

Advances in development of hypoallergenic latex immunotherapy

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Purpose of review

The characterization of clinically relevant latex allergens and the production of recombinant allergens is now well advanced, but this knowledge needs to be translated into new strategies for the safe and effective specific treatment of latex allergic diseases including asthma and anaphylaxis.

Recent findings

The current status of latex allergy is discussed indicating a changing demographic paradigm. A new wave of latex allergy is emerging outside the healthcare setting with the widespread use of latex products. An increased prevalence in developing countries is also reported. Limited studies on current specific immunotherapy for latex allergy are reviewed, confirming the feasibility but demonstrating an unacceptable risk of adverse events. The characterization of latex allergens and the identification of B and T-cell epitopes point to rational strategies for the generation of hypoallergenic preparations for specific immunotherapy. Results to date for latex allergens are reviewed, including recombinant, chemical modification and synthetic peptide approaches. Candidate hypoallergenic preparations for targeting sensitization to the major allergens Hev b 1, Hev b 3, Hev b 5 and Hev b 6.01 have been identified. Further investigations of optimal regimens for the delivery of specific immunotherapy to induce regulatory T-cell function are warranted.

Summary

The findings point to the selection of suitable hypoallergenic preparations for clinical trials of effective and safe latex allergy immunotherapy.

Keywords

hypoallergenic, IgE epitope, immunotherapy, latex allergy, T-cell epitope peptide

Abbreviations

ELISA	enzyme-linked immunosorbent assay
HCW	healthcare worker
NRL	natural rubber latex
SPT	skin-prick testing

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1528-4050

Introduction

Latex allergy remains an important allergic disease for which safe and effective immunotherapy is not currently available [1[•],2[•],3]. Sensitization to natural rubber latex (NRL) is common, with an estimated 40 000 medical and consumer products containing NRL [4,5,6[•]]. Latex allergens are highly effective at cross-linking effector cell-bound IgE on mast cells and basophils with subsequent degranulation. Clinical manifestations of IgE-mediated latex allergy include eczema, contact urticaria, rhinoconjunctivitis, asthma, food allergy, angioedema and anaphylaxis.

Latex allergy flared into an epidemic after the adoption of universal precautions for the avoidance of infections, the increased appreciation of latex allergy and the rise in atopic disease prevalence. After the recognition that glove-donning powder contributed to latex allergy pathogenesis, a decrease in the use of powdered latex gloves in the healthcare setting resulted in a marked decrease in the incidence of latex allergy in healthcare workers (HCWs) [7,8[•]]. However, there has now been a demographic paradigm shift from the healthcare industry to greenhouse workers, food handlers, construction workers, security personnel, postal workers and domestic consumers through the increased use of NRL gloves for barrier protection, of real or perceived necessity [2[•]].

This review provides a brief update on the latex allergens of clinical importance that are potential immunotherapy candidates, particularly because of their cross-reactivities with fruits/vegetables, and notes progress on B and T-cell epitope identification. It focuses on strategies for the development of hypoallergenic latex immunotherapy, including recombinant mutants, chemically modified molecules and T-cell epitope-based peptides, but includes recent advances pioneered with other allergens that have potential applicability to latex allergens. Earlier publications are cited when their content is pivotal to progress in this field.

Curr Opin Allergy Clin Immunol 5:544–551. © 2005 Lippincott Williams & Wilkins.

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Sponsorship: The work of the authors is supported by the National Health and Medical Research Council of Australia and the Alfred Hospital, Melbourne.

Current Opinion in Allergy and Clinical Immunology 2005, 5:544–551

Current status of IgE-mediated latex allergy

The reported prevalence of latex sensitization of HCWs remains at approximately 10% (range 0–40%) with an estimated prevalence rate of 1% for the wider, minimally exposed community [1[•],2[•]]. A recent community study explored the prevalence of latex allergy in the general UK paediatric population and detected a very low 0.2% prevalence in healthy 7-year-old children [9], but there is a high prevalence of latex sensitization and allergy in particular paediatric populations, such as those with spina bifida and frequent operative procedures. A recent study [10] showing a significant association between IL-13 and IL-18 promoter polymorphisms and latex allergy suggests that the identification of genetic factors that contribute to latex sensitization will further aid the identification of high-risk groups.

The recognition of latex allergy by the community shifted the diagnosis and management from allergy specialists largely to primary care physicians [2[•]], perhaps resulting in false perceptions of decreased clinical importance. More than 20 foods and plants are immunologically cross-reactive with latex, and the simple avoidance of NRL, if feasible, would not remove risks from these foods. In general, the primary sensitization to latex precedes the development of latex–fruit syndrome. Furthermore, a second epidemic of latex allergy is predicted to follow the technological revolution in countries including China, Taiwan, Turkey and Poland with marked increases in the usage of latex products [11[•],12–14]. The recognized high prevalence of latex allergy in certain sections of the population, together with the extremely high potency of latex allergens, emphasizes the desirability of a specific hypoallergenic immunotherapy preparation.

Conventional latex immunotherapy

To date, there have only been five reports of successful specific immunotherapy for latex allergy, all using unfractionated NRL extracts [15–19]. Two were controlled studies using subcutaneous protocols [17,19], one was a study of rush sublingual immunotherapy [16] and the other two were small case studies [15,18]. Although some clinical efficacy was demonstrated, there was a high incidence of local and systemic adverse events. Latex allergen desensitization by percutaneous exposure to latex gloves was recently reported [20], but further verification is indicated and the wider applicability to different patient subgroups and the sustainability of this approach over time would need evaluation.

Together, studies to date confirm the feasibility of specific immunotherapy for latex allergy to induce clinical tolerance, but reinforce the concern that the high anaphylactic potency of unfractionated NRL extracts presents an unacceptable risk of adverse events. There

is clearly a need for safer, hypoallergenic preparations of high efficacy.

Allergenic components of natural rubber latex

The refinement of latex preparations for safer and effective specific immunotherapy requires knowledge of the allergenic components of NRL. The improved specificity of latex allergy diagnosis would also allow improved patient management. Thirteen latex allergens have officially been designated to date (www.allergen.org) (Table 1) [21,22,23[•],24[•],25–28,29^{••}–31^{••},32–35,36^{••},37,38], but several other allergen candidates have been observed by IgE immunoblotting of two-dimensional gel electrophoresis of latex extracts [39[•]]. The reported frequencies of serum IgE binding to latex allergens differ widely, according to subject group, assay method and the use of natural or recombinant allergens [11[•]]. The recent identification of the highly IgE-reactive latex allergen Hev b 13, a 43 000 M_r lipolytic esterase [36^{••}], resolved conflicts regarding the similarly sized Hev b 7, now assigned as a minor latex allergen. As reports on the frequency of serum IgE binding to latex allergens by well-characterized subject groups are published, consensus patterns of reactivity are emerging [11[•],37]. Latex allergens of major clinical importance are Hev b 1 and Hev b 3 (cross-reactive with Hev b 1 [21]) for spina bifida patients, and Hev b 5, Hev b 6.02 and Hev b 13 for latex-allergic HCWs. The clinical importance of Hev b 2 and Hev b 4 remains unclear, requiring further studies. The different reactivity patterns between subject groups presumably reflect the different latex allergen content in latex products to which individuals are exposed, and possibly different exposure routes and cofactors. For example, Hev b 1 and Hev b 3 are rubber particle-bound proteins present in solid rubber products such as catheters [40], whereas Hev b 5, Hev b 6.02 and Hev b 13 are relatively abundant in dipped latex products, particularly latex gloves [41,42,43[•]].

Chemical characterization, cloning, sequencing and modeling of latex allergens allows B-cell [44[•],45] and T-cell [22,26,27,29^{••},46^{••}] epitope mapping, as well as the elucidation of the molecular basis for cross-reactivity between latex allergens and food and plant pollen allergens (Table 1). IgE cross-reactive components identified recently include the carbohydrate moiety of Hev b 13, which cross-reacts with a similar grouping of the potato tuber patatin [36^{••}]. Hev b 2, a 1,3- β -glucanase, cross-reacts with homologous proteins in bell pepper [23[•]], and is implicated in the cross-reactivity of latex with proteins of olive, ash and birch pollens, and of tomato, potato and banana [24[•],25]. Hev b 6.02, the N-terminal hevein-like domain of Hev b 6.01, cross-reacts with similar domains in banana and avocado [31^{••}]. The latter IgE reactivity was shown to be associated with the isolated domains rather than the intact endochitinases, emphasizing the

Table 1. Latex allergens: importance and hypoallergenic preparations.

Latex allergen	Mass (M _r)	IgE reactive glycan moiety	Other name/function	Molecular entities	IgE cross-reactivity		Allergen status ^a			CD4 T-cell epitopes mapped	Hypoallergenic recombinant variants produced
					Cross-reactive allergen sources	HCW/LAA	SB/LAC				
Hev b 1	14 600		Rubber elongation factor	Hev b 3 [21]	Latex		Minor	Major	Yes [22]		
Hev b 2	34 000	Yes	Plant β-1,3-glucanase and homologous proteins [23*,24*,25]	Plant β-1,3-glucanases and homologous proteins [23*,24*,25]	Bell pepper [23*] [24*,25] ^b	Olive	Minor/Major	Minor/Major			
Hev b 3	22 300			Hev b 1 [21]	Latex		Minor	Major	Yes [26]		
Hev b 4	50–57 000		Cyanogenic glucosidase				Minor/Major	Minor/Major			
Hev b 5	16 000						Major	Minor/Major	Yes [27]	Yes [28]	
Hev b 6.01	20 000		Prohevein	Class I chitinases [31**]	Avocado and banana [31**]		Major	Minor/Major	Yes [29**]	Yes [30**]	
Hev b 6.02	4 700		Hevein	Class I chitinases [31**]	Avocado and banana [31**]		Major	Minor/Major	Yes [29**]	Yes [30**,32]	
Hev b 6.03	14 000		C-fragment				Minor	Minor	Yes [29**]		
Hev b 7	46 000		Patatin-like				Minor	Minor			
Hev b 8	14 200, 14 000		Profilin	Profilins [23*,33]	Bell pepper [23*] pollen, timothy pollen, celery tuber [33]	Birch	Minor	Minor			
Hev b 9	47 600		Enolase	Mould enolases [34]	<i>Cladosporium herbarum</i> , <i>Alternaria alternata</i> [34]		Minor	Minor			
Hev b 10	22 900		Manganese superoxide dismutase	Mould manganese superoxide dismutase [35]	<i>Aspergillus fumigatus</i> [35]		Minor	Minor			
Hev b 11	33 000		Class I chitinase				Minor	ND			
Hev b 12	9 300		Lipid transfer protein				Minor	NR ^c			
Hev b 13	42–46 000	Yes	Early nodule-specific protein	Patatin [36**]	Potato [36**]		Major	Minor/Major			Yes ^d [36**]

^aMajor allergen: recognized by IgE from 50% or more of latex-allergic individuals in most recent studies. Minor allergen: recognized by less than 50% of latex-allergic individuals. Minor/Major: no clear consensus from various studies. Reviewed in detail in Wagner *et al.*, 2005 [11*]; Yeang, 2004 [37].

^bCross-reacting allergen within latex extract deduced from molecular entity.

^cNo IgE reactivity (NR) detected to this protein in latex-allergic children [38].

^dRecombinant Hev b 13 is non-IgE reactive when expressed in *Escherichia coli*. HCW, Healthcare workers; LAA, latex allergic adults; LAC, latex-allergic children; ND, no data; SB, spina bifida.

importance of molecular form for IgE binding. Recent, but as yet uncharacterized, cross-reactivities with latex include chickpea [47] and a Japanese cedar pollen chitinase [48]. This accumulating knowledge is guiding the development of latex allergy vaccines of appropriate allergen mix to target major clinical sensitizations as well as allergens responsible for the latex–fruit–pollen syndrome. The elucidation of dominant IgE and T-cell-reactive sites and the identification of critical residues for secondary, tertiary or quaternary structure on which conformational IgE epitopes depend will allow the rational design of hypoallergenic immunotherapy preparations.

Hypoallergenic latex immunotherapy strategies

Adverse events experienced during conventional immunotherapy are largely IgE mediated, suggesting that the abrogation of the IgE reactivity of allergens should generate safer reagents. Critically, to achieve efficacy, dominant CD4 T-cell epitopes should be retained. Identified mechanisms of immunotherapy include T-cell anergy, immune deviation from T helper type 2 to type 1-predominant response and the induction of regulatory T-cell subsets with the increased production of the regulatory cytokines IL-10 and transforming growth factor beta [49]. Different strategies for generating hypoallergenic preparations and for producing these for clinical use have been reviewed [50–53]. The reported approaches used to date for latex allergens are summarized in Fig. 1, and are discussed below together with other potential strategies that could be utilized in the development of latex allergen immunotherapy.

Hypoallergenic recombinant mutants

Recombinant DNA techniques can selectively alter allergen sequences to ablate IgE reactivity. As IgE epitopes are generally conformation dependent, one strategy is to

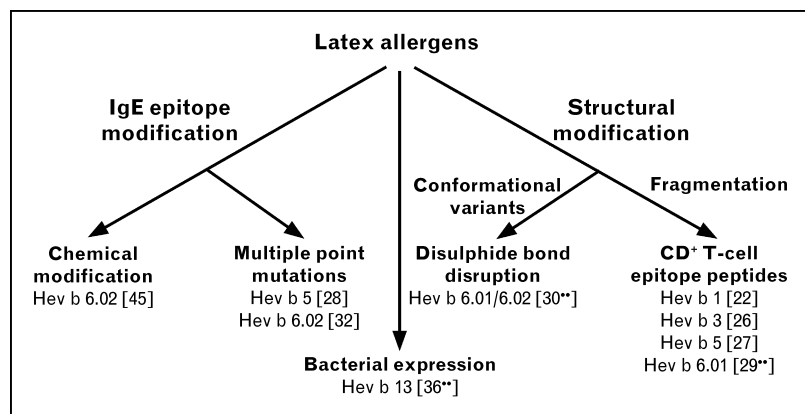
disrupt higher order structure. For allergens whose tertiary structure is stabilized by disulphide bonds, this can be achieved by the mutagenesis of cysteine residues. In a recent report [30^{**}], the alanine substitution of cysteine residues to disrupt the four disulphide bridges of the highly IgE-reactive Hev b 6.02 domain of rHev b 6.01 generated a hypoallergenic variant. On the basis of this result, a synthetic peptide comprising the substituted Hev b 6.02 was prepared and was shown to lack IgE reactivity completely in a basophil activation assay while retaining T-cell stimulatory ability.

Another recombinant approach to disrupt conformational IgE epitopes is to express fragments of allergens in cases in which the IgE epitope requires folding of the entire molecule for correct conformation. This has been applied successfully for Bet v 1 [54]. Alternatively, different allergens can be expressed together as one molecule. For example, a fusion protein of the bee venom allergens Api m 1 and Api m 2 failed to bind IgE as a result of a disrupted tertiary structure [55]. These approaches have not yet been described for latex allergens.

The IgE–allergen interaction site itself can be targeted by the co-mutation of specific residues. IgE-reactive regions are first identified by methods such as the phage display of random peptides [56], or a chimera-based strategy in which segments of an allergen molecule are inserted into a non-IgE-binding, structurally similar carrier molecule [57]. The latter approach was used to identify conformational B-cell epitopes of Hev b 6.02, and then critical amino acid residues of these epitopes were subsequently determined by scanning the Hev b 6.02 sequence using site-specific mutations [32]. A combinatory mutant with six critical residue substitutions showed markedly decreased IgE binding in inhibition enzyme-linked immunosorbent assay (ELISA) and no

Figure 1. Strategies for the generation of hypoallergenic latex preparations.

Summary of schema that have been utilised for reducing IgE reactivity of latex allergens, including targeting of the IgE antibody binding site as well as structural modification.



reaction on skin-prick testing (SPT). Of importance for potential as an immunotherapeutic agent would be whether the mutant stimulated T cells. As the substitutions were in previously identified T-cell-reactive sites of Hev b 6.02 [29^{••}], a key issue is whether these residues correspond to MHC class II anchor or T-cell receptor contact residues [58].

For allergens with a predominantly random secondary structure, IgE binding sites and critical residues for IgE binding can be mapped using short overlapping peptides followed by point mutations. The proline-rich Hev b 5 is one such molecule in which this approach has proved successful [28]. Alanine substitution of 14 residues within IgE epitopes decreased IgE binding 4500-fold, but T-cell reactivity of the hypoallergenic mutant was not tested. Again, many of the substituted residues are within T-cell epitopes of Hev b 5 [27], and a balance may be required between the substitution of residues to abolish IgE binding and the need to retain functional T-cell epitopes.

For the glycosylated allergen Hev b 13, bacterial expression alone may be sufficient, because predominant IgE reactivity resides with the carbohydrate moiety [36^{••}]. Natural Hev b 13 is reactive by SPT [37], but IgE reactivity is lost after periodate treatment [36^{••}]. In the case of Hev b 13, the carbohydrate component appears to be clinically significant, unlike the cross-reactive carbohydrate components of many other plant allergens. Hev b 2 is also a glycoprotein, with three reported isoforms of differing IgE reactivities and evidence of non-carbohydrate-dependent as well as carbohydrate-dependent IgE binding sites [59]. A recombinant Hev b 2 was produced in *Escherichia coli* with very low reactivity by SPT [60], but it is not clear which isoform was cloned. More investigation of the three Hev b 2 isoforms is required, including their cloning and testing for T-cell reactivity, before a clinically useful hypoallergenic form of Hev b 2 can be produced.

Whereas the above approaches aim to disrupt direct IgE binding by an allergen, a different strategy is to inhibit cell surface IgE cross-linking by an allergen. A recombinant trimer of Bet v 1 retained IgE binding but showed a marked decrease in the activation of allergic effector cells compared with the monomeric protein [61]. To date, this strategy has not been reported for a latex allergen.

Although many allergens have been altered in these ways, to date only one clinical study using a recombinant allergen has been published. In an important double-blind placebo-controlled clinical trial, recombinant fragments and trimers of Bet v 1 induced Bet v 1-specific IgG antibodies correlating with an improvement of

symptoms in birch pollen-allergic individuals [62]. This study supports the feasibility of successful immunotherapy with recombinant allergens. It is important to note that recombinant proteins for clinical use must meet strict manufacturing criteria [53].

One issue that should be considered for hypoallergenic preparations is whether the alteration of IgE epitopes prevents the induction of IgG₄ blocking antibodies and thus reduces specific immunotherapy efficacy. IgG₄ antibodies are believed to promote the clinical efficacy of specific immunotherapy by competing with IgE for allergen binding, skewing T-cell responses to T helper 1 type (via altered antibody-facilitated antigen presentation) and reducing inflammatory cell activation [49]. As proof of concept, Bet v 1 fragments lacking the ability to bind IgE because of conformation changes, did produce blocking IgG antibodies when used to immunize mice [63]. In addition, serum from patients in the clinical trial of rBet v 1 fragment immunotherapy inhibited allergen-induced basophil histamine release [62].

Chemically modified molecules

The chemical modification of amino acids, the addition of side chains, and the conjugation and polymerization of allergens have all been shown to mask IgE epitopes. For example, the conjugation of immunostimulatory DNA to the ragweed allergen Amb a 1 resulted in decreased allergenicity [64], and glutaraldehyde-induced polymerization of *Parietaria judaica* pollen extract showed marked reduction in IgE binding but the retention of human peripheral blood mononuclear cell proliferative capacity and IgG antibody induction in rabbits [65]. During a study of a potential conformational epitope of Hev b 6.02 [45], the chemical modification of two tryptophan residues resulted in a modified Hev b 6.02 with no SPT reactivity, but there was no report of testing for T-cell stimulatory activity.

T-cell epitope-based peptides

Pathogenic T cells may also be targeted without associated IgE-mediated side effects by using short synthetic peptides representing dominant T-cell epitopes. This strategy has been trialled for cat, bee venom and ragweed allergy with encouraging results [66]. There was evidence for the induction of T-cell tolerance and IgG₄ blocking antibodies [49,66]. To date, T-cell epitopes have been identified for Hev b 1 [22], Hev b 3 [26], Hev b 5 [27] and Hev b 6.01 [29^{••}]. As described previously, for molecules without a 'rigid' secondary or tertiary structure, short linear peptides containing T-cell epitopes may be IgE reactive [28,67], but these would be too short to cross-link mast cell or basophil surface IgE.

In general, allergens contain multiple T-cell reactive regions, and although sites of dominance are identifiable,

peptide 'cocktails' may be required. Encouragingly, population studies of T-cell epitope mapping for latex and other allergens support the promiscuity of peptide binding across a diverse array of MHC class II alleles (e.g. Eusebius *et al.* [68]). After screening using nested peptide sets, further testing with truncated peptide series is required to clearly define core T-cell epitopes (e.g. Burton *et al.* [58]). Candidate peptides should then be confirmed as lacking IgE binding by inhibition ELISA, and, importantly, by functional basophil activation tests [30**] before the consideration of clinical trials. Of concern after peptide immunotherapy for other allergens were reports of T-cell cytokine-mediated late reactions, but cat peptide studies suggested that these are avoidable by careful up dosing [66].

Conclusion

Latex allergy remains an important clinical condition. Latex allergens are potent and effective hypoallergenic-specific immunotherapy is clearly needed. Advances in the identification of clinically relevant latex allergens for particular high-risk groups, together with the characterization of dominant T and B-cell-reactive sites of these allergens provides a rational basis for the development of suitable preparations for immunotherapy. The first clinical trial of a recombinant hypoallergenic preparation for birch pollen-specific immunotherapy has yielded encouraging results, and peptide immunotherapy has been successful for several allergens.

Considerable progress has been made in developing hypoallergenic preparations of the major latex allergens Hev b 5 and Hev b 6.01. T-cell-reactive peptides of Hev b 1 and Hev b 3 have also been identified. Bacterially expressed recombinant Hev b 13 is likely to be a suitable hypoallergenic form for targeting reactivity to this more recently defined major allergen. Of particular importance for developing specific immunotherapy for the latex-allergic population is the targeting of allergenic components identified as cross-reacting with homologous proteins in fruits, vegetables and pollens to prevent adverse reactions on exposure to these substances.

The use of purified recombinant molecules or synthetic peptides from defined latex allergens offers advantages of the ease of standardization and preparation of clinical grade reagents. The risk of sensitization to minor allergens present in current unfractionated NRL extracts is also avoided. Given the current awareness of the importance of inducing regulatory T-cell function during specific immunotherapy [49], the final challenge will be to devise optimal strategies for administering hypoallergenic preparations to expand regulatory T cells to downregulate adverse T helper types 1 and 2 responses. The testing of new approaches using murine

models of latex allergy [69] should precede clinical evaluation.

Acknowledgements

The authors are members of the Cooperative Research Centre for Asthma and Airways, Australia.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 579–580).

- 1 Reunala T, Alenius H, Turjanmaa K, *et al.* Latex allergy and skin. *Curr Opin Allergy Clin Immunol* 2004; 4:397–401.
A review of the prevalence and outcome of latex allergy in HCWs including trials of specific immunotherapy using unfractionated NRL.
- 2 Taylor JS, Erkek E. Latex allergy: diagnosis and management. *Dermatol Ther* 2004; 17:289–301.
A comprehensive overview of latex allergy including clinical manifestations, epidemiology and risk factors, diagnosis and preventive measures.
- 3 Sutherland MF, Suphioglu C, Rolland JM, *et al.* Latex allergy: towards immunotherapy for health care workers. *Clin Exp Allergy* 2002; 32:667–673.
- 4 Taylor JS, Wattanakrai P, Charous BL, *et al.* Year book focus: latex allergy. In: *Theirs BH, Lang PG, editors. 2000 Year book of dermatology and dermatologic surgery.* St Louis: Mosby Inc; 1999. pp. 325–368.
- 5 Condemi JJ. Allergic reactions to natural rubber latex at home, to rubber products, and to cross-reacting foods. *J Allergy Clin Immunol* 2002; 110 (2 Suppl.):S107–S110.
- 6 Chiu AM, Kelly KJ. Anaphylaxis: drug allergy, insect stings, and latex. *Immunol Allergy Clin North Am* 2005; 25:389–405.
A clinical perspective on the diagnosis and therapy of anaphylaxis, featuring reactions associated with latex allergy.
- 7 Latza U, Haamann F, Baur X. Effectiveness of a nationwide interdisciplinary preventive programme for latex allergy. *Int Arch Occup Environ Health* 2005; 78:394–402.
- 8 Allmers H, Schmengler J, John SM. Decreasing incidence of occupational contact urticaria caused by natural rubber latex allergy in German health care workers. *J Allergy Clin Immunol* 2004; 114:347–351.
Documentation of the decreased incidence of occupational contact urticaria caused by NRL in HCWs correlating with decreased purchase of powdered NRL gloves in German acute-care hospitals. This study demonstrates the importance of donning powder as a vehicle for eliciting allergic reactions to NRL.
- 9 Roberts G, Lack G, Northstone K, *et al.* Prevalence of latex allergy in the community at age 7 years. *Clin Exp Allergy* 2005; 35:299–300.
- 10 Brown RH, Hamilton RG, Mintz M, *et al.* Genetic predisposition to latex allergy: role of interleukin 13 and interleukin 18. *Anesthesiology* 2005; 102:496–502.
- 11 Wagner S, Breiteneder H. *Hevea brasiliensis* latex allergens: current panel and clinical relevance. *Int Arch Allergy Immunol* 2005; 136:90–97.
A useful summary of the current knowledge on the frequency of reactivity to the 13 officially designated allergens of *Hevea brasiliensis* NRL in different subject groups.
- 12 Chen YH, Lan JL. Latex allergy and latex–fruit syndrome among medical workers in Taiwan. *J Formos Med Assoc* 2002; 101:622–626.
- 13 Ozkan O, Gokdogan F. The prevalence of latex allergy among health care workers in Bolu (Turkey). *Dermatol Nurs* 2003; 15:543–547; 554.
- 14 Dudek W, Walusiak J, Wittczak T, *et al.* Natural rubber latex allergy: antigen specific IgE in Polish blood donors, prevalence and risk factors—preliminary data. *Int J Occup Med Environ Health* 2005; 18:35–42.
- 15 Savi E, Maffini I, Burastero SE. A latex-containing hepatitis-B vaccine administered in a severely latex allergic paediatric patient after specific sublingual immunotherapy: a case report. *Allergy* 2004; 59:1014–1015.
- 16 Cistero Bahima A, Sastre J, Enrique E, *et al.* Tolerance and effects on skin reactivity to latex of sublingual rush immunotherapy with a latex extract. *J Invest Allergol Clin Immunol* 2004; 14:17–25.
- 17 Sastre J, Fernandez-Nieto M, Rico P, *et al.* Specific immunotherapy with a standardized latex extract in allergic workers: a double-blind, placebo-controlled study. *J Allergy Clin Immunol* 2003; 111:985–994.

- 18 Pereira C, Pedro E, Tavares B, *et al.* Specific immunotherapy for severe latex allergy. *Allerg Immunol (Paris)* 2003; 35:217–225.
 - 19 Leynadier F, Herman D, Vervloet D, *et al.* Specific immunotherapy with a standardized latex extract versus placebo in allergic healthcare workers. *J Allergy Clin Immunol* 2000; 106:585–590.
 - 20 Voza I, Ranghi G, Quaranta A. Allergy and desensitization to latex. *Clinical study on 50 dentistry subjects.* *Minerva Stomatol* 2005; 54:237–245.
 - 21 Banerjee B, Kanitpong K, Fink JN, *et al.* Unique and shared IgE epitopes of Hev b 1 and Hev b 3 in latex allergy. *Mol Immunol* 2000; 37: 789–798.
 - 22 Raulf-Heimsoth M, Chen Z, Rihs HP, *et al.* Analysis of T-cell reactive regions and HLA-DR4 binding motifs on the latex allergen Hev b 1 (rubber elongation factor). *Clin Exp Allergy* 1998; 28:339–348.
 - 23 Wagner S, Radauer C, Hafner C, *et al.* Characterization of cross-reactive bell pepper allergens involved in the latex–fruit syndrome. *Clin Exp Allergy* 2004; 34:1739–1746.
- Molecular characterization of an IgE-reactive bell pepper protein which cross-reacts with Hev b 2, expanding the number of latex allergens known to be involved in the latex–fruit syndrome.
- 24 Palomares O, Villalba M, Quiralte J, *et al.* 1,3- β -glucanases as candidates in latex–pollen–vegetable food cross-reactivity. *Clin Exp Allergy* 2005; 35:345–351.
- This study showed that the olive pollen major allergen Ole e 9, a 1,3- β -glucanase, cross-reacts with homologous proteins in birch pollen, tomato, potato, bell-pepper, banana and latex, again implicating Hev b 2 as an allergen responsible for the latex–pollen–fruit/vegetable syndrome.
- 25 Barral P, Batanero E, Palomares O, *et al.* A major allergen from pollen defines a novel family of plant proteins and shows intra- and interspecies cross-reactivity. *J Immunol* 2004; 172:3644–3651.
 - 26 Bohle B, Wagner B, Vollmann U, *et al.* Characterization of T cell responses to Hev b 3, an allergen associated with latex allergy in spina bifida patients. *J Immunol* 2000; 164:4393–4398.
 - 27 de Silva HD, Sutherland MF, Suphioglu C, *et al.* Human T-cell epitopes of the latex allergen Hev b 5 in health care workers. *J Allergy Clin Immunol* 2000; 105:1017–1024.
 - 28 Beezhold DH, Hickey VL, Sussman GL. Mutational analysis of the IgE epitopes in the latex allergen Hev b 5. *J Allergy Clin Immunol* 2001; 107:1069–1076.
 - 29 de Silva HD, Gardner LM, Drew AC, *et al.* The hevein domain of the major latex-glove allergen Hev b 6.01 contains dominant T cell reactive sites. *Clin Exp Allergy* 2004; 34:611–618.
- Demonstration of dominant T-cell-reactive sites of Hev b 6.01 in the Hev b 6.02 domain using overlapping 20-mer peptides. Dominant T-cell epitope peptides did not bind serum IgE from latex-allergic individuals consistent with candidate hypoallergenic latex preparation for specific immunotherapy.
- 30 Drew AC, Eusebius NP, Kenins L, *et al.* Hypoallergenic variants of the major latex allergen Hev b 6.01 retaining human T lymphocyte reactivity. *J Immunol* 2004; 173:5872–5879.
- Using a strategy of disruption of disulphide bonds, a hypoallergenic recombinant Hev b 6.01 mutant was produced. A synthetic peptide based on the cysteine-substituted Hev b 6.02 domain of this mutant lacked IgE binding as tested by inhibition ELISA and basophil activation assay but retained T-cell reactivity, consistent with a candidate hypoallergenic preparation.
- 31 Karisola P, Kotovuori A, Poikonen S, *et al.* Isolated hevein-like domains, but not 31-kd endochitinases, are responsible for IgE-mediated in vitro and in vivo reactions in latex-fruit syndrome. *J Allergy Clin Immunol* 2005; 115: 598–605.
- An evaluation of endochitinases from latex, avocado, banana and wheat germ and their corresponding hevein-like domains in causing cross-reactions between latex and fruit allergens. SPT of latex allergic individuals and inhibition ELISA demonstrated the importance of using isolated hevein-like domains rather than the endochitinases in the diagnosis of latex–fruit syndrome.
- 32 Karisola P, Mikkola J, Kalkkinen N, *et al.* Construction of hevein (Hev b 6.02) with reduced allergenicity for immunotherapy of latex allergy by computation of six amino acid residues on the conformational IgE epitopes. *J Immunol* 2004; 172:2621–2628.
 - 33 Ganglberger E, Radauer C, Wagner S, *et al.* Hev b 8, the *Hevea brasiliensis* latex profilin, is a cross-reactive allergen of latex, plant foods and pollen. *Int Arch Allergy Immunol* 2001; 125:216–227.
 - 34 Wagner S, Breiteneder H, Simon-Nobbe B, *et al.* Hev b 9, an enolase and a new cross-reactive allergen from hevea latex and molds. Purification, characterization, cloning and expression. *Eur J Biochem* 2000; 267: 7006–7014.
 - 35 Wagner S, Sowka S, Mayer C, *et al.* Identification of a *Hevea brasiliensis* latex manganese superoxide dismutase (Hev b 10) as a cross-reactive allergen. *Int Arch Allergy Immunol* 2001; 125:120–127.
 - 36 Arif SA, Hamilton RG, Yusof F, *et al.* Isolation and characterization of the early nodule-specific protein homologue (Hev b 13), an allergenic lipolytic esterase from *Hevea brasiliensis* latex. *J Biol Chem* 2004; 279:23933–23941.
- Report on the isolation and purification of a highly allergenic 42 980 Mr latex glycoprotein, Hev b 13, resolving previous controversy on IgE reactivity of the similarly sized Hev b 7. IgE reactivity of Hev b 13 was shown to reside with the carbohydrate moiety, which cross-reacted with a similar component of potato tuber patatin.
- 37 Yeang HY. Natural rubber latex allergens: new developments. *Curr Opin Allergy Clin Immunol* 2004; 4:99–104.
 - 38 Pamies R, Oliver F, Raulf-Heimsoth M, *et al.* No rHev b 12-specific IgE-response in children sensitized to natural rubber latex. *Allergy* 2005; 60:709–710.
 - 39 Yagami T, Haishima Y, Tsuchiya T, *et al.* Proteomic analysis of putative latex allergens. *Int Arch Allergy Immunol* 2004; 135:3–11.
- A method for the rapid and exhaustive analysis of putative allergens as applied to latex proteins. Non-ammoniated latex proteins were resolved by two-dimensional gel electrophoresis and 50 IgE-reactive proteins (five novel) were analysed by mass spectrometry and database searches.
- 40 Yeang HY, Cheong KF, Sunderasan E, *et al.* The 14.6 kd rubber elongation factor (Hev b 1) and 24 kd (Hev b 3) rubber particle proteins are recognized by IgE from patients with spina bifida and latex allergy. *J Allergy Clin Immunol* 1996; 98:628–639.
 - 41 Sutherland MF, Drew A, Rolland JM, *et al.* Specific monoclonal antibodies and human immunoglobulin E show that Hev b 5 is an abundant allergen in high protein powdered latex gloves. *Clin Exp Allergy* 2002; 32:583–589.
 - 42 Mitakakis TZ, Tovey ER, Yates DH, *et al.* Particulate masks and non-powdered gloves reduce latex allergen inhaled by healthcare workers. *Clin Exp Allergy* 2002; 32:1166–1169.
 - 43 Yeang HY, Arif SA, Raulf-Heimsoth M, *et al.* Hev b 5 and Hev b 13 as allergen markers to estimate the allergenic potency of latex gloves. *J Allergy Clin Immunol* 2004; 114:593–598.
- The use of a two-site immunoenzymatic assay to analyse the content of six latex allergens in latex gloves. Hev b 5 and Hev b 6.01/6.02 were detected in all gloves tested and the combination of Hev b 5 and Hev b 13 was shown to correlate best with the IgE binding capacity of latex gloves.
- 44 Beezhold DH, Hickey VL, Sutherland MF, *et al.* The latex allergen Hev b 5 is an antigen with repetitive murine B-cell epitopes. *Int Arch Allergy Immunol* 2004; 134:334–340.
- This study showed that Hev b 5 contains a repetitive linear B-cell epitope, potentially contributing to the high potency of this allergen. Critical residues for IgE binding were identified by site-directed mutagenesis.
- 45 Reyes-Lopez CA, Hernandez-Santoyo A, Pedraza-Escalona M, *et al.* Insights into a conformational epitope of Hev b 6.02 (hevein) *Biochem Biophys Res Commun* 2004; 314:123–130.
 - 46 Raulf-Heimsoth M, Rozynek P, Bruning T, *et al.* Characterization of B- and T-cell responses and HLA-DR4 binding motifs of the latex allergen Hev b 6.01 (prohevein) and its post-transcriptionally formed proteins Hev b 6.02 and Hev b 6.03. *Allergy* 2004; 59:724–733.
- A detailed analysis of HCW T-cell responses and IgE binding capacity of Hev b 6.01, Hev b 6.02 and Hev b 6.03 using recombinant maltose-binding fusion proteins and synthetic modified hevein peptides. Dominant IgE binding was observed for the Hev b 6.02 domain with discontinuous B-cell epitopes. In this study, Hev b 6.03 was shown to be more T-cell reactive than Hev b 6.02, in contrast to the findings of de Silva *et al.* [29**].
- 47 Branco Ferreira M, Pedro E, Meneses Santos J, *et al.* Latex and chickpea (*Cicer arietinum*) allergy: first description of a new association. *Allerg Immunol (Paris)* 2004; 36:366–371.
 - 48 Fujimura T, Shigeta S, Suwa T, *et al.* Molecular cloning of a class IV chitinase allergen from Japanese cedar (*Cryptomeria japonica*) pollen and competitive inhibition of its immunoglobulin E-binding capacity by latex C-serum. *Clin Exp Allergy* 2005; 35:234–243.
 - 49 Till SJ, Francis JN, Nouri-Aria K, *et al.* Mechanisms of immunotherapy. *J Allergy Clin Immunol* 2004; 113:1025–1034.
 - 50 Bhalla PL, Singh MB. Engineered allergens for immunotherapy. *Curr Opin Allergy Clin Immunol* 2004; 4:569–573.
 - 51 Niederberger V, Valenta R. Recombinant allergens for immunotherapy. Where do we stand? *Curr Opin Allergy Clin Immunol* 2004; 4:549–554.
 - 52 Vrtala S, Focke-Tejkl M, Swoboda I, *et al.* Strategies for converting allergens into hypoallergenic vaccine candidates. *Methods* 2004; 32: 313–320.
 - 53 Cromwell O, Suck R, Kahlert H, *et al.* Transition of recombinant allergens from bench to clinical application. *Methods* 2004; 32:300–312.
 - 54 Vrtala S, Hirtenlehner K, Vangelista L, *et al.* Division of the major birch pollen allergen, Bet v 1, into two non-anaphylactic fragments. *Int Arch Allergy Immunol* 1997; 113:246–248.

- 55 Kussebi F, Karamloo F, Rhyner C, *et al.* A major allergen gene-fusion protein for potential usage in allergen-specific immunotherapy. *J Allergy Clin Immunol* 2005; 115:323–329.
- 56 Hantusch B, Krieger S, Untersmayr E, *et al.* Mapping of conformational IgE epitopes on Phl p 5a by using mimotopes from a phage display library. *J Allergy Clin Immunol* 2004; 114:1294–1300.
- 57 Karisola P, Alenius H, Mikkola J, *et al.* The major conformational IgE-binding epitopes of hevein (Hev b 6.02) are identified by a novel chimera-based allergen epitope mapping strategy. *J Biol Chem* 2002; 277:22656–22661.
- 58 Burton MD, Blaher B, Suphioglu C, *et al.* T-cell receptor contact and MHC binding residues of a major rye grass pollen allergen T-cell epitope. *J Allergy Clin Immunol* 1999; 103:255–261.
- 59 Yagami T, Osuna H, Kouno M, *et al.* Significance of carbohydrate epitopes in a latex allergen with beta-1,3-glucanase activity. *Int Arch Allergy Immunol* 2002; 129:27–37.
- 60 Yip L, Hickey V, Wagner B, *et al.* Skin prick test reactivity to recombinant latex allergens. *Int Arch Allergy Immunol* 2000; 121:292–299.
- 61 Vrtala S, Hirtenlehner K, Susani M, *et al.* Genetic engineering of a hypoallergenic trimer of the major birch pollen allergen Bet v 1. *FASEB J* 2001; 15:2045–2047.
- 62 Niederberger V, Horak F, Vrtala S, *et al.* Vaccination with genetically engineered allergens prevents progression of allergic disease. *Proc Natl Acad Sci U S A* 2004; 101 (Suppl. 2):14677–14682.
- 63 Mahler V, Vrtala S, Kuss O, *et al.* Vaccines for birch pollen allergy based on genetically engineered hypoallergenic derivatives of the major birch pollen allergen, Bet v 1. *Clin Exp Allergy* 2004; 34:115–122.
- 64 Tighe H, Takabayashi K, Schwartz D, *et al.* Conjugation of immunostimulatory DNA to the short ragweed allergen Amb a 1 enhances its immunogenicity and reduces its allergenicity. *J Allergy Clin Immunol* 2000; 106:124–134.
- 65 Ibarrola I, Sanz ML, Gamboa PM, *et al.* Biological characterization of glutaraldehyde-modified *Parietaria judaica* pollen extracts. *Clin Exp Allergy* 2004; 34:303–309.
- 66 Larche M, Wraith DC. Peptide-based therapeutic vaccines for allergic and autoimmune diseases. *Nat Med* 2005; 11 (4 Suppl.):S69–S76.
- 67 Slater JE, Paupore EJ, O'Hehir RE. Murine B-cell and T-cell epitopes of the allergen Hev b 5 from natural rubber latex. *Mol Immunol* 1999; 36:135–143.
- 68 Eusebius NP, Papalia L, Suphioglu C, *et al.* Oligoclonal analysis of the atopic T cell response to the group 1 allergen of *Cynodon dactylon* (Bermuda grass) pollen: pre- and post-allergen-specific immunotherapy. *Int Arch Allergy Immunol* 2002; 127:234–244.
- 69 Hardy CL, Kenins L, Drew AC, *et al.* Characterization of a mouse model of allergy to a major occupational latex glove allergen Hev b 5. *Am J Respir Crit Care Med* 2003; 167:1393–1399.