

Absence of cross-reactivity of IgE antibodies from subjects allergic to *Hevea brasiliensis* latex with a new source of natural rubber latex from guayule (*Parthenium argentatum*)

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Background: Natural rubber latex sensitization is caused by proteins from the latex of *Hevea brasiliensis*, the Brazilian rubber tree. Alternative non-cross-reactive latex rubber sources are needed for the manufacture of nonallergenic medical devices (e.g., catheters) that require elasticity, which is not currently provided by synthetic materials.

Methods: We have studied the ability of IgE antibodies from subjects allergic to *Hevea* latex and IgG antibodies from hyperimmunized mice to detect proteins derived from guayule (*Parthenium argentatum*), an alternative rubber source, using a direct binding and competitive inhibition RAST and Western blot analysis.

Results: IgE antibodies from 62 subjects allergic to *Hevea* latex (46 adults and 16 children with spina bifida) and from serum pools of adults allergic to *Hevea* latex ($n = 183$), pediatric patients ($n = 101$), and patients with spina bifida ($n = 53$), as well as IgG antibodies from hyperimmunized mice, were unable to detect any proteins in guayule by RAST or Western blot analysis. No competitive inhibition of IgE anti-*Hevea* binding to *Hevea* solid phase was detected by the preincubation of sera from subjects allergic to *Hevea* latex with soluble guayule latex before RAST analysis.

Conclusions: These results demonstrate that proteins in guayule latex are not cross-reactive with *Hevea* latex allergens. Devices that require the elasticity provided by rubber may be manufactured with guayule latex as an alternative rubber source for patients with *Hevea* latex allergy. (*J Allergy Clin Immunol* 1996;98:895-902.)

Key words: Latex allergy, cross-reactivity, human IgE, mouse IgG, guayule latex

Proteins released from latex of *Hevea brasiliensis*, the Brazilian rubber tree, are now known to be highly allergenic. Since the first reports of latex allergy in the 1980s, IgE-mediated hypersensitivity

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Note on conflict of interest: Dr. Cornish has applied for a patent on some of the products described in this article (see reference 5).

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Abbreviations used

NAL:	Nonammoniated latex
PVDF:	Polyvinylidene difluoride
SDS-PAGE:	Sodium dodecylsulfate-polyacrylamide gel electrophoresis
TBS:	Tris-buffered saline
TTBS:	TBS containing 0.05% Tween

to *Hevea* latex proteins has appeared to increase, partly as a result of an increased use of latex gloves and other rubber products (e.g., condoms) and alterations in rubber manufacturing practices.^{1,2} Although efforts are being made to further reduce protein levels in *Hevea* latex products, even low protein products are unsuitable for those individuals already allergic to latex. At present, over 2500 species of plants are known to accumulate rubber in microscopic particles. Only *Hevea brasiliensis*

rubber has been used in the manufacture of medical and consumer goods. Some of these other plants may be commercially exploitable as alternative sources of latex, may fulfill the needs provided by rubber in terms of tensile strength and elasticity, and may be safe for use by individuals allergic to *Hevea* latex.

In this study we examine one attractive alternative rubber source, *Parthenium argentatum* or guayule, which is a desert shrub native to the Chihuahuan desert of north-central Mexico and southwest Texas. Guayule plants produce a natural rubber, which is comparable in quality to that of *Hevea*.³ However, unlike *Hevea*, which produces rubber particles in latex vessels that can be readily tapped, guayule accumulates rubber in bark parenchyma cells. A latex-like rubber particle suspension can be made from guayule bark, which is suitable for the production of dipped rubber goods such as gloves, catheters, and other medical and consumer devices.^{4,5} We demonstrate, using RAST and Western blot analyses, that serum IgE antibodies from individuals allergic to *Hevea* latex and IgG antibodies from *Hevea* latex-hyperimmunized mice do not recognize proteins from guayule latex, and thus *Hevea* and guayule latex proteins appear to be non-cross-reactive.

METHODS

Hevea latex

First, *Hevea* latex was obtained from two sources. J. R. Bugansky, a senior botanist in the Goodyear Tire & Rubber Co., provided nonammoniated latex (NAL) from clones PB260 and GYT577 of *H. brasiliensis* trees from a plantation in Sumatra. After tapping of the trees, the latex was mixed with two volumes of stabilization buffer (0.1 mol/L NaHCO₃, 50% glycerol, 0.3% [wt/vol] sodium azide, and 5 mmol/L cysteine) and transported at ambient temperature to Albany, California, where it was stored at -20° C until use. The latex was used for Western blots. Rubber particles were purified from *Hevea* latex clone PB260, as previously described.⁶ The rubber particle preparation was used for immunizing mice.

Second, the *Hevea* NAL used in direct binding and RAST inhibition studies was collected from *H. brasiliensis* trees (clone 600) by Revertex (Malaysia) and shipped to Baltimore at 40° C. This latex was collected in the same stabilization buffer without sodium azide. Processing of this latter NAL is discussed elsewhere.⁷

Guayule latex

Guayule plants were grown at the U.S. Water Conservation Laboratory in Phoenix, Arizona. Bark from mature shrubs was transported on ice to Albany, California. Rubber particle suspensions (purified latex) were made from bark tissue by repeated cycles of centrifuga-

tion, as previously described.⁶ The rubber particle suspensions were used for immunizing mice. Latex for Western blots and immunoadsorbents was made from guayule plants field-grown at the Maricopa Agricultural Center of the University of Arizona. The shrub was crushed with a hammer mill in an aqueous medium containing 0.1% sodium sulfite and 1% polyvinylpyrrolidone. After filtration, ammonia was added to a concentration of 0.2%, and the latex was purified from clarified homogenate by three cycles of phase separation, followed by four cycles of dilution of the latex with ammonium alginate, allowing the phases to separate and removing the lower nonlatex phase. The final latex contained 40% dry rubber, 0.05% ammonium alginate, and 0.2% ammonia and was stored at 4° C until use.

Protein determinations

Both guayule and *Hevea* latex protein levels were quantified by using the Micro-BCA protein assay (Pierce Chemical Co., Rockford, Ill.), as modified by Siler and Cornish.⁸

Polyclonal murine antibodies to guayule proteins

Female Swiss Webster mice were injected three times with purified *Hevea* or guayule latex rubber particle suspensions containing 60 µg of protein in RIBI adjuvant (ImmunoChem Research, Inc., Hamilton, Mont.), followed by one boost. Production of ascites fluid was induced, and fluid was collected by aspiration. Ascites fluid was clarified by centrifugation, and IgG antibodies were purified by chromatography on a 1 ml bed volume Protein G column (Hi-Trap; Pharmacia, Uppsala, Sweden) according to the manufacturer's recommendations.

Patient and control sera

Serum was obtained from two groups of subjects after informed consent was obtained. The test group included 46 adults with *Hevea* latex allergy, the majority of whom were health care workers, and 16 children with *Hevea* latex allergy and spina bifida. These subjects all had a positive clinical history for latex allergy and a positive IgE anti-*Hevea* latex antibody level greater than 2 ng/ml, as determined by RAST analysis.⁹ The control group included sera from four nonatopic adults, two atopic adults who were not allergic to *Hevea* latex, and two children with spina bifida who were not allergic to *Hevea* latex. The age, sex distribution, and total serum IgE levels for these subjects are presented in Table I. Analyses were also done with three serum pools containing individual sera from 101 children (pediatric serum pool), 53 children with spina bifida (spina bifida serum pool), and 183 adults (adult serum pool).

Guayule and *Hevea* latex allergosorbents

Guayule and *Hevea* latex proteins were individually coupled to CNBr-preactivated Sepharose 4B-CL (0.5 mg of protein per milliliter of packed particles; 2 hours at 23° C and 16 hours at 4° C). Unreacted sites on the

TABLE I. Study group demographics and direct binding RAST results

Study group	Adults not allergic to <i>Hevea</i> latex	Adults allergic to <i>Hevea</i> latex	Children not allergic to <i>Hevea</i> latex	Children with <i>Hevea</i> latex allergy and spina bifida
No. of subjects	6	46	2	16
Age (yr) (mean $\pm$ SEM)	41 $\pm$ 5	34 $\pm$ 1	9 $\pm$ 1	10 $\pm$ 1
Sex (Female/Male)	2F/4M	45F/1M	1F/1M	6F/10M
Total serum IgE (ng/ml) (mean $\pm$ SEM)	72 $\pm$ 35 (nonatopic, n = 4) and 3230 $\pm$ 110 (atopic, n = 2)	995 $\pm$ 362	8.5 $\pm$ 0.5	671 $\pm$ 200
IgE anti- <i>Hevea</i> latex				
No. positive	0/6	46/46	0/2	16/16
ng/ml (mean $\pm$ SEM)	all < 0.5	160 $\pm$ 63	all < 0.5	209 $\pm$ 91
IgE anti-guayule latex				
No. positive	0/6	0/46	0/2	0/16
ng/ml	all < 0.5	all < 0.5	all < 0.5	all < 0.5

guayule and *Hevea* latex sorbents were blocked with 1 mol/L ethanolamine, pH 8.0, (16 hours, 4° C), washed alternatively with high and low pH buffers, and stored at 50% vol/vol in phosphate-buffered saline–1% bovine serum albumin–0.05% Tween-20, 0.01% sodium azide (RAST buffer). Of the protein added, 92% and 89% of the guayule and *Hevea* proteins, respectively, were bound to activated Sepharose (Pharmacia) as determined by measurements of optical density at 280 nm on the fluid phase before and after coupling. The presence of immunoreactive protein on the guayule sorbent was confirmed by saturation analysis with increasing amounts of hyperimmunized mouse IgG anti-guayule control antibodies and a 1% vol/vol quantity of the guayule-Sepharose. Bound mouse antibody was subsequently detected with iodine 125-labeled staphylococcal protein A. Specificity of the guayule sorbent was confirmed by preincubation of the mouse IgG anti-guayule with soluble guayule protein before addition to the guayule sorbent. This competitive inhibition analysis demonstrated a 96% reduction in bound mouse anti-guayule by preincubation of guayule protein. Analogous quality control studies were performed on the *Hevea* sorbent by using human IgE anti-*Hevea* latex as the antibody source.¹⁰ The converse inhibition experiment was performed, in which guayule latex was preincubated with the IgE anti-*Hevea* latex reference serum before addition of *Hevea* sorbent. No detectable cross-inhibition of IgE anti-*Hevea* was observed with guayule latex.

IgE anti-latex assays

IgE anti-guayule latex and *Hevea* latex were measured in a Sepharose particle-based RAST by using previously described procedures.¹¹ In these analyses, 0.5 ml of 1% vol/vol guayule latex sorbent was incubated in triplicate 12 $\times$ 75 mm disposable test tubes with 0.1 ml of serum from control subjects (atopic but not allergic to latex) and subjects allergic to *Hevea* latex. Simultaneously, the

same sera were added to different tubes containing 1% vol/vol of *Hevea* latex sorbent. A reference serum dilution curve was constructed by analyzing 10 twofold dilutions of a potent IgE anti-*Hevea* latex serum that contained 80 ng/ml IgE anti-latex as determined by depletion analysis.¹² After a 16-hour incubation at 23° C, all tubes were washed with RAST buffer, and bound IgE antibody was detected with ¹²⁵I-labeled rabbit anti-human IgE Fc (16 hours at 23° C). Bound counts per minute were counted to statistical precision in a Capintec gamma counter (Capintec, Inc., Ramsey, N.J.) and interpolated from the reference curve in nanograms per milliliter of IgE anti-*Hevea* latex (equivalent). Analytical sensitivity of the IgE anti-*Hevea* latex assay was 1 ng/ml, intraassay precision was less than 6.1% coefficient of variation, and interdilutional percent coefficients of variation less than 14% confirmed assay parallelism.

Western blots

Sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli on 0.75 mm, 4% to 20% polyacrylamide gradient gels (Jule, Inc., New Haven, Conn.).¹³ After electrophoresis, the proteins were transferred to polyvinylidene difluoride (PVDF) or nitrocellulose membranes by using a Bio-Rad Trans Blot Cell (Bio-Rad, Richmond, Calif.), and membranes were cut into strips.¹⁴ Immunostaining with anti-*Hevea* latex IgE antibodies was performed as follows. Membrane strips were blocked overnight at 4° C in 0.2% I-Block (Tropix, Bedford, Mass.) in phosphate-buffered saline, pH 7.5, containing 0.1% Tween-20. All subsequent incubations were done at 23° C with gentle agitation on a platform shaker. Membrane strips were incubated sequentially in IgE anti-latex positive or control sera diluted 1:4 in blocker (2 hours), mouse IgG₁ monoclonal anti-human IgE Fc-biotin conjugate (clone HP6029¹²; Hybridoma Reagent Laboratory, Baltimore, Md.) diluted to 1

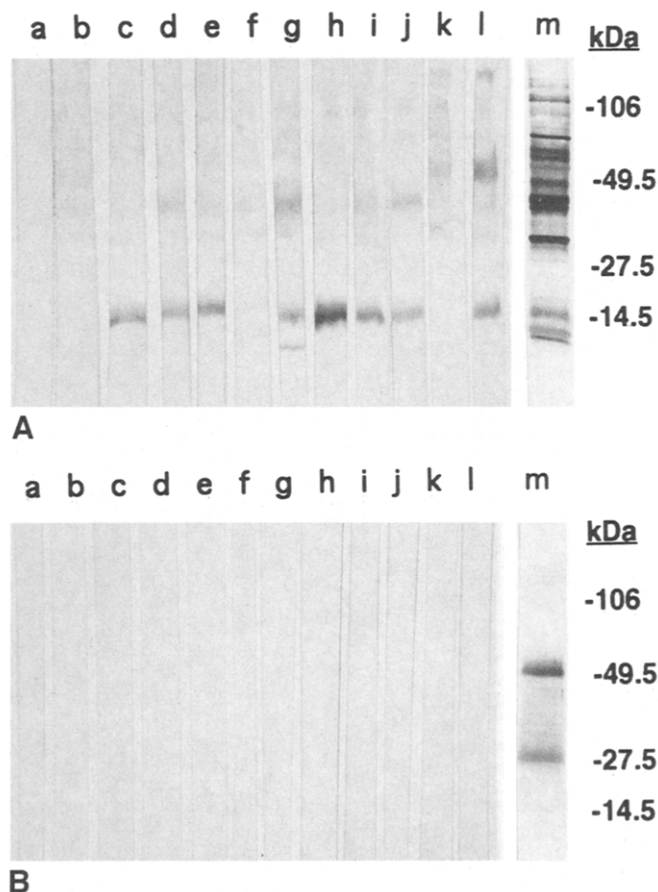


FIG. 1. Western blot analysis of *Hevea* clone PB260 (A) and guayule (B) latex proteins. The proteins, once transferred onto PVDF membranes, were incubated with IgE antibodies in sera from nonallergic control subjects (lane a) and subjects allergic to *Hevea* latex (lanes b to l). All bound IgE was then detected with a monoclonal anti-human IgE-biotin, avidin-alkaline phosphatase and substrate. Lane m was stained directly for total protein.

$\mu\text{g/ml}$ in blocker (2 hours), and ExtrAvidin-conjugated alkaline phosphatase (Sigma Chemical Co., St. Louis, Mo.) diluted 1:20,000 in blocker (1 hour). Membrane strips were washed three times for 20 minutes in blocker between each incubation step. Color was developed by addition of alkaline phosphatase substrate (Bio-Rad).

Immunostaining with mouse polyclonal anti-guayule IgG antibodies was done as follows. Membranes were blocked for 2 hours in 5% nonfat dry milk in Tris-buffered saline (TBS), rinsed in TBS containing 0.05% Tween (TTBS), incubated for 16 to 20 hours in a 1:500 dilution of mouse anti-guayule latex IgG, rinsed in TTBS, incubated for 2 hours in a 1:200 dilution of goat anti-mouse IgG alkaline phosphatase conjugate (Bio-Rad), and rinsed in TBS. Color was developed by addition of alkaline phosphate substrate. Membrane strips were also stained for total protein by using Colloidal Gold (Bio-Rad) as described by the manufacturer. Molecular weights were estimated by comparison with molecular weight standards (Bio-Rad).

ELISA

ELISAs were performed with standard methods as previously described.¹⁵ Microtiter plates were coated with proteins solubilized from guayule latex or extracts from *Hevea* latex gloves, blocked, then incubated with mouse anti-guayule latex IgG antibodies or antibodies raised in rabbits to *Hevea* latex film extracts (LEAP Assay Kit; Guthrie Research, Sayre, Pa.), followed by goat anti-mouse or anti-rabbit IgG alkaline phosphatase conjugate and substrate (Bio-Rad).

RESULTS

In the development phase of the IgE anti-guayule latex assay, the guayule protein sorbent was shown to contain bound guayule protein by the binding of the hyperimmunized mouse polyclonal IgG anti-guayule antibodies. A first indication of an absence of cross-reactivity was the observation that guayule latex proteins preincubated with the

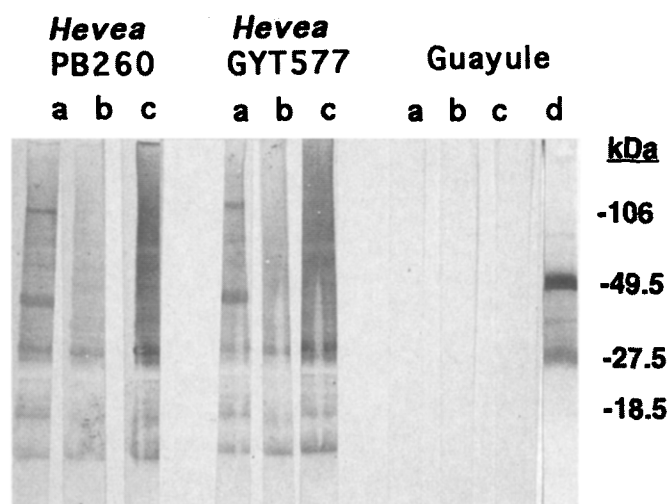


FIG. 2. Western blot analysis of *Hevea* (clones PB260 and GYT577) and guayule latex proteins. The proteins, once transferred onto PVDF membrane, were incubated with IgE antibodies from pediatric ($n = 101$, lane a), spina bifida ($n = 53$, lane b), and adult ($n = 183$, lane c) serum pools. All bound IgE was then detected with a monoclonal anti-human IgE-biotin, avidin-alkaline phosphatase and substrate. Lane d was stained directly for total protein.

IgE anti-*Hevea* latex reference serum produced no competitive inhibition of human IgE anti-*Hevea* latex binding to the *Hevea* sorbent. Also, *Hevea* latex preincubated with the hyperimmunized mouse antibodies produced no competitive inhibition of IgG anti-guayule latex to the guayule sorbent. Simultaneously performed homologous antigen inhibition controls produced more than 90% inhibition in both cases.

Table I summarizes the serologic results obtained in the IgE anti-*Hevea* and IgE anti-guayule assays with the adult and pediatric sera. No detectable nonspecific IgE binding was observed with any of the six adult or two pediatric control sera, even at total serum IgE concentrations of 3340 ng/ml. None of the total serum IgEs of the adult and pediatric subjects with latex allergy exceeded this level. None of the 62 IgE anti-*Hevea* positive sera from individuals with a clinical history of *Hevea* latex allergy produced any detectable binding in the guayule RAST. Furthermore, the adult serum pool ($n = 183$), with 459 ng/ml IgE anti-*Hevea*, produced no detectable binding in the guayule RAST. This indicated an absence of cross-reactivity between the guayule and *Hevea* latex proteins.

We further examined potential cross-reactivity by Western blot analysis. The SDS-PAGE revealed a spectrum of proteins in both the *Hevea* and guayule latex preparations (Fig. 1, A and B, lane m). Immunoblots with the adult, pediatric, and spina bifida serum pools (Fig. 2) and sera from 11 individual adults allergic to *Hevea* latex (Fig. 1,

lanes b to l) demonstrated no detectable binding of *Hevea* latex-specific IgE antibody to guayule proteins. IgE antibodies bound to many different *Hevea* latex polypeptides from clones PB260 and GYT577 (Fig. 2), as well as to clone 600 (data not shown); reactions to polypeptides of 14, 36, and 54 kd were most prominent in the serum samples from individuals (Fig. 1). The guayule latex contained two principal proteins at 30 and 52 kd (Fig. 1, B, lane m) as identified with the protein stain. None of the IgE antibodies from subjects allergic to *Hevea* latex reacted to these or other guayule latex proteins (Fig. 1, B, lanes b to l and Fig. 2).

To further examine the immunogenicity and possible cross-reactivity of the guayule and *Hevea* rubber particle proteins, mice were hyperimmunized with both preparations. Fig. 3 presents the Western blot analysis obtained with the polyclonal mouse IgG anti-guayule rubber particles and anti-*Hevea* rubber particles on nitrocellulose containing *Hevea* (lanes a, b, and c) and guayule (lanes d, e, and f) proteins. IgG antibodies raised in mice to guayule rubber particles recognized the 52 kd guayule protein (Fig. 3, lane e). The 30 kd protein present in field-processed guayule latex (Fig. 1, B, lane m) did not elicit an IgG antibody response, most likely because it is a soluble protein that was removed in the more purified rubber particle preparation used to hyperimmunize the mice (Fig. 3, lane d). Although there were multiple proteins on *Hevea* rubber particles (Fig. 3, lane a), IgG antibodies raised in mice to *Hevea* rubber particles

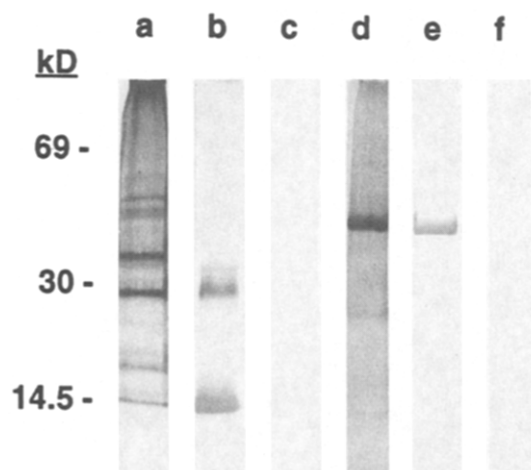


FIG. 3. Western blot analysis of *Hevea* (lanes a, b, and c) and guayule (lanes d, e, and f) rubber particle proteins. In these blots, latex proteins attached to nitrocellulose were incubated with IgG antibodies raised in mice against *Hevea* (lanes b and f) and guayule (lanes c and e) rubber particles. Bound IgG was detected with anti-mouse IgG-alkaline phosphatase and substrate. Lanes a and d were directly stained for total protein.

recognized only a few proteins, with major bands at molecular weights of 14 kD and 32 kD (Fig. 3, lane b). Most importantly, there was no cross-reactivity observed between the IgG antibodies raised in mice and the *Hevea* or guayule rubber particles (Fig. 3, lanes c and f). Mouse anti-guayule latex antibodies did not recognize proteins extracted from *Hevea* latex gloves (ELISA absorbance at 405 nm of -0.001 for $2 \mu\text{g/ml}$ protein). The same *Hevea* glove extract produced a positive ELISA reaction against antibodies raised against *Hevea* film extracts (ELISA absorbance at 405 nm of 0.383).

DISCUSSION

Sensitization to *Hevea* latex has occurred in children who receive multiple exposures during surgery and in occupationally exposed adults.^{1, 16, 17} At present, the only mode of treatment is avoidance of direct contact and exposure to airborne latex allergens (found on cornstarch donning powder). For health care workers, this means the personal use of synthetic examination and surgical gloves and the use of powder-free latex or synthetic gloves by coworkers. For individuals sensitized to *Hevea* latex, such as children with spina bifida who require frequent surgical procedures, avoidance has become more difficult because synthetic materials have not always possessed the physical properties required to re-

place *Hevea* rubber-containing medical products (e.g., catheters). Thus there are presently no ideal synthetic alternatives for some *Hevea* latex-containing medical devices. A rubber source that provides a nonallergenic alternative to *Hevea* latex would therefore be attractive for devices that must be highly elastic.

Of the approximately 240 different *Hevea* latex polypeptides that have been identified, 57 are reportedly allergenic.¹⁸⁻²⁰ IgE antibodies in the sera of children with *Hevea* allergy and spina bifida and occupationally exposed adults may recognize different proteins to different degrees, depending on their mode of initial sensitization. For instance, the 14.6 kD proteins Hev b 1 (rubber elongation factor or REF) and 24 kD Hev b 3, both of which reside principally on the rubber particle, require prolonged direct exposure to rubber particles to elicit sensitization.²¹⁻²³ Individuals who undergo multiple operations and a minority of adults who have dermatitis tend to develop IgE antibodies to these proteins. A different group of soluble *Hevea* latex proteins, which readily attach to cornstarch and can be readily aerosolized, appear to sensitize individuals who are occupationally exposed.²⁴ In this investigation we included sera from highly sensitized children and adults to ensure that a broad cross-section of IgE antibody was available for probing guayule proteins for potential cross-reactivity. The *Hevea* latex preparation used for making the immunoadsorbents is known to contain two primary rubber particle antigens.²¹

The sera containing anti-*Hevea* latex IgE from adults and children with spina bifida, which we used in our immunoassay and Western blot analyses to probe the guayule latex for cross-reactive proteins, have all been shown by in vitro and in vivo studies to bind readily to allergenic proteins in latex glove extracts.^{7, 10} Therefore the absence of reactive protein indicates that guayule does not contain allergenic proteins that are structurally similar to those in gloves and other latex products. These tests were done with field-processed guayule latex, which contains not only the rubber particle-bound proteins present in more purified rubber particle suspensions, but also soluble proteins such as the 30 kD protein (Fig. 1, B, lane m). Moreover, guayule latex proteins are not recognized by *Hevea* latex-specific polyclonal mouse IgG antibodies. However, proteins recognized by the mice may not be the same proteins that are recognized as allergens by human beings. These results with actual production lots of guayule latex confirm our previous limited observations with pilot lots^{25, 26} and

small samples (seven patients)²⁷ that guayule latex failed to produce skin reactivity in *Hevea* latex-sensitized individuals, and that IgG antibodies raised in rabbits to proteins extracted from *Hevea* latex films (LEAP Assay Kit, Guthrie Research) did not bind to guayule rubber particle proteins.¹⁵ These anti-*Hevea* film extract antibodies recognized many different *Hevea* latex proteins, including soluble and rubber particle-bound proteins.¹⁵ Thus guayule latex appears to represent an alternative source of rubber, which should be safe when used in or on *Hevea* latex-sensitized individuals.

Because guayule proteins have the potential to induce IgE immune responses, they must be considered potentially allergenic. Guayule is in the sunflower family of plants (Asteraceae), a family with over 20,000 species, which also includes the ragweeds of the genus *Ambrosia*.²⁸ Allergens from the pollen of some species of the genus *Parthenium* can cause allergic rhinitis, and there is a high degree of cross-reactivity between *Parthenium hysterophorus* (American feverfew) and ragweed pollen.²⁹ The possibility that proteins from guayule latex would cross-react with other allergens, such as those on ragweed pollen, has not been investigated. Risks of sensitization should be minimized by ensuring that guayule latex products have little or no leachable protein and are not used indiscriminately. Future developmental efforts should focus on the production of guayule latex products, such as catheters, that require the elasticity and collapsibility of rubber not matched by available synthetic materials.

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