

Original article

IgE reactivity to patatin-like latex allergen, Hev b 7, and to patatin of potato tuber, Sol t 1, in adults and children allergic to natural rubber latex

Background: Patients allergic to natural rubber latex (NRL) frequently show positive skin prick tests (SPT) and hypersensitivity reactions to various fruits, such as avocado, banana, and kiwi, as well as to vegetables such as potato.

Methods: Hev b 7 was purified from NRL “C-serum” and Sol t 1 from potato extract, and they were detected by immunoblotting. IgE antibodies to Hev b 7 and Sol t 1 were measured with ELISA in sera from 35 adults and 35 children allergic to NRL. ELISA inhibition and immunoblotting were used to study allergen cross-reactivity. The *in vivo* reactivity of Hev b 7 and Sol t 1 were demonstrated in the SPT.

Results: Seventeen (49%) of the 35 NRL-allergic adults had IgE antibodies to Hev b 7, in contrast to only one of the 35 NRL-allergic children. Fifteen (43%) of the NRL-allergic adults and 29 (83%) of the NRL-allergic children had IgE antibodies to Sol t 1. Ten (29%) of the adult sera showed IgE binding to both Sol t 1 and Hev b 7, and crosswise inhibition tests with pooled sera revealed marked cross-reactivity. In the SPT, both natural Hev b 7 and Sol t 1 were able to produce a wheal and flare reaction.

Conclusions: One-half of the NRL-allergic adults, but only one of the NRL-allergic children, had IgE antibodies to natural Hev b 7. These results suggest that Hev b 7 is an important NRL allergen for adults, but not for children. Elucidation of the clinical importance of the observed cross-reactivity between Hev b 7 and Sol t 1 requires further studies.

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IgE cross-reactivity between natural rubber latex (NRL) and fruits and vegetables, the so-called latex-fruit syndrome (1), has gained increasing interest in recent years (2–4). Like NRL allergens, many plant-food allergens belong to the group of pathogenesis-related (PR) proteins (5). Allergens from avocado (6) and banana (7, 8) have already been characterized and reported to belong to the PR-3 proteins, representing class-I plant endochitinases that cross-react with hevein (Hev b 6.02), a major NRL allergen (9).

Beezhold et al. (10) observed sequence homology and cross-reactivity between a patatin-like NRL allergen and patatins in potato and tomato. They found IgE antibodies to a patatin-like allergen by immunoblotting in 23% of the NRL-allergic adults. Kostyal et al. (11) and Sowka et al. (12) have cloned and expressed Hev b 7 in both *E. coli* and *Pichia pastoris*, and they studied by immunoblotting the IgE-binding properties of these recombinant proteins. The studies of Sowka et al. (12) and Breiteneder et al. (13) showed by immunoblotting that 11% of the NRL-allergic patients had IgE to both natural and recombinant Hev b 7.

Atopy is a significant risk factor for NRL sensitization, and the patients also frequently show a positive skin prick test (SPT) to various foods (2, 14, 15). The patatin of potato tuber (Sol t 1) was recently shown to be a novel food allergen for atopic children (16). Since

Abbreviations. ELISA: enzyme-linked immunosorbent assay; HIC: hydrophobic interaction chromatography; MALDI-TOF: matrix-assisted laser desorption/ionization time-of-flight; PAS: periodic acid-Schiff; SDS-PAGE: sodium dodecyl sulfate polyacrylamide gel electrophoresis; SPT: skin prick test.

Sol t 1 and Hev b 7 have remarkable sequence homology (10–13), we wanted to study the prevalence of IgE antibodies to natural Hev b 7 and Sol t 1 in NRL-allergic patients, both adults and children, and to assess the cross-reactivity of these antibodies.

Material and methods

Patient and control sera

Sera were obtained from 35 NRL-allergic adults (mean age 38, range 19–65 years), all of whom had a positive SPT (mean wheal diameter at least half that of histamine, 10 mg/ml) to a NRL glove extract (Triflex, Baxter, Valencia, CA, USA; size of the wheal mean diameter at least half that of histamine; at least 3 mm) and positive RAST (>0.4 kU/l; Pharmacia Biotech, Uppsala, Sweden) and/or Triflex glove challenge test. Thirty-three patients were prick-prick tested to raw potato as previously described (15) (health-care workers, $n=8$), and 24 (73%) had a positive SPT. Adult control sera were obtained from 25 atopic adults, with negative SPT for raw potato and NRL glove extract but positive SPT for various inhalant allergens, such as grass pollen, animal dander, or house-dust mites.

Sera were obtained also from 35 NRL-allergic children, including seven children with spina bifida or other congenital malformations (mean age $9\frac{1}{2}$, range 1–18 years, mean number of surgical operations 12, range 5–21 operations) (17). All children met three diagnostic criteria for NRL allergy:

- 1) a positive SPT to a commercial NRL allergen (Stallergènes SPT reagent, Antony, Cedex, France) (15) and/or to a NRL glove extract (Triflex, Baxter)
- 2) a positive RAST (>0.4 kU/l; Pharmacia Biotech)
- 3) a positive NRL glove (Triflex) challenge test performed as previously described (15).

The sera for this study were collected during a follow-up study, in which all children were prick tested with raw potato and 27 also with purified Sol t 1. Control sera were obtained from 11 atopic children (mean age 2.5 years, range 5 months to 6 years) with negative SPT for raw potato and NRL but positive SPT to various other allergens such as cow's milk, hen's egg, and/or cereals. The study was approved by the ethics committee of Tampere University Hospital (Finland). Every adult patient and all children and/or their parents gave informed consent to participate.

Isolation of the proteins

Patatin of potato tuber (Sol t 1) was purified as described elsewhere (16). Patatin-like NRL allergen, Hev b 7, was purified from NRL C-serum obtained from the Rubber Research Institute of Malaysia, Kuala Lumpur, Malaysia. For gel-filtration chromatography,

0.5 ml of 10 mM sodium phosphate buffer, 150 mM NaCl, pH 7.0 (running buffer), was added to 1.5 ml of latex C-serum, and the sample was subjected to 16×60 Superdex 75 fast-flow column (Pharmacia Biotech, Uppsala, Sweden) at a flow rate of 2.0 ml/min and monitored at 280 nm. Hydrophobic interaction chromatography (HIC) was performed in a 1-ml RESOURCETM PHE (phenyl) column (Pharmacia Biotech) equilibrated with 10 mM sodium phosphate buffer, pH 7.0, 1.5 M $(\text{NH}_4)_2\text{SO}_4$. The chosen fractions from gel filtration were pooled, brought to approximately 1.5 M $(\text{NH}_4)_2\text{SO}_4$, and applied to the column. The elution was performed with a decreasing linear gradient of $(\text{NH}_4)_2\text{SO}_4$ (1.5–0 M in 20 min). The flow rate was 2.0 ml/min, and elution was monitored at 280 nm. The fractions of interest were pooled and desalted in a 1×10 cm BioGel P-6DG column (Bio-Rad Laboratories, Hercules, CA, USA) in 50 mM Tris-Cl, pH 7.0.

Anion exchange chromatography of the desalted sample was performed on a Mono Q HR5/5 column (Pharmacia Biotech) equilibrated with 50 mM Tris-Cl, pH 7.0. Elution was performed with a linear gradient of NaCl (0–0.5 M in 20 min) in the equilibration buffer. The flow rate was 1.0 ml/min, and chromatography was monitored at 214 nm.

The buffer exchanges for ELISA experiments (to 50 mM carbonate buffer, pH 9.6), for SPT (to PBS, pH 7.5), and for immunoblot inhibition studies (to 10 mM Tris-Cl, 0.9% NaCl pH 7.5) were performed by gel filtration in the 1×10 cm BioGel P-6DG column (Bio-Rad Laboratories). The purified proteins were quantified by reversed-phase chromatography on a 0.21×10 cm TSK TMS 250 (TosoHaas Corporation, Japan) column. The column was equilibrated with 0.1% TFA (trifluoroacetic acid) and eluted with a linear gradient of acetonitrile (3–100%, in 60 min) in 0.075% TFA. The protein peaks observed were integrated, and the protein amount was calculated by comparison of the peak areas to areas obtained with known amounts of the proteins bovine serum albumin and ovalbumin.

Identification of the purified proteins

For peptide mass finger printing or internal sequencing, the protein to be identified was alkylated and digested with endoproteinase Lys-C (18). For mass finger printing, part of the protein digest was desalted by a microtip reversed-phase chromatography column (19). The mass-mapping analysis of the protein digest was performed by matrix-assisted desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) with a Biflex II instrument (Bruker Franzen Analytik, Bremen, Germany). Angiotensin II and ACTH fragment 18–39 (Sigma, A 0673, St Louis, MO, USA) were included in the peptide sample as internal standards.

The rest of the peptide mixture was separated on a 1.0 mm × 150 mm Vydac C8 column (MIC-15-05-C8, LC-Packings, Amsterdam, The Netherlands) with a linear gradient of ACN (0–40% in 120 min) in 0.1% TFA. The chromatography was performed at a flow rate of 50 µl/min and monitored at 214 nm. Separated peptides were automatically collected (SMARTTM Systems, Pharmacia Biotech). Peptide sequencing was performed by an Applied Biosystems 494 A ProciseTM sequencer (PerSeptive Biosystems, Foster City, CA, USA).

Electrophoresis and immunoblotting

Proteins were separated by SDS-PAGE on 12% gels, according to Laemmli (20), under reducing conditions. Separations on a Mini-Protein II device (Bio-Rad Laboratories) were performed at a constant current of 25 mA/gel. Molecular weight standards were either low molecular weight (LMW) (Pharmacia Biotech) or prestained SDS-PAGE standards, broad range (Bio-Rad Laboratories). Proteins were stained either with Coomassie brilliant blue (CBB) (PhastGel Blue R, Pharmacia Biotech), silver staining (21), or SYPRO Orange (Bio-Rad Laboratories) visualized by the Fluor-S image analyzer (Bio-Rad Laboratories). Glycoproteins were detected with PAS (periodic acid-Schiff) – staining, with 5 µg of transferrin as positive control (22).

For immunoblotting and immunoblot inhibition studies, the SDS-PAGE-separated proteins were transferred to a PVDF (polyvinylidene difluoride) membrane (Immobilon-P, Millipore Corporation, Bedford, MA, USA). The proteins were transferred at 100 V for 60 min in a Mini-Trans-Blot cell (Bio-Rad Laboratories) in 10 mM CAPS (3 [cyclohexyl-amino]-1-propanesulfonic acid), pH 11, 10% methanol (23).

Transferred proteins on the PVDF membrane were incubated for 45 min in a blocking buffer containing 5% nonfat milk powder in 10 mM Tris-Cl, 0.9% NaCl, pH 7.5 (TBS), and then placed into a Miniblotter (Biometra, Göttingen, Germany). For detection of the IgE response of the protein, the membrane was incubated overnight at 4°C with patient and control sera (diluted 1:5 in the blocking buffer, 120 µl/channel). The membrane was then washed with 0.1% Tween-20 in TBS, incubated for 30 min with biotinylated goat antihuman IgE antibodies (Vector, Burlingame, CA, USA) diluted 1:1000, removed from the blotter, and washed. Before the addition of streptavidin-conjugated alkaline phosphatase (Zymed, Camarillo, CA, USA) diluted 1:24 000 in TBS, the membrane was blocked again with buffer for 5 min and washed with 0.1% Tween-20 in TBS. The membrane was incubated for 30 min with streptavidin-conjugated alkaline phosphatase at room temperature. The membrane was placed in a color development solution (Bio-Rad Laboratories), which was stopped by rinsing with water.

For immunoblot inhibition studies, membranes were incubated in the blocking buffer for 45 min and placed in the Miniblotter. Pooled patient sera (diluted 1:5) together with inhibiting protein were incubated in a blocking buffer for 1 h at room temperature. The mixture was then placed on the membrane and incubated overnight at 4°C. The immunoblot was further developed as described above.

IgE ELISA

For ELISA, the concentrations of the purified proteins were adjusted to 2.0 µg/ml in 50 mM carbonate buffer, pH 9.6, applied onto 96-well polystyrene microtiter plates (100 µg/well) (Nunc, Roskilde, Denmark), and incubated for 3 h at room temperature and then overnight at 4°C. Wells were then emptied and post-coated for 1 h with 1% human serum albumin (HSA) in 100 µl of 50 mM carbonate buffer, pH 9.6. After rinsing three times with 0.05% Tween-20 in PBS (PBS-T), 100 µl of patient and control sera (diluted 1:10), with PBS-T containing 0.2% HSA (PBS-T-HSA), was added to the wells and incubated for 2 h at room temperature. Wells were then washed three times with PBS-T. Biotinylated goat antihuman IgE (Vector) diluted 1:1000 with PBS-T-HSA was incubated for 1 h on the plates. The wells were again washed three times with PBS-T and incubated for 1 h with streptavidin-conjugated alkaline phosphatase (Bio-Rad Laboratories), diluted 1:3000 with PBS-T-HSA. After three washes with PBS-T, the substrate development solution, containing *p*-nitro-phenyl phosphate (104 Sigma, St Louis, MO, USA) was added, and the developed color read at 405 nm with an automated ELISA reader (Titertek Multiskan, Eflab, Espoo, Finland).

In ELISA-inhibition studies, coating and postcoating of the microtiter plates were performed as described for IgE ELISA. Two pools were made from the sera of 10 adult patients (health-care workers, *n* = 2) and 10 children, respectively, and used as sources of IgE antibodies. One patient with high specific IgE binding against Hev b 7 was used as a reference serum. Sera (diluted 1:10 in PBS-T-HSA) were first incubated for 1 h at room temperature with an equal volume of the inhibitor solution. The final concentrations of the purified proteins used as inhibitors were 280, 100, 30, 10, 3, 1, 0.3, and 0.1 µg/ml. The mixtures containing the sera and the inhibitor were then incubated in the microplate wells for 2 h at room temperature, emptied, and washed three times with PBS-T, after which the procedure was continued.

SPT with purified allergens

The SPT was performed with purified Sol t 1 (30 and 200 µg/ml) and Hev b 7 (50 µg/ml), as previously described (15). Histamine dihydrochloride (10 mg/ml;

ALK, Copenhagen, Denmark) served as positive control and saline as negative control. A mean wheal diameter of at least 3 mm was considered positive for both Sol t 1 and Hev b 7.

Results

Purification and identification of patatin-like NRL allergen, Hev b 7

Gel-filtration chromatography was used as the first purification step to separate NRL C-serum proteins. Fractions were collected and analyzed by 12% SDS-PAGE with CBB staining. Since the molecular mass of Hev b 7 was known to be approximately 43 kDa (10–13), fractions consisting of proteins around the 43-kDa zone in SDS-PAGE were chosen for the next purification step, hydrophobic interaction chromatography (HIC). Peak fractions from HIC were desalted by gel filtration into 50 mM Tris-Cl, pH 7.0, and analyzed by 12% SDS-PAGE with silver staining. The strongest peak eluting in a rather high $(\text{NH}_4)_2\text{SO}_4$ concentration was seen to consist mainly of a 43-kDa protein. The fractions corresponding to this peak were further purified by anion exchange chromatography, yielding a pure protein with some charge heterogeneity (data not shown) but giving a single 43-kDa band in 12% SDS-PAGE with silver and SYPRO orange staining. In PAS staining with 5.0 μg of Sol t 1, Hev b 7, and transferrin, Sol t 1, but not Hev b 7, was seen to be glycosylated.

For ELISA, SPT, and immunoblot assays, the purified protein was brought into carbonate buffer, pH 9.6, PBS, or TBS.

The amino-terminal sequence analysis of the purified protein showed it to be blocked. Therefore, the protein

was identified by mass-mapping and internal sequence analysis. Mass-mapping of the 43-kDa protein after alkylation and digestion by endoproteinase Lys-C identified it as Hev b 7 (Fig. 1). In addition, the sequences obtained for four peptides after separation by reversed-phase chromatography gave 100% sequence identity to Hev b 7 (EMBL: AJ223039).

IgE antibodies to Hev b 7 and Sol t 1 in ELISA in NRL-allergic adults and children

Seventeen (49%) of the 35 NRL-allergic adults showed IgE antibody binding to Hev b 7 in ELISA (Fig. 2). Sera from 35 NRL-allergic children did not recognize Hev b 7, except for one borderline case (Fig. 3). IgE antibodies to Sol t 1 were seen in 15 (43%) of the NRL-allergic adults. Twenty-nine (83%) of the NRL-allergic children showed IgE binding to Sol t 1 in levels exceeding the mean + 3 SD of the 11 control children. A total of 10 (29%) of the 35 adult sera showed IgE antibodies against both Hev b 7 and Sol t 1 in levels exceeding the mean + 3 SD of the adult controls.

Cross-reactivity of Hev b 7 and Sol t 1 in ELISA and immunoblotting

IgE cross-reactivity between Hev b 7 and Sol t 1 in ELISA and immunoblotting was studied with pooled sera of the adult patients (Fig. 2) recognizing both Hev b 7 and Sol t 1. In ELISA, 60 $\mu\text{g}/\text{ml}$ of Sol t 1 inhibited 50% of IgE binding to solid-phase Hev b 7. Conversely, 10 $\mu\text{g}/\text{ml}$ of Hev b 7 inhibited 50% of IgE binding to solid-phase Sol t 1 when adult (Fig. 4) or children's sera were used (data not shown). In immunoblotting, specific binding to Hev b 7 was seen in 5/7 adult patients with IgE against Hev b 7 by ELISA. Four out of five adult

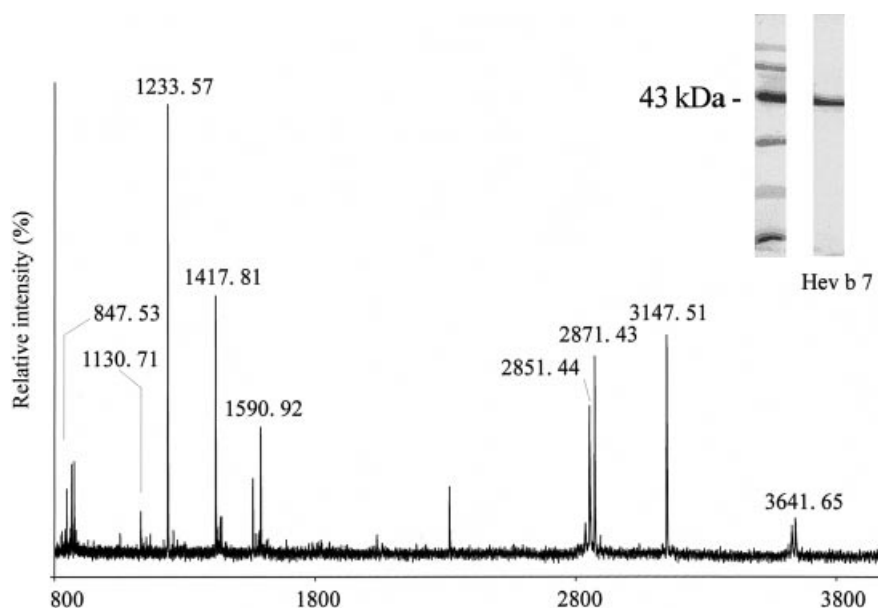


Figure 1. Mass finger-printing of Hev b 7 with MALDI-TOF mass spectrometry. In upper right corner is silver-stained 12% SDS-PAGE of Hev b 7 after anion exchange chromatography.

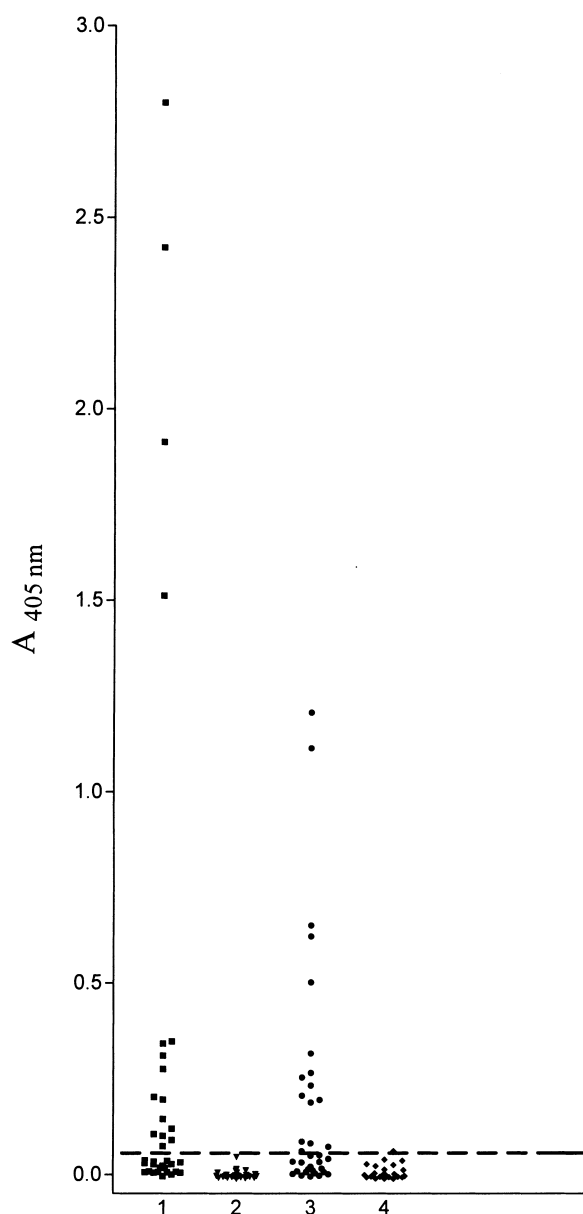


Figure 2. IgE binding to purified Hev b 7 and Sol t 1 for NRL-allergic adults ($n=35$) and controls ($n=25$) by ELISA. 1) Hev b 7; 3) Sol t 1; 2 and 4) controls. Cutoff level for positive response (mean OD + 3 SD of 25 controls) is indicated by horizontal line.

patient sera with specific IgE against Sol t 1 in ELISA showed IgE binding to Sol t 1 by immunoblotting. None of the children's sera showed IgE to Hev b 7. For immunoblot experiments with Sol t 1, sera from children with positive ELISA results were chosen. Eight out of nine showed IgE binding to Sol t 1. Crosswise immunoblot inhibition studies with purified Sol t 1 and Hev b 7, using pooled sera of adult patients with IgE antibodies to both Hev b 7 and Sol t 1, showed that 50 $\mu\text{g/ml}$ of Hev b 7 was able to inhibit all the IgE binding to Sol t 1, whereas 100 $\mu\text{g/ml}$ of Sol t 1 was not seen to inhibit any IgE binding to Hev b 7 (data not shown).

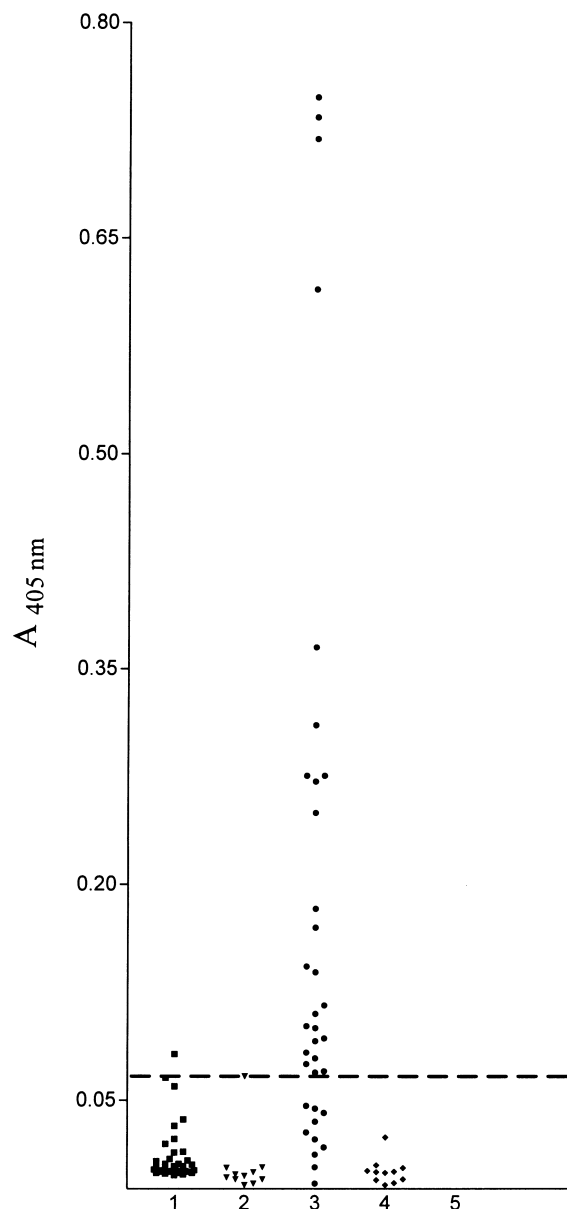


Figure 3. Occurrence of IgE antibodies to purified Hev b 7 and Sol t 1 for NRL-allergic children by ELISA. 1) Hev b 7; 3) Sol t 1; 2 and 4) controls. Cutoff level for positive response (mean OD + 3 SD of 11 control children) is indicated by horizontal line.

SPT results

Two out of four NRL-allergic adults with IgE antibodies to Hev b 7 in ELISA showed positive SPT to Hev b 7 (wheal mean diameters 6 and 7 mm, respectively). One of these two (who showed strong ELISA and immunoblot results to Hev b 7, but did not recognize Sol t 1 and had negative SPT to raw potato) was used as a reference patient for inhibition studies (Fig. 5). The other Hev b 7-positive patient (with positive ELISA and immunoblot results against both Hev b 7 and Sol t 1) also had positive SPT to Sol t 1 and raw potato. The SPT with Hev b 7 was not performed

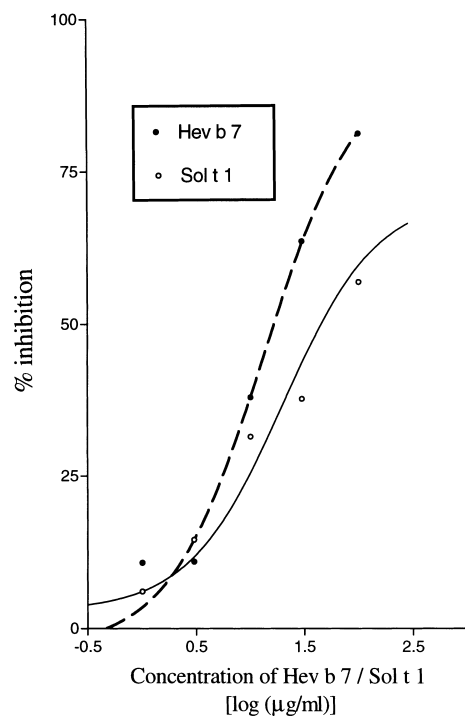


Figure 4. Crosswise inhibition ELISA result for Sol t 1 against solid-phase Hev b 7 with pooled sera of NRL-allergic adults having IgE antibodies binding to both Sol t 1 and Hev b 7.

on NRL-allergic children, since they showed no evidence of IgE antibodies to Hev b 7 in either ELISA or immunoblotting. The SPT with Sol t 1 was positive in eight (30%) children. All of these children showed IgE antibodies against Sol t 1 in ELISA.

Discussion

IgE binding to a natural patatin-like NRL allergen, Hev b 7, was revealed by ELISA in sera from 49% of adult NRL-allergic patients. In contrast, only one of the 35 NRL-allergic children showed IgE antibodies to Hev b 7. Concomitant IgE binding to both Hev b 7 and Sol t 1 was seen in 10 (29%) of the NRL-allergic adults, and crosswise inhibition studies by ELISA with pooled sera of these patients showed a high degree of cross-reactivity between Hev b 7 and Sol t 1. On the other hand, IgE antibodies to Sol t 1 were found in 83% of the NRL-allergic children, although they had no antibodies to Hev b 7. Both Hev b 7 and Sol t 1 were able to induce positive SPT responses in NRL-allergic adults, and 30% of the NRL-allergic children had a positive response to Sol t 1.

Sol t 1 and Hev b 7 both represent defense-related proteins which have been connected to cross-reactions between NRL and fruits and vegetables. Patatins of potato are encoded by two multigene families, one of which is mainly expressed in tubers and the other throughout the whole plant (24). Sol t 1 is located in

Cross-reactivity between latex and potato

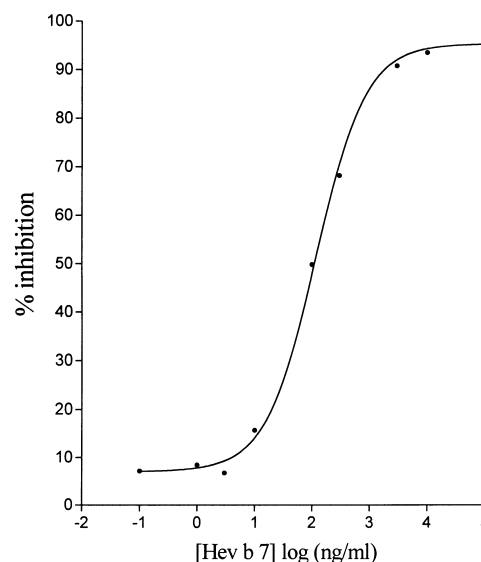


Figure 5. Homologous ELISA inhibition with serum of reference patient with Hev b 7-specific IgE antibodies. Decreasing amount of Hev b 7 was incubated with reference serum (final dilution 1:20), and ability of mixture to bind to solid-phase Hev b 7 (2.0 μg/ml) was assayed.

plant-cell vacuoles, is highly glycosylated, and shows some charge heterogeneity (25). Sowka et al. (12) have shown that natural Hev b 7 does not contain a typical hydrophobic signal sequence at its amino terminus, and although it presents two potential N-glycosylation sites, no N-glycans have been found in natural Hev b 7. In the present study, PAS staining revealed that Sol t 1, but not Hev b 7, is glycosylated, thus confirming the previous findings (12).

Cross-reactivity between plant allergens has been thought to be partly dependent on cross-reactive carbohydrate determinants that are found on a wide variety of nonmammalian glycoproteins. The structure of some strongly immunogenic, cross-reactive, non-mammalian, N-linked glycans has been elucidated, and differences in immunologic cross-reactivity have been found to depend on fucose and xylose residues (26). It is unlikely, however, that the IgE cross-reactivity observed in the present study between Hev b 7 and Sol t 1 could depend on cross-reactive carbohydrate determinants, since natural Hev b 7 lacks N-glycans (12) and no O-glycans have been detected in plant allergens (27).

We have no clear explanation of why NRL-allergic children, including the seven children with histories of multiple operations, did not show IgE binding to Hev b 7 while both children and adults frequently reacted to the structurally homologous Sol t 1. We observed, however, seven NRL-allergic adults who had IgE reactivity to Hev b 7, but not to Sol t 1, and five adult patients with IgE antibodies to Sol t 1, but not to Hev b 7. Therefore, it is possible that there are two types of IgE epitopes in Sol t 1, one which cross-reacts with Hev b 7, and another which does not. NRL-allergic

children's IgE reactivity could be restricted to the latter postulated Sol t 1 epitope. IgE antibodies to Sol t 1 are frequent also in atopic children with positive SPT to raw potato but negative tests to NRL (16). Therefore, both NRL-allergic and non-NRL-allergic children could have IgE antibodies to a common epitope in Sol t 1. This hypothesis is further substantiated by the observed high frequency of positive SPT reactions to Sol t 1 in both groups. It is also possible that some of such common epitopes bear cross-reactive carbohydrate determinants to which these atopic patients may have developed clinical tolerance (26). Food challenges with potatoes and with purified natural or recombinant patatins will further clarify the clinical importance of Sol t 1 as an allergen.

In crosswise ELISA-inhibition experiments with pooled sera from NRL-allergic adults, soluble Hev b 7 inhibited IgE binding to solid-phase Sol t 1 six times more efficiently than Sol t 1, suggesting that Hev b 7, and not Sol t 1, could be the primary sensitizer for development of these cross-reacting IgE antibodies. An important finding was that we could detect IgE antibodies to purified Hev b 7 with our ELISA method in as many as 49% of the NRL-allergic adult patients. Previous immunoblot studies using natural or recombinant Hev b 7 have found IgE antibodies in only 11–23% of the patients (10–13). It is therefore possible that some of the epitopes in Hev b 7, presented by the presumably native protein in ELISA, could become altered or destroyed under the conditions of immunoblotting. The frequent occurrence of IgE antibodies to Hev b 7 in adult NRL-allergic patients suggests that this allergen could be one of the major NRL allergens. Our preliminary

experiments have, however, failed to demonstrate immunoreactive Hev b 7 in several brands of NRL gloves, and, to our knowledge, no other data exist on its occurrence in manufactured rubber products. This suggests that primary sensitization could come from other sources found in the plant kingdom.

In conclusion, one-half of the present NRL-allergic adults had IgE antibodies to a patatin-like NRL allergen, Hev b 7, and these patients also frequently showed IgE binding to Sol t 1, a novel allergen of potato tuber (16). Consistently with their reported sequence homology (10–13), a considerable degree of allergen cross-reactivity between Hev b 7 and Sol t 1 was observed in ELISA inhibition. In contrast to adult patients, NRL-allergic children with one exception showed no IgE binding to Hev b 7 while frequently showing IgE antibodies to Sol t 1. This suggests that Hev b 7 is not an important NRL allergen for the NRL-allergic children. Considering the taxonomic distance between rubber trees and potatoes, the cross-reacting IgE antibodies to Hev b 7 and Sol t 1 form a new example of the existence of structurally homologous allergens in the plant kingdom.

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