

Clinical Experience with Recombinant Molecules for Allergy Vaccination

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Abstract Numerous allergens have been cloned and produced by the use of recombinant DNA technology. In several cases recombinant variants with reduced IgE-reactivity have also been developed as candidates for allergen specific immunotherapy. Only very few of these proteins have as yet been tested in the clinic, and the major focus has been on birch and grass pollen, two of the most common causes of IgE-mediated allergic disease. This article serves to justify the rational for using recombinant products and reviews the progress that has been made to date with their clinical assessment.

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The first publication describing the use of recombinant DNA technology to clone and express an allergen appeared in 1988 (Breiteneder et al. 1988, 1989) and since that time several hundred other allergens have been produced in recombinant form and characterized (Allergome 2009). There have also been numerous reports on the development of recombinant allergen variants with reduced IgE-reactivity as candidates for specific immunotherapy (Ferreira et al. 2006; Niederberger and Valenta 2006). The only substantial clinical studies conducted to date have been with recombinant allergens or allergen derivatives of grass and birch pollen, two of the most common causes of IgE-mediated allergic disease. Recombinant house dust mite and cat allergens have also been the focus of extensive preclinical development work. A small clinical study using recombinant Fel d 1 has already been reported and it seems likely that the group 1 and 2 allergens of house dust mites will soon be the subject of clinical trials. Before such products can be granted marketing licenses and placed on the market it will be necessary to demonstrate their clinical efficacy and safety. This article serves to justify the rationale for using recombinant products and review the progress that has been made to date with their clinical assessment.

1 Considerations for Optimal Allergen Specific Immunotherapy

Allergen specific immunotherapy is a causal treatment for IgE-mediated allergic disease, in other words the allergen responsible for sensitization and provoking symptoms of rhinoconjunctivitis, allergic asthma etc. is administered under controlled conditions in order to change the course of the immune response and thereby ameliorate symptoms. Characterization of the cytokine profiles of allergen specific human T cells revealed that the immune responses of healthy and allergic subjects to common environmental allergens can be defined in terms of a delicate balance between allergen specific T helper type 2 (Th2) and inducible type 1 T regulatory (Tr1) cells (Akdis et al. 2004). The Th2 cells of the allergic phenotype are associated with interleukin (IL)-4, IL-5 and IL-13, which promote IgE production and allergic inflammation, while Tr1 cells are particularly linked with the cytokine IL-10. Tr1 and Th3 regulatory cell phenotypes that secrete the suppressive cytokines IL-10 and TGF- β , respectively have been associated with induction of peripheral T cell tolerance in allergic subjects undergoing successful immunotherapy with bee venom and aeroallergens (Akdis et al. 1998;utel et al. 2003). whilst low allergen doses favor a Th2 cytokine response and a switch to IgE, high allergen doses favor induction of regulatory T cells and an enhanced Th1 cytokine profile together with modification or down-regulation of the Th2 phenotype (Larche et al. 2006). This is consistent with the data from studies in bee-keepers subjected to repeated bee stings which showed that high dose allergen tolerance is associated with clonal expansion of allergen specific IL-10-producing Tr1 cells.

Investigations with bee venom phospholipase (PLA) specific T cell clones from subjects either allergic, hyposensitized or immune (protected) to bee stings have shown that both absolute and relative amounts of secreted cytokines depend on the antigen concentration. Low antigen doses induced IL-4 production, but little or no IFN- γ , whereas significant amounts of both cytokines were obtained at higher PLA concentrations. T-cell clones from allergic and hyposensitized individuals required higher critical amounts of antigen for IFN- γ induction, and expressed increasing IL-4/IFN- γ ratios with increasing concentrations of PLA (Carballido et al. 1992). Therefore modulation of cytokine patterns is dependent on the dose of antigen, and high dose is important for induction of a protective immune response. These conclusions are supported by studies in mice (Ruedl et al. 2000) as well as by results for allergen specific immunotherapy in man which clearly show that a higher dose is more clinically effective (Frew et al. 2006).

Allergen specific immunotherapy is conducted using extracts of natural raw materials containing the appropriate allergens together with numerous other proteins and molecules from the plant or animal material in question. The concentrations of the allergens are dictated to a large extent by the raw material, and while some allergens may be well represented others may only be present in concentrations that are not sufficient to achieve an optimal clinical benefit. The use of allergens or allergen variants derived through the use of recombinant DNA technology provides an opportunity to create products which include only the relevant allergens in concentrations suitable for achieving optimal clinical benefit. The concentrations of all components can be declared in mass units and it will be possible to achieve excellent product consistency.

Allergen concentration is also relevant with regard to the possibility of inducing new sensitizations. Provided that the allergens or allergen derivatives are present in sufficiently high concentrations in a preparation administered for subcutaneous specific immunotherapy which by-passes the mucosa, it is unlikely that they will favor IgE production, but rather induction of peripheral T cell tolerance. By way of example, birch pollen allergic subjects who were sensitized predominantly to Bet v 1 and underwent treatment with a birch pollen extract developed new sensitivities to what are normally considered as minor birch pollen allergens that are not present in anything like the same concentration as Bet v 1 (Moverare et al. 2002). It is not yet clear if similar considerations apply for sublingual immunotherapy which has a clear booster effect on existing IgE responses (Didier et al. 2007; Dahl et al. 2008) and may well involve other mechanisms of action. These are areas that are worthy of further investigation.

It will not be realistic to produce every protein component of an allergen extract using recombinant DNA technology for inclusion in a therapeutic preparation. Therefore the first objective of a clinical development program has to be the identification of an adequate combination of proteins to achieve a clinically relevant benefit for the patients. In the case of birch pollen and cat allergy it appears that Bet v 1 and Fel d 1, respectively are sufficient, but in the cases of grass pollen and house dust mite allergy two or more allergens will be essential. It is important to establish these basic requirements before going ahead with studies with

hypoallergenic variants, since in the case of an unsuccessful study it would be difficult to decide if the result was attributable to the lack of an important allergen or an ineffective variant.

2 Allergen Variants with Reduced IgE-Binding Activity

One of the potential risks of this causal treatment is that it may induce allergen associated side effects, and at worst life-threatening anaphylactic reactions. This consideration was one factor that prompted the development of hypoallergenic derivatives produced by chemical modification of the allergen extracts (Maasch and Marsh 1987). Such preparations are intended to minimize the risk of inducing IgE-mediated reactions, while ensuring the possibility for administering an adequately high dose to favor a therapeutic effect. The reduced IgE-reactivity minimizes the potential to activate mast cells and basophils with the release of inflammatory mediators. Furthermore, IgE antibody-dependent uptake by antigen presenting cells, which would normally favor promotion of the allergic phenotype with production of Th2 cytokines and allergen specific IgE (van der Heijden et al. 1993), is also excluded (Akdis and Blaser 2001). Chemically modified allergen extracts (allergoids) have found widespread acceptance. The choice of chemical method used to produce the allergoids determines which types of chemical residues in the proteins will be subject to modification, but it is not possible to target specific sites within a protein. The advent of recombinant DNA technology provides the opportunity to use genetic engineering techniques to develop tailor-made hypoallergenic molecules which may present some additional advantages for allergen specific immunotherapy. Not only is it possible to compromise the IgE-reactivity of the proteins, but there is also the possibility to enhance the immunogenicity and introduce features that can influence the processing of an allergen by the immune system.

The design features of engineered hypoallergenic variants can be precisely defined and validated with respect to the intended specific immunotherapeutic application. Genetic detoxification of bacterial toxins by gene mutations at sites coding for the amino acids involved in the enzymatic sites, and thus the toxic effects, has already been achieved (Rappuoli et al. 1995), thus providing an alternative to the toxoids produced by chemical modification. In such cases the choice of mutation site is relatively straight-forward, but this is often not the case with allergens in which the IgE-binding epitopes rely principally on the conformation of the protein. Several strategies have been adopted in order to impart hypoallergenic characteristics to allergens without compromising T-cell reactivity and immunomodulatory potential, and experience has shown that such strategies have to be tailored to match the characteristics of each individual allergen. A bigger challenge is often presented by the search for a molecular variant that can be expressed and recovered in a soluble form in adequate amounts with consistent characteristics and quality.

It is important to define the criteria which a hypoallergenic variant has to fulfill before the decision can be made to take it into clinical testing. A variant must have advantages for a very large majority of potential recipients, that is to say it must have obvious hypoallergenic characteristics for all those patients. Allergic subjects differ widely in terms of the amounts of specific IgE antibody they produce against a particular allergen, and furthermore the spectrum and the number of epitopes recognized may vary. Consequently it is not sufficient to compromise reactivity of only one of several IgE-binding epitopes in an allergen, and it is important to screen new variants with a library of sera from allergic subjects in order to cover all IgE-binding epitopes. It is usually not appropriate to use pool sera to assess hypoallergenic characteristics. If only a small number of sera in the pool react strongly with a variant their contribution would be diluted by a large number of weak reactors. Furthermore various different methods should be used to assess the derivatives, including solid-phase and liquid-phase immunoassays and cellular assays such as basophil activation or histamine release. Skin or provocation testing may then be used to confirm hypoallergenic characteristics *in vivo*. Ideally hypoallergenic characteristics should be observed consistently with all test methods. Finally, the reduction in IgE-reactivity should be sufficient to confer a meaningful advantage.

3 Recombinant Grass and Birch Pollen Allergens

The grass *Phleum pratense* is a member of the sub-family *Pooideae*, which in turn belongs to the family *Poaceae*. It shows very substantial cross-reactivity with other members of the sub-family (Andersson and Lidholm 2003; Johansen et al. 2009) and can therefore be considered as representative of grasses found in temperate regions. The allergens Phl p 1, Phl p 2, Phl p 5a, Phl p 5b and Phl p 6 account for a substantial proportion of the specific IgE sensitization developed against grass pollen. Recombinant forms of these five allergens were the basis of a preparation investigated in a double blind placebo controlled clinical trial in 62 grass pollen allergic patients suffering from rhinoconjunctivitis with or without asthma (Jutel et al. 2005). Aluminium hydroxide adsorbates of the individual recombinant allergens were used as a mixture and administered by subcutaneous injection with increasing concentrations at 7-day intervals prior to the grass pollen season, starting with 0.02 µg total protein, followed by 0.16 µg and then doubling to 40 µg total protein (0.8 ml). The maximum dose contained 10 µg Phlp1, 5µg Phlp2, 10 µg Phlp5a, 10 µg Phlp5b and 5 µg Phlp6. Maintenance injections were then continued until after the subsequent pollen season, with a 50% reduction during each pollen season, so that the total period of treatment was 18 months.

The primary outcome measure to assess clinical efficacy was a combined symptom-medication score (SMS) derived from patients' diaries. Diaries were kept for 3 month periods encompassing each pollen season, and provided a record of the nature and severity of eye, nose and chest symptoms, together with the type

and dose of any rescue medication. A preprotocol analysis included 24 active treatment and 25 placebo patients, and showed a 39% improvement in the active treatment group relative to placebo ($p = 0.041$). Symptoms alone improved by 37% ($p = 0.015$) and the use of symptomatic medication decreased by 36.5% relative to placebo. The preparation showed a favorable safety profile.

A validated rhinitis quality of life questionnaire (RQLQ) (Juniper and Guyatt 1991) registered benefits for those subjects on active treatment by comparison with placebo during the first pollen season, and these increased still further during the second pollen season with an overall significant benefit ($p = 0.024$), providing further evidence of clinical efficacy. Significant effects were registered in 5 of 7 domains tested, with the mean differences between active and placebo treatment substantially in excess of a score of 0.5 that may be considered as a level of clinical relevance. Active treatment also increased allergen tolerance as judged in a conjunctival provocation test with a standardized 6-grass allergen extract. The effect failed to achieve statistical significance, very probably as a consequence of the relatively small number of patients. Taken together the results indicate that the mixture of five allergens is sufficient to achieve good clinical benefit.

Those subjects treated with the recombinant allergen preparation showed large and highly significant increases in both IgG1 and IgG4 allergen specific antibody concentrations together with a significant decrease in specific IgE. The specific IgG1 concentration increased approximately 60-fold, peaking during the first 12 months of the study. IgG4 showed a continuing upward trend, achieving an approximately 4000-fold increase by the end of the 18 month treatment period. Specific IgE levels were not significantly different between groups at the beginning of the study, but thereafter the active treatment group showed a downward trend with values significantly less than baseline. Phl p 5a/b specific IgE antibodies were not detected in four subjects from each group prior to the treatment, although all reacted to Phl p 1 and other grass pollen allergens. None of these subjects developed Phl p 5a/b reactive IgE antibodies during the study, although the 4 subjects receiving active treatment developed strong IgG4 and IgG1 Phl p 5a/b responses indicative of either pre-existing immunity to the allergen without class-switching to IgE or induction of immunity. This observation needs to be substantiated and is relevant to the possible prophylactic effects of specific immunotherapy and guarding against the development of new sensitizations.

The same five grass pollen allergens in the same relative concentrations were formulated with total protein concentrations of 20, 40, 80 or 120 μg per maximum dose and used in a randomized, double blind, placebo controlled dose finding study with 10 subjects per group. Grass pollen allergic patients were treated for approximately 3 months prior to the grass pollen season. Primary endpoint in this study was the number of grade III and IV systemic reactions graded according to Tryba (1994), and the main secondary endpoints the early and late phase reactions in intracutaneous tests. Despite the fact that the 120 μg dose contained 30 μg of each of Phl p 1, Phl p 5a and Phl p 5b, some of the highest concentrations tested to date for subcutaneous immunotherapy, safety was very good and there were no

drop-outs attributable to side effects. The use of progressive dosage increases probably contributes to this good safety record.

The early and late phase responses after a titrated intra-cutaneous test were able to discriminate between the different therapeutic dosage schedules with decreases in the magnitude of the skin reactions with increasing therapeutic dose (Sprung et al. 2009). Grass pollen specific IgG1 and IgG4 antibody responses were seen in all active treatment groups, but not in the placebo group. The *Phleum* preparation has now entered Phase III clinical testing.

One question that frequently arises is - how do the results with a recombinant allergen compare with those for a natural allergen? A study in birch pollen allergic subjects addressed this by comparing natural and recombinant Bet v 1 with placebo, and went one step further by including a group of subjects treated with a whole birch pollen preparation (Pauli et al. 2008). Treatment commenced 6 months prior to the expected peak of the birch pollen season, and the Bet v 1 major allergen dose was increased progressively from 0.05 to a maximum of 15.0 µg at weekly intervals. Treatment was extended over 2 years with monthly injections of the maximum dose. Significant reductions in rhinoconjunctivitis symptoms were seen after one year of treatment and these were increased still further to circa 50% in each of the three active treatment groups by comparison with placebo. The use of rescue medication was significantly reduced by the order of 65% in all active treatment groups. Skin test sensitivity also decreased significantly in all three active treatment groups, and interestingly the reduction in the group treated with recombinant Bet v 1 was significantly larger than those in the birch pollen and natural Bet v 1 groups. Marked increases in Bet v 1 specific IgG were seen in each of the treatment groups. No new IgE sensitizations were observed in the recombinant and natural Bet v 1 groups, but sensitivities to Bet v 2 were induced in 3/29 subjects treated with the pollen preparation.

4 Recombinant Hypoallergenic Variants of Birch Pollen Allergen Bet v 1

The IgE-binding reactivity of allergens such as Bet v 1 from birch pollen is very dependent on their 3-D structure. Cleaving the cDNA and expressing the two parts separately results in two allergen fragments (amino acid residues 1–73 and 74–159) showing random coil conformation (Vrtala et al. 1997). This loss of conformation almost certainly accounts for the loss of IgE-antibody binding activity. The cleavage point was chosen so as not to compromise recognized T cell epitopes (Vrtala et al. 2001a).

The birch pollen protein has also been used as a model system to investigate the potential of oligomerization to influence IgE-reactivity. Linking three copies of the Bet v 1 cDNA in sequence and expression in *E. coli* resulted in a trimeric form of the protein. IgE-reactivity was reduced, as judged by histamine release and skin

testing, but circular dichroism spectroscopy showed that secondary structure was essentially the same as monomeric Bet v 1 (Vrtala et al. 2001b). Basophil activation measured in terms of CD203c expression indicated that the trimer is hypoallergenic, but less so than the fragments (Kahlert et al. 2003). Steric hindrance of the IgE-binding sites is the probable explanation for the hypoallergenic characteristics.

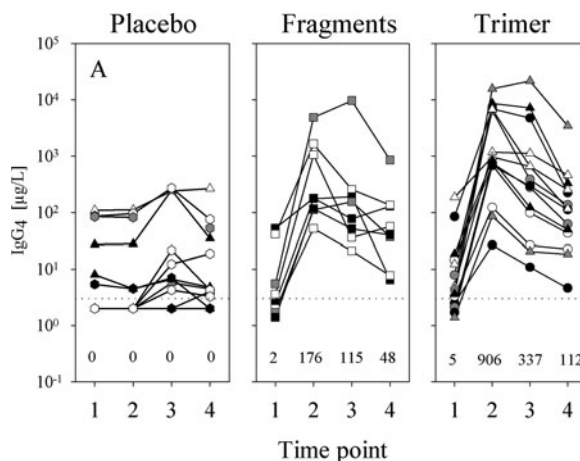
Skin prick tests with concentrations of 100 µg/ml of the fragments or the trimer in subjects with a positive response to native Bet v 1 showed that 18 of 23 and 15 of 23 subjects failed to react to the fragment mixture and the trimer, respectively (van Hage-Hamsten et al. 1999). A similar result emerged from intradermal testing, with 8 of 23 and 13 of 23 of the birch pollen allergic subjects failing to react to 1 µg/ml concentrations of the fragment mixture and the trimer, respectively. Clear dose response effects were seen with each of the allergen derivatives. The Bet v 1 fragments were tested separately and both were shown to be hypoallergenic in nature. A second study produced very similar results (Pauli et al. 2000). However it was observed that there was a very large inter-subject variation in the end-point of the intradermal tests, although both derivatives showed hypoallergenic characteristics to one degree or another. It is important to realize that such preparations are only going to offer advantages for safety and higher dosing if those advantages apply for all patients.

The first study of allergen specific immunotherapy with recombinant preparations investigated the clinical effects, immunological activity and tolerance of a mixture of the two Bet v 1 fragments and the Bet v 1 trimer in comparison to placebo. The recombinant preparations were adsorbed to aluminium hydroxide suspensions at concentrations of 100 µg/ml, and immunotherapy was conducted with a course of 8 pre-seasonal injections of increasing concentrations from 1 to 80 µg total protein, with further injections of the maximum concentration up until the beginning of the pollen season (Niederberger et al. 2004; Purohit et al. 2008). The results of the study were confounded to some extent by very different pollen counts in the three study centers and substantial counts of cross-reactive alder pollen several weeks in advance of the birch pollen season in one of the centers.

A combined symptom-medication score and a visual analogue score failed to reveal any significant differences between active and placebo treatments. Within-group comparisons, excluding those subjects from the center without a substantial pollen count, showed a significant improvement for the trimer group. Significant decreases in nasal sensitivity to allergen were seen in the fragment and trimer groups, but they did not differ significantly from placebo. All three study groups showed decreased skin test sensitivity. Local injection site reactions were most frequent in the trimer group and occurred soon after injection, whereas those in the fragment group generally occurred after several hours. Systemic reactions were elicited more frequently by fragments. These results together with the variations in hypoallergenic characteristics between birch pollen allergic subjects contributed to a decision not to pursue the clinical development of these preparations.

Withstanding the disappointing clinical data, both the fragment and trimer preparations of Bet v 1 proved to be strong immunogens inducing Bet v 1 specific

Fig. 1 Bet v 1 specific IgG4 antibody responses during the course of specific immunotherapy with a Bet v 1 trimer (n = 14), Bet v 1 fragments (n = 8) and placebo (n = 20). Data from one of three study centers (Vienna) for blood samples collected 1: before pre-seasonal immunotherapy; 2: after immunotherapy; 3: after the birch pollen season; and 4: 12 months after the first sample. (Purohit et al. 2008)



IgG1 and IgG4 antibody responses that peaked at the end of treatment with concentrations in the order of 100-fold more than those at baseline (Fig. 1). IgG2 and IgA antibody responses were also documented for subjects in both active treatment arms in one of the three study centers. Increases in Bet v 1 specific IgE during seasonal pollen exposure were blunted in the active treatment groups by comparison with placebo.

The serum antibodies were shown to be able to inhibit allergen induced histamine release in vitro from basophils of birch pollen allergic subjects (Niederberger et al. 2004). It was possible to show correlations between IgG1 antibody titers and both improvement in clinical symptoms, as judged by a ten-point interval scale, and reduction in skin test reactivity to Bet v 1.

Bet v 1 specific antibody responses were measured in nasal lavage fluids from a randomly selected sub-group of 23 subjects at the end of the birch pollen season following the course of immunotherapy and again 12 months after the time point at which treatment had commenced (Reisinger et al. 2005). Bet v 1 specific lavage fluid IgG1 levels were significantly raised at the end of the pollen season in those subjects that had received the active preparations (10 in trimer and 3 in fragment mixture groups) in comparison with placebo. Higher levels of IgG2 and IgG4 were also detected, as was the case in serum, but these were not significant. There were also no apparent differences in IgA levels. There were correlations between the various IgG subgroup concentrations in serum and lavage fluid. At the end of the birch pollen season there was a correlation between nasal IgG4 and reduced specific nasal sensitivity. Perhaps not surprisingly, the nasal antibody levels mirrored those in serum. The reduced nasal sensitivity may be accounted for by the inhibitory effect of the antibodies on basophil and mast cell mediator release as was demonstrated for the serum antibodies in vitro.

The possibility that immunotherapy with the fragments and trimer might also provide benefit for patients with birch pollen associated oral allergy syndrome (OAS) was also considered. A sub-group of 44 patients from one study centre who

suffered from symptoms of OAS attributable to apples, hazel nuts, carrot, celery or other plant derived foods showed various increases in IgG1 and IgG4 to major allergens from these foodstuffs (Niederberger et al. 2007). Antibody responses in other antibody classes appeared not to be affected. Seven of 25 actively treated subjects reported improvements in their OAS compared with only one from 19 in the placebo group. Two placebo and 2 actively treated subjects reported worsened OAS.

Investigations into cytokine responses showed that treatment with trimer resulted in significant reductions in IL-5 and IL-13 producing cells compared between pre- and post treatment, which was indicative of a suppression of the Th2 response (Gafvelin et al. 2005). There were also trends for decreased numbers of IL-4 producing cells and increased numbers of IL-12 producing cells, but differences were not significant, very probably because of the small subject numbers (8 in trimer, 10 in fragment mixture and 8 in placebo groups). The results from the various antibody and cytokine measurements provide an encouraging basis for pursuing the further development of hypoallergenic derivatives per se, but emphasis has to be placed on the generation of data to provide evidence of clinical efficacy.

An alternative strategy for developing a hypoallergenic derivative came from the observation that recombinant Bet v 1 can be induced to adopt a stable largely random coil structure which can be clearly distinguished from the secondary structure of the native molecule by circular dichroism spectroscopy. This folding variant, designated Bet v 1-FV, exhibits hypoallergenic properties as judged by immunoassay inhibition tests and basophil activation (Fig. 2) (Kahlert et al. 2008; Weber et al. 2003). An open, randomized comparative clinical study with recombinant Bet v 1-FV and a natural birch pollen extract was started in 2003 (Klimek et al. 2005; Narkus et al. 2009). Treatment with aluminium hydroxide adsorbed preparations of the allergens was administered to subjects with birch pollen rhinitis over a period of four months prior to the birch pollen season with injections at weekly intervals. The maximum dose of the hypoallergenic recombinant preparation was 80 µg, approximately four-fold higher than the Bet v 1 in the whole pollen preparation. The combined SMS for subjects receiving the recombinant preparation was favorably less than that for the subjects receiving the natural pollen extract, and the scores for both groups superior to those of a reference group with only anti-symptomatic treatment (parallel control group). A second course of preseasonal immunotherapy was administered prior to the next pollen season, and then both groups showed similar combined symptom/medication scores (Fig. 3) (Kettner et al. 2007a; Narkus et al. 2009). Improvements in specific nasal sensitivity, as judged by a nasal provocation test, were seen in both study groups, and the immunogenic activity of the preparations was confirmed by their ability to induce strong IgG1 and IgG4 antibody responses. Safety data indicated that the preparations were comparable with respect to the occurrence of adverse events. A subsequent double blind placebo controlled study in subjects with symptoms of rhinoconjunctivitis, and with more than 100 subjects in each arm, has confirmed the clinical efficacy of the recombinant preparation (Kettner et al. 2007b). Symptoms and use of rescue medication were documented

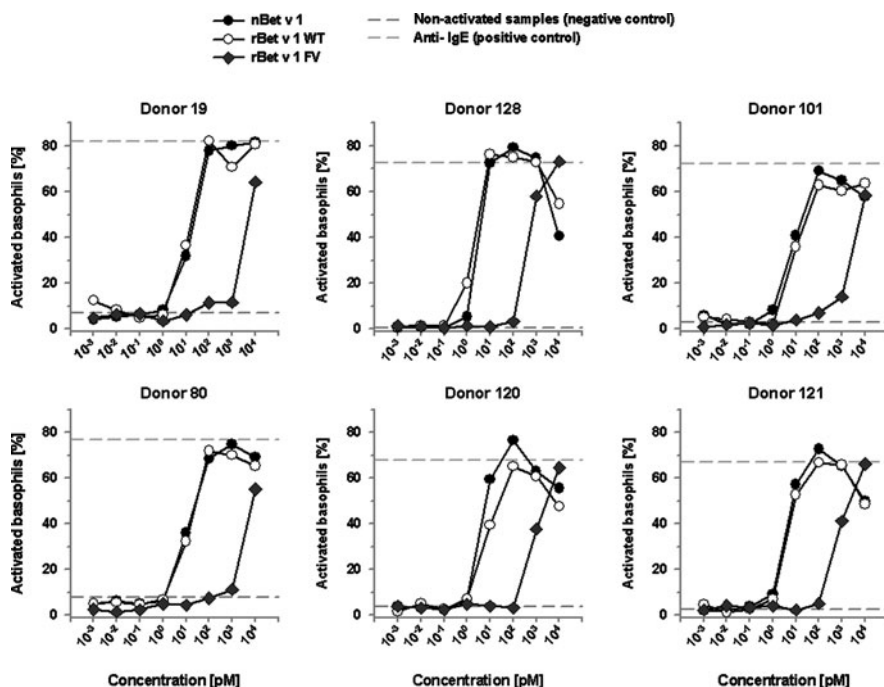


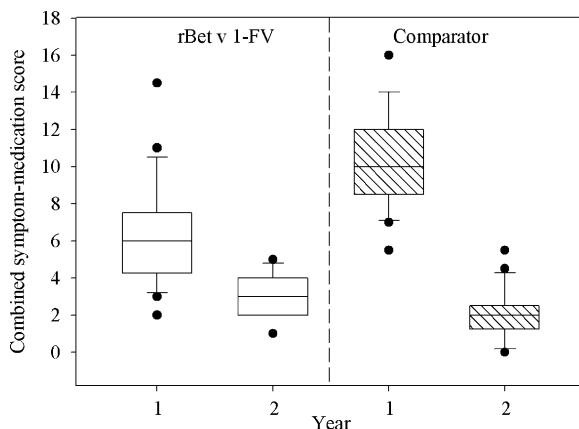
Fig. 2 Stimulation of basophils from whole blood with different concentrations of nBet v 1, rBet v 1-FV and rBet v 1-WT. Basophil activation was determined using CD203c expression. The proteins were tested in serial dilutions in a concentration range from 0.001 to 10,000 pM (Kahlert et al. 2008)

in a baseline year. Subjects received an 18 month course of immunotherapy starting four months prior to the expected pollen season such that they were established on a maximum maintenance dose of 80 μ g prior to pollen exposure. Therapy was continued through out the end of the subsequent pollen season during which the assessment of combined symptom/medication scores, expressed as median area under the curve, showed a significant benefit for the group treated with the recombinant hypoallergenic preparation by comparison with placebo with a score of 207.8 as opposed to 389.6. A sub-group of asthmatic subjects showed an even larger reduction in combined SMS. Strong allergen specific IgG4 antibody responses were observed in all subjects that received active treatment and no serious drug related events occurred. Further studies with this recombinant Bet v 1 folding variant are in progress.

5 Recombinant Cat Allergen Fel d 1

A recombinant form of the major cat allergen Fel d 1 has been engineered to include a linker sequence between the two chains of the protein (Grönlund et al. 2003). This has in turn been fused to a TAT-derived protein translocation domain

Fig. 3 Combined symptom-medication scores in birch pollen seasons following 1 or 2 years of personal subcutaneous immunotherapy with a recombinant Bet v 1 folding variant (Bet v 1-FV) or a birch pollen extract (Comparator). Boxes show median values with 25th and 75th percentiles, error bars the 10th and 90th percentiles, and points the outliers



and a truncated invariant chain for targeting the MHC class I pathway. This construct, designated MAT-Fel d 1 (Modular-Antigen-Translocation-Fel d 1), induces a shift away from a Th2 cytokine profile in cultures of human peripheral blood mononuclear cells (PBMC) (Crameri et al. 2007). This observation is supported by studies in mice which have shown that MAT-Fel d 1 stimulates higher IgG2a responses than Fel d 1 alone, leading to higher IgG2a:IgG1 ratios that are indicative of a bias toward a Th1 response (Martinez-Gomez et al. 2009). The MAT-Fel d 1 was also shown to have hypoallergenic characteristics in so far as it failed to induce anaphylaxis in sensitized mice and the stimulation of leukotriene generation by human basophils required 100-fold higher concentrations of MAT-Fel d 1 than recombinant Fel d 1 alone (Martinez-Gomez et al. 2009).

MAT-Fel d 1 has been tested in a first clinical study involving three intralymphnode injections of 1, 3 and 10 μ g of the MAT-construct adsorbed to alum at four week intervals (Senti et al. 2009). Efficacy has been assessed by provocation testing, as to have various immunological parameters, but at the time of writing detailed results have not been published.

6 Ragweed Amb a 1

The major allergen of short ragweed, Amb a 1, is not as yet available as a recombinant protein for clinical studies. A purified natural Amb a 1 in combination with immunostimulatory sequences (ISS) has however been tested in the clinic (Creticos et al. 2006). These ISS bind to Toll-like receptor 9 expressed predominantly on plasmacytoid dendritic cells, and thereby mediate the down-regulation of Th2 responses. The Amb a 1 was coupled with four ISS per molecule, with the result that immunogenicity was biased in favor of a Th1 response and the allergen showed reduced IgE-binding reactivity, factors which would be considered beneficial for a more effective and safer immunotherapy (Tighe et al. 2000). A pilot study with six

weekly injections prior to the pollen season showed better peak-season nasal symptom scores and improved quality of life scores by comparison with placebo (Creticos et al. 2006). There were only transient increases in allergen specific IgG, but seasonal increases in IgE were suppressed. A follow-up study gave disappointing results and the clinical development program was discontinued.

7 Where Do We Go from Here?

Recombinant DNA technology offers the prospect of a new generation of products for allergen specific immunotherapy. Products will be better defined than those derived from allergen extracts, containing highly purified active ingredients in defined concentrations that provide optimal clinical benefit. The advantages of hypoallergenic variants in respect of achieving adequate dosage and safety have already been established with chemically modified allergen extracts (allergoids), and the development of recombinant variants promises still further benefits. However it is clear that careful attention has to be paid to the selection of variants that are likely to be advantageous to a very large majority of patients selected for treatment.

The clinical development programs for products to treat some of the most common causes of allergic diseases are now gathering momentum and there are good grounds to believe that they will meet with success. The first products that are likely to be introduced on the market will provide an alternative to those derived from allergen extracts, and will be intended for the treatment of IgE sensitizations to birch and grass pollen. The prospect of recombinant products that are tailored to match the sensitization patterns to individual allergens from one source is something for the much longer term.

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