



Short communication

Immunogenicity studies of guayule and guayule latex in occupationally exposed workers

Robert G. Hamilton^a, Katrina Cornish^{b,*}^a Johns Hopkins University School of Medicine, Johns Hopkins Asthma and Allergy Center, 205501 Hopkins Bayview Circle, Baltimore, MD 21224, USA^b Yulex Corporation, 37860 West Smith-Enke Road, Maricopa, AZ 85238, USA

ARTICLE INFO

Article history:

Received 10 July 2009

Received in revised form

10 September 2009

Accepted 14 September 2009

Keywords:

*Hevea brasiliensis**Parthenium argentatum*

Guayule

Latex allergy

IgE antibody

IgG antibody

Occupational exposure

ABSTRACT

Type I *Hevea brasiliensis* rubber latex allergy is managed by avoidance, using synthetic and alternative latex (such as *Parthenium argentatum*, guayule) products. This study investigates the ability of high-dose occupational exposure to guayule shrub/homogenate/latex to induce guayule-specific antibody responses in employees (Yulex Corporation). Participants completed an allergy history/guayule exposure questionnaire and provided annual blood samples from 2006 to 2008. Sera were analyzed for IgG and IgE anti-guayule (protein from homogenate, commercial-grade latex and non-ammoniated total plant proteins) using solid phase immunoassays (negative = IgG < 1 µg/ml, IgE < 1 ng/ml). Guayule-specific IgG antibody (range: 2.0–9.7 µg/ml) was detected in 3 of 16 (19%) highly exposed employees in the pilot plant and R&D/applications laboratory. Antibody levels related to relative cumulative-years (e.g. >3) of reported guayule homogenate/latex exposure. Equivocal IgG antibody responses (1.0–2.0 µg/ml) were detected in 2 of 5 (40%) of administrators with infrequent guayule homogenate/latex contact. No guayule-specific IgE antibody or guayule-associated allergic reactions were detected. We conclude that protein from guayule and in guayule latex can be immunogenic but not allergenic in occupationally exposed workers.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

Natural rubber latex is extensively used in commercial products ranging from airplane tires, toy balloons and condoms to protective medical gloves. While over 2500 plants accumulate rubber in microscopic particles, the principal source of commercially available natural rubber latex is from the Brazilian rubber tree, *Hevea brasiliensis* (Jacob et al., 1993). In the 1980s, reports of severe immune responses to *Hevea* latex proteins began appearing in the literature. By the 1990s, allergic reactions to *Hevea* latex had reached epidemic proportions (Charous et al., 1994; Grzybowski et al., 1996; Sussman et al., 2002). Sensitization, as evidenced by a positive *Hevea*-specific IgE antibody skin test or serology, was estimated to be 2.9–8.2% in the general healthcare worker population and as high as 12.1% in operating room workers and 56% in children with spina bifida (Grzybowski et al., 1996). The causes of the meteoric rise in the prevalence of latex allergy included increased use of protective medical gloves due to Universal Precautions and the resultant accelerated latex production and manufacturing of *Hevea* rubber gloves. An increase in *Hevea* allergenic protein exposure

led to heightened sensitization among high risk populations and an explosion in *Hevea* latex allergy symptoms ranging from hives, rhinitis and asthma to systemic anaphylaxis and death (Charous et al., 1994; Grzybowski et al., 1996).

Since pharmacotherapy, immunotherapy and anti-IgE therapy are ineffective, avoidance is the only effective means of managing latex allergies (Hamilton and Brown, 2000). *Hevea* product manufacturers have worked to reduce the allergenic protein content and alternative non-*Hevea* materials are being increasingly used. These include petroleum-based synthetic elastomers and non-*Hevea* natural rubber derived from alternative sources such as *Parthenium argentatum*, common name guayule (Mooibroek and Cornish, 2000). Guayule is a shrub that is native to the Chihuahuan desert of north-central Mexico and southwest Texas and generates a natural rubber similar in quality to *H. brasiliensis*. Unlike *Hevea* that produces rubber particles in latex vessels which are readily tapped, *Parthenium* accumulates rubber particles in its bark parenchyma cells (Bonner and Arreguin, 1949). A latex-like rubber particle suspension is made from the guayule bark by homogenizing the whole plant and extracting the latex fraction (Yulex[®] natural rubber emulsion). Purified guayule latex possesses <1% of the protein content of *Hevea* latex (Cornish et al., 2006, 2008). Moreover, about 90% of the trace protein that remains is allene oxide synthase (Pan et al., 1995) a cytochrome P450 oxidase, that belongs to the P450-protein family which has not been associated

* Corresponding author. Tel.: +1 520 381 2233; fax: +1 520 413 2629.
E-mail address: kcornish@yulex.com (K. Cornish).

with allergic reactions in humans (personal communication, Dr. HP Rihs, BGFA, Ruhr-University-Bochum, Germany).

Because guayule latex contains 1% of the protein content of *Hevea* latex, Yulex rubber products contain <2 µg of extractable protein/g dry weight product. This level is below achievable standards and quantifiable levels (<50 µg extractable protein/g dry weight product) established for *Hevea* latex products (ASTM D 3577-01 Standard Specification for Rubber Surgical Gloves; ASTM D 3578-01 Standard Specification for Rubber Examination Gloves; ASTM D6499-03 Standard Test Method for Immunological Measurement of Antigenic Protein in Natural Rubber and its Products; ASTM D5712-05 Standard Test Method for Analysis of Aqueous Extractable Protein in Natural Rubber and Its Products Using the Modified Lowry Method).

At the height of the *Hevea* latex allergy epidemic, in the mid-1990s, guayule latex was shown to lack protein cross-reactivity with *Hevea* allergens. *In vitro* studies showed that *Hevea* latex-specific human IgE antibodies did not bind to guayule latex protein (Siler et al., 1996). Positive mouse and rabbit IgG anti-guayule and human IgE anti-*Hevea* latex negative control sera confirmed the specificity of these analyses. *In vivo* studies involving puncture skin testing confirmed guayule latex to be non-allergenic in *Hevea*-sensitized healthcare workers (Carey et al., 1995). Thus, Yulex® rubber emulsion-based products are considered safe for use by *Hevea*-sensitized individuals.

“Immunogenic” products are defined as those that elicit an antibody response, principally of the immunoglobulin IgG isotype, that has minimal clinical consequences with respect to allergies. IgG antibodies are primarily involved in defense against pathogens. In contrast, an “allergenic” product elicits IgE antibody than binds or “sensitizes” mast cells and basophils, which are the effector cells that mediate immediate human allergic reactions through the release of vasoactive mediators (Matsson et al., 2009). People produce much less IgE than IgG, but the presence of IgE antibodies can be dangerous since it can trigger Type I or immediate-type hypersensitivity reactions following subsequent exposure to allergens. This can lead to anaphylactic shock and death. The objective of the current prospective study was to investigate the immunogenic (IgG antibody) and allergenic (IgE antibody) potential of guayule in a group of occupationally exposed factory, research laboratory and administrative workers. The study group includes Yulex Corporation workers who have the highest known direct guayule exposure of any single human group.

2. Materials and methods

2.1. Study population

Twenty-two Yulex Corporation employees completed questionnaires and provided sera during annual physical examinations over a 3-year period from 2006 to 2008. Participants worked in the agriculture-processing plant with daily exposure to guayule plants ($n=7$), R&D-applications laboratory where the processed latex was handled and applied to rubber product production ($n=9$) and administration with only occasional guayule exposure ($n=6$). Years of employment and relative exposure at the time of blood collection were recorded as well as the atopic status (history of multiple allergies or not) and job descriptions of the participants (Table 1).

2.2. Guayule latex preparations

Two to three year guayule shrubs (line AZ2) were processed to produce a homogenate of the whole plant, a non-ammoniated guayule latex (purified rubber particles) and a purified ammoni-

ated guayule latex protein fraction (Backhaus et al., 1991; Pan et al., 1995). Initial homogenates were made in a four gallon Waring blender and all secondary homogenizations were performed using a Polytron rotor-stator, unless specified otherwise. Homogenates were centrifuged 15 min at 6500 rpm (Sorvall RC5B centrifuge).

2.3. Guayule plant homogenate

Guayule shrubs, without roots, were harvested from fields in Arizona, homogenized in 0.2% ammonium hydroxide–0.1% Na₂SO₃ and filtered through eight layers of cheesecloth. Homogenate (1 l) was stirred (3 h) with 50 ml of 1 M Tris buffer (pH 7.5) and 50 ml of 20% SDS, homogenized for 2 min, centrifuged and then the clarified supernatant was decanted and stored (4°, 16 h). Homogenate (450 ml) was mixed with deoxycholate (DOC) (4.5 ml at 15 mg/ml) and particulates were allowed to settle for 10 min. Phosphotungstic acid/trichloroacetic acid (90 ml) (PTA/TCA) was then added and the mixture was incubated (30 min). The homogenate mixture was then centrifuged, decanted and the protein pellet was stored (4°C, 16 h). The pellet (43.69 g) was resuspended in 0.2 M NaOH (227 ml) and centrifuged for clarification, yielding a final protein content of 78 µg/ml.

2.4. Non-ammoniated guayule latex

Guayule shrubs (792 g, 60% stem, 40% leaf) were homogenized in 2500 ml of 100 mM Tris (pH 7.5) and 5 mM MgSO₄ buffer. The homogenate (3500 ml) was mixed with 200 ml 20% SDS, homogenized for 5 min and stored at 4°C overnight. 1 l was mixed with 15 mg/ml of DOC (10 ml, 10 min), and PTA/TCA (200 ml, 30 min), then centrifuged, decanted and the pellet stored (16 h, 4°C). The pellet (133.65 g) was resuspended in 143 ml phosphate-buffered saline containing 1% SDS and centrifuged to clarify. The final protein content was 50 µg/ml.

2.5. Purified guayule protein

Guayule latex (2.8 l, lot 10/24/2005) was mixed with 75 ml 2 M Tris buffer (pH 7.5) and 150 ml 20% SDS, stirred overnight, homogenized and centrifuged. Clarified homogenate (1040 ml) was mixed with DOC (10.4 ml at 15 mg/ml, 10 min), then mixed with PTA/TCA (208 ml, 30 min) and centrifuged. The pellet (83.91 g) was stored (16 h, 4°C), resuspended in 151 ml 0.2 M NaOH and centrifuged, producing a final protein content of 52 µg/ml.

2.6. Guayule latex allergosorbents

Each guayule latex preparation was individually coupled to CNBr-activated Sepharose-CL-4B (0.5 mg protein/ml pack particles. 2 h, 23°C; 16 h, 4°C) as previously described for *Hevea* latex (Hamilton et al., 1995). Unreacted sites were blocked with 1 M ethanolamine (pH 8.0, 16 h, 4°C). Each sorbent was then washed alternatively with high and low pH buffers and stored at 50% (v/v) in phosphate-buffered saline containing 1% bovine serum albumin, 0.05% Tween 20, 0.01% sodium azide [assay buffer].

2.7. Serological studies

Sera were analyzed for human IgG and IgE anti-guayule using methods previously reported for detecting *Hevea*-specific IgG and IgE antibodies in human serum (Hamilton et al., 1995). In brief, serum (1:50-IgG; neat-IgE) were rotated separately with the three guayule allergosorbents (16 h, 23°C). Following a buffer wash to remove unbound serum protein, bound human IgG and IgE were detected with radioiodinated Protein-G or anti-human-IgE, respectively (16 h, 23°C). Following a second assay buffer wash, bound

Table 1
Employee IgG anti-guayule serology.

Work group ^a	Subject code ^b	Atopic history ^c	Job title ^d	Maximum years of exposure ^e	Purified latex IgG-a-guayule (µg/ml) ^f	Homogenate IgG-a-guayule (µg/ml) ^g	Non-ammoniated latex IgG-a-guayule (µg/ml) ^h	Purified latex IgE-a-guayule (ng/ml) ⁱ	Homogenate IgE-a-guayule (ng/ml) ^j	Non-ammoniated latex IgE-a-guayule (ng/ml) ^k	
Field	CT	Negative	Research Geneticist	10	<1	<1	<1	<1	<1	<1	
	FM	Negative	VP Agriculture	3	<1	<1	<1	<1	<1	<1	
	JL	Positive	Agriculture Director	0.5	<1	1.3	<1	<1	<1	<1	
Factory	NA	Negative	VP Manufacturing	2	<1	<1	<1	<1	<1	<1	
	MR	Negative	Pilot plant manager	7	<1	<1	<1	<1	<1	<1	
	CG	Negative	Shift Supervisor	3	2.0	8.9	3.7	<1	<1	<1	
	CJ	Positive	Maintenance	2	<1	<1	<1	<1	<1	<1	
	CV	Negative	Engineer	0	<1	<1	<1	<1	<1	<1	
	HJ	Positive	Engineer	2	<1	<1	<1	<1	<1	<1	<1
				3	<1	10.4	4.7	<1	<1	<1	<1
Lab	WJ	Positive	Sn. Scientist	4	<1	9.7	3.6	<1	<1	<1	
				3	<1	8.0	4.3	<1	<1	<1	
				4	<1	5.2	2.8	<1	<1	<1	
	NK	Positive	Scientist	5	1.0	4.4	2.3	<1	<1	<1	
				2	<1	<1	<1	<1	<1	<1	
	WR	Negative	Support Scientist	3	<1	<1	<1	<1	<1	<1	
	BR	Negative	Intern	0.5	<1	<1	<1	<1	<1	<1	
	FB	Positive	QA Technician	1	<1	<1	<1	<1	<1	<1	
	LU	Positive	Technician	1	<1	<1	<1	<1	<1	<1	
	BM	Positive	Support Scientist	0.5	<1	<1	<1	<1	<1	<1	
	Admin	MJ	Negative	CEO	10	<1	<1	<1	<1	<1	<1
		CK	Positive	Sn. VP R&D	20	1.1	1.0	1.1	<1	<1	<1
		DJ	Positive	Exec Assistant	2	<1	<1	<1	<1	<1	<1
MJ		Positive	VP Tech Development	1.5	<1	<1	<1	<1	<1	<1	
GT		Positive	VP Finance & Admin	1	<1	<1	<1	<1	<1	<1	

Note: Multiple years of testing are shown only for subjects (HJ and WJ) with a positive IgG response and who volunteered for each test cycle. All other subjects had ≤3 years of testing depending on length of service.

^a Volunteers are grouped by their primary work function.

^b Subject codes to allow internal tracking.

^c A positive history indicates that the subject has a history of multiple allergies, and may, therefore, have a greater propensity for immunogenic response than those without such a history (negative rating).

^d Job title gives an indication of current responsibilities but does not necessarily reflect exposure history.

^e No attempt has been made to determine an actual cumulative exposure. Exposure to any form of guayule materials was included.

^f The amount of human IgG-a-guayule that recognized hydrolyzed proteins purified from ammoniated guayule latex.

^g The amount of human IgG-a-guayule that recognized total plant proteins (hydrolyzed) extracted from ammoniated guayule shrub homogenate.

^h The amount of human IgG-a-guayule that recognized proteins purified from non-ammoniated (non-hydrolyzed) purified guayule rubber particles.

ⁱ The amount of human IgE-a-guayule that recognized hydrolyzed proteins purified from ammoniated guayule latex.

^j The amount of human IgE-a-guayule that recognized total plant proteins (hydrolyzed) extracted from ammoniated guayule shrub homogenate.

^k The amount of human IgE-a-guayule that recognized proteins purified from non-ammoniated (non-hydrolyzed) purified guayule rubber particles.

radioactivity was measured in a gamma counter. Specificity of positive IgG anti-guayule responses was confirmed with homologous guayule competitive inhibition as previously described (Siler et al., 1996). The analytical sensitivities of the IgG and IgE antibody assays were 1 µg/ml and 1 ng/ml, respectively.

3. Results

Participants were divided into employment groups (Table 1). Table 1 presents the participants' work group, years of exposure to guayule or guayule materials, atopic history, job title, and levels of IgG and IgE antibody specific for proteins extracted from purified latex, ammoniated guayule homogenate (hydrolyzed total plant protein) and non-ammoniated guayule homogenate (total plant protein). The field group grew and harvested the guayule shrub which involved skin contact and inhalation of airborne plant materials. The factory group took the plant and processed it into a fine homogenate from which the latex was purified and concentrated. The laboratory group performed quality assurance and product development with the concentrated latex which was the highest anticipated direct contact to guayule latex proteins. The research laboratory scientists empirically varied latex formulations and produced prototype Yulex rubber products which involved direct hand contact with the guayule products. The administration group worked primarily in offices, however, the Senior Vice-President of R&D (KC) had extensive skin and inhalation exposure to guayule plants, homogenate and latex at various times over a 18-year period. Some employees had worked in multiple areas. For instance, engineer HJ had direct guayule experience in the field, in the pilot plant and in the research and development laboratory, and the pilot plant manager MR had direct guayule latex contact in the laboratory for a period of months.

The factory (pilot plant) workers all had extensive daily skin contact with guayule latex due to the nature of the latex purification process. Of the six factory employees in the study, CG had the highest estimated exposure to guayule latex, with frequent contact with equipment containing or coated with guayule materials. However, the laboratory workers, in general, had the greatest direct contact with guayule latex and latex products, especially with their hands, over the longest time period.

In this study, guayule-specific IgG antibody ranged from <1 to 10.4 µg/ml (Table 1). No IgE anti-guayule responses were detected in any of the sera tested, i.e. all serological analyses of IgE-a-guayule were <1 ng/ml.

Serological results of the study show that guayule can be immunogenic as evinced by the induction of guayule-specific IgG antibody. This required high levels of occupational exposure in the laboratory and pilot plant, but detectable allergenicity was absent since no IgE antibody was detected.

4. Discussion

The development of allergic symptoms results from a combination of interacting factors, including (1) the dose of the allergen, in this case guayule proteins, (2) the duration of exposure, which is related to the individual worker's assignment, (3) the individual's genetic make-up or propensity to develop allergies (atopic tendency), and (4) the immunogenicity of the particular protein or guayule component in question. Guayule-related immunogenicity and allergenicity studies in humans are difficult to perform because few individuals naturally encounter guayule. Furthermore, deliberate exposure for the sake of a research investigation of immunogenicity is irresponsible. This prospective study examined the only existing highly exposed human population for possible guayule latex protein immunogenicity (IgG antibody) and aller-

genicity (IgE antibody). The study group of 21 included 12 of 21 (57%) with a history of atopic tendencies: these atopic subjects had a history of multiple allergies and would, therefore, have been expected to be the most likely members of the group to show an immunogenic and allergenic response to guayule.

Results of the study confirm that given sufficient exposure, guayule plant proteins can elicit a benign IgG antibody response in humans (Table 1, columns 6–8). In sera from workers showing a specific IgG antibody response, the amount of quantifiable IgG antibody was highest in concentration against the guayule homogenate proteins. This protein preparation contains the totality of guayule protein that is extractable from the plant. Workers in all Yulex departments would be expected to have exposure to some of these proteins, even though field workers should not be exposed to latex proteins, and laboratory workers would rarely be exposed to leaf or flower proteins. Thus, worker exposure to the homogenate protein group would likely be higher than to the two forms of purified latex (non-ammoniated and ammoniated). Low IgG anti-guayule responses of 1–2 µg/ml were observed against the residual proteins in the purified latex. However, when these proteins were concentrated, they were capable of eliciting an IgG response in mice and rabbits (Siler and Cornish, 1994; Siler et al., 1996). The lack of a detectable humoral immune response in workers to latex protein, who otherwise developed an IgG antibody response to the homogenate (total plant) proteins, probably reflects the absence of a sensitizing dose of protein. In other words, the latex protein levels were too low to induce an immunogenic response. Moreover, the proportion of latex protein in the homogenate was too low to elicit an immune response, or the immunogenicity of this protein preparation had been severely reduced by hydrolysis. This contrasts with the history of Type I latex allergy development which involved widespread sensitization to high levels of soluble proteins in unwashed *Hevea* latex products. IgE antibodies responses are readily elicited to all 13 known allergenic *Hevea* proteins. This includes the rubber particle bound proteins Hev b 1 and 3 (Siler and Cornish, 1994) which, by themselves in well leached *Hevea* products, were not able to induce sensitization even with repeated *Hevea* glove exposures of health-care workers (Charous et al., 1994).

In contrast to the IgG antibody response detected in three Yulex workers to the homogenate and non-ammoniated latex proteins, no IgE antibody or clinical evidence of allergic responses were detected in these workers (Table 1, columns 9–11). Thus, the current study was unable to find evidence for guayule protein allergenicity, even in atopic workers who experienced extraordinarily high occupational exposures that greatly exceed the trace quantities of guayule protein in finished Yulex® natural rubber products.

In conclusion, guayule latex products, which are safe for use by *Hevea*-sensitized individuals because of their lack of cross-reactive protein, would be expected to remain non-allergenic in spite of long term use of guayule products that involves multiple exposures. The reason for this is the extremely low protein content and the general lack of allergenicity associated with any trace proteins in guayule natural rubber-containing products.

Acknowledgements

The authors gratefully acknowledge J.L. Williams at Yulex Corporation and Dr. W. Xie at the USDA-ARS in Albany, CA for their assistance in the preparation of the purified *P. argentatum* proteins used in this study. The authors also extend their appreciation to J. Wisenauer and C. Bronzert at the Johns Hopkins Dermatology, Allergy and Clinical Immunology (DACI) Reference Laboratory for their analyses of human IgG and IgE anti-guayule. The serology portion of this study was funded by an internal grant from the Johns Hopkins University School of Medicine, Baltimore, MD, USA.

References

- Backhaus, R.A., Cornish, K., Chen, S.-F., Huang, D.-S., Bess, V.H., 1991. Purification and characterization of an abundant rubber particle protein from guayule. *Phytochemistry* 30, 2493–2497.
- Bonner, J., Arreguin, B., 1949. The biochemistry of rubber formation in the guayule. I. Rubber formation in seedling. *Arch. Biochem. Biophys.* 21, 109–124.
- Carey, A.B., Cornish, K., Schrank, P.J., Ward, B., Simon, R.A., 1995. Cross reactivity of alternate plant sources of latex in subjects with systemic IgE mediated sensitivity to *Hevea brasiliensis* latex. *Ann. Allergy Asthma Immunol.* 74, 317–320.
- Charous, B.L., Hamilton, R.G., Yunginger, J.W., 1994. Occupational latex exposure: characteristics of contact and systemic reactions in 47 workers. *J. Allergy Clin. Immunol.* 94, 12–18.
- Cornish, K., McMahan, C.M., Xie, W., Williams, J., Nguyen, K.C., Kostyal, D., Kelly Horton, K., Marsh, D.T., 2006. ASTM D1076 Category 4 Latex and Quantifying Guayule (NRG) and Hevea (NR) Latex Protein. No 15. In: Proceedings of the 9th International Latex Conference, Charlotte, NC, July 25–26, 16 pp.
- Cornish, K., Williams, J.L., Hall, J.L., McCoy Jr., R.G., 2008. Production and properties of Yulex(r) – the natural solution to latex allergy. *Rubber Chem. Technol.* 81, 709–723.
- Grzybowski, M., Ownby, D.R., Peyser, P.A., Johnson, C.C., Schork, M.A., 1996. The prevalence of anti-latex IgE antibodies among registered nurses. *J. Allergy Clin. Immunol.* 98, 535–544.
- Hamilton, R.G., Brown, R.H., 2000. Impact of personal avoidance practices on health care workers sensitized to natural rubber latex. *J. Allergy Clin. Immunol.* 105, 839–841.
- Hamilton, R.G., Charous, B.L., Adkinson, N.F., Yunginger, J.W., 1995. Serological methods in the laboratory diagnosis of latex rubber allergy: study of non-ammoniated, ammoniated and glove extracts as reagent sources. *J. Lab. Clin. Med.* 123, 594–604.
- Jacob, J.L., d'Auzac, J., Prevôt, J.C., 1993. The composition of natural latex from *Hevea brasiliensis*. *Clin. Rev. Allergy* 11, 325–332.
- Matsson, P.N.J., Hamilton, R.G., Esch, R.E., Halsey J.F., Homburger, H.A., Kleine-Tebbe, J., Mari, A., Ownby, D.R., Reeves, J.P., Renz, H., Vogt, Jr., R.F., Williams, P.B., 2009. Analytical Performance Characteristics and Clinical Utility of Immunological Assays for Human Immunoglobulin E (IgE) Antibodies and Defined Allergen Specificities; Approved Guideline. Second Edition. CLSI: 29, No 9, Document No. I/LA20-A2.
- Mooibroek, H., Cornish, K., 2000. Alternative sources of natural rubber. *Appl. Microbiol. Biotechnol.* 53, 355–365.
- Pan, Z., Durst, F., Werck-Reichhart, D., Gardner, H.W., Camara, B., Cornish, K., Backhaus, R.A., 1995. The major protein of guayule rubber particles is a cytochrome P450: characterization based on cDNA cloning and spectroscopic analysis of the solubilized enzyme and its reaction products. *J. Biol. Chem.* 270, 8487–8494.
- Siler, D.J., Cornish, K., 1994. Hypoallergenicity of guayule rubber particle proteins compared to *Hevea* latex proteins. *Ind. Crops Prod.* 2, 307–313.
- Siler, D.J., Cornish, K., Hamilton, R.G., 1996. Absence of cross-reactivity of IgE antibodies from *Hevea brasiliensis* latex allergic subjects with a new source of natural rubber latex from guayule (*Parthenium argentatum*). *J. Allergy Clin. Immunol.* 98, 895–902.
- Sussman, G.L., Beezhold, D.H., Liss, G., 2002. Latex allergy: historical perspective. *Methods* 27, 3–9.