

Monoclonal antibody against a major latex allergen reacts with latex products and ammoniated natural latex

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Spina bifida patients have been shown to be a high-risk group for latex allergy. Of the several latex proteins identified from nonammoniated natural latex, a polypeptide with a molecular mass of about 23 kDa was shown to be one of the major allergens reacting with IgE in the sera from such patients. Using hybridoma technology, a monoclonal antibody (mAb) 1E2 against the 23-kDa latex allergen was produced. The presence of the 23-kDa or related allergens in finished latex products was evaluated using 1E2. Of the 16 extracts of finished latex products, 12 reacted with 1E2. The other four products are not used in health care. Since the majority of latex products used in health care are manufactured from ammoniated latex and ammoniation may alter latex proteins, we also investigated the effect of ammoniation on the 23-kDa allergen. Although ammoniation degraded latex proteins, the 23-kDa antigen could still be detected by using mAb 1E2 on immunoblot of ammoniated latex proteins. Furthermore, ammoniation resulted in the appearance of additional proteins in high molecular weight positions not present in nonammoniated natural latex, which retained the 23-kDa epitope. The 23-kDa allergen could also be detected on surgical gloves after extensive washing.

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Immediate hypersensitivity reactions have been elicited following exposure to latex products such as gloves, condoms, and surgical devices. The reactions are IgE-mediated and include contact urticaria, angioedema, rhinitis, conjunctivitis, asthma, anaphylaxis, and even death (5, 16, 17, 22, 23). Several groups at high risk for latex allergy are well defined and include health-care workers, rubber industry workers, and patients with spina bifida or urogenital abnormalities (4, 9, 15, 27, 28). Repeated exposure to latex products during daily use or multiple surgical operations and procedures at an early age are postulated to lead to latex sensitivity (19).

Latex proteins are responsible for latex allergy as evidenced by skin test, basophil histamine release, IgE RAST, ELISA, and immunoblot assays (3, 13, 18, 20, 25). Manufacturing procedures including ammoniation have been suspected to alter latex proteins (20). Previous investigators have used several methods to characterize latex antigens from different sources (6, 24). We and other investigators also used hybridoma technology to obtain pure relevant latex antigens (14, 21).

In the present study, we report the development of a monoclonal antibody (mAb) 1E2 against a latex allergen having an approximate molecular mass of 23 kDa. We have also investigated the presence of the antigen reacting with 1E2 in finished latex products and the effects of ammonia treatment on the 23-kDa allergen. The results demonstrated that the 23-kDa allergen was present in a majority of finished latex products. Ammonia treatment altered most of the 23-kDa allergen and revealed additional high molecular mass proteins with reactivity to the mAb against the 23-kDa allergen.

Material and methods

Nonammoniated natural latex protein (NAL)

Natural rubber sap collected by tapping rubber trees (*Hevea brasiliensis*) in Malaysia was frozen immediately and shipped on dry-ice. It was thawed at 4°C, and the clear supernatant from the clotted latex was collected and put through a 0.45-µm filter to remove small rubber particles. The filtrate contained non-ammoniated natural latex proteins (NAL).

To determine the effect of incubation at room temperature for a prolonged period of time on the 23-kDa antigen, NAL was left in a sterile tube for up to 7 days. This resulted in a precipitate. After centrifugation, the precipitate was collected and washed five times with phosphate-buffered saline (PBS) to remove soluble proteins. After prolonged incubation at room temperature, both supernatant and precipitate of NAL were studied in SDS-PAGE and immunoblot.

Ammoniated natural latex protein (AL)

To study the effect of ammonia on the latex antigens, particularly the 23-kDa polypeptide, NAL was treated with 0.7% ammonia (26) for 1, 3, and 7 days separately at room temperature. All three treatments resulted in two phases: a supernatant and a precipitate. The supernatants were collected by centrifugation and were dialyzed against PBS across 1000-Da cutoff dialysis tubes (Spectrum Medical Industries, Inc, Los Angeles, CA, USA) at 4°C for 48 h with frequent changes of PBS. The precipitate obtained after 7 days' treatment was washed five times with PBS to remove soluble proteins. All three supernatants and the precipitate from 7 days of ammonia treatment were used in SDS-PAGE and immunoblot studies.

Extracts of finished latex products

The finished latex products used in this study included latex gloves, surgical supplies, balloons, pencil erasers, finger protectors, and rubber cement (Table 1), extracted with PBS for 24 h at room temperature. The protein contents and the allergenicity of these extracts were reported previously (29).

Unextractable antigens of latex gloves

Latex glove pieces were washed repeatedly after extraction with PBS to remove remaining elutable antigens from the gloves. The glove pieces were then cut into circles (6.4 mm in diameter) and used directly in ELISA as antigens. Three brands of surgical gloves, Ansell (Ansell International, Melbourne, Australia), MEDyX (Steubenville, OH, USA), and Perry (Style 332, Massillon, OH, USA), were used in this study.

Production and purification of monoclonal antibody

The fusion procedure for the production of B-lymphocyte hybridomas was as previously described (10–12, 14). Briefly, 1 mg NAL was mixed with the same volume of TiterMax Adjuvant (CytRx Corp., Norcross, GA, USA) to immunize BALB/c

Table 1. ELISA results of monoclonal antibody 1E2 reacting with extracts of finished latex products

Latex products	OD
Glove 1 (Desert, NJ, USA)	0.278*
Glove 2 (Seamless, FL, USA)	0.256
Glove 3 (Johnson, TX, USA)	2.4
Glove 4 (Perry, Style42, OH, USA)	0.221
Glove 5 (Perry, Style73, OH, USA)	0.167
Glove 6 (Perry, Style89, OH, USA)	0.276
Penrose Drain (Davol, Inc, RI, USA)	0.144
Foley Cath (Bard, Inc, NJ, USA)	0.644
Dick Valve (Burron Medical, Inc, PA, USA)	0.165
Back Check Valve (Burron, Medical, Inc, PA, USA)	0.203
Extension Set (Abbott Hospital, Inc, USA)	0.168
Suction Tubing (Davol, Inc, RI, USA)	0.296
Balloon (USA)	0.094
Eraser (USA)	0.105
Finger protector (USA)	0.101
Rubber cement (USA)	0.075
Negative control	0.071

* Mean OD value at 490 nm. Result was considered to be positive when mean OD was greater than twice negative control.

mice twice. Spleen cells from immunized mouse were fused with mouse myeloma cells (P3-NS1/Ag4-1). Of the several mAbs produced against latex proteins, mAb 1E2 reacted with the 23-kDa allergen in NAL on immunoblot was selected for the present study. The isotype of the heavy chain of 1E2 was found to be IgG1, as determined by ELISA using rabbit antimouse heavy-chain specific antibodies (Zymed Laboratories, South San Francisco, CA, USA). The mAb from ascitic fluid was purified by using a protein A column (Pierce, Rockford, IL, USA), according to the method of Harlow & Lane (8).

SDS-PAGE

The protein profiles of various latex antigens were evaluated in a 15% polyacrylamide gel containing 0.1% SDS with Bio-Rad Mini Protean II Electrophoresis equipment (Bio-Rad, Richmond, CA, USA). Electrophoresis was carried out for 45 min at a constant voltage of 200 V. The gel was then stained with Coomassie brilliant blue or transferred to a nitrocellulose membrane for use in the immunoblot assay.

Two-dimensional electrophoresis (2-DE)

The protein profile of NAL was evaluated by 2-DE where isoelectric focusing was used in the first and SDS-PAGE in the second dimension. The procedure of 2-DE was the same as described before (1).

Immunoblot

The mAb 1E2 was tested for reactivity with antigens by immunoblots. The procedure of immunoblot was the same as described before (1, 2). Purified mAb 1E2 was used at a concentration of 2 µg/ml. The molecular weight and isoelectric points of the latex polypeptides reacting with 1E2 were determined by comparing with the standards run at the same time. A mAb (AspB12) against *Aspergillus* having an IgG1 isotype which had been evaluated previously (10) served as a negative control.

ELISA with extracts of finished latex products

ELISA was used to demonstrate the presence of the specific antigen reacting with mAb 1E2 in extracts of finished latex products. Ninety-six-well plates (Microtiter, Dynatech, Chantilly, VA, USA) were coated with various extracts (10 µg protein/ml, 100 µl/well). mAb 1E2 (2 µg/ml), biotinylated antimouse IgG1 (1:2000, Zymed), and streptavidin-HRP (1:5000, Sigma) were added (100 µl/well) sequentially, and each was incubated for 1 h at room temperature. The plates were washed with PBS containing 0.01% Tween 20 (PBS-T) after each incubation. Then the substrate, orthophenyl diamine (OPD), was added to each well. The color developed was read with an automatic ELISA Reader (Dynatech Laboratories, Inc, Chantilly, VA, USA) at 490 nm. Appropriate controls were used in each plate. The results were expressed as positive when the mean OD value was greater than twice the negative control.

ELISA with unextractable antigens of latex gloves

ELISA was performed with washed glove pieces to detect unextractable antigens reacting with the mAb 1E2 present in the latex gloves. The glove pieces (6.4 mm diameter) were put on the bottoms of the wells of 96-well plates (Dynatech) and blocked in PBS-T for 2 h. This was followed by addition of 100 µl mAbs (2 µg/ml) diluted in PBS-T. After incubation at 4°C overnight, biotinylated antimouse IgG1, streptavidin-HRP, and OPD were added as described above. The glove pieces were washed between the addition of the reagents. Finally, equal volumes of reaction mixtures were removed to another ELISA plate, and the OD at 490 nm was measured as described above. mAb AspB12 served as a negative control.

Results

Antigens reacting with mAb 1E2 in NAL

More than 25 distinct protein bands in NAL were detected by SDS-PAGE stained with Coomassie

brilliant blue (Fig. 1, lane 1). mAb 1E2 showed predominant reactivity with a 23-kDa protein of NAL in immunoblot of SDS-PAGE and a minor faint band at the 19-kDa position (Fig. 2, lane 1). Fig. 3 shows over 100 polypeptides in NAL resolved by a 2-DE gel reacting with rabbit anti-NAL antibodies. In an immunoblot of 2-DE, eight polypeptides reacted with this mAb. The approximate molecular masses and isoelectric points of these polypeptides are shown in Fig. 4. One polypeptide (Fig. 4, number 3) with approximate molecular mass of 23 kDa (pI 4.8) showed a strong reaction on immunoblot. This 23-kDa polypeptide had significant allergenicity as it bound to serum IgE from about 80% of spina bifida patients with latex allergy (10).

The effect of ammonia treatment on the antigens of mAb 1E2

Although the 23-kDa protein in the supernatant and precipitate of NAL treated with ammonia could not be detected by SDS-PAGE gel stained with Coomassie brilliant blue (Fig. 1), it was present in both kinds of samples and showed a weak reaction with 1E2 in immunoblot (Fig. 2). Ammonia treatment also resulted in additional bands reacting with 1E2 at high molecular weight regions compared with the pretreatment sample. The supernatant of NAL treated with ammonia for 1 day showed a significant band at the 72-kDa position reacting with 1E2 in

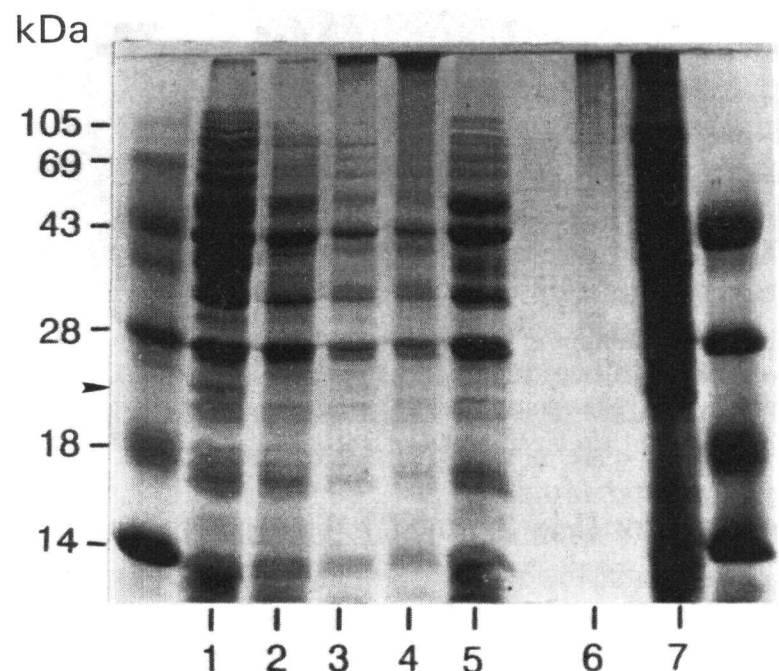


Fig. 1. SDS-PAGE stained with Coomassie brilliant blue. Lane 1: nonammoniated latex (NAL); 2: supernatant of NAL treated with ammonia for 1 day; 3: supernatant of NAL treated with ammonia for 3 days; 4: supernatant of NAL treated with ammonia for 7 days; 5: supernatant of NAL incubated at room temperature for 7 days without ammonia; 6: precipitate of NAL treated with ammonia for 7 days; 7: precipitate of NAL incubated at room temperature for 7 days. Arrow indicates 23-kDa polypeptide. High molecular mass standards in far left lane and low molecular mass standards in far right lane.

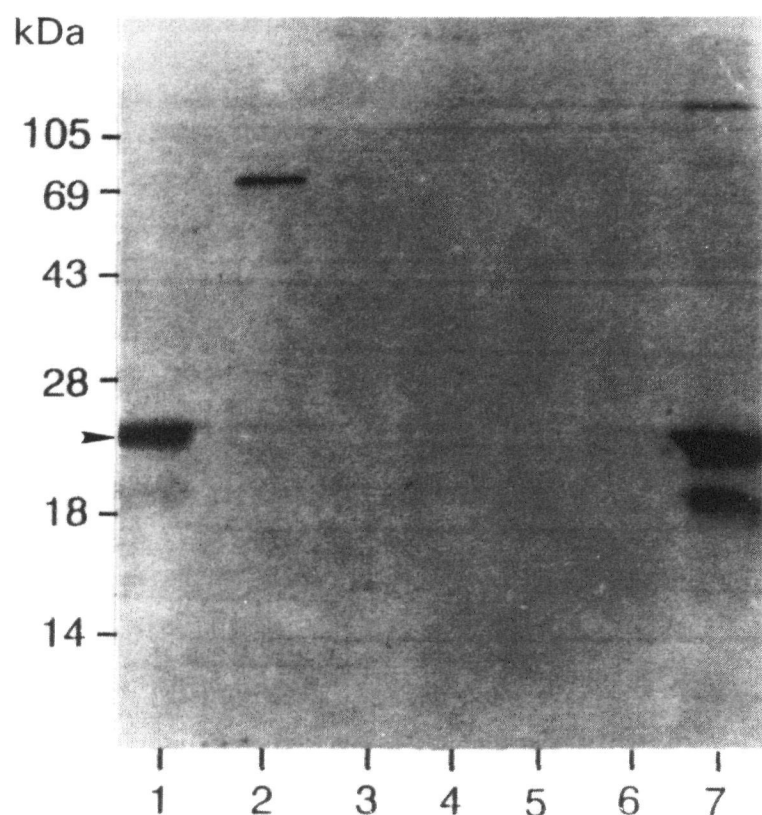


Fig. 2. Immunoblot showing various latex antigens reacting with monoclonal antibody 1E2. Lanes 1–7 are same as in Fig. 1. Arrows indicate 23-kDa band.

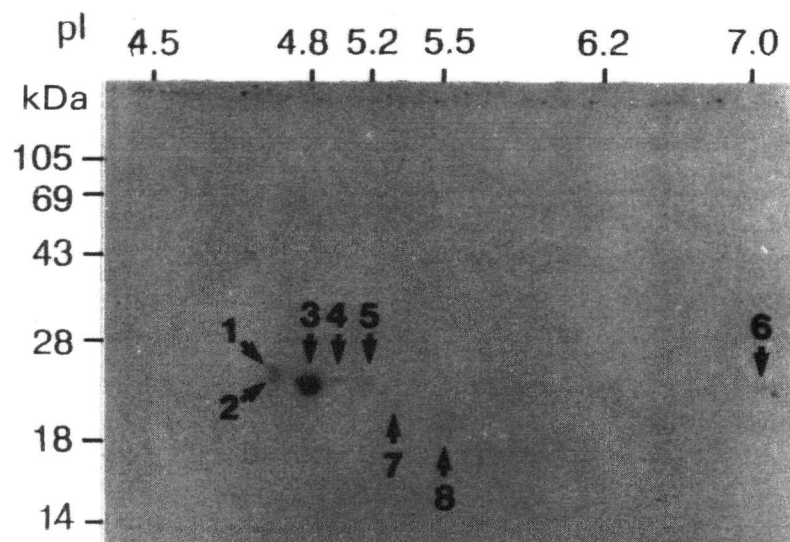


Fig. 4. Reactivity of monoclonal antibody 1E2 to peptides of NAL transferred from 2-DE gel. 1: 24 kDa, 4.7 pI; 2: 23 kDa, 4.7 pI; 3: 23 kDa, 4.8 pI; 4: 23 kDa, 5.0 pI; 5: 23 kDa, 5.2 pI; 6: 23 kDa, 7.0 pI; 7: 21 kDa, 5.3 pI; 8: 19 kDa, 5.5 pI.

reaction with 1E2 was noted on the immunoblot (Fig. 2, lane 5). However, the precipitate reacted strongly with the mAb and showed four major bands at about 120, 23, and 19 kDa and a band at the top of the immunoblot membrane (Fig. 2, lane 7).

Reaction of the mAb 1E2 with extracts of finished latex products

The reactivity of 1E2 against antigens extracted from finished latex products is shown in Table 1. Of the 16 samples investigated, 12 samples used in health care showed positive reactions, while the other four samples extracted from balloons, erasers, finger protectors, and rubber cement separately failed to react with 1E2.

Reaction of the mAb 1E2 with unextractable antigen of latex gloves

The reactivity of extensively washed gloves with 1E2 was detected by ELISA; the results are shown in Fig. 5. All the brands of gloves investigated in the present study showed significant reaction with mAb 1E2 ($P < 0.01$) in comparison with the reactivities of the antigen with a control mAb AspB12 produced against *Aspergillus*. Of the three gloves, the Perry gloves showed stronger reactivity with 1E2 than did the other two brands of gloves.

Discussion

The early detection of latex allergy, particularly in high-risk populations such as patients with spina bifida and health-care workers, is essential to avoid untoward reactions in these individuals. However, the allergens used in diagnosis are currently pre-

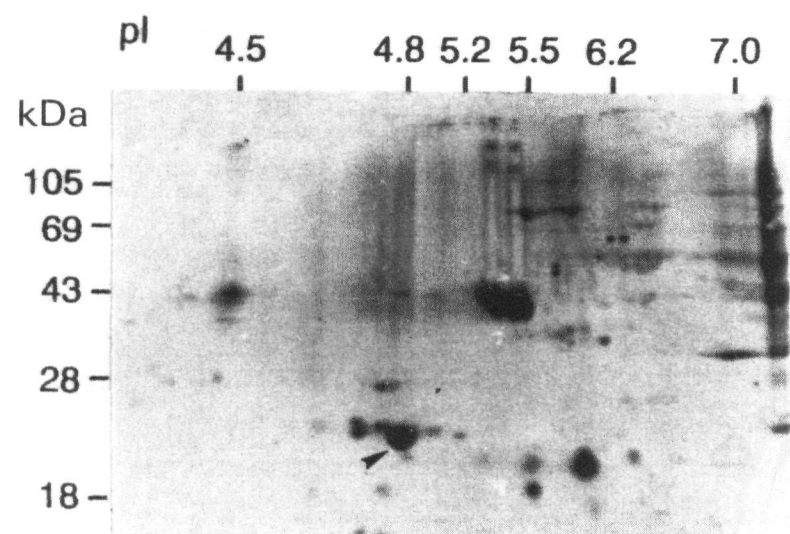


Fig. 3. Immunoblot of NAL reacting with rabbit anti-NAL antibodies after 2-D electrophoresis. Large number of NAL proteins reacted with rabbit antibodies. Arrow indicates 23-kDa spot.

immunoblot (Fig. 2, lane 2). This polypeptide only showed a weak band in immunoblot of the sample treated with ammonia for 3 days (Fig. 2, lane 3), but not for 7 days (Fig. 2, lane 4). Meanwhile another band at the top of the SDS-PAGE gel appeared first in the supernatant of 3 days of ammonia treatment (Fig. 1, lane 3) and became stronger in the supernatant of 7 days of ammonia treatment (Fig. 1, lane 4). This protein also reacted with 1E2 on the immunoblot. (Fig. 2, lanes 3 and 4).

The supernatant of NAL incubated at room temperature for 7 days showed a faint band at the 23-kDa position in SDS-PAGE (Fig. 1, lane 5), but no

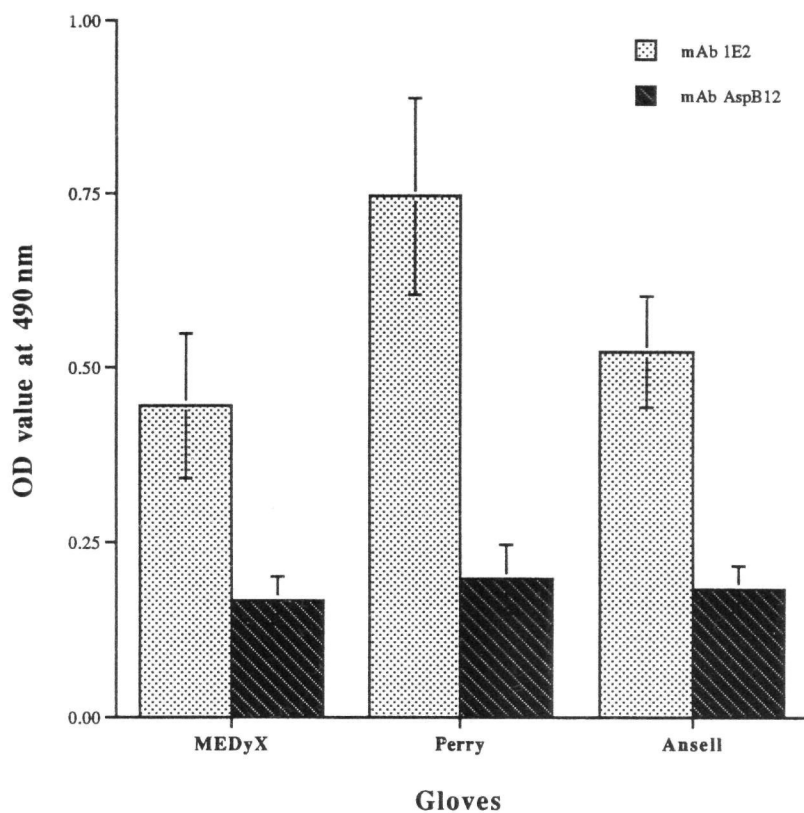


Fig. 5. Reactivity of monoclonal antibody 1E2 to extensively washed latex gloves by ELISA. AspB12, irrelevant monoclonal antibody, served as control. Mean OD values were plotted. Bars indicate standard error of mean.

pared from different latex products by various techniques in different laboratories. These allergens vary considerably in their protein composition, reactivity, and dependability. Even the latex allergens commercially available in some countries are not standardized. Hence, the results obtained by different laboratories are not comparable. Therefore, standardized latex allergens are needed for the reliable and dependable clinical diagnosis of latex allergy.

A large number of latex proteins have been shown to react with human IgE in patients with latex allergy. A 23-kDa polypeptide present in NAL has demonstrated significant binding to IgE antibody from about 80% of spina bifida patients having latex allergy (10). Although the monoclonal antibody has been recognized as a powerful tool in characterizing and purifying antigens, it has not yet been used to characterize important latex allergens. In the present study, we evaluated a mAb (1E2) reacting with the 23-kDa allergen and its potential use in purifying relevant latex allergens which may be helpful in the diagnosis of latex allergy.

The antigen reacting with mAb 1E2 was widely present in the extracts of finished latex products. Of the 16 extracts of finished latex product, 12 showed positive reaction with 1E2, and these 12 products, including surgical gloves and intravenous (iv) tubings, are frequently used in health care. Furthermore, all of the six glove extracts (four brands) used in this study showed positive reactions with 1E2, while a previously reported murine mAb LA-E3 reacted with only two of 10 glove extracts (14). Because of the

presence of the antigen reacting with 1E2 in a number of medical devices, spina bifida patients are likely to be exposed frequently. Hence, the demonstration of the 23-kDa allergen in latex products may help in the avoidance of such products with the expectation of reducing anaphylactic reactions.

Natural rubber latex coagulates spontaneously within the first few hours of collection if no anti-coagulants are added. One of the earliest and most widely used methods of preservation is the addition of ammonia to the latex to a concentration of 0.7–1.6% (7, 19, 26). It has been reported that treatment of latex sap with ammonia degrades antigenic polypeptides (20). This was verified in our study by comparing the latex protein profiles before and after ammoniation. Fewer protein bands could be detected in SDS-PAGE of an ammoniated latex protein sample than in untreated NAL (Fig. 1, lanes 1 and 4). It was also observed that the amount of 23-kDa polypeptide in AL was much less than in NAL, indicating that ammoniation degraded this protein.

Ammoniation also resulted in modification of the latex peptides in some instances. It is significant that a 72-kDa protein appeared in the supernatant after 1 day's treatment with ammonia but disappeared on prolonged treatment (3 days or 7 days), while a protein appearing on top of the SDS-PAGE after ammonia treatment became more distinct as the duration of ammonia treatment was increased. The 69-kDa protein and the new protein that appeared on top of the gel showed reactivity with the mAb 1E2, indicating that ammoniation may have altered the 23-kDa allergen present in NAL to another form with a large molecular size while retaining the 23-kDa allergen epitope. The 23-kDa protein in extracts of finished latex products has not previously been reported in SDS-PAGE studies. The 23-kDa allergen is probably present as a modified or degraded protein in extracts of finished latex products and may not always have a 23-kDa molecular size.

Ammoniation of natural latex results in two phases, one liquid and the other solid. The solid product is about 50–60% of the total latex and is used to make dipped latex products (7). In the present study, we observed that ammoniation also induced water-insoluble proteins. We assume that the water-soluble proteins of ammoniated latex are present in the extracts of finished latex products, and the water-insoluble latex proteins of ammoniated latex may still remain as unextractable antigens of latex products. This is supported by the present study demonstrating that latex antigens reacting with mAb 1E2 exist in both the supernatant of ammoniated latex (Fig. 2, lane 4) and the extracts of finished latex products (Table 1), and that mAb 1E2 reacts with both the precipitate of ammoniated latex (Fig. 2, lane

6) and extensively washed latex gloves (Fig. 5). Since the unextractable antigens contain the epitope of the 23-kDa allergen, they may also be involved in latex allergic reactions, especially those resulting from contact.

The same latex sample incubated at room temperature without ammonia also resulted in the precipitation of latex proteins, and treatment with or without ammonia had seemingly different effects on the proteins. NAL incubated at room temperature failed to show the 23-kDa polypeptide in the supernatant (Fig. 2, lane 5), but the antigens reacting with 1E2 constituted a major proportion of the precipitate, as evidenced by the immunoblot results (Fig. 2, lane 7). The new proteins which appeared in the precipitate at a high molecular weight region also reacted with 1E2, indicating that the 23-kDa polypeptide may have aggregation characteristics. This characteristic of the polypeptide may be the reason why our attempts, using an affinity column of 1E2, to obtain pure 23-kDa antigen free of other peptides from NAL have been unsuccessful.

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