

FORUM

Latex Allergy in the Workplace

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Received May 8, 2000; accepted July 25, 2000

While less than 1% of the general population is sensitized to latex, the U.S. Occupational Safety and Health Administration estimates that 8–12% of health-care workers are sensitized. The major source of workplace exposure is powdered natural rubber latex (NRL) gloves. NRL is harvested from *Hevea brasiliensis* trees and ammoniated to prevent coagulation resulting in the hydrolysis of the latex proteins. Prior to use in manufacturing, the latex is formulated by the addition of multiple chemicals. Thus, human exposure is to a mixture of residual chemicals and hydrolyzed latex peptides. Clinical manifestations include irritant contact dermatitis, allergic contact dermatitis (type IV), and type I immediate hypersensitivity response. Type I (IgE-mediated) NRL allergy includes contact urticaria, systemic urticaria, angioedema, rhinitis, conjunctivitis, bronchospasm, and anaphylaxis. Taking an accurate history, including questions on atopic status, food allergy, and possible reactions to latex devices makes diagnosis of type-I latex allergy possible. To confirm a diagnosis, either *in vivo* skin prick testing (SPT) or *in vitro* assays for latex-specific IgE are performed. While the SPT is regarded as a primary confirmatory test for IgE-mediated disease, the absence of a U.S. Food and Drug Administration-licensed *Hevea brasiliensis* latex extract has restricted its use in diagnosis. Serological tests have, therefore, become critically important as alternative diagnostic tests. Three manufacturers currently have FDA clearance for *in vitro* tests, to detect NRL-specific IgE. The commercially available assays may disagree on the antibody status of an individual serum, which may be due to the assay's detecting anti-NRL IgEs to different allergenic NRL proteins. Sensitized individuals produce specific IgE antibody to at least 10 potent *Hevea* allergens, Hev b 1–Hev b 10, each of which differs in its structure, size, and net charge. The relative content and ratios of Hevs in the final allergen preparation most probably could effect diagnostic accuracy. The Hev proteins have been cloned and expressed as recombinant proteins. Sequencing demonstrates both unique epitopes and sequences commonly found in other plant proteins. Sequence homology helps to explain

the cross reactivity to a variety of foods experienced by latex allergic individuals. The development of recombinant allergens provides reagents that should improve the diagnostic accuracy of tests for latex allergy. Although clinical and exposure data have been gathered on the factors affecting response in latex-allergic individuals, less is known regarding the development of sensitization. Coupled with *in vitro* dermal penetration studies, murine models have been established to investigate the route of exposure in the development of latex sensitization. Time-course and dose-response studies have shown subcutaneous, intratracheal, or topical administrations of non-ammoniated latex proteins to induce IgE production. Both *in vitro* penetration and *in vivo* studies highlight the importance of skin condition in the development of latex allergy, with enhanced penetration and earlier onset of IgE production seen with experimentally abraded skin. The diagnosis of latex allergy is complicated by these variables, which in turn hinder the development of intervention strategies. Further epidemiological assessment is needed to more explicitly define the scope, trends, and demographics of latex allergy. Diagnostic accuracy can be improved through greater knowledge of proteins involved in the development of latex allergy, and better documentation of the presently available diagnostic tests. *In vivo* and *in vitro* models can elucidate mechanisms of sensitization and provide an understanding of the role of the exposure route in latex allergy-associated diseases. Together, these efforts can lead to intervention strategies for reducing latex allergy in the workplace.

Key Words: natural rubber latex (NRL); latex allergy; contact dermatitis; hypersensitivity.

Introduction

Natural rubber latex (NRL) allergy has become an important occupational health concern, particularly among health-care workers. Shoup (1998) noted that latex allergy did not appear in the literature until 1979. Although references to allergic-type reactions to rubber appeared over 65 years ago (Downing, 1933; Stern, 1927), there has been little interest in the topic until the 1990s. Figure 1 illustrates the escalating number of publications appearing in the literature in the past decade. It is not known to what extent the interest in latex allergy coincides

Workshop presented at the 39th Annual Meeting of the Society of Toxicology, Philadelphia, PA, March 22, 2000.

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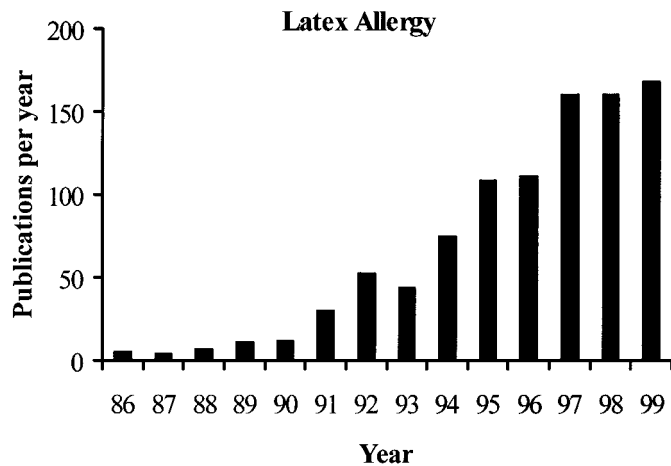


FIG. 1. Number of publications using the key words "latex allergy." Bars are total publications for a given year.

with prevalence of the disease. It is generally believed that the U.S. Centers for Disease Control and Prevention's recommendation (CDC, 1987) for the use of latex gloves to prevent HIV transmission was a seminal event. In addition, the U.S. Occupational Safety and Health Administration has required employers to provide gloves and other protective measures against blood-borne pathogens since 1992. Use of latex gloves increased from 12 billion pairs used in 1987 to 200 billion pairs used in 1997 (Valliere, 1999). Interestingly, latex gloves have been used in the health-care industry for over 100 years (Shoup, 1998). What has changed in the last decade is unprecedented increase in the use of latex gloves, changes in the manufacturing process by some manufacturers, and the switch to one-time-use disposable gloves (Shoup, 1998). Also, latex gloves are widely used today outside the health-care field by such diverse professionals as hairdressers, police officers, painters, and food-service workers (NIOSH, 1997). As NRL is used in the manufacture of a wide variety of products, allergic individuals face considerable difficulty in minimizing exposure to latex in the workplace once sensitization has occurred. Therefore, the common use of latex in household items and in medical environments has increased the possibility of sensitization to latex. Although there is no consensus on the prevalence of latex allergy, it is nonetheless considered a serious medical problem for a growing number of patients and a disabling occupational disease among health-care workers.

Processing of NRL

NRL is harvested from *Hevea brasiliensis* trees, primarily in Southeast Asia. Upon collection, chemicals, including ammonium hydroxide, formaldehyde, or zinc oxide are added to prevent coagulation, deterioration, and bacterial growth. Treatment with ammonia causes the proteins within the latex to undergo hydrolysis. The raw latex is centrifuged to separate the large rubber particles from aqueous "serum" layers containing

very small rubber particles, soluble and insoluble proteins, carbohydrates, and lipids. Prior to manufacturing, the latex is further formulated to enhance the barrier properties of latex film by the addition of anti-oxidants and accelerators (thiurams, carbamates, and/or mercapto compounds) and other chemicals. Dipped latex products are produced by immersing molds into tanks of compounded rubber. In the case of latex gloves, lubricants that facilitate donning and removal, such as cornstarch, may be added by dipping the gloves into a powder slurry. Proteins, liberated from the latex, attach to the powder as the slurry dries onto the gloves. This powder can act as a carrier for latex proteins and may potentially have adjuvant effects (Ruhl *et al.*, 1994). In addition, protein-laden powder particles can become aeroallergens during donning and removal of gloves (Tarlo *et al.*, 1994). Therefore, exposure to latex involves a mixture of residual chemicals and hydrolyzed latex peptides. The hydrolyzed nature of the allergens complicates efforts to identify major allergens and the development of reagents for *in vivo* and *in vitro* diagnostic tests.

Symptoms of NRL Allergy

Powdered NRL gloves have long been known to be responsible for irritant contact dermatitis, which is a relatively common reaction, perhaps related to occlusion, a moist environment, and other irritants such as residual soap. The most common immunologic reaction resulting from the use of latex gloves is allergic contact dermatitis, caused by rubber additives including thiurams, carbamates, and mercaptobenzothiazoles. This is due to a type-IV or delayed hypersensitivity immunologic response (Heese *et al.*, 1991).

Since 1988, an increasing number of cases of type-I allergic reactions have been reported (Sussman and Beezhold, 1995). Between 1988 and 1992, the FDA received 1000 reports of allergic reactions to NRL. The typical clinical presentation includes contact urticaria, allergic rhinoconjunctivitis, and asthma. The most serious type-I response is anaphylaxis, which is characterized, in severe cases, by airway obstruction. Life-threatening anaphylactic shock occurs during surgery in sensitized patients, due to absorption of latex protein allergens from NRL surgical gloves. Fifteen deaths have been attributed to latex-allergic reactions from exposure to NRL barium enema tips, which subsequently have been removed from the marketplace.

Prevalence of NRL Allergy

The reported prevalence of NRL sensitization in the general population is considered to be less than 1% (Liss and Sussman, 1999; NIOSH, 1997). High risk groups for sensitization and the development of clinical NRL allergy include health-care workers with reported prevalence from 6 to 17% (ACAA, 1995; Kaczmarek *et al.*, 1996; Kibby and Akl, 1997; Lagier *et al.*, 1992; Yassim *et al.*, 1994). The U.S. Occupational Safety and Health Administration estimates the range from 8 to 12%.

Children with spina bifida have reported prevalences of 28 to 67% (Slater, 1992; Sussman *et al.*, 1991). Health-care workers are exposed to latex proteins from routine wearing of NRL gloves or inhaling airborne NRL-laden glove powder. The dermal and inhalation routes of exposure are believed to lead to the development of sensitization. Spina-bifida children are exposed to NRL through multiple surgical procedures predisposing them to latex hypersensitivity. It is thought that exposures to NRL early in life makes these children more vulnerable to development of latex-specific IgE antibody and type-1 latex hypersensitivity.

In addition to symptoms of contact sensitivity to NRL gloves, work related latex allergic complaints, anaphylactic events during surgery, and the presence of atopy and food allergy are critical elements of a clinical history (Liss *et al.*, 1999). Latex-allergic patients have a high prevalence of food allergy, particularly to certain fruits, which has been recognized as a "latex-fruit syndrome" (Brehler *et al.*, 1997). Patients with food allergy may be at increased risk for developing latex allergy. In 1991, latex and banana were reported to cross-react (M'Raihi *et al.*, 1991). The most common cross-reacting foods in addition to banana include avocado, chestnut, kiwi, potato, and tomato (Beezhold *et al.*, 1996) while less commonly cross reacting are apple, apricot, celery, cherry, fig, grape, melon, papaya, and peach. Clinically, the presentation may include local perioral itching but occasionally also food-induced life-threatening anaphylactic shock (Fernandez de Corres *et al.*, 1993). While the occurrence of symptomatic cross-reactions to foods is commonly associated with latex sensitization, whether sensitization to food is a significant route for latex allergy or *vice versa* remains to be determined.

Diagnosis

The identification of susceptible individuals can be ascertained with a medical-history questionnaire. Although a latex skin-prick test (SPT) is a sensitive indicator of IgE sensitization, a Food and Drug Administration-approved skin test extract is not currently available in the USA. "Home-made" NRL glove extracts are unreliable and unpredictable. Where extracts are available, latex skin testing requires qualified specialists familiar with the correct technique and interpretation. This includes, but is not exclusive to, the initiation of testing with well-diluted solutions and the availability of emergency equipment for treatment of possible systemic reactions (Kelly *et al.*, 1994). Blood tests for latex protein-specific IgE antibodies using radioallergosorbent tests (RAST) or enzyme-linked immunosorbent assays (ELISA) and Western blots can identify about 75% of persons with IgE sensitivity (Kelly *et al.*, 1993). While the SPT is regarded as the primary test for IgE-mediated disease, the absence of U.S. FDA-licensed *Hevea brasiliensis* latex extract has restricted SPT use in the U.S. This has led to increased importance and use of serological tests as an alternative diagnostic tool.

Assessment of Diagnostic Kits

Clinical accuracy of serological tests is based on the ability to discriminate between individuals who score positive or negative on SPT, which serves as a "gold standard." In the absence of an FDA-licensed extract, there is a need to compare performance of diagnostic tests on sera from a population that has had SPT testing using a validated non-ammoniated latex extract, and has undergone evaluation with a medical history questionnaire (Hamilton *et al.*, 1999; Hamilton and Adkinson, 1996, 1998). Three manufacturers have currently obtained 510K (safe medical device) clearance from the FDA for their latex *in vitro* reagents: the CAP System (Pharmacia-UpJohn), the AlaSTAT (Diagnostic Products Corporation), and the HY-TEC assay (HYCOR Biomedical). Overall, the currently FDA-cleared latex IgE serological assays produce 25–28% false negative and 27% false positive IgE antibody results (Hamilton *et al.*, 1999).

Although commercial assays are based on non-ammoniated latex as their primary allergen, there are differences in their performance. A comprehensive analysis of paired performance of the available FDA-cleared latex anti-IgE assays indicates that systematic biases within the assays results in poor association between the serological assays and SPT for the diagnosis of latex allergy. This was established using the results of non-ammoniated latex SPT as the diagnostic standard to evaluate 311 individuals of whom 131 were positive and 180 negative, based on questionnaire results and SPTs for latex allergy. Sera from these individuals were tested for non-ammoniated latex IgE antibody concentration using the 3 serological kits. The kits were compared using Receiver Operating Characteristic (ROC) curves and commercially available software (GraphROC for Windows, 2.0) to evaluate test outcomes. ROC plots graphically display the entire spectrum of a test's performance for a particular sample group of allergic and non-allergic subjects. With an ROC curve, the accuracy of a test is described by plotting all the sensitivity vs. specificity pairs resulting from varying the IgE antibody concentration cut-off point for accepting or rejecting the outcome as positive.

Many factors go into the selection of a cut-off point that are independent of the performance of a diagnostic kit. These factors are properties of the circumstances of the clinical application and include the outcomes and the relative values of those outcomes, the costs to the patient (and others) of incorrect information (false-positive and false-negative classifications), and the costs and benefits of various treatment options. These characteristics are not properties of the tests themselves but interact with test results to affect usefulness. As a consequence, selection of cut-off points for a diagnostic kit, which favor sensitivity over specificity or *vice versa*, varies, depending on the application of the results. Therefore, using sensitivity or specificity as the only criteria for selection of a diagnostic test may not be optimal. ROC analysis provides information on the relationship between specificity and sensitivity so that, the

greater area under the ROC curve, the more optimal the relationship between sensitivity and specificity.

Using ROC curve analysis and a standardized SPT outcome for 311 individuals as a “gold standard”, the HY-TEC system demonstrated significantly greater ($p < 0.01$) area under the curve (AUC, 0.924 ± 0.017) than CAP (0.869 ± 0.024) or AlaSTAT (0.858 ± 0.024), suggesting it is the most accurate of the 3 FDA-cleared latex-specific IgE antibody tests. Results of these analyses indicate that the HY-TEC system, at a maximum diagnostic efficiency cut off of 0.11 kIU/L of IgE yields a significantly greater AUC than CAP or AlaSTAT. At this cutoff, the HY-TEC system has an increased sensitivity of as much as 9.2% over CAP and AlaSTAT (at their respective maximally efficient cutoffs of ≥ 0.35 κ IU/L and ≥ 0.35 κ IU/L), with a reduction in specificity of only 4.4%, clearly indicating that it is the most sensitive of the 3 tests. It should be kept in mind that the HY-TEC assay, using the 0.11 κ IU/L cutoff, will still mis-classify 16.8% of SPT-positive individuals as being negative, and 7.2% of SPT-negative individuals as being positive. For comparison, the HY-TEC test using a 0.05 κ IU/L cutoff yields a diagnostic sensitivity of 91.6%, with a diagnostic specificity of 73.3%. Comparing the two HY-TEC cutoffs (0.05 and 0.11) indicates a loss of sensitivity at the higher cutoff of 8.4%, with a gain in specificity of 20.5%

In addition to its application to the evaluation of diagnostic kits, ROC analyses also provide support for the proposed hypothesis (Matsson *et al.*, 1998) that IgE antibody assays can detect different subsets of IgE antibody of a given specificity, possibly as a result of differential specificities of their allergen-containing reagents. There are several possible reasons for this: different batches of source NRL are known to vary up to 25-fold in their total allergen content (Yeang and Hamilton, 1998); sensitized individuals produce specific IgE antibody to at least 10 potent *Hevea* allergens, Hev b 1–Hev b 10; each of these allergens differs in its structure, size, net charge (pI), relative allergenicity, and abundance in natural rubber latex. Moreover, aqueous latex extracts vary widely in their relative content of rubber particle-associated proteins Hev b 1 (rubber elongation factor [14.6 or 58 kD tetramer]) and Hev b 3 (prenyltransferase or small rubber-particle protein [23–27 kD]). The relative content and ratios of all hevein proteins in the final allergen preparation most probably could alter the diagnostic accuracy of a specific test. Other potential causes of allergen-containing reagent heterogeneity include variable stability during storage and variable binding of allergen to labels (e.g., biotinylated co-polymer in AlaSTAT) or solid supports (sponge in CAP; cellulose disc in HYTEC)(Yeang and Hamilton, 1998).

Animal Models

Animal models are frequently used to conduct mechanistic studies that, due to test article toxicity or sensitization potential, would be unethical to perform in humans. Additionally intervention strategies can be developed and evaluated in ani-

mal models. To this end several animal models of latex allergy have been developed.

Historically, guinea pigs have been used to investigate systemic and dermal anaphylaxis (Kuby 1992) and recent investigations by Hayes *et al.* (2000) have shown hairless guinea pig skin to be a good surrogate for human skin with respect to *in vitro* penetration of latex proteins. Aamir, *et al.* (1996) demonstrated that guinea pigs immunized subcutaneously with non-ammoniated latex or components of NRL mixed with Freund’s complete adjuvant developed latex-specific antibodies capable of mediating dermal and systemic responses. The major shock organ for the guinea pig is the lung, which makes it a good model for evaluating pulmonary responses; however, the major reaginic antibody for the guinea pig is IgG₁, in contrast to IgE for the human.

Rabbit models of latex allergy have also been evaluated, due to the size of the animal and the advantages it offers in the conduct of physiological studies. In these studies, Reijula, *et al.* (1994) demonstrated an increase in latex-specific IgG in serum and lung lavage fluid of immunized animals, with all sensitized animals developing an immediate wheal and flare reaction following intradermal skin testing with latex. Histological evaluation of the lungs of sensitized rabbits following intratracheal exposure revealed a granulomatous interstitial and bronchial inflammation with eosinophils and histiocytes.

Due to the increased knowledge of the immunology of the mouse and its similarity to the human immune system, the immunological reagents and transgenic and genetic knockout animals available, and the reduced cost associated with purchasing and housing mice, mouse models have become widely used in investigations of hypersensitivity responses. Kurup, *et al.* (1994) first demonstrated the immunological responses in mice following either intraperitoneal or intranasal exposure to latex proteins in BALB/c and C57BL/6 mice. Mice showed elevations in total IgE and latex-specific IgG of all subclasses, peripheral blood, and lung eosinophilia as well as elevated serum levels of IL-4 and IL-5. Lung histology, after pulmonary challenge, showed chronic inflammatory infiltrates, most notably around bronchioles and small vessels. Knockout mice have been used in evaluating the role of IL-4 in the pathogenesis of latex allergy (Xia *et al.* 1999). Following pulmonary challenge with latex proteins, IL-4 knockout mice did not show elevations in IgE or eosinophils. However, these animals exhibited increased airway resistance (although reduced as compared to wild-type mice) after challenge as compared to controls. Pulmonary pathology was found to be similar between wild-type and IL-4 knockout mice. The authors conclude from these studies that other factors in addition to IgE and eosinophils may be responsible for pulmonary response in latex allergy. Care must be taken in interpreting these data as they may relate to humans, in that IgG₁ can function to sensitize mast cells in mice.

Although clinical and exposure data have been gathered on the factors affecting the elicitation of responses in latex allergic

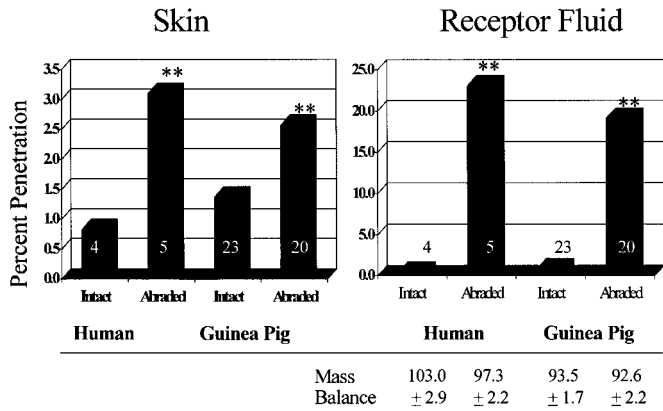


FIG. 2. *In vitro* percutaneous penetration of non-ammoniated latex protein into and through human and hairless guinea-pig skin. Values for skin, receptor fluid, and mass balance represent the mean percentage of the applied ^{125}I -labeled latex protein dose. The number shown in each bar represents the total number of skin samples combined from all penetration studies. Mass balance percentages were calculated by adding together the counts for the skin, receptor fluid, and soap and water washes. Unpaired *t*-tests were performed to compare data obtained from abraded skin versus intact skin where $**p < 0.01$.

individuals, less is known regarding the development of sensitization. Coupled with *in vitro* dermal penetration studies, murine models were established to investigate the role of the route of exposure in the development of latex sensitization. Mice were shown to develop increased levels of total and latex-specific IgE following exposure to latex proteins by respiratory (intranasal and intratracheal), subcutaneous (sc), and dermal exposure. Time-course and dose-response studies have shown that multiple subcutaneous administrations of as little as $0.19 \mu\text{g}$ of non-ammoniated latex proteins (NAL) elicited IgE production in 5 weeks. Animals exposed sc, intratracheally, or topically to $12.5 \mu\text{g}$ of non-ammoniated latex demonstrated IgE production within 2, 3, and 5 weeks, respectively (Woolhiser *et al.*, 2000). Immunoblot analysis of sera from these animals demonstrated differing patterns of protein recognition with sera from sc-exposed mice showing a unique recognition of a 27 kD protein and mice exposed via the intratracheal route demonstrating a high degree of recognition of a 92 kD protein. As discussed in the following section, different patterns of sero-recognition for specific proteins have been demonstrated between health-care workers and children with spina bifida. Data from these mouse models suggests that route of exposure may be involved in determining the primary proteins responsible for sensitization.

Much of the focus on intervention strategies and mouse models has been on respiratory exposure. The use of powdered latex gloves has been demonstrated to increase the amount of latex allergen recovered by air and dust sampling and intervention strategies where powdered latex gloves have been replaced with non-latex or powder-free latex gloves have been effective in decreasing aeroallergen levels. Recent investigations have focused on dermal exposure as a route of sensitiza-

tion. Both *in vitro* penetration and *in vivo* studies highlight the importance of skin condition in the development of latex allergy, with enhanced penetration and earlier onset of IgE production seen with experimentally abraded skin.

Hayes *et al.* (2000) demonstrated an increase in percutaneous penetration through abraded human skin. A strong correlation exists between the degree of skin abrasion and the amount of proteins penetrating, with less than 1% penetrating through intact skin and up to 30% penetrating abraded skin (Fig. 2). Proteins penetrating the skin ranged from approximately 4 to 27 kDa in size. These molecular weights correspond to several of the major latex allergens. *In vivo* studies in mice showed an earlier elevation in IgE in mice where the skin was abraded prior to exposure; however, following 53 days of repeat exposure, IgE levels were comparable in mice regardless of skin condition (Fig. 3). This data indicates that skin condition is important in cases of acute exposure but may not be as significant with chronic exposure.

Decreased pulmonary conductance and compliance following challenge with methacholine (ip) or latex protein (iv) have been demonstrated in mice previously exposed to latex via the intranasal route (Thakker *et al.*, 1999). Increased bronchoconstriction has also been demonstrated in mice sensitized by the dermal route and challenged with methacholine and latex proteins by the pulmonary route (unpublished results).

Animal models are also being used to investigate the role of concurrent exposure to other chemicals and agents in the workplace on the development of latex allergy. Intranasal co-exposure to endotoxin with recombinant Hev b 5 increases the Hev b 5-specific IgE response without altering total IgE

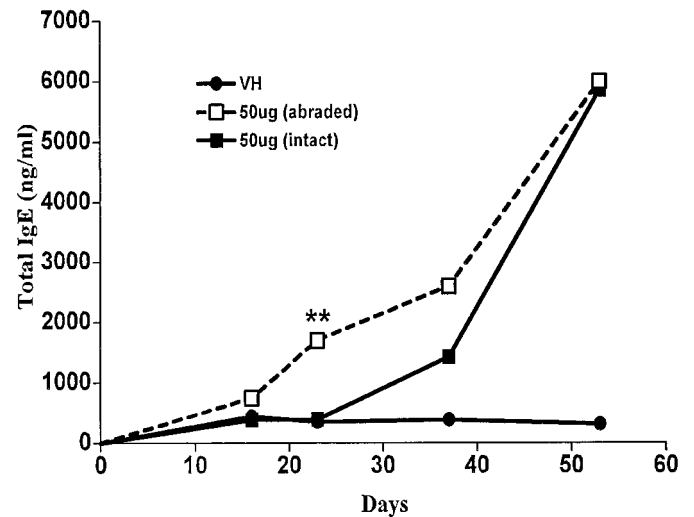


FIG. 3. Time course of total IgE response following topical application of non-ammoniated latex to intact or abraded skin. BALB/c female mice were topically exposed to vehicle (glycerol buffer/acetone) or 50-g, non-ammoniated latex proteins 5 days/week. Blood was collected via tail vein on the days shown, and was analyzed for total IgE by ELISA. Data points represent means for each group ($n = 5$). $**$ Indicates statistical difference from vehicle-exposed mice at $p < 0.01$ using a Dunnett's test.

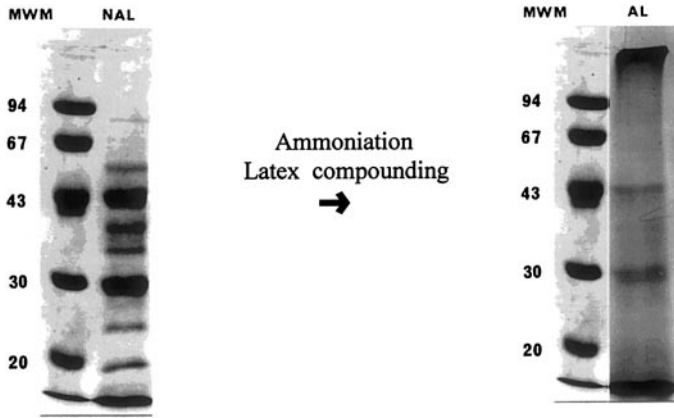


FIG. 4. SDS-PAGE analysis of latex proteins. Non-ammoniated latex (NAL) protein shows a discrete banding pattern, while protein isolated from ammoniated latex (AL) demonstrates a smear of peptides indicating hydrolysis.

(Slater *et al.*, 1998). However, other laboratories have found that intranasal co-exposure of endotoxin with a mixture of non-ammoniated latex proteins suppresses the elevation in total and latex specific IgE compared to the response observed to non-ammoniated latex alone (Meade *et al.*, unpublished). Concurrent with the decrease in latex specific IgE, these animals had elevated IgG_{2a} levels. Investigation of the effects of co-exposure of latex with glutaraldehyde, a common contaminant in health-care environments, revealed an augmentation of the latex-specific IgE response at concentrations of glutaraldehyde near the OSHA-permissible exposure limit. These observations underscore the complexity of the mechanisms involved in the development of an allergic response to a complex mixture.

Molecular Characterization of Latex Proteins

NRL is a cytoplasmic exudate of the laticifer layer of the tree and contains most of the cytosolic organelles and proteins found in any plant cell. Immediately upon tapping, NRL is further complexed by the addition of ammonium hydroxide to prevent coagulation. This allows products to be manufactured

by dipping molds into the liquid latex (gloves, condoms, catheters, and balloons). Products manufactured from a dry rubber, rubber that is allowed to coagulate at the tree, have significantly lower protein levels than dipped products.

The ammonia treatment causes the proteins found in latex to undergo hydrolysis (Fig. 4). Prior to manufacturing, the latex is further formulated by the addition of chemicals such as thiurams or carbamates to accelerate coagulation times and enhance the barrier properties of latex film. As noted above, these added chemicals are responsible for the type-IV allergic reactions. Therefore, exposure to latex involves a complex mixture of residual chemicals and the hydrolyzed latex peptides. The roles of the chemicals in type-1 allergic responses to latex proteins are not known, but they have been postulated to function as haptens. The hydrolyzed nature of the protein allergens complicates efforts to identify major allergens and the development of appropriate reagents for *in vivo* and *in vitro* diagnostic tests.

To better understand latex allergy, it is important to identify the major protein allergens in latex. When analyzed by 2-D electrophoresis, non-ammoniated latex contains more than 250 peptides of which at least 30 bind IgE from latex allergic patients (Kurup *et al.*, 1994). Multiple investigators, using a variety of methodologies, have succeeded in characterizing a number of these latex protein allergens. Typically, Western blot analysis with patient sera has been used to identify the IgE-binding proteins. Analysis indicates that patient reactivity is very heterologous (Fig. 5). To identify the allergens, proteins are isolated from non-ammoniated latex and characterized using routine biochemical characterization.

Many of NRL proteins have been cloned, sequenced, and expressed as recombinant proteins. Currently, 10 proteins have been characterized and given allergen designations (Table 1) by the International Union of Immunological Societies. There appears to be selective reactivity to certain proteins. Although the reasons for this are unclear, it is hypothesized to be due to the different routes of exposure. For example, Hev b 1 and 3 are more common allergens for patients with spina bifida or

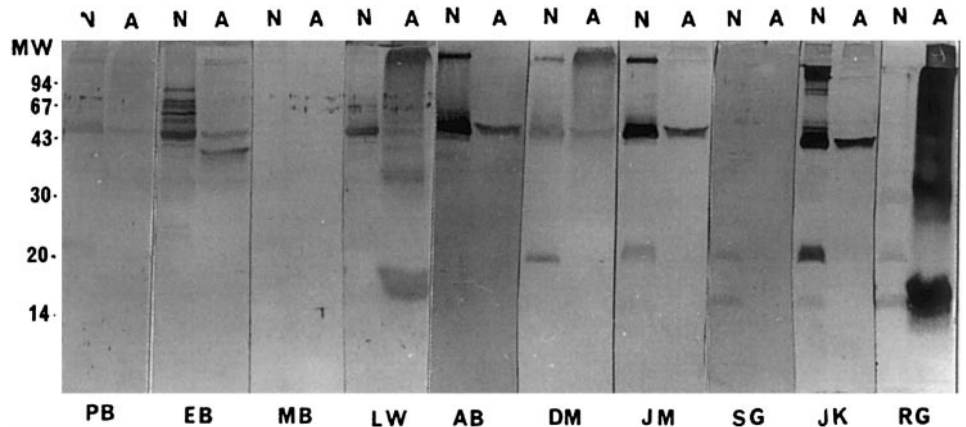


FIG. 5. IgE Western blot of serum from 10 latex allergic patients (2-letter designations) demonstrating variable reactivity to multiple latex proteins in non-ammoniated latex (N) and peptides in ammoniated latex (A).

TABLE 1
NRL IgE-Binding Allergens

Allergen	Common name	MW (kDa)	SPT reactivity to rHev b (13)	Epitopes identified
Hev b 1	Rubber elongation	58/14.6	nd	T and B cells
Hev b 2	β 1-3-glucanase	34-36	7% (2/29)	No
Hev b 3	Small rubber particle protein	24-27	7% (2/29)	No
Hev b 4	Microhelix protein	~100	nd	No
Hev b 5	Acidic protein	16-24	62% (18/29)	T and B cells
Hev b 6	Hevein preprotein	20, 14, 5	65% (19/29)	B cells
Hev b 7	Patatin homologue	43	41% (12/29)	B cells
Hev b 8	Profilin	14	3% (1/29)	No
Hev b 9	Enolase	51	nd	No
Hev b 10	Mn-superoxide dismutase	26	nd	No

Note. Allergen names designated by IUIS Allergen Nomenclature Subcommittee.

other urogenital malformations (medical exposure), while other proteins are more common allergens in health-care workers (occupational exposure). In a recent study (Yip *et al.*, 2000), 6 recombinant proteins (Hev b 2, 3, 5, 6, 7, and 8) were used for skin testing in latex-sensitive health-care workers. This study revealed Hev b 5, 6, and 7 to be the most common allergens for health-care workers. Reactivity to Hev b 5 was 62%, for Hev b 6 was 65%, and for Hev b 7, 41%, in this group of 29 subjects. Twenty-seven (27/29) patients reacted to at least one of the recombinant proteins, with 11 subjects being mono-sensitized. Of the mono-sensitized patients, 5 were sensitized to Hev b 5, 3 to Hev b 6, and 3 to Hev b 7. Of the 27 patients reacting to at least 1 recombinant protein, all reacted to Hev b 5, 6, or 7; thus, a combination of Hev b 5, 6, and 7 produced a positive reaction in 27/29 (93%) of the latex allergic patients. Mono-sensitized patients had significantly lower anti-latex IgE levels (AlaSTAT), suggesting that the reason *in vitro* diagnostic tests have lower sensitivity than skin testing, may be due to insufficient quantities of these specific proteins in the non-ammoniated latex preparations used for producing the diagnostic kits (Beezhold *et al.*, 1999). The use of recombinant allergens should provide reagents that greatly improve the diagnostic accuracy of tests for latex allergy.

Patients with food or plant allergies, while they commonly test positive for latex by *in vitro* diagnostic tests (i.e., AlaSTAT or CAP), generally are asymptomatic upon latex exposure (Garcia-Ortiz *et al.*, 1998; Vallier *et al.*, 1995). Plant protein sequence homology or a limited number of common epitopes could explain the cross reactivity to a variety of the foods. Using overlapping synthetic peptides, linear IgE-binding epitopes have been determined for several latex allergens. At least 4 latex allergens have been epitope-mapped using synthetic peptides (Banerjee *et al.*, 1997; Beezhold *et al.*, 1997, 1999; Chen *et al.*, 1996; Slater *et al.*, 1999). Hev b 1 was found to contain 2 major epitopes, with protein sequence homology and limited cross reactivity to papain from papaya (Chen *et al.*, 1996). Hev b 5 was shown to contain at least 6 epitope regions (Slater *et al.*, 1999, Beezhold *et al.*, 1999). Hev b 5 has

sequence homology with a protein from kiwi, but the epitopes appear in regions of limited homology reducing the likelihood that this protein is responsible for cross reactivity with kiwi (Beezhold *et al.*, 1999). Hev b 6 contains at least six epitopes (Banerjee *et al.*, 1997; Beezhold *et al.*, 1997) the most important of which are found in the N-terminal mature hevein. However, the epitopes in Hev b 6 are in regions of high homology to hevein-like proteins and class-I chitinases that are widely distributed in plants. Recent evidence suggests that Hev b 6 is responsible for much of the cross reactivity to fruits (Sanchez *et al.*, 1999; Blanco *et al.*, 1999; Posch *et al.*, 1999; Hanninen *et al.*, 1999; Chen *et al.*, 1998; Mikkola *et al.*, 1998). Hev b 7 has structural homology to a potato phospholipase called patatin, although 15 epitomes have been found (Beezhold *et al.*, unpublished; Kostyal *et al.*, 1998), the experimental evidence suggests that little of the cross-reactivity with potato is due to cross reactivity with patatin (Sowka *et al.*, 1999). Hev b 8 was identified as a member of the profilin family, a well known pan-allergen, but few latex allergic individuals react to Hev b 8 (Yip *et al.*, 2000) suggesting that it is not responsible for extensive cross-reactivity. It appears that the hevein family of proteins is a previously unrecognized pan-allergen responsible for much of the cross reactivity of latex with other plant and food allergens (Blanco *et al.*, 1999).

Prevention

Individuals develop sensitivity to NRL by multiple routes including inhalation and compromised skin. Repeated physical contact with latex enhances the potential for developing a response. This is especially problematic for health-care workers who may wear dozens of gloves daily. Once the immune system is sensitized there may be no safe level of latex exposure and workers are at risk for severe allergic reactions. The only treatment is avoidance. The American Academy of Allergy and Asthma Immunology (AAAAI, 1993; AAAAI and ACAAI, 1997; ACAAI, 1995) have developed guidelines for diagnosing and managing care to persons with latex allergy

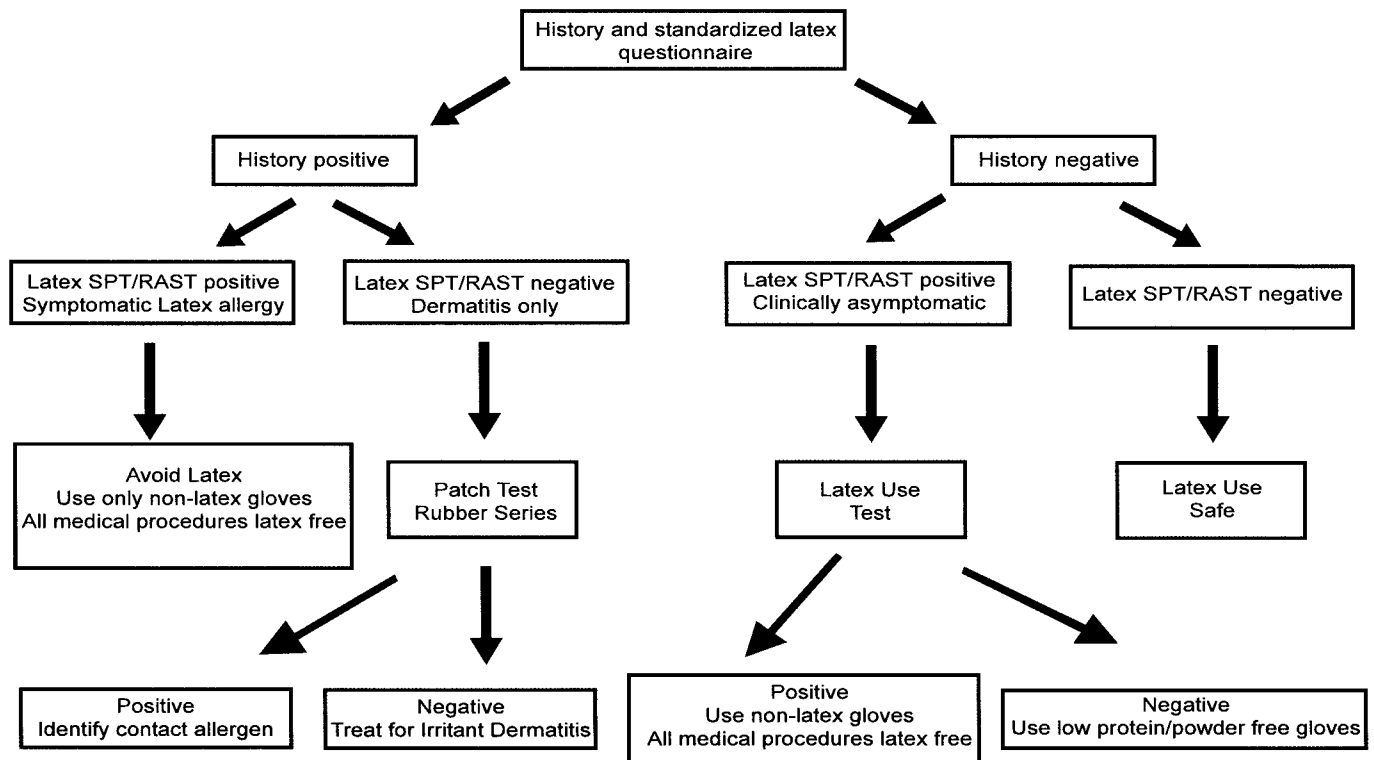


FIG. 6. Overview of the identification and management of persons with or at risk for latex allergy. Individuals are divided into those with positive or negative histories and those with positive or negative results from skin tests, and a management outcome for these patients is given. Individuals with negative histories do not receive skin testing unless they are categorized at high risk. RAST = radioallergosorbent testing, SPT, kin-prick test.

(Fig. 6). Important points are: (1) the recording of a medical history focusing on symptoms, (2) availability of clinical testing for latex allergy in high-risk individuals, and (3) avoidance of NRL by identified latex-allergic individuals. For allergic patients, medical and dental procedures should be performed in a latex-safe environment with no direct NRL exposure from gloves, catheters, adhesives, or tourniquets. Only non-latex gloves should be used. Patients with irritant contact dermatitis should avoid excessive washing, hand occlusion, and the use of irritants such as soaps. Type-IV hypersensitivity should be diagnosed by appropriate patch tests and the implicated allergen avoided. Workers with severe latex-induced rhinoconjunctivitis and asthma may require workplace relocation. But avoidance needs to be part of prevention. Although it is known that the latex proteins are the responsible allergens, the corn-starch powder used with latex gloves easily absorbs and disperses aerosolized latex dust when gloves are snapped on and off. Low-allergen powder-free NRL gloves are available and manufacturers are working to lower allergenic protein levels. As these products become widely used the incidence of new sensitization is expected to decline. Additional effective workplace practices should include identification of areas contaminated with latex. Monitoring of workplace environments where latex gloves are used has revealed that latex particle concentrations can exceed $200 \mu\text{g}/\text{m}^3$, and concentrations as low as

$0.6 \mu\text{g}/\text{m}^3$ have been linked to NRL-specific allergic response (Baur *et al.*, 1998). Therefore, it may be beneficial if workers use good housekeeping to remove latex-containing dusts. Indeed, removal of NRL gloves has been shown to reduce aeroallergens below the detection limit (Allmers *et al.*, 1998) and allowed severely symptomatic individuals to return to their original workplace (Tarlo *et al.*, 1994). To establish effective remediation, education programs and training materials about latex allergy need to be provided to workers. High-risk workers should also be screened for latex allergy symptoms. Detecting symptoms early is essential for prevention of long-term health effects (NIOSH, 1997).

REFERENCES

- AAAAI (1993). Task force on allergic reactions to latex. American Academy of Allergy, Asthma and Immunology, committee report. *J. Allergy Clin. Immunol.* **92**, 16–18.
- AAAAI and ACAAI (1997). AAAAI and ACAAI Joint statement concerning the use of powdered and non-powdered natural latex gloves. *Ann. Allergy Asthma Immunol.* **79**, 487.
- Aamir, R., Safadi, G. S., Mandelik, J., Cornish, K., Melton, A. L., Pien, L. C., Wagner, W. O., Battisto, J. R. (1996). A guinea pig model of hypersensitivity to allergenic fractions of natural rubber latex. *Int. Arch. Allergy Immunol.* **110**, 187–194.
- ACAAI (1995). Latex allergy: An emerging health-care problem. American

- College of Allergy, Asthma, and Immunology position statement. *Ann. Allergy Asthma Immunol.* **75**, 19–21.
- Allmers, H., Brehler, R., Chen, Z., Raulf-Heimsoth, M., Fels, H., and Baur, X. (1998). Reduction of latex aeroallergens and latex-specific IgE antibodies in sensitized workers after removal of powdered natural rubber latex gloves in a hospital. *J. Allergy Clin. Immunol.* **102**, 841–846.
- Banerjee, B., Wang, X., Kelly, K., Fink, J. N., Sussman, G. L., and Kurup, V. P. (1997). IgE from latex-allergic patients binds to cloned and expressed B-cell epitopes of prohevein. *J. Immunol.* **159**, 5724–5732.
- Baur, X., Chen, Z., and Allmers, H. (1998). Can a threshold-limit value for natural rubber latex airborne allergens be defined? *J. Allergy Clin. Immunol.* **101**, 24–27.
- Beezhold, D. H., Hickey, V. L., Slater, J. E., and Sussman, G. L. (1999). Human IgE binding epitopes of the latex allergen Hev b 5. *J. Allergy Clin. Immunol.* **103**, 1166–1172.
- Beezhold, D. H., Kostyal, D. A., and Sussman, G. L. (1997). IgE epitope analysis of a major latex allergen, hevein preproteins. *Clin. Exp. Immunol.* **108**, 114–121.
- Beezhold, D. H., Sussman, G. L., Liss, G., Chang, N.-S. (1996). Latex allergy can induce clinical reactions to specific foods. *Clin. Exp. Allergy* **26**, 416–422.
- Blanco, C., Diaz-Perales, A., Collada, C., Sanchez-Monge, R., Aragoncillo, C., Castillo, R., Ortega, N., Alvarez, M., Carrillo, T., and Salcedo, G. (1999). Class-I chitinases as potential pan-allergens involved in the latex fruit syndrome. *J. Allergy Clin. Immunol.* **103**, 507–513.
- Brehler, R., Theissen, U., Mohr, C., and Luger, T. (1997). "Latex-fruit syndrome": Frequency of cross-reacting IgE antibodies. *Allergy* **52**, 404–410.
- Center for Disease Control and Prevention (CDC) (1987). Recommendations for prevention of HIV transmission in health-care settings. *MMWR* **36**, 32.
- Chen, Z., Posch, A., Cremer, R., Raulf-Heimsoth, M., and Baur, X. (1998). Identification of hevein (Hev b 6.02) in Hevea latex as a major cross-reacting allergen with avocado fruit in patients with latex allergy. *J. Allergy Clin. Immunol.* **102**, 476–481.
- Chen, Z., Van Kampen, V., Raulf-Heimsoth, M. R., and Baur, X. (1996). Allergenic and antigenic determinants of latex allergen Hev b 1: Peptide mapping of epitopes recognized by human, murine and rabbit antibodies. *Clin. Exp. Allergy* **26**, 406–415.
- Downing, J. G. (1933). Dermatitis from rubber gloves. *New Eng. J. Med.* **208**, 196–198.
- Fernandez de Corres, L., Moneo, I., Munoz, D., Bernaola, G., Fernandez, E., Audicana, M., and Urrutia, I. (1993). Sensitization from chestnuts and bananas in patients with urticaria and anaphylaxis from contact with latex. *Ann. Allergy* **70**, 35–39.
- Garcia-Ortiz, J. C., Moyano, J. C., Alvarez, M., and Bellido, J. (1998). Latex allergy in fruit-allergic patients. *Allergy* **53**, 532–536.
- Hamilton, R. G., and Adkinson, N. F., Jr. (1996). Natural rubber latex diagnostic skin testing reagents. Safety and diagnostic accuracy of non-ammoniated latex, ammoniated latex, and latex rubber-glove extracts. *J. Allergy Clin. Immunol.* **98**, 872–883.
- Hamilton, R. G., and Adkinson, N. F., Jr. (1998). Diagnosis of natural rubber latex allergy: Multicenter latex skin testing efficacy study. (Multicenter Latex Skin Testing Study Task Force). *J. Allergy Clin. Immunol.* **102**, 482–490.
- Hamilton, R. G., Biagini, R. E., and Krieg, E. F. (1999). Diagnostic performance of Food and Drug Administration-cleared serologic assays for natural rubber latex-specific IgE antibody. (The Multi-Center Latex Skin Testing Study Task Force). *J. Allergy Clin. Immunol.* **103**, 925–930.
- Hanninen, A.-R., Mikkola, J. H., Kalkkinen, N., Turjanmaa, K., Ylitalo, L., Reunala, T., and Palosuo, T. (1999). Increased allergen production in turnip (*Brassica rapa*) by treatments activating defense mechanisms. *J. Allergy Clin. Immunol.* **104**, 194–210.
- Hayes, B. B., Afshari, A., Millecchia, L., Willard, P. A., Povoski, S. P., and Meade, B. J. (2000). Evaluation of percutaneous penetration of natural rubber latex proteins. *Toxicol. Sci.* **56**, 262–270.
- Heese, A., von Hintzenstem, J., Peters, K. P., Kock, H. U., and Horstein, O. P. (1991). Allergic and irritant reactions to rubber gloves in medical health services. Spectrum, diagnostic approach, and therapy. *J. Am. Acad. Dermatol.* **25**, g31–39.
- Kaczmarek, R. G., Silverman, B. G., Gross, T. P., Hamilton, R. G., Kessler, E., Arrowsmith-Lowe, J. T., and Moore, R. M., Jr. (1996). Prevalence of latex-specific IgE antibodies in hospital personnel. *Ann. Allergy Asthma Immunol.* **76**, 51–56.
- Kelly, K. J., Kurup, V. P., Reijula, K. E., and Fink, J. N. (1994). The diagnosis of natural rubber latex allergy. *J. Allergy Clin. Immunol.* **93**, 813–816.
- Kelly, K. J., Kurup, V., Zacharisen, M., Resnick, A., and Fink, J. N. (1993). Skin and serologic testing in the diagnosis of latex allergy. *J. Allergy Clin. Immunol.* **91**, 1140–1145.
- Kibby, T., and Akl, M. (1997). Prevalence of latex sensitization in a hospital employee population. *Ann. Allergy Asthma Immunol.* **78**, 41–44.
- Kostyal, D., Hickey, V., Noti, J., Sussman, G., and Beezhold, D. (1998). Cloning and characterization of a latex allergen (Hev b 7): Homology to patatin, a plant PLA₂. *Clin. Exp. Immunol.* **112**, 355–362.
- Kuby, J. (1992). *Immunology*. Freeman, New York.
- Kurup, V. P., Kumar, A., Choi, H., Murali, P. S., Resnick, A., Kelly, K. J., and Fink, J. N. (1994). Latex antigens induce IgE and eosinophils in mice. *Int. Arch. Allergy Immunol.* **103**, 370–377.
- Lagier, F., Vervloet, D., Lhermet, I., Poyen, D., and Charpin, D. (1992). Prevalence of latex allergy in operating room nurses. *J. Allergy Clin. Immunol.* **90**, 319–322.
- Liss, G., and Sussman, G. L. (1999). Latex sensitization: Occupational versus general population prevalence rates. *Amer. J. Ind. Med.* **35**, 196–200.
- Matsson, P., Hamilton, R. G., Adkinson, N. F., Jr., Esch, R., Homburger, H., Maxim, P., and Williams, P. (1998). *Evaluation Methods and Analytical Performance Characteristics of Immunological Assays for Human Immunoglobulin E (Ige) Antibodies of Defined Allergen Specificities*, Vol 16-LA20-A, p. 19. National Committee on Clinical Laboratory Standards, Wayne, PA.
- Mikkola, J. H., Alenius, H., Kalkkinen, N., Turjanmaa, K., Palosuo, T., and Reunala, T. (1998). Hevein-like protein domains as a possible cause for allergen cross-reactivity between latex and banana. *J. Allergy Clin. Immunol.* **102**, 1005–1012.
- M'Raihi, L., Chapin, D., Pons, A., Bongrand, P., and Vervlet, B. (1991). Cross-reactivity between latex and banana. *J. Allergy Clin. Immunol.* **87**, 129–130.
- NIOSH (1997). National Institute for Occupational Safety and Health Alert: Preventing allergic reactions to natural rubber latex in the workplace. DHHS (NIOSH) Publication No. 97–135.
- Posch, A., Wheeler, C. H., Chen, Z., Flagge, A., Dunn, M. J., Papenfuss, F., Raulf-Heimsoth, M., and Baur, X. (1999). Class-I endochitinase containing a hevein domain is the causative allergen in latex-associated avocado allergy. *Clin. Exp. Allergy* **29**, 667–672.
- Reijula, K. E., Kelly, K. J., Kurup, V. P., Choi, H., Bongard, R. D., Dawson, C. A., and Fink, J. N. (1994). Latex-induced dermal and pulmonary hypersensitivity in rabbits. *J. Allergy Clin. Immunol.* **94**, 891–902.
- Ruhl, C. M., Urbancic, J. H., Foresman, P. A., Cox, M. J., Rodeheaver, G. T., Zura, R. D., and Edlich, R. F. (1994). A new hazard of cornstarch, an absorbable dusting powder. *J. Emerg. Med.* **12**, 11–14.
- Sanchez-Monge, R., Blanco, C., Diaz-Perales, A., Collada, C., Carrillo, T., Aragoncillo, C., and Salcedo, G. (1999). Isolation and characterization of major banana allergens: Identification as fruit class-I chitinases. *Clin. Exp. Allergy* **29**, 673–680.

- Shoup, A. (1998). Why latex allergy now? *Semin. Perioper. Nurs.* **7**, 222–225.
- Slater, J. E. (1992). Allergic reactions to natural rubber. *Ann. Allergy Asthma Immunol.* **68**, 203–209.
- Slater, J. E., Paupore, E. J., Elwell, M. R., and Truscott, W. (1998). Lipopolysaccharide augments IgG and IgE responses of mice to the latex allergen Hev b 5. *J. Allergy Clin. Immunol.* **102**, 977–983.
- Slater, J. E., Paupore, E. J., and O’Hehir, R. E. (1999). Murine B-cell and T-cell epitopes of the allergen Hev b 5 from natural rubber latex. *Mol. Immunol.* **36**, 135–143.
- Sowka, S., Hafner, C., Radauer, C., Focke, M., Brehler R., Astwood, J. D., Am Arif, S., Kanani, A., Sussman, G. L., Scheiner, O., Beezhold, D. H., and Breiteneder, H. (1999). Molecular and immunological characterization of new isoforms of the *Hevea brasiliensis* latex allergen Hev b 7. Evidence of no cross-reactivity between Hev b 7 isoforms and potato patatin and proteins from avocado and banana. *J. Allergy Clin. Immunol.* **104**, 1302–1310.
- Stern, G. (1927). Ueberempfindlichkeit gegen kautschuk als ursache von urticaria und quinkeschem odem. *Klinische Wochenschrift* **6**, 1096–1097.
- Sussman, G. L., and Beezhold, D. H. (1995). Allergy to Latex Rubber. *Ann. Intern. Med.* **122**, 43–46.
- Sussman, G. L., Tarlo, S., and Dolovich, J. (1991). The spectrum of IgE-mediated responses to latex. *JAMA* **265**, 2844–2847.
- Tarlo, S. M., Sussman, G. L., Contala, A., and Swanson, M. C. (1994). Control of airborne latex by use of powder-free latex gloves. *J. Allergy Clin. Immunol.* **93**, 985–989.
- Thakker, J. C., Xia, J. Q., Rickaby, D. A., Krenz, G. S., Kelly, K. J., Kurup, V. P., and Dawson, C. A. (1999). A murine model of latex allergy-induced airway hyperreactivity. *Lung* **177**, 89–100.
- Vallier, P., Baland, S., Harf, R., and Deviller, P. (1995). Identification of profilin as an IgE-binding component in latex from *Hevea brasiliensis*: Clinical implications. *Clin. Exp. Allergy* **25**, 332–339.
- Valliere, R. (1999). Severe latex allergies said to afflict one-tenth of health-care employees. *BNA’s Occupational Safety and Health Reporter* **28**, 216.
- Woolhiser, M. R., Munson, A. E., and Meade, B. J. (2000). Immunological responses of mice following administration of natural rubber latex proteins by different routes of exposure. *Toxicol. Sci.* **55**, 343–351.
- Xia, J. Q., Rickaby, D. A., Kelly, K. J., Choi, H., Dawson, C. A., and Kurup, V. P. (1999). Immune response and airway reactivity in wild and IL-4 knockout mice exposed to latex allergens. *Int. Arch. Allergy Immunol.* **118**, 23–29.
- Yassin, M. S., Lierl, M. B., Fischer, T. J., O’Brien, K., Cross, J., and Steinmetz, C. (1994). Latex allergy in hospital employees. *Ann. Allergy Asthma Immunol.* **72**, 245–249.
- Yeang, H. Y., and Hamilton, R. G. (1998). Impact of biologic variation on latex allergenicity. *J. Allergy Clin. Immunol.* **101**, 145–146.
- Yip, L., Hickey, V., Wagner, B., Liss, G., Slater, J., Breiteneder, H., Sussman, G., and Beezhold, D. (2000). Skin prick-test reactivity to recombinant latex allergens. *Int. Arch. Allergy Immunol.* **121**, 292–299.