

Immunotherapy of the futur

Variants of allergen Phl p 5b and reduction of anaphylactogenic potential

Mutants de l'allergène Phl p 5b et réduction du pouvoir anaphylactogène

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Abstract

One new approach to improved immunotherapy for type 1 allergy might be the use of modified allergens with reduced IgE reactivity but retained T cell reactivity. By site- directed mutagenesis outside the dominant T cell epitopes, we generated deletion mutants of the grass pollen allergen Phl p 5b. Some of these variants revealed significantly reduced IgE reactivity and histamine releasing capacity compared to the wild-type allergen. Furthermore, in vivo skin prick tests showed that the variants had up to 100 fold lower potency to induce cutaneous reactions than the wild-type allergen. On the other hand, T cell clones and T cell lines from grass pollen-allergic patients showed comparable proliferation after stimulation with these variants and wild-type allergen. Thus, variants of the allergen Phl p 5b with reduced anaphylactogenic potential but retained T cell reactivity could be valuable tools for improved allergen-specific immunotherapy.

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Résumé

Une nouvelle approche afin d'optimiser l'immunothérapie des allergies de type 1 peut consister à utiliser des allergènes modifiées par réduction de la réactivité des IgE tout en conservant la réactivité des cellules T. Par mutagenèse directe en dehors des épitopes dominants des cellules T, nous avons obtenu des mutants de l'allergène Phl p 5b du pollen de graminées. Quelques variants révèlent une activité IgE et une capacité d'histaminolibération réduites par rapport à l'allergène sauvage Phl p 5b. De plus, in vivo, les prick-tests cutanés montrent que les mutants ont une capacité 100 fois plus basse pour provoquer des réactions cutanées que l'allergène sauvage Phl p 5b. Par ailleurs, les clones et les lignées T issues de patients allergiques subissent une prolifération comparable après stimulation par les allergènes Phl p 5b mutants et sauvages. Ainsi, les mutants de l'allergène Phl p 5b de potentialité anaphylactogène diminuée mais de pouvoir immunogène conservé pourraient représenter des outils utiles pour améliorer l'immunothérapie spécifique.

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Since 1911 conventional allergen-specific immunotherapy of type I allergy is based on subcutaneous application of increasing amounts of crude allergen extracts [1]. Although the mechanism by which immunotherapy works is not yet known in detail, large amounts of relevant allergens are believed to lead to immunomodulation on T cell level and to the induction of blocking IgG antibodies [2]. However, administration of high amounts of complete crude allergen extracts bears the risk of unwanted side effects such as additional sensitization to further components of the crude extracts and anaphylactogenic reactions to the complete IgE-reactive allergens.

One possibility to make immunotherapy safer and more effective might be to apply well-defined modified recombinant allergens with reduced IgE reactivity but retained T cell reactivity. Such variants were produced for example by Takai et al. of the house dust mite allergen Der f 2 [3]. To test the suitability of this concept for immunotherapy with grass pollen allergens we generated variants of the group V major grass pollen allergen Phl p 5b by deleting IgE-binding epitopes outside the dominant T cell epitopes.

Earlier epitope mapping of the homologous group V allergen of velvet grass pollen, Hol l 5, using overlapping recombinant fragments, had allowed to identify at least four continuous IgE-binding epitopes (Fig. 1) [4]. Since Hol l 5 and Phl p 5b display high similarity (78%) [5], an analogous distribution of IgE-binding epitopes was assumed for both allergens. Dominant T cell epitopes of Phl p 5b were identified using a series of overlapping synthetic dodeca-peptides (Fig. 1). By stepwise site-directed mutagenesis outside the T cell epitope regions, point (PM) and deletion (DM) mutants were generated to reduce the IgE reactivity but not the T cell reactivity (Fig. 1). Allergen variants were analysed for IgE-

binding capacity with sera of different grass pollen allergic patients by Western blotting, dot blotting, and EAST inhibition test. Histamine releasing capacity was analysed in vitro with peripheral blood basophils from different patients and in vivo by skin prick test in five different patients. T cell reactivity was analysed with T cell clones and T cell lines established from 25 different grass pollen allergic patients.

The point mutations established by us did not have an effect on IgE reactivity. Thus, the two amino acid exchanges ($D^{49} \rightarrow L$ and $K^{50} \rightarrow A$) in point mutant PM1 had the same IgE reactivity as wild-type Phl p 5b as shown by Western blot, Dot blot and EAST inhibition test. In point mutant PM3 a new cysteine residue ($A^{13} \rightarrow C$) was introduced in addition to wild-type cysteine residue C^{186} to induce formation of an intramolecular disulphide bond resulting in a change of the molecular conformation. However, also this modification did not affect IgE reactivity. In contrast, reduced IgE reactivity was found in three out of four deletion mutants lacking various stretches of the internal sequences. The most significant results were observed in case of deletion mutants DM2 and DM4. The histamine-releasing capacity was completely abolished in these two deletion mutants. Compared to wild-type Phl p 5b, both DM2 and DM4 caused up to 100-fold weaker skin prick reactivity. The T cell reactivity, however, was retained in most of the Phl p 5b variants. DM4 was considered most suitable for immunotherapy since it showed significant reduction of IgE-binding capacity in vivo and in vitro with an overall conserved T cell reactivity. This mutant DM4 is currently under investigation in an extended skin prick test study to confirm the in vivo results. Recently, Swoboda, I., et al. described similar variants of another group V grass pollen allergen, Lol p 5, derived by introduction of a series of point mutations, which showed also reduced IgE reactivities [6]. Together, these results suggest that allergen engineering by recombinant DNA technology is a suitable method to generate non-IgE-reactive, but T cell-reactive variants also for grass pollen allergens. Therefore the project was continued with the construction of variants of the second major allergen of timothy grass pollen, Phl p 1. The availability of hypoallergenic variants of both important allergens of timothy grass pollen should allow to use them for allergen-specific immunotherapy in a patient-tailored combination.

In conclusion, engineered allergens with reduced anaphylactogenic potential but conserved T cell reactivity could be important tools for efficient and safe allergen-specific immunotherapy.

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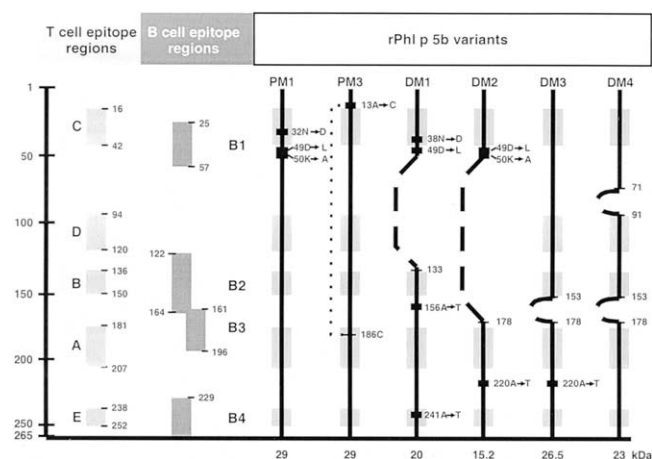


Fig. 1 from [7]. Schematic map of the identified T cell epitope regions (light grey) and putative IgE-binding regions (dark grey) of rPhl p 5b and the allergen variants generated by site-directed mutagenesis. PM, point mutants; DM, deletion mutants. Point mutations are indicated by arrows; dotted line shows the putative disulfide bond in PM3; dashed lines indicate deleted stretches.

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