Chemically modified recombinant Fel d 1: a good alternative to hypoallergenic mutants

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Rationale: Hypoallergenic mutants of recombinant major allergens are proposed to make immunotherapy safer and more efficient. Their development, however, is laborious and requires detailed structural information. The aim of this study was to develop chemically-modified recombinant Fel d 1 as an alternative to a hypoallergenic mutant.

Methods: Fel d 1 was cloned and expressed in *Pichia pastoris* as a hybrid molecule of both chains of the allergen. The resulting recombinant (r)Fel d 1 was compared to purified natural (n)Fel d 1 by CD and mass spectroscopy, RAST(-inhibition), competitive RIA, ELISA, immunoblot, basophil histamine release (BHR) and T-cell proliferation assays. Chemical modifocation was carried out using glutaraldehyde. Hypoallergenicity was assessed by RAST-inhibition (RI) and BHR.

Results: rFel d 1 was expressed at ~500 mg/L and was shown to be glycosylated by the yeast. Physico-chemical and immunological comparison with nFel d 1 showed that the recombinant molecule is well-folded and that the immune-reactivity of both molecules is very similar. Deglycosylation did not influence immune-reactivity. Chemical modification resulted in at least a 200-fold decrease in IgE reactivity (RI) and a 1000-fold decrease in biological activity (BHR).

Conclusions: Chemically modification of recombinant major allergens is a good and easy alternative to the development of hypoallergenic mutants.