

Hypoallergenic Forms of the Ryegrass Pollen Allergen Lol p 5 as Candidates for Immunotherapy

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Key Words

Grass pollen allergy · Recombinant allergens · Allergen mutants · Immunotherapy

Almost 25% of the world's population suffer from the symptoms of type I allergy (e.g. allergic rhinoconjunctivitis, asthma, dermatitis, food allergy, and anaphylactic shock). Allergic individuals exhibit an increased tendency to produce IgE antibodies against otherwise harmless environmental antigens (allergens).

Whereas the symptoms of allergic diseases can be controlled by pharmacotherapy, allergen-specific immunotherapy is the only approach towards a causative treatment of allergy. This form of therapy involves the step-wise subcutaneous injection of increasing concentrations of disease-eliciting allergen extracts to achieve a state of immunological tolerance [1]. Modulation of the immune response at the level of T helper cells [2] and induction of

blocking antibodies [3, 4] represent some of the immunological mechanisms underlying specific immunotherapy. One major disadvantage of allergen-specific immunotherapy is that the allergen extracts in use consist of difficult to standardize mixtures of allergenic and nonallergenic components. Recent advances in the field of recombinant DNA technology offer new avenues for the production of pure and well-characterized allergens. However, administration of such pure allergens poses the risk of anaphylactic side effects in patients. A major improvement of allergen-specific immunotherapy may be achieved by using genetically modified hypoallergenic allergen derivatives [5, 6].

The most important cause of seasonal hay fever and allergic asthma is pollen of various grass species. We engineered the most potent ryegrass pollen allergen Lol p 5 to reduce its IgE-binding capacity, but retain the T cell epitopes essential for immunotherapy. In contrast to a previous study where the authors prepared extensive changes in group 5 allergens [7], we performed point mutations

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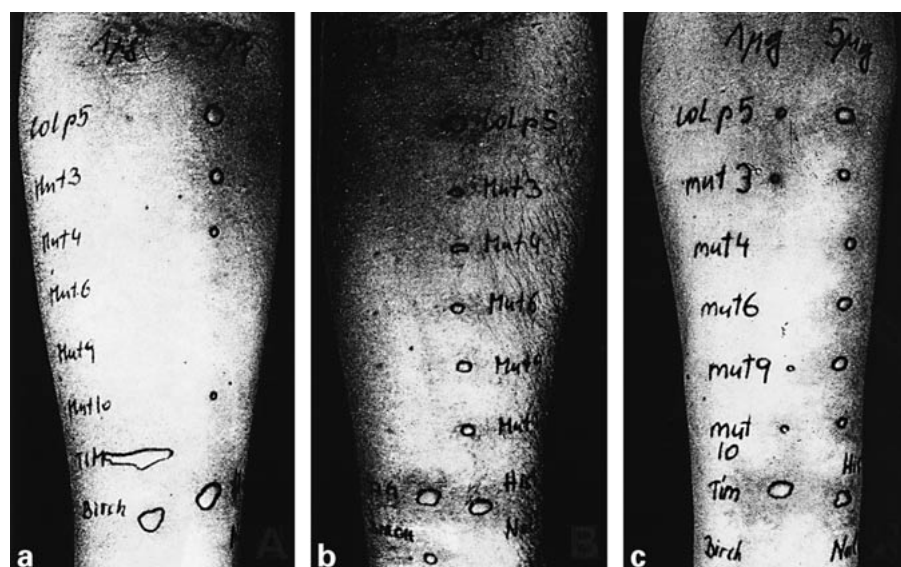
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1018–2438/01/1243–0380\$17.50/0

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Fig. 1. Induction of type I skin reactions with rLol p 5 and rLol p 5 mutants. Three pollen-allergic patients (a–c) were skin prick-tested with rLol p 5 and rLol p 5 mutants (mut 3, mut 4, mut 6, mut 9, mut 10) using two different allergen concentrations (1 and 5 µg/ml). Commercially available test solutions of birch pollen (Birch), timothy grass pollen (Tim), histamine hydrochloride (Hist) and sodium chloride (NaCl) served as controls. Wheal reactions were surrounded with a ball point pen.



based on previous B and T cell epitope mapping studies [8, 9] and on sequence comparison of group 5 allergens from different grasses. Ten Lol p 5 variants were generated by site-directed mutagenesis containing point mutations in highly conserved protein domains outside identified T cell epitope regions. The IgE-binding capacity of purified recombinant Lol p 5 mutants compared to the recombinant wild-type Lol p 5 protein was evaluated using different in vitro methods: Western blotting, slot/dot blotting and ELISA competition experiments. Since most of the variants showed a significant reduction in IgE reactivity, we concluded that with the exchanged amino acids we had been able to identify 'hot spots' for IgE recognition in the Lol p 5 sequence.

To assess the biological activity of the mutants, we also analyzed their capacity to induce basophil histamine release and immediate-type skin reactions. We found that those variants which exhibited a reduction in IgE binding were also less potent in inducing histamine release and cutaneous responses (fig. 1). Although we noticed inter-subject variability regarding the allergenic reactivity of the mutants, the overall most consistent reduction of allergenic reactivity was observed for mut 9 (unpubl. results) and this mutant was, therefore, considered as the most suitable candidate for therapeutic application. Analysis of T cell reactivity revealed that the variants retained the ability of Lol p 5 to induce proliferation of group 5-specific T cell clones and T cell lines. We, therefore, suggest to use recombinant hypoallergenic Lol p 5 mutants for safer forms of grass pollen immunotherapy.

Acknowledgments

Financial assistance from International Collaboration Program of the University of Melbourne and the Australian Research Council is gratefully acknowledged. We thank Ute Siemann and Minoo Ganadan for excellent technical assistance. The Austrian team was supported in part by grant Y078 GEN of the Austrian Science Fund and by the ICP program of the Austrian Ministry for Research and Transport.

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