

Natural latex, grass pollen, and weed pollen share IgE epitopes

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Background: Because of the frequent use of natural latex products, IgE-mediated reactions to latex proteins represent an important health threat in industrialized countries. Although several latex allergens have been characterized and IgE cross-reactivities with allergens present in plant-derived food have been described, limited information is available regarding the presence of common IgE-binding components in latex and plant pollen.

Methods: By using serum IgE from 56 individuals with latex allergy, the IgE-binding components in ammoniated latex milk and latex glove extracts were characterized by immunoblotting. The presence of cross-reactive IgE-binding components in the different latex extracts, extracts from mugwort, ragweed, timothy grass pollen, and recombinant birch pollen allergens (Bet v 1 and Bet v 2 [birch profilin]) was studied by immunoblot inhibitions and quantitative competition experiments. The involvement of carbohydrates in the constitution of cross-reactive IgE epitopes was studied by periodate treatment of extracts.

Results: Although sera from certain individuals with latex allergy showed IgE reactivity with protein bands of different molecular weights in Western-blotted latex milk and glove extracts, both extracts contained common IgE epitopes. Although preincubation with recombinant Bet v 1 and Bet v 2 did not significantly inhibit IgE binding to latex proteins, weed and, in particular, timothy grass pollen extract strongly inhibited IgE binding to latex allergens. The cross-reactive IgE epitopes were sensitive to periodate treatment.

Conclusions: Mugwort, ragweed, and timothy grass pollen share IgE epitopes with glycoprotein latex allergens. The presence of common epitopes might in part explain clinical symptoms in patients allergic to pollen on contact with latex. (*J Allergy Clin Immunol* 1997;100:356-64.)

Key words: Latex allergy, timothy grass pollen, mugwort pollen, ragweed pollen, IgE, cross-reactivity

Abbreviations used

kUA/L:	Kilounits of antigen per liter
SDS-PAGE:	Sodium dodecylsulfate-polyacrylamide gel electrophoresis

Proteins from natural latex (*Hevea brasiliensis*) have been recently recognized as a potent source for type I allergic reactions (cutaneous, nasal, bronchial, and systemic reactions).¹⁻⁹ Because of the frequent use of latex products, IgE-mediated reactions to latex represent a health problem of increasing importance.¹⁰ Allergic reactions are frequently observed in certain occupations (medical staff, gardeners)¹¹⁻¹³ and in children with chronic urogenital and neurologic disorders (e.g., spina bifida) that routinely expose them to latex products.^{14, 15} The prevalence of skin test responses to natural latex was 4.2% in Germany,¹⁶ was nearly 4.5% in a Canadian study¹⁷ (estimated in allergy units), and very recently was estimated up to 6.4% in a group of 1000 volunteer blood donors.¹⁸

Because of the relatively high prevalence of latex allergy, considerable effort has been spent in the characterization of latex allergens. The allergenic components found in latex products represent water-soluble proteins and/or glycoproteins that originate from *Hevea brasiliensis*.^{19, 20} Starch powder used for the manufacturing of certain latex products (e.g., gloves) was found to contain no allergenic components but can act as a carrier for latex allergens.^{21, 22} Latex allergens can cause either local reactions (e.g., contact urticaria) or systemic reactions (asthma) when distributed by powders or anaphylaxis during surgery or when inhaled.²⁰ Among the latex allergens studied so far, proteins that seemed to be rather specific for latex such as the rubber elongation factor,²³⁻²⁵ hevein,²⁶ papain,²⁴ and a lysozyme were described.²⁷ On the other hand, several studies report the presence of common IgE-binding structures in certain fruits (banana, avocado, and pear) and latex, pointing to the possibility that allergic symptoms to each of the sources could be partly the result of IgE cross-reactivities.²⁸⁻³⁴ The cross-reactive allergen, profilin, representing a cytoskeletal actin-binding protein, could be purified from latex as an IgE-binding component, but it is present in small amounts in latex extracts.³⁵ A more recently identified complementary DNA clone coding for a low-molecular-weight latex allergen, Hev b 5, might

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represent another component with similarity to fruit proteins.^{36,37} Although several investigators have studied the phenomenon of IgE cross-reactivity between latex and plant food, limited information on the presence of cross-reactive allergens in latex and pollen is available.³⁸

The aim of this study was the characterization of latex allergens in ammoniated latex milk extract and latex glove extracts by IgE immunoblotting with a representative panel ($n = 56$) of well-documented sera from patients with latex allergy. The presence of common IgE epitopes in latex milk and latex glove extracts was studied by IgE inhibition experiments. In addition, IgE-immunoblot inhibition studies with recombinant birch pollen allergens (Bet v 1,³⁹ Bet v 2⁴⁰), mugwort, ragweed pollen extracts, and timothy grass pollen extracts were performed to investigate the presence of cross-reactive allergens in latex and plant pollen.

METHODS

Characterization of patients with latex allergy

Patients with latex allergy ($n = 56$) were characterized by a case history indicative of latex allergy, positive skin prick test results, and positive RAST results. Sera from six individuals without latex allergy (sera 21, 22, 30, 35, 43, and 45) were included as negative controls. The 56 patients with latex allergy (39 women and 17 men) were included in the study in chronological order according to their appearance in the clinics as evidenced by positive anamnesis and skin prick test results. The average age of the patients was 34 years (<20 years, 7 patients; 21 to 30 years, 20 patients; 31 to 40 years, 15 patients; 41 to 60 years, 8 patients; >60 years, 6 patients). Thirty-five patients (62.5%) were health care workers, four patients were children with congenital urogenital or neurologic disorders (e.g., spina bifida), and the remaining 17 patients had occupations that are not normally known to be frequently associated with latex allergy. In 14 patients (25%) the latex sensitization was discovered accidentally during routine skin prick testing (three of these patients were tested because of a congenital disorder known to be frequently associated with latex allergy). The other 42 patients reported immediate symptoms after contact with latex products. Among these patients, 15 showed only contact urticaria when wearing latex gloves, and seven patients had contact urticaria and rhinoconjunctivitis and/or Quincke's edema after blowing into balloons. Twenty patients displayed contact urticaria and/or systemic reactions (conjunctivitis, dyspnea, bronchospasm, asthma, and collapse). Five patients in the group with systemic reactions had reactions (rhinitis, conjunctivitis, urticaria in different location, bronchospasm, collapse) during skin testing. In addition to immediate skin reactions, a type IV allergy (eczema) to latex products could be verified by skin testing in one patient. According to anamnesis and skin testing, 38 (73.1%) of the patients were sensitized against aeroallergens, displaying rhinoconjunctivitis and asthma. Fifteen patients had food allergy as diagnosed by positive case history and RAST results. Skin prick tests were done with different aeroallergens and with a natural latex extract (1:20 wt/vol; ALK, Horsholm, Denmark). The tests were conducted in accordance with the recommendations of the European Academy of Allergy and Clinical Immunology.⁴¹ Table I describes the serologic parameters (total IgE, RAST values, and results from IgE immunoblotting with latex extracts). RAST

classes and kilounits of antigen per liter (kUA/L) are related as follows: <0.35 kUA/L = RAST class 0; 0.36 to 0.7 kUA/L = RAST class 1; 0.8 to 3.6 kUA/L = RAST class 2; 3.7 to 17 kUA/L = RAST class 3; 18 to 50 kUA/L = RAST class 4; 51 to 99 kUA/L = RAST class 5; >100 kUA/L = RAST class 6.

Protein extracts and recombinant allergens

Extracts from ammoniated latex milk. Ammoniated latex milk was kindly provided by Semperit Technical Products, Vienna, Austria. Approximately 0.5 ml of latex milk was extracted in 0.5 ml of sodium dodecyl sulfate (SDS)-sample buffer,⁴² boiled for 5 minutes, and centrifuged at 10,000 g for 5 minutes; and the supernatant was recovered for analysis.

Extracts from latex gloves. Latex examination gloves (Sempermed EXAM; Semperit Technical Products) were cut into pieces and extracted in distilled water (six gloves in 500 ml of water) containing 1 mmol/L of phenylmethylsulfonyl fluoride for 1 hour at 37° C under continuous shaking. Extracts were centrifuged at 10,000 g to remove insoluble particles and were then lyophilized.

Plant pollen extracts. Timothy grass (*Phleum pratense*), mugwort (*Artemisia vulgaris*), and ragweed (*Ambrosia elatior*) were purchased from Allergon, Valinge, Sweden. Pollen was pure as determined by light microscopy. Two grams of pollen were extracted in 50 ml of distilled water under continuous shaking at 37° C for 1 hour. Extracts were centrifuged at 10,000 g to remove debris, and supernatants were lyophilized. The amount and quality of the extracts was estimated by sodium dodecyl-sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)⁴³ and Coomassie Blue⁴⁴ staining with a Rainbow Marker (Amersham, Buckinghamshire, U.K.) as a standard.

Recombinant allergens. Recombinant Bet v 1³⁹ and Bet v 2 (birch profilin)⁴⁰ were expressed in *Escherichia coli* DE3 (BL21) using plasmid pET (Novagen, Madison, Wis.) as nonfusion proteins. Recombinant Bet v 1 was purified by conventional chromatography to homogeneity (Susani et al., unpublished data). Recombinant Bet v 2 was purified by poly(L-proline) affinity chromatography as described by Susani et al.⁴⁵

SDS-PAGE and immunoblotting

Approximately 100 µg/cm protein extract (latex milk, glove, mugwort, ragweed, and timothy grass pollen extract) were separated by preparative 12.5% SDS-PAGE.⁴³ Proteins were then blotted onto nitrocellulose (Schleicher & Schuell, Dassel, Germany).⁴⁶ Nitrocellulose strips approximately 5 mm wide were blocked two times for 5 minutes and once for 30 minutes in buffer A (50 mmol/L Naphosphate, pH 7.5, 0.5% vol/vol Tween-20, 0.05% weight/volume NaN₃). After blocking, strips were incubated with 1:10 in buffer A-diluted sera from patients with latex allergy and a nonallergic control subject overnight at 4° C. After washing in buffer A, bound IgE was detected with 1:10 in buffer A diluted iodine 125-labeled anti-human IgE antibodies (Pharmacia RAST; Uppsala, Sweden) overnight at room temperature. After washing in buffer A, strips were dried and exposed to Kodak XOMAT film with intensifying screens (Kodak, Heidelberg, Germany) at -70° C between 24 hours and 5 days.

Immunoblot inhibition and quantitative CAP inhibition experiments

Sera from patients with latex allergy and a nonallergic control subject were diluted 1:10 in buffer A. One milliliter of diluted serum was preincubated overnight at 4° C with 5 µg of rBet v 1, 5 µg of rBet v 2, 50 µg of latex glove extract, 30 µg of mugwort pollen extract, 30 µg of ragweed pollen extract, and 30 µg of

TABLE I. Serologic characterization of patients with latex allergy

Patient No.	Total IgE (kU/L)	Latex CAP (kUA/L)	Latex milk Wb (MW kd)	Latex Glove Wb (MW kd)	g6CAP (kUA/L)	w1CAP (kUA/L)	w6CAP (kUA/L)	rBet v 1 (kUA/L)	rBet v 2 (kUA/L)
1	529	82.5	15	69 30 15 10	5.2	0.4	2.9	3.3	0
2	196	13.7	50/60 30 14	90 60 30	15.9	16.1	14.6	0.9	0.9
3	18	3.9		10	0	0	0	0	0
4	197	1			1.1	0.4	0	0	0
5	503	3.7	50/60 30		100	4.6	4.9	4.8	0
6	133	15.7	15	90 60 50 30 15	ND	0	0	0	0
7	458	10		10	100	0.9	0.8	28.8	0.4
8	161	1.2	50/60		91.2	18	10.8	100	0
9	101	19.3		10	0	0	0	0	0
10	211	4.5	50/60 30	90 60	5.3	4.3	4.1	0	0
11	2000	24.4	50/60 30	90 60 30	97	26	20.1	100	1.7
12	2000	48	50 15	10	100	40.3	39.1	100	55.8
13	ND	8.5		90 60 50	16.3	0	0	86.4	0
14	251	1.7	50		7.1	2.1	1.6	0	0
15	37	0.7			0	0	0	0	0
16	964	4.4			5.6	0	0	28.9	0
17	80	0		90 50	0	0	0	0	0
18	ND	9.8	60 15	15	ND	0	0	0	0
19	22	0.9			0	0	0	0	0
20	339	6.5		10	1	2.9	6.6	4	5.3
21	ND	0			0	0	0	0	0
22	ND	0			ND	0	0	0	0
23	189	4.4	50/60 30	60 50	4.9	4.6	4.1	0.5	0.6
24	ND	38.4		90 60 30 10	28.1	0	0	60.8	0
25	69	10		10	6	0	0	0	0
26	144	0.5			33	1.5	0.9	10.9	0.6
27	489	5.1	15		0.4	0.4	4	0	0
28	313	47.6	60 15	90 60 30	0	0	0	0	0
29	206	34.1		90 60 30	6.7	0	0	0	0
30	ND	0			2.1	0	0	6.3	0
31	358	7.1		90 60 30 10	5.7	1.7	0	2.5	2.5
32	12684	100	50/60 30 10	30 10	100	5.1	8.2	100	1
33	610	100	90 50/60 30 15	90 60 30 15 10	0	0	0	3.1	0
34	124	15.6	50/60 30	90	17.2	15.9	14.7	0.5	0.9
35	ND	0			0.5	0.4	0	0	0
36	ND	3.9		10	4	3.1	3.5	0	0
37	376	0			0.9	0	0	12.8	0
38	1500	21.4	50/60 30	90 40	ND	27.8	18.8	1	1.2
39	356	2.1		90 60 30	88.1	0	0.5	40	0
40	ND	2.8			2.1	0	0	0	0
41	ND	3.6		30 10	0	0	0	28.3	0
42	1328	2.3	10	30 10	100	39.1	18.3	4.6	0
43	ND	0			ND	0	0	0	0
44	141	0			0	0	0	2.2	0
45	ND	0			0	0	0	0	0
46	2000	62.8	10	30 10	0.5	0	0	0	0
47	3377	2.2			64	0	0.4	0	0
48	31	8.1	15	90 50	0	0	0	0	0
49	ND	4.5		90 60 30	0	0	0	38.7	0
50	512	62.6	60		0	0	0	0	0
51	6	1.2			0	0	0	0	0
52	13	0.8			0	0	0	0	0
53	64	1.4		10	2	0	0	3.1	0
54	ND	13.4	15	90 50 30	2.6	3.3	0.7	0	0
55	134	35.7	10	10	0	0	0	0	0
56	996	6.7	15		ND	2.8	8.7	100	0
57	1240	100	50/60 15	90 60 30 10	100	2.2	2	0.8	0
58	140	6.2	15		43.1	1	0.7	0	0
59	372	0.8			100	0	1.2	0	0
60	348	3.9	15	10	1.6	0	0	19	0
61	ND	3.6			18.2	0	0.4	63.5	0
62	68	2.1	15	90 60	0	0	0	0	0

Wb, Western blot; MW, molecular weight; g6, timothy grass pollen; w1, ragweed pollen; w6, mugwort pollen; ND, not done.

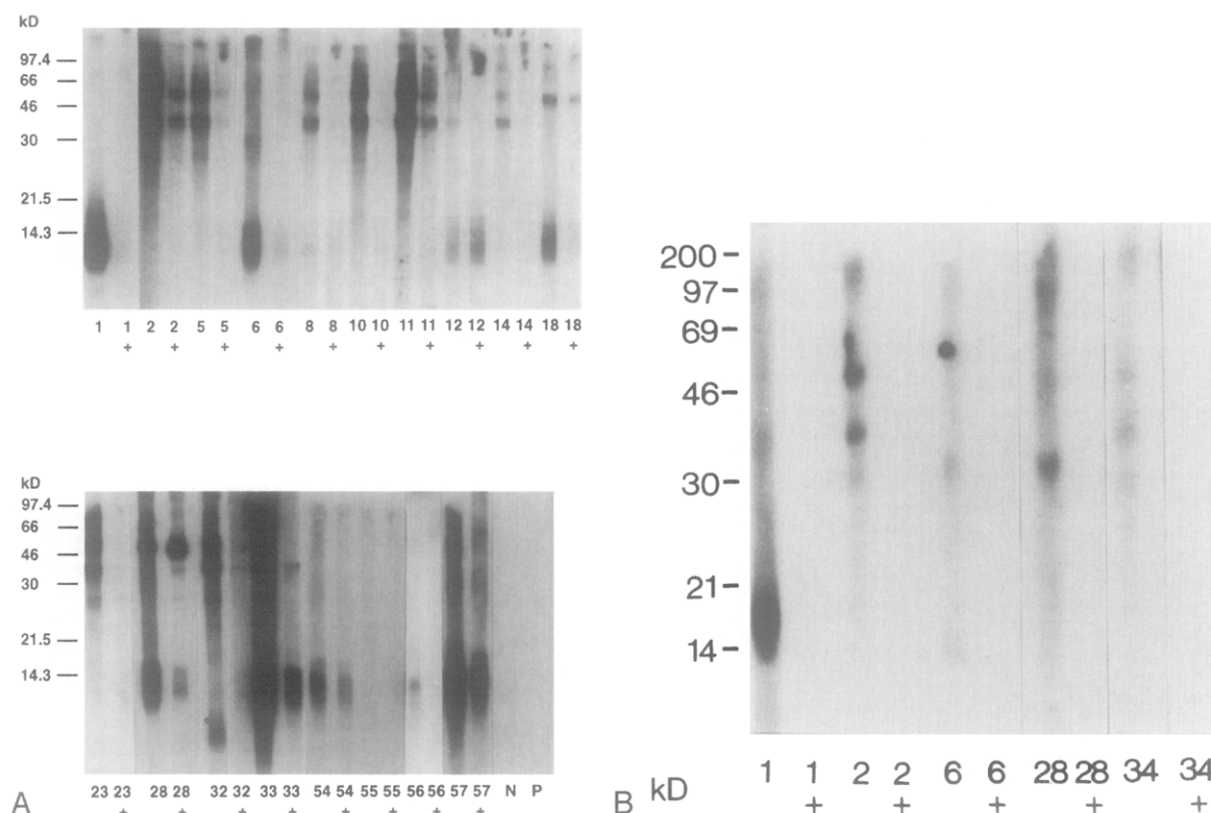


FIG. 1. Sera from 23 patients with latex allergy (numbered as in Table I) were preincubated with bovine serum albumin or latex glove extract (+) before probing with nitrocellulose-blotted latex milk extracts (**A**). Sera were preadsorbed with nitrocellulose-blotted latex milk proteins (+) or bovine serum albumin before incubation with blotted glove extract (**B**). Bound IgE was detected with ^{125}I -labeled anti-human IgE antibodies and visualized by autoradiography. Lane N represents a control with serum from a nonallergic individual, and lane P represents the buffer control without addition of serum.

timothy grass pollen, respectively. Preadsorbed sera were then used to detect nitrocellulose-blotted latex allergens as described for immunoblotting. A nonspecific inhibition of IgE binding on preincubation with the recombinant allergens and extracts was excluded by control experiments, demonstrating that IgE binding to unrelated nitrocellulose-blotted allergens (cat hair/dander extract) was not affected by the procedure. To preadsorb sera with latex milk extracts, preincubation was done with nitrocellulose strips containing blotted milk extract (approximately 50 μg) or, for control purposes, a comparable amount of bovine serum albumin. Quantitative IgE inhibitions were done by using the CAP system (Pharmacia). Sera were preadsorbed with 50 μg of ragweed, mugwort, or timothy grass pollen extract and, for control purposes, with a comparable volume of 0.9% sodium chloride solution overnight at 4°C. Latex-specific IgE levels were then measured in the CAP system.

Periodate treatment

To determine whether carbohydrates may play a role in the constitution of IgE epitopes of latex allergens, blotted extracts were treated with sodium m-periodate (Sigma, St. Louis, Mo.) before incubation with serum IgE.⁴⁷ Nitrocellulose strips containing latex milk and glove extract were incubated in 50 mmol/L sodium acetate buffer pH 4.5, 10 mmol/L sodium m-periodate, or, for control purposes, without sodium m-

periodate at room temperature for 1 hour. Nitrocellulose strips were then blocked in buffer A two times for 5 minutes and once for 30 minutes and exposed to serum IgE as described for immunoblotting.

RESULTS

RAST analysis of sera from patients with latex allergy

In 53 of the 56 patients with latex allergy IgE antibodies against latex could be measured by the CAP system. Among the patients with positive CAP results, 14 had a RAST class of greater than 4, 23 had a RAST class of 3, 14 had a RAST class of 2, and two had a RAST class of 1. Only three patients with positive skin prick test results (patients 17, 37, and 44) had negative results in the CAP assay. In 37 patients additional RAST testing with kiwi, banana, walnut, and avocado extracts was done. IgE antibodies against kiwi were found in 13 patients, against banana in 19 patients, against walnut in 13 patients, and against avocado in 16 patients. Twenty-five patients with latex allergy had positive results on the ragweed RASTs (11 with a RAST class >3) and 26 had positive results on the mugwort RASTs (14 with a RAST class >3). Thirty-eight patients displayed IgE reactivity in the timothy

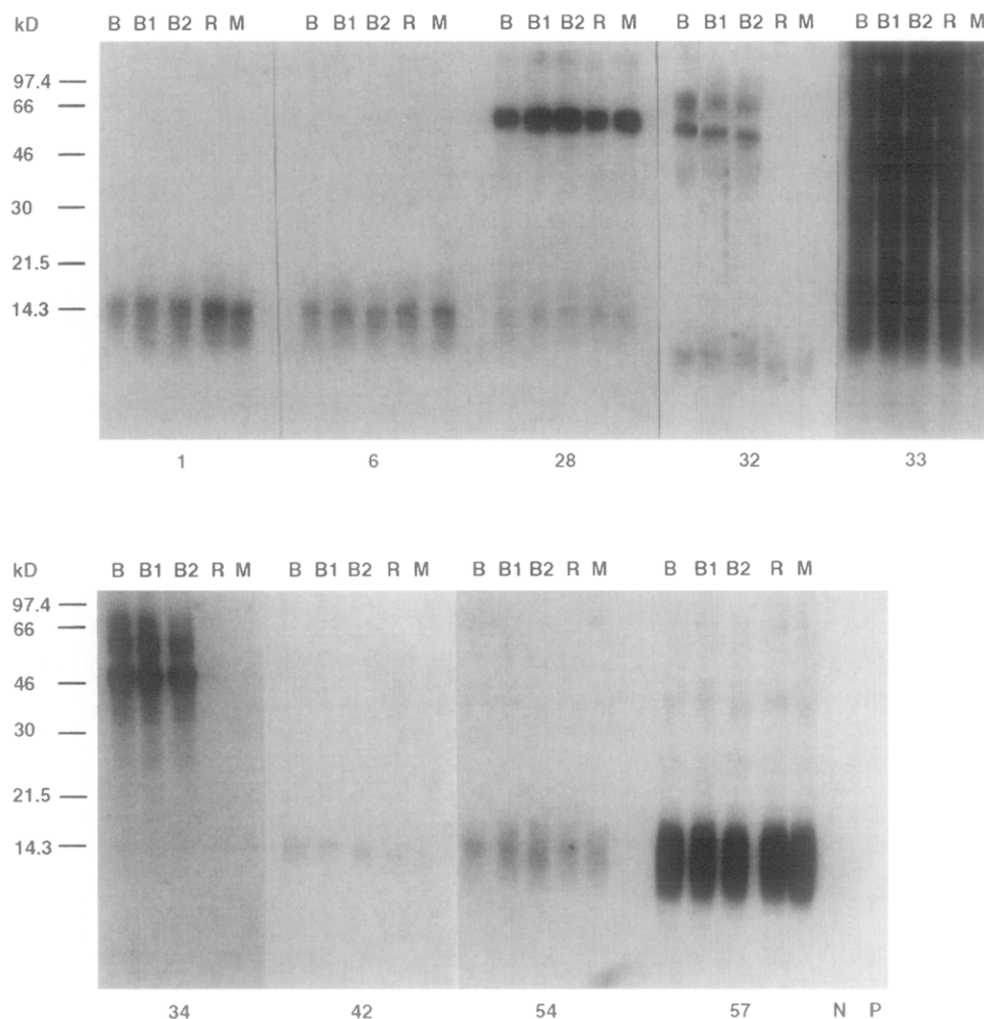


FIG. 2. Inhibition of IgE binding to milk proteins by rBet v 1, rBet v 2, mugwort, and ragweed extract. Sera from nine individuals with latex allergy (numbered as in Table I) were preincubated with bovine serum albumin (*lane B*), rBet v 1 (*B1*), rBet v 2 (*B2*), ragweed pollen extract (*R*), or mugwort pollen extract (*M*), before probing for IgE reactivity with nitrocellulose-blotted latex milk proteins. *Lane N* represents a control with the serum from a nonallergic individual, and *lane P* represents the buffer control without addition of serum.

grass pollen RAST, 30 were positive for rBet v 1, and 11 were positive for rBet v 2. No significant association of elevated serum IgE and latex-specific IgE was found.

IgE reactivity profile of sera from individuals with latex allergy to nitrocellulose-blotted latex milk and glove extracts

The IgE reactivity profile of the patients with latex allergy with nitrocellulose-blotted ammoniated latex milk proteins and glove extracts is summarized in Table I. Components with a molecular weight of approximately 90, 50 to 60, 30, 15, and 10 kD were detected by using patient's IgE antibodies. In most cases sera bound to components of different molecular weights in ammoniated latex milk and glove extracts. For example, serum 1 bound to a 15 kD component in latex milk and to 60, 30, 15, and 10 kD components in glove extract. Serum 12 reacted with a 50 and 15 kD component in latex milk and a 10 kD component

in glove extract. Forty-four of the 56 patients with latex allergy reacted to nitrocellulose-blotted latex allergens. Those sera that showed no detectable binding had a latex-specific RAST less than 3. However, one serum that tested negative in the RAST assay displayed IgE reactivity to nitrocellulose-blotted latex allergens (serum 17). Certain sera bound to glove extract but not to latex milk extracts (e.g., sera 3, 7, 9, 17, 20, 25, 36, 39, 41, 49, 53), whereas other sera reacted with milk proteins but not with glove extract (e.g., sera 8, 14, 27, 50, 56, and 58). These results indicated the presence of common, and also different, IgE epitopes in latex milk and glove extracts.

Ammoniated latex milk and latex glove extracts share IgE epitopes

The recognition of components with different molecular weight in latex milk and glove extract by serum IgE, as well as the selective reactivity of certain sera to milk or glove

extract, led us to investigate whether milk extract and glove extract contain similar or different IgE epitopes. Sera from 23 patients with latex allergy that had detected components of different molecular weight in milk and/or glove extracts were preadsorbed with glove extract before probing with nitrocellulose-blotted milk extract (Fig. 1, *A*). Latex glove extract completely inhibited IgE binding to milk proteins in six sera and substantially reduced IgE binding in the other sera tested. Although these results indicate that latex milk and glove extract share most IgE epitopes, certain epitopes appeared to be present in lower amounts or not at all in the glove extract, perhaps because of the manufacturing process. This result was confirmed by the reverse experiment. Preadsorption of sera with nitrocellulose-blotted latex milk extracts completely inhibited IgE binding to blotted glove extract in all (5 of 5) sera tested (Fig. 1, *B*).

Mugwort and ragweed pollen contain allergens that share IgE epitopes with latex

To investigate the presence of common IgE epitopes in latex and weed pollen, immunoblot inhibition experiments were performed (Fig. 2). Six representative sera from individuals with latex allergy that had low to high RAST classes in response to ragweed and mugwort pollen and three latex sera without RAST-detectable IgE to ragweed and mugwort were selected. The sera were preincubated with ragweed pollen extract, mugwort pollen extract, recombinant Bet v 1 (the major birch pollen allergen), and recombinant birch profilin (rBet v 2). In two of the six sera a substantial inhibition of IgE binding to high-molecular-weight (30 to 90 kD) latex allergens was obtained with ragweed and mugwort pollen extract. Recombinant Bet v 1 did not inhibit IgE binding to latex allergens in any of the sera tested, although sera 1, 32, 33, 34, 42, and 57 contained Bet v 1-specific IgE. No inhibition of IgE binding to latex allergens was observed after preincubation with rBet v 2; however, only sera 32 and 34 contained profilin-specific IgE (RAST <2).

Timothy grass pollen extract strongly inhibits IgE binding to nitrocellulose-blotted latex milk proteins

Six latex sera that contained high levels of timothy grass pollen-specific IgE were selected for immunoblot inhibition experiments. Sera were preadsorbed with timothy grass pollen extract before probing for IgE binding to nitrocellulose-blotted latex milk proteins (Fig. 3). Preadsorption with timothy grass pollen extract completely inhibited IgE binding of four sera to latex allergens of high molecular weight (30 to 90 kD). Only serum 54, which reacted with the 15 kD component of latex, was not inhibited. A nonspecific inhibition of IgE binding by the timothy grass pollen extract was excluded by a control experiment, showing that IgE binding to unrelated cat hair or dander allergens was not inhibited (data not shown). No IgE reactivity to the nonallergic control serum or the buffer control was observed. Preincubation of sera with latex extract did not lead to a

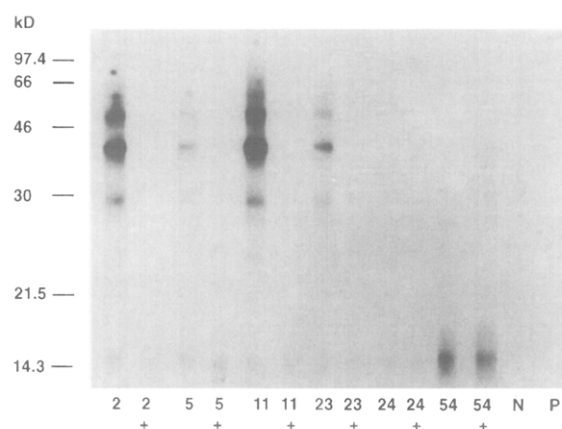


FIG. 3. Inhibition of IgE binding to latex milk with timothy grass pollen extract. Sera from six individuals with latex allergy (numbered as in Table I) were preincubated with bovine serum albumin or timothy grass pollen extract (*lane +*) before probing with nitrocellulose-blotted latex milk extract. *Lane N* represents a control with serum from a nonallergic individual, and *lane P* represents the buffer control without addition of serum.

substantial inhibition of IgE binding to nitrocellulose-blotted timothy grass pollen extract, indicating that timothy grass pollen extract contained more or other IgE epitopes (data not shown).

Preincubation of sera from patients with latex allergy with weed and grass pollen extracts inhibits a high percentage of latex-specific IgE

To estimate the amount of latex-specific IgE that cross-reacts with weed or grass pollen extracts, quantitative CAP inhibition studies were performed (Table II). Although in certain sera (19, 25, and 54) IgE binding to latex was almost not inhibited, a high or almost complete inhibition of IgE reactivity to latex was observed after preincubation of other sera (2, 5, 8, 11, 23, and 34) with weed or grass pollen allergens. In the latter sera, the high degree of inhibition of IgE binding to latex was not associated with high or low levels of latex-specific IgE antibodies (Table I). It was noted that a high percentage of the sera (5 of 7 sera; 2, 5, 8, 11, and 23), that were significantly inhibited by pollen extracts, was derived from patients with accidentally diagnosed latex allergy. Only sera 32 and 34 were obtained from patients with primary contact urticaria in response to latex. In contrast, all of the sera that did not cross-react between latex and pollen allergens were obtained from patients with primary clinical symptoms of latex allergy (sera 1, 6, 19, 25, 28, 33, 42, and 54).

Sensitivity of IgE epitopes present in natural latex to periodate treatment

Several cross-reactive plant allergens are known to represent glycoproteins.⁴⁸ To investigate the effects of deglycosylation on IgE binding to latex milk and glove proteins, nitrocellulose-blotted extracts underwent periodate treatment. IgE-binding components of 30 to 60

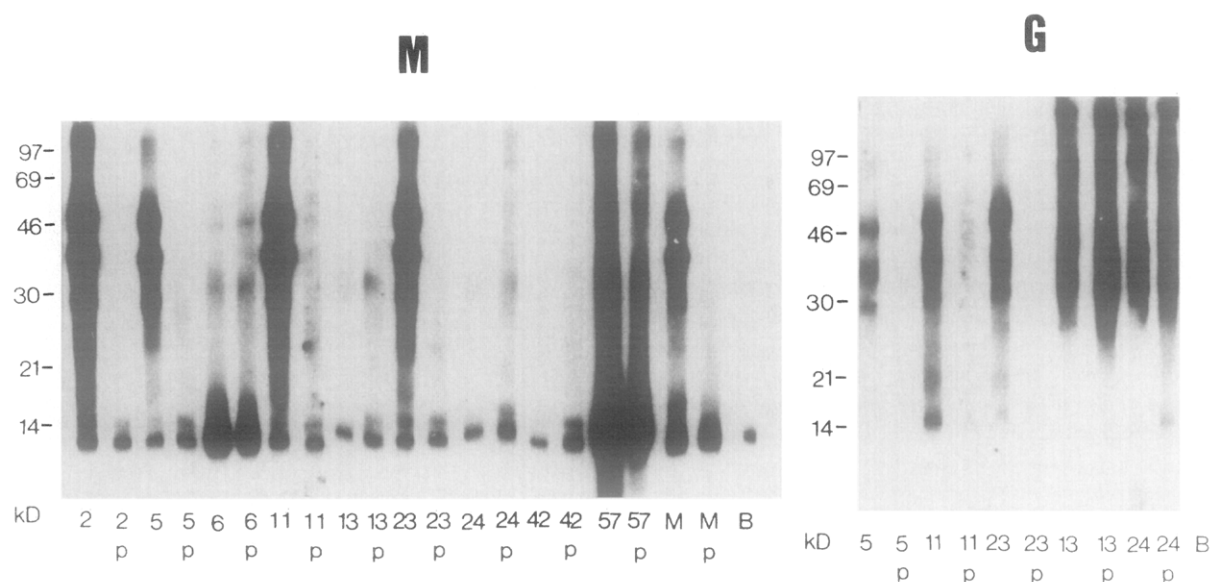


FIG. 4. Influence of periodate treatment on IgE binding to nitrocellulose-blotted latex milk extract and latex glove extract. Latex milk extract (M) and latex glove extract (G) were separated by SDS-PAGE and blotted onto nitrocellulose. Strips were preincubated with periodate (p) or sodium acetate buffer before incubation with sera from patients with latex allergy (numbered as in Table I), serum from an individual with mugwort allergy (M), or buffer (B). Bound IgE was detected with ^{125} I-labeled anti-human IgE antibodies.

TABLE II. Inhibition of IgE binding to latex allergens after preadsorption with ragweed, mugwort, or timothy grass pollen extract determined by quantitative CAP measurements

Serum No.	Latex CAP (kUA/L)	Latex CAP + ragweed (kUA/L)	Inhibition (%)	Latex CAP + mugwort (kUA/L)	Inhibition (%)	Latex CAP + timothy (kUA/L)	Inhibition (%)
2	13.7	<0.35	100	<0.35	100	<0.35	100
5	3.8	<0.35	100	<0.35	100	<0.35	100
8	1.3	<0.35	100	<0.35	100	<0.35	100
11	27.9	4.2	85	5.8	79	2.9	90
19	0.6	0.6	0	0.6	0	0.6	0
23	3.9	<0.35	100	<0.35	100	<0.35	100
25	7.2	6.8	6	6.8	6	6.8	6
34	17.8	<0.35	100	<0.35	100	<0.35	100
54	10.2	10.0	2	8.5	17	10.2	0

Inhibition of IgE binding to <0.35 kUA/L was considered 100% inhibition.

kd present in latex milk and glove extracts detected by the cross-reactive sera 2, 5, 11, and 23 contained IgE epitopes that were sensitive to periodate treatment (Fig. 4). Serum from a patient with mugwort allergy (M) containing IgE specific for a previously described 60 kd cross-reactive mugwort allergen⁴⁹ bound to similar bands in milk extract, which also contained periodate-sensitive IgE epitopes. Certain high-molecular-weight components detected by serum 57 in milk extract and sera 13 and 24 in glove extract and low-molecular-weight allergens (10 to 14 kd) identified by sera 6, 57, and M were not sensitive to periodate treatment. In the case of three sera (13, 24, and 42) IgE binding to milk proteins was slightly increased after periodate treatment.

DISCUSSION

Type I allergy to latex proteins has been recognized as an important health threat in industrialized countries.¹⁰ In certain groups of patients (children with congenital disorders) and occupations (health care workers) an increased prevalence of latex allergy, possibly related to the frequent exposure to latex, has been noticed.¹³⁻¹⁵ On the other hand, a relatively high prevalence of latex allergy was also reported in a group of 1000 volunteer blood donors.¹⁸ Water-soluble proteins from *Hevea brasiliensis* are easily extracted from latex products and are known to lead to local and systemic anaphylactic reactions in sensitized individuals.^{19, 50-52} While several latex allergens, such as the rubber elongation factor²³ and

prohevein,²⁶ were characterized by protein chemical techniques and appear to be rather specific for latex, other latex allergens were found to share IgE epitopes with plant-derived food, in particular banana and avocado.²⁸⁻³³ These findings imply that sensitivity to latex may at least be in part because of sensitization against homologous allergens present in other plant products. Using IgE immunoblotting, we studied the IgE-reactivity profile of a representative number of patients with latex allergy ($n = 56$) with latex milk and glove extracts. No association of a certain IgE-reactivity profile to blotted latex allergens and the frequency of systemic reactions or certain symptoms on contact with latex was noted.

A considerable variability regarding the molecular weights of the IgE-binding components in latex milk and glove extract was observed. This finding is in agreement with a previous study performed with one serum pool of patients with latex allergy and extracts from nonammoniated latex, ammoniated latex, and latex glove extracts.⁵³ Despite the great variability in the molecular weights of IgE binding components in milk and glove extracts, IgE-immunoblot inhibition experiments demonstrated that latex milk and glove extracts shared IgE epitopes. The fact that glove extract only partially inhibited IgE binding to latex milk extract, whereas milk extract completely inhibited IgE binding to glove extract, indicated that latex milk contains more IgE-binding determinants. One explanation for this finding would be that IgE epitopes may be lost during the rather harsh manufacturing process. This explanation supports the hypothesis that sensitization to latex proteins may be caused primarily by the original natural plant products rather than by the manufactured latex products.

Several studies have demonstrated the presence of common IgE epitopes in latex and plant-derived food (fruits and vegetables) and point to the possibility that certain patients might have been sensitized originally against either latex or plant food proteins and show IgE cross-reactivity as a consequence of this sensitization. Although several studies have demonstrated the presence of common IgE epitopes in pollen and plant-derived food,⁵⁴ little is known regarding the presence of cross-reactive allergens in pollen and latex. We have therefore studied the presence of IgE epitopes in latex, grass, and weed pollen by IgE immunoblot inhibitions and have estimated the degree of cross-reactivity by quantitative CAP inhibitions. Recombinant birch pollen allergens (rBet v 1,³⁹ birch profilin, and rBet v 2⁴⁰), which were shown to share IgE epitopes with homologous allergens in plant-derived food (fruits and vegetables) and mugwort, ragweed, and timothy grass pollen extracts containing a broadly cross-reacting component of approximately 60 kd^{49,55} were used to preadsorb sera. Although profilin could be purified as an IgE binding component from latex,³⁵ no significant inhibition of IgE binding was observed when rBet v 2 was used for preadsorption, presumably because of the low amount of profilin in latex extracts. Recombinant Bet v 1 also did not lead to an inhibition of IgE binding to latex milk extracts. In contrast, a substantial inhibition of IgE binding to high-

molecular-weight latex allergens (30 to 60 kd) was observed on preincubation of sera with mugwort, ragweed, and timothy grass pollen extract. Timothy grass pollen extract almost completely inhibited IgE binding to latex allergens, whereas latex extract did not lead to a substantial inhibition of IgE binding to timothy grass pollen extract (data not shown). The high degree of cross-reactivity as evidenced by an up to 100% inhibition of IgE binding to latex CAPs was, however, observed preferentially in a group of patients in whom latex allergy was diagnosed accidentally (patients 2, 5, 11, and 23) compared with patients who were frequently exposed to latex products. The more frequent occurrence of cross-reacting antibodies in sera of the accidentally diagnosed patients suggests the existence of two types of individuals with latex allergy. One group of patients might be sensitized preferentially against pollen and other plant products and display symptoms of allergy to latex caused by cross-reactive IgE antibodies, whereas a second group of patients might be originally sensitized to latex and display a low degree of cross-reactivity to pollen allergens.

The further investigation into whether the IgE epitopes of the high-molecular-weight (30 to 60 kd) cross-reactive latex allergen might be sensitive to periodate treatment showed that carbohydrates contribute to the IgE-binding capacity of these epitopes. A final conclusion as to whether the glycan moieties represent direct targets for the cross-reactive IgE antibodies or are necessary to maintain the polypeptide backbone of the cross-reactive structures cannot be drawn from the experiments. The similar molecular weights of the cross-reactive latex allergen and a previously described cross-reactive 60 kd mugwort glycoprotein allergen⁴⁹ and the fact that one patient's serum IgE, which recognized the 60 kd mugwort allergen, also bound to comparable component in latex indicate that both components might be related.

In conclusion, we have identified a high-molecular-weight cross-reactive glycoprotein allergen in timothy grass pollen, weed pollen, and latex and have provided an explanation for sensitivity to latex in certain individuals with pollen allergy. The substantial inhibition of IgE binding to latex allergens after preadsorption with pollen extract even indicates that these patients constitute a group of patients with latex allergy who were initially sensitized against pollen.

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