

IgE immune response to rubber proteins in adult patients with latex allergy

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In the present immunoblot study we examined IgE immune responses to natural rubber latex proteins in sera from 27 adult patients with latex allergy. We demonstrated at least 30 proteins in the rubber tree sap, of which 19 bound IgE antibodies in a heterogeneous manner in patient sera. Twenty-two of the 27 patient sera (81%) showed positive immunoblot test results. IgE antibodies in the patient sera bound most frequently to a 20 kd allergen, which was recognized by 17 of the 22 (77%) patient sera with positive immunoblot test results. These results suggest that a 20 kd rubber protein could be a major allergen in adult patients with latex allergy. Immunoblotting with a representative set of allergens as antigen would offer a sensitive, confirmatory in vitro test to demonstrate hypersensitivity to latex. (J ALLERGY CLIN IMMUNOL 1994;93:859-63.)

Key words: IgE, natural rubber, latex, allergy, immunoblotting

The importance of IgE-mediated allergy to natural rubber latex (NRL) has emerged from the increasing number of reported cases of latex allergy during the past 10 years. There is general agreement that in patients with latex allergy, proteins eluted from different natural rubber products can cause in type I hypersensitivity reactions, such as contact urticaria, rhinitis, asthma, anaphylaxis, and even death.¹⁻⁵ In addition to hospital employees who become sensitized by occupational exposure,⁶ other individuals can become sensitized to nonmedical natural rubber products such as balloons, condoms, dummies, and household gloves.⁷⁻¹⁰ Skin prick testing with surgical glove eluates and extracts of natural rubber has been shown to be sensitive for identification of patients with latex allergy. Previous in vitro studies for latex allergy have mainly been based on latex RAST, although commercial latex RAST (Phar-

Abbreviation used

NRL: Natural rubber latex

macia, Uppsala, Sweden) detects IgE antibodies in only about 50% to 70% of patients with latex allergy.¹¹ We have previously demonstrated IgE and IgG₄ antibodies against several rubber proteins in patients with latex allergy by immunoblotting.^{12, 13} The aim of the present study was to study latex-binding IgE antibodies in a larger group of patients and to further characterize allergenic rubber proteins.

METHODS

Patients and control subjects

Sera were obtained from 27 adult patients with latex allergy, ranging in age from 29 to 65 years, and 30 control subjects with no history of latex allergy. The patients had positive and the control subjects had negative reactions to a prick test with an eluate of latex gloves (Exona; Semperit, Vienna, Austria). The glove extract was prepared and the prick test was performed as previously described.⁶ Clinical histories of the patients are outlined in Table I.

Rubber extract

The sap of the rubber tree *Hevea brasiliensis* was collected in Malaysia and prepared as previously described.¹² Total protein concentration of the extract

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TABLE I. Clinical manifestations and laboratory test results of 27 patients with latex allergy

Patient No.	Sex	Age (yr)	Main clinical manifestations	Latex skin prick test*	Serum total IgE (kU/L)	Latex RAST (kU/L)	IgE immunoblot test†
1	F	38	CU,E,FE,GU,A	4+	396	20,0	+
2	F	65	E	3+	2442	11,0	+
3	F	45	E	3+	2479	21,0	+
4	F	39	CU,ES	3+	256	<0,4	+
5	F	49	CU,ES,FE,GU	3+	64	1,4	+
6	F	47	CU,E	4+	797	5,0	+
7	M	31	CU	2+	17200	2,2	+
8	F	37	AR	2+	641	3,3	+
9	F	32	CU,E	4+	1842	3,0	+
10	F	43	CU,E,FE	4+	102	4,7	+
11	F	46	CU	3+	414	1,6	+
12	M	32	CU	2+	1890	<0,4	+
13	F	37	CU,E	4+	7075	18,0	+
14	M	46	CU,E	3+	560	2,9	+
15	F	32	CU,FE,GU,AR	3+	2867	18,0	+
16	F	37	E	2+	28	<0,4	+
17	F	32	CU,FE	4+	259	8,3	+
18	F	35	CU,ES,FE	3+	47	<0,4	+
19	F	29	ES,FE	3+	82	3,4	+
20	F	43	R	3+	287	<0,4	+
21	F	42	CU,ES,FE,R	3+	1546	3,9	+
22	F	32	CU	3+	136	2,7	+
23	F	34	CU	2+	19	<0,4	—
24	F	45	CU,E	3+	11	<0,4	—
25	F	63	CU,R,A	3+	12	<0,4	—
26	F	35	CU,E	3+	165	<0,4	—
27	F	46	CU,E,ES	3+	6	<0,4	—

CU, Contact urticaria; E, eczema; FE, facial edema; GU, generalized urticaria; A, asthma; ES, eye symptoms; AR, anaphylactic reaction; R, rhinitis.

*Latex skin prick test scores 0 to 4+.

†Patient showing binding of IgE to one or several latex proteins in immunoblotting is indicated as +, and patient showing binding of IgE to none of the latex proteins is indicated as —.

(NRL) was determined with Lowry's method¹⁴ with bovine γ -globulin as the standard (Bio-Rad Laboratories, Chemical Div., Richmond, Calif.).

Immunoblot and total protein staining assays

The immunoblot method used in the present study has been described in detail earlier.¹² Briefly, the dissolved rubber samples were electrophoresed through a 12% sodium dodecylsulfate-polyacrylamide gel and then transferred electrophoretically from the gel to nitrocellulose membranes (Trans-Blot, Bio-Rad Laboratories, Chemical Div.). Thereafter, the membranes were cut into strips that were incubated first with the patient and control sera (diluted 1:5) and then with biotinylated anti-human IgE antibody (Vector, Burlingame, Calif.; diluted 1:2000) and finally with streptavidin-conjugated alkaline phosphatase (Bio-Rad Laboratories, Chemical Div.; diluted 1:5000). Finally, color

development solution was added, and the strips were incubated for 6 minutes.

Total protein staining of the rubber extract was performed with a commercial kit (Blotting Detection Kit for Total Protein; Amersham, Amersham, U.K.) according to the manufacturer's instructions. Protein concentrations of the rubber protein samples were adjusted to 450 μ g/ml before immunoblotting and total protein staining.

Latex RAST

A commercially available latex RAST CAP kit (Pharmacia) was used according to the manufacturer's instructions, and the results were expressed as kilounits per liter. The cutoff level for positivity was 0.4 kU/L.

RESULTS

At least 30 protein bands, with apparent molecular weights ranging from 14 to 200 kd, were

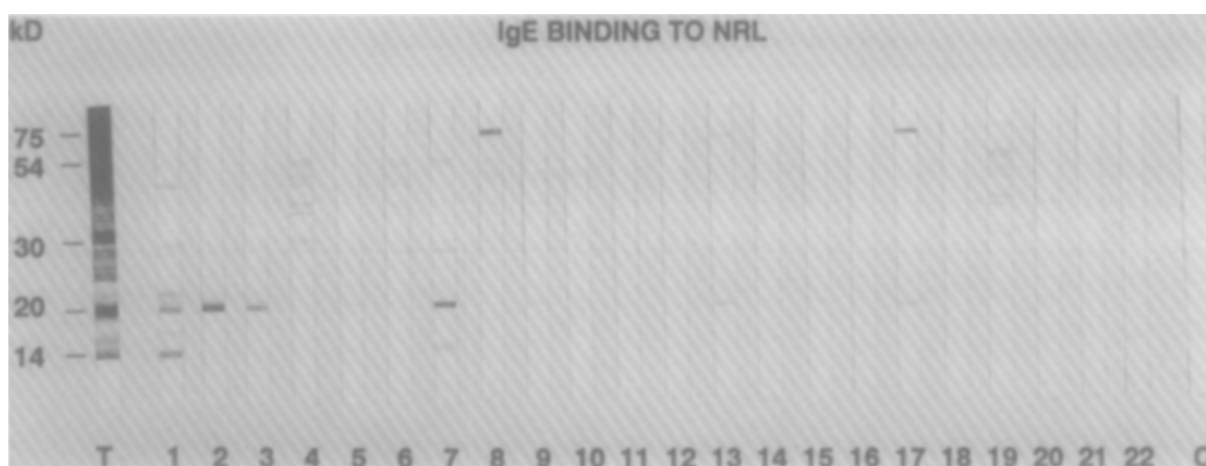


FIG. 1. Binding of IgE antibodies from 22 immunoblot-positive patient sera (*strips 1 to 22*) and one control serum (*C*) to NRL. A strip used for total protein staining is denoted as *T*. Molecular weights (in kilodaltons) of some of the significant allergens are given on the left side of the column.

demonstrated in NRL by total protein staining (Fig. 1). IgE antibodies from the patient sera bound in a heterogeneous manner to 19 proteins in NRL (Fig. 1). An immunoblot test result was considered positive if binding of IgE to at least one protein was demonstrated. Twenty-two of the 27 patient sera (81%) and 1 of 30 control sera showed positive immunoblot test results. Positive test results were found most frequently against a 20 kd allergen that bound IgE antibodies in 17 of the 22 (77%) patient sera with positive test results in immunoblotting. Common allergens were also 14, 30, 36, 39, 44, 46, 54, and 75 kd proteins. A pattern of binding to six allergens (30, 36, 39, 44, 46, and 54 kd) was found in five patient sera (patients 3, 4, 6, 8, and 19 in Table II). However, no particular clinical symptoms were evident in this group. IgE antibodies from the one control serum showing a positive immunoblot test result bound the 20 kd protein in NRL. Results of commercial latex RAST with the CAP method were positive in 17 of the 27 (63%) patients (Table I) and negative in all seven of the control subjects tested. Five patients with negative RAST results showed IgE antibodies to several rubber proteins in immunoblotting (patients 5, 12, 16, 18, and 20 in Table II). All five patients with negative immunoblot test results also had negative RAST results (Table I).

DISCUSSION

In the present immunoblot study, comprising 27 patient and 30 control sera as the source of IgE

antibodies, we could identify 19 allergenic rubber proteins in NRL. IgE antibodies from 17 patient sera bound to a 20 kd allergen. Other common allergens were 14, 30, 36, 39, 44, 46, 54, and 75 kd proteins. In previous studies by other investigators more than 15 natural rubber allergens were detected in various rubber preparations,^{12, 15, 16-20} but only a few of these allergens (10, 14, 20, 30, and 100 kd proteins) have been demonstrated by more than one group. We have suggested earlier, on the basis of the results with sera from five patients with latex allergy¹² that the 14 and 21 kd proteins could be the major allergens. In the present study it seemed that the 20 kd allergen (reported as a 21 kd allergen in our previous study) might be more significant than the 14 kd allergen, at least in this particular patient group. Slater^{19, 21} reported that IgE from all of his 14 patients bound to a 14 kd natural rubber allergen, whereas only six of these (40%) bound to a 20 kd allergen, which could be the same as the 20 kd allergen that we have identified in the present study. All patients in Slater's^{19, 21} study were children with spina bifida who had experienced severe allergic symptoms and had undergone multiple operations, whereas our patients in the present study were adults, most of whom had relatively mild symptoms. In addition to differences in the patient materials, these discrepant findings could also be due to differences in laboratory methods and sources of rubber antigens.

In the present study 22 of the 27 patient sera (82%) and one of the 30 control sera showed

TABLE II. Binding of IgE antibodies from 22 immunoblot-positive patient sera to NRL proteins

MW of allergens (kd)	Patient No.											
	1	2	3	4	5	6	7	8	9	10	11	12
111												
75				+		+		+++				+
67				+								
63				+								
54	+	+	+	++		+	++	+	+			
46	++		+	++		+		+				
44			+	++		+		+				
39		+	+	++		+		+				
36		+	+	++		+		+				
35			+									
33								+				
30	++	+	+	++		+		+				
29							++					
26		+										
23	++	+	+									
20	+++	+++	+++		+	+	+++	+	++	+	+	
19							+					
18	++											
14	+++	+			+		++		+		+	

MW, Molecular weight; + + +, + +, and +, intensity of the bands in immunoblotting.

positive immunoblot test results. It is possible that the control subject whose serum showed positive test results in immunoblotting had been sensitized against the 20 kd rubber protein, although the results of the skin prick test were negative, it may also be that cross-reacting IgE antibodies bound to this protein; however, no clinical symptoms were evident in this case. Five patients with latex allergy had negative test results in immunoblotting. This could be due to lack of relevant allergen in the antigen preparations used in this study or lack of sensitivity of the immunoblot method. It is noteworthy that commercial latex RAST detected latex-binding IgE antibodies in only 17 of 27 patient sera (63%), in agreement with previous studies, whereas immunoblotting results were positive in 22 of 27 patient sera (81%). It is possible that allergen source materials in commercial RAST discs do not carry an optimal selection of relevant allergens. Certain in-house RASTs have provided better sensitivities, suggesting that method improvement might also enhance performances of commercial RASTs.²²

IgE antibodies from the patient sera bound in a heterogeneous manner to proteins in the NRL. We also found a certain pattern of binding to six

allergens (30, 36, 39, 44, 46, and 54 kd proteins) in the sera of five patients. These results show that IgE response against natural rubber proteins varies considerably in individual patients with latex allergy. However, we could not detect any correlation between clinical symptoms and various IgE patterns in the present patient group.

In conclusion, in the present study we showed that IgE antibodies from sera of patients with latex allergy bound in immunoblotting in a heterogeneous manner to 19 proteins in NRL. Eighty-one percent of the 27 patient sera showed positive results in immunoblotting and 17 of them detected a 20 kd protein, suggesting that this could be a major rubber allergen in adult patients with latex allergy. Immunoblotting with a representative set of allergens as antigen would offer a sensitive, confirmatory in vitro test to demonstrate hypersensitivity to latex.

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Patient No.										Patients with positive immunoblot test	
13	14	15	16	17	18	19	20	21	22	No.	Percent
								+		1	5
++				+++		++				7	32
										1	5
										1	5
						+				9	41
						+				6	27
						+				5	23
						+				6	27
						+				6	27
										1	5
										1	5
						+				7	32
										1	5
										1	5
										3	14
++	+	+		++	+	+	+			17	77
										1	5
										1	5
		+							+	8	36

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