Validation of recombinant allergens: ELISA reactivity, IgE antibody binding, and skin test reactivity.

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In order for recombinant allergens to be useful as diagnostics and in therapy, they must react with mAb and with IgE antibodies from allergic individuals to a comparable degree as natural allergenic products. In this study, two site mAb ELISA were used to compare natural and recombinant allergens. A chimeric ELISA was used to correlate allergen specific IgE ab by linear regression analysis in >250 sera from allergic patients. Recombinant forms of cat, mite and cockroach proteins, many of which have been difficult to express in the past, were successfully produced in four different systems. Recombinant Bla g 1, Bla g 2, and ProDer f 1 were produced in Pichia pastoris; rFel d 1 in Baculovirus and Pichia; ProDer p 1 in CHO cells and Pichia; and rDer p 2 in E. coli. Allergens were purified using ion exchange, or mAb affinity chromatography, and gel filtration HPLC. Natural allergens were purified from extracts of house dust, spent mite culture or cockroach frass.
All recombinant allergens showed parallel binding curves and had comparable levels of sensitivity to natural allergens in mAb ELISA. Allergens were then used at 0.5μg/ml in a serum based ELISA to correlate IgE antibody binding to natural versus recombinant protein. Results were quantified (ng/ml IgE) using a chimeric mouse anti Der p 2 standard curve.

Allergen	System	Sera	r value	p value
Bla g 2	P. past.	54	0.96	<0.001
Bla g 1	P. past.	34	0.92	< 0.001
Fel d 1	Bacul.	68	0.98	< 0.001
Fel d 1	P. past.	57	0.91	< 0.001
Der p 1	P. past.	33	0.93	< 0.001
Der p 1	CHO	33	0.92	< 0.001
Der f 1	P. past.	98	0.96	< 0.001
Der p 2	E. coli	50	0.92	< 0.001

A significant correlation ($p > 0.001$) was also obtained between IgE ab binding to recombinant Fel d 1 and Der f 1 and Pharmacia CAP score for cat epithelium and D. farinae respectively. Recombinant Derp 2 and Bla g 2 gave skin test reactivity from 10-6 to 10-2μg/ml respectively. With the exception of rBla g 1 and rDer p 2, the recombinant allergens were glycosylated, showing diffuse banding at a higher molecular weight than the natural allergen on SDS-PAGE. However, glycosylation had no effect on IgE ab binding. The results show that the immunoreactivity of recombinant allergens can be successfully validated using a combination of in vitro tests (mAb and IgE) and in vivo skin tests. These validated reagents will provide new tools for allergy diagnosis and treatment.