

Immunologic responses to conjugates of antigen E in patients with ragweed hay fever

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Allergens conjugated with several simple repeating polymers have reduced allergenicity in man, but large doses retain the ability to suppress ongoing allergen-specific IgE synthesis in strains of high-responder mice. To determine whether suppression of IgE antibodies could be induced in man, preliminary trials of immunologic responses to conjugates in man were carried out in ragweed hay fever patients treated with antigen E (AgE) coupled to methoxypolyethylene glycols (MPEGs) of 5000 and 2000 daltons, lauryloxypolyethylene glycol of 1200 daltons, and a random copolymer of D-lysine and D-glutamic acid of 69,000 daltons. In varying degrees all these conjugates had reduced allergenicity by basophil histamine release when these conjugates were compared with native AgE and could suppress IgE response in mice. Patients received one of these conjugates or native AgE in a series of subcutaneous injections and were observed for allergic reactions. The conjugates induced a lower rate of systemic reaction than native AgE but failed to induce early suppression of IgE antibodies. Instead, early rises in IgE antibody occurred in the several groups and were followed by a slow decline during a year or more that was similar to that observed with standard immunotherapy. Because the conjugates eventually caused local and systemic allergic reactions as the dose was raised, it was not possible to test the IgE-suppressive effects at doses similar to those used in mice. In contrast, rapid sustained rises in IgG antibodies occurred in all groups. The MPEG conjugates appeared to be more effective than native AgE in this regard. The reduced rate of systemic reaction and rapid rise in IgG antibody that was noted with MPEG conjugates make them worth further exploration as agents for immunotherapy. (J ALLERGY CLIN IMMUNOL 73:782-9, 1984.)

Animal experiments mainly in rodents have indicated that antigens that are modified by conjugation to simple repeating polymers have reduced allergenicity yet retain the property of the native antigen in that large doses result in suppression of an ongoing specific-IgE response. These experiments have been performed with haptens such as DNP¹ and penicilloyl² groups and with protein allergens such as ovalbumin and ragweed AgE.^{3, 4} Suppression is specific and is maintained over many weeks. Polymers have in-

Abbreviations used

DNP:	Dinitrophenol
AgE:	Antigen E
PEG:	Polyethylene glycols
DGL:	Copolymer of D-lysine and D-glutamic acid
MPEG:	Methoxypolyethylene glycol
BRIJ:	Lauryloxypolyethylene glycol
SPRIA:	Solid-phase radioimmunoprecipitation assay
RIA:	Radioimmunoassay

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cluded various alkoxyPEG of different molecular weights,⁵⁻⁷ a random copolymer of D-glutamic acid and D-lysine (DGL),^{1,3-5} and others. Several methods of covalent linkage between polymer and antigen have been tried. In general, the longer the chain and the greater the degree of substitution, the greater the reduction in allergenicity.

Although the mechanism of suppression has not been fully elucidated, the favored hypothesis is that antigen-specific, T-suppressor cells are stimulated and in turn down-modulate the proliferation of B cell clones engaged in IgE synthesis. It has been suggested repeatedly that the principle might be applicable to allergic man so that specific undesirable IgE antibodies could be abrogated with resultant amelioration of allergic symptoms. This subject has recently been reviewed by Schon.⁸

This article describes our attempts to detect suppression of IgE antibodies in ragweed-allergic individuals with ragweed AgE coupled to several polymers including MPEG of 5000 and 2000 daltons, lauryloxyPEG (BRIJ) of 1200 daltons, and DGL of 69,000 daltons. Control patients were given unaltered AgE because immunotherapy with native allergens is well-known to suppress annual rises in IgE antibody that occur during natural respiratory exposure and to result in eventual partial reduction in serum levels of IgE antibodies. The results reveal little hope that the conjugates tried can achieve clinically useful suppression but do suggest that the properties of these conjugates might be used successfully in conventional immunotherapy to raise IgG-blocking antibodies.

MATERIAL AND METHODS

MPEG-5K-AgE. MPEG having an average molecular weight of about 5000 daltons was activated with cyanuric chloride by the method of Abuchowski et al.⁹ and then coupled to AgE as described previously.⁵ Chemical analysis indicated that about six of the total 18 lysyl residues of AgE were modified with MPEG side chains. Electrophoresis in 4% polyacrylamide gel containing sodium dodecylsulfate demonstrated the MPEG-5K-AgE to contain one broad band of 250K to 600K daltons. The broadness of the band is due partly to dissociation of AgE into its two polypeptide chains.¹⁰

MPEG-2K-AgE and BRIJ-1K-AgE. MPEG of average molecular weight of 2000 daltons and BRIJ of average molecular weight of 1200 daltons were converted to their respective O-(alkoxyPEG)-S-carboxamidomethyldithiocarbonates by the method of King and Weiner.¹¹ The activated alkoxyPEG derivative (10mM) was then coupled to AgE (0.2 to 0.3 mM) in sodium borate buffer of pH 9.2. After 16 to 20 hr at 25° C, the mixture (25 ml or less) was separated on a column (200 by 2.6 cm) of Sephacryl S-200 (Pharmacia, Piscataway, N.J.) with 0.1M NH_4HCO_3 as eluent. To avoid micelle formation of the BRIJ-dithiocarbonate, the reaction mixture was diluted eightfold before separation. Both conjugates, MPEG-AgE or BRIJ-AgE, were eluted at about 480 ml (column void volume at about 380 ml), whereas the native AgE and the excess dithiocarbonate were eluted at about 620 and 900 ml, respectively. Chemical and spectral analyses demonstrated the conjugates to contain 6

to 8 alkoxyPEG groups per mole of AgE, and the concentration of AgE conjugates was established by amino acid analysis after acid hydrolysis. Electrophoresis in 5% polyacrylamide gel containing sodium dodecylsulfate demonstrated the MPEG-2K-AgE to contain one broad band of 40K to 90K daltons, whereas the BRIJ-1K-AgE contained a broad band of 40K to 70K daltons. Two batches of MPEG-2K-AgE and three batches of BRIJ-1K-AgE were prepared during the course of the study.

Acute safety tests of each batch of conjugate were done in mice by intraperitoneal injection of 0.2 ml of a 0.1 mg per milliliter solution and in guinea pigs by injection of 4 ml. No unusual symptoms or weight changes were observed; normal histopathologic results were found when the animals were killed on day 7; no long-term ill effects were found in mice that had been treated with the first batch of each conjugate (200 μg per mouse) and observed for 3 to 6 mo. These studies served to demonstrate the suppressive effects of MPEG-AgE and BRIJ-AgE in animals.^{5, 6}

The methods used for gel electrophoresis and for measuring the relative antigenicity of the conjugates have been described previously.¹¹

AgE. Unaltered AgE to be administered to control patients was furnished by Miles Laboratories, Elkhart, Ind. The sample was verified by gel electrophoresis and by quantitative rocket immunoelectrophoresis with specific rabbit antisera. By these tests the sample contained $60 \pm 15\%$ of the stated AgE concentration.

DGL-AgE. AgE from the same batch was coupled by Miles Laboratories to a random copolymer of DGL with a ratio of 60 D-glutamic acid/40 D-lysine, and an average molecular weight of 69,000 daltons was furnished. AgE/DGL = 0.36 so that about three side chains were attached to each molecule of AgE. The single batch of DGL-AgE used in these studies was tested in mice by Dr. David G. Katz and suppressed IgE responses to AgE (personal communication).

Diluent. Phosphate-buffered saline with 0.4% phenol and 0.03% human serum albumin was furnished by Miles Laboratories.

Standardization. All materials were labeled according to their content of AgE in micrograms; hence, all doses are reported in AgE equivalents. The AgE concentration after conjugation was determined by amino acid analysis (see description of conjugates) and by ultra violet absorption. Ultra violet absorption of AgE at 280 nm was only slightly changed by the coupling process so that the AgE equivalency of conjugates could be redetermined directly from protein content.⁶ Stock solutions of 500 μg AgE Eq/ml of MPEG-2K-AgE and BRIJ-1K-AgE were furnished in phosphate-buffered saline containing 0.2% methylparaben. AgE and DGL-AgE were furnished as 1000 μg AgE Eq/ml of stock solutions in phosphate-buffered saline containing 0.4% phenol. As the Miles Laboratories AgE restandardized in our laboratory gave a value 60% of that stated on the label, dosages of AgE and DGL-AgE administered to patients were calculated by use of the corrected figure. New dilutions were made from stock concentrates at regular intervals and were stored at 4° C. They were kept in an

TABLE I. Relative activity of conjugates of AgE

	By histamine release*		Reaction with rabbit anti-AgE†
	Before treatment	After treatment	
DGL-AgE	1.54	1.26	ND
MPEG-2K-AgE	1.61	2.17	‡ > 4.7 ± 2.3
BRIJ-1K-AgE	3.39	5.58	‡ > 3.7 ± 0.8
MPEG-5K-AgE	312	—	130

ND = not done.

*Ratio concentration of conjugated AgE/AgE for 50% histamine release.

†Ratio concentration of conjugated AgE/AgE for 50% inhibition of binding of labeled AgE to rabbit anti-AgE.

‡Averages of three preparations (September 1980; April 1981; September 1981).

ice chest on the day of injections to maintain storage temperature.

Patients. Individuals between the ages of 18 and 55 yr with histories of autumnal hay fever were chosen from volunteer responders to a newspaper article describing the study. Patients were accepted if they were in good health except for allergy, could conform to the needs of the study, and had a positive intradermal skin reaction (10 mm wheal and >20 mm erythema) to 0.01 PNU/ml or less of whole ragweed extract. Patients who had previously received immunotherapy with ragweed extract were excluded. Patients were informed as to potential risks and benefits and gave consent on a form approved by the Johns Hopkins Joint Committee on Clinical Investigation.

Histamine release. Leukocyte histamine release was performed on the basis of the method of Lichtenstein and Osler¹² as modified by May et al.¹³ Briefly, washed leukocytes resuspended in a Ca^{++} containing buffer are exposed to various concentrations of native or modified allergen at 37°C for 45 min. The cells are then pelleted by centrifugation, and the released histamine measured by use of an automated fluorometric method.¹⁴ The released histamine is expressed as a percentage of the total histamine content of aliquots of perchloric acid-lysed cells after subtraction of the cell blank ($\leq 5\%$ of total) from all samples. Dose-response curves (percent versus allergen concentration in AgE equivalents) are plotted, and the allergen dose required for 50% histamine release determined.

IgE antibodies. A highly sensitive RAST by use of Sepharose 4B to which purified ragweed AgE was coupled was used as previously described.¹⁵ All IgE antibody results are reported in nanograms per milliliter and calculated on the basis of a standard serum pool that was quantified by use of a RAST elution method.¹⁶ All sera from an individual patient were run in duplicate at two dilutions in the same assay and were repeated if the coefficient of variation between dilutions exceeded 10%.

IgG antibodies. The sera obtained in 1978 and 1979 were analyzed by use of highly sensitive double antibody radioimmunoassay.¹⁷ After the development of a SPRIA by use of radiolabeled *Staphylococcus aureus* A as the detection protein,¹⁸ this system was used to measure AgE-specific IgG antibodies in 1980 and 1981. An excellent correlation exists between the results obtained by RIA and

SPIRA when purified allergens are used $r = 0.981$, $p < 0.001$.¹⁸ Numerically, a 100-fold ratio exists between the results obtained by RIA versus SPRIA. Therefore for consistency, the early RIA values were multiplied by 100 and are reported in terms of arbitrary SPRIA units.

Symptom diaries. Patients filled out daily symptom diary forms as described previously¹⁹ for 8 wk during the ragweed pollination season. The forms report the duration of sneezing, rhinitis, conjunctivitis, and cough for each 12-hour period, along with antihistamine and other medication used. An average daily symptom and medication score for each patient and an average score for each group of patients for each day of the season were calculated.

Reactions. Patients were observed for 30 min after each injection, and the size of any wheal and erythema at the site of injection and any systemic symptoms were recorded. Each patient was given a sheet to record the size of any local swelling at 24 hr and on subsequent days, if any swelling was still present. These forms were returned by mail.

RESULTS

In 1978 a preliminary trial of MPEG-5K-AgE was carried out in four volunteers. A study of the allergenicity of MPEG-5K-AgE and AgE by basophil histamine release demonstrated in six untreated ragweed-sensitive donors that the ratio of concentration required for 50% histamine release (MPEG-5K-AgE/AgE) ranged from 180 to 666 with a mean of 312. The antibody-combining activity of MPEG-5K-AgE relative to that of AgE is 130 (Table I) as determined by comparing the concentrations of conjugate of AgE required for 50% inhibition of the binding of horseradish peroxidase-labeled AgE to plastic tube-bound rabbit antibody specific for AgE.

On the basis of this low allergenicity, a brief course of injections was planned involving four preseasonal visits with two or three injections per visit starting in late June when the compound became available.

On the first day the planned schedule was 0.1, 1.0, and 10.0 μg AgE equivalents administered at 30-minute intervals. Three patients received these doses with only a mild early wheal and erythema reactions,

TABLE II. Treatment with MPEG-5K-AgE

Patient	Cumulative dose (microgram AgE equivalents)	IgE antibody (nanogram per milliliter)		IgG antibody (Arbitrary units)		Symptom score
	Preseason	Before treatment	After treatment*	Before treatment	After treatment*	
1978						
R. W.	1111	86	17	<156	10000	3.5
J. W.	226	55	53	<156	16100	11.0
C. M.	861	76	73	<156	141290	1.4
K. C.	96	375	>670	<156	714300	8.6
1979						
R. S.	2171	<1	<1	<156	<156	0.5
B. R.	476	<1	<1	<156	<156	3.9
P. Mc.	241	<1	<1	<156	4300	11.9
M. D.	831	27	23	<156	3500	6.2
T. M.	58	56	83	<156	4500	14.5
E. P.	225	136	136	<156	10000	9.3
D. R.	66	161	230	<156	8300	2.9
C. M. (second year)	360	60	75	27700	140000	1.4

*Approximately September 15 (midseason).

but one patient had a larger wheal and erythema after 1.0 μ g. The final dose was limited to 5.0 μ g. At 24 hr, this patient had developed a large swelling extending to the elbow that lasted for 3 days. Subsequent doses were administered in single injections weekly for the next 5 wk, and dosage was limited again by a second late swelling 12 cm in size. The other three patients received their injections in the planned three additional visits and did not have large, late local reactions, although immediate wheal and erythema reactions were a source of caution that limited dosage in one patient. There were no systemic reactions.

Table II details cumulative doses administered, pre and posttreatment IgE and IgG antibodies to AgE, and mean daily symptom scores in these patients. Large rises in IgG antibody accompanied by little or no rise in IgE antibodies were encouraging immunologic responses. Symptom scores were variable and not remarkably different from those obtained in other treated groups of patients.

This result encouraged a further trial in 1979. Only one patient of the 1978 group was available for study, but eight additional patients were recruited to receive MPEG-5K-AgE for the first time. One of these patients failed to keep appointments and did not receive any shots. The other seven patients had an initial series of injections in four weekly visits in late April and May with one to three injections per visit depending on previous reactions. There was one systemic reaction in one patient requiring antihistamine only. One large, late local reaction occurred in one patient,

and three such reactions occurred in another patient. After a period during which immunologic responses were evaluated, the patients received a final booster injection on August 15, and another serum was taken for antibodies about 30 days later.

Table II illustrates dosages, before and after booster-antibody levels to AgE, and symptom scores. Despite positive skin tests to whole ragweed extract, three patients were found to have undetectable pre-treatment levels of IgE antibody to AgE after the study had been started. It was not surprising that there was no increase in IgE antibody in any of the three and that there was no IgG response in two as other studies have demonstrated that patients with no detectable IgE antibody to a specific allergen are unlikely to respond strongly to injections of that allergen. The remaining patients received total doses in the same general range as in 1978 but had less striking IgG responses. Again there was little rise in IgE but no evidence of suppression. Symptom scores were variable and not revealing without controls.

The above results prompted us to study conjugated allergens in a more systematic fashion. With several additional polymerconjugates having been reported to induce specific-IgE suppression in mice, it seemed appropriate to try several compounds. These were compared with unaltered AgE in patients matched for sensitivity. The availability of BRIJ-1K, MPEG-2K, and DGL (69K) allowed us to try several chain lengths and at the same time, varying degrees of substitution. Accordingly, 48 patients were selected for a

TABLE III. Dosages* and reactions

Group	No. patients†	No. injections	Highest dose‡	Cumulative dose‡	4-Hour reactions§	Systemic reactions
1980						
AgE	10/2	22	190 (27 to 360)	880 (190 to 1640)	9/4	3/4/4
DGL-AgE	10/3	22	310 (150 to 600)	1480 (600 to 2620)	6/5	2/0/2
MPEG-2K-AgE	9/1	22	330 (90 to 730)	1640 (400 to 2410)	5/3	0/2/1¶
BRIJ-1K-AgE	9/2	22	440 (30 to 930)	2220 (200 to 4530)	10/4	1/0/1#
1981						
AgE	7	11	380 (30 to 600)	2240 (290 to 3530)	4/1	1/1/1
DGL-AgE	7	12	460 (210 to 600)	3360 (820 to 6350)	1/1	0/0/0
MPEG-2K-AgE	7	15	550 (75 to 1000)	3480 (600 to 6250)	3/2	0/0/0
BRIJ-1K-AgE	5	13	580 (300 to 1000)	4260 (2620 to 4726)	3/2	0/0/0

*Microgram AgE equivalents.

†Number of patients who completed year/number of patients who dropped out during year. Patients who dropped out not included in number of injection or dosage tabulations.

‡Mean and range.

§Number of reactions larger than 10 cm/number of patients who had reactions.

||Number of reactions *not* requiring epinephrine/number of reactions requiring epinephrine/number of patients who had reactions.

¶One patient who dropped out had two reactions requiring epinephrine.

#One patient who dropped out had one reaction *not* requiring epinephrine.

preseasonal study in 1980 and matched into quartets on the basis of skin tests to whole ragweed extract. Members of each quartet were assigned at random to one of the possible treatments (three conjugates and unaltered AgE).

In the patients to be treated with each conjugate, an estimate was made by histamine-release studies on blood basophils of the relative allergenicity of the conjugate. Table I demonstrates the geometric mean ratio of the relative allergenicity of each conjugate in each group of patients as determined before and after the first year of treatment. Table I also demonstrates the relative antibody-combining activity of conjugate as determined with rabbit antibodies specific for AgE. There appears to have been little change with treatment and fair agreement between methods by use of different antibodies. The ratios of these conjugates are clearly much less than that of the MPEG-5K-AgE used in 1978 and 1979 (see discussion).

Several patients found that they could not meet the schedule of injections and blood samples after being selected and dropped out before receiving any treatment. Forty-six patients began treatment of whom 38 completed the protocol. All material became available later than anticipated, but treatment was initiated in 1980 nevertheless because the main purpose was to examine immunologic rather than clinical responses. Injections were initiated on July 11, and several shots were given at each of six weekly visits until August 15. Thereafter a single injection was given every 2 wk until December 29 at which time injections were discontinued temporarily to evaluate immunologic responses and the usefulness of further study. Eight

patients dropped out before completion of the series. So far as could be ascertained by personal contact, seven of these were due to personal reasons. One patient receiving MPEG-2K-AgE desired to discontinue injections after two reactions with systemic symptoms treated with epinephrine.

Table III demonstrates the number of patients in each group who completed the first series of injections, the dosages administered, and the rate of reactions. The mean dose administered to patients receiving conjugates was somewhat larger than the dose given to those receiving AgE. Systemic reactions occurred with all four materials. Counting both patients who completed the first course of treatment and those who dropped out before completing it, the rates of systemic reaction (number of reactions/number of injections) were 7/241 for AgE, 2/253 for DGL-AgE, 2/216 for MPEG-2K-AgE, and 1/217 for BRIJ-1K-AgE. Because the dose was reduced after systemic reactions in 4/10 patients on AgE, the mean dose of AgE was lower than that of the three conjugates. Despite that fact, three reactions rated as 4+ (requiring more than 0.5 ml epinephrine) occurred in the AgE group, and none so severe occurred in the other groups so that it appears that the reduced allergenicity of the modified allergens demonstrated by histamine release reduced the tendency to elicit systemic reactions. Local swellings larger than 10 cm were infrequent in all four groups but did result in limitation of dosage in a number of patients. There does not appear to be a difference in the rate of 24-hour local reactions.

An assessment of IgE antibodies to AgE in sera taken from these patients during and just after the first

series of injections demonstrated that all except one patient had an initial rise in IgE antibody and that in many the titer had begun to decline by the time the final serum was received 2 to 3 wk after the last injection. Every patient also had a striking rise in IgG antibody titer to AgE. Symptom scores taken during the season of ragweed pollination failed to demonstrate any differences between the groups.

Because of the possibility that further treatment with larger doses might begin to produce the desired decline in IgE antibodies, a second year of treatment was planned and all 38 patients were offered the opportunity to continue with a course of further injections. Twenty-six responded favorably; the other 12 cited difficulty in making appointments or lack of progress as reasons for not continuing with the study. Patients were administered single injections at 2-week intervals from early June until the end of November 1981. The initial dose has one tenth the final dose of the previous series, and dosage was raised according to the physician's judgement as to tolerance. One person receiving AgE had two systemic reactions. No systemic reactions occurred in the other three groups. Dosages and reactions are demonstrated in Table III. Larger doses were achieved in all patients, but two patients remained at single-dose levels lower than 250 μ g of AgE equivalents, one receiving AgE because of several systemic reactions and one receiving MPEG-2K-AgE because of several large local reactions. As is common in the second year of immunotherapy, reactions of both types occurred less frequently.

Antibody responses were determined in detail only on those patients who completed both years of study. Fig 1 demonstrates serial specific-IgE antibody determinations on these patients (a sixth patient who received BRIJ-1K-AgE and who dropped out only 2 mo before the completion of the study was added for completeness). An initial rise in IgE-antibody titer followed by a subsequent decline toward pretreatment levels appears to be the general rule. In 1980 the early rise in IgE antibody is similar to that observed usually when immunotherapy is initiated. Since it occurred concomitantly with seasonal exposure, the effects of injection and natural inhalation are not readily distinguished. After a 5-month rest, several patients had a modest secondary rise after the second course of treatment was begun. Three patients (one on DGL-AgE and two on MPEG-2K-AgE) started with low levels and had a continuing rise in titer over the period of study, although their final IgE-antibody level was still low. The responses to conjugated AgE preparations do not appear to be significantly different from the response to unaltered AgE.

Table IV demonstrates the IgG antibody 2 wk after the last injection in the same patients. All four agents

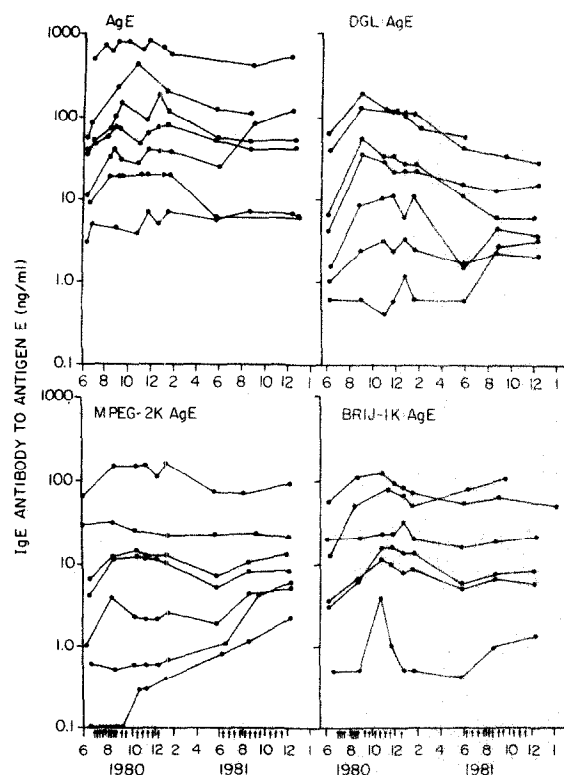


FIG. 1. IgE antibody to AgE (nanogram per milliliter) by quantitative RAST during two seasons of treatment in patients followed for entire period of study. Injections of conjugates or native AgE are indicated by arrows.

produced sizable antibody responses that increased with the larger doses administered the second year. PEG-conjugated AgE appears to be somewhat more efficient at raising IgG antibody levels than the other agents in this trial, but the difference is of borderline significance. Symptom scores in both years were similar in the four groups and lower than usually reported by placebo treated groups (Table V). As in other studies, we were unable to discern any quantitative relationship between symptom scores and in-season IgG antibody (not shown).

DISCUSSION

The addition of several synthetic polymers of simple structure to allergens appears not to carry any increased risk of toxicity. Both long-term animal studies and administration to humans over several years have been without evidence of hematopoietic or hepatic toxicity. The structure of these materials suggests that they will be readily metabolized or excreted. Studies with radiolabeled MPEG-5K conjugate of bovine plasma albumin in rabbits demonstrated that more than 90% of the conjugate appeared in urine after 4 days and that less than 4% was still retained by the animal (chiefly in the liver) after 31 days.⁹

TABLE IV. IgG antibodies to AgE*—geometric means and ranges

Group	No. patients	Before treatment	After treatment (1980)†	After treatment (1981)†
AgE	7	958 (300 to 3760)	19920 (12560 to 39300)	33980 (18170 to >80000)
DGL-AgE	7	896 (<156 to 4550)	22260 (10750 to >40000)	43550 (19700 to >80000)
MPEG-2K-AgE	7	456 (>156 to 999)	38550 (23090 to >80000)	65334 (35880 to >80000)
BRIJ-1K-AgE	6	904 (459 to 1758)	15100 (<170 to 45260)	46020 (19290 to >80000)

*Arbitrary units.

†Approximately 2 wk after last injection.

TABLE V. Mean daily symptom-medication scores

Group	1980		1981	
	No. patients	Mean score	No. patients	Mean score
AgE	10	4.2	7	5.1
DGL-AgE	10	6.2	7	6.7
MPEG-2K-AgE	9	5.6	7	6.3
BRIJ-1K-AgE	9	5.7	5	4.5

The reduced allergenicity of these materials in inducing immediate skin test reactions or basophil histamine release in vitro appears to be reflected in a lower rate of systemic reactions when they are administered by injection. In the comparison of native AgE with three conjugates, systemic reactions were more frequent and more severe in the AgE patients despite a lower mean dose. Among the compounds tried, the conjugate MPEG-5K-AgE appears to be most successful in this regard as it was the least allergenic and induced a considerable IgG-immune response with only four visits for injections. Although the allergenicity ratio of the other three compounds was much less, in the first year of treatment when the risk of systemic reactions is the highest, these compounds appeared to cause fewer reactions than did unaltered AgE.

The higher reduction of allergenicity and antibody-combining activity of MPEG-5K-AgE relative to that of the MPEG-2K-AgE or to BRIJ-1K-AgE is not solely a result of the increased size of MPEG-5K groups or the different degrees of substitution of alkoxyPEG groups. It is more a consequence of the two different chemical methods used for the preparation of these conjugates, since samples of MPEG-5K-AgE prepared by the two methods differ in their size distribution. When MPEG-5K-AgE was prepared by the dithiocarbonate method¹¹ its antibody-combining activity was only slightly lower than that of the MPEG-2K-AgE prepared by the same method, but it was much higher than that of MPEG-5K-AgE prepared by

the cyanuric chloride method to a similar degree of substitution.

Despite the reduction in allergenicity, there was sufficient tendency in about half the patients to cause reactions, particularly late, local swellings at the site of injection to limit dosage below the rather modest top dose planned of 1000 μ g of AgE equivalent per injection, and this dose was achieved with some regularity only near the end of the second year of treatment. This dose is far below that administered to mice to suppress IgE synthesis. For example, ragweed alkoxyPEG conjugates have been given to mice in doses of 50 to 1000 μ g per mouse to induce suppression, 70 to 1400 times the dose on a weight basis that we have administered to man. It appears that a conjugate for suppression in man will be useful only if the doses required are at least tenfold less.

When the alkoxyPEG-AgE conjugates were used in low doses in mice,¹¹ they demonstrated greatly reduced immunogenicity for primary antibody responses, but they were just as effective as native AgE in inducing secondary antibody responses. Only when the conjugates or the native antigen were used in high doses in mice did they demonstrate suppression of specific IgE responses with the conjugates being slightly more effective than the native antigen.⁵ The immunologic findings in man when limited doses were used are, therefore, quite similar to the findings in mice. It appears therefore that in man these materials should not be thought of as uniquely capable of suppressing IgE antibodies but rather as relatively conventional immunizing agents. Such agents regularly cause first a rise in IgE antibodies and only later cause a modest decline that appears to be clinically irrelevant. An earlier study suggests that a rise in IgG antibodies may have more to do with clinical efficacy in immunotherapy.²⁰

Conjugates such as these might therefore achieve the immunologic response and the degree of relief obtained from immunotherapy with unaltered extracts but in fewer injections with a reduced risk of untoward allergic reactions. Our results suggest that max-

imum efficiency in this regard would come from higher degrees of substitution with longer chain polymers than were used in our studies. The degree of substitution, however, is limited by the problem of denaturation that produces proteins so extensively modified as to be poorly immunogenic for antibody responses specific for the native proteins.²¹

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