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Recombinant latex allergens Les allergènes recombinants du latex

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Abstract

In the last few years many efforts have been made to reduce the incidence of latex allergy. The identification of the major latex allergens and their recombinant production are useful steps towards the understanding of latex allergy and its management. A clear advantage of the recombinant proteins in general is the possibility to produce large-scale quantities at highly reproducible quality. Recombinant allergens also facilitate the study of the molecular bases of the immune reactivity of the proteins. Nevertheless, they have to be validated against native proteins for equivalence in allergenicity before they can be more widely adapted for clinical use. Currently 13 latex allergens have been included in the latest nomenclature list of the International Nomenclature Committee of Allergens and assigned the official names Hev b 1–13. With the use of single native and/or recombinant allergens the determination of specific sensitization profiles for different patient groups dependent on their various routes of exposure is possible. The specific IgE-diagnostic was further improved significantly by addition of recombinant Hev b 5 to natural latex extract, which was underrepresented in the initial test extract.

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Résumé

Les travaux de ces dernières années se sont attachés à diminuer l'incidence de l'allergie au latex. L'identification des allergènes majeurs du latex et la production d'allergènes recombinants constituent des étapes utiles à la compréhension et à la prise en charge de l'allergie au latex. Un avantage évident des protéines recombinantes est de permettre une grande production de molécules parfaitement reproductibles. Les allergènes recombinants permettent d'étudier les bases moléculaires des réactions immunitaires envers ces allergènes. Il reste cependant indispensable de valider au plan clinique ces allergènes recombinants en les comparant aux allergènes natifs. Actuellement 13 allergènes du latex ont été inscrits au registre du Comité international de nomenclature des allergènes (Hev b 1–13). Le recours aux allergènes isolés (natifs et/ou recombinants) permet de préciser le profil spécifique de sensibilisation en fonction des types d'exposition au latex. La valeur diagnostique des IgE spécifiques circulantes a été significativement accrue par l'addition de l'allergène recombinant Hev b 5 à l'extrait naturel de latex, palliant ainsi sa sous-représentation antérieure.

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Keywords: Latex allergy; Recombinant latex allergens; Sensitization profile; In vitro diagnostics; Cross-reactive carbohydrate determinants

Mots clés : Allergie au latex ; Allergènes recombinants au latex ; Profil de sensibilisation ; Réaction aux carbohydrate déterminants

1. Introduction

Natural rubber latex (NRL) is the cytoplasm obtained from the laticifers of the rubber tree *Hevea brasiliensis*. Dried NRL is a raw material of a wide range of rubber products such as vehicle tyres and shoes. Alternatively NRL can be stabilized and concentrated for processing into “dipped” latex products

such as examination, surgical and household gloves, catheters, condoms, baby pacifiers, balloons etc. Besides rubber elastomer, the NRL cytoplasm contains a multitude of metabolites and proteins. A small proportion of these proteins is responsible for the NRL-allergy, which is an IgE-mediated type I-hypersensitivity. Allergy to NRL is prevalent among health care workers and other personnel who routinely wear protective gloves in the cause of their work. Today, 13 NRL-proteins (Hev b 1–13) are recognized by the International Union of Immunological Societies (IUIS) as causative agents of NRL-allergy [1]. Knowledge of the causative allergen is

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essential to develop reliable diagnostic for NRL-allergy and to develop methods for determination of allergenicity of NRL-products [2,3].

2. Diagnostic of latex allergy

The diagnostic of latex allergy should be based on both, a positive clinical history and a positive in vivo-test result. The determination and quantification of NRL-specific IgE-antibodies determined by use of in vitro-tests are not always associated with clinical NRL-protein sensitivity. Based on the specific symptoms (urticaria, acute erythema, respiratory symptoms from allergic rhinitis to asthma) of the patients challenge test may be also performed. As prerequisites for correct in vivo- and in vitro-testing, standardized skin test extracts and well-characterized allergen mixtures for IgE-determination are necessary tools. Studies have been demonstrated cases of disagreement between results of latex-specific IgE analysis and skin-prick test with latex extract. This phenomenon may be explained by the occurrence of nonspecific skin reactivity to allergen extract, failure of in vitro-test to detect certain latex-specific IgE-antibodies or a combination of both. False negative IgE-test results may be due to the absence or shortness of some allergen components in the allergen extract used for antibody capture. Indeed, 8 out of 16 subjects with clear latex allergy but negative IgE-test results show IgE-reactivity to Hev b 5. This finding suggested that the latex allergen Hev b 5 might play a special role in the diagnostic test showing false negative IgE-results. Drawing from these results latex ImmunoCAP with rHev b 5 added to the latex extract was developed and used to retest a panel of serum samples of confirmed latex-allergic individuals. With the Hev b 5-complemented test a significantly higher IgE-antibody level was obtained for a proportion of this sera and a small number of sera converted from negative to positive test results. These results indicate a new approach of the improvement of an allergen extract used for in vitro-test if a relevant allergen component is found to be present in suboptimal amount in a natural allergen extract that component can be added as a stable recombinant protein to the extract preparation during production. Therefore the new latex allergen ImmunoCAP spiked with rHev b 5 is an improvement of in vitro-test for an IgE-related sensitization [4,5].

3. Recombinant single latex allergens

Substantial progress has been made over the years in the molecular- and immuno-characterization of NRL-allergens. A clear advantage of the recombinant proteins in general is a possibility to produce large-scale quantities of highly produced reproducible qualities. Since the appearance of the first recombinant latex allergen Hev b 1, more than a dozen latex allergens have been produced in recombinant form. Most of the recombinant latex allergens are produced in *E. coli* due to the fact that they contain no or minor posttranslational modification. Recombinant latex allergens also facilitate the study of the molecular bases of the immune reactivity of the

proteins but they have to be validated against native proteins for equivalence of allergen reactivity before they can be more widely adapted for clinical use. With respect to validation, our data demonstrate a very good correlation between recombinant and native Hev b 6.02 tested with the ImmunoCAP-assay [6]. Among latex proteins, the two major allergens Hev b 2 and 13 are the known glycosylated ones, and especially for Hev b 2 we demonstrated previously that the recombinant form produced in *E. coli* was not able to bind specific IgE. Nevertheless, the IgE-reactivity of the glycosylated latex allergens seems not to be restricted to their glycan chains. Our data demonstrated that only a minority of sera of patients with clinical relevant latex allergy recognized cross-reactive carbohydrate determinates (CCDs). In the case of these glycosylated allergens a combined binding is conceivable, comprising of a peptide and a carbohydrate epitope on the same allergen molecule. In contrast to our patients with clear latex-induced latex-sensitization, in patients who are not originally sensitized to the allergen test, the carbohydrate epitopes recognized by the IgE of the patient are highly cross-reactive, and the clinical relevance of the positive test results have to be considered [7].

4. Properties of individual latex allergens

The rubber particle proteins comprise two allergens, Hev b 1 and Hev b 3, both major allergens strongly associated with latex allergy in spina bifida patients. Hev b 1, the first latex allergen, described by Czuppon et al. [8], is the so-called rubber elongation factor. Hev b 1 is a latex-specific allergen without relevant homology to other plant proteins. Depending on the study 54–100% of spina bifida (SB) patients with IgE-allergy reacted to Hev b 1, whereas a frequency of only 13–32% was observed in healthcare workers [9].

In addition, B- as well as T-cell epitopes of Hev b 1 had been identified. Hev b 3 (23 kD) forms an integrated part of the small rubber particles and is free of posttranslational modification like glycosylation. In vitro-measurements with recombinant Hev b 3 coupled to ImmunoCAPs revealed a comparable frequency of 12.5 in 40 latex-allergic healthcare workers tested. A further important latex allergen is Hev b 5, an acidic and heat stable 16–24 kD-protein. Hev b 5 may be the missing allergen to fill the diagnostic gap for some allergic patients with clear clinical latex allergy, but with native serology reactivity. One of the most important NRL-allergens of healthcare workers (HCW) appears to be prohevein (20 kD preprotein designated as Hev b 6.01). Posttranslational cleavage generates two further proteins, the 4.7 kD hevein (Hev b 6.02) and the 14 kD C-terminal domain Hev b 6.03. All three allergens exist in the plant, although the ratio between Hev b 6.01 and Hev b 6.03 is about 32:1. Hevein comprises the most important part of the IgE-binding epitope in the prohevein molecule. In addition, hevein shows homology to several chitin-binding lectin domains and may be responsible for certain cross-reactivities with several other plants and foods.

Based on the analysis of the sensitization profiles, Hev b 2, 5, 6.01, and 13 are major allergens for HCW and SB and

together with Hev b 1, the major allergen for SB, this allergen panel should be included in sufficient amounts in a standardized latex diagnostic extract. Further allergens like Hev b 7, 8, 9, 10, 11, and 12 have to be considered for testing cross-reactivity in individual cases. CCDs are only of minor relevance in patients with clinical relevant latex allergy. Component-resolved diagnostic for latex allergy sets the stage for specific immunotherapy strategies.

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