Development of Polymerized Allergens for Immunotherapy*

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Immunotherapy has been used for the treatment of allergic rhinitis since its description in 1911 by Noon and Freeman. The efficacy was only demonstrated within the past few decades, however, when double-blind clinical trials were performed.[1] In spite of the known efficacy in properly selected patients, there are still difficulties with conventional immunotherapy. The most important medical problem in administration of current immunotherapy is that there is a risk of systemic allergic reactions and even death.

Another problem with currently available allergens is the time involved. This is usually 3 to 5 years if patients are given perennial therapy and is possibly longer if patients are given annual preseasonal therapy. A related difficulty is the cost involved. Injection fees, parking fees, time lost from work, baby sitter fees, and other associated outlays over 3 to 5 years can amount to substantial sums.

As a consequence of the difficulties with conventional immunotherapy, several investigators have attempted to improve upon usual aqueous allergens. The goal of these investigators has been to alter allergens so that specific IgE synthesis would be halted[2,3] or so that the immunogenicity of the allergen would be retained while its allergenicity would be reduced. At present, there have been no successful human trials in which altered allergens have eliminated specific IgE. There have been several modifications including oil emulsion repository therapy,[4] formaldehyde treatment,[5] and glutaraldehyde treatment/tyrosine absorption[6] that have reduced allergenicity. Oil emulsion therapy has been abandoned because it could result in sterile abscesses and because sarcomas were found to be caused in mice by mineral oil, which used in the emulsion therapy. Trials are continuing with the other two altered allergens.

Polymerization of allergens is an extensively documented and highly successful method of producing allergens with reduced allergenicity and retained immunogenicity.[7] Polymerization of allergens results from cross-linking protein with glutaraldehyde to form high molecular weight polymers. Fractionation of the cross-linked proteins by gel filtration chromatography allows selection of the desired molecular weight range.[8]

A number of desirable new properties result from the increased molecular weight of polymerized allergens. First, because a higher molecular weight cross-linked polymer of 100 allergen molecules, for example, has less surface area than the surface area of 100 individual allergen molecules, the polymer would have fewer exposed antigenic determinants to react with IgE antibody on mast cells. Secondly, because of distance between mast cells and other steric factors, it would be very likely that a polymer of 100 allergen molecules would cross-link far fewer pairs of IgE molecules on the surface of a mast cell than the 100 individual allergen molecules would be expected to cross-link. Finally, high molecular weight polymers have been demonstrated to diffuse more slowly from the injection site than would individual allergen molecules and therefore polymer has less opportunity to interact with IgE sensitized mast cells. As a consequence of the above, the allergenicity of polymerized allergens would be expected to be less than that of their monomeric counterparts. By cutaneous endpoint titrations of weight equivalent amounts of monomeric and polymeric allergens, it has been confirmed that the allergenicity of polymers is approximately 1/100,-000 of the weight equivalent monomer.[9] This allows higher safe starting doses, fewer systemic reactions and fewer injections for a course of polymer immunotherapy than for conventional immu-

notherapy.[10,11,12,13] Another probable ramification of polymerized allergen immunotherapy is that a greater number of patients will desire immunotherapy because of the reduced number of injections and the earlier onset of symptom amelioration. Significantly, even though polymerized allergens have reduced allergenicity they have been demonstrated to be as immunogenic in man as weight equivalent amounts of monomeric allergens.

In preparation of polymerized allergens, the first model was ragweed antigen E.[11] The study patients had histories of classic ragweed rhinitis confirmed by skin tests. The first trial compared no treatment, polymerized antigen E, and monomer antigen E. Symptom scores were recorded by patients during the ragweed season. Untreated patients had significantly higher symptom scores than patients in either of the treated groups. Patients treated with monomer antigen E had scores similar to patients treated with polymerized antigens. Very importantly, the incidence of systemic and local allergic reactions was significantly lower in the polymer treated group than in the monomer group; eight systemic reactions occurred in monomer ragweed patients while only 2 occurred in the polymer group. We have subsequently tallied our total experience with all polymerized allergens and found that the incidence of systemic reactions per patient course is 0.5 per cent which is markedly superior to quoted figures for conventional immunotherapy of 8-30 per cent per patient course.[14]

Because of the success of the trial of polymerized antigen E therapy, whole pollens were polymerized. For example, whole crude ragweed was partially purified, polymerized and fractionated to produce polymerized ragweed (PRW), a material that has been extensively standardized and studied in humans.[15] Currently, it is standardized by two criteria. PRW must have a molecular weight between 150,000 and 20

million as determined by gel filtration chromatography. It must be 4 to 6 logs less allergenic than monomer as determined by cutaneous endpoint titration. It has also been demonstrated that preparations so standardized will fall within 0.8 log of each other in a radioimmunoassay. The first trial of PRW immunotherapy compared PRW, monomer ragweed (MRW), and no therapy.[10] PRW and MRW treated patients had similar improvement in symptom scores as compared to untreated patients and they had similar rises in blocking antibody. Significantly, while none of the PRW patients experienced systemic reactions, 7 of the MRW patients experienced systemic allergic reactions; with regard to local reactions, MRW patients had 15 times as much induration and 40 times as much erythema as PRW patients. It is also of note that 3 of 9 MRW patients were unable to tolerate the target maintenance dose of 2500 PNU due to large local or systemic reactions while all 10 PRW patients tolerated 2500 PNU without difficulty.

In a multi-institutional trial of PRW[12] involving 80 patients in 4 cities, 90 per cent of the patients reported a reduction in symptoms as a result of PRW therapy. The treated patients in this study had an average 12 fold increase in blocking antibody to antigen E. Several of those patients were studied and found to have blocking antibody to antigen K, Ra 3[16] and Ra 5 demonstrating that the 4 allergens studied had been retained in the polymerization process. In this group, there were no systemic allergic reactions and there were no changes in usual routine laboratory tests after therapy with PRW.

Most recently, in a double-blind histamine placebo controlled trial of PRW,[13] efficacy, immunogenicity and safety were again confirmed. PRW treated patients had significantly lower symptom scores than placebo patients, a 40 fold rise in blocking antibody, no systemic reactions and no changes in usual labora-

tory parameters. In addition to the PRW and histamine placebo groups, there was an untreated group whose symptom scores were almost identical to those of the histamine placebo group (p = 0.42).

Grass pollens were partially purified in a fashion similar ragweed; initally the pollens were mixed and then polymerized.[17] These preparations were found to be less allergenic than unmodified grass pollen and to be as immunogenic in humans as unmodified grass pollen.

In our most recent study of polymerized grass, each individual pollen was purified and then polymerized.[18] Then, after individual polymerization of grass pollens, mixtures were made. The mixtures were individualized so that a patient only received those pollens to which the patient had skin reactivity. In a double-blind histamine placebo controlled trial of individually polymerized grass (IPG), efficacy was demonstrated.[19] Symptom scores were significantly lower (p< 0.02) in the IPG group than in the histamine placebo group. In the IPG treated group there was a significant 9 fold rise in blocking antibody to Rye Grass Group I, a major allergen in northern grasses. There was no change in usual laboratory parameters after IPG therapy and there were no systemic reactions to IPG.

Because PRW and polymerized grass are less allergenic than monomer ragweed or monomer grass, we studied six patients who were unable to be advanced to usual maintenance doses of standard extracts because of severe local or systemic allergic reactions or both.[20] Each of these patients was converted to PRW and polymerized grass and then was advanced to usual maintenance doses with no allergic reactions. All had marked rises in blocking antibody to antigen E and Rye Grass Group I.

We have also prepared polymerized whole partially purified tree pollens.[21] Just as grass pollens were initially mixed and then were polymerized, tree pollens were so prepared. This polymerized prepara-

tion was demonstrated in humans to be four logs less allergenic than the corresponding monomer. Subsequently, we have prepared individually polymerized tree (IPT) pollens: white oak, elm and box elder.[22] It is planned that *in vitro* and *in vivo* characterization of IPT will be followed by human trials with the IPT.

Individual hymenoptera venoms have been polymerized, but do not form soluble polymers as pollens do but form insoluble precipitates.[23] When polymerized insoluble bee venom (PIBV) is injected into rabbits or humans, it is immunogenic. We have also polymerized all of the other hymenoptera venoms: yellow jacket, yellow hornet, white faced hornet, and wasp. All form insoluble precipitates and are immunogenic in animals but better preparations are necessary based on initial human studies.

Several questions about polymerized allergens are asked often. Among these are the following:

Is there any risk in the administration of polymerized allergens?

Systemic reactions to polymerized antigen E and to PRW have occurred: they were mild and easily reversed. Compared to the risk per patient course of immunogenically equivalent dose of unmodified extract (8-30%), the risk of systemic reactions to polymerized allergens is definitely reduced (0.5%). However, the same precautions taken for standard aqueous extracts, will be required. The managing physician must observe for and treat any systemic reaction.

Would the availability of PRW and IPG alter the practice of allergy?

It is very likely that more patients will be treated with immunotherapy because the reduced number of injections and reduced time to amelioration of symptoms might make immunotherapy more acceptable to patients.

When available, of what value would PRW and IPG be in a patient with sensitivities to multiple aeroallergens?

Patients with multiple aeroallergen seansitivites could be treated with standard mixtures of aqueous inhalant allergen extracts. However, as ragweed and grass pollens tend to cause more symptoms than other inhalant allergens and as ragweed and grass extracts tend to contribute to local and systemic reactions more than other inhalants, the physician might choose to give patients IPG, PRW and standard aqueous extracts of other aeroallergens.

Summary

Polymerized allergens, the model being ragweed, are safe and effective. The number of injections is 1/5 that of conventional therapy and the systemic reaction rate is 0.5 per cent per patient course. Studies of ragweed and grass polymers are complete and studies of tree polymers are in progress.

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