

IgE epitope analysis of the hevein preprotein; a major latex allergen

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SUMMARY

We have previously identified the hevein preprotein as a common allergen for latex allergic healthcare workers. The B cell epitopes in the hevein protein that are recognized by IgE of latex-allergic individuals have not been identified. In this study, we examined the hevein preprotein using epitope mapping. Overlapping synthetic peptides of 10 amino acids (two aa overlap) were synthesized on a derivatized cellulose membrane using Fmoc chemistry. The peptide spots were probed with pooled sera from 10 latex-allergic patients, and the IgE-reactive peptides identified with anti-IgE MoAbs. We identified six B cell epitopes within the full length hevein preprotein which bound IgE from latex-allergic patients. Two were located in the N-terminal 5-kD hevein domain and four were observed in the 14-kD C-domain. A broad epitope was located between the N-terminal amino acids 13–24. This epitope had nearly complete homology to wheat germ agglutinin (WGA). Immunological cross-reactivity to WGA was confirmed by Western blot analysis with purified WGA, and this reactivity could be inhibited by latex proteins or WGA. Of the five remaining epitopes, four had homologies to other proteins in the pathogenesis-related family of plant proteins (PR-4). The data demonstrate that hevein has multiple IgE epitopes. The significant homology of these epitopes to a broad family of plant defence proteins further explains the increased prevalence of food allergies in latex-allergic individuals.

Keywords allergy antigens IgE epitopes latex hevein

INTRODUCTION

Immediate hypersensitivity to natural rubber latex (NRL) presents as a clinical spectrum of manifestations from local contact dermatitis, allergic rhinoconjunctivitis, asthma, to life-threatening anaphylaxis [1]. Prolonged contact or repeated exposure to NRL gloves and other medical products is thought to result in latex sensitization. Healthcare workers (5–15%) and spina bifida children (40–68%) have the highest reported prevalence of latex allergy [2–5]. Type I allergic reactions to latex occur in individuals who develop specific IgE antibodies to proteins originating from the *Hevea brasiliensis* tree. Proteolytic breakdown products of latex protein(s) remain as residuals on most NRL products [6].

For a better understanding of latex allergy it is necessary to characterize the important allergens. NRL, being a cytosolic exudate, contains many different proteins [7]. A number of latex proteins have been isolated and identified by amino acid (aa) sequence analysis or by recombinant DNA technology, including prenyltransferase, rubber elongation factor, hevein, hevamine, and β -glucanase [8–12]. Other proteins including chitinases, profilin, papain, and other enzymes have also been identified in latex [13–15]. Because of the large number of proteins in latex, identification

of the major allergens has been difficult. This is further complicated because field latex is treated with ammonium hydroxide to prevent coagulation. This results in hydrolysis of the proteins, making it difficult to identify the source of the allergenic peptides in end-use consumer products. Using non-ammoniated latex (NAL), a number of IgE binding proteins have been identified as allergens, but there is little agreement as to which are the major allergens [16–19].

Previously, we observed that 50% of a latex-allergic population had IgE antibodies to protein bands identified as the hevein preprotein by aa sequence analysis [17]. Hevein is a 5-kD chitin-binding protein that has structural homology to allergens from ragweed and to wheat germ agglutinin (WGA) [20]. Hevein is synthesized as a 20-kD preprotein that is post-translationally processed into the 5-kD amino-terminal hevein (aa 1–43) and a 14-kD carboxyl-terminal domain [10]. The 5-kD mature hevein appears to have an important role in latex coagulation [21], while the function of the C-domain is unknown. In this study, we used overlapping synthetic peptides to identify the IgE-reactive epitopes in both the N-terminal hevein and the C-domain.

MATERIALS AND METHODS

Patient sera

Sera were pooled from 10 latex-allergic healthcare workers who

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Table 1. Characteristics of latex-allergic population used for epitope analysis

Patient	Latex symptoms	Western blot and SPT results
1. Atopic dental asst	CD, CU, ILA, OLS	Positive; 46, 29, 18, 14, 5 kD SPT ⁺ at 1:1 000 000
2. Atopic or HCW	CD, CU, OLS, ILA	Weak positive, 14 kD SPT ⁺ at 1:1 000 000
3. Atopic lab. worker	CD, CU, ILA, OLS, LIAS	Positive; 46, 29, 14 kD SPT ⁺ at 1:100 000
4. Atopic lab. worker	CD, CU, ILA, OLS, LIAS, LIAN	Positive, nearly all proteins SPT ⁺ at 1:100 000
5. Atopic HCW	CD, CU, OLS, ILA	Positive, 29, 14 kD SPT ⁺ at full strength
6. Atopic dental asst	CD, CU, ILA, OLS, LIAS	Weak positive, 18 kD SPT ⁺ at 1:1000
7. Atopic dental asst	CD, CU, ILA, OLS, LIAS, LIAN	Positive, nearly all proteins SPT ⁺ at 1:100 000
8. Atopic RN	CD, CU, ILA, OLS, LIAS	Positive, 29, 18, 14, 5 kD SPT ⁺ at 1:1000
9. Atopic RN	CD, CU, ILA, OLS, LIAS	Positive, 110, 68, 46, 18, 14 kD SPT ⁺ at 1:10 000
10. Atopic dental asst	CD, CU, ILA, OLS, LIAS, LIAN	Positive, 68, 35, 14 kD SPT ⁺ at 1:1000

CU, Contact urticaria; CD, contact dermatitis; ILA, inhalant latex allergy; sneezing rhinorrhoea, nasal pruritus; OLA, ocular latex symptoms, itchy watery eyes; LIAS, latex-induced asthma; LIAN, latex-induced anaphylaxis.

Anaphylaxis was defined as patients presenting with multi-system involvement including at least two of the following skin (itching, urticaria, angioedema, respiratory (shortness of breath and wheezing)) or cardiovascular (lightheadedness and loss of consciousness).

Western blots were performed as previously described [17].

Skin prick testing (SPT) was performed with a low ammonia commercially available latex skin test reagent (Bencard, Mississauga, Ontario, Canada).

were chosen based on their immunoblot reactivity to 14- and/or 18-kD hevein polypeptides [17]. Characteristics of this latex-allergic population are given in Table 1, and represent a range of symptoms and levels of skin prick test reactivity. None of the latex-allergic patients was known to have cross-reacting allergies to wheat. Sera from three atopic non-latex-allergic patients with high IgE levels were pooled for control sera.

Reagents

Purified lectins from *Lycopersicon esculentum* (tomato), *Persea americana* (avocado), *Arachis hypogaea* (peanut), *Solanum tuberosum* (potato) and *Triticum vulgaris* (wheat germ) were purchased from Sigma Chemical Co. (St Louis, MO). The NAL protein preparation, ammoniated latex (AL) preparation, and rabbit anti-latex antisera were isolated as previously described [17,22].

Synthetic peptides

Peptides spanning the entire hevein preprotein were synthesized as a series of 10 aa peptides which overlapped by two aa. The peptides were synthesized on a derivatized cellulose membrane (SPOTs; Genosys Biotechnologies Inc., The Woodlands, TX) according to the manufacturer's instructions. The N-terminal hevein domain (aa 1–42) was synthesized in 17 overlapping peptides, while the C-domain (aa 44–187) required 68 separate peptides (Table 2).

Synthesis was performed using Fmoc chemistry on an 8 × 12 matrix of coloured spots in which coupling reactions are followed by a colour change as an indicator that the reaction has occurred. Briefly, 0.9 µl of the appropriate aa was spotted to the membrane (twice) and allowed to react for 15 min. The membrane was washed three times in 20 ml dimethyl formamide (DMF) for 2 min, with 800 µl acetic anhydride being added to the last wash. The membrane was again washed three times with 20 ml DMF for 2 min, and then placed in 20 ml of 20% piperidine in DMF for 5 min. The membrane was washed five times in 20 ml DMF for 2 min. Next, 200 µl of 1% bromophenol blue were added to DMF and the membrane was incubated until the spots turned blue. These steps were repeated until the final aa in the sequence was added. The membrane was washed and then placed in 20 ml DMF with 400 µl acetic anhydride to acetylate the peptide. The membrane was washed three times with 20 ml DMF, three times with 20 ml methanol and air-dried. Finally, the membrane was incubated for 1 h in a solution of 5 ml dichloromethane, 5 ml trifluoroacetic acid and 250 µl triisobutylsilane, washed four times with 20 ml dichloromethane, three times with 20 ml DMF and four times with 20 ml methanol. The membrane was air-dried before use in immunoblotting.

Epitope immunoblotting

The membrane was blocked overnight with 20 ml 5% non-fat dry milk protein in PBS. The blocking buffer was removed and the membrane washed once in 0.1% Tween 20 in Tris buffer saline (T-TBS). It was then incubated for 4 h with pooled human sera (1:10) obtained from either high IgE control or latex-sensitive individuals. The membrane was then washed three times with 20 ml T-TBS and reacted with mouse monoclonal anti-human IgE for 4 h, washed three times in 20 ml T-TBS and placed in B-galactosidase-conjugated polyclonal sheep anti-mouse antibody for 4 h. After washing, the membrane was placed in development solution (5-bromo-4-chloro-3-indoyl-B-D galactopyranoside) and colour allowed to develop for 40 min. A blue reaction product was indicative of a positive reaction, pink staining indicated a non-specific interaction (manufacturer's instructions). Regions were considered epitopes if three or more consecutive blue spots had a signal intensity greater than any spot on the control membrane. The membrane was washed in PBS and photographed after each probing.

To regenerate the membrane for additional probing, it was rinsed in 20 ml of MilliQ H₂O, followed by DMF and then a second MilliQ H₂O. The membrane was then washed three times in regeneration buffer A (8 M urea, 1% SDS, 0.1% β-mercaptoethanol (β-ME)), and three times in regeneration buffer B (50% EtOH, 10% acetic acid in MilliQ H₂O). After two final washes with 20 ml EtOH the membrane was air-dried and ready to be re-probed. The membrane was probed with pooled sera from latex-allergic patients, high IgE control sera, a single latex-allergic patient, and a pooled polyclonal rabbit anti-latex serum. The membrane was treated with development solution after stripping to ensure complete removal of the antibodies.

Immuno cross-reactivity

Western blot using a triple antibody sandwich technique was used to assess immunological cross-reactivity between hevein and WGA. Non-ammoniated latex proteins (100 µg/lane) or purified lectins (5 µg/lane) were separated by SDS-PAGE on 15% gels. The proteins were transferred to nitrocellulose (0.1 µm; Schleicher and Schuell Keene, NH) and the membrane was blocked for 1 h

A. Amino acid Sequence of Prohevein

1 E Q C G R Q A G G K L C P N N L C C S Q W G W C G S T D E Y C S P D H N C Q S N C K D S G E G V G
10 20 30 40

50 G G S A S N V L A T Y H L Y N S Q D H G W D L N A A S A Y C S T W D A N K P Y S W R S K Y G W T A F
60 70 80 90

100 C G P V G A H G Q S S C G K C L S V T N T G T G A K T T V R I V D Q C S N G G L D L D V N V F R Q L
110 120 130 140

150 D T D G K G Y E R G H I T V N Y Q F V D C G D S F N P L F S V M K S S V I N
160 170 180 187

Start of Mature C-Domain

End of mature hevein

End of mature C-domain

Note: Arrows indicate sites of post-translational processing as determined in reference 25.

B. Overlapping Synthetic Peptides of Hevein

[illegible]

Note: The C-Domain was synthesized in a similar manner starting with AA 45 and continuing through 187, this includes the N-terminal 44-49, and the C-terminal 174-187 which are cleaved during processing of the prohevein.

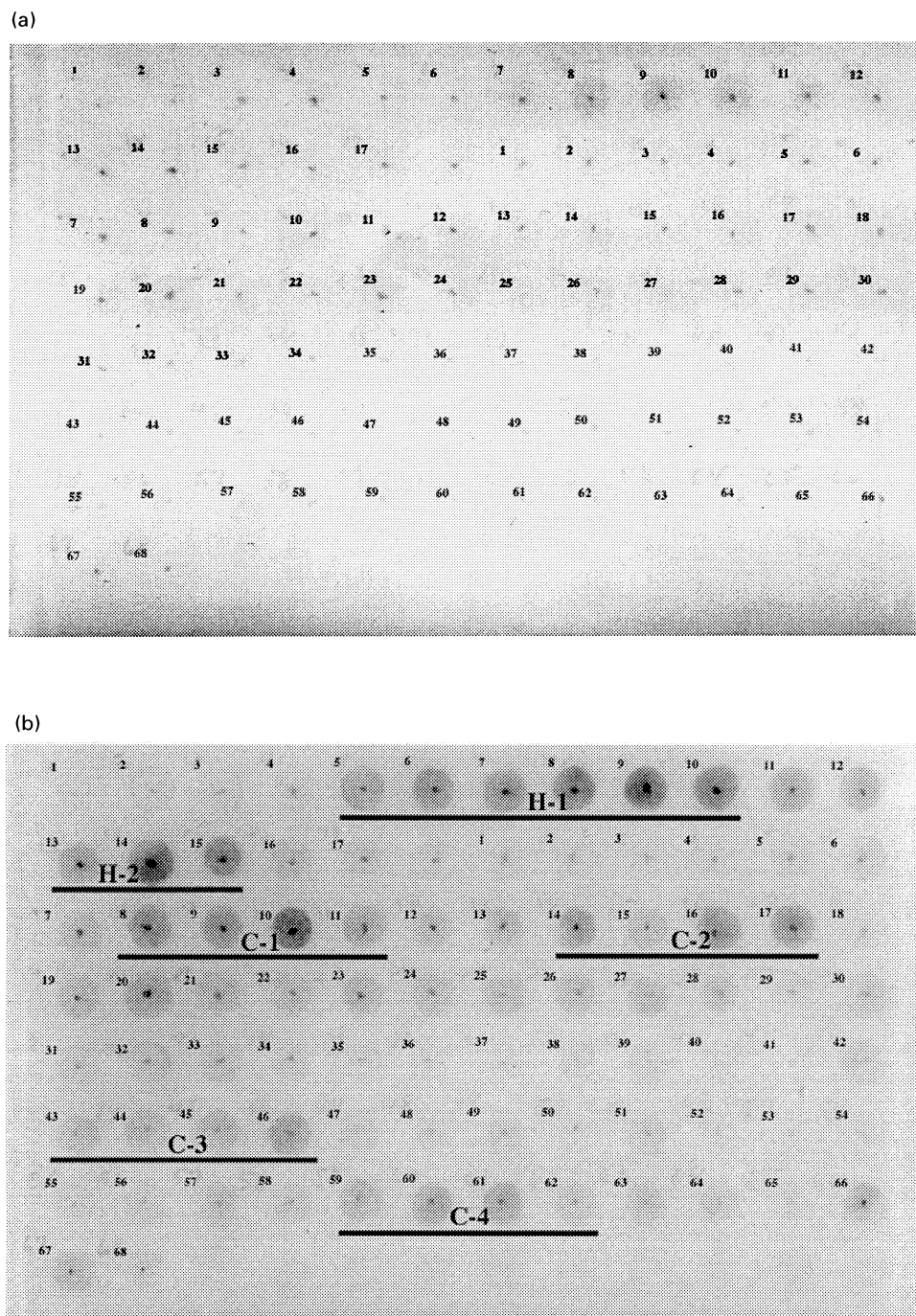


Fig. 1. Reactivity of latex-specific IgE with synthetic peptides of hevein. Each spot represents a separate 10 amino acid (aa) synthetic peptide. Sequential peptides were synthesized on a derivatized cellulose membrane and the sequences were overlapping by two aa. The N-terminal hevein was synthesized in the first 17 spots while the C-domain was represented in 68 spots. The membranes were reacted with high IgE control sera (a) or with sera pooled from 10 latex-allergic patients (b). Regions of IgE binding (underlined) were identified by reaction with galactopyranoside to produce a blue reaction product. Slight staining of spots 8–10 (a, control) and spots 19–28 (b, patients) was pink rather than blue, indicating a non-specific interaction.

with 2% dry milk. The membrane was reacted overnight with a 1:10 dilution of patient sera with or without the addition of proteins being tested for inhibition. Following washing with T-TBS, the blot was reacted for 1 h with a 1:2000 dilution of monoclonal anti-human IgE (clone GE-1; Sigma), washed and then reacted with a 1:5000 dilution of alkaline phosphatase-labelled goat anti-mouse

IgG (Promega, Madison, WI). IgE reactivity was visualized by developing the membrane in the alkaline phosphatase substrate NBT/BCIP (Promega). Immunoblot inhibition studies were performed as previously described [23]. Antisera were incubated with each inhibitor for 1 h at room temperature before exposure to the membranes.

Table 3. Epitope mapping: reactivity with synthetic latex peptides

Anti-sera	Hevein		C-domain			
	H1	H2	C1	C2	C3	C4
Control sera	—	—	—	—	—	—
Pooled human anti-latex IgE	5–10	13–16	8–12	14–17	42–46	59–62
Single latex-allergic patient IgE	5–6, 8–10	13–16	9–11	16–17	44–46	59–62
Rabbit anti-latex IgG	8–10	—	8–10	14–16	48–49	53–58
Epitope location*	13–24	29–36	62–69	74–81	134–139	164–171

Numbers indicate the spots that were reactive with the antisera.

*Amino acid location based on reactivity of pooled human anti-latex IgE.

Epitope identification

Homology searching was performed using the BLAST program at the National Center for Biotechnology Information (Bethesda, MD) [24]. The antigenic index was determined using the MacVector program which models protein structure using Chou–Fasman and Robson–Garnier analysis of secondary structure to predict exposed surfaces of the protein.

RESULTS

Epitope analysis

When the synthetic peptides were probed with high IgE control sera, no IgE binding was observed (Fig. 1a). With sera pooled from 10 latex-allergic patients, six areas of reactivity within the full length hevein preprotein bound IgE (Fig. 1b). Two were located in the N-terminal 5-kD hevein domain (H1 and H2) and four were observed in the 14-kD C-domain protein (C1–C4). IgE reactivity to H1, H2 and C1 appeared with the greatest intensity (Fig. 1b).

The membrane was stripped and re-probed with sera from a single patient included in the pooled sera and with a polyclonal rabbit antisera, and the data are summarized in Table 3. Serum from the single latex-allergic patient had a similar reactivity profile to the pooled sera. A notable difference was in the H1 region, where this individual reacted to spots 5–6 and 8–10, suggesting that there may be two epitopes within this region. Polyclonal anti-latex protein sera obtained from rabbits immunized with

ammoniated latex only recognized five regions. H2 was not recognized by these sera, while C3 was shifted towards the C-terminus (from 42–46 to 48–49) and C-4 was shifted towards the N-terminus (from 59–62 to 53–58).

The aa sequence for each epitope was determined from the overlapping areas of the sequential peptides which reacted with IgE. Using the BLAST program, the aa sequences for each epitope were searched for homology to other known proteins. Homologies are shown in Table 4. Epitope H1 had 100% homology to WGA and a lectin from barley. Significant structural similarity was also found to a lectin and a chitinase from rice. Four epitopes (H2, C2–C4) had homologies to defence-related proteins in potato (WIN1, WIN2) and tobacco (CPB20, PRP4). The C1 epitope did not have homology to these defence-related proteins, but it did have limited homology to a metalloproteinase from *Bacillus subtilis*.

The antigenicity profile for prohevein was generated using the MacVector program (Fig. 2). The antigenicity profile indicates the regions of the molecule likely to be exposed at the surface and accessible for antibody recognition. Of the six epitopes, four were found in highly antigenic regions, while two (C2, C4) were found in non-antigenic regions.

Immunoblot reactivity with WGA

Because epitope H1 was identified as having significant homology to WGA, we screened WGA and several commercially available lectins for immunological cross-reactivity with latex-specific IgE. Only WGA was recognized by sera from latex-allergic patients,

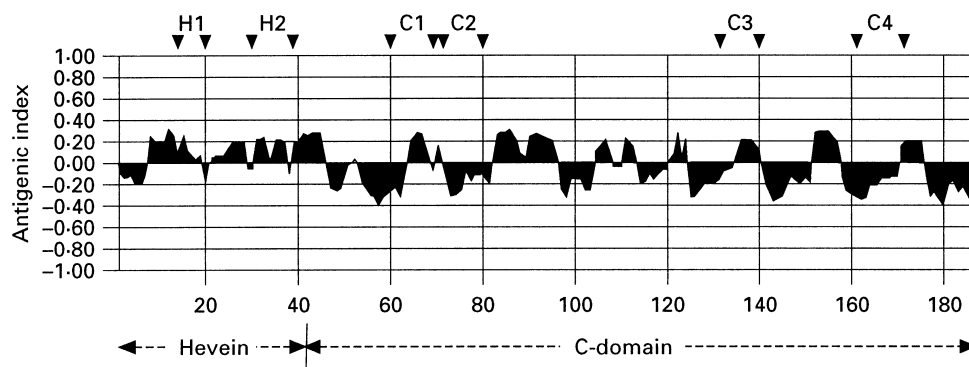


Fig. 2. Antigenic index for hevein preprotein. Locations of the six epitopes determined by reaction to synthetic peptides are indicated by the arrows at the top of the figure. The post-translational cleavage site that divides the molecule into the mature hevein and the C-domain is indicated by the vertical bar below the amino acid designations at the bottom.

Table 4. N-terminal hevein epitopes

13 PNNLCCSQWGW C24	Hevein epitope H1
PNNLCCSQWG * C	WGA (wheat)
PNNLCCSQWG * C	BLYLEC (barley root lectin)
PN * LCCSQ * G * C	LECTIN (rice)
PN * LCCSQ * WG * C	CHITINASE (rice)
* NNLCCSQ * GWC	WIN 2 (potato)
** NLCCSQ * GWC	WIN 1 (potato)
29 EYCSPDHN36	Hevein epitope H2
EYCSP***	WIN 1 (potato)
EYCSP***	WIN 2 (potato)
EYCSP***	CBP 20 (tobacco)
<i>C-terminal domain epitopes</i>	
62 L YNSQDHG69	Hevein epitope C1
***SQDHG	Metalloproteinase (<i>B. subtilis</i>)
74 AASAYCST81	Hevein epitope C2
A * SAYCST	WIN 1 (potato)
A * SAYCST	WIN 2 (potato)
A * SAYCST	CBP 20 (tobacco)
AASA * C * T	PRP 4A (tobacco)
134 CSNGGL139	Hevein epitope C3
CSNGGL	WIN 2 (potato)
CSNGGL	CBP 20 (tobacco)
CSNGGL	PRP 4a (tobacco)
CSNGGL	DRP (<i>Zea mays</i>)
CSNGGL	GLY M (soybean)
164 NYQFVDCG171	Hevein epitope C4
NYQFVDCG	GLY M (soybean)
NYQFVDCG	WIN 1 (potato)
NYQFV * CG	WIN 2 (potato)
NY * FV * CG	CBP 20 (tobacco)
NYQFV * CG	PRP 4 (<i>Sambucus nig</i> , elder tree)

Numbers indicate the amino acid location within the hevein preprotein.

while peanut, avocado, tomato, and potato lectins were negative (Fig. 3). To address the issue of specificity, inhibition Western blots were performed. In Fig. 4 it can be seen that control sera failed to react to WGA or latex, while latex-allergic sera strongly reacted to both preparations. Inhibition with NAL completely removed IgE reactivity to both the WGA and latex. Inhibition with WGA reduced reactivity to WGA and reduced but did not eliminate reactivity to latex. As IgE contains 18% carbohydrates, it was necessary to rule out the possibility that IgE was binding to WGA because of its lectin properties. The inhibition immunoblot was repeated using N-acetyl glucosamine as an inhibitor (Fig. 5). The N-acetyl glucosamine failed to block IgE binding, further indicating that the latex-specific IgE reactivity to WGA was a cross-reactivity rather than a non-specific recognition of the IgE carbohydrate moiety.

DISCUSSION

Understanding latex allergy is complicated because multiple NRL proteins are allergens. Before standardized skin test reagents or effective immunotherapy regimens can be implemented, latex allergens must be further characterized. Several latex allergens

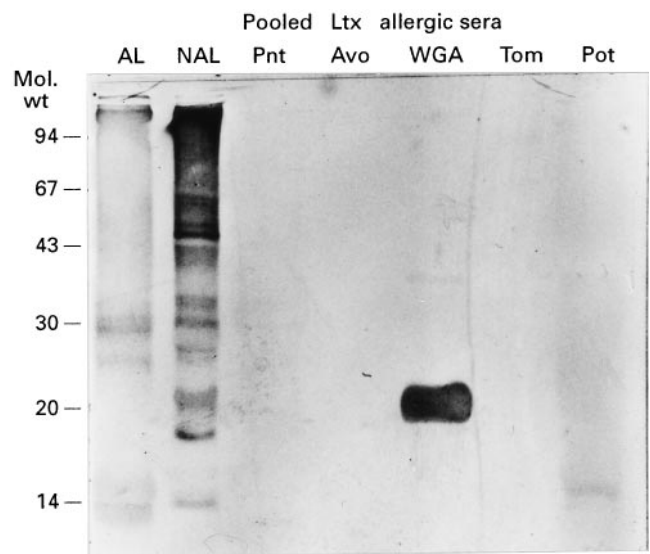


Fig. 3. Latex-specific IgE cross-reactivity with wheat germ agglutinin (WGA). Western blot analysis was performed to assess the possible cross-reactivity to plant lectins. Ammoniated latex proteins (AL) and non-ammoniated latex proteins (NAL) were used as positive controls. Commercially available lectins were screened for cross-reactivity with latex-specific IgE. Lectins were from peanut (Pnt), avocado (Avo), wheat (WGA), tomato (Tom), and potato (Pot).

have been identified, while additional IgE binding proteins are known to occur but remain to be identified. Previously, we reported IgE binding to the hevein protein in 50% of our latex-allergic healthcare workers, suggesting that this is a common allergen [17].

Hevein, a member of a family of 'pathogenesis-related' (PR-4) plant proteins, is synthesized as a 20-kD preprotein and subsequently post-translationally cleaved into the 5-kD mature hevein

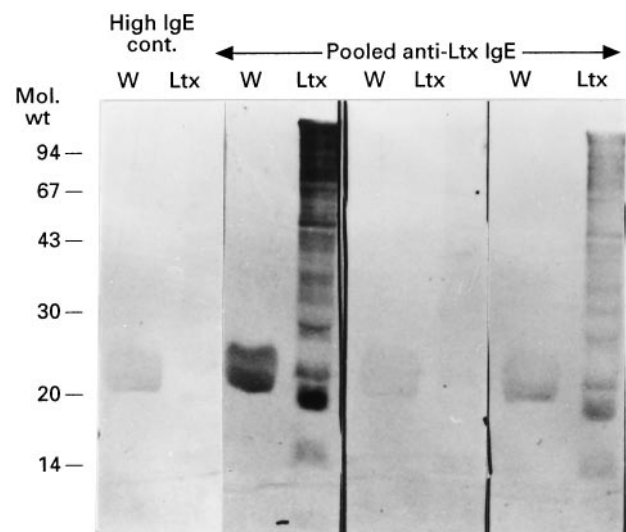


Fig. 4. Specificity of the cross-reaction of anti-latex IgE to wheat germ agglutinin (WGA). Inhibition immunoblots were performed as described in Materials and Methods. Each panel containing WGA (5 µg/lane) and non-ammoniated latex proteins (NAL; 100 µg/lane) was reacted with high IgE control sera (panel 1) or with pooled anti-latex IgE (panels 2–4). Before addition to the membrane, anti-latex IgE was incubated with 100 µg ammoniated latex proteins (AL; panel 3) or with 100 µg WGA (panel 4).

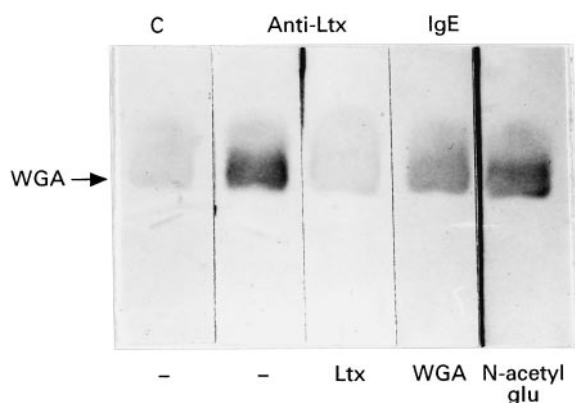


Fig. 5. Failure of N-acetyl-glucosamine to inhibit reactivity to wheat germ agglutinin (WGA). Immunoblot inhibition was performed as described in Fig. 4. Lane 1 represents the high IgE control sera, lane 2 is anti-latex IgE with no inhibitor, lane 3 is anti-latex IgE with latex protein as the inhibitor, lane 4 is anti-latex IgE with WGA as the inhibitor, and lane 5 is anti-latex IgE with N-acetyl-D-glucosamine as the inhibitor. N-acetyl-D-glucosamine (500 mM) was added to the membrane during the blocking step and to the latex-specific sera 1 h before addition of the sera to the membrane.

and the 14-kD C-domain [10]. The cleavage products of prohevein have been identified in latex [25] and can be purified from latex gloves [26]. We identified two hevein cleavage products at 14 and 18 kD that reacted with patient IgE [17]. Both peptides had the N-terminal hevein aa sequence, indicating that the 14-kD protein was not the C-domain of hevein. Our data suggested that there may be another cleavage site that is C-terminal to the previously identified site which cleaves the mature hevein from the C-domain [10]. The existence of an additional cleavage site in the C-domain has been demonstrated by others [25]. The observation that IgE was recognizing peptides containing the N-terminus sequence suggested that IgE epitopes were primarily in the N-terminal hevein [17]. Recently, Alenius *et al.* [19] confirmed our observation that hevein is a major allergen, and also reported that most IgE reactivity is within the N terminal hevein [26].

In this study, we identified six epitopes. Two were in the mature hevein, while four were found within the C-domain. The three epitopes (H1, H2, C1) with the most intense IgE reactivity were found in the N-terminus of the molecule, confirming previous observations that the allergenic regions are located in this region of the molecule [17,19]. Antigenic index was determined using MacVector to predict regions of the molecule that may be accessible on the surface. Two of the four epitopes in the C-domain (C2 and C4) had a negative antigen index, suggesting that these sites may be internal epitopes. Internal epitopes are likely because latex protein is degraded by ammoniation, a process common to all dipped latex products. While we identified the location of the epitopes, further studies are in progress to define the minimal aa sequence of the epitopes. Studies are also ongoing to evaluate synthetic peptides containing these epitopes for skin prick testing (SPT) reactivity in latex-allergic patients.

The N-terminal epitope (H1) had 100% homology to WGA as well as to other plant proteins in the chitinase family. Chitinase molecules are present in many plant species and function as defence proteins, protecting the plant against attack by insects and fungus. We confirmed immunological cross-reactivity with WGA by Western blot and inhibition analysis. IgE binding to WGA was not the result of the carbohydrate residues of IgE

binding to the lectin, since reactivity was not inhibited by N-acetyl-D-glucosamine at a concentration sufficient to inhibit > 50% of the known low-affinity binding of IgE to WGA [27]. The reactivity was specific, in that high IgE control sera did not react with WGA, and the latex-specific sera could be specifically inhibited by both latex and WGA. Soluble latex proteins inhibited the cross-reactivity of latex-allergic sera to WGA more strongly than did WGA. WGA did not completely inhibit reactivity to the 14- and 18-kD bands in latex. This was not unexpected, as only one of the six epitopes had homology to WGA. Although there is a definite immunological cross-reactivity, it is not known if latex-allergic patients have clinical reactions to wheat.

The epitopes identified in the hevein molecule were primarily in regions that were conserved among the PR-4 family of plant proteins. Of the six epitopes identified, four were common to proteins in this family. The homology of hevein epitopes to the wound-inducible proteins of potato (WIN1, WIN2) as well as previously observed homology of a 46-kD latex protein to the potato storage protein patatin [23] explain the finding that 40% of latex-allergic patients had skin test reactivity to potato, an otherwise rare allergen [28,29]. The existence of multiple epitopes for IgE in conserved regions of common plant defence proteins provides the mechanism for multiple cross-reactivities. Developing IgE to these conserved epitopes in latex predisposes one to reactivity to similar proteins in other plants and helps to explain the surprisingly high degree of food allergies in latex-allergic patients [23].

Epitope C-1 appeared to be unique, in that no homology was found to other plant proteins of the PR-4 family. There was, however, a limited region of homology to a metalloproteinase from *B. subtilis*. Metalloproteinases from *B. subtilis* are used in detergents, and were identified as the allergen causing occupational asthma in detergent manufacturing plant employees [30,31].

Latex allergy is characterized by IgE reactivity to a seemingly large number of protein allergens. By defining the epitopes for the specific allergens, it was hoped that a common epitope could be identified. Although we identified several epitopes, we were unable to identify epitopes with a repeated sequence or common motif within the hevein molecule. The hevein epitopes had no homologies to the recently defined epitopes in the rubber elongation factor (Hev b 1) allergen [32]. Whether the epitopes identified in hevein contain common motifs found in other latex allergens awaits their further identification and epitope mapping of these other molecules.

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