

The allergenic properties of fresh and preserved *Hevea brasiliensis* latex protein preparations

R. KEKWICK, S. BHAMBRI, M. H. CHABANE*, J.-E. AUTEGARDEN*, D. A. LEVY* & F. LEYNADIER* School of Biochemistry, University of Birmingham, Birmingham, UK, and *Centre d'Allergie, Hôpital Rothschild, Paris, France

(Accepted for publication 24 January 1996)

SUMMARY

The allergenic properties of the proteins of two lyophilized fractions of fresh natural rubber latex obtained by ultracentrifugation, the C serum and the sedimented bottom or lutoid fraction, have been compared with those of the serum proteins of two samples of high ammonia latex (HAL), [A]HALS obtained from HAL stored for more than 1 year, and [M]HALS derived from HAL stored for 6 weeks before ultracentrifugation and lyophilization. The most potent source of allergenic polypeptides both for skin prick testing of latex-sensitive patients and for immunoblots of their blood serum was the lutoid fraction of fresh latex. Skin prick tests and immunoblots of patients' sera showed that the allergenicity of the ammoniated latex decreased during storage. Skin prick tests using fractions of [A]HALS, C serum and lutoid proteins obtained after passage through a Sephacryl S300 column showed that the components of all three preparations which eluted in the largest volumes were almost equally effective in provoking the largest number of responses. Immunoblots of the sera of 43 latex-sensitive individuals showed that the majority (66%) of sera of the adult allergic patients reacted with a polypeptide of 19 kD. No characteristic pattern of binding latex polypeptides could be recognized in the sera from patients who were also asthmatic or from those who had had an anaphylactic response to latex proteins.

Keywords latex allergens skin prick tests latex maturation

INTRODUCTION

Rubber gloves for surgical use have been made by dipping a former into a solution of natural rubber in a hydrocarbon solvent for about a century. This process has been largely superseded by the use of an aqueous concentrate of natural rubber latex, a technique developed in the 1930s. It was not until 1979 that contact urticaria attributed to gloves made from latex was described by Nutter [1]; however, a report in 1927 of an apparent type 1 allergy referred to the development of urticaria resulting from wearing a dental prosthesis said to have been made of rubber [2]. In the intervening period, delayed hypersensitivity reactions (type 4) of an eczematous nature attributed to rubber compounding ingredients have been widely recognized [3] and largely overcome, resulting in the marketing of products labelled 'hypoallergenic'. However, the spate of reports published since Nutter's 1979 description and the establishment of universal precautions for barrier protection from blood and body fluids in 1987 by the US Centres for Disease Control [4], suggests that type 1 allergy to latex products has reached almost epidemic proportions in groups with high exposure

in some countries. This has raised the question why, during the 40 or more years of use of dipped latex articles before Nutter's observation, did the condition remain unremarked?

Natural rubber latex, the exudate of the specialized laticifers of the rubber tree *Hevea brasiliensis* (Euphorbiaceae), consists of a suspension of rubber particles (diameter 0.01–1 µm) coated with a phospholipid- and polypeptide-containing half unit membrane (reminiscent of a high density lipoprotein), embedded in which are a number of polypeptides, the major component being the 14.5-kD so-called rubber elongation factor. These particles are dispersed in a cytoplasmic serum containing about 1% protein. Latex also contains some subcellular organelles, the most important of which, from the viewpoint of the potential allergenic protein content of manufactured latex goods, is the lutoidic tonoplast which has a single membrane and which shows the properties of a lysosome and a tonoplast. Ultracentrifugation of freshly tapped latex yields three principal zones, a supernatant layer of rubber particles, a clear cytoplasmic or C serum, and a bottom fraction consisting largely of lutoid particles. The lysed lutoid fraction contains about 25% of the total latex proteins [5].

Latex for use in the manufacture of dipped goods (gloves, toy balloons, catheters and condoms) is ammoniated after tapping from

Correspondence: R. Kekwick, School of Biochemistry, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK.

the tree and the rubber content enriched from 30% to 60% by centrifugation in a cream separator. The ammonia content is then adjusted either to 0.7% (high ammonia latex (HALS)) or to 0.2% (low ammonia latex fortified with thiuram and zinc oxide (LATZ)). During the course of storage and centrifugation the lutoid particles lyse and the proteins are liberated into the latex serum. The latex is then allowed to mature for about 4–6 weeks during which its mechanical stability increases sufficiently for an adequate coating to be obtained on dipping a former coated with a coagulant (almost invariably $\text{Ca}(\text{NO}_3)_2$). Latex currently used for manufacture in S.E. Asia is normally matured for 4–6 weeks. Before the mid 1970s most gloves in use in Europe and the USA were manufactured in these regions from ammoniated latex concentrate transported from S.E. Asia, which involved far longer maturation periods of from 3 to 12 months or more during transport, distribution and storage [6].

Allergic sensitivity is invariably reported to manufactured articles, indeed there appear to be no reports of the sensitization of rubber estate workers to fresh latex. Whilst the manufactured goods are of necessity made from ammoniated latex, there is some evidence from *in vitro* studies that the proteins thereof may be less allergenic than those of fresh latex [7,8]. As a result, a standardized commercial allergen preparation based on fresh latex has recently been described [9].

Amongst the latex preparations which can be used as a potential source of allergens are: the total serum from either the aged HALS as used in Europe and the US ([A]HALS), or the matured 4–6-week-old [M]HALS currently in use in S.E. Asia, the F serum obtained by freezing and thawing fresh latex [10], the C serum and the lutoid fraction obtained by ultracentrifugation of fresh latex. These may all contain sensitizing polypeptides. (The F serum suffers from the slight disadvantage that the proteins are exposed to proteolytic action during the period of thawing the latex and extrusion of the serum therefrom.)

In the most extensive study of *in vitro* reactions so far published [11], a preparation of F serum obtained from frozen fresh latex was used to detect IgE antibodies to 19 different latex polypeptides in 22 latex-allergic persons, clearly establishing the allergenicity of the polypeptides of fresh latex.

The aim of this study was to compare the allergenicity of the soluble proteins from ammoniated latex, [A]HALS and [M]HALS, and from fresh latex, C serum and lutoid proteins, and to identify the major allergenic polypeptides.

MATERIALS AND METHODS

Description of patients

The 43 adults (39 females, four males) who volunteered to take part in this study had been seen previously in consultation at the Centre d'Allergie of the Hôpital Rothschild with a clear-cut history of immediate sensitivity to latex goods as well as a positive skin prick test (SPT) to latex. Most of the subjects complained either of contact urticaria ($n = 10$), or of angioedema ($n = 7$) or both ($n = 5$), and at least 11 had experienced one or more episodes of anaphylactic shock due to latex. Five patients were suffering from asthma caused by latex, with crises occurring at work, and three patients had rhinitis. In four patients, latex allergy was diagnosed after anaphylactic shock or angioedema after ingestion of fruit (avocado, $n = 3$; banana, $n = 1$). Of the remaining patients the case histories revealed that five had previously experienced angioedema following the eating of fruit (avocado, $n = 2$; banana, $n = 1$;

walnuts, $n = 1$; hazelnuts, $n = 1$). In 20 cases latex allergy became apparent during surgery.

The age of the patients ranged from 21 to 71 years, 67% of whom were under 40 years, the modal decennial class (37%) being 30–39. The group included 17 healthcare workers, and two others who were involved in hospital administration. Atopy, including either asthma or rhinitis to pneumallergens and atopic dermatitis, was reported in 16 patients.

Latex preparations

The two protein fractions of fresh latex used, the C serum and the lutoid fraction, were obtained from latex of unidentified *Hevea* clones prepared in the laboratories of the Rubber Research Institute of Malaysia by the ultracentrifugation procedure of Archer & Sekhar [10], lyophilized immediately after separation, and stored at -20°C . The soluble proteins of the lutoid fraction were extracted by stirring with PBS and the solid material was removed by centrifugation.

Two preparations of the rubber-free serum of ammoniated latex were used. The first, [A]HALS, was obtained by ultracentrifugation (1 h, 40 000 *g*) of a commercial preparation of HALS shipped from Malaysia to be used in rubber manufacture in Europe. The second, [M]HALS, was similarly prepared from ammoniated (0.7%) latex concentrate prepared in the laboratories of the Rubber Research Institute of Sri Lanka, flown to England and stored at approx. 20°C for 6 weeks before ultracentrifugation. Both preparations were lyophilized and stored at -20°C .

Skin prick testing

Skin prick tests were carried out on all patients with a commercial latex preparation marketed for allergen testing (Stallergenes SA, Fresnes, France) at a concentration of 10 μg protein/ml. SPTs of laboratory prepared fractions of latex proteins derived from the sera of [A]HALS, fresh C serum and the lysed lutoid fraction of fresh latex (see below) were sterilized by filtration through a 0.22- μm membrane filter (Millipore, Watford, UK) after adjustment of the protein concentration to 10 μg /ml. A solution of 0.3% human serum albumin in 0.9% NaCl containing 50% glycerol and 0.04% phenol formed the negative control and 9% codeine phosphate was added to this solution to make the positive control. A weal diameter of 3 mm which was also greater than or equal to half the positive control weal was considered positive.

Protein fractionation

Soluble proteins of [A]HALS, C serum and the lutoid preparation were fractionated by gel filtration on a Sephacryl S300 column, 92×2.6 cm, in 0.01 M phosphate buffer pH 7.4 at 4°C .

The protein content of each fraction was estimated by the Bradford [12] procedure. The column was calibrated with standard preparations of fibrinogen IgG, bovine serum albumin (BSA), ovalbumin, carbonic anhydrase, myoglobin, lactoglobulin ribonuclease, lysozyme and cytochrome C. The polypeptide composition of the fraction was analysed by SDS-PAGE [14].

Estimation of total and latex-specific IgE in blood serum

Total IgE was assayed by the CAP system (Pharmacia, St Quentin-Yvelines, France) according to the manufacturer's instructions. Results are expressed as kU/l. All the samples were tested for

latex-specific IgE antibodies by using the commercially available CAP system. These results are also expressed as kU/l, the cut-off of positivity being 0.35 kU/l.

Detection of latex-specific serum IgE by immunoblotting

Samples of latex proteins (100 µg) were subjected to SDS-PAGE in 12% polyacrylamide with 0.32% bisacrylamide gels and were electroblotted by the Towbin *et al.* [13] procedure on to nitrocellulose membranes (Schleicher & Schuell; Anderman, Kingston on Thames, UK; 0.2 µm). The membrane binding sites were blocked with 5% low fat skimmed milk powder (Marvel, Stafford, UK) in Tris-buffered saline (TBS) pH 7.4 containing 0.1% Tween 20. Blots were incubated with sera from allergic patients diluted 1:5 with TBS. After washing the blot with TBS containing 5% Marvel, bound IgE was detected with biotin-labelled anti-IgE (Diagnostics Products Corp., Los Angeles, CA) dissolved in TBS containing 5% Marvel followed by avidin conjugated with alkaline phosphatase dissolved in TBS. The enzyme binding was detected with the chromogenic substrate 5-bromo-4-chloro-3-indolyl phosphate.

RESULTS

Skin prick tests with latex protein preparations

A comparison of the responses of some of the latex-allergic subjects with SPTs using the four different latex protein preparations is shown in Table 1. Whilst 88% of the subjects in a group of 33 patients responded to the lutoid protein preparation and 82% of the same group to C serum, only 15% of 27 members of this group responded to [A]HALS, but 56% of 18 of these patients responded to the fresher [M]HALS.

Fractions obtained by gel filtration of [A]HALS, C serum and lutoid fraction proteins were used in SPTs on 15 patients (Fig. 1). The parent [A]HALS gave only a small number of responses (1/15). Protein from [A]HALS eluting in the largest volume from the column (which would be expected to have the lowest molecular weight) produced a response in most of the patients in the group (13/15). Analogous fractions from C serum and lutoid preparations also gave a response with nearly all the members of the group (15/15 and 14/15, respectively). Theoretically, it would be expected that these fractions would contain the polypeptides of lowest molecular mass; however, SDS-PAGE (Fig. 2) indicates that this was not entirely true, a number of polypeptides of relative molecular mass far greater than that expected from the K_{av} values of the calibrating proteins being eluted in these fractions. This effect must be attributed to interactions of the proteins with the column matrix. It is also evident from the SDS-PAGE analysis showing a comparison of the allergenically most active fractions of the three latex preparations (Fig. 2) that their polypeptide

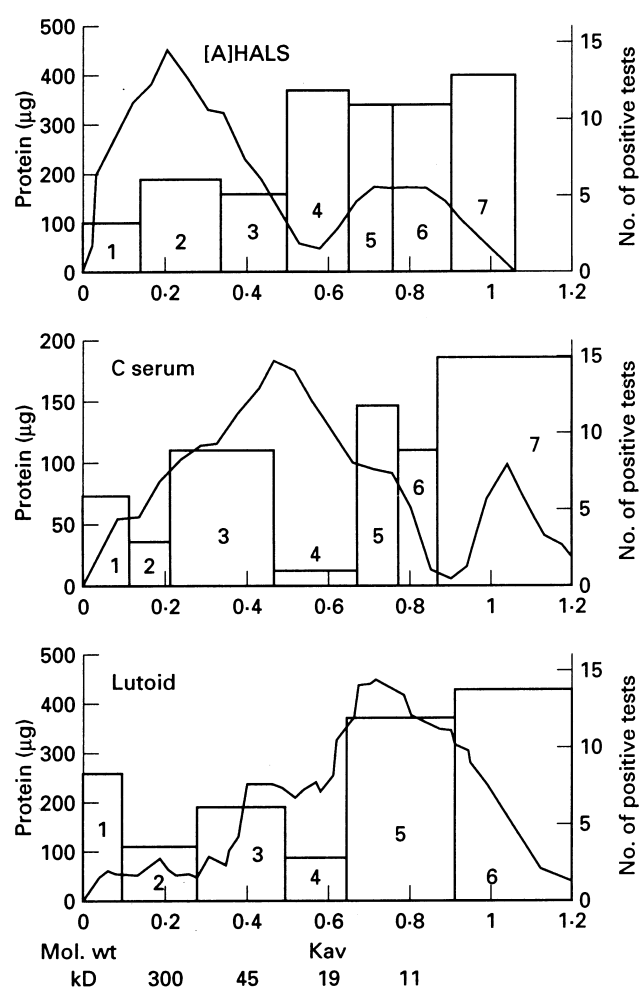


Fig. 1. Fractionation of latex protein preparations on Sephacryl S300. Fractions were bulked to give numbered samples used for skin prick tests (SPTs) of a group of 15 patients. The histogram shows the number of responses to each sample.

composition was similar. Bands corresponding to polypeptides of mol. wt 43, 32, 29, 24 and 19 kD could be detected in all three fractions, but the lutoid fraction had particularly high concentrations of 27 and 24 kD polypeptides.

Resolution of reactive polypeptides by immunoblotting

A comparison of the numbers of positive immunoblots obtained with the four protein preparations (Table 2) shows as in the SPTs that the lutoid preparation was the richest source of reacting polypeptides. Whilst 80% of the sera of 43 patients tested had sera reacting with at least one polypeptide of the lutoid fraction, when 11 of these sera were tested against latex C serum only five (31%) reacted. Again, of the two ammoniated latex preparations, [M]HALS, although much less effective than the lutoid preparation, was a better source of IgE binding polypeptides than [A]HALS. Of the group of 43 sera, 37% reacted with at least one polypeptide of [M]HALS, whilst only 9% of 22 of the group reacted with [A]HALS polypeptides.

Table 3 shows the relative molecular masses of the polypeptides bound by the different patient sera. In general these coincided in the four latex preparations. Binding polypeptides were more evident in the lutoid fraction, and all of the sera giving a positive

Table 1. Comparison of the responses to fresh and ammoniated latex protein preparations in skin prick tests

	Fresh latex		Ammoniated latex	
	Lutoid	C serum	[M]HALS	[A]HALS
Positive response/no. sera Tested	29/33 88%	27/33 82%	10/18 56%	4/27 15%

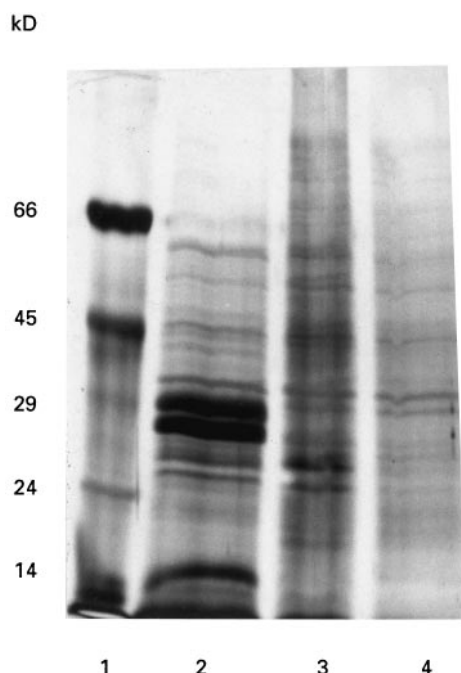


Fig. 2. SDS-PAGE of the most reactive Sephacryl S300 latex protein fractions shown in Fig. 1. Lane 1, Mol. wt markers; lane 2, lutoid fraction 6; lane 3, C serum fraction 7; lane 4, [A]HALS fraction 7.

immunoblot with any of the preparations reacted with this fraction. Frequently polypeptides in the lutoid fraction binding IgE were not detected in any other preparation. The five polypeptides most frequently binding IgE had relative molecular masses of 59, 39, 31, 19 and 15 kD, respectively, and more of the sera (66%) reacted with the 19-kD polypeptide than any other. Of the 13 sera reacting with only one polypeptide, 10 reacted with the 19-kD polypeptide. An immunoblot of one of the sera against SDS-PAGE of proteins extracted from a commercial examination glove also showed a binding of IgE to a 19-kD polypeptide.

In general, sera with high latex-specific IgE levels reacted with a larger number of latex polypeptides and those with lower specific IgE with fewer. Thus, of the 17 sera giving a CAP class III or above, 11 reacted with more than one polypeptide in the immunoblots. There were exceptions, however. Whilst one of the group having only a class I reaction in the CAP test reacted with four polypeptides and two reacted with three polypeptides in the immunoblots,

five CAP class II and two CAP class I sera failed to give detectable immunoblots. On the other hand, two of the CAP class 0 sera gave positive immunoblots, one reacting with two polypeptides and the other with one. Sera of CAP class IV reacted with an average of three, whilst those of CAP class II reacted with 2.4 and those of CAP class I, 1.4 polypeptides.

DISCUSSION

The highest response rate for both the *in vivo* and *in vitro* tests was obtained with the native proteins of the lutoid particle extract. In SPTs, the response to the [A]HALS both in the series reported here and also in another series of latex-allergic patients (unpublished observation) was quite poor, but the response to the [M]HALS was much greater. The differences seem to be due to the concentrations of allergenic proteins in the different latex preparations. Gel filtration of [A]HALS produced a fraction eluting in the highest volumes which evoked almost as high a response at the same protein concentrations as the analogous fractions derived from the fresh latex preparations. It is noteworthy that the gel filtration elution patterns, which are to an extent indicative of the molecular mass distribution of the proteins, are different for the preparations studied. The [A]HALS showed a much higher proportion eluting in smaller volumes (which would have a higher relative molecular mass) than the preparation from fresh latex. The fractions corresponding to a higher molecular mass were poorly allergenic, whether derived from HAL C serum or lutoid. Thus the lower allergenicity of the parent [A]HALS could be attributed to the increase in the apparently high molecular mass (presumably aggregated) material formed in storage in the presence of ammonia.

Having regard to the imprecision of the estimation of the relative molecular mass of a polypeptide by SDS-PAGE, it is likely that the polypeptide to which a molecular mass of 19 kD has been attributed in this study corresponds to the 20-kD allergen of Alenius *et al.* [11], to which a 77% response was reported. About 63% of the patients in this study and 70% of a group of blood donors having antibodies to latex proteins in another study reacted with this polypeptide (unpublished observation). If indeed the same polypeptide is being recognized by the sera in both studies, this component becomes a prime candidate for consideration as the major allergen of latex.

Although the greater sensitivity of latex-allergic persons to fresh as opposed to ammoniated latex proteins in SPTs does not seem to have been previously reported, higher response rates to the proteins of non-ammoniated latex *in vitro*, both in the Radio-allergosorbent test (RAST) [7] and in the immunoblot tests [8], have been observed.

No relation could be discerned between the patients' symptoms and the pattern of bound polypeptides in asthmatics, nor did the sera of the healthcare workers or of the patients who had had anaphylaxis react with a particular array of polypeptides. A high proportion of responses (80%) to the polypeptides of the lutoid fraction of fresh latex was given by the sera of a group of 43 patients (Table 2), whilst only 30% of the same group bound peptides of [M]HALS. When sera of some of the 43 patients were tested against C serum, few positive immunoblots (31%) were obtained and only 9% of a group of 22 sera taken randomly from the 43 bound any polypeptides of the [A]HALS. The sera also on average bound more of the lutoid polypeptides than those of [M]HALS. All of the sera giving a positive reaction with any of

Table 2. Polypeptides bound in immunoblots of fresh and ammoniated latex preparations

	Fresh latex		Ammoniated latex	
	Lutoid	C serum	[M]HALS	[A]HALS
No. patients' sera tested	43	16	43	22
No. of latex-specific IgE sera (CAP) positive	41	16	41	22
No. of positive immunoblots	35	5	16	2
Total polypeptides bound no. latex positive sera	3.1	—	0.97	—

Table 3. IgE levels of and latex polypeptides bound by sera of latex-sensitive patients

Patient no.	Age (years)	Sex	HCW	Surgery	AS	A	AT	SPT	Latex specific IgE (kU/l)	Total IgE (kU/l)	Reacting polypeptides (rel. mol. mass kD)														
											59	48	39	32	30	27	24	22	19	16	14				
CAP class V																									
1	47	M	–	+	+	–	+	7–45	60.3	616	+	+	–	–	–	–	–	–	+	–	–				
CAP class IV																									
2	28	F	–	–	–	+	+	8–35	37.3	>2000	–	–	–	–	–	–	–	–	+	+	–				
3	23	F	–	+	+	–	–	8–20	36.0	116	–	–	+	+	–	+	+	+	–	–	–				
4	38	F	+	–	–	–	+	7–30	36.0	298	–	+	+	+	+	–	–	–	+	–	–				
5	39	F	–	–	–	–	+	8–15	35.8	253	+	–	–	–	–	–	+	+	+	–	–				
6	40	F	–	+	–	+	–	6–18	35.4	>2000	–	–	–	–	–	–	–	–	–	–	+				
CAP class III																									
7	50	F	+	–	–	+	–	10–40	16.2	721	+	+	+	–	–	–	–	–	+	–	–				
8	27	F	+	–	–	+	+	7–40	12.0	1118	–	–	–	–	–	+	–	+	+	–	–				
9	31	F	–	–	–	–	–	5–30	7.7	301	–	+	–	+	+	+	–	+	+	+	–				
10	37	F	–	–	–	–	–	15–20	7.4	ND	–	–	–	–	–	–	–	–	+	–					
11	21	F	(+)	–	–	+	–	5–20	6.5	245	+	–	+	–	–	–	–	–	–	–					
12	29	F	+	–	–	–	–	6–32	6.1	361	–	–	+	+	–	–	–	–	–	+					
13	34	F	+	–	–	–	+	8–30	5.6	171	–	–	–	–	–	–	–	–	+	+					
14	45	F	+	–	–	–	+	6–25	5.6	260	–	–	–	–	–	–	–	–	+	–					
15	31	M	+	–	–	–	–	6–30	5.1	ND	–	–	–	–	–	–	–	–	+	–					
16	24	F	(+)	+	–	–	–	4–20	5.0	55	–	–	–	–	–	–	–	–	+	+					
17	35	F	+	+	+	+	–	15–20	5.0	54	–	–	+	+	+	–	–	–	–	–					
18	31	F	–	–	–	–	–	11–45	4.1	136	–	–	–	–	–	–	–	–	+	–					
CAP class II																									
19	43	F	–	+	+	+	+	6–35	3.5	>2000	–	+	+	+	+	–	–	–	+	–	–				
20	35	F	+	+	–	+	+	7–35	3.5	350	–	–	–	–	–	–	–	–	+	–	–				
21	63	F	–	+	–	–	+	5–42	3.5	36	–	–	–	–	–	–	–	–	–	–					
22	54	F	–	+	–	–	–	8–30	3.5	54	+	–	+	+	+	–	–	–	–	–					
23	NA	F	–	–	–	–	–	10–40	3.4	51	–	–	–	+	+	+	–	–	+	+					
24	71	M	–	+	–	–	–	7–25	3.4	413	–	–	–	–	–	–	–	–	–	–					
25	29	F	–	+	–	–	+	4–25	3.2	561	–	–	–	–	–	–	–	–	+	–					
26	33	F	+	+	–	–	–	15–40	3.2	36	+	–	–	–	–	–	–	–	–	+					
27	27	F	+	–	–	–	–	10–50	2.8	92	–	–	–	–	–	–	–	–	+	–					
28	25	F	+	–	–	–	–	6–26	2.5	217	–	–	–	–	–	–	–	–	+	–					
29	46	F	+	+	–	–	+	15–50	2.2	131	–	–	–	–	–	–	–	–	–	–					
30	36	F	–	–	+	–	–	10–45	2.2	62	–	–	–	–	–	–	–	–	–	–					
31	48	F	–	+	–	–	–	5–30	1.9	21	–	–	–	–	–	–	–	–	+	–					
32	24	M	–	–	–	–	+	5–30	1.5	ND	–	–	–	–	–	–	–	–	–	+					
33	68	F	–	+	+	–	–	8–20	1.4	21	–	–	–	–	–	–	–	–	+	–					
34	38	F	–	+	+	–	+	7–40	1.1	245	–	–	–	–	–	–	–	–	–	–					
35	28	F	+	–	–	–	–	6–45	1.1	105	+	–	–	–	–	–	–	–	–	–					
CAP class I																									
36	22	M	+	–	–	+	–	9–32	0.9	54	–	–	–	–	–	–	–	–	–	–					
37	39	F	+	–	–	–	–	8–30	0.67	93	–	–	–	–	–	–	–	–	+	–					
38	54	M	–	+	–	–	–	15–45	0.53	109	–	–	–	–	–	–	–	–	–	–					
39	27	F	+	–	–	+	–	7–35	0.48	10	–	–	–	–	+	–	–	–	+	+					
40	36	F	–	–	–	–	–	5–20	0.38	262	–	–	+	+	+	–	–	–	–	–					
CAP class 0																									
41	40	F	–	–	–	–	+	4–20	<0.35	148	–	+	–	–	+	–	–	–	–	–					
42	34	F	–	+	–	+	+	5–25	<0.35	3000	–	–	–	–	–	–	–	–	+	–					
43	59	F	–	–	–	–	+	10–32	<0.35	67	–	–	–	–	–	–	–	–	–	–					
% active sera binding											20	17	25	25	25	11	3	6	66	17	14				

HCW, Healthcare worker; +, other hospital worker; surgery, reaction apparent at surgery or dental surgery; AS, reaction involved anaphylaxis; A, asthmatic; AT, atopic; SPT, skin prick test, weal and flare diameters in mm; IgE, concentration in kU/l; ND, not determined; NA, not available.

the latex protein preparations reacted with the lutoid polypeptides, and in general the molecular weights of the polypeptides bound by the different preparations corresponded. Frequently, however, polypeptides detected in the lutoid fraction by the sera were not detected in the other preparations.

SPTs with preparations of latex proteins fractionated on Sephacryl S300 show that both for the fresh latex-derived C serum and lutoid proteins and for the ammoniated latex serum [A]HALS, the fractions eluted in the largest volumes have the highest allergenic activity. Notwithstanding the poor specific allergenic activity of the total HALS protein, it is clear that allergenic activity remains in fractions analogous to those of the fresh latex proteins having a high specific allergenic activity.

It is remarkable that the potent allergenic properties of fresh latex proteins observed in this study were recognized in one of the earliest reports of allergy to rubber gloves. In 1933 Downing [15], in an article largely describing delayed hypersensitivity to rubber gloves, quoted a personal letter from the chief chemist of a glove manufacturing company, stating that 'some individuals are so hypersensitive that they will get a marked reaction from the protein materials in the latex as it comes from the tree'.

In conclusion, it seems that, although the primary source of sensitizing allergens in Europe and North America cannot be fresh latex, it is the epitopes of the native latex proteins, particularly those of the lutoid particles, which are in the main allergenic. Prolonged storage of latex containing ammonia results in a progressive loss of these epitopes, accompanied by an accumulation of weakly allergenic aggregated polypeptides. The relatively recent emergence of the problem of allergy to dipped latex goods may be due in part to the use of a fresher ammoniated latex which retains a higher concentration of the allergenic epitopes than that found in the latex preparations formerly used.

ACKNOWLEDGMENTS

We wish to thank the London International Group plc for financial assistance to R.K., the Rubber Research Institutes of Malaysia and Sri

Lanka for the supply of latex preparations, and Stallergenes SA for the sterilization of the samples.

REFERENCES

- 1 Nutter AF. Contact urticaria to rubber. *Br J Dermatol* 1979; **101**:597–8.
- 2 Stern G. Überempfindlichkeit gegen Kautschuk als Ursache von Urticaria und quinkezschem Ödem. *Klin Wochenschrift* 1927; **6**:1096–7.
- 3 Taylor JS. Rubber. In: Fischer AA, ed. *Contact dermatitis*, 3rd edn. Philadelphia: Lea & Febiger, 1983:603–43.
- 4 Centres for Disease Control. Recommendation for prevention of HIV transmission in healthcare settings. *MMWR* 1987; **36** (Suppl. 2S): 2S–18S.
- 5 d'Auzac J, Jacob J-L. In: d'Auzac J, Jacob J-L, Chrestin H, eds. *Physiology of rubber tree latex*. Boca Raton: CRC Press, 1989:59.
- 6 Gazeley KF, Gorton ADT, Pendle TD. Latex concentrates: properties and composition. In: Roberts AD, ed. *Natural rubber science and technology*. Oxford: OUP, 1988:63–98.
- 7 Slater JE, Chhabra SK. Latex allergens. *J Allergy Clin Immunol* 1992; **89**:673–8.
- 8 Alenius H, Turjanmaa K, Palosuo T, Mäkinen-Kiljunen S, Reunala T. Surgical latex gloves: characterisation of rubber protein allergens by immunoblotting. *Int Arch Allergy Appl Immunol* 1991; **96**:376–80.
- 9 Levy D, Autegarden JE, Leynadier F, Turjanmaa K, Andre C, Tran TX. *In vitro* and *in vivo* standardisation of a latex extract. *J Allergy Clin Immunol* 1995; **149**: Abstr. 36.
- 10 Archer BL, Sekhar BC. The proteins of *Hevea brasiliensis*. 1. The protein constituents of fresh latex serum. *Biochem J* 1955; **61**:503.
- 11 Alenius H, Turjanmaa K, Palosuo T, Mäkinen-Kiljunen S, Reunala T. IgE immune response to rubber proteins in patients with latex allergy. *J Allergy Clin Immunol* 1994; **93**:859–63.
- 12 Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein-dye binding. *Anal Biochem* 1976; **72**:248.
- 13 Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* 1979; **76**:4350–4.
- 14 Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T₄. *Nature* 1970; **227**:680.
- 15 Downing JG. Dermatitis from rubber gloves. *New Eng J Med* 1933; **208**:196–8.