 patients, fruit reactivity was not noted. In the latest International Union of Immunological Societies nomenclature list, the *Hevea* LTP was denominated as allergen Hev b 12.⁶ More recently, Pamies et al⁷ studied rHev b 12 specific IgE reactivity in 31 latex-sensitized children with spina bifida, including 25 with clinical symptoms to NRL. None of these patients showed a positive IgE response.⁷ So far, 13 allergens of NRL are characterized, and some of them, such as hevein (Hev b 6.02), have been shown to play a dominant role in food cross-reactivity to certain fruits, especially avocado, kiwi, and banana.⁸ This association is called latex-fruit syndrome, and it shows an IgE-binding prevalence of 30% to 50% in NRL-allergic patients,⁹ whereas Beezhold et al¹⁰ showed that only approximately 7% are associated with clinical symptoms after ingestion of foods. Although several studies^{8,9,11} have shown that there is general cross-reactivity between NRL and the previously mentioned fruits, the present study aimed to elucidate IgE reactivity of the *Hevea* LTP in fruit-allergic adult patients with and without NRL

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allergy and in a group of 10 adult NRL-allergic patients displaying no symptoms to fruit.

MATERIALS AND METHODS

Criteria for the Selection of Human Sera

Serum samples were obtained from 10 German NRL-allergic patients who had no symptoms to fruit (8 women and 2 men; mean age, 30.5 years; age range, 22–45 years) and from 48 fruit-allergic patients (14 Spanish and 34 German patients; 34 women and 14 men; mean age, 39 years; age range, 11–71 years) (Table 1). All the fruit-allergic patients had a history of immediate-type symptoms to 1 or more of the following fruits: almond, apple, apricot, avocado, banana, cherry, hazelnut, kiwi, nectarine, orange, peach, pear, plum, raspberry, strawberry, and walnut. Thirty-six of the 48 fruit-allergic patients had oral allergy syndrome, whereby patients showed 1 or more of the following symptoms: itching or swelling of the lips, tongue, or throat. The other 12 fruit-allergic patients showed systemic reactions such as eczema, rhinitis, conjunctivitis, urticaria, facial angioedema, asthma, and anaphylaxis but no oral allergy syndrome. Fruit-allergic patients were divided into 3 subgroups: 11 fruit-allergic patients with an additional NRL allergy (patients 1–11), 14 fruit-allergic patients with an additional NRL-specific sensitization but without clinical symptoms to NRL (patients 12–25), and 23 fruit-allergic patients without sensitization to NRL (patients 26–48) (Table 1).

Sensitization to NRL was demonstrated by NRL-specific IgE antibodies in the serum or positive skin prick test reactions to at least 1 NRL extract. Skin prick tests were performed using at least 1 commercial NRL extract (100 HEP, ALK-Scherax, Hamburg, Germany; Bencard 1%, Bencard Allergie GmbH, Munich, Germany; and Alyostal, Staller-genes, Rheinberg, Germany) and a commercial peach test extract (Allergopharma, Reinbek, Germany). Alternatively, extracts from fresh peaches were prepared directly before testing (dissolved in phosphate-buffered saline). Cutaneous reactions were read after 15 minutes and were considered positive when the wheal diameter was at least 3 mm. Skin tests were disregarded if there was a reaction to the negative control or no reaction to the positive control. The NRL specific IgE antibodies and total IgE were examined using a commercially available kit (Pharmacia CAP-FEIA System; Phadia GmbH, Freiburg, Germany).

Polymerase Chain Reaction Amplification of Complementary DNA

Complementary DNA (cDNA) was obtained from total RNA of *H brasiliensis* leaves as described previously.¹² Amplification of the *LTP* gene was performed in a 50- μ L reaction mixture containing 4 ng of *Hevea* cDNA, 50-mmol/L potassium chloride, 10-mmol/L Tris hydrochloride (pH 9.0), 1.5-mmol/L magnesium chloride, 0.01% (wt/vol) gelatin (Sigma, Deisenhofen, Germany), 500 μ M of each deoxynucleotide triphosphate, 25 pmol of each of the primers H12Sfo5' (5'-CGCC GGCGCC²GCA³CTT⁴AAG⁵ATG⁶-3') and H12Hind3' (5'-

GGCAAGCTTCACTTGACGGTGGC-3') derived from EMBL Accession No. AY057860,⁵ and 2.5 U of Taq polymerase (Eppendorf, Hamburg, Germany). Reaction products were obtained using a thermal cycler (Applied Biosystems, Weiterstadt, Germany) with an initial denaturation step (95°C for 3 minutes) and a total of 40 cycles of denaturation (95°C for 1 minute), annealing (55°C for 1 minute), and extension (72°C for 2 minutes), followed by 10 minutes at 72°C and produced a single 363-base pair (bp) fragment including an open reading frame of 345 bp that corresponds to 115 amino acid residues and starts with the second amino acid residue alanine of its signal peptide.

Subcloning and Sequencing of Polymerase Chain Reaction Products

The 363-bp polymerase chain reaction (PCR) product, including the signal peptide coding sequence without its first residue methionine, was subcloned into the pDrive cloning vector (Qiagen, Hilden, Germany). Clones carrying inserts were characterized by means of restriction analysis and sequencing on an ABI 310 sequencer (Applied Biosystems). Sequencing analysis revealed that the PCR product corresponds to a 345-bp open reading frame and was named NRL-LTP, whereby the first 69 bp correspond to the signal peptide, followed by 276 bp corresponding to the sequence of the mature LTP.⁵

Computer analysis using the antigenic index of the Protean 6.1 program (DNASTAR Inc, Madison WI) revealed no antigenicity for the signal peptide. For the efficient expression of the recombinant NRL-LTP in *Escherichia coli* TB-1, the cDNA coding for the complete LTP sequence and its N-terminal signal peptide lacking the initial start codon was isolated after a *SfoI*-*HindIII* digest from the pDrive cloning vector on 1% agarose gel. Finally, the gel-purified *SfoI*-*HindIII* fragment was subcloned into the expression vector pMAL-c2 (New England Biolabs, Frankfurt, Germany) downstream from the *malE* gene coding for the maltose-binding protein (MBP). Integrity of the sequence was confirmed by sequencing as described previously herein.

Expression and Purification of the MBP-rHev b 12 Fusion Protein

One liter of 2xYT medium containing 100 μ g/mL (wt/vol) of ampicillin and 2 mg/mL (wt/vol) of glucose was inoculated with 10 mL of fresh recombinant *E coli* cells from an overnight culture and was incubated at 30°C for 2 hours in a laboratory shaker (New Brunswick Scientific GmbH, Nuertingen, Germany). Expression of the fusion protein was induced by shaking in the presence of 0.3-mmol/L isopropyl β -D-1-thiogalactopyranoside (Roth, Freiburg) for 4 hours at 30°C. The *E coli* cell lysates containing the expressed fusion protein were prepared by means of sonification in an ice water bath using a Branson sonifier-250 (Branson, Danbury, CT) (8 intervals of 20 seconds on level 4 followed by a time stop of 30 seconds) followed by centrifugation at 10,000g for 30 minutes. The supernatant was loaded onto a 1.5 $\times$ 20.0-cm column filled with a 7-mL bed volume of amylose

Table 1. Characteristics of the 58 Study Patients

Patient No./sex/age, y	Total IgE, kU/L	Symptoms to peach (other fruit)	Symptoms to NRL	Peach (other fruit) specific IgE, kU/L	NRL specific IgE, kU/L	SPT to peach	SPT to latex
Fruit Allergy and NRL Allergy							
1/F/34	192	R	U	2.99	1.19	ND	ND
2/F/33	374	(OAS: a, k)	A	<0.35 (a: 5.50)	40.70	ND	ND
3/F/35	988	OAS (U: av)	A	0.82	3.71	ND	Positive
4/F/51	950	OAS (OAS: a, b, pl)	U, R	3.37	11.80	ND	Positive
5/F/44	266	(OAS: a, av, b)	An	<0.35 (a: 0.49)	4.98	Negative	Positive
6/F/34	865	(OAS: a)	U, R	<0.35 (a: 2.90)	12.30	Negative	Positive
7/F/42	51	(OAS: a, b, k)	A, C, R, U	<0.35 (a: 0.80)	4.00	Negative	Positive
8/F/32	4,267	(OAS: a, o)	A, U	0.40 (a: 8.70)	>100	Negative	Positive
9/M/51	165	(OAS: a, av, b)	A, U	<0.35 (a: 1.40)	1.20	ND	Positive
10/M/37	533	(OAS: a, h, w)	A, C, R, U	2.40 (a: 4.10)	4.00	ND	Positive
11/F/64	317	(OAS: a, b, h, w)	A, C, R, U	0.92 (a: 7.90)	12.10	ND	Positive
Fruit Allergy and NRL Sensitization but Without Symptoms to NRL							
12/M/17*	425	U	None	31.90	0.56	Positive	ND
13/F/26	353	R	None	1.83	4.42	ND	ND
14/F/39	121	R	None	2.36	0.51	ND	ND
15/M/50	693	OAS (OAS: k)	None	0.80	0.39	Negative	Negative
16/F/68	105	An (An: r)	None	0.60	0.40	ND	Negative
17/F/24	248	(OAS: k)	None	0.49 (k: 26.5)	0.99	Positive	Negative
18/F/19	720	(OAS: a, c, w)	None	6.90 (a: 13.10)	2.23	Positive	Positive
19/M/44	399	OAS (OAS: al, b)	None	7.41	1.04	Negative	Negative
20/M/11	946	OAS (OAS: a)	None	3.20	0.40	ND	ND
21/F/31	851	(OAS: a)	None	1.12 (a: 2.09)	6.60	ND	Negative
22/F/24	518	OAS (An: c, h)	None	1.46	1.00	ND	Negative
23/M/21	400	OAS (OAS: h, w)	None	0.50	0.72	ND	Negative
24/M/28*	716	(OAS: c, h)	None	14.30 (c: 1.3, h: 11.8)	2.17	ND	ND
25/F/53*	337	(OAS: c, U: h)	None	19.10 (c: 0.8, h: 3.17)	0.99	ND	ND
Fruit Allergy Without Sensitization to NRL							
26/F/19	69	FA, U	None	9.20	<0.35	Positive	ND
27/F/27	294	FA, U	None	3.70	<0.35	Positive	ND
28/F/24*	367	A, U	None	3.60	<0.35	Positive	ND
29/F/24	1,656	FA, U	None	39.50	<0.35	Positive	ND
30/M/19	425	A, U	None	10.60	<0.35	Positive	ND
31/M/24	233	A, U, OD	None	29.30	<0.35	Positive	ND
32/F/30*	142	OD (OAS: h)	None	25.20 (h: 7.50)	<0.35	ND	ND
33/F/25	286	OAS (OAS: h)	None	17.60 (h: 6.75)	<0.35	ND	ND
34/F/30	72	(U: h)	None	14.60 (h: 5.69)	<0.35	ND	ND
35/F/34*	211	(OAS: h)	None	40.70 (h: 11.50)	<0.35	ND	ND
36/F/31	190	(OAS: a, al)	None	<0.35 (a: 2.00)	<0.35	Negative	ND
37/F/30	60	OAS, U	None	3.10	<0.35	Positive	Negative
38/M/34	2,824	OAS (OAS: n)	None	1.58	<0.35	Positive	Negative
39/M/33	25	An	None	3.30	<0.35	ND	ND
40/M/26	122	OAS, U	None	0.98	<0.35	Negative	Negative
41/F/43	515	OAS, U	None	2.48	<0.35	Negative	ND
42/F/43	251	(OAS: a, h, s)	None	<0.35 (a: 2.20)	<0.35	ND	ND
43/F/38	271	(OAS: a, p)	None	0.36 (a: 7.30)	<0.35	Positive	Negative
44/F/49	251	(OAS: h)	None	<0.35 (h: 0.46)	<0.35	Positive	Negative
45/F/41	733	(OAS: a, ap, c)	None	<0.35 (a: 0.40)	<0.35	Negative	Negative
46/F/62	44	(OAS: a, ap)	None	<0.35 (a: 1.20)	<0.35	ND	Negative
47/F/52	29	OAS (An: h)	None	1.50	<0.35	Negative	Negative
48/M/71	295	OAS, R, C	None	7.60	<0.35	Positive	ND
NRL Allergy Without Symptoms to Fruit							
49/F/22	24	None	Ec, C, R	<0.35	1.3	ND	Positive
50/F/35	154	None	R	<0.35	30.3	ND	Positive
51/F/32	72	None	Ec, C, R, U	<0.35	5.7	ND	Positive
52/F/23	343	None	A	<0.35	35.0	ND	Positive
53/F/45	617	None	Ec, U, R	<0.35 (fx21: 0.53)	99.7	ND	Positive
54/M/26	221	None	A, R	<0.35 (fx21: 3.26)	>100	ND	Positive
55/F/23	362	None	A, C, R	<0.35 (fx21: 1.52)	70.1	ND	Positive
56/F/36	210	None	C, R, U	<0.35	14.3	ND	Positive
57/M/29	225	None	A, C, R, U	<0.35	43.6	ND	Positive
58/F/34	118	None	U	<0.35	2.3	ND	Positive

Abbreviations: A, asthma; a, apple; al, almond; AD, atopic dermatitis; An, anaphylaxis; ap, apricot; av, avocado; b, banana; C, conjunctivitis; c, cherry; Ec, eczema; FA, faciales angiodema; fx21, fruit mixture including kiwi, melon, banana, peach, and pineapple; h, hazelnut; k, kiwi; n, nectarine; ND, not determined; NRL, natural rubber latex; o, orange; OAS, oral allergy syndrome; OD, edema; p, pear; pl, plum; R, rhinitis; r, raspberry; s, strawberry; SPT, skin prick test; U, urticaria; w, walnut.

* Patients showing rHev b 12 specific IgE antibodies (Table 2).

(New England Biolabs). Equilibration, washing, and elution were performed as recommended by the supplier (New England Biolabs). Finally, the isolated fusion protein with a calculated molecular mass of 54.5 kDa was concentrated using Centricon YM-30 concentrators (Millipore Corp, Bedford, MA).

Determination of rHev b 12 Specific IgE Antibodies by Means of the ImmunoCAP Method

To determine rHev b 12 specific IgE, the MBP-rHev b 12 fusion protein was bound onto an activated cellulose solid phase by means of covalent binding. The coupling concentration was adjusted to attain a linear measurement range and a background below 0.35 kU/L. The capture antigen (ImmunoCAP of rHev b 12) was used to determine rHev b 12 specific IgE antibodies by means of the Pharmacia CAP system. A specific IgE value greater than 0.35 kU/L was regarded as positive. The carrier protein MBP was also bound onto a cellulose solid phase (ImmunoCAP) and served as a negative control in all experiments.

Determination of rPru av 3 and rCor a 8 Specific IgE by Means of Enzyme Allergosorbent Testing

The allergens rPru av 3 (0.5 µg per disk) and rCor a 8 (0.3 µg per disk) were coupled to filter paper disks activated with cyanogenbromide (Hycor, Kassel, Germany).^{13,14} The enzyme allergosorbent test was performed using the Allergopharma specific IgE enzyme-linked immunosorbent assay according to the manufacturer's instructions.

IgE Inhibition Experiments

In specific cases, IgE inhibition experiments were performed with MBP-rHev b 12 ImmunoCAP as solid phase and 10 µL of nonammoniated NRL C-serum (10 mg/mL) added to 50 µL of serum as inhibitor. Inhibitions greater than 20% were defined as relevant. The MBP served as a negative control.

RESULTS

*PCR Amplification of the Hevea LTP cDNA, Expression, Purification, and Analysis of the Recombinant LTP of *H brasiliensis* (rHev b 12)*

For the efficient expression of rHev b 12, the cDNA fragment encoding the LTP from *H brasiliensis* leaves was sequenced and subcloned into the pMAL vector system. The expression induced by isopropyl β-D-1-thiogalactopyranoside generated the fusion protein MBP-rHev b 12 with an estimated molecular mass of 54.53 kDa corresponding to 42.7 kDa for the carrier protein MBP and 11.83 kDa for the target protein rHev b 12, including its signal peptide corresponding to the 23 N-terminal amino acid residues. This stretch served as a spacer between target and carrier protein. The purified protein on 10% Bis-Tris gel showed a mass of approximately 57 kDa (Fig 1). The calculated isoelectric point of rHev b 12 was 9.24. The total yield of affinity-purified recombinant MBP-rHev b 12 fusion protein was up to 4 mg/L according to the analysis using the Bradford method with bovine serum albumin as a standard. Analytical expression experiments showed

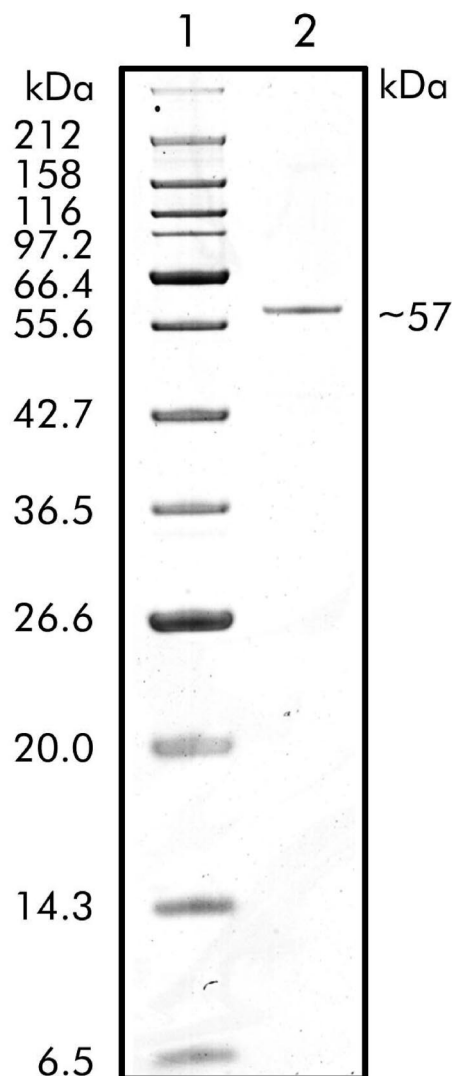


Figure 1. Analysis of purified maltose-binding protein (MBP)-rHev b 12 on 10% NuPAGE Bis-Tris gel after concentration by YM-30 concentrators. Lane 1 represents the molecular mass marker; lane 2, an aliquot (2 µg) of the concentrated MBP-rHev b 12 fusion protein after affinity chromatography.

that induction at 37°C led to less efficient protein synthesis regardless of which *E coli* host strain was used (data not shown). This observation was also made in the case of rCor a 8 representing the recombinant LTP of the hazelnut.¹⁴

IgE-Binding Reactivity of the Recombinant Hevea LTP (rHev b 12)

The 6 serum samples showing positive rHev b 12 specific IgE-binding reactivity (≥ 0.35 kU/L) together with the available corresponding clinical data are summarized in Table 2. The serum sample from patient 12 displayed an rHev b 12 specific IgE value of 0.88 kU/L and a low level of NRL specific IgE antibodies (0.56 kU/L). Also, the 2 serum samples from German patients 24 and 25 displayed rHev b 12

Table 2. Clinical Data of the 6 Fruit-Allergic Patients With and Without NRL Sensitization Showing Positive rHev b 12 Specific IgE Reactivity but no Symptoms to NRL

Patient No./sex/age, y	Total IgE, kU/L	Symptoms to peach (other fruit)	SPT to peach	Peach (other fruit) specific IgE, kU/L	NRL specific IgE, kU/L	MBP-rHev b 12 specific IgE, kU/L*	rHev b 12 specific inhibition reactivity with nonammoniated NRL C-serum, %
Fruit-Allergic Patients With NRL Sensitization but Without NRL Symptoms							
12/M/17	425	U	Positive	31.90	0.56†	0.88‡§¶	15
24/M/28	716	(OAS: c, h)	ND	14.30 (c: 1.32, h: 11.8)	2.17 (1.00)	0.68‡§	0
25/F/53	337	(OAS: c, U: h)	ND	19.10 (c: 0.82, h: 3.17)	0.99 (12.22)	0.96‡§	1
Fruit-Allergic Patients Without NRL Sensitization							
28/F/24	367	A, U	Positive	3.60	<0.35	2.27‡¶	0
32/F/30	142	OD (OAS: h)	ND	25.20 (h: 7.50)	<0.35	2.25‡§	5
35/F/34	211	(OAS: h)	ND	40.70 (h: 11.50)	<0.35	1.58‡§	ND

Abbreviations: A, asthma; c, cherry; h, hazelnut; MBP, maltose-binding protein; ND, not determined; NRL, natural rubber latex; OD, edema; OAS, oral allergy syndrome; SPT, skin prick test; U, urticaria.

* All sera tested with MBP-rHev b 12 were also tested with MBP alone and revealed negative results (<0.35 kU/L) in all cases.

† Negative to the mixed ImmunoCAP (rHev b-Mix) with rHev b 1, rHev b 5, rHev b 6.01, and rHev b 8 and to the single allergens rHev b 3, rHev b 7, rHev b 9, rHev b 10, and rHev b 11 (CAP).

‡ rPru av 3 positive (enzyme allergosorbent testing)

§ rCor a 8 positive (enzyme allergosorbent testing).

¶ rPru p 3 positive (enzyme-linked immunosorbent assay).¹⁵

|| Positive to the rHev b-Mix.

specific IgE values of 0.68 kU/L and 0.96 kU/L, respectively, in addition to NRL specific IgE values of 2.17 kU/L and 0.99 kU/L, respectively. Both patients showed symptoms to cherry and hazelnut. Patients 28, 32, and 35 belonged to the subgroup of fruit-allergic patients without sensitization to NRL and had symptoms to peach alone (patient 28) or to peach and hazelnut (patients 32 and 35). Serum samples from patients 28, 32, and 35 displayed rHev b 12 specific IgE-binding reactivity (1.58, 2.25, and 2.27 kU/L). Serum samples from the 10 NRL-allergic patients without symptoms to fruits (patients 49–58) and the 11 fruit-allergic patients with an additional latex allergy (patients 1–11) displayed no rHev b 12 specific IgE binding. Also, the MBP specific IgE value was less than 0.35 kU/L in all the sera tested. In summary, 6 (12.5%) of the 48 serum samples from fruit-allergic patients with and without latex allergy tested displayed rHev b 12 specific IgE values.

Recognition of rHev b 12 With Recombinant LTPs of Cherry (rPru av 3) and Hazelnut (rCor a 8)

The 6 serum samples that showed IgE binding to rHev b 12 were tested for the presence of specific IgE antibodies to the recombinant LTPs of cherry (rPru av 3) and hazelnut (rCor a 8) by using the enzyme allergosorbent test. All 6 serum samples showed rPru av 3 specific IgE, and 5 also showed rCor a 8 specific IgE (data not shown). Serum samples from patients 12 and 28, strong peach-allergic patients with severe symptoms, recently demonstrated IgE reactivity to native and recombinant peach LTP (Pru p 3)

by using an enzyme-linked immunosorbent assay method.¹⁵

Sera of Fruit-Allergic Patients With Sensitization to NRL and rHev b 12 Specific IgE Antibodies: Reactivity With Other Recombinant NRL Allergens

The 3 fruit-allergic patients with additional NRL sensitization, who displayed rHev b 12 specific IgE antibodies, were also tested for IgE-binding reactivity with further NRL allergens using the ImmunoCAP method. Patients 24 and 25 showed positive specific IgE values (1.00 and 12.2 kU/L) when tested with a mixed ImmunoCAP coupled with the single recombinant allergens rHev b 1, rHev b 5, rHev b 6.01, and rHev b 8. In contrast, serum samples from patient 12 yielded a negative value (<0.35 kU/L) to the mixed ImmunoCAP and to the tested single allergens rHev b 3, rHev b 7, rHev b 9, rHev b 10, and rHev b 11. Additional NRL allergens were not tested.

IgE Inhibition Experiments With rHev b 12 ImmunoCAP as Solid Phase and Nonammoniated Latex C-Serum as Inhibitor

Serum samples from patients 12, 24, 25, 28, and 32 were chosen to prove the ability to inhibit rHev b 12 specific IgE reactivity with latex C-serum. Only the serum sample from patient 12 showed marginal inhibition reactivity of 15%. The other 4 serum samples tested displayed inhibition reactivity of 0% to 5% (Table 2).

DISCUSSION

Clinical cross-reactivity to fruits such as avocado, kiwi, and banana is often observed in NRL-allergic individuals.^{8,9,16} The responsible NRL allergen in most of these patients is hevein (Hev b 6.02) itself¹⁷ or proteins carrying a hevein-like domain, such as the class I chitinase (Hev b 11) of *H brasiliensis*.^{18,19}

Cross-reactivity to fruits belonging to the Rosaceae family, such as apple, peach, cherry, apricot, and plum, are frequent in Central Europe but occur more often in adults in Mediterranean countries.²⁰ The cause of cross-reactivity to these fruits in the Mediterranean area are allergens of the LTP family comprising a group of ubiquitous heat- and protease-resistant plant proteins with a molecular mass of 9 to 10 kDa and an isoelectric point of approximately 9.0 that are involved in the defense against pathogens and environmental stress.^{14,20–25}

The present study aimed to elucidate the role of the recombinant LTP of *H brasiliensis*, a protein not characterized in its native form to date. In an initial study, Beezhold et al⁵ demonstrated by means of immunoblot experiments specific IgE binding to the recombinant LTP of *H brasiliensis* (rHev b 12) in 9 (24%) of 37 NRL-allergic individuals. In the present study, rHev b 12 specific IgE determination was performed in sera of 14 Spanish and 34 German fruit-allergic patients using ImmunoCAP. In total, the frequency of rHev b 12 specific IgE binding was only 12.5%. In only 3 serum samples, rHev b 12 specific IgE binding was accompanied by binding of specific IgE antibodies to NRL. Nevertheless, none of these patients showed clinical symptoms to NRL. Furthermore, none of the fruit-allergic patients with latex allergy (n = 11, patients 1–11) and none of the patients with latex allergy and without symptoms to fruit (n = 10; patients 49–58) displayed rHev b 12 specific IgE antibodies. These results indicate that rHev b 12 specific IgE binding is restricted to some fruit-allergic patients as a result of co-recognition to corresponding fruit LTPs.

This hypothesis was supported by a study by Garcia-Casado et al,²⁴ who identified 3 IgE-binding epitopes of Pru p 3, the LTP of peach, representing the major allergen in peach-allergic individuals in the Mediterranean area.^{21–25} One of these epitopes comprising residues 71 to 80 (GKCGV-SIPYK) displays a close homology with the corresponding epitope of Hev b 12 (GKCGVNIPYK), differing only in amino acid position 76, where S (serine) is replaced by N (asparagine). Furthermore, this epitope sequence of rHev b 12 matches perfectly with the hazelnut LTP rCor a 8. Also, the corresponding epitope of cherry LTP (GKCGVNVPYK) displays close homology with that of rHev b 12. The only difference is that the second V (valine) in position 77 is changed to an I (isoleucine) in the case of rHev b 12. In contrast, the other 2 described major IgE-binding epitopes of peach LTP and the corresponding sequences of cherry LTP show larger differences with respect to those of rHev b 12. All rHev b 12–positive sera in this study showed rPru av 3

specific IgE antibodies. In the case of rCor a 8, 5 of 6 serum samples also displayed specific IgE binding to the hazelnut LTP. In addition, 2 of the serum samples (patients 12 and 28) are known to contain Pru p 3 specific IgE antibodies.¹⁵ A further common property of all rHev b 12–positive sera is the relative high yield of peach specific IgE (mean, 18.8 kU/L; range, 3.6–40.7 kU/L).

Taking into account the current knowledge of the different fruit LTPs, we conclude that the IgE-binding reactivity of rHev b 12 could be the result of common epitopes in the LTP of *Hevea*, peach, cherry, and hazelnut, whereby residues 71 to 80 showed the highest homology among each another and could play a major role. Furthermore, latex LTP seems not to be included in its native form in the extract used as solid phase (k82) in the ImmunoCAP assay used to determine NRL specific IgE antibodies because serum samples from patients 28, 32, and 35 showed negative results. In addition, we observed inhibition reactivity of only 0% to 15% when rHev b 12 specific ImmunoCAPs were used to test serum samples from patients 12, 24, 25, 28, and 32 preincubated with a nonammoniated latex C-serum (Table 2). In summary, these results indicate that the LTP of *H brasiliensis* (Hev b 12) itself seems to have only a limited role in NRL allergy.

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