

Clinical aspects of allergic disease

Characterization of a monoclonal antibody against latex protein associated with latex allergy

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Several hybridomas were produced against antigens extracted from the latex sap of Hevea brasiliensis. One of the monoclonal antibodies (LA-E3) reacted with antigens demonstrating binding to patient sera on crossed enzyme immunoelectrophoresis. This monoclonal antibody reacted with 2 of 10 glove extracts studied and with both Malaysian and Indian latex plant extracts. The antigens purified with monoclonal antibody affinity chromatography demonstrated specific IgE in the sera of patients with latex allergy as determined by ELISA. This monoclonal antibody can thus be utilized to obtain reliable antigens useful in the diagnosis of latex sensitivity, although additional antigens will likely be necessary to enhance sensitivity and specificity. (J ALLERGY CLIN IMMUNOL 1993;92:638-43.)

Key words: Monoclonal antibody, latex protein, latex allergy, rubber plant extract

Although contact hypersensitivity to rubber products has been known for a long time, immediate hypersensitivity has not been considered a major concern until recently.¹⁻⁴ The marked increase in the use of latex products, particularly gloves, in recent years has been attributed to acquired immunodeficiency syndrome and has likely contributed to the increase in latex sensitization and the resulting allergic reactions in susceptible individuals. Severe generalized anaphylactic reactions resulting in death, as well as urticaria, rhinitis, and respiratory symptoms such as asthma have been described as a result of latex sensitization.⁵⁻⁸ Increased sensitivity to latex has been particularly noted in patients with meningo-myelocele or spina bifida, in patients undergoing barium enemas with latex balloon catheters, and in certain health care workers.⁹⁻¹³

The early detection of latex allergy is essential

Abbreviations used:

CIE:	Crossed immunoelectrophoresis
MoAb:	Monoclonal antibody
PBS:	Phosphate-buffered saline
RPE:	Rubber plant extract
SDS-PAGE:	Sodium dodecylsulfate-polyacrylamide gel electrophoresis

to avoid untoward reactions, particularly in high-risk patients. Skin prick test reactivity to latex allergens constitutes a major diagnostic tool, which is used to identify such patients. However, the allergens/antigens used to demonstrate IgE-mediated skin hypersensitivity are crude glove and latex sap extracts prepared by a number of techniques by different laboratories. These antigens vary considerably in their potency, reactivity, and dependability, and hence the results obtained are not comparable.¹⁴⁻¹⁷ Similarly, skin tests that are useful in the diagnosis of latex allergy may be hazardous in susceptible patients and may be contraindicated in some instances.¹⁸ In such patients the diagnosis rests on information from available in vitro assays. Currently, RAST and ELISA are used to measure latex-specific IgE levels in these patients.^{14-16, 19, 20} However, because of the lack of defined antigens, the results are not completely dependable and are often not reproducible.

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To address these problems it is essential to have relevant and dependable antigens available for skin testing and in vitro antibody detection assays. The development of monoclonal antibodies (MoAbs) to significant latex allergens would allow purification of relevant antigens for diagnosis. One of the MoAbs (LA-E3) produced against latex in this study was used in affinity chromatography to purify antigen that showed a high degree of reactivity with specific IgE antibodies in the sera of latex-sensitive patients. In this report we present the characterization and evaluation of an antigen purified with an MoAb produced against latex antigen in vitro assays to demonstrate latex-specific IgE antibodies in the sera of patients.

METHODS

Latex antigen for immunization

The sap from *Hevea brasiliensis* was collected in India and transported to the laboratory in 0.5 formalin to suppress microbial growth and to preserve latex proteins. The sap was extracted with equal volumes of phosphate-buffered saline (PBS) (pH 7.4) overnight at 4° C and centrifuged at 15,000 rpm for 30 minutes. Three layers could be clearly identified after centrifugation. The clear fluid in the middle layer was collected with a needle and syringe and again extracted as described above. The clear PBS extract was dialyzed against deionized water at 4° C and frozen at -70° C in aliquots. The reactivity of this rubber plant sap extract (RPE) was ascertained by ELISA with known positive and negative sera. The extract was further characterized by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for its protein profile and by crossed immunoelectrophoresis (CIE) with rabbit anti-latex serum for the number of reactive components.

Other latex antigens

Glove extracts prepared from 10 different brands and batches of surgical and examination gloves as described previously were used to evaluate cross-reactivity of the MoAb produced as described below.^{20, 21} We have also studied the cross-reactivity of the MoAb against sap extract obtained from Malaysian *Hevea brasiliensis* (a gift from Dr. Kristina Turjanmaa, Finland).

Patients

Serum samples from 42 patients with immediate wheal and flare skin test reactivity to latex allergens were studied for specific IgE antibodies. Nine of these patients had a history of anaphylaxis to latex products. This group included patients with spina bifida and health care workers of both sexes and various ages. Seventeen normal control serum samples were obtained from laboratory workers with casual exposure to

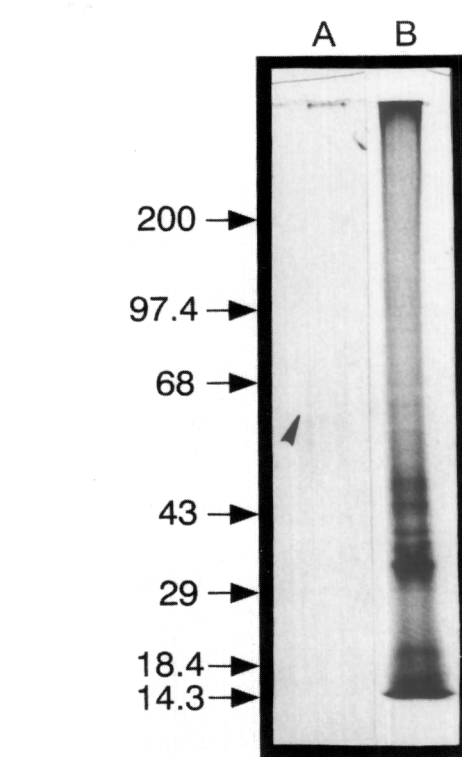


FIG. 1. SDS-PAGE of latex proteins. Lane A, Latex protein purified with MoAb (LA-E3); lane B, crude RPE. Molecular weight standards are marked on the left.

latex gloves and other latex products who had no symptoms of latex allergy. Only seven of these subjects volunteered for latex skin testing with crude antigens, and all results were negative.

Production of MoAbs

BALB/c mice were inoculated subcutaneously with 200 µg of the latex protein mixed with 1 mg of Immunotone (Southern Biotechnology Co., Rockville, Md.).²² Weekly injections were given to each animal for 4 weeks. One week after the last injection, the animals were bled and the specific IgG antibody against latex was tested by ELISA. Animals demonstrating elevated levels of latex-specific IgG compared with preinoculation levels were given boosters of the same antigen. Animals were killed 3 days after the last immunization, and their spleen cells were isolated and fused with myeloma cells (P3-NS-1/Ag-4) as previously described.²² Hybridomas were obtained by hypoxanthine, aminopterin, and thymidine selection. Antibody production by hybridomas was tested by ELISA, and clones that secreted relevant MoAbs were purified by successive cloning with the use of limiting dilution. These hybridomas were expanded and used for ascites production in pristane-primed syngeneic mice. The ascitic fluids were collected, purified, and used in immunologic studies.²²

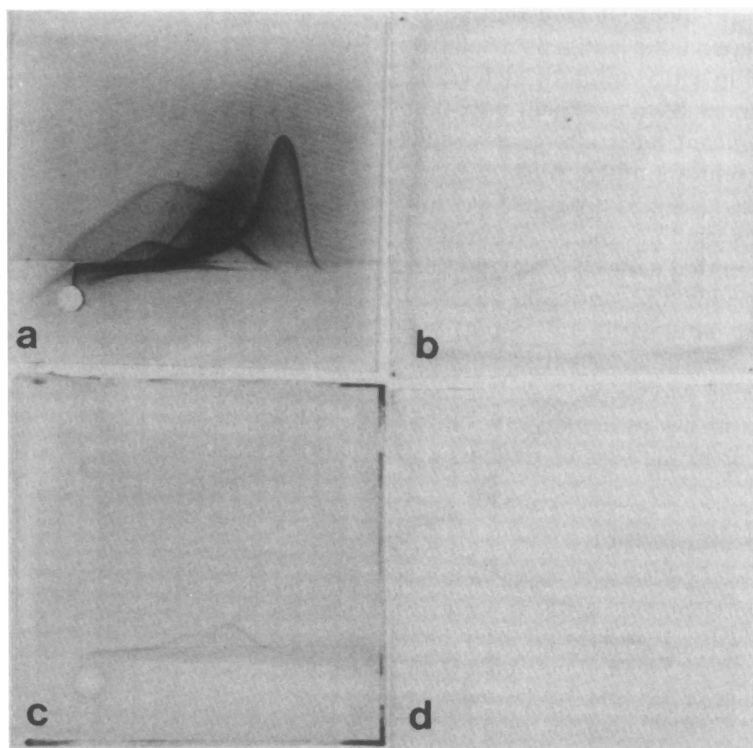


FIG. 2. CIE of RPE and rabbit anti-latex serum (**a**) and crossed enzyme immunoelectrophoresis with LA-E3 and patient sera (**b-d**). **a**, CIE of RPE and rabbit anti-latex antibody showing 12 to 16 precipitin arcs; **b**, crossed enzyme immunoelectrophoresis with LA-E3 showing three stained bands, **c**, latex-sensitive patient serum showing identical reaction as in **b**, indicating reactivity of the same three bands with IgE antibody, and **d**, negative reaction with a normal control serum sample.

Characterization of MoAb

The MoAb was characterized by means of isotype-specific ELISA. The reactivity of the MoAb was tested against additional latex antigens prepared as described above. Two RPEs and 10 glove extracts were tested against the MoAb.

CIE and crossed enzyme immunoelectrophoresis

The number of reactive antigenic components in the crude latex extract was determined by CIE. To determine whether human antibody to latex can bind to the precipitin arcs, crossed enzyme immunoelectrophoresis was carried out. The precipitin arcs formed in the gels were incubated with individual serum samples from patients with latex allergy. Human IgE binding to the latex antigens present in the antigen-antibody complex was visualized with the use of enzyme-conjugated second antibody and a chromogenic substrate as previously reported.²³ Similarly, the MoAb binding to the precipitate in CIE was also studied by incubation with the MoAb followed by goat anti-mouse IgG. The gels were then incubated with an enzyme-coupled second antibody and a chromogenic substrate, and stained precipitin arcs were compared with those formed with patient sera.²³

Purification of specific proteins with MoAb affinity chromatography

The MoAb (LA-E3), as well as the IgE antibodies, from patients with latex allergy demonstrated binding to identical precipitin arcs formed in CIE. Hence, we used LA-E3 to purify specific IgE-binding antigens. The LA-E3 was precipitated with 50% ammonium sulfate and bound to Sepharose-4B beads (Pharmacia, Uppsala, Sweden) and used in a column to obtain pure antigens from the crude extract as previously described.²⁴ The antigens thus purified were then subjected to SDS-PAGE and ELISA. The gels were stained with Coomassie Brilliant blue R250 (Imperial Chemical Industries, Ltd., Macclesfield, Cheshire, England), and the molecular weight of the purified protein was determined by comparison with crude antigen and molecular weight standards.

ELISA

Wells of polystyrene microplates (Immulon 2, Dynatech Laboratories, Chantilly, Va.) were coated with 100 μ l of a 1 μ g/ml concentration of the purified (LA-E3; affinity purified) latex protein diluted in PBS.²⁰ The plates were incubated at room temperature for 3 hours followed by overnight incubation at 4° C. After washing and blocking the wells, different serum samples were

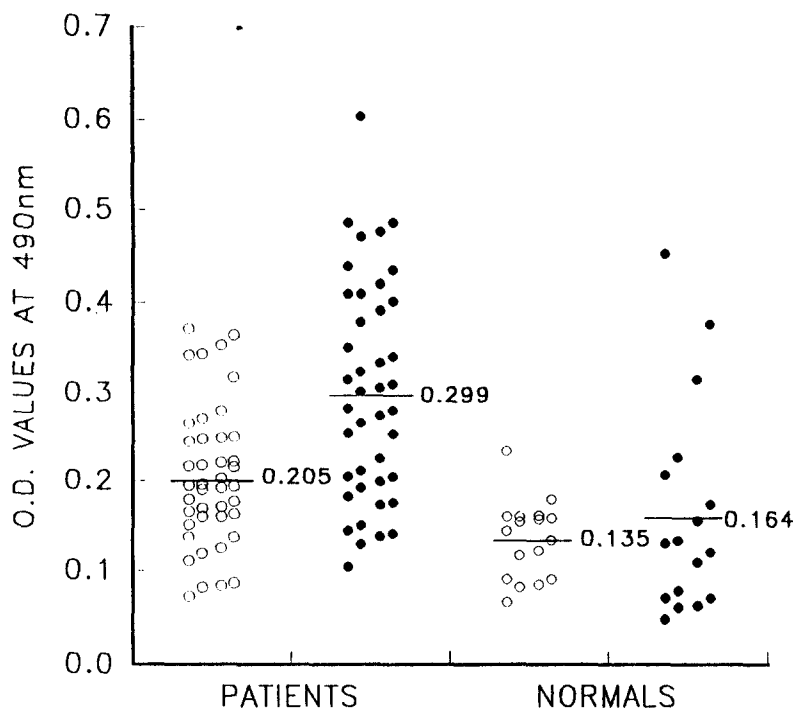


FIG. 3. IgE antibodies in the sera against LA-E3-purified antigen and crude antigens. *Open circles* are optical density values with LA-E3 antigen, and *closed circles* are values with crude antigen. *Horizontal bars* represent mean optical density values of each group.

added and incubated for 2 hours at room temperature. This was followed by the addition of goat anti-human IgE, biotinylated rabbit anti-goat IgG, and streptavidin peroxidase. The plates were incubated at room temperature for 1 hour each with different reagents and washed with PBS-Tween in between the addition of reagents. Ortho-phenylenediamine substrate was added, and the color developed was measured in an automatic ELISA reader with a 490 nm filter. The optical density values were compared with known positive and negative samples run with each plate. Similarly processed crude antigen-coated plates (1 µg/ml) were also compared. All of the reagents were standardized by checkerboard titration with the use of positive and negative sera.²⁰

RESULTS

MoAbs

A number of clones secreting latex-specific antibodies were obtained from fusions performed from the splenocytes of two mice immunized with latex antigens. On the basis of the reactivity and high titer in ELISA and the specific reactivity to human IgE-binding antigens in crossed enzyme immunoelectrophoresis, one MoAb (LA-E3) was selected for further study. As determined by isotyping, this MoAb contained IgG1 heavy chain and "k" light chains.

The latex protein reacting with this monoclonal antibody was purified by means of affinity chro-

matography as described above. When subjected to SDS-PAGE, this protein demonstrated three faint bands in close proximity to one another, which were similar to the protein bands seen with RPE (Fig. 1). When compared with standards, these peptides showed a molecular weight size range of 55 to 65 kd. Reactivity of LA-E3 to different antigen extracts from gloves and RPE indicates considerable diversity. Of the 10 glove extracts studied, 8 showed no reactivity with LA-E3, whereas the two remaining glove extracts and both Malaysian and Indian RPE reacted with the MoAb.

In CIE the crude RPE showed 12 to 16 precipitin arcs when tested against sera from rabbits immunized with either Malaysian or Indian RPE (Fig. 2, *a*). Glove antigens usually showed only two to eight weak precipitin arcs against these sera. In crossed enzyme immunoelectrophoresis LA-E3 reacted with three precipitin arcs (Fig. 2, *b*). These three arcs also showed reactivity with IgE antibody from patients with latex allergy but not with normal control subjects (Fig. 2, *c* and *d*).

Demonstration of specific IgE against antigens purified with the MoAb

Both RPE and purified antigen demonstrated IgE antibody in the sera of patients with skin test reactivity to latex antigen. Of the 42 patients, 30%

showed "significant" levels of latex-specific IgE antibodies (2 standard deviations above the mean of control subjects), whereas none of the normal subjects showed latex-specific IgE. Crude RPE, on the other hand, produced elevated IgE levels in 26% of patients and 6% of normal control subjects (Fig. 3). Of these patients only 16% had elevated IgE antibodies to both crude and purified antigens.

DISCUSSION

We have obtained several hybridomas that secrete MoAbs against latex proteins. The antigen purified with one MoAb in affinity chromatography resolved in SDS-PAGE and demonstrated 55 to 65 kd peptides. This MoAb (LA-E3) also reacted in crossed enzyme immunoelectrophoresis and demonstrated three precipitin arcs. These three arcs reacted with patient sera but not with any of the normal sera studied. The three bands obtained in the SDS-PAGE may be breakdown products of the same antigen or may represent partially homologous peptides recognized by the MoAb. Nevertheless, these antigens, purified with the MoAb, represent a significant antigen associated with latex allergy.

The peptides purified with the MoAb demonstrated antibodies in all sera from all patients and normal subjects but in varying titers. However, only 2 of 10 crude glove antigen preparations tested in the present study reacted with the MoAb. It is not surprising that the protein content of gloves and other end products varies considerably. Hence, to represent most of the latex antigens, additional MoAbs are essential. We have several other hybridomas that show different reactivities with glove antigens, and RPE from India and Malaysia (unpublished results). It is interesting to note that patients and normal subjects demonstrated latex-specific IgE to the purified antigen, indicating the possibility of exposure to diverse latex products.

The antigen purified with MoAb affinity chromatography reacted with latex-specific IgE in the sera of patients with positive skin test results. However, several patients who demonstrated skin test reactivity failed to show elevated levels of IgE against this antigen. Therefore because of the likely diversity in the immune response, additional antigens are essential to increase the reliability of the technique. With pure antigens high specificity, but not high sensitivity, can be expected because some of the epitopes may not be available to react with antibody. However, sensitivity can be im-

proved by the addition of newer relevant purified antigens.

All patients and normal subjects showed recognizable levels of IgE against latex as determined by ELISA, although levels in normal subjects were much lower than those in patients. In a previous article, we demonstrated the specificity of these antibodies with a dose-dependent inhibition ELISA assay.²⁰ Furthermore, we have also found that there are no detectable IgE antibody levels in cord sera (unpublished observations). This suggests that universal exposure to latex is likely the reason for the diverse reactivity noted.

In Western blot analysis Alenius et al.¹⁶ have shown that an antigen with a molecular weight of 53 kd from both ammoniated and frozen rubber plant sap extracts reacted with patient sera. In Western blots, Slater and Chhabra²⁵ and Jaeger et al.²⁶ demonstrated proteins with molecular weights greater than 43 kd and 45 kd, respectively. It may be possible that some of these proteins are identical to the antigen presently purified with the MoAb. In conclusion, a latex antigen has been isolated by means of MoAb affinity chromatography and can be of value in demonstrating IgE antibody in some of the sera from patients with latex allergy.

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