

Review article

The spectrum of immunologic sensitization in latex allergy

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Natural rubber latex (NRL) allergy is a type I respiratory hypersensitivity recognized in individuals exposed to latex allergens (1–9). This form of hypersensitivity has affected a large number of individuals in recent years and has become an important health concern in several occupational groups, including health-care workers, workers in the latex factories, and plantation workers tapping *Hevea brasiliensis* trees and collecting and processing latex (1, 3, 7, 10). It is assumed that the sensitization usually occurs through skin contact with latex or inhalation of antigen from latex gloves and devices containing allergens (11, 12). In hospitals and other health-care facilities, these products include gloves, balloons, barium enema catheters, tubing, and other accessories (13). However, recent evidence indicates that the exposure to latex allergen results from proteins bound to cornstarch powder used as a lubricant in latex gloves (11, 12).

Latex allergy

The typical allergic reaction to latex protein is characterized by pruritis, erythema, and edema, as is common with other allergen-IgE, mast-cell sensitivities (14–16). As sensitivity and exposure to allergen increases, urticaria may also develop, initially restricted to the site of the latex contact, but it may eventually spread to the contiguous areas of the skin and finally become systemic (15–18). Like skin contact with latex allergens, oral, vaginal, or rectal exposure also results in the development of

localized latex allergic manifestations, which may lead to generalized involvement. Airborne exposure may lead to nasal, ocular, and pulmonary symptoms (11, 12, 19–23). The ocular symptoms usually start with pruritis and progress to tearing, chemosis, and edema (5, 14, 16, 24). Frequently, direct contact with latex products leads to sudden swelling of the eyelids. Nasal symptoms include sneezing, watery rhinorrhea, and congestion. Patients may develop sore throat, irritation of the larynx, or cough (22, 23). Pulmonary symptoms may range from coughing to life-threatening asthma (14, 15). Lung-function changes and chest symptoms may or may not be present in patients exposed to airborne latex allergens (7). Gastrointestinal, cardiovascular, and genitourinary symptoms from latex allergy have been well documented (5, 16, 25, 26). The severe forms of latex allergy are characterized by wheezing, stridor, sneezing, rhinorrhea, ocular itching, urticaria, hypotension, and anaphylaxis (16). Although not very common, latex-induced anaphylaxis occurs soon after exposure to the allergens, and even with prompt intervention latex-induced anaphylaxis can be fatal (27–29).

Latex-fruit syndrome

Latex allergy has been reported to be associated with hypersensitivity to a number of fruit and nut allergens, and this cross-reactivity of latex-allergic patients to phylogenetically distant plants is called the “latex-fruit syndrome” (30). These fruits and nuts include banana,

avocado, kiwi, papaya, passion fruit, fig, melon, mango, pineapple, peach, and chestnut (30–35). In addition, patients with allergy to other food materials such as potato and tomato, food grains such as wheat and barley, and pollens such as ragweed and grass also may show cross-reactivity to latex allergens (36–41).

This widespread cross-reactivity with other plant allergens may be explained by the presence of common epitopes (40–42). However, no detailed study has been conducted to ascertain the clinical and immunologic finding in patients with latex-fruit syndrome. By RAST and RAST inhibition studies, about 70% of latex-allergic patients demonstrate fruit-specific IgE antibody in their sera (43). A number of latex-allergic patients also demonstrate symptoms after ingestion of various fruits, and several of them also show intolerance reactions to the fruits (16). The chitin-binding proteins present in latex have been recognized as allergenic and have been detected in a number of grains, potato, and other tubers (40, 42, 44). Similarly, the latex allergen, profilin, shares similarity with other plant profilins and may be involved in cross-sensitivity and cross-reactivity, as may carbohydrates present in the allergens (46, 47). Another group of proteins implicated in cross-reactivity are defense-related proteins produced by a number of unrelated plants. Some of these significant proteins have enzymatic activities and include hydrolases, enolases, and proteases (48, 49).

Allergic contact dermatitis

Contact dermatitis and irritant reactions have been associated with latex products for over 50 years (14, 50). The relevant contact dermatitis is a delayed-type hypersensitivity or cellular immune reaction, typically involving the hands after wearing rubber gloves. The reaction includes scaly eczematous lesions appearing 48–72 h after contact with allergen. The lesions become chronic if the exposure persists, while the reaction disappears gradually if exposure is discontinued. Occasionally, contact dermatitis has been reported with the use of rubber products used in cosmetic application, prosthetics, etc. (51, 52). The hypersensitivity is the result of immune responses to residual low-molecular-weight chemical additives and accelerators including thiurams, carbamates, amines, and benzothiazoles, rather than latex proteins, used in the manufacture of gloves and other rubber products. Such reactions should not be confused with the more common irritant reaction resulting from occlusion of the skin and irritant responses to glove powder or chemicals.

Prevalence of latex allergy

The exact prevalence of latex allergy is unknown. It has been reported that the prevalence of latex allergy in a

nonatopic normal population is around 1%, while that among those in health-care professions ranges from 3% to 12% (53). However, a wide difference in the prevalence has been reported in various occupational groups. Even within the broad occupational group of hospital employees, 2.9–17% sensitization has been reported (54, 55). The presenting symptoms and evaluation methods frequently influence the accuracy in determining the prevalence. The prevalence also depends on the glove-wearing time and the concentration of latex allergens in the environment. For example, the concentration of latex allergens in surgical areas in hospitals has been determined to be 10–100-fold more than that of nonsurgical areas (56, 57). Atopy and eczema have been considered major contributing factors to allergy to latex. Balloons, toys, and several other latex-containing material have been sporadically involved in asthma due to latex allergy.

Studies conducted in rubber-manufacturing plants showed a large number of workers to be sensitized to latex proteins (7). The incidence varied from 6% to 11%, although the reported studies are not conclusive due to the small number of subjects evaluated.

The prevalence of NRL allergy has been shown to be significantly higher among patients with spina bifida than in any other patient group. About 18–65% of spina bifida patients showed latex sensitivity as detected by IgE serum antibody and symptoms with latex contact (53, 58). Multiple mucosal and visceral exposures are considered the reason for the high incidence (59). Patients undergoing multiple surgical procedures have shown an enhanced prevalence; however, paraplegic patients with frequent exposure to latex products failed to show any remarkable increase in the incidence. Individuals with no known underlying risk also have experienced allergic reactions to NRL-induced asthma. Individuals with other IgE-mediated reactions such as asthma or allergic rhinitis have also been shown to have an increased risk of developing latex allergy (58).

Diagnosis and treatment of latex allergy

The clinical presentation of latex allergy results from the exposure to antigens by cutaneous, mucosal, and parenteral routes (1, 14, 16). Diagnosis depends mainly on the clinical history of IgE/mast-cell-mediated reactions in patients allergic to latex (14, 56, 60). Clinical history, although important, will not always identify all latex-allergic patients. It is essential to ascertain underlying conditions and disease such as the presence of spina bifida, atopy, multiple surgeries, congenital abnormalities, history of allergy to fruits and mold, and occupational predilections. However, despite advances in the laboratory diagnosis of latex allergy, a detailed history may yield a more definitive diagnosis. A history of redness, itching, or swelling after contact with latex

products or unexplained episodes of urticaria may indicate latex allergy. Skin tests with latex allergens, either from crude NRL or from extracts of latex products, may be helpful. Most of the crude preparations are not completely dependable. Although purified allergens are available, no studies have yet been undertaken to evaluate them. The use of exposure tests has also been evaluated, but caution should be exercised in employing the *in vivo* tests with certain patient groups with underlying diseases such as spina bifida. Clinical diagnosis can be confirmed with laboratory diagnosis (61, 62). Although no standardized antigens are currently available for skin or *in vitro* testing, purified and well-characterized reagents will be available in the near future for the immunodiagnosis of latex allergy.

The treatment of latex allergy follows the same course as for other allergies and includes avoidance as the most important precaution. However, because of the severity of the reaction, more stringent practices of avoidance of exposure to latex allergens are essential (57). The utility of premedication is not clear at this time due to conflicting efficacy reports. It is recommended that corticosteroids with or without ephedrine be used to reduce the severity of intraoperative latex allergic reactions. Patients who have systemic symptoms from latex should have injectable epinephrine readily available at all times. Asthma symptoms are treated with standard anti-inflammatory agents and bronchodilators. Urticaria most often responds to the elimination of the antigen or antihistamine therapy. Refractory symptoms may require corticosteroids.

Allergens associated with latex allergy

Natural rubber latex (NRL)

NRL is made up of the processed rubber particles present in the specialized cells (laticifers) of the rubber tree *H. brasiliensis*. Although over 2000 plants produce latex, over 99% of the latex used commercially comes from *H. brasiliensis* (63). The protein content varies from 1% to 1.8% according to the clonal origin of the rubber plants, climatic factors, soil types, and fertilizers (63). Particles of the rubber hydrocarbon, polyisoprene, constitutes 25–45% of the latex content. Although the actual number of the individual proteins may exceed more than 200, hevein and hevamine constitute the majority of the proteins (64, 65). The latex also contains lipids, carbohydrates, and many inorganic constituents including potassium, magnesium, calcium, sodium, zinc, manganese, copper, and iron (63).

A number of proteins with an apparent role in latex allergy have been isolated from NRL. The genes encoding several of these allergens have been cloned, sequenced, and expressed in appropriate vectors. A list of latex allergens approved by the IUIS Nomenclature Committee is given in Table 1 (66, 67).

A majority of the proteins detected in NRL have also appeared in the finished latex products in the natural form, or occasionally in an altered configuration. Chemical treatment during manufacture results in the fragmentation of latex proteins. In nonammoniated latex, over 240 separate polypeptides have been detected by two-dimensional electrophoresis (64, 68). However, only 25% of these peptides showed binding to IgE from latex-allergic patients (68). The protein content of NRL depends on a number of factors including genetic background and the physiologic characteristics of the rubber plant.

The analysis of NRL proteins in SDS-PAGE demonstrated a wide range of peptides with molecular masses of 5–200 kDa (68–73). The IgE antibody in the sera of latex-allergic patients showed variable reactivity in immunoblot against these peptides. The size of the allergens reacting with IgE varied from 5 to 100 kDa. According to the specificity of the reactivity in Western blots, the allergens with molecular sizes of 11, 14, 18, 24, 27, 35, 66, and 100 kDa have been found to be significant in latex allergy (74, 75). New proteins which were not present in NRL have also been reported in the finished products (74). Significant IgE binding was reported with latex proteins of 14, 20, 23, and 28 kDa by allergic patients with spina bifida in immunoblots (75). Several specific latex proteins affecting only health-care workers have also been reported and may play a role in their disease (72).

The immune responses have been studied with purified recombinant allergens in allergic patients (62). The results confirm the specificity and reliability of the purified allergens in the immunodiagnosis of latex allergy. With these recombinant allergens, the reagents can be standardized to obtain more reliable results and better comparison between laboratories (62).

Table 1. Latex allergens and their characteristics

Allergen	Subcellular localization	Molecular mass (kDa)	Accession no.	Significance as allergen
Hev b 1	Large rubber particles	14.6	X56535	Minor/major
Hev b 2	Lutoids	35.1	U22147	Major
Hev b 3	Small rubber particles	22.3	AF051317 AJ223388	Major
Hev b 4	Lutoids	50–57	N/A*	Major
Hev b 5	Cytoplasm	16	U51361 U42640	Major
Hev b 6.01	Lutoids	20	M36986	Major
Hev b 6.02	Lutoids	4.7		
Hev b 6.03	Lutoids	14		
Hev b 7	Cytoplasm	42.9	AJ223038 U80598	Minor
Hev b 8	Cytoplasm	42.9	Y15402 AJ243325	Minor
Hev b 9	Cytoplasm	47.7	AJ132580	Minor
		47.5	AJ132381	
Hev b 10	Mitochondria	22.9	L11707 AJ249148	Minor

*N/A: not available.

Latex proteins show strong cross-reactivity with a number of other proteins, and it is useful to understand the cross-reactivity to avoid serious anaphylaxis after skin testing or other *in vivo* testing for latex allergy. The list of allergens potentially cross-reacting with latex is increasing rapidly and includes a number of fruits, vegetables, and grains (30–46). The cross-reactivity among these antigens may be explained in terms of the unique metabolic processes seen in plants. The chitin-binding protein present in a number of plants shows marked homology to the sequences from different plants (48, 49). It has been suggested that the chitin-binding domain is the building block of many proteins with diverse specificity. However, it is not clear whether the cross-reactivity detected between allergens from fruits, nuts, beans, ornamental plants, and the latex from *H. brasiliensis* is due to chitin-binding proteins or lectins, and this question can only be answered by further studies (40, 42, 43).

Typically, the cross-reactivity between two or more allergens can be ascertained by competitive inhibition of the antibody titers of the sera from patients with latex allergy. Specific IgE obtained from affinity chromatography of various food allergens showed only a moderate degree of cross-reactivity (33, 37, 45). Similar results have been obtained with immunoblot and RAST inhibition using fruit allergens. Hence, because of the association of fruit and latex allergy, it has been recommended that patients be cautioned regarding potential cross-reactions when allergy to one antigen is present (43).

A reliable standardized skin test antigen to detect latex allergy is currently not available. In most instances, crude NRL latex or proteins extracted from gloves or other commercial products are used as skin test reagents. Recently, several antigens have been proposed for standardization (76, 77), but none have the reliability or purity for universal acceptance. With the availability of major recombinant latex allergens for universal use, there is a possibility of developing standardized reagents (66, 67). As recombinant proteins can be obtained in quantity, such allergens should be evaluated in skin test reactivity.

IgE and IgG antibodies to latex antigens

In all the earlier studies, IgE antibody against latex allergens has been demonstrated with crude latex proteins or extracts from gloves or other latex products

(61, 64, 65, 68–70, 74). In most latex-allergic patients, including the two major groups representing health-care workers and spina bifida, elevated levels of latex-specific IgE have been detected in their sera. IgE-reactive bands have been demonstrated by Western blot (64, 65, 68, 72, 74). The predominantly reactive latex protein bands have been detected at regions of 5, 14, 15, 19, 21, 23, 27, 35, and 45 kDa. However, the chemical treatment and manufacturing process determine the preservation of the residual or neolates allergens in the finished products (74). Thus, the sensitization to different products may result in the involvement of different antigens. The differences in the sensitization, variation in the presence of proteins, and other related factors such as allergenicity could be attributable to the lack of a standardized antigen to demonstrate IgE in the sera of latex-allergic patients. In recent years, more advanced techniques have been employed to obtain pure relevant allergens from latex. These efforts have resulted in purification of most of the major and minor allergens by cloning the genes and expressing the proteins (66). Similarly, interest has focused on determination of the reliability of these allergens to diagnose latex allergy. Currently, attempts are being made to use these allergens in automated techniques to demonstrate IgE in the sera.

The allergen Nomenclature Subcommittee of the the International Union of Immunological Sciences has approved 10 allergens and named them in the sequence Hev b 1 to Hev b 10 (67). Some of these allergens are major latex allergens with strong reactivity with a majority of patients, while others react less frequently with patients' sera.

Hev b 1 (rubber elongation factor)

Rubber elongation factor (REF) was the first well-characterized allergen of *H. brasiliensis* (78). This protein was isolated, purified, and sequenced even before its role in allergy was recognized (79, 80, 81). REF binds to large rubber particles and constitutes the upper layer after centrifugation (Table 2). It is a 137-amino-acid-long protein and has a tetrameric form with a molecular mass of 58 kDa. Czuppon et al. (78) evaluated latex-allergic patients and found specific IgE in high levels against REF in all 13 patients compared to 9/13 patients with IgE against crude extracts. The antibody was inhibited by competitive

Table 2. Localization of relevant *H. brasiliensis* allergen in latex sap

Designated fractions after centrifugation	Subcellular components	Latex allergens
White upper layer	Large rubber particles	Hev b 1
	Small rubber particles	Hev b 3
Aqueous middle layer	Cytoplasm from cells of latex vessels	Hev b 5, Hev b 7, Hev b 8
Bottom layer (B-serum)	Vacuole-like luteoids	Hev b 2, Hev b 4, Hev b 6.01, Hev b 6.02, Hev b 6.03, heveamine, class I and II chitinases

inhibition assays using glove powder containing REF. No inhibition was seen when powder without allergen was used. Another study showed Hev b 1 to be a major allergen reacting with 81% of spina bifida patients with latex allergy. Over 50% of health-care workers showed strong IgE reactivity to this allergen (82). In a multicenter study, 13–32% of health-care workers and 54–100% of spina bifida patients with latex allergy showed strong reactivity by RAST or ELISA. When Hev b 1 was used in PBMC stimulation, approximately 52% of the patients showed stimulation (62). In a recent study, we found strong PBMC stimulation in 57% of patients, but no correlation was detectable between PBMC stimulation and the presence of high IgE in the serum (83).

Hev b 2 (β -1,3-glucanase)

Hev b 2 was isolated from B serum containing leutoids of NRL (Table 1). This basic protein, β -1,3-glucanase, showed binding to IgE in the sera of latex-allergic patients (84–86). A cDNA clone encoding a mature protein of 35 kDa was obtained (87). However, the expressed protein failed to bind to the serum IgE from latex-allergic patients. It is not known whether the lack of reactivity was due to nonglycosylation of the expressed protein or to conformational changes. However, both spina bifida and health-care workers with latex allergy showed strong reactivity to the native Hev b 2 (62). Depending on the methods used, the reactivity varied from 20% to 61% of the patients. Antigen-induced PBMC stimulation indicated that over 56% of the patients showed strong stimulation to Hev b 2 (62). There was over 70% correlation between the IgE levels against Hev b 2 in the sera and the PBMC stimulation with this allergen (83).

Hev b 3

The Hev b 3 protein forms an integral part of the small rubber particles of 70 nm or less (87). Hev b 3 showed strong IgE-binding reactivity with latex-allergic patients with spina bifida (64, 87, 88). The reactivity of Hev b 3 with serum IgE in health-care workers was less frequent and weaker than in spina bifida patients (64, 88). This protein has a tendency to break down into smaller fragments. The comparison of different proteins from the small rubber particles showed complete identity and demonstrated cross-reactivity with Hev b 1, a component of the large rubber particles. This allergen has been cloned and expressed, but the reactivity was not consistent compared to the native protein (87). Strong stimulation of PBMC from spina bifida patients with latex allergy was reported, although our studies with PBMC from health-care workers indicate that only 25%

of patients demonstrate stimulation by Hev b 3 (62). No correlation between IgE responses and PBMC stimulation was detectable with this protein (83, 89).

Hev b 4

Hev b 4 was reported by Sundaresan et al. to be a microhelix component with a molecular size of approximately 50–57 kDa (85). With Hev b 4 in ELISA, about 65% of the health-care workers and 77% of the spina bifida patients with latex allergy demonstrated IgE binding (62). However, a few normal subjects also demonstrated IgE to Hev b 4 in their sera. The results of RAST varied considerably among both patients and normal controls (62). Although this allergen detected IgE in over 65% of health-care workers, only 14% of those patients showed antigen-induced PBMC stimulation (83).

Hev b 5

Hev b 5 was cloned independently by two investigators (90, 91). It has an apparent molecular mass of 16 kDa, a PI of 3.5, and a sequence homology with kiwi protein. Hev b 5 demonstrated strong histamine release from basophils of latex-allergic patients. The amount of histamine released showed a strong correlation with the IgE levels in the sera against Hev b 5. This latex protein is considered a major latex allergy-associated protein due to its strong IgE binding in both health-care workers and spina bifida patients with latex allergy. Hev b 5 also demonstrated strong T-cell responses in patients with latex allergy, particularly health-care workers (92).

Hev b 6 (prohevein)

The Hev b 6 gene was cloned and the protein expressed by Broekaert et al. (93) in 1990. This protein has strong homology with wound-inducible and defense-related proteins of potato, wheat-germ agglutinins, and tobacco (93, 94). It has an amino terminal domain of 43 amino acids and a carboxyl terminal domain of 144 amino acids. Prohevein showed strong reactivity with IgE from health-care workers and spina bifida patients with latex allergy (71, 74, 94, 95). Both immunoblots and ELISA using sera from latex-allergic spina bifida patients showed more patients reacting with the N-domain than the C-domain of Hev b 6. The results of skin test reactions correlated favorably with the *in vitro* IgE to latex allergens (94). Epitope mapping of the prohevein molecule revealed more IgE-binding regions at the N-terminal end (94). Hev b 6 reacted strongly with IgE from both health-care workers and spina

bifida sera with high specificity and sensitivity (62). This allergen also showed strong stimulation of PBMC from latex-allergic health-care workers (83).

Hev b 7

The patatin-like protein Hev b 7 with a molecular size of 42.9 kDa has been cloned and expressed (44, 96, 97). This protein showed IgE-binding reactivity in only a small percentage of health-care workers with latex allergy. Spina bifida patients and health-care workers with latex allergy reacted equally to this allergen. In a recent study, we found that strong IgE binding to Hev b 8 was seen in patients with less reactivity to other allergens, particularly Hev b 2 (62). PBMC stimulation was detected in 15% of the patients with latex allergy, and no correlation could be made between the IgE content in the sera and stimulation of PBMC (83).

Hev b 8 (latex profilins)

Plant profilins are frequently identified allergens with varying molecular sizes (Table 1) (98–100). The *H. brasiliensis* profilin showed cross-reactivity with IgE from 36 patients with ragweed allergy (99). The proteins were purified and used in skin prick testing of 24 spina bifida patients, who all showed a positive reaction. However, only 6/17 health-care workers tested showed reactivity to this allergen (99, 100). Definite skin test reactivity and T-cell responses to this allergen have not yet been established.

Hev b 9 (enolase)

Hev b 9 is a 51-kDa enzyme with very high homology with *Ricinus communis* enolase and *Cladosporium* enolase (65, 101). Our studies with 26 health-care workers and 12 spina bifida patients with latex allergy failed to show any IgE binding with this protein (Kurup et al., unpublished results). This protein has not been evaluated for cross-reactivity with other enolases and their IgE responses. In view of the homology between fungal enolase and Hev b 9, some degree of cross-reactivity is expected, but this needs to be elucidated by future studies.

Hev b 10 (manganese superoxide dismutase) (26 kDa)

Manganese superoxide dismutase (MnSOD) has been detected in a number of fungi, bacteria, and man (102, 103). *Aspergillus fumigatus* MnSOD has been recognized as a major allergen (104). These allergens also showed strong PBMC stimulation (103). In a recent study, we found a strong IgE binding of this antigen in one health-care worker with latex allergy out of 26 patient sera studied (Kurup et al., unpublished results).

This antigen showed strong homology with *A. fumigatus*, *E. coli*, and human MnSOD. The results from these few studies indicate that in spite of the homology with other MnSODs, this allergen showed only a low degree of cross-allergenicity with mold-allergic sera (103).

There are several other minor components that may bind to IgE from patients with latex allergy (66, 105–107). As there are over 200 polypeptides discernible in latex, additional useful allergens may exist (64, 68). Some of them may prove to be useful in the diagnosis, while others may be of value in understanding the sensitization and immune mechanism of the disease.

T-cell responses to latex allergens

Exposure to latex allergen results in the production of mainly IgE antibody in latex-allergic patients (1, 66, 108). The presence of IgE and Th2 cytokines in latex-allergic patients indicates that the immune mechanism is T-cell mediated. This was further substantiated by the results obtained in a murine model of latex allergy (109, 110). However, not much attention has been paid to studying lymphocyte responses in latex-allergic patients or to elucidating the immunopathogenesis of latex allergy. The results of the few studies indicate that the crude antigens, such as ammoniated or nonammoniated latex sap or extracts obtained from finished latex products used in demonstrating the antibody responses, are toxic to lymphocytes (83). Hence, purified or less toxic crude antigens are essential to study T-cell responses (66, 108, 111).

When purified antigens were used, significant T-cell stimulation has been demonstrated. HPLC-purified fractions with a molecular size of 3–10 kDa demonstrated PBMC stimulation *in vitro* (112). Significant stimulation of PBMC from health-care workers with latex allergy has been detected with a 30-kDa latex protein purified by HPLC and Sephadex G-200 chromatography (111). Although there was no correlation between IgE levels and PBMC stimulation with latex allergens, there was no stimulation with this latex antigen when cells from normal subjects were studied. Raulf-Heimsoth et al. evaluated latex glove extract, latex sap, and Hev b 1 in PBMC stimulation and obtained 47.8%, 65%, and 52% stimulation, respectively, in latex-allergic patients, compared to only 25%, 37%, and 25% in nonallergic subjects exposed to latex allergens (113). There was, however, no correlation between specific IgE levels and the PBMC stimulation with these allergens (83, 113). With crude latex allergens, a stimulation index much higher than in normal subjects and atopic controls was seen in patients with latex allergy. However, no correlation was detected with specific IgE levels in the serum and stimulation, although less than 40% of the patients showed PBMC stimulation (114).

Our studies using six purified latex allergens demonstrated significant PBMC stimulation with some of the latex allergens (83). Of the 28 health-care workers studied, 12 failed to stimulate the PBMC with any of the six antigens, while the remaining 16 patients showed considerable variation in their responses. Hev b 2 stimulated the PBMC of all the remaining 16 patients, followed by Hev b 6, which stimulated the PBMC of 75% of patients. The other purified allergens tested showed less reactivity. Crude latex extract (MNA) showed the least response of the allergens. This study also failed to demonstrate any correlation with IgE binding and T-cell stimulation. Recently, significant T-cell stimulation was demonstrated with Hev b 5 in 5/6 health-care workers with latex allergy (92). The two major epitopes identified in the stimulation of T-cell clones and lines include the peptides with amino acids 46–65 and 109–128. A predominantly Th2-type response was detected by the two peptides, as shown by the IL-5, but not IFN- γ , secretion by the two T-cell lines stimulated with the peptides (92).

Epitopes of latex allergens

The specificity of the allergens binding to IgE or to T cells depends on the amino-acid sequence of the allergens. Information on epitopes and their interaction with receptors may be of immense value in devising specific immunodiagnostic and immunotherapeutic reagents for more reliable diagnosis and patient care. The T-cell epitopes are linear and MHC class 2 restricted, while the B-cell epitopes are usually conformational, rarely linear, and not MHC controlled. However, some of the linear B-cell epitopes may adequately block IgE binding and cross-linking of allergens on the surface of basophils and mast cells. Beezhold et al. detected six IgE-binding epitopes spanning the whole sequence of the prohevein molecule, of which four were in the C-domain of the Hev b 6 molecule (115). The N-terminal epitope had 100% homology with wheat-germ agglutinins and other plant proteins belonging to the chitinase family (116).

On evaluation of the amino-acid sequence of the Hev b 6 molecule with overlapping synthetic peptides, 10 IgE-binding regions were identified (94). Some of these epitopes were specific for IgE binding to spina bifida patients, while the others showed specificity to health-care worker patients. In addition, epitopes binding equally to the IgE of both patients have also been detected. The two N-terminal epitopes showed similar sequence homology with the WIN-1 and WIN-2 proteins of potato (36, 38, 94, 117).

The IgE-binding epitopes of Hev b 1 and 3 were studied with sera from both health-care workers and spina bifida patients with latex allergy (118). Hev b 1 showed eight IgE-binding epitope regions when spina bifida patients were studied, while Hev b 3 showed 11

IgE-binding epitopes. Three epitopes from Hev b 1 and two from Hev b 3 reacted similarly with both groups of patients. The similar binding features noted with Hev b 1 and Hev b 3 allergens may be due to the presence of similar epitopes and sequence homology. Of the eight epitopes identified in Hev b 1, six were in the N-terminal end, while only two were located in the C-terminal end. Hev b 3 showed 11 epitopes binding to IgE, of which the four N-terminal epitopes spanning amino acids (aa) 1–114 were highly conserved. All eight epitopes from Hev b 1 reacted with spina bifida sera, but only three showed binding with sera from health-care workers. It is interesting to note that aa 16–25 at the N-terminal end was specific for spina bifida patients, while the two epitopes at the C-terminal end of Hev b 3 reacted with both health-care worker and spina bifida patients. The epitopes at the N-terminal end of Hev b 3 were specific to spina bifida patients. The results of epitope analysis also demonstrated specificity for IgG or IgE, although epitopes binding to both IgG and IgE have also been detected.

By spleen cell stimulation in a murine model of latex allergy, Slater et al. identified four T-cell-binding regions in Hev b 5 (119). Hev b 5, a proline-rich acidic protein of latex, showed four major IgE-binding regions representing aa 1–38, 55–74, 109–128, and 132–151. Two of the four T-cell epitopes failed to bind IgE, suggesting the usefulness of the sequence as an immunotherapeutic peptide. After study of the T-cell response in six latex-allergic patients, two immunodominant regions were identified in Hev b 5 (92). On careful evaluation of the epitopes, it was found that certain specific amino acids were highly conserved, and the sequences of the epitopes were represented by KXEE or KEXE, where “X” is empty or represented by threonine or alanine (119).

Diagnostic assays for latex allergy

IgE assay

Latex-specific IgE in the sera of patients has been studied by RAST or ELISA using crude NRL extracts or purified latex allergens (62). In recent years, a number of commercial methods such as ImmunoCAP and Ala STAT have become available for evaluating patient sera for specific IgE. Although these methods are not standardized, when followed according to the manufacturers' instructions to evaluate IgE from patients, the results were consistent, with few variations from test to test. However, because of the cross-reactivity of latex allergens with fruits, molds, and other allergens, the interpretation of the results was difficult.

Other antibody assays, such as Western blot and RAST, have also shown encouraging results. Two-dimensional electrophoresis blots yield significant and specific results, but are usually time-consuming and expensive (64, 68, 71, 72). Histamine release from

basophils can be studied with peripheral blood basophils. Several purified allergens showed significant histamine release by this method (112).

Future directions

Although there are currently several purified and well-characterized allergens available, no concerted effort has yet been made to standardize them for *in vitro* diagnosis of latex allergy or in developing a dependable skin test antigen. Crude preparations contain reactive materials, and the use of such materials is discouraged in skin testing and in *in vitro* immunoassays due to the presence of toxic materials and the difficulty of interpreting the results. These factors are major deterrents to their use as standard safe reagents. However, for reliable results, it may be necessary to use more than one purified recombinant allergen to

achieve the desired specificity and sensitivity. Similarly, well-characterized and specific allergens may be essential for developing immunotherapeutic reagents.

Defined peptides that have promising applications must be identified and evaluated. In an experimental animal model, a suitable peptide with the expected characteristics has been identified by Slater et al. (119). However, additional studies and peptides are needed before any human trial begins. Similarly, the naked DNA vaccine and appropriate synthetic peptides may help in altering the Th2-type response to a Th1 response.

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