

Hypoallergenicity of guayule rubber particle proteins compared to *Hevea* latex proteins

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Abstract

Hevea brasiliensis Muell. Arg. is currently the sole commercial source of natural rubber. However, products made from *Hevea* latex are responsible for causing allergies affecting an increasing number of people world-wide. In this paper we test the hypothesis that latex allergy can be circumvented by using rubber of low hypoallergenicity from other species. We also report studies to determine the feasibility of ameliorating latex allergy in *Hevea*.

We show that antibodies raised against proteins extracted from films of ammoniated *Hevea* latex did not recognize latex proteins from two other rubber producing plant species, guayule (*Parthenium argentatum* Gray) and *Ficus elastica* Roxb., indicating that *Hevea* latex allergy can be circumvented using rubber from other species. The hypoallergenicity of guayule and *F. elastica* latex was confirmed in preliminary clinical trials (reported elsewhere). *F. elastica* rubber is mostly of low molecular weight and short chain, whereas guayule rubber is comparable in quality to that of *Hevea*. Guayule latex should be suitable for the manufacture of high-quality, hypoallergenic natural rubber products for the *Hevea*-hyperallergic individual.

The *Hevea* latex protein antibodies recognized not only proteins from raw *Hevea* latex, but also rubber particle preparations from which the soluble latex proteins were removed. Hence extensive removal of rubber particle-bound proteins, in addition to the soluble latex proteins, would be required to produce a safe *Hevea* latex product. Latex samples from different commercial lines of *H. brasiliensis* reacted similarly to the antibodies, indicating that clonal selection is unlikely to be helpful in eliminating latex antigens.

Keywords: Guayule; *Parthenium argentatum*; *Hevea brasiliensis*; *Ficus elastica*; Latex allergy; Latex protein; Natural rubber; *cis*-1,4-polyisoprene

1. Introduction

Latex allergy is triggered by proteins present in products made from natural rubber. The prevalence and severity of symptoms have greatly increased in recent years along with increased use of latex gloves and other products. The allergy can

cause immediate (Type I) hypersensitivity, which in its most serious manifestation can lead to severe anaphylactic reactions. The Food and Drug Administration responded to this public health problem by issuing a medical alert concerning use of latex products (March 1991) (Tomazic et al., 1992).

The protein allergens come from the latex of *Hevea brasiliensis* Muell. Arg., the Brazilian rub-

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ber tree, at present the sole commercial source of natural rubber. Latex is a complex and complete cytoplasm, which contains about 30% rubber in the form of microscopic particles. Some of the latex proteins are soluble, some are associated with organelles, and some are bound to the surface of the rubber particles. The most serious allergens in a natural rubber product could originate from any or all of these protein groups. Attempts to identify latex allergens have implicated proteins with sizes ranging from 2 to 100 kD, including a 14.6 kD rubber particle-bound protein (Hamann, 1993; Czuppon et al., 1994).

Over 2000 species of plants make and accumulate rubber. Other rubber-producing species may contain proteins different from those of *H. brasiliensis* and perhaps provide a source of hypoallergenic rubber. We have examined two plant species, *Ficus elastica* Roxb. and guayule (*Parthenium argentatum* Gray).

F. elastica, the Indian rubber tree, is used mainly as an ornamental and was one of the first plants of tropical Asia to be grown for rubber production (Polhamus, 1962). Like *Hevea*, *F. elastica* accumulates rubber in latex vessels. Although *F. elastica* produces predominantly poor quality, low molecular weight rubber, small amounts of high molecular weight rubber are produced (Hager et al., 1979; Cornish et al., 1993).

Guayule, a desert shrub native to the Chihuahuan desert of north-central Mexico and southwest Texas, produces high molecular weight rubber comparable to *Hevea* rubber. Guayule is the only crop other than *Hevea* that has been utilized, in the past, for rubber production on a commercial scale, and guayule rubber has been used for fabricating automobile tires (Hammond and Polhamus, 1965). Unlike the laticiferous species, guayule produces rubber in root and stem parenchyma cells. However, a latex-like material can be prepared from guayule that is suitable for the manufacture of dipped goods such as gloves and medical devices (Jones, 1948; Cornish, 1993). Germplasm improvement is being conducted to increase rubber yield and make guayule rubber competitive with that from *Hevea* (Thompson and Ray, 1989). However, the identification of high value markets such as hypoallergenic rubber prod-

ucts would make the immediate development of guayule feasible.

In this paper we report studies to determine the feasibility of ameliorating latex allergy in *Hevea*, and as an alternative, to test the hypothesis that latex allergy can be circumvented by using rubber from other species.

2. Materials and methods

2.1. Plant materials

Guayule plants (line 11591) were field-grown at the U.S. Water Conservation Laboratory, Phoenix, AZ. Bark from mature plants was air-transported on ice to Albany, CA. The bark (100 gm) was homogenized in 400 ml of 100 mM Tris-HCl, pH 7.5, 50 mM KF, 1% ascorbic acid, 5 mM MgSO₄, 5 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride, and 40 gm of polyvinylpyrrolidone, and filtered through cheesecloth. The resultant rubber particles were purified by three cycles of centrifugation and resuspension using 100 mM Tris-HCl, pH 7.5, 2.5 mM MgSO₄, as previously described (Cornish and Backhaus, 1990).

F. elastica plants were obtained from a local nursery and grown in a greenhouse at Albany, CA. *F. elastica* latex was obtained by excising the stems and petioles and collecting the exudate. The latex was analyzed immediately as described below. Rubber particles were purified from the latex as previously described (Siler and Cornish, 1993).

Hevea latex was provided by J.R. Bugansky, Senior Botanist, Plantations Operations, The Goodyear Tire & Rubber Co., from *H. brasiliensis* trees (lines PB260, PB330 and GYT577) at the Goodyear Plantations in Sumatra. All are commercial clones. PB260 is a cross between PB5/51 and PB49, and PB330 is a cross between PB5/51 and PB32/36. GYT577 is of unknown parentage (J.R. Bugansky, pers. commun., 1993). Latex was collected by tapping, mixed with two volumes of stabilization buffer (0.1 M NaHCO₃, 50% glycerol, 0.3% (w/v) NaN₃, and 5 mM cysteine) and air-transported as cabin luggage (ambient temperature) to Albany, CA. Rubber particles were purified from the *Hevea* latex as previously de-

scribed for guayule (Cornish and Backhaus, 1992) by four cycles of centrifugation and resuspension in 100 mM Tris-HCl, pH 7.5, 2.5 mM MgSO₄.

2.2. Protein determinations

Latex and purified rubber particle preparations were incubated in 1% sodium dodecyl sulfate (SDS) for 1 h at room temperature to solubilize membrane and rubber particle-bound proteins. The remaining rubber was removed by filtration through a 0.22 μ m membrane. The filtrates containing the proteins were diluted in distilled water, and protein concentrations were determined using the Micro-BCA protein assay (Pierce, Rockford, IL), as described by the manufacturer.

2.3. Enzyme-linked immunosorbent assay (ELISA)

Latex proteins were analyzed using the Guthrie LEAP Latex ELISA for Antigenic Proteins (Guthrie Research Institute, Sayre, PA). The LEAP assay is based on an antiserum from rabbits immunized against proteins extracted from films of ammoniated latex. The assay was performed as described by the manufacturer with slight modification. All incubations were done at 37°C. Protein samples from latex and purified rubber particles were serially diluted with 0.1 M sodium carbonate, pH 9.6, and bound to wells (100 μ l/well) of polystyrene microtiter plates (Corning) by incubating the plates for 4 h. Control wells were coated with dilutions of protein from latex film extracts (latex protein standard). Wells were rinsed once with wash buffer (phosphate-buffered saline (PBS) containing 0.1% Tween 20), and blocked (200 μ l/well rather than 100 μ l/well as recommended) for 1 h in 5% bovine serum albumin in PBS. Wells were rinsed three times with wash buffer, and incubated overnight with rabbit antiserum raised against proteins extracted from films of ammoniated *Hevea* latex (100 μ l/well), followed by three rinses with wash buffer and a 2 h incubation in anti-rabbit IgG conjugated to the enzyme horse radish peroxidase (HRP) (100 μ l/well). After a final rinse (five times with wash buffer), HRP substrates were added (100 μ l/well), and color development was allowed to proceed for

20 min. The reaction was stopped by the addition of 50 μ l/well of 4 N sulfuric acid, and the absorbance read at 490 nm using a microplate reader (Bio-Tek Instruments, Inc.). Color development is proportional to the amount of HRP present, and thus to the amount of antibody bound. This is a measure of the amount of antigen present in the samples. The microtiter plates, the rabbit anti-latex protein antiserum, the HRP-anti-rabbit IgG conjugate, the control latex protein, and the HRP substrates were provided with the LEAP assay kit.

ELISAs using glutaraldehyde-activated microtiter plates were done as follows. Immulon-2 plates (Dynatech Laboratories, Inc.) were incubated in 0.25% glutaraldehyde (100 μ l per well) for 1 h at 4°C, and rinsed with PBS. Protein samples were serially diluted in PBS, and allowed to bind to the wells overnight at 4°C. Wells were rinsed three times with PBS. All subsequent steps followed the procedure previously described, with the exception of incubation times (1 h in blocker, 2 h in antibody) and temperature (room temperature).

2.4. Western analysis

SDS polyacrylamide gel electrophoresis was done according to the method of Laemmli (1970), on 0.75 mm, 4–20% polyacrylamide gradient gels (Jule, Inc., New Haven, CT). Following electrophoresis, the proteins were transferred to nitrocellulose by electroblotting for 1.5 h at 300 mA in 50 mM Tris-HCl, 384 mM glycine, 0.01% SDS and 20% methanol (Towbin et al., 1979), using a Bio-Rad Trans Blot Cell cooled to 2.2°C. Nitrocellulose membranes were cut in half. One half was stained for total protein using Colloidal Gold (Bio-Rad) as described by the manufacturer. The other half was blocked with 5% nonfat dry milk and immunostained using the LEAP antibody, followed by goat anti-rabbit IgG conjugated to alkaline phosphatase (Bio-Rad). Color was developed by addition of alkaline phosphatase substrates, BCIP (5-bromo-4-chloro-3-indolyl phosphate) and NBT (nitroblue tetrazolium) (Bio-Rad). Biotinylated molecular weight standards (Bio-Rad) were detected with avidin conjugated to alkaline phosphatase (Bio-Rad) followed by alkaline phosphatase substrates.

3. Results

Proteins purified from *Hevea* latex and from *Hevea* rubber particles reacted strongly in the LEAP assay, indicating that antibodies raised against proteins in the latex film extracts recognized antigens in both *Hevea* preparations (Fig. 1). However, latex from guayule and *F. elastica* gave no reaction (Fig. 1).

It appears from the results shown in Fig. 1 that the protein solution from *Hevea* rubber particles, without the soluble latex proteins, had a higher titer than the protein solution from *Hevea* latex. However, it seemed possible that this difference reflects a difference in the binding of the proteins to the wells of the microtiter plates, rather than a difference in the concentration of antigenic proteins. Binding of proteins to the wells is due in large part to hydrophobic interactions between the proteins and the plastic material of the well. The rubber particle-bound proteins are likely to be much more hydrophobic than soluble latex proteins, and therefore would bind more

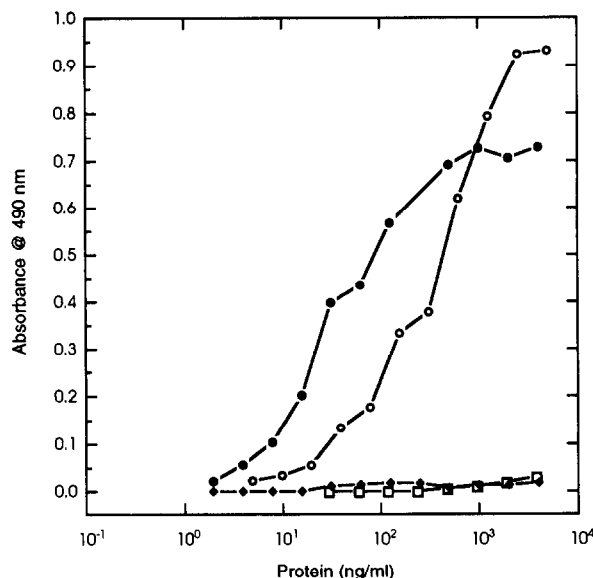


Fig. 1. LEAP assay of latex samples from three plant species. Antibodies raised against proteins extracted from films of *Hevea* latex were reacted against proteins from *Hevea* latex (circles), *Hevea* purified rubber particles (dots), *F. elastica* latex (squares), and guayule latex (diamonds).

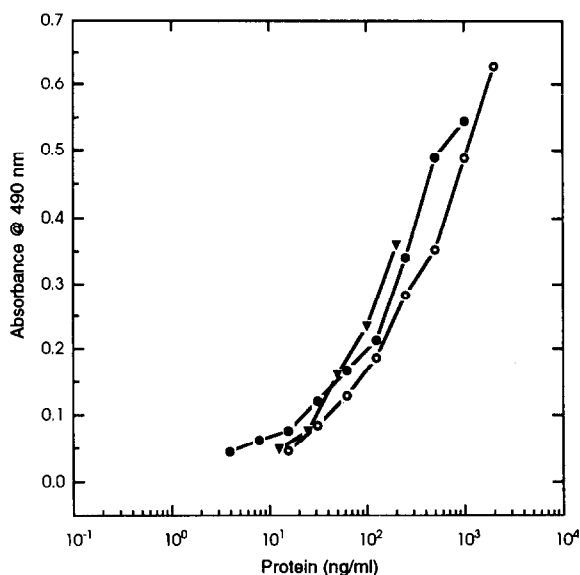


Fig. 2. LEAP assay of *Hevea* latex samples on glutaraldehyde-activated microtiter plates. Antibodies raised against proteins extracted from films of *Hevea* latex were reacted against proteins from *Hevea* latex (circles), *Hevea* purified rubber particles (dots), and control latex protein standard (triangles).

readily to the microtiter plate wells. To minimize differences in binding, assays were made using glutaraldehyde-activated microtiter plates. Glutaraldehyde can be used to activate materials through the $-NH_2$ groups (Harlow and Lane, 1988). The glutaraldehyde forms a chemical linkage between the plastic and the protein.

Proteins from *Hevea* latex and *Hevea* rubber particles exhibited similar titers when assayed on glutaraldehyde-activated plates (Fig. 2). This indicates that the differences previously observed (Fig. 1) were due to differences in the degree of binding of the proteins to the plastic.

Proteins from latex samples of the three *Hevea* lines PB260, PB330, and GYT577 showed similar reactions in the LEAP assay (Fig. 3). All three samples had similar protein concentrations before dilution, indicating comparable protein concentrations in the original latices. Therefore, the similar titers observed reflect similar concentrations of antigenic proteins.

Western analysis followed by staining with colloidal gold showed similar, but not identical, pro-

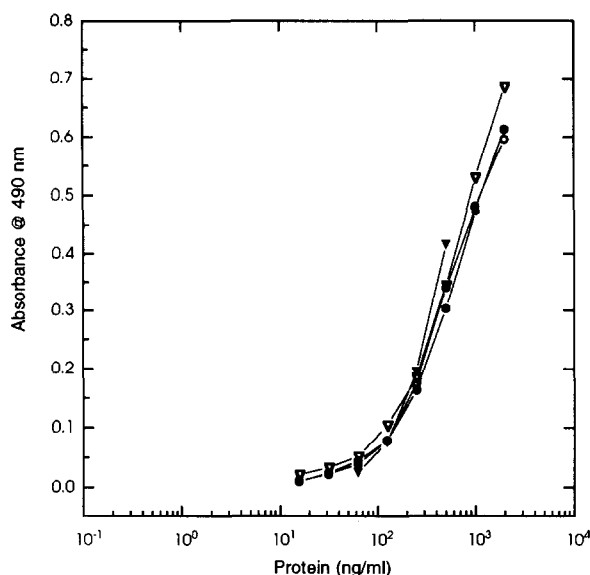


Fig. 3. LEAP assay of latex samples from three lines of *Hevea brasiliensis*. Antibodies raised against proteins extracted from films of *Hevea* latex were reacted against latex proteins from *Hevea* lines PB260 (circles), PB330 (dots), and GYT577 (white triangles), and control latex protein standard (black triangles).

tein profiles in latex and purified rubber particles of the three *Hevea* lines (Fig. 4). As expected, purified rubber particles contain far fewer proteins than latex. Immunostaining revealed numerous antigenic proteins in both latex and purified rubber particles from all three *Hevea* lines (Fig. 4). No cross-reactivity was apparent in Western analyses of the rubber particle proteins from guayule or *F. elastica* with the *Hevea* LEAP antibody. However, the predominant protein on *F. elastica* rubber particles is 376 kD (Siler and Cornish, 1993), too large to transfer efficiently in electroblotting. Therefore, weak cross-reactivity of this large protein with the *Hevea* LEAP antibody cannot be ruled out. Nonetheless, the ELISA using *F. elastica* latex (Fig. 1) demonstrates that none of the *F. elastica* proteins, including the 376 kD protein, were allergenic in the LEAP assay.

4. Discussion

Serious concerns about the safety of natural rubber products have made the development of

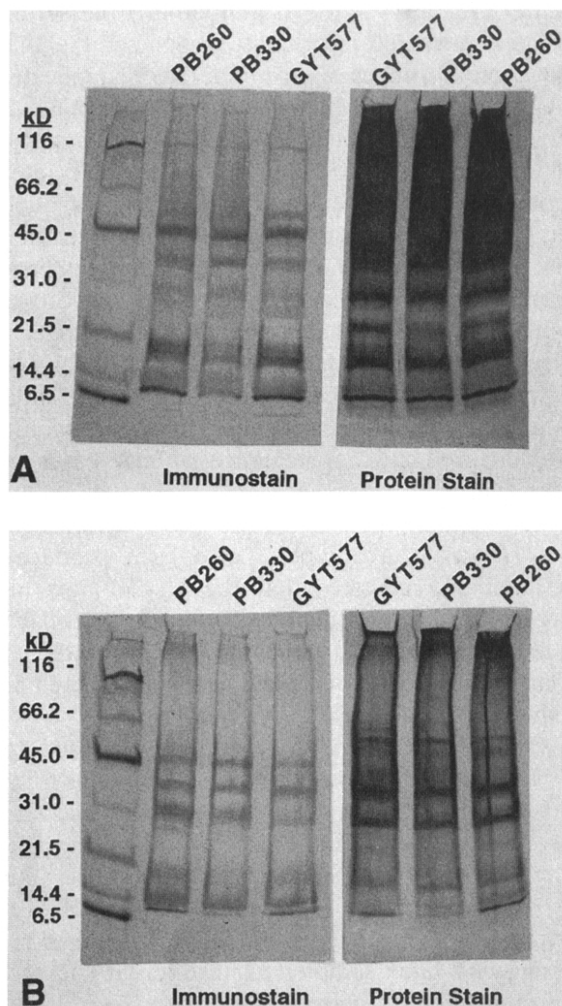


Fig. 4. Western analysis of *Hevea* lines PB260, PB330, and GYT577. Latex proteins (50 µg) (A) and rubber particle proteins (3 µg) (B) were stained with colloidal gold or immunostained with antibodies raised against proteins extracted from films of *Hevea* latex. The first lane in each gel is molecular weight standards.

hypoallergenic latex imperative. Although it appeared possible that the allergenicity of *Hevea* latex products could be ameliorated by reducing overall protein concentrations (Siler and Cornish, 1992), we have now demonstrated (Fig. 4) that even highly purified *Hevea* rubber particle preparations contain numerous proteins, which reacted strongly with antibodies raised against proteins in *Hevea* latex film extracts. The recent report (Czup-

pon et al., 1994) that IgE antibodies from serum of latex-sensitized individuals recognized the 14.6 kD protein rubber elongation factor implicates this rubber particle-bound protein as a major allergen in *Hevea* latex. Thus extensive removal of both rubber particle-bound and soluble latex proteins would be required to produce a hypoallergenic *Hevea* latex material. Our demonstration that latex samples from different commercial lines reacted similarly indicates that clonal selection is unlikely to be helpful in eliminating latex antigens. Clearly, even products made from highly purified *Hevea* latex would not be suitable for those individuals already allergic, although their use would probably reduce the incidence of new cases of latex allergy.

The hypoallergenicity of *F. elastica* latex which, like *Hevea*, is a complete cytoplasm produced in laticifers, indicates that the *Hevea* latex allergens are species-specific. Thus, sources of hypoallergenic natural rubber are not restricted to non-laticiferous rubber-producing species such as guayule.

Our demonstration that antibodies raised to *Hevea* proteins do not recognize the proteins from guayule or *F. elastica* latex provides evidence that *Hevea* latex allergy can be circumvented using rubber from other species. These results agree with preliminary medical tests where individuals with severe hypersensitivity to *Hevea* latex were skin-tested with latex samples from different sources. All these subjects reacted strongly to *Hevea* latex, whereas none responded to latices from guayule and *F. elastica* (Schränk et al., 1993; Ber et al., 1993). The inferior quality of *F. elastica* rubber precludes this species as a possible commercial source. Large-scale production of guayule latex is feasible (Cornish, 1993) and the high quality of guayule natural rubber should make it suitable for hypoallergenic product manufacture.

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