

REVIEW

Latex allergy: towards immunotherapy for health care workers

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Summary

Latex allergy is an important allergic disease for which safe and readily available immunotherapy is currently lacking. Despite advances in latex glove technology and reduction in allergen content, there remains a core of severely allergic health care workers (HCW), particularly with concomitant food allergy, for whom allergen avoidance is insufficient. Current experience with immunotherapy using crude latex extracts has shown an unacceptable level of local and systemic side-effects. Latex allergens are extremely potent with a heightened capacity to cross-link effector cell-bound IgE and induce anaphylaxis. The predominant pattern of allergen reactivity among HCW is different from that among children with spina bifida, perhaps due to exposure to latex glove proteins, particularly via inhalation, rather than particle bound latex proteins present in urinary catheters. Recent studies using purified skin testing reagents have indicated that the most clinically important latex allergens amongst HCW are Hev b 5, 6 and 7. Elucidation of the molecular and cellular mechanisms of the immune response to these allergens is pivotal to facilitate the search for safer immunotherapy of latex allergy among HCW.

Background

Latex allergy is principally a late 20th century phenomenon, rising to prominence through the convergence of a number of factors, including the rise of atopy in general, increased awareness by both patients and clinicians, and most importantly, the Human Immunodeficiency Virus (HIV) epidemic which resulted in an exponential increase in the use of latex gloves as a barrier protection among HCW. The disorder has attracted attention for its potentially catastrophic consequences when mucosal surfaces are breached by latex proteins in allergic individuals. This happened with the sentinel barium enema deaths [1], and is seen in intraoperative anaphylaxis to latex [2]. Moreover, latex allergy causes considerable morbidity in affected individuals and may be associated with asthma [3], rhinoconjunctivitis [4], urticaria [5] and food allergy [6].

This article will focus on the current and possible future approaches towards safer immunotherapy of latex allergy,

concentrating on HCW. It will discuss the current status of immunotherapy in latex allergy and review the latex allergens of clinical importance among HCW, which are thus potential immunotherapy candidates. Finally, it will look at the possible future approaches to immunotherapy including T cell epitope peptides, hypoallergenic mutants, allergens conjugated to immunostimulatory sequences (ISS) of DNA and DNA vaccination.

Traditional immunotherapy for latex allergy

Three studies have reported immunotherapy in latex allergy, and all have used crude ammoniated latex preparations. The most important of these studies was a randomized, placebo-controlled trial using the Stallergenes low ammoniated latex (LAL) preparation [7]. This is a standardized extract used widely in Europe and on a restricted basis in Australia for skin testing in the diagnosis of latex allergy. It is manufactured from Malaysian rubber (RRIM clone 600), has a 100% specificity and 93% sensitivity when used at 100 index of reactivity (IR) units (22 µg/mL concentration) in skin testing for latex allergy diagnosis and contains all the known IgE binding latex components [8]. This was a multicentre study in which 17 HCW with latex allergy were randomized to immunotherapy or placebo. The immunotherapy regimen involved a two day rush protocol where patients were up-dosed to their maximum tolerated dose with the 10 IR or 100 IR vial depending on sensitivity. This was followed by a 12-month maintenance phase of injections at the maximum tolerated dose, initially fortnightly and then monthly. The active treatment group reported a statistically significant decrease in symptoms of rhinoconjunctivitis and urticaria but not of asthma, though it was noteworthy that the placebo group had an increased incidence of asthma at the commencement of the study compared with the active group. Although efficacy for the treatment was shown, side-effects were noted. These included a local reaction rate of over 40% in the active treatment group compared with 15% in the placebo group. In addition, 15% of injections in the active group induced episodes of rhinitis, 2.7% induced asthma, 0.6% angioedema and 0.3% systemic anaphylaxis. Most importantly, these reactions occurred just as frequently in the maintenance phase of injections as compared with the up-dosing phase of treatment.

The two other reports of immunotherapy in latex allergy are case reports. In the first, Pereira et al. [9] described the desensitization of a 31-year-old-radiology technician using a low

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ammoniated latex extract (ALK-Abello). During the up-dosing phase of immunotherapy she had a systemic reaction with hypotension and voice hoarseness requiring adrenaline. Again, once in the maintenance phase, symptoms upon latex exposure were reported by the patient to be much reduced and this was validated by a reduction in reactivity on latex provocation testing and skin testing. Interestingly, there were also decreases in *in vitro* reactivity to banana, kiwi and chestnut. The second of the case reports described the sublingual desensitization of a latex-allergic medical student using the ALK-Abello LAL extract [10]. There were no systemic side-effects reported up to a cumulative dose of 500 µg of the extract, while there was a reduction in symptoms and reactivity to latex-specific provocation of the subject.

These studies indicate some of the inherent difficulties with immunotherapy of latex allergy. Firstly, paradoxically, those patients who are the most latex-sensitive, and thus have the greatest potential benefit from desensitization, are most at risk from therapy. Patients with mild or moderate latex allergy may be managed successfully with allergen avoidance measures alone [11]. Immunotherapy, to be of real benefit, must safely help subjects with severe allergy for whom allergen avoidance is more difficult because of their propensity to get symptoms on exposure to even minute quantities of latex protein. Latex is a ubiquitous material within our modern day environment and these severely affected individuals may get symptoms where mildly affected individuals have no difficulty. In fact, they may be put at risk of anaphylaxis through everyday activities in the community [12, 13]. The studies to date have indicated that there is a high risk of anaphylaxis, or at least severe local side-effects with immunotherapy using crude latex extracts. Thus, highly latex-sensitive individuals may be exposed to considerable risk by immunotherapy using crude extracts. At present, immunotherapy with crude extracts can only be considered experimental and must be performed by an experienced allergist in a hospital setting where intensive care backup is readily available. Further trials of immunotherapy with crude extracts are awaited with interest and are underway in Europe with the Stallergenes extract and in the USA with the Greer non-ammoniated latex (NAL) extract.

Latex allergens are extremely potent and have a high capacity to cross-link effector cell-bound IgE

The immunotherapy experience with crude extracts combined with the instances of barium enema and intraoperative anaphylaxis indicate that latex proteins are extremely potent allergens in their ability to induce severe IgE-mediated reactions. Other experimental evidence further supports this claim. For example, in a skin testing study using recombinant and natural latex extracts, Yip et al. found that the most latex-sensitive patient reacted to a Bencard LAL extract on simple skin testing down to a concentration of 70 pg/mL [14]. In addition, it has been recently shown that latex proteins eluted from rubber medication vial closures are enough to elicit weal and flare reactions when used in skin testing of latex-allergic individuals [15]. Latex is also the most common allergen to induce systemic reactions on skin testing, when compared with all others including drugs, foods and pollens [16]. The exact molecular mechanisms for this potency are yet to be fully elucidated. It has been suggested that

the major latex allergen Hev b 5, may have enhanced ability through multivalency to cross-link multiple effector cell-bound IgE molecules [17]. Other possible reasons for potency of latex allergens may relate to their bio-availability or enhanced ability to traverse mucous membranes, but little firm data is available.

The latex–fruit syndrome makes allergen avoidance difficult for some latex-allergic individuals

Up to 52% of latex allergy sufferers have sensitivity to various fruits and vegetables [6]. Also, latex-allergic individuals have four times the risk of the general population of food allergy. Food allergy amongst latex-sensitive subjects frequently manifests as anaphylaxis (36% in the Blanco study) [6]. Therefore, the latex–fruit syndrome is of considerable clinical importance. A Medline search identified more than 20 foods, fruits or plants that have been reported to be immunologically cross-reactive with latex [6, 18–32]. The most commonly described are banana, avocado, kiwifruit and chestnut. Figure 1 however, shows the fruits and vegetables reported cover a wide proportion of the plant kingdom. This is indicative of two important issues: firstly, that a pan-allergen (or allergens) is likely important, and secondly, the breadth of dietary components that may put severely latex-allergic individuals at risk of food allergy symptoms. It is not currently advised for latex-allergic patients to avoid all such foods unless they have specific symptoms. However, it is incumbent upon clinicians to warn patients of the potential for these reactions. The major latex allergen Hev b 6 has been identified as being the principal latex allergen responsible for this cross-reactivity [33]. The hevein molecule (Hev b 6.02) has striking homology with Class 1 chitinases [34] which have a hevein-like domain and occur widely in plants, thus fitting the description of a 'pan-allergen'. RAST inhibition and skin testing studies have provided further correlation of this molecular observation. Although primary sensitization via fruit exposure has been reported and is a risk factor for latex allergy [26], current evidence indicates that primary sensitization to latex accounts for the vast bulk of the latex–fruit syndrome. Whether other latex allergens are responsible for food cross-reactivity is not so clear. Hev b 5 has sequence homology to an acidic protein in kiwi fruit [35] and also in sugar beet [36] but no evidence for cross-reactivity has been found. Hev b 7 has sequence homology to patatin, which can cause positive skin test results and inhibition of IgE binding to Hev b 7 among Hev b 7-sensitized individuals [29]. Hev b 8 is a profilin; homologues of which are identified as allergens in many fruits and vegetables. Clear evidence of primary sensitization to Hev b 8 via latex exposure with subsequent food allergy has not been demonstrated and therefore for Hev b 8-sensitive individuals, primary sensitization to grass pollen profilins are the likely cause [37].

The most clinically important allergens in HCW are Hev b 5, 6 and 7

As described in the previous section, the qualification of Hev b 6 as a pan-allergen cements its clinical importance. IgE-binding, and skin test data also add support. The 4.7 kDa Hev b 6.02 (hevein) is cleaved from the *N*-terminus of Hev b 6.01

Kingdom	Sub-kingdom	Super-division	Division	Class	Sub-class	Order	Family	Genus, species (Common name)
Plantae (Plants)	Tracheobionta (Vascular plants)	Spermatophyta (Seed plants)	Magnoliophyta (Flowering plants)	Magnoliopsida (Dicotyledons)	Rosidae	Apiales	Apiaceae (Carrot family)	<i>Apium</i> L. (Celery) [27]
						Euphorbiales	Euphorbiaceae (Spurge family)	<i>Hevea brasiliensis</i> (Willd. Ex Adr. Juss) Muell. Arg. (Rubber tree)
						Rosales	Rosaceae (Rose family)	<i>Malus</i> P. Mill. (Apple) [27] <i>Prunus</i> (L.) (Plum) [30] <i>Prunus avium</i> (L.) L. (Sweet cherry) [23] <i>Prunus persica</i> (L.) Batsch (Peach) [26] <i>Prunus persica</i> (L.) Batsch var. <i>nucipersica</i> (Suckow) C. Schneider (Nectarine) [30]
						Sapindales	Anacardiaceae (Sumac family)	<i>Managifera indica</i> L. (Mango) [25]
					Magnoliidae	Lauraceae	Lauraceae (Laurel family)	<i>Persea americana</i> P. Mill. (Avocado) [19]
						Magnoliales	Annonaceae (Custard-apple family)	<i>Annona cheirimola</i> P. Mill. (Cherimoya) [28] <i>Asimina triloba</i> L. (Pawpaw) [6]
					Hamamelidae	Fagales	Fagaceae (Beech family)	<i>Castanea sativa</i> P. Mill. (European chestnut) [6]
						Urticales	Moraceae (Mulberry family)	<i>Artocarpus altilis</i> (Parkinson) Fosberg (Breadfruit) [24] <i>Ficus benjamina</i> L. (Weeping fig) [24]
					Dilleniidae	Ebenales	Ebenaceae (Ebony family)	<i>Diospyros virginiana</i> L. (Persimmon) [21]
						Theales	Actinidiaceae (Chinese gooseberry family)	<i>Actinidia chinensis</i> Planchon. (Kiwi fruit) [6]
						Violales	Cucurbitaceae (Cucumber family)	<i>Cucumis</i> L. (Melon) [26] <i>Cucurbita pepo</i> L. (Zucchini) [31]
					Caryophyllidae	Polygonales	Polygonaceae (Buckwheat family)	<i>Eriogonum Michx.</i> (Buckwheat) [20]
						Solanales	Solanaceae (Potato family)	<i>Solanum lycopersicum</i> L. (Tomato) [20] <i>Solanum tuberosum</i> L. (Potato) [29]
				Liliopsida (Monocotyledon)	Zingiberidae	Bromeliales	Bromeliaceae (Bromeliad family)	<i>Ananas comosus</i> (L.) Merr. (Pineapple) [27]
						Zingiberales	Musaceae (Banana family)	<i>Musa acuminata</i> Colla (Banana) [18]
					Com- melin- idae	Cyperales	Poaceae (Grass family)	<i>Phleum pratense</i> L. (Timothy grass) [32]

Fig. 1. Taxonomic classification of fruits, vegetables and other plant tissues reported to cross-react with latex. References are cited by numbers in square brackets following common names.

(prohevein). Being hydrophilic, hevein is easily leached from gloves during wear and forms a large percentage of extractable protein of gloves [38]. Purified hevein has also been found on skin testing to induce a positive reaction in 81% of patients with latex allergy [39].

Hev b 5 is also an important allergen amongst HCW. Our group has shown that Hev b 5 is relatively more abundant in high protein powdered glove extracts than in raw latex extracts [40]. This is likely due to its ability to survive the harsh conditions of glove manufacture and, as for hevein, its hydrophilicity. Latex allergy among HCW may result from both sensitization through the respiratory tract by inhalation of latex proteins adsorbed to carrier particles such as cornstarch donning powder, and sensitization through direct glove contact via the transcutaneous route. Therefore, this results in the predominant reactivity to Hev b 5, 6 and 7 rather than the so-called rubber particle bound proteins Hev b 1 and 3, that are important allergens among children with spina bifida. Slater et al. found that 92% of latex-allergic HCW reacted with the fusion protein of rHev b 5 with maltose binding protein (MBP) by RAST [41].

The skin testing study performed by Yip et al. used recombinant and purified latex allergens in skin testing of latex allergic patients. This was a valuable study since it employed an *in vivo* method and the allergens were systematically evaluated in serial dilutions. The allergens tested were Hev b 2, 5, 6, 8 (recombinant) and Hev b 3 and 7 (purified natural protein). All allergens were skin tested at 10-fold serial dilutions in 31 latex-allergic individuals (predominantly HCWs), two of whom were excluded from the analysis (one due to dermatographism, the other due to non-reactivity to the LAL-positive control), and 10 non-latex allergic controls. The important findings of the study were that 93% of subjects with a positive skin test to the raw latex extract (Bencard, LAL) reacted to one or more of the recombinant or purified latex allergens. Furthermore, the most frequent reactivity was to Hev b 6 (66%), Hev b 5 (62%) or Hev b 7 (41%) and all subjects who reacted to one or more recombinant or purified latex allergens reacted to one or more of Hev b 5, 6 and 7, giving a 93% diagnostic sensitivity by using these three recombinant allergens alone. None of the 10 control individuals reacted to the recombinant or purified allergens, giving 100% specificity. More importantly, no adverse reactions were reported on skin testing with the recombinant allergens, though detailed analysis of adverse events was not given. Another interesting finding of the study was the presence of monosensitization, particularly to Hev b 5 (17%), 6 (10%) and 7 (10%); again making immunotherapy with a combination of these allergens more attractive. One weakness of the study was that not all the cloned and sequenced latex allergens were used; particularly Hev b 1 and Hev b 4. Hev b 1 appears to be more important among the *spina bifida* population and shows cross-reactivity with Hev b 3 (which was used) while Hev b 4 may be an important allergen among HCW but further skin testing studies are currently underway. Also, only a small number of controls were used, and larger studies will be needed to establish the sensitivity and safety of these recombinant and natural latex allergens.

These studies, combined with the fact that Hev b 5 and 6 are the relatively abundant latex glove proteins, qualify Hev b 5, 6 and 7 as the most clinically important allergens among HCW. Therefore, the successful development of immunotherapeutic

reagents representing these allergens would be, at the very least, a considerable advance, and may be sufficient to ensure effective treatment for the majority of HCW.

Reducing the potential for anaphylaxis: (I) peptide immunotherapy for latex allergy

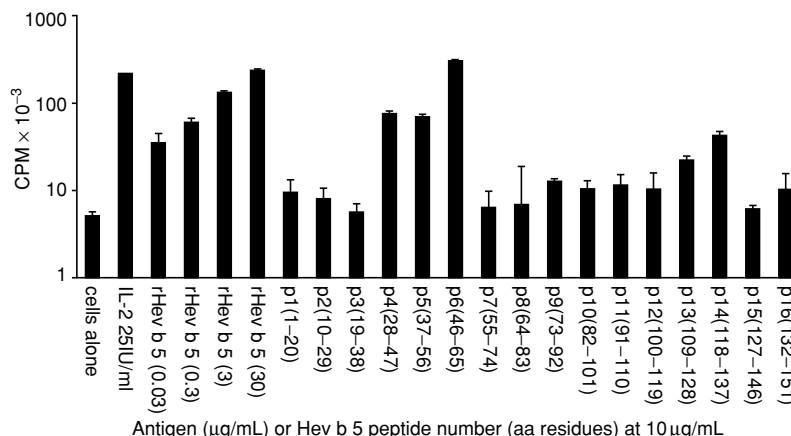
There has been a renewal of interest in peptide immunotherapy of allergy following two encouraging reports on cat [42] and honey bee venom allergy [43].

T cell epitope peptides are intrinsically attractive for use in immunotherapy because of their ability to stimulate T cells and thus modulate the cellular immune response, without cross-linking effector cell-bound IgE. It is already known that immunotherapy for latex and other diseases is effective, however, the risks of IgE mediated morbidity and possibly mortality makes immunotherapy for latex allergy in its current form unacceptable [7]. Honey bee venom allergy has some parallels to latex allergy in that traditional immunotherapy with natural antigens has, at times, a high risk of systemic side-effects [44]. In honey bee venom allergy, however, this risk is warranted because of the difficulty of absolute avoidance and the measurable and real risk of fatality amongst unprotected honey bee allergic individuals. In an effort to reduce systemic side-effects, Muller et al. administered a mixture of the three dominant T cell epitopes of phospholipase A₂ (PLA₂) to five honey bee venom-allergic subjects. There were no systemic side-effects from the subcutaneous injections of the peptides, and the patients were protected from challenge with PLA₂ in all cases and in 3/5 against sting challenge. The authors demonstrated decreased *in vitro* T cell proliferation and cytokine secretion consistent with anergy to the whole PLA₂ molecule.

Tempering this enthusiasm has been the knowledge that peptide injections have been reported to induce adverse events in cat allergic patients [45]. More recently, Haselden et al. showed that a mixture of Fel d 1 T cell epitope peptides in equimolar amounts was able to induce MHC-restricted T cell-dependent late asthmatic responses independent of IgE [42]. While these responses are of concern they indicate that Fel d 1 specific T cells are being activated without effector-cell IgE cross-linking. More encouragingly, the same group has reported both safety and efficacy of immunotherapy with Fel d 1 T cell epitope peptides in a double-blind, placebo-controlled trial [46], and in addition, developed a dosage schedule that avoids the induction of late asthmatic responses [47]. The use of a dominant peptide or small number of peptides may be advantageous in avoiding some of the potential problems of aggregation of a complex mixture of 20 or more peptides. The dominant peptides, being better immunogens may be better tolerogens and there is evidence from animal models that a dominant peptide can induce tolerance against the whole molecule through linked suppression [48]. This was also seen in the honey bee venom peptide immunotherapy trial [43].

What of peptide approaches in latex allergy? Our work with short-term LAL- and rHev b 5-specific T cell lines has shown that Hev b 5 has a dominant T cell epitope [49]. Hev b 5 peptide 46–65 caused highly significant T cell proliferation and increased IL-5 cytokine secretion in 5/6 highly latex-allergic HCW studied. A T cell proliferation assay to Hev b 5 peptides from a HCW with high IgE-specific ELISA response to rHev b 5

Fig. 2. T cell proliferation assay from short-term T cell line of latex-allergic HCW. PBMCs were stimulated for 1 week with a Hev b 5-rich powdered examination glove extract (GE) at 20 µg/mL. Cells were then recovered, autologous irradiated PBMC added in 1 : 1 ratio and restimulated for a further week with GE 20 µg/mL. Suboptimal IL-2 (25 IU/mL) was added on day 1 and 3 following restimulation. T cells were then recovered once more, autologous irradiated PBMC added in 1 : 1 ratio and the cells stimulated with different concentrations of rHev b 5 or Hev b 5 peptides at 10 µg/mL for 72 h. Proliferation was measured by tritiated thymidine incorporation and expressed as counts per minute (CPM). Mean CPM (+ SEM) for triplicate cultures are shown.



is shown (Fig. 2). Whether a single, highly dominant peptide will be sufficient to induce T cell anergy against the whole Hev b 5 molecule is at present unknown. Hev b 5 is an attractive initial target for immunotherapy strategies due to the appreciable incidence (17%) of monosensitization seen amongst latex allergic HCW [14]. However it is likely, as already stated, that effective immunotherapy for latex allergy in HCW would need to target at least Hev b 6 and 7 in addition to Hev b 5. Apart from Hev b 5, T cell epitope mapping has only been reported for Hev b 1 [50] and 3 [51] in children with spina bifida, and as discussed, these allergens appear much less important in the HCW population.

Reducing the potential for anaphylaxis: (II) hypoallergenic mutants

Another approach to decrease the effector cell-bound IgE cross-linking with the aim of reducing potential side-effects, is to modify the primary, secondary or tertiary structure via engineering of recombinant allergens. To maintain efficacy, T cell reactivity must be preserved. This has been reported in other allergen systems, notably house dust mite [52], birch pollen [53] and timothy grass pollen [54].

With regard to the clinically relevant latex allergens in HCW, Hev b 5 has been modified by Beezhold et al. to reduce IgE binding [17]. They found that a total of seven alanine substitutions were required within three of the linear IgE binding epitopes of Hev b 5 to obtain a 100-fold reduction in IgE binding to the whole molecule by inhibition ELISA. With a total of 14 alanine substitutions in 8 epitopes, IgE binding could be decreased by 4500 fold. Figure 3 shows schematically the combination of the major T cell [49] and linear B cell [17, 55] epitopes of Hev b 5 that have been reported. As can be seen there is some overlap. Consequently for the generation of successful candidate allergen mutant vaccines, future studies will need to address whether such mutants retain T cell reactivity in addition to the demonstration of decreased IgE binding.

Hev b 6 is an attractive target for modification of tertiary structure via mutagenesis, particularly in the highly reactive hevein domain which is cysteine rich and contains four disulphide bridges [56]. At this stage there are no published studies describing mutation of Hev b 6 and its effect on IgE binding. Similarly, mutational studies of Hev b 7 have not been reported.

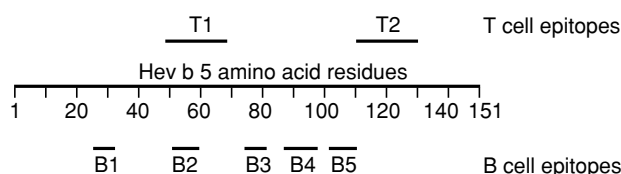


Fig. 3. Schematic diagram of major T cell and B cell epitopes for Hev b 5. Epitope sequences were classified as major if they reacted with greater than 50% of Hev b 5 reactive patients [17, 49]. Amino acid residues: T1 = 45–65, T2 = 109–128; B1 = 27–34, B2 = 50–59, B3 = 75–82, B4 = 86–98, B5 = 103–111.

Reducing the potential for anaphylaxis: (III) DNA vaccination and conjugation of allergens to immunostimulatory DNA

Finally, in addition to mutant recombinant allergen vaccines, DNA vaccination or conjugation of allergens to immunostimulatory sequences (ISS) of DNA hold promise as alternative methods of delivering allergen immunotherapy. Animal studies [57, 58] and *in vitro* human studies [59] using ISS in ragweed pollen allergy and DNA vaccination in peanut allergy [60] have demonstrated decreased allergenicity with increased immunogenicity and the ability to modulate established allergic states, by the up-regulation of allergen-specific Th1 responses and concomitant reduction in Th2 responses. In latex allergy, studies have thus far been limited to a murine model utilizing DNA vaccination for Hev b 5 [61]. This study showed successful distribution of the Hev b 5 transcript in the lungs, spleen, lymph nodes, blood and tongue and the mounting of both an allergen-specific cellular and humoral immune response of Th1 type. Toxicity was not seen, but severe local reactions had been observed by the same author during optimization experiments [61]. It is likely that safety and efficacy of DNA vaccination or ISS conjugate vaccines in humans will be established in other allergen systems such as ragweed pollen allergy, where trials of an ISS conjugated Amb a 1 have begun [62], prior to its possible application in the immunotherapy of latex allergy.

Summary and conclusions

Latex allergy is a significant health hazard for HCW. As yet, specific therapy that is both safe and effective is lacking.

Immunotherapy with crude extracts has been shown to be effective in symptom reduction, but has an unacceptable risk of local and systemic side-effects. Latex allergy among HCW is characterized by a different pattern of reactivity to latex allergens from that seen in spina bifida children. The pattern of reactivity among HCW is predominantly to Hev b 5, 6 and 7, and Hev b 5 and 6, appear to be the important allergens in latex gloves, the main route of sensitization and symptoms in HCW. While allergen avoidance may be sufficient for mildly latex-allergic individuals, for more severely affected individuals, particularly with associated food allergy, adequate avoidance may be difficult or impossible and safe immunotherapy is desirable. Hev b 5, 6 and 7 are attractive candidates for immunotherapy approaches both for their frequency of reactivity and also because of the significant incidence of mono-sensitization. Novel approaches to therapy include peptide immunotherapy or modifications of whole allergens such that T cell reactivity is maintained but IgE binding is abrogated. Further studies of *in vitro* T cell reactivity, basophil histamine release and *in vivo* skin testing of candidate peptides and whole allergen mutants are awaited with great interest.

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