Industrial Crops and Products 31 (2010) 197–201

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Short communication

Immunogenicity studies of guayule and guayule latex in occupationally exposed workers

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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.

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

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doi:10.1016/j.indcrop.2009.09.012

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 ammoniated 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% Na_2SO_3 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 settled 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 $MgSO_4$ 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