

Variants of the timothy grass allergen Phl p 5b: new concept for a more efficient immunotherapy

G. SCHRAMM, H. KAHLERT, R. SUCK, B. WEBER, H.T. STÜWE, W.D. MÜLLER, A. BUFE, M.W. SCHLAACK, L. JÄGER, O. CROMWELL, H. FIEBIG, W.M. BECKER

BACKGROUND

Conventional allergen-specific immunotherapy of type I allergy is based on s.c. application of increasing amounts of crude allergen extracts as introduced by Noon 1911 [1]. Although the mechanism by which immunotherapy works is not known in detail until now, large amounts of relevant allergens are believed to lead to immunomodulation on T cell level and to induction of blocking IgG antibodies. However, administration of such 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 anaphylactic reactions to the complete IgE-reactive allergens.

OBJECTIVE

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 [2, 3]. To check out 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.

METHODS

By epitope mapping of the homologous group V allergen of velvet grass pollen, Hol 1 5, using overlapping recombinant fragments, at least four continuous IgE-binding epitopes (B1-B4, fig. 1) were identified (G. Schramm et al., manuscript in preparation). Both allergens, Hol 1 5 and Phl p 5b display such high homology (78%) [4] that similar distribution of IgE-binding epitopes was assumed. Dominant T cell epitopes of Phl p 5b were identified using a series of overlapping synthetic dodeca-peptides (A-E, 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

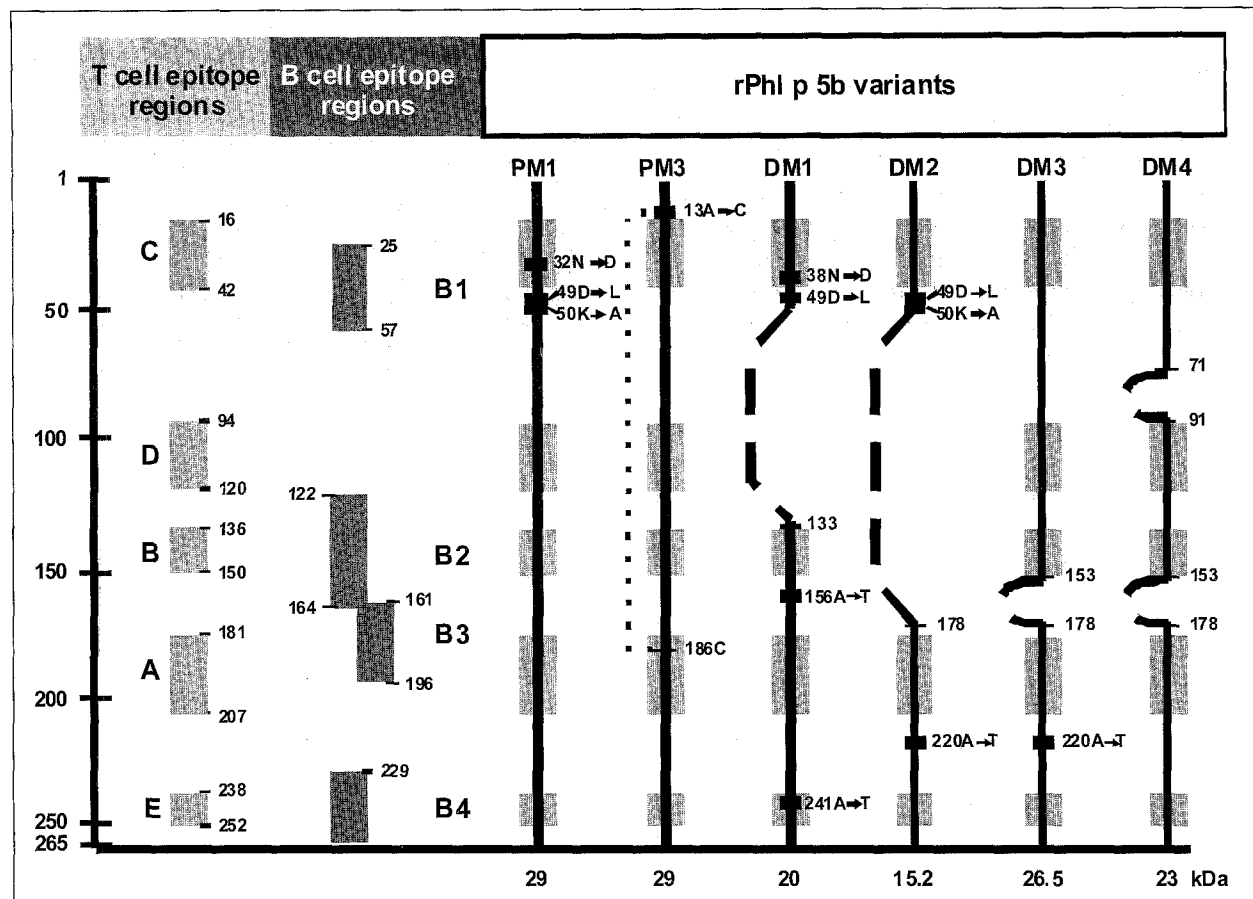


Fig. 1. – Schematic map of the identified T cell epitope regions (light grey) and putative IgE-binding regions (dark grey) of rPhl p 5b and the allergen variants generated by site-directed mutagenesis. PM, point mutants; DM, deletion mutants. Point mutations are indicated by arrows; dotted line shows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

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 with five different patients. T cell reactivity was analysed with T cell clones and T cell lines established from 25 different grass pollen allergic patients.

RESULTS

The point mutant PM1 contained two amino acid exchanges ($D^{49} \rightarrow L$ and $K^{50} \rightarrow A$). However, the introduction of these point mutations had no effect on IgE reactivity of this variant as shown by Western blot, Dot blot and EAST inhibition test. The point mutant PM3 displayed a new cysteine residue ($A^{13} \rightarrow C$) in addition to wild-type cysteine residue C^{186} . We expected that the formation of

an intramolecular disulphide bond between these cysteine residues would change the molecular conformation of this mutant resulting in a reduced IgE reactivity, but IgE reactivity was not affected. Furthermore, various deletion mutants were constructed (DM1-DM4), which lacked various stretches of the internal sequences. Three of these deletion variants showed reduced IgE reactivity; 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. Both, DM2 and DM4 also show up to 100fold reduced 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.

CONCLUSION

Our 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. Considering their reduced anaphylactogenic potential together with their conserved T cell reactivity, the engineered allergens could be important tools for efficient and safe allergen-specific immunotherapy.

REFERENCES

1. Noon L. - Prophylactic inoculation against hay fever. *Lancet*, 1911, 1, 1572-1573.
2. Smith A.M., Chapman M.D. - Reduction in IgE binding to allergen variants generated by site-directed mutagenesis: contribution of disulfide bonds to the antigenic structure of the major house dust mite allergen Der p 2. *Mol. Immunol.*, 1996, 33, 399-405.
3. Takai T., Yokota T., Yasue M., Nishiyama C., Yuuki T., Mori A., Okudaira A.H., Okumura Y. - Engineering of the major house dust mite allergen Der f 2 for allergen-specific immunotherapy. *Nat. Biotech.*, 1997, 15, 754-759.
4. Schramm G., Bufe A., Petersen A., Schlaak M., Becker W.M. - Molecular and immunological characterization of group V allergen isoforms from velvet grass pollen (*Holcus lanatus*). *Eur. J. Biochem.*, 252, 200-206.

