

Characterization of Latex Allergens and Correlation of Serum IgE/IgG Antibody Ratio with Clinical Symptoms

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ABSTRACT

Enzyme-linked immunosorbent assay (ELISA) was used to study latex-specific IgE and IgG. Levels of these immunoglobulins were correlated with latex allergy severity. Total protein content and relative immunoreactivity of 12 different brands of disposable gloves were compared. Specific latex proteins were extracted and characterized by immunoblotting. Blots revealed that the major latex allergen, reactive with both IgE and IgG, was a 34 KD component. Protein content varied among brands of gloves but correlated with immunoreactivity ($R = 0.683$; $P < 0.017$). The ratio of IgE/IgG correlated positively with symptom severity ($R = 0.689$; $P < 0.001$) and was useful to predict the severity of latex allergy. (Allergy and Asthma Proc 17:143–148, 1996)

Latex allergy was associated with a wide spectrum of symptoms,^{1–4} yet the prediction of symptom severity has been difficult. The importance of allergy to latex protein was highlighted by the life-threatening reactions that might occur in some patients during surgical operations. Despite the gravity of the problem, the nature of latex allergens has not been well studied.

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Although not as sensitive as radioallergosorbent assay (RAST), enzyme-linked immunosorbent assay (ELISA) offers the advantages of a long shelf life and nonhazardous reagents. ELISA has been used successfully to detect IgE antibodies in susceptible individuals.^{5–7} In this study, we attempted to develop an ELISA to measure latex-specific IgE and IgG in allergic and nonallergic persons. Levels of IgE, IgG, and the ratio of IgE to IgG were evaluated and compared to severity of allergy. Because there are contradictory reports regarding protein content and allergenicity of latex gloves,^{8,9} a competitive inhibition ELISA with patients' sera was used to measure immunoreactivity, and the results were compared with total protein content of 12 different brands of gloves. Due to its unlimited supply, a mouse monoclonal antibody¹⁰ may be advantageous to use in the competitive inhibition assay. However, mouse IgG and human IgE may not necessarily bind the same epitopes.

Immunoblotting has been used to identify and characterize serum IgE and IgG₄ binding to latex allergens.^{11,12} From the latex-specific IgE and IgG binding patterns, we determined the frequency of antibody response to latex components in order to identify possible common allergen(s).

MATERIALS AND METHODS

Patients and Sera

The allergic group consisted of 20 patients, including four health care workers and one laboratory technician. The patients all had a history of latex allergy. Among them, 17 had a history of multiple surgeries. The disease states of the patients included spina bifida, cerebral palsy, reflux due to

urethral anomaly, hydrocephalus, and tracheo-esophageal fistula. The five controls, having no symptoms of latex allergy, included two laboratory technicians who were often exposed to latex gloves, and two healthy children and one healthy adult who were not exposed to latex gloves. Serum samples from the 20 patients and five control subjects were studied for specific IgE and IgG antibodies against extracted latex antigens.

Classification of Symptom Severity

In order to conduct statistical analysis and correlate symptom severity with other factors, the symptom scores were classified into four categories.⁷ Minor modifications were made to this classification system.^{13,14} Briefly, mild reactions limited to reaction of skin were given a score of 1; moderate conditions involving congestion of upper respiratory tract were classified as 2; severe conditions with additional systemic reactions (edema of eyes, face, or lips) were classified as 3; systemic complications that occurred during surgery (unstable blood pressure, hypotension, arrhythmia, generalized erythematous rash, asthma, or shock) were given a score of 4.

Gloves

A total of 12 different brands of latex gloves currently in use in our hospital were studied: five sterile types, and seven nonsterile types.

Protein Content

The protein content of the glove extracts from different brands was determined according to the Lowry method with bovine serum albumin (BSA) as a standard.¹⁵

Extraction of Glove Proteins

Latex proteins from 20 g of glove from each brand were prepared as described previously¹¹ in 0.02 M phosphate-buffered saline (PBS), pH 6. Extracts were filtered through an ultrafiltration unit containing a 3K molecular weight cutoff membrane. Final volume and protein content were measured, and the glove extracts aliquoted into individual vials and lyophilized.

Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA was used to detect latex-specific IgE and IgG from sera of latex-sensitive patients. Competitive inhibition of ELISA was used to compare immunologic activity of latex allergens from different brands of gloves. Optimal concentrations of latex protein and serum to detect IgE titer were chosen by checkerboard titration with alkaline phosphatase-labeled goat anti-human IgE (Kirkegaard & Perry Laboratories Inc.; Gaithersburg, MD) diluted 1:5K in PBST (0.2% Tween 20 in 0.02 M PBS, pH 7.2). This optimal serum concentration was then used to determine the appropriate concentration of alkaline phosphatase-labeled goat anti-human IgG (Kirkegaard & Perry Laboratories

Inc.; Gaithersburg, MD). The wells of ELISA plates were coated with 200 μ L of glove extracts (Sime; 2 μ g/well) diluted in carbonate buffer, pH 9.6, and incubated overnight at 4° C. This was followed by blocking with 3% dry milk in PBST for 1 hour at 37° C. The plates were washed 4 minutes four times with PBST. Two hundred microliters of sera diluted 1:50 (in PBST) from allergic and nonallergic subjects were incubated at 37° C for 3 hours. In the competitive inhibition assay, 100 μ L of 1:35 diluted pooled sera and 100 μ L of different protein doses (0.125, 0.25, 0.5, and 1 μ g/100 μ L) from different brands were incubated. Control wells consisted of nonspecific binding without serum added and total binding without allergens. Wells were washed, followed by the addition of 200 μ L of alkaline phosphatase-labeled goat anti-human IgE (1:5K dilution in PBST) or anti-human IgG (1:20K dilution in PBST) and incubated at 37° C for 3 hours. After a final wash, 200 μ L of substrate (p-nitrophenylphosphate; 1 mg/mL in diethanolamine buffer, pH 9.8) were added. The optical density (OD) of the color developed was read at 405 nm.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Immunoblotting

SDS-PAGE was performed in a vertical electrophoresis system (Bio-Rad Laboratories; Richmond, CA). Latex protein (Sime), 5.33 mg, was run on 12% gel using the method of Laemmli,¹⁶ and then transferred electrophoretically from the gel to Immobilon P membrane (Millipore; Bedford, MA) according to manufacturer's instruction. The membrane for IgE detection was blocked with 10% BSA for 1 hour and then incubated with Blotto/Tween Tris buffer (3% dry milk powder, 0.2% Tween 20 in 0.05 M Tris, pH 7.5) for another hour at room temperature before placement in a miniblitter instrument (Immunetics; Cambridge, MA). Separate channels of the membrane were incubated with sera of patients, nonallergic subjects, and control (normal mouse serum NMS, normal rabbit serum NRS, and buffer containing no serum) diluted 1:5 in Blotto/Tween Tris buffer and next with alkaline phosphatase-labeled goat anti-human IgE diluted 1:10K in Blotto/Tween Tris buffer. Membrane processing for color development was used following a standard procedure.¹⁷ For detection of IgG, the operative procedure was the same as that of IgE, except that the membrane was blocked with 0.3% human serum albumin in borate buffer (0.125 M, pH 8.2), then in Blotto/Tween borate buffer. The first antisera was diluted to 1:10, and the incubation time of antibody reaction was reduced to 1 hour.

Statistical Analyses

Titers of serum immunoglobulin were expressed by OD value observed subtracted from the value of nonspecific binding. Statistical significance of the difference in immunoglobulin titers between allergic and nonallergic subjects was done using the Student's *t*-test. *P* < 0.05

was taken as significant. Correlation between protein content and immunoreactivity, and correlation between symptom severity and IgE, IgG, or IgE/IgG were analyzed using linear regression, with the Fisher's least significant difference procedure to test homogeneity at a significance level of 0.05.

Immunoreactivity of the Glove Extracts from Different Brands

Relative immunoreactivity from different brands of gloves was determined using Sime latex protein as a reference standard. Coefficients of variation for ELISA of Sime protein were 3.4% ($N = 3$) for intra-assay and 5.7% ($N = 8$) for inter-assay. Relative immunoreactivity of latex allergens from each brand of glove was determined by competitive inhibition binding at 20%, 35%, and 50% against Sime latex protein. The average of the three values was used as relative immunoreactivity.

RESULTS

Latex-Specific IgE and IgG in the Sera

The net mean OD of latex-specific IgE and IgG in the sera of allergic and nonallergic subjects were summarized in Table I. The mean OD for latex-specific IgE of 20 allergic subjects and 5 nonallergic subjects were 0.399 ± 0.099 (mean $\pm$ standard error of mean) and 0.010 ± 0.008 , respectively, and those for IgG were 1.099 ± 0.167 and 0.485 ± 0.187 , respectively. Both IgE and IgG showed significantly ($P < 0.001$) higher titers in allergic than in nonallergic subjects (Fig. 1).

Symptoms

Table I showed the clinical history, severity classification, and IgE/IgG for 20 allergic patients. IgG titers alone did not correlate with severity of symptoms ($R = -0.178$; $P > 0.32$), nor did IgE titers alone ($R = 0.406$; $P > 0.08$). However, the OD ratio IgE/IgG (ranging from

TABLE I
General Characteristics of Latex Allergic Patients and Nonallergic Persons

Name	Severity Score*	IgE	IgG	IgE/IgG Ratio	Diagnosis	No. of Surgeries
O.D.						
AL	4	0.656	0.258	2.543	spina bifida	40
GN	4	0.055	0.054	1.019	cerebral palsy, neurogenic bladder	40
FK	4	1.347	1.756	0.767	spina bifida	7
CD	4	0.262	0.464	0.565	spina bifida, nephritic syndrome	10
GM	3	1.230	1.008	1.220	post-urethral valve (bilateral urethrostomy)	35
HS	3	0.312	0.326	0.957	pruritus	2
CL	3	0.098	0.143	0.685	eczema	(-)
FS	3	1.161	1.971	0.598	hydrocephalus	16
OE	3	1.091	2.059	0.530	spina bifida	5
CK	3	0.394	0.824	0.478	spina bifida	9
CE	3	0.085	0.185	0.459	pruritus, rhinitis	3
LM	3	0.322	0.769	0.419	spina bifida	5
SA	2	0.159	0.948	0.168	spina bifida	8
FC	2	0.188	1.812	0.104	spina bifida	17
WD	2	0.079	1.308	0.060	spina bifida	12
CS	2	0.053	1.841	0.029	tracheal esophageal fistula	2
KK	1	0.138	0.564	0.245	cerebral palsy	8
GT	1	0.165	1.381	0.119	pruritus	(-)
SC	1	0.091	1.944	0.047	Klippel-Feil syndrome	22
CH	1	0.085	2.362	0.036	pruritus	(-)
KS	**NA	0.042	0.703	0.060	laboratory technician	
GU	**NA	0.004	0.115	0.035	laboratory technician	
SN	**NA	0.005	1.118	0.004	healthy child	
LC	**NA	0	0.21	0	healthy child	
WJ	**NA	0	0.28	0	healthy adult	

* Symptoms of latex allergy are classified 1-4 according to the severity of symptoms. Score 1: mild reactions of skin; score 2: moderate conditions involving congestion of upper respiratory tract; score 3: systemic reactions; score 4: surgical complications (hypotension, arrhythmia, unstable blood pressure, asthma, or shock during surgery).

**NA: nonallergic.

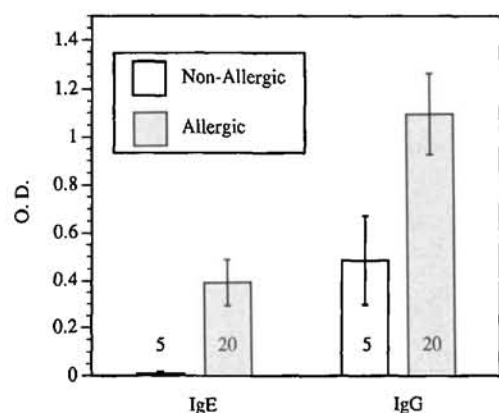


Figure 1. Comparison of latex-specific IgE and IgG titers between the nonallergic and allergic groups. Number of people in this study appears in each histogram. Histograms represent average titers. Vertical error bars represent standard error of the mean.

0.029 to 2.543) correlated well with severity of symptoms ($R = 0.689$; $P < 0.001$). Five nonallergic control subjects showed significantly low IgE/IgG (ranging from 0 to 0.06).

Analysis of IgE Binding by Immunoblotting

IgE immunoblot showed that all resolved polypeptides ranged from molecular weight of 10 to 58 KD (Fig. 2). Sera from nonallergic subjects (Lane GU, WJ, KS, LC, and SN), normal mouse (Lane NMS), and normal rabbit (Lane NRS) either only showed a marginal binding or did not react at all with the transferred polypeptides. Additionally, no binding was observed when alkaline phosphatase-labeled goat anti-human IgE antibody was used alone (Lane C). The majority of sera (14/20) had a reaction pattern that formed a strong band near 34 KD. We also observed that binding of IgE at 34 KD was almost always associated with binding near 12 KD on the same lane.

Analysis of IgG Binding by Immunoblotting

Similar to the IgE immunoblot, IgG showed the same binding pattern (Fig. 3). The band near 34 KD was also recognized by most sera (17/20). Sera from some of nonallergic controls also bound certain components. It was apparent from Figure 3 that no binding was found at lane NMS and C, except for the 12 KD band at lane NRS.

Protein Content of the Glove Extracts

Table II showed protein content of the glove extracts ranging from 4 μ g (Dextren) to 511 μ g (Travenol-Triflex) per gram of glove.

Immunoreactivity of the Glove Extracts

Table II showed the relative immunoreactivity against latex-specific IgE of the glove extracts from different brands as compared to the Sime standard. In regression analysis, protein content and relative immunoreactivity were significantly correlated ($R = 0.683$; $P < 0.017$).

DISCUSSION

Our serological data showed that allergic subjects had significantly ($P < 0.001$) elevated IgE and IgG against latex protein than nonallergic subjects. We believe that the clinical significance of antibody response may not be restricted to IgE isotype.

A previous study¹³ demonstrated a positive correlation between symptoms and the concentration of specific IgE antibodies in their patients. However, our study of 20 patients showed that the relationship between the severity of symptoms and latex IgE titers was not evident ($R = 0.406$; $P > 0.08$). Two recent studies^{7,18} reported similar findings. Symptom severity was even less correlated to IgG titers alone ($R = -0.178$; $P > 0.32$). Our results indicated that the ratio of specific IgE to IgG was more useful in predicting severity or outcome of symptoms than the use of absolute levels of either IgE or IgG. We found that IgE/IgG had

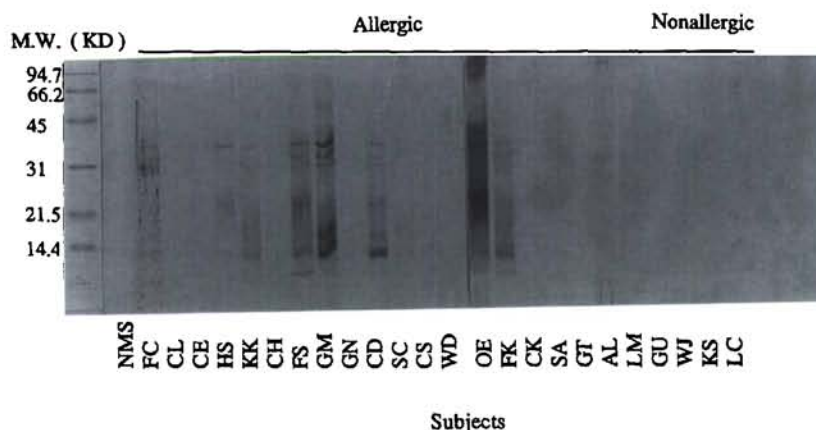


Figure 2. IgE-binding components in the glove extracts (Sime) on membrane, detected by 27 sera in 1:5 dilution. Lane C: buffer without serum; Lane NMS: normal mouse serum; Lane NRS: normal rabbit serum.

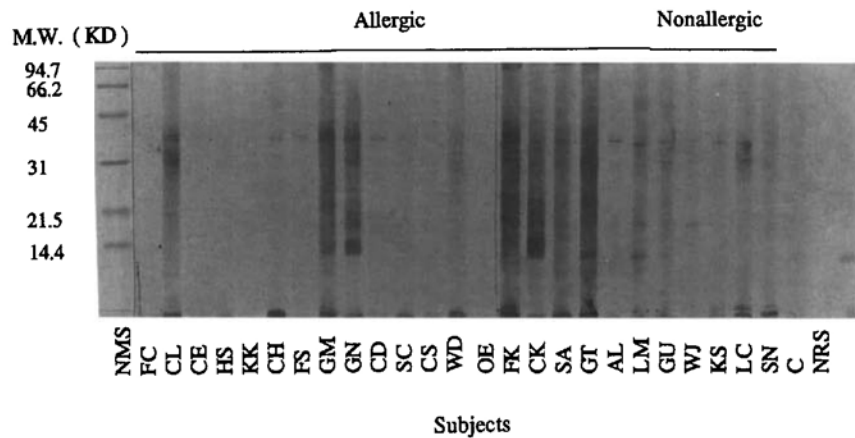


Figure 3. IgG-binding components in the glove extracts (Sime) on membrane, detected by 27 sera in 1:10 dilution. Lane C: buffer without serum; Lane NMS: normal mouse serum; Lane NRS: normal rabbit serum.

TABLE II

The Protein Content and Relative Immunoreactivity Against IgE of the Glove Extracts from Different Brands

Brands of Gloves	Lot No.	Manufacturer	Protein Content* ($\mu\text{g/g}$ of Glove)	Relative Immunoreactivity
Sime**	MD506912	Sime Health (Seattle, WA)	334.60 ± 10.36	1.000
Travenol-Triflex+	ONG203W	Travenol (Deerfield, IL)	511.40 ± 8.58	1.904
Ansell Sensi-Touch+	211102605	Ansell (Dothan, AL)	83.24 ± 3.12	1.638
Ultra Care	R8A23023	HPI Inc. (Fayette, AL)	176.50 ± 16.65	1.350
Perry X/AM	103657	Smith & Nephew Perry (Massillon, OH)	157.50 ± 2.52	1.095
Derma Thin	M309042N	Best Manufacturing (Menlo, GA)	126.40 ± 7.29	0.963
Fisherbrand	M316043N	Fisher Scientific (Pittsburgh, PA)	170.18 ± 7.24	0.799
Baxter-Triflex+	PGS2P046N	Baxter Healthcare (Valencia, CA)	186.49 ± 2.21	0.697
Safe Skin	1GA4186AC	Safe Skin (Boca Raton, FL)	16.26 ± 1.24	0.634
Aladan	2264413	Aladan (Dothan, AL)	41.86 ± 0.96	0.595
Dextren+	02072155M	Becton Dickinson (Franklin Lakes, NJ)	4.00 ± 1.37	0.562
Baxter-Ultraderm+	K3A174	Baxter Healthcare (Valencia, CA)	36.66 ± 3.90	0.406

* $N = 3$.

** Used as a reference standard.

+ Sterile surgical gloves.

a positive correlation with symptoms ($R = 0.689$; $P < 0.001$), indicating that both IgE and IgG may play a regulatory role in the manifestation of the clinical symptoms.

Results from the immunoblotting study showed that the ranges of the molecular weights of allergic latex components were similar to those reported by Jaeger et al.¹³ Our data showed that the 34 KD component might be the major allergen. This result differed from other reports, which indicated that the major allergens were at 14.6¹⁹ or 21 KD.¹² The discrepancy in identification of the major allergens from different studies can be attributed either to the different source of latex,²⁰ to the electrophoresis condition,²⁰ or to the use of sera from different patient populations.⁶ In our study, IgE from some patients bound to both the 34 KD band and the 12 KD band, suggesting that these proteins might possess shared allergenic determinants. We speculate that the 12

KD protein is possibly a degraded product from a higher molecular weight component. A similar explanation has been offered in a study of cat flea allergens.²¹

IgG antibody to latex detected in the immunoblotting study demonstrated that its binding pattern was similar to that observed for IgE. Our study clearly indicated that the major antigen for both IgE and IgG was at the 34 KD position. There was no direct relationship between IgE or IgG binding patterns in the immunoblot and clinical symptoms in latex allergy. IgG from the sera of some nonallergic subjects also bound to some latex components. At present, the role of IgG to latex remains speculative. In the IgG immunoblot, a 12 KD band appeared in the NRS lane. This nonspecific binding may be due to lectin-like interaction that has been described for several allergens.²²

We found that latex protein content correlated well with immunoreactivity ($R = 0.683$; $P < 0.017$), which was in

agreement with a previous report.⁸ The Food and Drug Administration of the U.S. Government recommends that companies which produce latex products lower the total elutable protein content of their products.¹⁰ We believe, based on our study, that reduction of protein content of latex products may reduce immunoreactivity, thus reduce the risk of sensitization. Furthermore, in a longitudinal assessment of the status of latex sensitization, a periodical measurement of IgE/IgG ratio is useful for predicting the severity of symptoms and for assessing the effectiveness of avoidance.

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REFERENCES

1. Axelsson JGK, Johansson SGO, Wrangsjö K. IgE-mediated anaphylactoid reactions to rubber. *Allergy* 42:46–50, 1987.
2. Swartz J, Braude BM, Gilmour RF, et al. Intraoperative anaphylaxis to latex. *Can J Anaesth* 7:589–592, 1990.
3. Tarlo SM, Wong L, Roos J, Booth N. Occupational asthma caused by latex in a surgical glove manufacturing plant. *J Allergy Clin Immunol* 85:626–631, 1990.
4. Nguyen DH, Burns MW, Shapiro GG, et al. Intraoperative cardiovascular collapse secondary to latex allergy. *J Urol* 146:571–574, 1990.
5. Ownby DR, Tomlanovich M, Sammons N, McCullough J. Anaphylaxis associated with latex allergy during barium enema examination. *Am J Roentgenol* 156:903–908, 1991.
6. Kurup VP, Kelly KJ, Turjanmaa K, et al. Immunoglobulin E reactivity to latex antigens in the sera of patients from patients from Finland and the United States. *J Allergy Clin Immunol* 91:1128–1134, 1993.
7. Kwittken PL, Sweinberg SK, Campbell DE, Pawlowski NA. Latex hypersensitivity in children: Clinical presentation and detection of latex-specific immunoglobulin E. *Pediatrics* 95:693–699, 1995.
8. Yunginger JW, Jones RT, Fransway AF, et al. Extractable latex allergens and proteins in disposable medical gloves and other rubber products. *J Allergy Clin Immunol* 93:836–842, 1994.
9. Alenius H, Makinen-Kiljunen S, Turjanmaa K, et al. Allergen and protein content of latex gloves. *Ann Allergy* 73:315–320, 1994.
10. Slater JE, Trybul DE. Immunodetection of latex antigens. *J Allergy Clin Immunol* 93:825–830, 1994.
11. Alenius H, Turjanmaa K, Palosuo T, et al. Surgical latex glove allergy: Characterization of rubber protein allergens by immunoblotting. *Int Arch Allergy Appl Immunol* 96:376–380, 1991.
12. Alenius H, Reunala T, Turjanmaa K, Palosuo T. Detection of IgG₄ and IgE antibodies to rubber proteins by immunoblotting in latex allergy. *Allergy Proc* 13:75–77, 1992.
13. Jaeger D, Kleinhaus D, Czuppon AB, Baur X. Latex-specific protein causing immediate-type cutaneous, nasal, bronchial, and system reaction. *J Allergy Clin Immunol* 89:759–768, 1992.
14. Bock SA, Sampson HA, Atkins FM, et al. Double-blind, placebo-controlled food challenge (DBPCFC) as an office procedure: A manual. *J Allergy Clin Immunol* 82:986–997, 1988.
15. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with Folin Phenol reagent. *J Bio Chem* 193:265–275, 1951.
16. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680–685, 1970.
17. Harlow E, Lane D. Antibodies: A manual. Ch. 12. In: *Immunoblotting*. Cold Spring Harbor Laboratory, 1988, p. 505.
18. Charous BL, Hamilton RG, Yunginger JW. Occupational latex exposure: Characteristics of contact and systemic reactions in 47 workers. *J Allergy Clin Immunol* 94:12–18, 1994.
19. Czuppon AB, Chen Z, Rennert S, et al. The rubber elongation factor of rubber trees (*Hevea brasiliensis*) is the major allergen in latex. *J Allergy Clin Immunol* 92:690–697, 1993.
20. Ownby DR. Is rubber elongation factor the major allergen of latex? *J Allergy Clin Immunol* 92:633–635, 1993.
21. Greene WK, Carnegie RL, Shaw SE, et al. Characterization of allergens of the cat flea, *Ctenocephalides felis*: Detection and frequency of IgE antibodies in canine sera. *Parasite Immunol* 15:69–74, 1993.
22. Barnett D, Howden MEH. Lectins and the radioallergosorbent test. *J Allergy Clin Immunol* 80:558–561, 1987. □

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