

Characterization of cross-reactive bell pepper allergens involved in the latex-fruit syndrome

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Summary

Background Between 30% and 50% of individuals who are allergic to latex products are also allergic to specific plant foods, a fact that is well documented as the latex-fruit syndrome. Simultaneous sensitization to latex and bell pepper has been previously reported. Although bell pepper fruits are frequently consumed raw, cooked or as a spice, little is known about the cross-reactive allergens.

Objective In this study we wished to identify bell pepper allergens involved in the latex-fruit syndrome.

Methods Sera of four patients who displayed clinical symptoms to latex and bell pepper were used in immunoblot studies on protein extracts of three different cultivars of fresh bell pepper and fresh *Hevea* latex. Cross-reactive allergens were identified by inhibition experiments using recombinant Hev b 8 (latex profilin), and natural Hev b 2 (latex β -1,3-glucanase) in addition to the protein extracts. A novel cross-reactive IgE-reactive 30 kDa protein was subjected to sequence analysis.

Results Three patients displayed IgE to profilins from bell pepper fruits and latex. Two patients possessed IgE to Hev b 2, a latex β -1,3-glucanase, and a homologous protein in bell pepper. One patient possessed IgE reactive with a protein of 30 kDa identified by N-terminal sequencing as an L-ascorbate peroxidase and another patient to a protein of 38 kDa. Additionally, IgE binding proteins in two higher molecular weight ranges showed cross-reactive capacities.

Conclusion Our findings show on the molecular level that bell pepper is part of the latex-fruit syndrome. For the first time we have identified the major latex allergen Hev b 2, a β -1,3-glucanase, and the bell pepper L-ascorbate peroxidase as cross-reactive allergens. We were also able to show that profilins are responsible for some of the IgE cross-reactivity.

Keywords bell pepper, food allergy, *Hevea brasiliensis*, L-ascorbate peroxidase, latex-fruit syndrome, profilin, β -1,3-glucanase

Submitted 16 January 2004; revised 22 June 2004; accepted 22 July 2004

Introduction

Bell peppers (*Capsicum annuum*) are consumed throughout the world in most countries. Fresh bell pepper and dried bell pepper fruits cause adverse allergic reactions. Especially, dried and powdered bell pepper was described as one of the spices eliciting the highest number of reactions in patients allergic to spices [1, 2]. The role of bell pepper fruit in food allergy was investigated with protein extracts of eight different cultivars [3]. IgE reactivity of patients' sera was shown to profilin in all eight cultivars, to a homologue of Bet v 1, the major birch pollen allergen, in four out of eight cultivars, and also to a thaumatin-like protein homologue in four out of eight cultivars [3]. Recently, a cDNA sequence from a profilin of bell pepper was cloned and produced as a recombinant protein, and named Cap a 2 [4]. Furthermore, it

was described that pollen of bell pepper plants is also a source of sensitization and that two-thirds of employees working in bell pepper horticulture suffer from work-related respiratory symptoms associated with an IgE-mediated allergy as a result of the high exposure to bell pepper pollen [5, 6].

The first evidence of simultaneous sensitization to latex and bell pepper was observed in a patient who first became allergic to latex and then developed an oral allergy syndrome to sweet pepper [7]. A protein of 20 kDa, possibly prohevein, was then characterized as the responsible allergen for the cross-reactivity between latex and bell pepper in this patient [8]. Tücke et al. [9] investigated co-sensitization to latex and foods in a study with children with atopic dermatitis. They observed that 18% of the investigated children displayed IgE to proteins of sweet pepper and more than 90% of these children were also sensitized to latex. The association of allergy to a plant food and latex allergy is well documented as the latex-fruit syndrome [10, 11]. Nowadays, about 30–50% of patients who are allergic to latex are also allergic to certain plant foods and several allergens are described as the

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responsible cross-reactive proteins (reviewed in [12]). Class I chitinases, containing a small N-terminal hevein domain are described as the most important pan-allergens associated with the latex-fruit syndrome [13–17]. Hev b 2, a β -1,3-glucanase, was associated with the latex-fruit syndrome based on the recognition of the protein by IgE of atopic patients [18]. Hev b 7, a patatin-like protein, was recently identified as an important cross-reactive allergen in latex-associated potato allergy [19], and the latex profilin, Hev b 8, was associated with cross-reactivities to pollen and fruit [20, 21].

In this study, we aimed to elucidate the role of bell pepper in the latex-fruit syndrome. We provide insights into the cross-reactive allergens by characterizing and defining the responsible proteins in bell pepper and latex extracts using sera of patients with clinical symptoms to bell pepper fruits as well as latex products.

Methods

Patients

Four patients were selected based on the presence of allergy to *Hevea brasiliensis* latex as well as to bell pepper. All patients displayed clinical symptoms to latex and to raw bell pepper fruit ingestion. Latex allergy was assessed by open latex glove provocation test. In addition, sensitization was confirmed by positive skin prick test (SPT) to latex extract (ALK, Horsholm, Denmark) and positive prick-to-prick test to bell pepper, and by positive CAP to latex and bell pepper (UniCAP/Pharmacia CAP System, Pharmacia Diagnostics, Uppsala, Sweden). Positive results for SPT and prick-to-prick tests were scored in three classes: (+) when the weal diameter was from equal to one and a half the histamine weal, (++) when it ranged from one and a half to twice the histamine weal, and (+++) greater than twice the positive control.

Latex protein extracts and latex allergens

Fresh *Hevea* latex was collected in chilled containers from rubber trees (*H. brasiliensis*, clone RRIM 600). The latex was centrifuged at 44 000 g at 4 °C for 1 h to separate it into three main fractions: a top fraction containing the rubber particles, an aqueous phase, the C-serum, and a bottom fraction containing specialized organelles, called lutoids. The aqueous phase was collected and centrifugation repeated. The clear aqueous C-serum was freeze dried and stored at –20 °C. The bottom fraction containing the lutoids was resuspended in 0.4 mol/L mannitol, resedimented and subjected to repeated alternate freezing and thawing to rupture the lutoids. The fluid content of the lutoids, the B-serum, was then recovered by centrifugation [22]. Recombinant (r) Hev b 8 was obtained from Biomay (Vienna, Austria), and natural (n) Hev b 2 was a kind gift from H. Y. Yeang from the Rubber Research Institute of Malaysia (Kuala Lumpur, Malaysia).

Protein extracts of different bell pepper cultivars

Three different cultivars of fresh bell pepper (bell pepper extract 1 (BP1) = Luteus F1, high yellow; bell pepper extract 2 (BP2) = We 880 Block OHZ, big green; and bell pepper extract 3 (BP3) = Marras, big red) were purchased from a

local retail shop. Raw fruits were homogenized and extracted by overnight shaking at 4 °C in 10 mM potassium phosphate buffer (pH 7.0), 2% (w/v) suspended solid polyvinylpyrrolidone, 2 mM EDTA, 10 mM sodiumdiethyldithiocarbamate, and 3 mM sodium azide. After centrifugation at 40 000 g for 60 min at 4 °C, supernatants were extensively dialysed against 10 mM potassium phosphate buffer (pH 7.0), 3 mM sodium azide. The dialysates were subsequently freeze dried and stored at –20 °C.

Immunoglobulin E immunoblot studies

Fifty micrograms latex-B or latex-C extracts and 20 µg BP1, BP2 or BP3 per lane were separated on 12% SDS/PAGE gels to ensure good separation and visibility of protein bands and blotted to nitrocellulose membranes (Pall Corporation, Pensacola, FL, USA). Membranes were cut into strips 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) for 30 min. Strips were then incubated with patients' sera diluted 1 : 5 in blocking buffer overnight at 4 °C. After three washing steps blots were incubated with ¹²⁵I-labelled anti-(human IgE) IgG (IBL, Hamburg, Germany) overnight. Patients' IgE bound to the proteins was visualized on BiomaxTM MS film (Kodak, New York, NY, USA). A serum pool from seven atopic individuals with high IgE levels for house dust mite, but negative SPT, and negative CAP results to bell pepper and latex was used as negative control.

Immunoglobulin E inhibition studies with protein extracts and Hevea allergens

For inhibition of IgE binding to latex allergens, patients' sera diluted 1 : 5 in blocking buffer were incubated with 50 µg BP1, BP2 or BP3 overnight at 4 °C. For inhibition of IgE binding to bell pepper allergens, sera were incubated with 100 µg latex C- or latex B-serum proteins overnight at 4 °C. For the inhibition studies with specific allergens, sera were incubated with 50 µg rHev b 8 or nHev b 2 overnight at 4 °C. These sera were then allowed to react with nitrocellulose membrane strips containing separated latex or bell pepper proteins. Binding of IgE was detected as described above.

N-terminal amino acid sequencing

N-terminal sequencing of purified proteins was performed with an Applied Biosystems Procise 491 sequencer (Applied Biosystems, Foster City, CA, USA). Twenty micrograms of BP3 were separated on a 12% SDS/PAGE gel and proteins transferred to a PVDF (polyvinylidene difluoride) membrane (Schleicher & Schuell, Dassel, Germany). One portion of the membrane was used for IgE binding with serum 1 as described above. After localization of the IgE reactive 30 kDa band, the corresponding protein band on the remaining portion of the membrane was excised and subjected to sequence analysis using the pulsed liquid cleavage programme with the manufacturer's chemistry version 1.1.1. Sequence data were compared with the databases (Swissprot and translated EMBL) using the basic local alignment search tool (BLAST) programme.

Results

Patients

All four subjects included in the study had clinical histories consistent with type I allergy to latex products and bell pepper fruits. Urticaria was the most common reported symptom for latex allergy. One patient showed additional contact urticaria to latex gloves and another reported asthmatic symptoms when exposed to latex products. All patients suffered repeatedly from oral allergy syndrome immediately after eating raw bell pepper fruits. Three patients also had allergic symptoms to unrelated pollen sources. Sensitizations were confirmed by specific IgE detection for both sources, latex extract SPT, and prick-to-prick test with fresh bell pepper fruits. Case histories and diagnostic data are summarized in Table 1.

Reactivity to profilin

IgE reactivity of patients 1, 2, and 4 to latex and bell pepper profilins is illustrated in Fig. 1. Arrows indicate protein bands representing profilins. IgE of patient 1 bound to a protein present in latex C-serum of approximately 12 kDa (patient 1, lane 1). This reaction was completely inhibited by rHev b 8 (patient 1, lane b8) confirming that this band represented the latex profilin. IgE binding to the same band was greatly reduced by pre-incubating the sera of patients 1, 2, and 4 with BP1, BP2, and BP3 (C-serum, lanes 2–4, respectively).

IgE-binding to profilins present in the extracts of the three bell pepper cultivars (blots BP1, BP2, BP3, lanes 5) was strongly inhibited by rHev b 8 (blots BP1, BP2, and BP3, lane 6) and diminished or completely inhibited by pre-incubating the patients' sera with latex C-serum proteins (blot BP1, BP2,

and BP3, lane 8). Interestingly, patient 1 showed IgE reactivity to two isoforms of profilin in BP2 (blot BP2). For patient 2, inhibition of IgE-binding to profilin of BP1 and for patient 4 to profilin of BP1, BP2, and BP3 was also achieved by pre-incubating with latex B-serum (patient 2: blot BP1, lane 9; patient 4: blots BP1, BP2, and BP3, lane 9). Latex B-serum proteins were not able to diminish IgE binding of patient 1 to profilins of all three bell pepper extracts and of patient 2 to profilins of BP2 and BP3 (blot BP1, BP2, and BP3, lanes 7 and 9).

Reactivity to β -1,3-glucanase

Figure 2 shows the cross-reactivity of β -1,3-glucanases of latex and bell pepper indicated by arrows. A double band in latex B-serum was identified as Hev b 2, the *Hevea* latex β -1,3-glucanase, by inhibition of this protein band with nHev b 2 (patient 1, lane b2). IgE-binding to the Hev b 2 double band (blots B, lane 1) was detected for patients 1 and 2 and could be completely inhibited by pre-incubating the sera with BP1, BP2, and BP3 (blots B, lane 2, 3, and 4, respectively). IgE binding to a 35 kDa protein band in BP1 was only detectable for patient 1 and could be greatly reduced by pre-incubating the serum with nHev b 2 or with latex B-extract (blot BP1, lanes 7 and 9).

Reactivity to additional allergens of latex and bell pepper

As indicated by brackets in Fig. 1, IgE binding of patient 2 to proteins in the range of 30–70 kDa of BP2 was weakly diminished and to those of BP3 was almost completely inhibited by latex B-serum (Fig. 1, patient 2, blots BP2 and BP3, lane 5 and 9).

As indicated by brackets in Fig. 2, IgE reactivity of patients 1 and 2 to proteins in the range of 40–50 kDa of latex B-serum could be completely inhibited by all three bell pepper extracts (Fig. 2, patients 1 and 2, blot B). For patient 1, IgE binding to proteins in BP1 in the same molecular weight range could be reduced by nHev b 2 and latex B-serum (Fig. 2, patient 1, blot BP1, lanes 7 and 9). No IgE reactivity to 40–50 kDa allergens could be detected for patient 1 for BP2 and BP3 and for patient 2 for all three bell pepper extracts.

In Fig. 3, IgE binding of patient 1 to a protein band of approximately 30 kDa in latex C-serum and BP2 and BP3 is shown (Fig. 3, patient 1, blots C, BP2, BP3, lanes 1 and 5). In BP2 the protein appears as two bands. IgE binding to this protein in latex C-serum could be completely inhibited by BP1, BP2, and BP3 (Fig. 3, patient 1, blot C, lanes 2–4). Pre-incubation with latex C-serum inhibited IgE binding to the protein in BP2 and BP3 (Fig. 3, patient 1, blots BP2 and BP3, lane 8). In addition, in Fig. 3, a weak IgE binding of patient 3 to a protein of approximately 38 kDa in latex C-serum and BP2 is shown (Fig. 3, patient 3, blots C and BP2, lanes 1 and 5). Patient 3 did not display IgE recognizing any other allergens. Pre-incubation with all three bell pepper extracts inhibited the IgE binding to latex C-serum (Fig. 3, patient 3, blot C, lanes 2–4), and latex C-serum inhibited binding to the corresponding protein band in BP2 (Fig. 3, patient 3, blot BP2, band 8).

Table 1. Characteristics of patients

Patient	1	2	3	4
Sex	M	F	F	F
Age	21	24	27	51
Profession	Student	HCW	HCW	HCW
Symptoms				
Latex allergy	U	U	A, U	U, CU
Bell pepper allergy	OAS	OAS	OAS	OAS
Additional	R, SR	SR		SR
SPT to other foods	A, C, K, P, N	Z	A, Av, L, S	
Total IgE (kU/L)	592	220	413	1869
CAP-RAST (kU/L)				
Latex	10.6	13.7	22.9	8.3
Bell pepper	21.1	14.9	21.4	4.2
SPT latex	+	+	+	+
Prick-to-prick test				
Yellow bell pepper	+	+	+	+
Green bell pepper	+	+	+	+
Red bell pepper	++	++	+	+

M, male; F, female; HCW, health care worker; U, urticaria; A, asthma; CU, contact urticaria; OAS, oral allergy syndrome; R, rhinoconjunctivitis; SR, seasonal rhinitis; SPT, skin prick test; A, Apple; Av, Avocado; C, celery; K, Kiwi; L, lychee; N, nuts; P, potato; S, spinach; Z, zucchini; +, the weal diameter is equal to one and a half the histamine weal; ++, the weal diameter is equal to one and a half to twice the histamine weal.

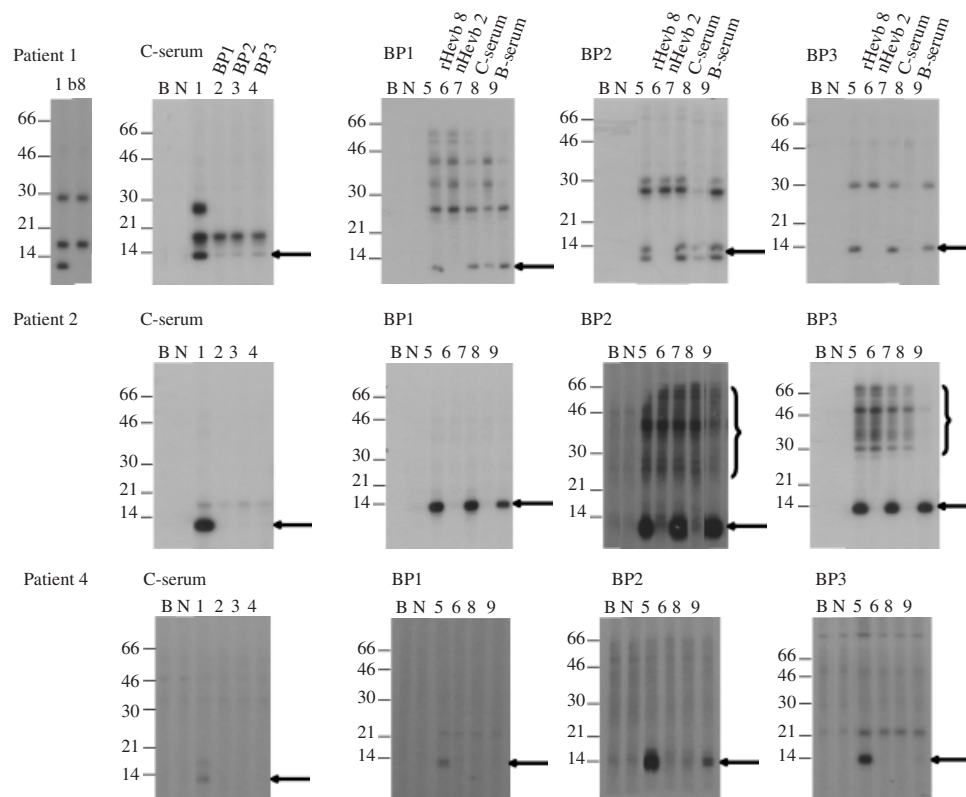


Fig. 1. Immunoblot determination of the cross-reactivity of profilins and allergens of higher molecular weights. Proteins of latex C-serum, and of bell pepper extracts (BP1, BP2, and BP3) were separated on 12% SDS-PAGE and transferred to nitrocellulose membranes. Blot strips were incubated with buffer (lane B), a serum pool of atopic control patients (lane N), untreated sera of patients 1, 2, and 4 (lanes 1 and 5), or pre-incubated with recombinant Hev b 8 (rHev b 8) (lanes b8 and 6), with BP1 (lane 2), BP2 (lane 3), BP3 (lane 4), natural Hev b 2 (nHev b 2) (lane 7), latex C-serum (lane 8), or latex B-serum (lane 9).

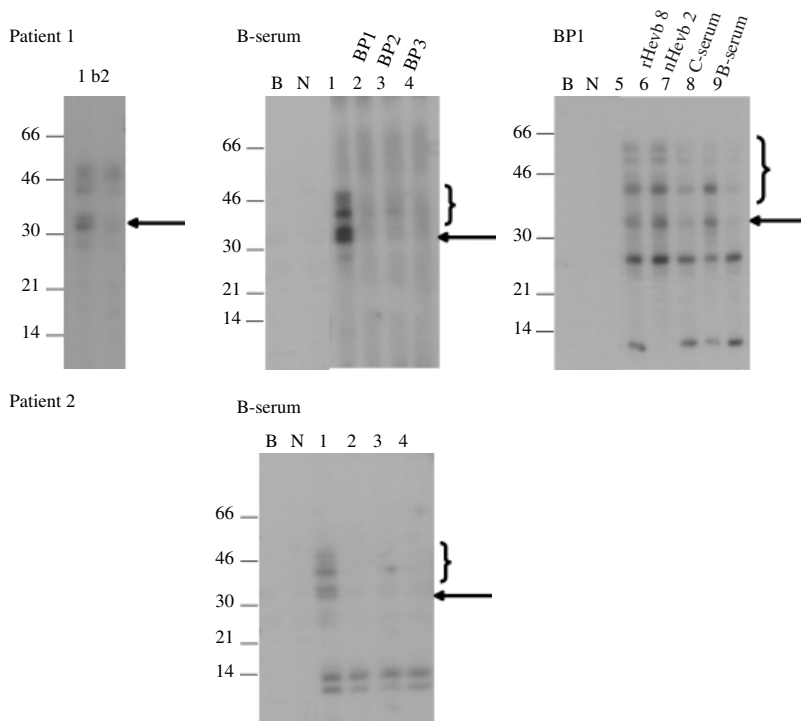


Fig. 2. Immunoblot determination of the cross-reactivity of a β -1,3-glucanase and allergens of higher molecular weights. Proteins of latex B-serum, and of bell pepper extract 1 (BP1) were separated on 12% SDS-PAGE and transferred to nitrocellulose membranes. Blot strips were incubated with buffer (lane B), a serum pool of atopic control patients (lane N), untreated sera of patients 1 and 2 (lanes 1 and 5), or pre-incubated with natural Hev b 2 (nHev b 2) (lanes b2 and 7), with BP1 (lane 2), BP2 (lane 3), BP3 (lane 4), recombinant Hev b 8 (rHev b 8) (lane 6), latex C-serum (lane 8), or latex B-serum (lane 9).

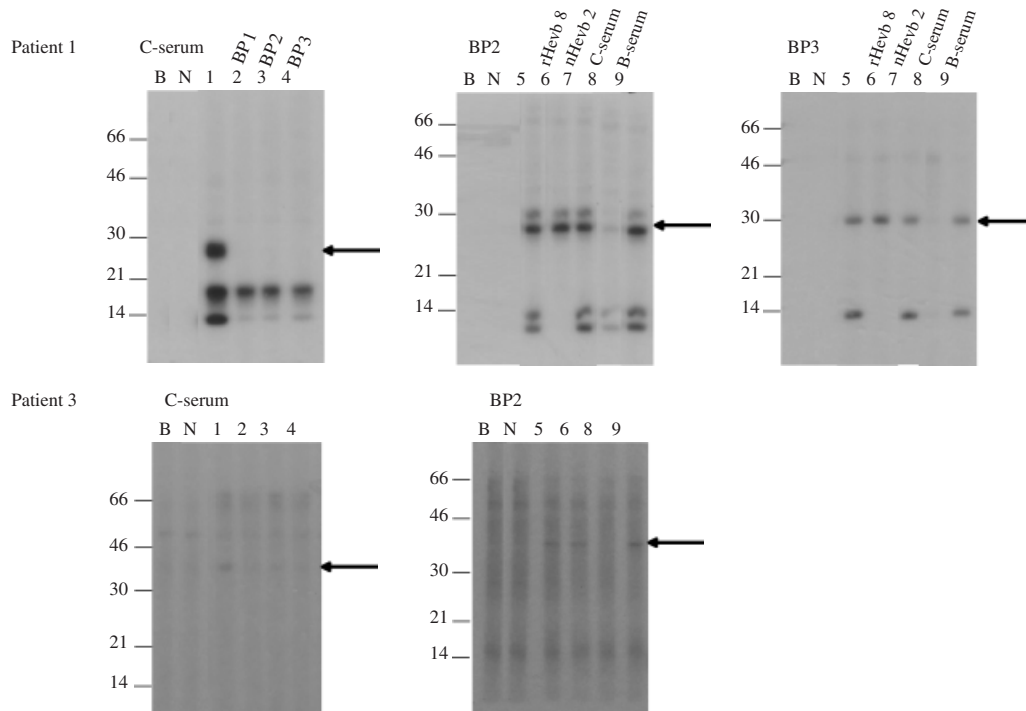


Fig. 3. Immunoblot determination of cross-reactivities of a 30 and a 38 kDa allergen. Proteins of latex C-serum (C), and of bell pepper extracts (BP2 and BP3) were separated on 12% SDS-PAGE and transferred to nitrocellulose membranes. Blot strips were incubated with buffer (lane B), a serum pool of atopic control patients (lane N), untreated sera of patients 1 and 3 (lanes 1 and 5), or pre-incubated with BP1 (lane 2), BP2 (lane 3), BP3 (lane 4), recombinant Hev b 8 (rHev b 8) (lane 6), natural Hev b 2 (nHev b 2) (lane 7), latex C-serum (lane 8), or latex B-serum (lane 9).

Table 2. IgE reactivity to latex and bell pepper allergens

Patient	Profilin, latex C, BP1–3	β -1,3-glucanase		30 kDa protein, latex C, BP2, BP3	38 kDa protein, latex C, BP2	40–50 kDa		30–70 kDa, BP2, BP3
		Latex B	BP1			Latex B	BP1	
1	+	+	+	+	–	+	+	–
2	+	+	–	–	–	+	–	+
3	–	–	–	–	+	–	–	–
4	+	–	–	–	–	–	–	–

BP, bell pepper. +, IgE reactivity; –, no IgE reactivity.

Cross-reactive latex and bell pepper allergens

Table 2 summarizes the IgE reactivity of the sera of the four patients directed against cross-reactive allergens in latex B- and C-serum and in bell pepper extracts. Profilins were recognized by patients 1, 2, and 4 in latex C-serum and all three bell pepper extracts. Patients 1 and 2 displayed IgE to β -1,3-glucanase in latex B-serum and patient 1 to a homologous protein in BP1. Specific IgE to an approximately 30 kDa protein was detectable for patient 1 in latex C-serum and BP2 and BP3. An approximately 38 kDa protein was recognized by patient 3 in latex C-serum and BP2. Patient 1 displayed IgE to proteins in a molecular weight range of 40–50 kDa in latex B-serum and BP1 and patient 2 to proteins in the range of 30–70 kDa in BP2 and BP3.

N-terminal amino acid sequencing

The IgE reactive 30 kDa protein band in BP3 was subjected to N-terminal amino acid sequence analysis. The resulting

sequence comprising 16 amino acid residues (KCYPTV-SEELYLKAVDK) showed 100% identity to the N-terminus of an L-ascorbate peroxidase (EC 1.11.1.11) from bell pepper (EMBL/GenBank/DBJ accession no. X81376), a protein of 250 amino acid residues with no N-glycosylation site.

Discussion

Since the first report of a systemic allergic reaction caused by banana in a latex-allergic patient [23], only few of the actual allergens responsible for the latex-fruit syndrome have been described (reviewed in [12]). In this study, we identified several additional cross-reactive allergens of bell pepper and latex (summarized in Table 2). In IgE immunoblot experiments performed with sera of four patients allergic to proteins from bell pepper fruits and latex products profilin was identified as a cross-reactive allergen in three patients. Two patients displayed IgE to Hev b 2, a β -1,3-glucanase, and one

of them to a homologous protein in bell pepper. A protein of 30 kDa and one of 38 kDa could be detected as cross-reactive allergens each in one patient. The 30 kDa bell pepper allergen was identified as an L-ascorbate peroxidase by N-terminal sequencing. Additionally, IgE-binding proteins in two ranges of higher molecular weight showed cross-reactive capacities.

Three of four patients displayed IgE antibodies against a protein of approximately 12 kDa in all three bell pepper extracts and in latex C-serum (Fig. 1). We demonstrated that this bell pepper protein represented profilin by inhibition of IgE binding with a recombinant latex profilin (rHev b 8). rHev b 8 has been previously described as an equivalent to the natural latex profilin in latex C-serum [21]. According to the observations from Jensen-Jarolim et al. [3], profilins were the predominant allergens in all bell pepper extracts investigated. In a recent study by Willeroider et al. [4] a recombinant bell pepper profilin was able to bind IgE of 11 out of 34 (32%) of bell pepper allergic patients. Interestingly, in our BP2 two isoforms of profilins were detected. The fact that profilins exist in several isoforms is well known [24] and was recently observed in profilins from several sources [25]. All three bell pepper extracts were able to inhibit IgE-binding to the latex profilin. Moreover, bell pepper profilin-specific IgE was inhibited by rHev b 8 and latex C-serum containing Hev b 8 (Fig. 1). For patients 2 and 4 inhibition of IgE binding to bell pepper profilins was also achieved by latex B-serum. This indicates that a certain amount of latex profilin was also present in latex B-serum. Inhibitions were almost complete for all three patients indicating that the IgE epitopes of bell pepper profilins and Hev b 8 are highly similar. These data, however, do not allow identifying the primary sensitizing allergen source. In addition, since all three patients suffer from pollen allergy, inhalation may also represent the route of primary sensitization. Nevertheless, clinical relevance of IgE binding to profilin has to be distinguished from cross-reactive IgE binding without evoking allergic symptoms. Profilin as a relevant allergen has been described in recent studies for patients sensitized to latex, banana, pineapple, melon, hazelnut, celery, carrot, and peach [21, 26–32], and bell pepper was reported to cause clinical food allergy supporting possible clinical relevance of anti-profilin IgE in selected cases [33].

Two of the four patients showed IgE reactivity to a double band at approximately 35 kDa in latex B-serum. This double band could be characterized as Hev b 2, the latex β -1,3-glucanase, by inhibition of IgE binding to the protein with nHev b 2 (Fig. 2). This IgE binding could also be reduced by all three bell pepper extracts. Additionally, IgE binding to a 35 kDa protein in the yellow bell pepper extract by patient 1 could be inhibited by nHev b 2 and latex B-serum containing Hev b 2. Inhibition experiments with nHev b 2 were only performed with sera of patients recognizing Hev b 2. Although all three bell pepper extracts were able to inhibit IgE binding to the protein in latex B-serum, the corresponding protein was detected only in BP1 by patient 1. This observation could be based on the phenomenon that bell peppers of different cultivars express allergens in different amounts [3]. Nevertheless, the fact that inhibition of IgE binding to Hev b 2 was achieved with high amounts of all three bell pepper extracts indicates that the corresponding allergen is present in all three extracts. Interestingly, IgE

binding of patient 1 to proteins in the range of 50–60 kDa could be inhibited by Hev b 2 (Fig. 2). This observation could be because of IgE of patient 1 directed against carbohydrate determinants of bell pepper proteins cross-reactive with the carbohydrate epitope described for Hev b 2 [34].

Up to now proteins with β -1,3-glucanase activity have been described as allergens in latex and in olive pollen [22, 35]. Yagami [18] has hypothesized that defence-related proteins including β -1,3-glucanases are a major cause of latex allergy and the latex-fruit syndrome. The same group described that carbohydrate epitopes of questionable clinical relevance in Hev b 2 homologues were responsible for IgE binding [34]. In a recent study Hev b 2 was identified as a relevant latex allergen producing skin reactions in more than 60% of health care workers with histories of latex allergy [36]. Here, we describe for the first time that the latex allergen Hev b 2 is capable of greatly reducing the IgE binding to Hev b 2 homologous proteins in a plant food extract, namely bell pepper.

In the publication of Jensen-Jarolim et al. [3] that described bell pepper allergens in different cultivars, Fig. 2 showed IgE immunoblots of 11 patients on a bell pepper extract. Seven of the patients displayed IgE against allergens in the range of 28–31 kDa. Our extract BP3 corresponds to the extract prepared by Jensen-Jarolim et al. [3]. Our patient 1 displayed IgE to a 30 kDa band in this extract BP3, in extract BP2, and also a possible cross-reactive 30 kDa protein in latex C-serum (Fig. 3). For characterization of this allergen we performed inhibition experiments with a recombinant latex class I chitinase, Hev b 11 [13], and western blot experiments with a chicken anti-natural thaumatin IgY (kindly provided by K. Hoffmann-Sommergruber) with no results identifying the protein as the respective allergen (data not shown). N-terminal amino acid sequencing of the protein from BP3 identified the protein as an L-ascorbate peroxidase.

Ascorbate peroxidase has not been described as an allergen but it is a well-documented fact that housekeeping enzymes like Hev b 9 and Hev b 10 from latex can act as allergens with cross-reactive capacities [37, 38].

Patient 3 showed weak IgE reactivity to only one protein in latex C-serum at 38 kDa cross-reactive with the possibly corresponding protein in BP2 (Fig. 3). Interestingly, the patient shows clinical symptoms to latex and bell pepper fruits indicating either a monosensitization to a protein of 38 kDa of clinical relevance or that other relevant allergens were below the detection level under the conditions employed.

The binding of cross-reactive IgE of patients 1 and 2 to proteins in two ranges of high-molecular-weight proteins in latex B-serum and the bell pepper extracts is possibly because of cross-reactive carbohydrate determinants. These are present on glycosylated proteins from organelles including the lutoids found in latex B-serum. Carbohydrate determinants can bind human IgE and may play a significant role in cross-reactivity between allergens from unrelated sources as it has been shown for the alliin lyase from garlic [39]. The importance of carbohydrate determinants as IgE epitopes and their biological activity is the cause of an ongoing discussion. Van der Veen et al. [40] observed poor biological activity of carbohydrate determinants in their study with grass pollen-allergic patients with significant levels of anti-peanut IgE but no symptoms of peanut allergy. On the other hand,

biologically relevant carbohydrate-specific IgE antibodies were observed for the tomato allergen Lyc e 2 or the celery allergen Api g 5 [41, 42].

From our data it seems that bell pepper is involved in the latex-fruit syndrome. Our work showed that the cross-reactivity between bell pepper and latex experienced by the patients studied was based on four proteins including profilin, β -1,3-glucanase, and L-ascorbate peroxidase. With regard to the fact that Hev b 2, the latex β -1,3-glucanase, may be a major latex allergen, patients sensitized to this allergen are at risk to experience allergic symptoms when they ingest β -1,3-glucanases from plants like bell pepper.

Acknowledgement

This work was supported by the Austria Science Fund grant SFB F01802.

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