

Regulation of the human immune response to ragweed pollen by immunotherapy. A controlled trial comparing the effect of immunosuppressive peptic fragments of short ragweed with standard treatment

A. LITWIN*, A. J. PESCE†, T. FISCHER‡, M. MICHAEL‡ and J. G. MICHAEL*

Departments of *Molecular Genetics, Biochemistry and Microbiology, †Pathology, and ‡Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH, U.S.A.

Summary

A new allergenic preparation consisting of peptic fragments of short ragweed has been tested for its clinical effectiveness. Such enzymatically derived fragments have been shown in prior murine studies to retain the T epitopes of the original allergen but to have a severe reduction in the number of B epitopes. Three groups of ragweed hayfever patients were placed on pre-seasonal immunotherapy. One group received a conventional ragweed preparation that had been enriched for antigen E (*Amb a* I), designated as Pool 2. The second group was given fragments of Pool 2 (fSRW) prepared by peptic digestion and the third group was injected with histamine as a placebo. Groups treated with the fSRW and Pool 2 had significantly reduced symptom-medication scores compared with the placebo-treatment group. However, fSRW-treated patients fared significantly better than Pool 2 patients ($P < 0.02$). fSRW injections caused a significant rise in preseasonal specific IgG antibodies as well as suppression of the seasonal anamnestic specific IgE increase. Similar, but not quite as marked changes occurred with Pool 2 treatment. fSRW was well tolerated and non-toxic. Thus, allergen modification by enzymatic degradation, as demonstrated here, appears to be a promising new approach for allergen immunotherapy.

Clinical and Experimental Allergy, Vol. 21, pp. 457-465. Received 1 June 1990; revised 11 January 1991; accepted 17 January 1991.

Introduction

Allergen immunotherapy has been in clinical use since 1911 [1]. It consists of the repeated subcutaneous injection of an allergen into a specifically sensitized individual inducing alleviation of allergic symptoms. Well-controlled studies have left little doubt as to its efficacy in the treatment of allergic rhinitis [2,3], allergic asthma [4] and insect venom anaphylactic hypersensitivity [5]. However, as presently administered, immunotherapy has several disadvantages including the cumbersome need for frequent repeated subcutaneous injections, local and systemic reactions, and as an end result, at least in the case of

allergic rhinitis and asthma, only a partial decrease in symptoms rather than a 'cure.' One approach to overcome many of these problems has been the endeavour to develop modified allergens. These attempts have included preparation and clinical trials with urea-denatured allergens [6], allergoids created by formalin treatment of allergens [7], allergens bound to tolerogens such as D-glutamic acid-D-lysine [8] or polyethyleneglycol [9] and glutaraldehyde-polymerized allergens [10]. These modifications have as their primary aim an effect on immunological events that occur during immunotherapy. Such events include a rise in specific 'blocking' IgG antibodies, the blunting of the seasonal increase in specific IgE antibodies, eventual reduction in specific IgE antibodies below pretreatment levels and finally, the induction of suppressor T cells. A secondary goal for allergen modifi-

Correspondence: Allen Litwin, MD, University of Cincinnati, Molecular Genetics, Biochemistry & Microbiology, MSB-M.L. 524, 231 Bethesda Avenue, Cincinnati, OH 45267, U.S.A.

cation has been to decrease the need for frequent injections.

Studies in our laboratory have shown that an ongoing IgE response to a ragweed antigen E (*Amb a 1*) enriched fraction could be suppressed in mice by intravenous injection of peptic fragments of this fraction (unpublished observations). In similar studies, an ongoing murine IgE response to honey bee venom phospholipase A₂ (PLA₂), the major allergen present in honey bee venom, could also be suppressed by intravenous injections of P1, a large peptide fragment of PLA₂ [11]. In a subsequent human study, injections of P1 were shown by us to partially suppress IgE antibodies to PLA₂ in honey-bee venom-allergic patients as well as causing fewer local and systemic reactions than intact PLA₂ [12].

The purpose of the present study was to extend these observations to the treatment of humans with ragweed hayfever. Peptic fragments of a short ragweed fraction enriched for *Amb a 1* (fSRW) were used. In our prior human pilot study fSRW was shown to be clinically effective and, with higher doses, produced 'desirable' immunological benefits, such as partial abrogation of the seasonal anamnestic rise in ragweed specific serum IgE antibodies [13]. In this study, we compare the efficacy of high-dose treatment with these fragments, with results obtained with a regimen of a conventional ragweed allergen preparation.

Subjects and methods

Subjects

All subjects were volunteers recruited through ads placed in a local newspaper. To be entered in the study, each individual was required to be aged between 21 and 50 years, have a history of allergic rhinitis limited to August and September for at least 2 consecutive years, not to have received immunotherapy for at least 2 years, have a negative history and physical examination for cardiovascular disease, and, if a woman, not be pregnant nor expect to become pregnant during the course of the study. In addition, each subject had to have a positive prick test to short ragweed with appropriate positive and negative control skin tests. A physical examination, complete blood count, sedimentation rate, urinalysis, and a biochemical profile were done prior to initiation of the study, repeated at 5 months and again at the end of the study. The study was approved by the Human Research Committee of the University of Cincinnati College of Medicine, as well as the Human Research Committee of the Jewish Hospital, Cincinnati, Ohio. All patients signed an informed consent.

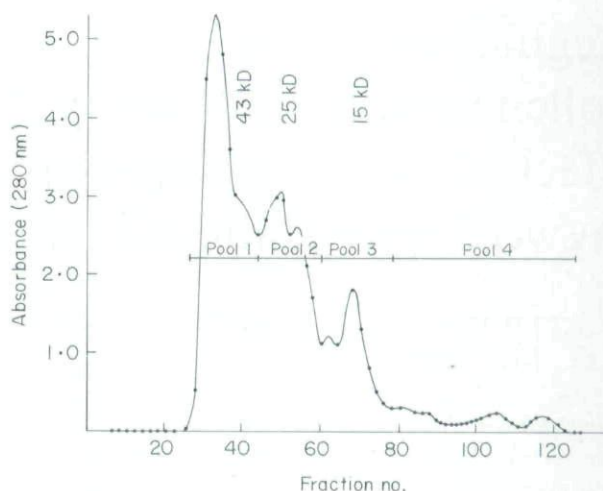


Fig. 1. Separation of fraction A in Sephadex G-50.

Allergen preparations

Defatted short ragweed pollen (Greer Laboratories, Lenoir, NC) was extracted with distilled water for 22 hr. Fraction A of short ragweed was prepared by precipitation of this extract with 90% (NH₄)₂SO₄. The re-dissolved precipitate (containing 5% *Amb a 1* by the Mancini technique [14]) was chromatographed on Sephadex G50 (Fig. 1). The fractions between 25 and 43 kD, Pool 2, were collected, dialysed against saline, Millipore-filtered and lyophilized. The proteins in Pool 2 contained about 26% *Amb a 1*. Pool 2 was dissolved in water and enzymatically digested as follows: Pool 2 at a concentration of 2 mg/ml was incubated with porcine gastric pepsin (activity 2660 U/mg), 1 mg/ml, at 150:1 protein:pepsin ratio in water for 4 hr at pH 3.0. After incubation, the pH of the digest solution was raised to 8.6 with NH₄HCO₃ and lyophilized. The pepsin was spontaneously denatured at this step. The lyophilized digest was rehydrated and fractionated on a Sephadex G50 column with 0.1 M ammonium acetate (pH 7.0) (Fig. 2). The second peak, fSRW, which contained the small peptides from the degradation of Pool 2 proteins, was collected, concentrated to 10 mg/ml, and lyophilized. fSRW and Pool 2 were the two allergen preparations used in this study. fSRW had an average molecular weight of about 10 kD or less as measured on a calibrated Sephadex G50 column. It was shown to be free of pepsin by PAGE in the presence and absence of SDS. The precise *Amb a 1* content in fSRW could not be determined as fSRW would not precipitate in agar when reacted with anti-*Amb a 1* antiserum. Rehydration of Pool 2 and fSRW was done with human serum albumin saline solution (Albay; Hollister-Stier, Spokane, WA). A single lot of lyophilized fSRW and Pool 2 was used throughout the study.

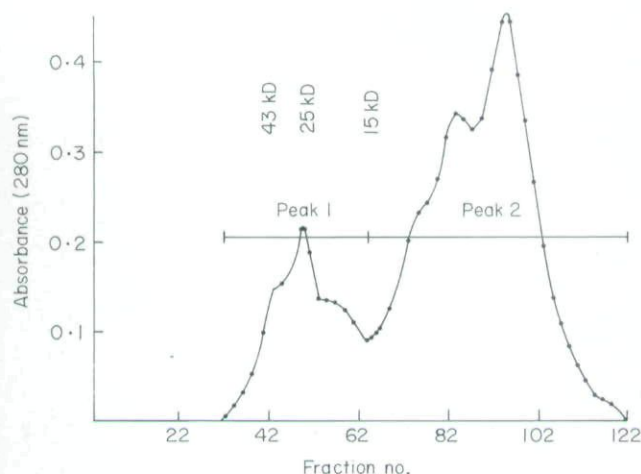


Fig. 2. Separation of pepsin digest of Pool 2 on Sephadex G-50.

Skin tests

Prick tests were done with both short ragweed 1/10 and *Alternaria tenuis* 1/20 in glycerin-saline (Hollister-Stier). Glycerin-saline was used for a negative control and histamine (1.0 mg/ml) as a positive control. The criterion for a positive prick test was the presence of induration of ≥ 3 mm in cross-diameter greater than that observed with the negative control 15 min after application of the test.

Intradermal end-point titrations. End-point titrations were performed by intradermal injection of 0.02 ml into the volar surface of the forearm of 10-fold dilutions of Pool 2, fSRW and Fraction A. Again, histamine (0.001 mg/ml) and saline were used as positive and negative controls. An intradermal skin test was considered positive when it exhibited cross-diameter induration of ≥ 6 mm greater than that observed with the saline control 15 min after application. End-point titrations of all allergens were done prior to the study and were repeated only with Pool 2 at the completion of the study.

Serological assays

Blood was drawn prior to initiation of therapy, prior to the onset of ragweed season, and in mid-October at the end of the ragweed season.

Specific IgE antibodies were determined by a RAST assay using filter paper-disks to which the Pool 2 fraction of short ragweed was coupled [15]. ^{125}I -labelled anti-human IgE (Pharmacia Fine Chemicals, Piscataway, NJ) was used to quantify the assay. Serum was serially diluted so that the c.p.m. were in the range of 1500–5000. The linearity of the response was confirmed using a single high titred human anti-short ragweed serum as a control in

each analytical run. The constancy of the control dilution was used to validate assay reproducibility. All sera for each individual were batched and run simultaneously in duplicate. The intra-assay variation was 14% and the interassay variation was 10%.

Specific IgG antibodies were measured by ELISA on polystyrene plates (Dynatech Immulon; Baxter Healthcare Corporation, Oletz, OH) coated with Pool 2. The test serum was diluted 1/20 to 1/1028 in two-fold serial dilutions. A goat anti-human IgG antibody coupled to alkaline phosphatase (Zymed, Burlingame, CA) was used as the indicator [16]. A standard serum was given the value of 100 U. Test sera and the standard serum results were calculated with selected dilution giving a color in the linear range. Then, the unit equivalent of the test serum was calculated by comparing the ratio of dilutions. The intra-assay variation was 16% and the interassay variation was 15%.

Experimental plan. All patients included in the study were selected from individuals replying to our advertisement. Patients were matched by their end-point skin test titration results to Pool 2 and placed into one of three groups by one of the investigators who had no contact with the patients at any time during the study (J.G.M.). The study was double-blinded throughout. One physician was responsible for assigning the doses of allergen or placebo to be administered and another who was unaware of which treatment each patient was receiving was responsible for clinical management of the patient during the ragweed pollination season. One patient group received subcutaneous injections of Pool 2 weekly beginning with 0.10 ml of 0.001 $\mu\text{g/ml}$ Pool 2 with a goal of achieving a final dose of 0.50 ml of 100 $\mu\text{g/ml}$ Pool 2 (approximately 13 μg *Amb a I*). The second group received subcutaneous injections of fSRW beginning with 0.10 ml of 1 μg fSRW/ml with the goal of achieving a final dose of 0.50 ml of 1 mg/ml fSRW (approximately the equivalent of 130 μg of *Amb a I* based on the Pool 2 content of *Amb a I*). The third group received graded doses of histamine phosphate 1 mg/ml in sufficient dosage to produce local erythema and a small weal. Injections in each group of patients were given weekly or bi-weekly when the patients had achieved a maintenance dose. The amount of allergen injected on each visit was doubled from the previous visit unless the patient incurred a significant local or systemic reaction (urticaria, respiratory tract symptoms, etc.). When solutions with the highest concentration of Pool 2 (100 $\mu\text{g/ml}$) or fSRW (1 mg/ml) were used, the rate of increase was decreased to 0.05 ml increments with each injection. Each patient filled out a reaction report after every injection recording any symptoms or signs of an injection reaction for a 48-hr

Table 1. Clinical characteristics of patient groups

Treatment Group	Subjects (n)	Mean age (years)	Mean end-point skin test titre to Pool 2	Mean end-point skin test titre to fSRW	Prick test to Alternaria (Positive/total)
Pool 2	9	35.9	$10^{-3.8}$	$10^{-1.0}$	6/9
fSRW	10	34.8	$10^{-4.2}$	$10^{-1.2}$	6/10
Placebo	8	37.8	$10^{-4.2}$	$10^{-0.9}$	3/8

period. All treatment in each group was discontinued prior to the onset of the ragweed season. The effect of treatment on ragweed hayfever symptoms was assessed from 1 August to mid-October, 1987. The initial 2 weeks of recording were to acquaint the patients with the accurate recording with the symptom-medication cards [17]. Each subject graded his symptoms for each 12-hr period, 12 noon until midnight and from midnight until 12 noon. Separate ratings were given for sneezing; stuffy nose; red itchy eyes; coughing; and number of antihistamine tablets taken. The antihistamines included chlorpheniramine 4 mg or tripelemine 50 mg and/or pseudoephedrine 60 mg which could be taken with one of the antihistamines. A third alternative antihistamine-decongestant was RondecTM tablets. The choice of an antihistamine was dictated by the patient's preference based on side-effects encountered, efficacy, etc. Each system was graded on a scale of 0-3: 0, no symptoms; 1, symptoms for less than 30 min; 2, symptoms for 30 min to 2 hr; and 3, symptoms for more than 2 hr. The number of antihistamine tablets taken was added to the total symptom score to arrive at a daily total symptom-medication score for each patient. This is the same scoring scheme utilized by the Johns Hopkins group in their numerous investigations of allergen immunotherapy [17]. The potential total daily symptom-medication score ranged from 0 to 30. Efficacy of blinding was assessed by questioning each patient as to which treatment group he or she thought they were assigned in mid-August when the injections in all groups had ceased.

Pollen counts were measured daily from 1 August to 10 October 1987, using a rotorod counter which was placed on the roof of Children's Hospital Medical Center, Cincinnati, Ohio.

Statistical analysis

Statistical analyses were performed using the CLINFO system and SASS. The Wilcoxon two-sample test and the Wilcoxon rank sum test were employed for evaluating the data. When applicable, Student's *t*-test was used, as stated in the text.

Results

Clinical results

Homogeneity of groups. Table 1 depicts the mean age and end-point skin test results in all three groups as well as the skin-prick test response to Alternaria. There were no statistically significant differences between the three groups when comparing the initial endpoints to Pool 2 or fSRW. The fSRW skin test end-point was approximately 3 logs less than that found with Pool 2 in all patient groups, indicating that peptic fragments of Pool 2 had markedly diminished skin test reactivity. This implied that patients might tolerate very high doses of fSRW compared to Pool 2. In our previous human studies using fSRW [13], this proved to be the case.

Immunotherapy regimen. Injections were initiated in late October of 1986 and continued until mid-August, 1987. Initially 10 patients received Pool 2, 10 patients received fSRW, and nine patients received placebos. Two patients dropped out of the study, one was receiving Pool 2, and one was receiving placebo. Only one patient treated with Pool 2 reached the highest planned dose (50 µg). The range of final doses in this group was 1-50 µg, with a mean final dose for the group of 19.1 µg (containing about 5 µg Amb a 1). Three patients treated with fSRW reached a final dose of 500 µg. The range of final doses of fSRW varied from 5 µg to 500 µg with a mean final dose of 265.5 µg. This was 14 times the mean final dose achieved in Pool 2-treated patients on a weight basis and obviously was much greater on a molar basis. The reason for patients not achieving the highest dose was most frequently non-compliance with the injection regimen and, secondly, the occurrence of local and systemic reactions to the injections which resulted in a reduction of dosage and modifications of the incremental increase of dosage with each injection. Thirteen local reactions (weal > 2.5 cm in cross-diameter) and one systemic reaction occurred in Pool 2-treated patients, while fSRW-treated patients incurred 10 local reactions and one systemic reaction. Placebo-treated patients had two large local reactions and

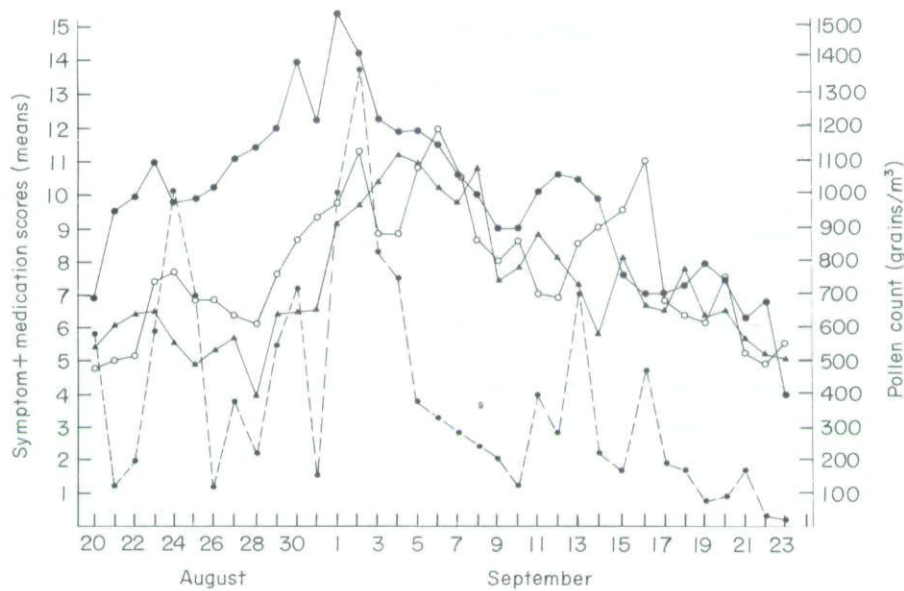


Fig. 3. Mean daily symptom-medication scores of each treatment compared with pollen counts (●—●) in the 1987 ragweed season. ●, placebo; ○, Pool 2; ▲, fragment.

no systemic reactions. In spite of the reactions, good blinding was achieved as the patients' responses to the question of which treatment they thought they were receiving were entirely random.

Response to immunotherapy in each patient group. The subjects were essentially asymptomatic at the initiation of the study which was confirmed on the initial physical examination. Figure 3 shows the mean total daily symptom-medication scores in each treatment group and the daily pollen count for the 35-day period 20 August 1987 to 23 September 1987. This period of time encompassed the onset and decline of ragweed-induced symptoms in the three groups of patients (pollen counts prior to 20 August were less than 100 grains/m³ and symptom-medication scores were very low in all three groups). When the pollen counts were high (20 August to 5 September and 11 September to 15 September), there was a marked separation of scores between the three groups of patients. However, when pollen count dropped, the scores tended to merge. Thus, the merging of symptom-medication scores between the three groups of patients in the latter portion of the ragweed pollination season correlates with and seems to be the result of the sudden and sustained drop of airborne ragweed pollen.

The mean total symptom-medication scores for the entire study in Pool 2-treated patients was 7.85 and the median was 8.0; for fSRW-treated patients the mean was 7.47 and the median 5.0, and for placebo-treated patients, the mean was 9.97 and the median 8.0. Both active

treatment groups' scores were significantly different from placebo patients, with Pool 2 *vs* placebo $P=0.4$, and fSRW *vs* placebo $P=0.0001$. There was also a statistically significant difference between the scores of the two active treatment groups with fSRW treatment scores significantly lower than Pool 2 scores, $P<0.02$. Restricting the statistical analysis only to the period of high pollen challenge, i.e. August 20 to September 5, the results are similar except that now both active treatment groups' symptom-medication scores show even greater statistical significance when compared to placebo treatment. Thus, Pool 2 patients (mean score for this period 7.71, median score 7) compared with placebo patients (mean score 11.4, median score 10) achieves $P=0.001$ and fSRW treatment (mean score 7.09, median score 5) *vs* placebo has $P<0.0001$. The two active treatment groups are still significantly different from each other, with $P=0.048$ (fSRW scores < Pool 2).

Toxicity studies. Repeat physical examinations and clinical laboratory studies remained normal in all patients throughout the study including at the termination.

Specific IgE responses

Effect of immunotherapy regimens. As shown in Table 2, a small increase in specific IgE occurred in both treatment groups prior to the ragweed season with Pool 2-treated patients having an increase of +14.2% and fSRW patients +3.9%. A small decline in specific IgE of -9%

Table 2. Mean percentage of change in antibody level

Group	Specific IgE		Specific IgG	
	October–August (effect of immunotherapy injections)	August–September (effect of seasonal challenge)	October–August (effect of immunotherapy injections)	August–September (effect of seasonal challenge)
Pool 2	+14.2%	+14.7%*	+106.4%*	–3.2%*
fSRW	+3.9%	+9.9%*	+59.1%*	–10.1%*
Placebo	–9.0%	+89.0%	+1.0%	+26.4%

* Statistically significant from placebo, $P < 0.05$.

occurred among placebo-treated subjects. Presumably the rise in the two treatment groups was caused by immunotherapy. The differences between all the groups were not statistically significant when compared with each other ($P > 0.05$ in each instance).

Effect of seasonal exposure to ragweed. Placebo-treated patients showed a mean rise in specific IgE of +89.0%. Both allergen treatment regimens abolished this expected increase. fSRW-treated patients showed the most profound effect with a mean increase in specific IgE of only +9.9%. An actual seasonal decline of specific IgE was found in four out of 10 of these patients and there was an increase of +11% or less in eight out of 10 patients. This difference as compared with controls was statistically significant ($P < 0.01$). Pool 2-treated patients also experienced a diminished anamnestic response of specific IgE as compared with placebo-treated subjects with a mean increase of +14.7%. An actual decline of specific IgE was found in three out of nine patients, and five out of nine had an increase of +11% or less. These results were statistically significant compared with controls, $P < 0.01$. Although fSRW treatment seemed to produce a more suppressive effect than Pool 2 treatment, the difference between the two groups was not significant ($P > 0.05$).

Specific IgG

Effect of immunotherapy regimen. Prior to the onset of the ragweed pollination season, all patients in each of the two active treatment groups, Pool 2 and fSRW, showed a significant increase in specific IgG antibodies ($P < 0.05$, Student's *t*-test) which presumably was secondary to their injections. The mean increase of specific IgG was 106.4% in Pool 2-treated patients and 59.1% in fSRW-treated patients. This difference between the two groups was not statistically significant ($P > 0.05$, Student's *t*-test). Specific IgG increased 1% in placebo-treated patients.

Effect of seasonal exposure. Placebo-treated patients showed an increase in specific IgG antibodies during the ragweed season (+26.4%). Treatment with either fSRW or Pool 2 prevented a similar seasonal rise in specific IgG antibodies in both of these treatment groups. fSRW-treated patients actually experienced a fall of –10.1% with a lesser fall noted in Pool 2-treated patients, of –3.2%. The decreased seasonal specific IgG found in each of these two groups was statistically significant when compared with the placebo-treated patients ($P < 0.05$) but not when compared with each other ($P > 0.05$).

Skin test end-points. Skin test end-points to Pool 2 were repeated in each patient after completion of the study. There were no significant changes from the pretreatment end-point titre.

Discussion

This study demonstrates convincingly that fSRW is an effective immunotherapeutic agent for use in the management of ragweed hayfever. Furthermore, as an immunotherapeutic agent, it displayed several potential advantages over conventional allergens which were represented in this study by Pool 2. First, injections of fSRW could be tolerated at doses substantially higher (14-fold) than those that could be achieved with Pool 2. Secondly, because very high doses of fSRW could be tolerated, the opportunity to 'immunoregulate' ragweed hayfever more effectively was presented. In the present study this seemed to occur, as small but definite differences were detected clinically and serologically between patients treated with fSRW and those with Pool 2. Symptom-medication scores were statistically significantly lower with fSRW treatment compared with Pool 2 treatment, when patients underwent their seasonal ragweed challenge. Also, levels of specific IgE and IgG antibodies were affected in presumably a more favourable direction by fSRW treat-

ment, as compared with Pool 2. Thus, while patients were receiving injections of fSRW, their levels of specific IgE remained virtually unchanged, while specific IgG antibody levels, which may act as 'blocking' antibodies, rose significantly. Then, when the seasonal ragweed challenge occurred, the expected anamnestic increase in both specific IgE and IgG antibodies was significantly suppressed. Similar serological changes were found in patients treated with Pool 2, but they were not as marked except for levels of specific IgG antibodies, which rose higher in these patients while they were receiving their immunotherapy injections. Thus, the differential help by fSRW for induction of IgE and IgG antibodies with suppression of the former and stimulation of the latter is suggested but not proven by our data. The higher increase in specific IgG with Pool 2 treatment was associated with a lesser clinical benefit than that observed with fSRW. This would imply that so-called 'blocking antibodies' are not the sole cause for clinical improvement with immunotherapy. Other investigators have found a similar lack of correlation between levels of IgG antibodies and clinical benefit [18]. Indeed, a recent study of immunotherapy in ragweed hayfever found that attenuation of nasal reactivity was present very early in the course of immunotherapy before any change in specific IgG antibody levels was induced in the majority of patients [19]. These apparent serological differences between the two active treatment regimens did not reach statistical significance, primarily because of the small number of subjects in each group. Further studies will be required to explore the full potential of fSRW in immunoregulation of ragweed-sensitive patients, and to determine whether these clinical and serological effects can be amplified by sustained high-dose treatment for considerably longer periods of time.

The obvious expectation engendered by the above results, that fSRW might be a more useful immunotherapeutic agent than the presently available intact ragweed proteins, has an immunological basis. We believe that the immunological effects of the molecular alterations induced by enzymatic degradation of ragweed proteins account for this improvement. Specific IgE responses are extremely T cell dependent [20]. The T helper and suppressor epitopes on ragweed proteins control the immune response to inhaled ragweed pollen as well as the modulating effects of injections of ragweed proteins when they are given for immunotherapy. One of the several limiting factors in the use of unaltered allergens in immunotherapy is that intact proteins retain B epitopes which cause them to bind to specific IgE on mast cells and basophils causing allergic reactions. This in turn limits the quantity of allergen that can be injected safely. As high-dose immunotherapy has been shown to be considerably more effective than low-dose treatment, this dosage

restriction may limit the 'favourable' stimulatory effect on T cells by immunotherapy [21]. Limited pepsin degradation of ragweed proteins is an attempt to bypass this problem. B epitopes seem to be altered or partially destroyed in fSRW, as shown by the difference in skin test end-point titration results between fSRW and Pool 2 in all of the patients included in this study (approximately 3 logs) (Table 1). However, T epitopes in fSRW are preserved and remain functional as was illustrated in our prior study in mice by finding equivalent lymphoproliferative responses to Pool 2 and fSRW. In this same murine study, the B epitopes of fSRW were undetectable, as shown by lack of fSRW binding to specific murine IgE and IgG antibodies (unpublished observations). Thus, enzymatically derived allergen fragments have the potential to provoke T cell responses more effectively as large doses can be administered. Standring *et al.* [22] confirmed these findings independently using chymotrypsin treatment of rye grass pollen in their murine study. Their enzymatically derived allergenic fragments also retained T determinants but lost all B determinants.

The nature of the T cell response also can be influenced when using enzymatically derived allergenic fragments. In our prior studies with pepsin-derived fragments of bovine serum albumin (BSA), the preserved T epitopes in such BSA fragments favoured immune suppression rather than help. Thus, the BSA peptic fragments while poor immunogens themselves primed for immunosuppression to a subsequent antigen challenge [23]. This suppression was mediated by T cells.

In selecting a method to create a new immunotherapeutic agent, enzymatic degradation of ragweed proteins was chosen over other methods to isolate small immunoregulatory peptides. Such peptic-derived fragments presumably contain multiple T epitopes with decreased B epitopes. Another approach being pursued by some investigators involves isolation of peptides that contain only a single T epitope. This latter approach still has to be proven effective. As it has been shown that individuals in heterogenous populations may respond to different unique T epitopes on the same protein [24,25], a single T epitope might not suffice for effective allergen immunotherapy in outbred human populations.

The general applicability of our concept to other allergens has already been demonstrated by our experiments with honey bee venom PLA₂ [11,12] and as mentioned above by Standring *et al.* [22] with rye grass pollen. In studies of humans with spontaneous immediate hypersensitivity to honey bee stings and in studies of mice with induced specific IgE antibodies to PLA₂, injections of a peptic fragment of PLA₂ caused a fall in specific IgE antibodies. Also in our experiments in mice, murine-specific IgE antibodies to ragweed using fSRW as an

inhibitor as well specific IgE antibodies to PLA₂ using a peptic fragment of PLA₂ as the inhibitor showed that both fragments had no detectable B determinants.

The number of systemic reactions to allergen injections encountered in this study was small. One followed an injection of fSRW, and one Pool 2 injection. This rate of systemic reactions (10.5%) is comparable to that found in other immunotherapy studies in which a rate of 0.67–15% [reviewed in 26] was observed. It should be reemphasized that far larger doses of fSRW than Pool 2 were used. This relatively low incidence of systemic reactions to fSRW despite the large amounts injected (potentially the equivalent of 130 µg *Amb a I*/500 µg total protein based on Pool 2 content of *Amb a I*) was anticipated by our skin test data and is consistent with a diminished number of B determinants on fSRW. We administered very high doses of fSRW because we wished to determine the clinical and serological effects of such large injection doses. It is quite likely that definite clinical benefit could have been conferred by smaller doses of fSRW which may produce fewer or no reactions in highly sensitive patients. Further studies are needed to resolve this issue.

An additional virtue of the fSRW preparation was that it was non-toxic as physical examinations and a battery of laboratory tests remained normal in all patients treated with this allergen preparation throughout this study as well as in our previous pilot studies [13].

Acknowledgments

We wish to acknowledge the kind assistance of Katherine Dunn, Katherine Jackson, Victoria Hassert, and Margaret Riordan. We thank Ms Barbara Cromer and Mrs Charlotte Creamer for skillfully typing the manuscript. This study was supported by a research grant from the Jewish Hospital, Cincinnati, Ohio and PHS grant AI 25957.

References

- Noon L. Prophylactic inoculation against hayfever. *Lancet* 1911; i:1572–3.
- Lowell FC, Franklin W. A double-blind study of the effectiveness and specificity of injection therapy in ragweed hayfever. *N Engl J Med* 1965; 273:675–9.
- Norman PS, Winkenwerder WL, Lichtenstein LM. Immunotherapy of hayfever with ragweed antigen E: comparisons with whole pollen extract and placebos. *J Allergy* 1968; 42:93–108.
- Van Metre TE Jr, Marsh DG, Adkinson NF, Kagey-Sobotka A, Khattignavong A, Norman PS, Rosenberg GL. Immunotherapy for cat asthma. *J Allergy Clin Immunol* 1988; 82:1055–68.
- Hunt KJ, Valentine MD, Sobotka AK, Benton AW, Amodio FJ, Lichtenstein LM. A controlled trial of immunotherapy in insect hypersensitivity. *N Engl J Med* 1978; 299:157–61.
- Ishizaka K, Okudaira H, King TP. Immunogenic properties of modified antigen E. II. Ability of urea-denatured antigen and α -polypeptide chain to prime T cells specific for antigen E. *J Immunol* 1975; 114:100–15.
- Norman P, Lichtenstein LM, Kagey-Sobotka A, Marsh DG. Controlled evaluation of allergoid in the immunotherapy of ragweed hayfever. *J Allergy Clin Immunol* 1982; 70:248–60.
- Katz D. Recent studies on the regulation of IgE antibody synthesis in experimental animals and man. *Immunology* 1980; 41:1–24.
- Sehon AH, Lee WY. Suppression of immunoglobulin E antibodies with modified allergens. *J Allergy Clin Immunol* 1979; 64:242–50.
- Metzger WJ, Patterson R, Zeiss CR, Irons JS, Pruzansky JJ, Suszko IM, Levitz D. Comparison of polymerized and unpolymerized antigen E for immunotherapy of ragweed allergy. *N Engl J Med* 1976; 295:1160–4.
- Michael JG, Pesce AJ, Litwin A, Freisheim J. Immunosuppressive properties of a phospholipase A₂ antigen fragment. *J Allergy Clin Immunol* 1985; 75:200. (Abstract).
- Litwin A, Pesce AJ, Michael JG. Regulation of the immune response to allergens by immunosuppressive allergenic fragments. I. Peptic fragments of honey bee venom Phospholipase A₂. *Int Arch Allergy Appl Immunol* 1988; 87:361–6.
- Litwin A, Pesce AJ, Michael JG. Use of peptic fragments of short ragweed in treatment of ragweed hayfever. (Abstract). *J Allergy Clin Immunol* 1988; 81:294.
- FDA Potency test on Amb a 1. Section 680.4 of Title 21 of the Food and Drug Administration.
- Hoffman D. The use and interpretation of RAST to stinging insect venoms. *Ann Allergy* 1979; 42:224–30.
- Enguall E, Perlman P. Enzyme-linked immunosorbent assay, ELISA. III. Quantitation of specific antibodies by enzyme-labeled anti-immunoglobulin in antigen-coated tubes. *J Immunol* 1972; 109:129–35.
- Lichtenstein LM, Norman PS, Winkenwerder WL, Osler AG. *In vitro* studies of human ragweed allergy; changes in cellular and humoral activity associated with specific desensitization. *J Clin Invest* 1966; 45:1126–36.
- Lichtenstein LM, Norman PS, Winkenwerder WL. A single year of immunotherapy for ragweed hay fever. Immunologic and clinical studies. *Ann Intern Med* 1971; 75:663–71.
- Hedlin G, Silber G, Naclerio R, Proud D, Eggleston P, Adkinson NF. Attenuation of allergen sensitivity early in the course of ragweed immunotherapy. *J Allergy Clin Immunol* 1989; 84:390–9.
- Michael JG. Thymus dependence of reaginic antibody formation in mice. *J Immunol* 1973; 111:1600–1.
- Franklin W, Lowell FW. Comparison of two dosages of ragweed extract in the treatment of pollenosis. *JAMA* 1967; 201:915–17.
- Strandberg R, Lavender EA, Wheeler AW, Spackman VM, Moran DM. Induction of T-helper cell activity by fragments

- of rye grass pollen extract produced by digestion with chymotrypsin. *Int Arch Allergy Appl Immunol* 1988; 87:337-41.
- 23 Ferguson TA, Peters T Jr, Reed R, Pesce AJ, Michael JG. Immunoregulatory properties of antigenic fragments from bovine serum albumin. *Cell Immunol* 1983; 78:1-12.
- 24 Berzofsky JA, BenSussan A, Cease KB, *et al.* Antigenic peptides recognized by T-lymphocytes from AIDS viral envelope-immune humans. *Nature* 1988; 334:706-8.
- 25 Roy S, Scherer MT, Briner TJ, Smith JA, Gefter ML. Murine MHC polymorphism and T cell specificities. *Science* 1989; 244:572-5.
- 26 Greenberg MA, Kaufman CR, Gonzalez GE, Rosenblatt CD, Smith LJ, Summers RJ. Late and immediate systemic-allergic reactions to inhalant allergen immunotherapy. *J Allergy Clin Immunol* 1986; 77:865-70.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.