

CLINICAL EXPERIENCE WITH RECOMBINANT POLLEN ALLERGENS

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Recombinant DNA technology enables allergens to be produced to high pharmaceutical standards resulting in preparations with improved quality in terms of purity, consistency and the relative concentrations of different allergens. There is also the possibility to formulate vaccines to include only the most relevant allergens in defined concentrations and to match composition to allergen sensitization profiles of individual patients (1).

GRASS POLLEN

We have cloned and expressed five major allergens from the grass *Phleum pratense* (Timothy grass) in *E. coli*. Purification protocols using various chromatographic techniques have been developed for each allergen (2), and the resulting active pharmaceutical ingredients have been subjected to detailed physicochemical and immunological characterization. The IgE-reactivity, determined by basophil activation and histamine release, and specific T cell reactivity compare very well with the natural proteins. Each of the five allergens has been adsorbed to aluminium hydroxide gel and the resulting adsorbates have been mixed in approximately equimolar concentrations. The resulting preparation has been tested in a pilot randomized double-blind placebo controlled study with patients suffering from grass pollen induced allergic rhinitis, with or without asthma, with the objective of determining efficacy and safety. The preparation was administered by subcutaneous injection in increasing doses to a maximum of 40µg total protein comprised of 10µg each of Phl p 1, Phl p 5a and Phl p 5b, and 5µg of Phl p 2 and Phl p 6. Treatment commenced in January 2002 and the dosage was increased progressively with 10 subcutaneous injections at 7-day intervals. Once the maximum or maintenance dose had been achieved the interval was increased step-wise to 14, 28 and finally 42 days. Immunotherapy was continued for a period of approximately 20 months encompassing two pollen seasons

during which the maintenance dose was reduced by 50%. Symptom Medication Score in the second year of the study was the primary outcome measure to assess efficacy. A validated rhinitis quality of life questionnaire (RQLQ) was a secondary endpoint and was completed by subjects during the baseline visit, prior to each pollen season and every 2 weeks during the seasons. The questionnaire following the maximum pollen count was used for analysis. Conjunctival provocation tests with half-log increments in concentration were performed before therapy (inclusion criterion) and after the second grass pollen season (2003) using a standardized lyophilized 6-grass allergen extract. *Phleum pratense* specific IgE, IgG1 and IgG4 antibodies were quantified using microtitre plates coated with either *Phleum pratense* pollen extract 10 µg/ml (Allergopharma), or purified natural allergens (1 µg/ml) and appropriate monoclonal antibodies for detection. Evaluation of symptoms alone for the per protocol set showed a 36.5% lower median average symptom score for active treatment compared with placebo (3.38 versus 5.32; $p = 0.015$). There was also a reduction in the need for medication reflected in a 36.5% lower medication score. The combined symptom medication score showed a significant difference between groups after the 18 month treatment period, with median average daily scores of 4.92 versus 8.05 for active and placebo treatment respectively, a difference of 38.9% ($p = 0.044$). This result is of the same order as the mean 45% additional improvement in disease severity above the response to placebo seen in 43 rhinitis studies including 1,120 actively treated patients (3).

The RQLQ was evaluated in respect of seven domains and also as a whole (28 questions). Assessment completed in the second pollen season (2003) showed significant and clinically relevant differences in favour of active treatment for the whole questionnaire and 5 of seven domains. These differences were improvements on those already seen in the first pollen season. The conjunctival provocation test conducted at the end of the study showed a clear trend to a higher threshold allergen dose with 21/28 treated subjects tolerating

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higher allergen concentrations, although this was not statistically different from placebo ($p = 0.081$). IgG1 concentrations showed a median increase of approximately 60-fold, peaking during the first 12 months of the study. IgG4 concentrations showed a continuing upward trend, achieving an approximately 4000-fold median increase by the end of treatment. Comparisons between the groups showed statistically significant differences at all time points following the commencement of immunotherapy ($p < 0.001$). All subjects in the active treatment group showed specific IgG isotype responses to the individual natural grass allergens. Responses to Phl p 5a/b were stronger than those to Phl p 1, medians being 2.9×10^2 (2) and $2.3 \times 10^1 \mu\text{g/ml}$ respectively for IgG4, and 1.3×10^1 and $2.1 \mu\text{g/ml}$ for IgG1. A favourable safety profile was demonstrated. The majority of the reactions were local and involved transient erythema and swelling in the vicinity of the injection sites consistent with allergic responses or mild trauma. Reactions to placebo were probably attributable in large part to histamine included to mimic allergen induced reactions. Systemic reactions were isolated in nature and typical of those normally associated with immunotherapy. The fact that all subjects continued therapy with either the same or higher doses without further problems indicated that the preparation was generally well tolerated.

BIRCH POLLEN

Hypoallergenic variants produced by the chemical modification of natural allergen extracts have been used successfully for specific immunotherapy for a number of years. The clinical efficacy of such preparations has been demonstrated in several studies, and a recent clinical trial with a grass pollen allergoid (Allergovit®) showed a 48.4% improvement in a combined symptom medication score over and above that of a placebo group after two pre-seasonal courses of treatment (4). Such preparations offer various advantages including the possibility to administer high concentrations with relatively aggressive dosage schedules, and with a reduced risk of inducing IgE-mediated adverse reactions. Recombinant DNA technology offers the opportunity to develop genetically engineered hypoallergenic derivatives. In the case of the birch pollen major allergen Bet v 1 this has been achieved by either expressing the molecule in two fragments or through the construction of oligomeric forms, typically a trimer (5). A hypoallergenic form of the allergen now has been created by inducing the protein to adopt a different folding pattern. Again the characteristics can be demonstrated by reduced activity in basophil activation and histamine release tests, and the immunomodulatory potential of the variant has been confirmed by

its ability to activate specific T cells at least to the same extent as the natural allergen. The birch pollen allergen has been expressed in *E. coli* and used to produce the rBet v 1-FV hypoallergenic form. The protein has been tested in a randomized controlled study using a natural birch pollen extract for comparison. Treatment was conducted using pre-seasonal courses of injections, eight in the case of the recombinant protein and 14 for the natural allergen preparation. The cumulative dose of rBet v 1-FV was $157.5 \mu\text{g}$, and all subjects achieved the maximum concentration of $80 \mu\text{g}$. Fifty-one subjects commenced therapy, 24 with rBet v 1-FV and 27 with the natural allergen extract. The combined symptom medication score used as the primary end-point showed superiority of the recombinant preparation after one pre-seasonal course of treatment. Comparison of both groups with the baseline data from a parallel study showed that both preparations caused significant improvement in symptom medication score. Nasal provocation tests showed that improvement in allergen tolerance was comparable in the two study groups. There were no significant changes in IgE antibody levels measured before and after immunotherapy and the again after the pollen season. Both allergen specific IgG1 and IgG4 were significantly increased following treatment. The incidence of side reactions was comparable between the two groups. In both cases the majority of the systemic reactions occurred late in the dosage schedule, and it is speculated that concomitant exposure to pollen from cross-reacting early-pollinating trees, such as hazel, may have been a major contributory factor. The results of the two clinical studies described here are seen as an endorsement for the development and application of recombinant allergens and derivatives for allergen specific immunotherapy. The level of efficacy that can be achieved appears to be at least comparable to that with native allergen derivatives. It remains to be seen whether or not improvements can be made, possibly through modification of dosage schedules or eventually the inclusion of additional allergens.

References

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