

Plant defense-related enzymes as latex antigens

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Background: Latex allergy is an increasing hazard to people who frequently come into contact with latex products. Of interest concerning this immediate-type allergy is the cross-reactivity to various vegetable foods and pollen. Despite its high prevalence, no adequate explanation has been provided for the cross-reactive antigens.

Objective: We have hypothesized that a series of plant defense-related proteins act as latex allergens, as well as vegetable food allergens. To evaluate this hypothesis, hydrolytic enzymes that are very likely to take on defensive roles in rubber trees were examined for their antigenicity.

Methods: By applying chromatographic procedures, defense-related enzymes were separated from nonammoniated latex (NAL). Their antigenicity was examined by immunoblotting and ELISA with sera containing IgE antibodies to crude latex proteins.

Results: Three kinds of hydrolytic enzymes (basic β -1,3-glucanases [35, 36.5, and 38 kD], a basic chitinase/lysozyme [29.5 kD], and an acidic esterase [44 kD]) were separated from NAL. They were recognized by IgE antibodies from a significant number of patients allergic to latex. The basic β -1,3-glucanases and the acidic esterase were also strongly recognized by IgE antibodies from several atopic subjects who were allergic to various vegetable foods rather than latex products.

Conclusion: It was ascertained that the three defense-related enzymes separated from NAL constituted part of the latex antigens. Taking together the well-known serologic or immunologic relationships and amino acid sequence similarities of defense-related proteins coming from phylogenetically distant plant species, we can suspect their universal antigenicity and cross-reactivity. (*J Allergy Clin Immunol* 1998;101:379-85.)

Key words: Latex allergy, antigen, cross-reactivity, serologic relationship, defense-related protein, hydrolytic enzyme, immunoblotting, ELISA

Latex allergy is an immediate-type allergy caused by proteins eluting from diverse latex products prepared from natural rubber.¹ Persons most susceptible to this

Abbreviations used

HPLC:	High-performance liquid chromatography
MALDI-TOF-MS:	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
NAL:	Nonammoniated latex
SDS-PAGE:	Sodium dodecylsulfate-polyacrylamide gel electrophoresis
TBS:	Tris-buffered saline
TBS-T:	Tris-buffered saline containing 0.1% Tween 20

type of allergy are health care workers and pediatric patients with congenital abnormalities. They inevitably come into contact with latex products in daily life. Latex allergy is provoked in sensitized people when they are in direct contact with latex products or inhale glove powder-adsorbing latex allergens. Rubber particles in urban air originating from automobile tires are regarded as another potential latex allergen source.² Of interest concerning latex allergy is the cross-reactivity of patients allergic to latex to phylogenetically distant plants, which is often called the "latex-fruits syndrome." Structurally similar proteins in the respective plants may be responsible for such extensive cross-reactivity. However, no adequate explanations for the cross-reactive antigens in these plants have been documented.

Cross-reactivity of plant-derived allergens has been recognized for many years. Some of the cross-reactions were attributed to profilins,³ and others were attributed to carbohydrate cross-reactive determinants. As far as latex allergy is concerned, however, the extensive cross-reactivity cannot be fully explained by these classical cross-reactive antigens alone. Although Vallier et al.³ detected a profilin in a nonammoniated latex (NAL) extract, they failed to prove it to be an allergen eluting from latex products. Moreover, Kurup et al.⁴ and Moneo et al.⁵ could not detect any glycoproteins in their latex extracts. It is rational to think that plant proteins that are different from these conventional cross-reactive antigens should be involved in latex allergy and the accompanying cross-reactivity.

We have hypothesized that a series of plant defense-related proteins⁶⁻⁹ is a cause of latex allergy and cross-reactivity. Serologic or immunologic relationships and amino acid sequence similarities among the pathogenesis-, or rather, defense-related proteins from taxonomi-

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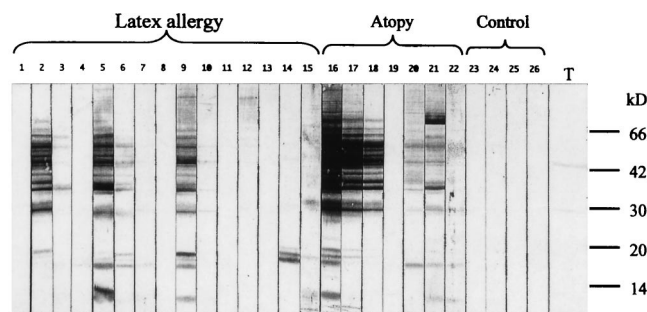


FIG. 1. Immunoblotting of crude NAL protein. Denatured protein (12 μ g/cm) was applied to SDS-PAGE and then transferred onto Immobilon-P membrane. Membrane was cut into strips, blocked, and incubated overnight with respective sera from patients allergic to latex (lanes 1 to 15). Some strips were incubated with respective sera from atopic subjects who had IgE antibodies to crude latex proteins but had subjective symptoms to vegetable foods rather than latex (lanes 16 to 22). In control experiments each strip was incubated with a serum from healthy individuals having no allergic predisposition (lanes 23 to 25) or IgE myeloma serum (lane 26). Strip number corresponds to number of the serum applied to it. After complete washing, IgE antibodies that had bound to antigens on strips were reacted with phosphatase-labeled goat anti-human IgE. IgE-positive antigens were then visualized by soaking strips in substrate solution. For comparison, all proteins transferred onto membrane were stained with Amido Black 10 B stain (lane 7). Migrated positions of molecular weight markers are shown at right edge.

cally distant plant species are well known. Provided that these ubiquitous stress-inducible plant proteins are latex allergens, we can easily explain the extensive cross-reactivity. The readily predictable stresses for rubber trees are repeated wounding and phytohormone applications for economically effective latex production.^{10, 11} Genetic selection of agronomically advantageous rubber trees may be another factor that causes hypersensitive induction of such proteins.¹² Indeed, we detected the activities of the defense-related enzymes (e.g., β -1,3-glucanase, chitinase/lysozyme, and esterase) in the extracts from ammoniated latex and latex gloves.¹³ These hydrolytic enzymes must have been induced, and they played defensive roles in rubber trees suffering from the stressful conditions.¹⁴⁻¹⁸ Moreover, a lysozyme separated from a brand of household gloves and an esterase/protease separated from the ammoniated latex were recognized by IgE antibodies in sera from patients allergic to latex.^{19, 20} These previous data certainly support our hypothesis that plant defense-related proteins are responsible for latex allergy and the accompanying cross-reactivity. To evaluate its validity further, we newly separated the above-mentioned three kinds of defense-related enzymes from NAL. Their affinities for IgE antibodies in a larger number of patients' sera were examined with immunoblotting and ELISA. These experimental results are reported herein.

METHODS

Sera

Sera from patients allergic to latex (sera nos. 1 to 15) were provided by PlasmaLab International (Everett, Wash.) and local hospitals in Japan. These patients had been diagnosed with latex allergy based on their clinical histories and positive IgE responses to crude latex proteins, which had been determined by a RAST. The sera from allergic subjects who had IgE

antibodies to the crude latex proteins but did not claim definite subjective symptoms to latex products were classified as sera from atopic subjects (nos. 16 to 22). Some of the patients allergic to latex (nos. 2, 3, 5, and 6) and all the atopic subjects had been confirmed to have IgE antibodies to proteins from various vegetable foods such as banana, potato, and tomato. Human IgE myeloma serum (no. 26; The Binding Site, Ltd., Birmingham, England) and normal sera (nos. 23 to 25 and three additional sera for ELISA) from healthy individuals who have no allergic predisposition were used in control experiments.

Analyses of protein samples

The total protein in a sample solution was quantified by BCA Protein Assay Reagent (Pierce Chemical Co., Rockford, Ill.) according to the manufacturer's instructions. The precise molecular weight of a separated protein was measured by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). Each protein for the measurement was mixed with sinapinic acid matrix on a stainless probe tip and introduced into a mass spectrometer (Kompact MALDI IV; Shimadzu Co., Kyoto, Japan). The isoelectric point of a separated protein was determined with isoelectric focusing on a polyacrylamide slab gel containing carrier ampholyte (Bio-Lyte 3/10; Bio-Rad Laboratories, Hercules, Calif.). Protein test mixture 9 (Serva Feinbiochemica, Heidelberg, Germany) and isoelectric point-marker proteins (Oriental Yeast Co., Osaka, Japan) were used as the isoelectric point standards.

Extraction of crude protein from NAL

NAL collected from rubber trees (*Hevea brasiliensis*, clone RRIM 600, Malaysia) was frozen and thawed repeatedly to obtain a liquid called F-serum. The protein in the F-serum was settled by adding $(\text{NH}_4)_2\text{SO}_4$ to 90% saturation. This precipitate was collected by vacuum filtration and desalted by passing through a Sephadex G-10 column (2.1 $\times$ 28 cm; Pharmacia Biotech, Uppsala, Sweden). The crude NAL protein obtained in this way was the source for further purification of defense-related enzymes.

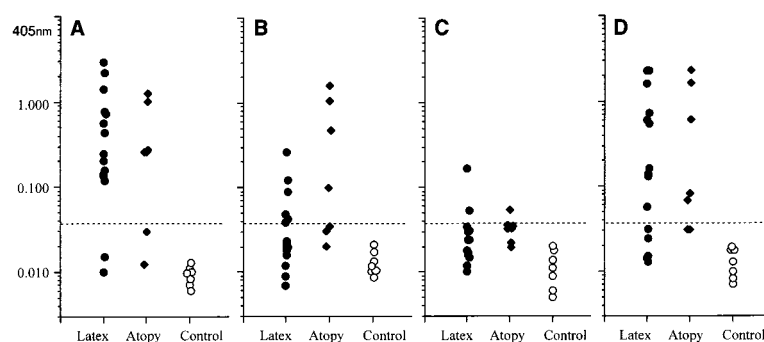


FIG. 2. ELISA of crude NAL protein (A) and three kinds of defense-related enzymes (β -1,3-glucanases [B], chitinase/lysozyme [C], and esterase [D]) that were separated from crude NAL protein. For its antigenicity analysis, protein was adsorbed onto microtiter plates by incubating overnight at room temperature. After blocking of unoccupied sites, diluted serum was poured into respective wells and incubated overnight. Each applied serum was donated by patients allergic to latex (*Latex*) or by atopic subjects (*Atopy*) who had IgE antibodies to crude latex proteins but had subjective symptoms to vegetable foods rather than latex. In control experiments (*Control*), each serum from healthy individuals or IgE myeloma serum was used. After complete washing, IgE antibodies binding to antigens on plates were reacted with phosphatase-labeled goat anti-human IgE for 1 hour at room temperature. Final color development was continued for 2 hours in *p*-nitrophenyl phosphate solution, and absorbance at 405 nm was measured. Every assay on a serum was done in triplicate, and average values are depicted. Border line (broken line) was arbitrarily set at 0.036 for definition of IgE-positive sera. This blank-corrected absorbance corresponds to mean + 5 $\times$ SD of all control sera.

Separation of basic β -1,3-glucanases

The crude NAL protein was applied to a Macro-Prep (Bio-Rad Laboratory) anion-exchange column (1.5×22 cm) equilibrated with 20 mmol/L carbonate buffer (pH 10.5). The basic protein that had not been adsorbed on the exchanger possessed strong β -1,3-glucanase activity. The purity of this protein fraction was examined with native PAGE that was available for cationic proteins. All the proteins on the gel were stained using a 2D-Silver Stain II reagent kit (Daiichi Pure Chemicals, Tokyo, Japan). Concurrently, β -1,3-glucanase activity was detected directly on the gel according to the method of Pan et al.²¹ This protein fraction was further analyzed with sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Separation of a basic chitinase/lysozyme

This bifunctional enzyme was separated from the crude NAL protein as described previously.¹⁹ The resulting chitinase/lysozyme was purified finally with reverse-phase high-performance liquid chromatography (HPLC). The enzyme was applied to a Resource RPC column (Pharmacia Biotech) that had been equilibrated with 20 mmol/L acetate buffer (pH 4.5). The elution was carried out with a linear gradient of acetonitrile (0% to 70% during 30 minutes), and the main peak at a retention time of 10 minutes was fractionated. The purity and molecular weight of chitinase/lysozyme thus obtained were examined by SDS-PAGE followed by protein dyeing. Chitinase activity was detected directly on a gel after SDS-PAGE in which glycol chitin was included as a substrate. Similarly, lysozyme activity was detected directly on a gel after SDS-PAGE according to the method described by Audy et al.²²

Separation of an acidic esterase

An esterase was separated from the crude NAL protein according to our previous procedure.²⁰ The separated enzyme was purified further by using anion-exchange HPLC. The esterase was applied to a Resource Q column (Pharmacia Biotech) that had been equilibrated with 20 mmol/L Tris-HCl

buffer (pH 9.0). Eluting with a linear gradient of NaCl (0 to 0.5 mol/L during 15 minutes), the most intense peak (0.3 to 0.4 mol/L NaCl concentration) was fractionated. The purity of the separated esterase was evaluated by a native PAGE that was available for anionic proteins. All the proteins on the gel were visualized by silver staining, and the proteins having esterase activity were detected directly on it as stated previously.²⁰ Bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.) was used as a substrate to examine the proteolytic activity of the esterase.

Amino acid sequencing and BrCN cleavage

The *N*-terminal amino acid sequence of a protein or peptide was analyzed by automated Edman degradation (PSQ-1 Protein Sequencer; Shimadzu Co.). The separated basic β -1,3-glucanases were fragmented by BrCN cleavage without further purification.²³ The resultant BrCN peptides were applied to reverse-phase HPLC on a Capcell PAK C₁₈ column (4.6×150 mm; Shiseido Company, Ltd., Tokyo, Japan) and eluted with a linear gradient of acetonitrile (1.6% to 30% during 60 minutes followed by 30% to 60% during 30 minutes) in 0.05% trifluoroacetic acid. One of the fractionated peptides (retention time: 73 minutes) was submitted to the *N*-terminal sequence analysis.

SDS-PAGE and immunoblotting

Each of the proteins for antigenicity examination was dissolved in a sample buffer containing 1% SDS and 2.5% 2-mercaptoethanol. The solution was then boiled for 3 minutes. The denatured protein was applied to a 12.5% polyacrylamide slab gel and electrophoresed at a constant current of 15 mA. After completion of the SDS-PAGE, the separated protein was transferred onto an Immobilon-P membrane (Millipore Co., Tokyo, Japan) by applying a constant current of 65 mA for 1 hour. The resulting protein-binding membrane was cut into 5 mm strips. One of the strips was stained with Amido Black 10B stain for total protein detection. The other strips were incubated with 0.5% casein dissolved in Tris-buffered saline (TBS)



FIG. 3. Immunoblotting of basic β -1,3-glucanases separated from NAL. Denatured enzyme (2.4 μ g/cm) was applied to SDS-PAGE. Refer to legend of Fig. 1 for experimental conditions and description.

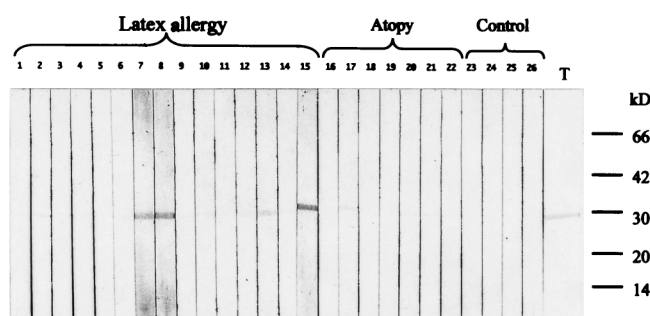


FIG. 4. Immunoblotting of bifunctional chitinase/lysozyme separated from NAL. Denatured enzyme (2.3 μ g/cm) was applied to SDS-PAGE. Refer to legend of Fig. 1 for experimental conditions and description.

at 40° C for 1 hour to block the unoccupied area. Each strip was then incubated overnight with a patient or control serum that was diluted 1:10 with the blocking buffer. After successive washing (twice for 2 minutes and then three times for 5 minutes with TBS containing 0.1% Tween 20 [TBS-T]) the strips were gathered together and incubated again in the blocking buffer for 10 minutes. The IgE antibodies binding to the antigens were then reacted for 1 hour with phosphatase-labeled goat anti-human IgE (Kirkegaard & Perry Lab., Inc., Gaithersburg, Md.) that was diluted 1:5000 with the blocking buffer. After complete washing with TBS-T, the IgE-binding antigens were visualized by soaking the strips in a substrate solution (Western Express; Promega Co., Madison, Wis.).

ELISA

Each separated enzyme was dissolved in 0.1 mol/L carbonate buffer (pH 10.0) at a concentration of 2.0 μ g/ml. In the case of the crude protein extracted from NAL, the concentration was adjusted to 20 μ g/ml. Each sample solution (100 μ l) was poured into the respective wells of microtiter plates (EIA/RIA Plate 3590; Costor, Cambridge, Mass.) and incubated overnight at room temperature. After five washings with TBS-T, the blocking buffer (200 μ l) was distributed into every well and kept for 2 hours at room temperature. After aspiration of the buffered solution, the patient or control serum (100 μ l) that was diluted 1:10 with the blocking buffer was poured into the respective wells. The plates were then kept overnight at room temperature and washed out the next morning. The IgE antibodies binding to the antigens were reacted for 1 hour with the phosphatase-labeled goat anti-human IgE (Kirkegaard & Perry) that was diluted 1:500 with the blocking buffer (100 μ l). After successive washing, 100 μ l of *p*-nitrophenyl phosphate solution (Vector

Lab., Inc., Burlingame, Calif.) was distributed into every well. The color development was continued for 2 hours at room temperature, and the absorbance at 405 nm was measured by a microplate reader (Titertek Multiskan Plus MK-II; Dainippon Pharmaceutical Co., Osaka, Japan). Every assay on a serum was done in triplicate and averaged. A patient's serum that resulted in a blank-corrected absorbance higher than 0.036 was arbitrarily judged to be IgE-positive to the antigen on the plate. This absorbance corresponds to the mean + 5 $\times$ SD of all the control sera.

RESULTS

Separation and characterization of defense-related enzymes

The basic proteins that had not been adsorbed on an anion-exchange resin at pH 10.5 showed multiple bands on a native PAGE gel after silver staining. However, almost all of them were also detected on a zymogram for β -1,3-glucanase activity (data not shown). This indicated that the enzyme fraction was composed of multiple β -1,3-glucanase isoenzymes. When this enzyme fraction was analyzed by SDS-PAGE, three protein bands at 35, 36.5, and 38 kd were detected. Likewise, three broad peaks were recorded at 35,100, 36,700, and 38,000 d in its MALDI-TOF-MS spectrum. Their isoelectric points were estimated to be higher than 10 (multiple bands). Although the *N*-terminus of the enzyme was blocked, one of the BrCN peptides had an *N*-terminal sequence of FDENXKQXEVEKHFGFLFFPD. This internal sequence coincided with that of Hev b 2 (³²³F to ³⁴²D)

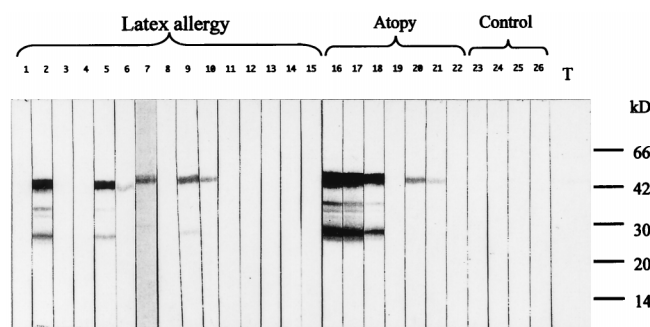


FIG. 5. Immunoblotting of acidic esterase separated from NAL. Denatured enzyme (0.6 μ g/cm) was applied to SDS-PAGE. Refer to legend of Fig. 1 for experimental conditions and description.

except for the X positions in which the constituent was not strictly determined.^{17, 23}

The separated chitinase/lysozyme was displayed as a single protein band (29.5 kD) on a gel after SDS-PAGE. Both the chitinase and lysozyme activities were detected at the same position as this protein band on respective zymograms (data not shown). These results clearly proved the purity and bifunctionality of the separated enzyme. The molecular weight of this enzyme was precisely determined to be 29,600 d with MALDI-TOF-MS, and its isoelectric point was conjectured to be 10 (three bands). The determined *N*-terminal sequence of this protein, GGIAIYWQGNGNEGTLTQTSTRK, matched completely with the *N*-terminal sequence of hevamines.²⁴

On a gel for native PAGE applicable to anionic proteins, the acidic esterase was detected as a single band after silver staining. Its position strictly coincided with the sole band on a zymogram for esterase activity (data not shown). Despite this purity confirmation, there were a few stained protein bands on a gel after SDS-PAGE. The position of the most intense 44 kD band agreed with that of an esterase/protease separated previously from the ammoniated latex.²⁰ Several weaker bands (28 and 35 to 39 kD) always appeared below the main 44 kD band in their SDS-PAGE patterns. A molecular weight of 44,200 d (broad peak) and an isoelectric point of 4.6 (multiple bands) were assigned to the separated acidic esterase by MALDI-TOF-MS and isoelectric focusing, respectively. The *N*-terminus of this enzyme was blocked, and we did not succeed in BrCN cleavage of it. This esterase did not exhibit appreciable proteolytic activity to bovine serum albumin.

Immunoblotting and ELISA

A number of IgE-binding latex antigens were shown on immunoblot strips where the crude NAL protein was the antigen source (Fig. 1). Many of the bands were commonly present in several lanes. In contrast, some of the patients' sera gave no distinct IgE-positive bands on the strips. The IgE-positive rates in this immunoblotting were 11 of 15 (73%) for patients allergic to latex (lanes 1 to 15) and 6 of 7 (86%) for atopic subjects (lanes 16 to

22). Neither the sera from three healthy individuals nor the IgE myeloma serum showed any IgE-positive bands (lanes 23 to 26). From the ELISA of the crude NAL protein, 13 of 15 (87%) of the patients allergic to latex and 5 of 7 (71%) of the atopic subjects were classified as IgE-positive (Fig. 2, A). The false-negative ratio (13%) in this ELISA was very near the reported figure.²⁵

When the antigenicity of the basic β -1,3-glucanases was examined by immunoblotting, 6 of 15 (40%) of the sera from the patients allergic to latex and 5 of 7 (71%) of the sera from atopic subjects showed clear IgE-positive bands (Fig. 3). Similar IgE-positive rates were noted in the corresponding ELISA; 6 of 15 (40%) for the patients allergic to latex and 4 of 7 (57%) for the atopic subjects (Fig. 2, B).

The separated chitinase/lysozyme exhibited relatively low affinities for IgE antibodies in the patients' sera. We observed thin IgE-binding bands in 4 of 15 (27%) of the immunoblot strips from patients allergic to latex and only a faint band in 1 of 7 (14%) of the strips from atopic subjects (Fig. 4). On the other hand, 2 of 15 (13%) of the patients allergic to latex and 1 of 7 (14%) of the atopic subjects were classified as IgE-positive by the corresponding ELISA (Fig. 2, C). However, most of the absorbances leading to these IgE-positive determinations, especially those of the atopic subjects, were just above the borderline.

The separated acidic esterase was most frequently recognized by IgE antibodies in the patients' sera. IgE-positive rates in the immunoblotting were found for 6 of 15 (40%) of the patients allergic to latex and 5 of 7 (71%) of the atopic subjects (Fig. 5), whereas in the ELISA the IgE-positive rates were 10 of 15 (67%) for the patients allergic to latex and 5 of 7 (71%) for the atopic subjects (Fig. 2, D). These positive rates were close to those recorded in the ELISA in which the crude NAL protein was fixed on the plates (Fig. 2, A).

DISCUSSION

In our continuing research on the relevance of plant defense-related proteins to latex allergy and the accompanying cross-reactivity,^{13, 19, 20, 26} the responsibilities of defense-related enzymes in NAL were investigated in

this study. By combining several chromatographic procedures, we obtained three kinds of defense-related enzymes with sufficient purity for their antigenicity examination. Unfortunately, the number of the atopic subjects' sera used is too small to draw a definitive conclusion about the cross-reactivity. Nevertheless, we could present a clue to understand the cross-reactivity between rubber latex and vegetable foods.

The molecular weights, the isoelectric points, and the internal peptide sequence of the basic β -1,3-glucanases were very close to those reported for Hev b 2 (34 and 36 kd; isoelectric point, 9.5).²³ On the other hand, the properties of the basic chitinase/lysozyme corresponded to those of hevimins (29.6 kd; isoelectric point, 9.5).²⁴ Although the separated chitinase/lysozyme would be a low-affinity latex antigen, we cannot neglect it as a minor latex allergen. Alenius et al.²⁷ also reported the low IgE-positive rates of a hevimin in their immunoblot experiments, whereas Lavaud et al.²⁸ described that a 30 kd latex lysozyme was an important cross-reactive latex allergen. The characteristics of the acidic esterase were comparable with those of hevamin I and a serine-centered esterase/protease separated from the ammoniated latex.^{20, 29} Beezhold et al.³⁰ have reported a similar size latex allergen (46 kd) with a patatin-like sequence, but its N-terminus had not been blocked. By contrast, an acidic latex protein (43 kd) documented by Subroto et al.³¹ had a blocked N-terminus and an internal sequence homologous to that of patatin. The esterase we described herein might correspond to the patatin-like acidic protein reported by Subroto et al.

In their recent article, Alenius et al.³² emphasized that hevein was the major epitope shared by latex allergens. Hevein and its precursors, proheveins or hevein preproteins, can be classified as defense-related proteins of a rubber tree.^{10, 11, 33, 34} The patatin-like latex allergen reported by Beezhold et al.³⁰ may also have a defensive role in a rubber tree. It is reported that patatin had serine-centered hydrolytic-enzyme activities and inhibited the growth of invertebrate pests' larvae.^{14, 35} Moreover, another major latex allergen (Hev b 4) from the microhelix that is induced by ethephon treatment²³ also could be a defense-related protein. Together with our findings presented here, the relevance of plant defense-related proteins to latex allergy and the accompanying cross-reactivity is becoming clear.

Some of the plant defense-related proteins have generally been called pathogenesis-related proteins because of their marked induction after phytopathogen attacks.⁶⁻⁸ Interestingly, their documented biochemical features are compatible with those reported for most allergen molecules: stable to acidic conditions and resistant to endogenous or exogenous proteolytic activities.³⁶ In addition, many cereal allergens have sequence homologies to plant α -amylase/protease inhibitors.³⁷ Such enzyme inhibitors are also considered to participate in plant-defense responses, at least to some extent.³⁸ Moreover, recent research revealed that the Bet v 1-related proteins were important cross-reactive allergens in var-

ious vegetable foods and pollen.³⁹ They have sequence homologies to many plant defense-related proteins belonging to the PR-10 class.⁴⁰ Several notable pieces of evidence suggesting the role of Bet v 1 in plant defense responses have also been reported.⁴¹⁻⁴⁴ Now Bet v 1 can be seen as more of a defense-related protein that is especially rich in pollens because of its developmental induction⁴⁵ than the unique plant protein causing extensive cross-reactions in patients allergic to pollen. Taking the serologic relationships of the ubiquitous plant defense-related proteins into consideration,⁶⁻⁸ it may be reasonable to suspect their universal antigenicity and cross-reactivity.

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REFERENCES

1. Turjanmaa K, Alenius H, Mäkinen-Kiljunen S, Reunala T, Palosuo T. Natural rubber latex allergy. *Allergy* 1996;51:593-602.
2. Williams PB, Buhr MP, Weber RW, Volz MA, Koepke JW, Selner JC. Latex allergen in respirable particulate air pollution. *J Allergy Clin Immunol* 1995;95:88-95.
3. Vallier P, Balland S, Harf R, Valenta R, Deviller P. Identification of profilin as an IgE-binding component in latex from *Hevea brasiliensis*: clinical implications. *Clin Exp Allergy* 1995;25:332-9.
4. Kurup VP, Kelly KJ, Resnick A, Bansal NK, Fink JN. Characterization of latex antigen and demonstration of latex-specific antibodies by enzyme-linked immunosorbent assay in patients with latex hypersensitivity. *Allergy Proc* 1992;13:329-34.
5. Moneo I, Llamazares A, Curiel G, Martinez J. Characterization of latex and chestnut antigens by immunoblotting. *Ann Allergy Asthma Immunol* 1995;75:440-4.
6. Bol JF, Linthorst HJM, Cornelissen BJC. Plant pathogenesis-related proteins induced by virus infection. *Annu Rev Phytopathol* 1990;28:113-38.
7. Linthorst HJM. Pathogenesis-related proteins of plants. *Crit Rev Plant Sci* 1991;10:123-50.
8. Stintzi A, Heitz T, Prasad V, Wiedemann-Merdinoglu S, Kauffmann S, Geoffroy P, et al. Plant 'pathogenesis-related' proteins and their role in defense against pathogens. *Biochimie* 1993;75:687-706.
9. Shewry PR, Lucas JA. Plant proteins that confer resistance to pests and pathogens. *Adv Bot Res* 1997;26:135-92.
10. Broekaert W, Lee H-I, Kush A, Chua N-H, Raikhel N. Wound-induced accumulation of mRNA containing a hevein sequence in laticifers of rubber tree (*Hevea brasiliensis*). *Proc Natl Acad Sci USA* 1990;87:7633-7.
11. d'Auzac J, Bouteau F, Chrestin H, Clément A, Jacob JL, Lacrotte R, et al. Stress ethylene in *Hevea brasiliensis*: physiological, cellular and molecular aspects. *Curr Plant Sci Biotechnol Agric* 1993;16:205-10.
12. Kush A, Goyvaerts E, Chye M-L, Chua N-H. Laticifer-specific gene expression in *Hevea brasiliensis* (rubber tree). *Proc Natl Acad Sci USA* 1990;87:1787-90.
13. Yagami T, Sato M, Nakamura A. Plant defense-related proteins eluting from latex gloves and ammoniated latex: potential latex allergens. *J Nat Rubb Res* 1995;10:100-7.
14. Bolter T. Hydrolytic enzymes in plant disease resistance. In: Kosuge T, Nester EW, editors. *Plant-microbe interactions*. Vol. 2. New York: Macmillan; 1987. p. 385-413.
15. Düring K. Can lysozymes mediate antibacterial resistance in plants? *Plant Mol Biol* 1993;23:209-14.
16. Churngchow N, Suntaro A, Wititsuwannakul R. β -1,3-Glucanase

- isozymes from the latex of *Hevea brasiliensis*. *Phytochemistry* 1995;39:505-9.
17. Chye M-L, Cheung K-Y. β -1,3-Glucanase is highly-expressed in laticifers of *Hevea brasiliensis*. *Plant Mol Biol* 1995;29:397-402.
18. d'Auzac J, Prévôt J-C, Jacob J-L. What's new about lutoids? A vacuolar system model from *Hevea* latex. *Plant Physiol Biochem* 1995;33:765-77.
19. Yagami T, Sato M, Nakamura A, Shono M. One of the rubber latex allergens is a lysozyme. *J Allergy Clin Immunol* 1995;96:677-86.
20. Yagami T, Sato M, Nakamura A, Shono M. Esterase from ammoniated latex: biochemical characterization and antigenicity. *Food Agric Immunol* 1996;8:121-36.
21. Pan S-Q, Ye X-S, Kuc J. Direct detection of β -1,3-glucanase isozymes on polyacrylamide electrophoresis and isoelectrofocusing gels. *Anal Biochem* 1989;182:136-40.
22. Audy P, Grenier J, Asselin A. Lysozyme activity in animal extracts after sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *Comp Biochem Physiol* 1989;92B:523-7.
23. Sunderasan E, Hamzah S, Hamid S, Ward MA, Yeang HY, Cardoso MJ. Latex B-serum β -1,3-glucanase (*Hev b* II) and a component of the microhelix (*Hev b* IV) are major latex allergens. *J Nat Rubb Res* 1995;10:82-99.
24. Jekel PA, Hartmann JBH, Beintema JJ. The primary structure of hevamine, an enzyme with lysozyme/chitinase activity from *Hevea brasiliensis* latex. *Eur J Biochem* 1991;200:123-30.
25. Kelly KJ, Kurup V, Zacharisen M, Resnick A, Fink JN. Skin and serologic testing in the diagnosis of latex allergy. *J Allergy Clin Immunol* 1993;91:1140-5.
26. Yagami T, Sato M, Nakamura A, Shono M. One of the rubber latex allergens has lysozyme activity. Do the allergens correspond to defense-related proteins? In: *Proceedings of the Latex Allergy Symposium*. Toronto, Canada: 1994. Poster No. 15.
27. Alenius H, Kalkkinen N, Lukka M, Reunala T, Turjanmaa K, Mäkinen-Kiljunen S, et al. Prohevein from the rubber tree (*Hevea brasiliensis*) is a major latex allergen. *Clin Exp Allergy* 1995;24:659-65.
28. Lavaud F, Prevost A, Cossart C, Guerin L, Bernard J, Kochman S. Allergy to latex, avocado pear, and banana: evidence for a 30 kd antigen in immunoblotting. *J Allergy Clin Immunol* 1995;95:557-64.
29. Lynn KR, Clevette-Radford NA. Hevains: serine-centred proteases from the latex of *Hevea brasiliensis*. *Phytochemistry* 1986;25:2279-82.
30. Beezhold DH, Sussman GL, Kostyal DA, Chang N-S. Identification of a 46-kD latex protein allergen in health care workers. *Clin Exp Immunol* 1994;98:408-13.
31. Subroto T, Van Koningsveld GA, Schreuder HA, Soedjanaatmadja UMS, Beintema JJ. Chitinase and β -1,3-glucanase in the lutoid-body fraction of *Hevea* latex. *Phytochemistry* 1996;43:29-37.
32. Alenius H, Kalkkinen N, Reunala T, Turjanmaa K, Palosuo T. The main IgE-binding epitope of a major latex allergen, prohevein, is present in its N-terminal 43-amino acid fragment, hevein. *J Immunol* 1996;156:1618-25.
33. Van Parijs J, Broekaert WF, Goldstein IJ, Peumans WJ. Hevein: an antifungal protein from rubber-tree (*Hevea brasiliensis*) latex. *Planta* 1991;183:258-64.
34. Lee H-I, Raikhel NV. Prohevein is poorly processed but shows enhanced resistance to a chitin-binding fungus in transgenic tomato plants. *Braz J Med Biol Res* 1995;28:743-50.
35. Strickland JA, Orr GL, Walsh TA. Inhibition of *Diabrotica* larval growth by patatin, the lipid acyl hydrolase from potato tubers. *Plant Physiol* 1995;109:667-74.
36. Astwood JD, Leach JN, Fuchs RL. Stability of food allergens to digestion in vitro. *Nature Biotechnol* 1996;14:1269-73.
37. Sandiford CP, Tee RD, Newman-Taylor AJ. Identification of cross-reacting wheat, rye, barley and soya flour allergens using sera from individuals with wheat-induced asthma. *Clin Exp Allergy* 1995;25:340-9.
38. Ryan CA. Protease inhibitors in plants: genes for improving defenses against insects and pathogens. *Annu Rev Phytopathol* 1990;28:425-49.
39. Vanek-Krebitz M, Hoffmann-Sommergruber K, Laimer da Camara Machado M, Susani M, Ebner C, Kraft D, et al. Cloning and sequencing of Mal d 1, the major allergen from apple (*Malus domestica*), and its immunological relationship to Bet v 1, the major birch pollen allergen. *Biochem Biophys Res Commun* 1995;214:538-51.
40. Van Loon LC, Pierpoint WS, Boller T, Conejero V. Recommendations for naming plant pathogenesis-related proteins. *Plant Mol Biol Report* 1994;12:245-64.
41. Swoboda I, Scheiner O, Kraft D, Breitenbach M, Heberle-Bors E, Vicente O. A birch gene family encoding pollen allergens and pathogenesis-related proteins. *Biochim Biophys Acta* 1994;1219:457-64.
42. Swoboda I, Dang TCH, Heberle-Bors E, Vicente O. Expression of Bet v 1, the major birch pollen allergen, during anther development: an in situ hybridization study. *Protoplasma* 1995;187:103-10.
43. Swoboda I, Scheiner O, Heberle-Bors E, Vicente O. cDNA cloning and characterization of three genes in the *Bet v 1* gene family that encode pathogenesis-related proteins. *Plant Cell Environ* 1995;18:865-74.
44. Swoboda I, Hoffmann-Sommergruber K, O'Riordáin G, Scheiner O, Heberle-Bors E, Vicente O. Bet v 1 proteins, the major birch pollen allergens and members of a family of conserved pathogenesis-related proteins, show ribonuclease activity in vitro. *Physiol Plant* 1996;96:433-8.
45. Dickinson H. Simply a social disease? *Nature* 1994;367:517-8.