

Effect of immunotherapy on immunoglobulin E and immunoglobulin G antibodies to ragweed antigens: a six-year prospective study

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The effect of immunotherapy with aqueous short ragweed (SRW) extract on IgE and IgG antibodies was tested over a 6 yr period in 47 adults with ragweed hay fever. Sera were collected each year in July and October from 1973 through 1979. In May 1976, 23 patients began immunotherapy with a lyophilized standardized SRW extract. From 1976 through 1979, treated patients received an average total dose of 4.8×10^5 protein nitrogen units (1039 μ g of AgE). IgE antibodies to SRW and ragweed AgE were measured by the radioallergosorbent test (RAST) in antigen excess using allergens bound to Sepharose. Blocking antibodies primarily of the IgG class were measured by RAST interference. In response to inhalation of ragweed pollen, untreated patients showed seasonal rises (July to October) and postseasonal falls (October to July) of IgE antibodies during the entire study period. IgE antibody levels in the untreated patients decreased with time and from 1974 to 1979 fell 41% ($p < 0.003$) for an average halftime of 6.2 yr. Before immunotherapy, treated patients also showed seasonal rises and postseasonal falls. Treatment with SRW extract in 1976 produced an abrupt increase in IgE and IgG antibodies and a clear-cut suppression of seasonal rises of IgE antibodies without an effect on postseasonal falls through 1978. From 1974 to 1979, IgE antibodies to AgE and SRW decreased more in the immunized group than in the control group, and by 1979 these levels showed a mean fall of 73%. Blocking antibodies increased in the treated patients and reached maximal levels by July 1978. In 1978 and 1979, the levels of IgG blocking antibodies to AgE were inversely related to the IgE antibody levels to AgE. These results indicate that adults with ragweed hay fever show regular seasonal and postseasonal changes in IgE antibodies and that IgE antibodies spontaneously decrease with time. Immunotherapy magnifies these decreases by suppression of the seasonal rises, but it does not affect the postseasonal falls. (J ALLERGY CLIN IMMUNOL 70:261, 1982.)

Pollen-sensitive patients have been treated with injections of pollen extracts since the early part of this century.^{1, 2} For ragweed pollen the efficacy of this treatment has been established by several double-blind

studies.³⁻⁵ However, the mechanisms responsible for the treatment effect remain obscure. Injection of ragweed pollen extract elevates IgG blocking antibodies,⁶⁻⁸ reduces the magnitude of seasonal rises of IgE antibodies,⁹⁻¹² in some cases reduces or abolishes the ability of peripheral blood leukocytes to release histamine,^{11, 13-15} and reduces lymphocyte proliferation to ragweed AgE.¹¹

In a prior study, we established a calibrated RAST for measurement of the absolute levels of IgE antibodies to SRW and presented evidence that immuno-

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Abbreviations used

AgE:	Antigen E
RAST:	Radioallergosorbent test
SRW:	Short ragweed
PNU:	Protein nitrogen unit

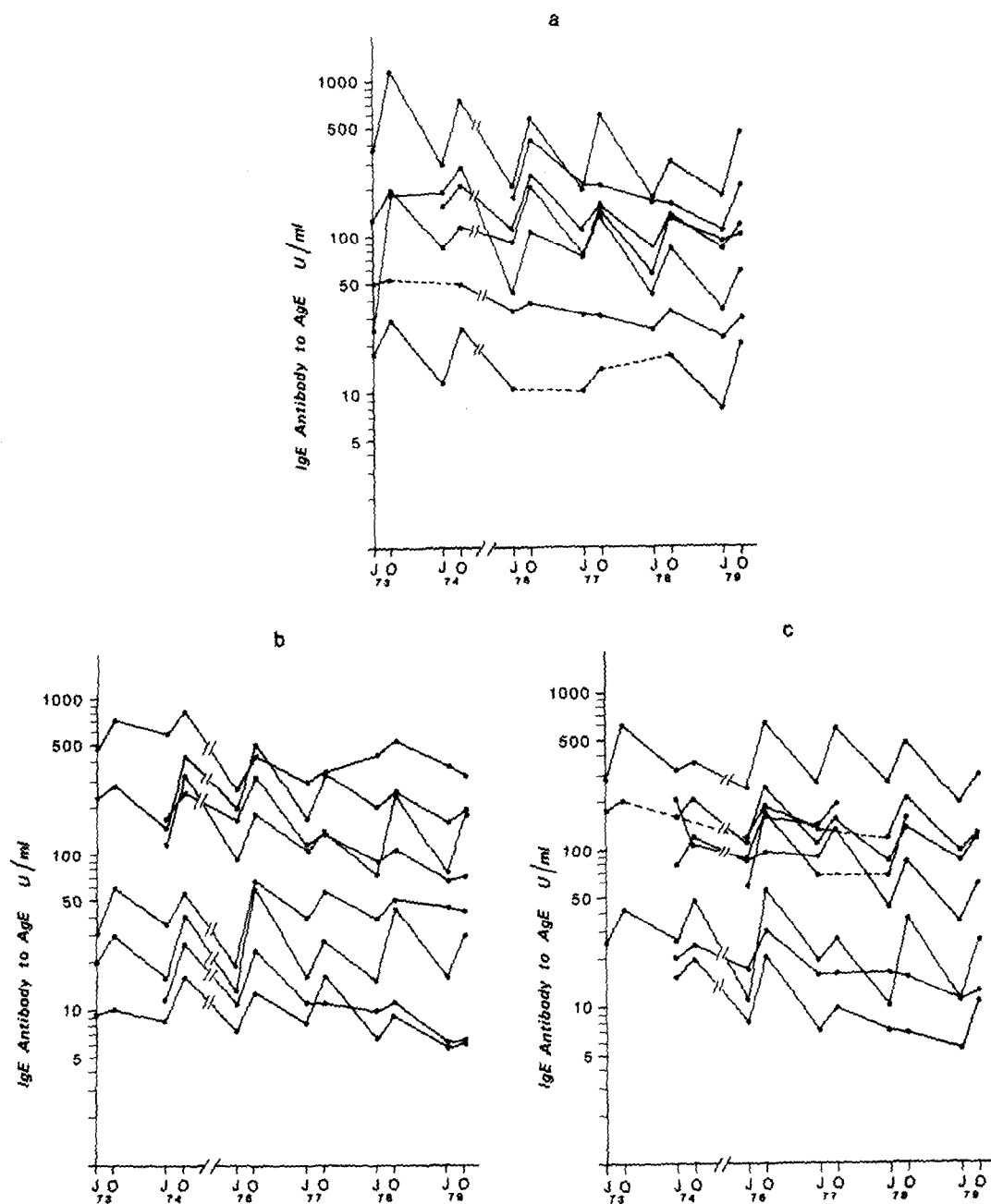


FIG. 1. Levels of IgE antibodies to AgE during July (J) and October (O) in untreated hay fever patients. Dashed lines connect points between which serum samples were unavailable. Each line represents one patient. The results in the 24 patients are shown in three subgroups (a, b, and c).

TABLE I. Characteristics of the patient groups*

	No.	Sex		Age		Duration of hay fever	
		F	M	Mean	Range	Mean	Range
Control group	24	15	9	39	19-60	28	13-50
Treated group	23	8	15	38	19-69	22	10-40

*In 1976 at the initiation of immunotherapy.

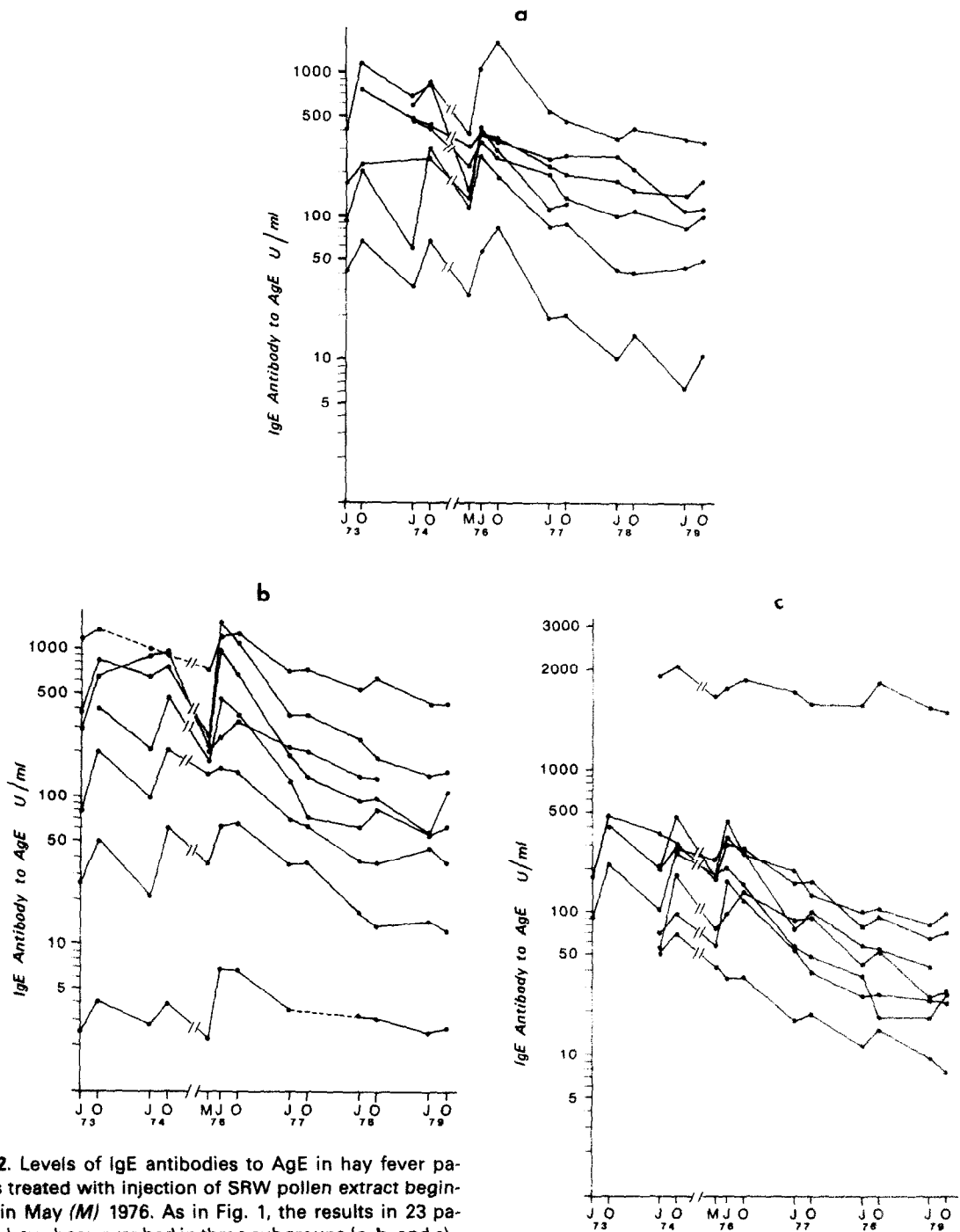


FIG. 2. Levels of IgE antibodies to AgE in hay fever patients treated with injection of SRW pollen extract beginning in May (M) 1976. As in Fig. 1, the results in 23 patients have been graphed in three subgroups (a, b, and c).

therapy suppressed the seasonal rises of IgE antibodies to SRW.¹² However, because the patients had received injections of pollen extract before the study period, we could not determine the magnitude of the treatment effect. Therefore, in a 6 yr prospective study we analyzed the changes of IgE and IgG blocking antibodies to AgE and SRW in patients before and after immunotherapy to determine the degree of suppression of the IgE antibody level.

MATERIALS AND METHODS

Ragweed-allergic patients

Forty-seven patients sensitive to ragweed were studied. All gave typical histories of hay fever during the ragweed pollination season and all had elevated IgE antibody levels to SRW and AgE. Thirty-six patients were skin tested with SRW extract and all had positive reactions. None of the patients had received injections of SRW extract in the 5 yr before 1976. Serum samples were obtained in July and Oc-

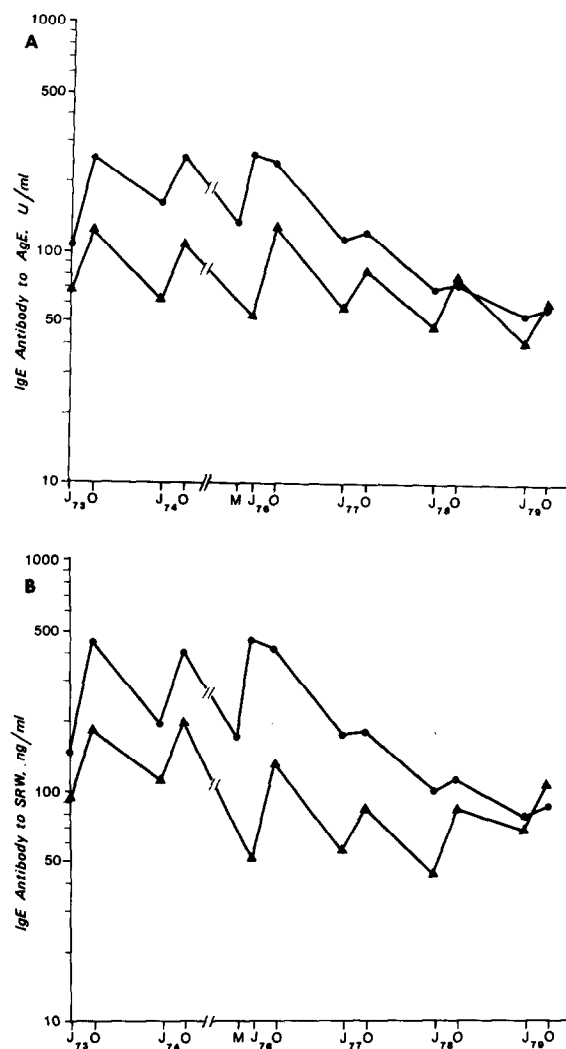


FIG. 3. Geometric mean levels of IgE antibody to AgE and SRW in untreated (Δ) and treated patients ($\bullet$). Note that May 1976 samples were not obtained from the untreated patients. **A**, AgE; **B**, SRW.

tober of 1973 (26 patients) and 1974 (44 patients) prior to the initiation of immunotherapy. All sera were stored at -20°C . The patients were assigned to control and treatment groups based on the levels of IgE antibodies to SRW and on the expected exposure to SRW pollen, e.g., whether or not the patient had air-conditioning at home or work and lived in a rural or urban area. Initially, the immunized group had higher levels of IgE protein as well as IgE antibody to AgE and SRW. The characteristics of the two groups are shown in Table I.

Immunotherapy regimen

Treated patients received injections of SRW pollen extract beginning in May 1976. The extract was a lyophilized preparation from Greer Laboratories (Lot No. L56-38) labeled as containing 495,000 PNU per vial and 900 μg of AgE per vial. After reconstitution with 10 ml of phosphate-

buffered saline, pH 7.4, containing 0.5% human serum albumin, we found that the vials contained 108 μg AgE/ml, 26 μg Ra3/ml, and 9.4 μg Ra5/ml by RAST inhibition.¹⁶ As described previously,¹⁶ these three allergens are not cross-reactive with IgE antibodies by RAST inhibition. Over the 4 yr treatment period, the lyophilized extract was stored at 4°C .

Patients were injected with increasing quantities of the ragweed pollen extract to the maximally tolerated dose. Subcutaneous injections were given twice weekly for the first 6 wk and weekly thereafter until the maximally tolerated dose or a dose of 10,000 PNU/injection (21.8 μg of AgE) was reached. The injections were given less frequently, usually at monthly intervals, thereafter. Serum samples were obtained in July and October of each year from both groups, since our prior studies had shown that the nadir and zenith, respectively, of IgE antibody to ragweed is reached during these months.^{12, 17} An additional blood sample from the treated patients only was obtained in May 1976 just prior to beginning immunotherapy. Sera were not obtained in October 1975; therefore, data for that year are not presented.

Measurement of IgE antibody

IgE antibody to SRW extract and AgE was measured by a calibrated RAST as described previously,¹² with the following modification. To eliminate the possibility that blocking factors might interfere in the RAST, we coupled ragweed fraction A¹⁸ (the void volume peak obtained from Sephadex G-25) and AgE to Sepharose 4B. The details of the SRW Sepharose 4B preparation have been presented previously.¹⁹ AgE (Resource Reagents Branch, National Institutes of Health, Catalog No. A601-902-185, 2.5 mg in 0.5 ml buffered glycerol) was coupled to Sepharose 4B, using a ratio of 10 mg protein/ml of packed volume of Sepharose. Both solid-phase antigens were tested to determine that amount which removed all the IgE antibody from 0.05 ml of serum containing a high IgE antibody level and which prevented interference by IgG antibodies in sera containing elevated levels of blocking activity.¹⁹ In the case of SRW-Sepharose, 0.5 ml of a 1:40 dilution (v/v) in RAST diluent¹² was used, whereas 0.5 ml of a 1:100 dilution (v/v) of AgE-Sepharose was used.

The RAST for IgE antibodies to SRW was calibrated with a pool of sera from five nonimmunized, ragweed-sensitive patients. The IgE antibody content to SRW antigens in the pool was determined by immunosorption with solid-phase SRW as described previously^{12, 20} and expressed as nanograms per milliliter. The RAST for IgE antibodies to AgE was calibrated with the same serum pool; the results were expressed in arbitrary units per milliliter. Each RAST assay for IgE antibodies to AgE and SRW included sera from nonallergic controls and a standard curve constructed with the pool of sera from ragweed-sensitive patients. In the RAST to AgE, two sera from normal subjects gave a mean value of $1.09\% \pm 0.27\%$ (mean $\pm$ 1 SD, $n = 8$) of counts bound for 0.05 ml of undiluted serum in four assays. The pool of sera from sensitive patients gave linear log-log dose-response curves, with correlation coefficients ranging

TABLE II. Seasonal changes on IgE antibody

Group		IgE antibody to:			
		AgE		SRW	
		r _s	p value	r _s	p value
Seasonal rise vs July antibody level					
1973	T + C	0.621	<0.002	0.718	<0.001
1974	T + C	0.358	<0.05	0.382	<0.02
1976	C	0.899	<0.001	0.836	<0.001
	T	0.034	NS	0.187	NS
1977	C	0.641	<0.002	0.210	NS
	T	-0.311	NS	-0.246	NS
1978	C	0.608	<0.005	0.706	<0.001
	T	-0.237	NS	-0.146	NS
1979	C	0.477	<0.05	0.492	<0.05
	T	0.034	NS	0.187	NS
Postseasonal fall vs October antibody level					
1973-1974	T + C	0.692	<0.001	0.753	<0.001
1974-1975	T + C	ND	ND	0.945	<0.001
1976-1977	C	0.918	<0.001	0.946	<0.001
	T	0.851	<0.001	0.648	<0.005
1977-1978	C	0.704	<0.001	0.848	<0.001
	T	0.677	<0.002	0.610	<0.005
1978-1979	C	0.947	<0.001	0.415	NS
	T	0.920	<0.001	0.948	<0.001

T = treated patients; C = control patients; NS = not significant; ND = not done.

TABLE III. Changes of IgE antibody and IgE protein with time in untreated patients

	IgE antibody to AgE		IgE antibody to SRW		IgE protein	
	July	October	July	October	July	October
Slope of regression line	-0.031	-0.049	-0.065	-0.086	-0.035	-0.043
(mean $\pm$ SEM)	± 0.018	± 0.010	± 0.012	± 0.009	± 0.009	± 0.010
Halftime (yr)	9.7	6.1	4.6	4.4	8.4	7.0

from 0.987 to 0.998 in four assays, and the dose responses ranged from 1.05% to 32.6% of counts bound. The coefficient of variation for the four assays for IgE antibodies to AgE was 19%. In the RAST to SRW, two sera from normal subjects gave a mean value of $2.84\% \pm 0.51\%$ (mean $\pm$ 1 SD, $n = 8$) of counts bound for 0.05 ml of undiluted serum in four assays. The pool of sera from sensitive patients gave linear log-log dose-response curves, with correlation coefficients ranging from 0.992 to 0.996 in four assays, and the dose responses ranged from 2.71% to 31.2% of counts bound. The coefficient of variation for four assays was 10%.

Measurement of blocking antibodies

Blocking antibodies were measured by RAST interference as described previously.^{19, 21} In brief, sera were heated at 56°C for 4 hr to destroy IgE heat-labile antigenic determinants. Heated serum was added to the solid-phase AgE or

SRW followed by the addition of a constant amount of the unheated pooled serum from the allergic patients. After incubation and washing, radiolabeled anti-IgE was added. After a second incubation and wash, the radioactivity bound was determined in a gamma scintillation counter. We employed 0.05 mg of microcrystalline cellulose SRW or AgE, 0.05 ml of heated test serum, and 0.005 ml of a 1:10 dilution of the unheated pooled serum from the allergic patients in a total volume of 0.4 ml in the first step of the procedure. In the second step, 0.1 ml radiolabeled anti-IgE containing 5 ng of antibody in 0.4 ml total volume was employed. Solid-phase AgE was prepared as described previously¹⁶ by exposing 3.75 gm of cyanogen bromide-activated microcrystalline cellulose to 7.5 mg of AgE and 7.5 mg of human serum albumin. The preparation of solid-phase SRW has been described previously.¹²

In the RAST interference assay for AgE the serum pool from the allergic patients gave 9.8% and 11.8% and serum

TABLE IV. Effects of immunotherapy on rises and falls of IgE antibody: statistical comparison of results in treated and untreated groups

	p values	
	AgE	SRW
Seasonal rise from July to October		
1973	NS	NS
1974	NS	NS
1976	<0.002	<0.002
1977	<0.0002	<0.0002
1978	<0.0004	<0.0002
1979	<0.02	<0.001
Postseasonal fall from October to July		
1973-1974	NS	NS
1976-1977	NS	NS
1977-1978	NS	NS
1978-1979	<0.01	NS

NS = not significant.

from normal subjects gave 0.40% and 0.43% counts bound, respectively, in two assays. A pool of serum from three patients allergic to SRW who had received immunotherapy for 3 yr with SRW was heated at 56° C for 4 hr and used to calibrate the RAST interference procedure.²¹ Addition of increasing quantities of heated serum reduced binding of IgE antibody. The largest volume of heated serum added, 0.1 ml, reduced binding from 9.8% to 1.9% and from 11.8% to 2.6% of counts bound in two AgE RAST interference assays, respectively. When these results were plotted on a semilog scale, the resulting regression lines had correlation coefficients of 0.996 and 0.999. In the two assays for blocking activity to AgE the coefficient of variation was 30%. Blocking activity for SRW was determined in a similar manner with comparable results, and in two assays the coefficient of variation was 8.3%.

Measurement of IgE protein

Serum IgE protein was measured by double-antibody radioimmunoassay as described previously.²²

Statistical evaluation

Levels of IgE antibody to SRW and AgE and IgE protein were logarithmically transformed ($\log_{10}$) and regression analyses were performed for July and October, respectively, of each year by means of a Hewlett-Packard 9845B desktop computer. Slopes of regression lines were tested to determine whether IgE antibodies decreased with time and as a consequence of treatment. Correlations were tested by Spearman's rank correlation coefficient. The sign test and the Mann-Whitney rank sum test were used for analysis of changes in antibody levels.

RESULTS

The changes of IgE antibody to AgE in the patient groups are shown in Figs. 1 to 3. In 1973 and 1974

TABLE V. Regression analyses of changes of IgE antibody to AgE*

Group	Mean slopes regression line		p value†
	1973-1974	1976-1979	
Control	0.055	-0.021	NS
Treatment	0.047	-0.223	0.008
p value‡	NS	<0.0002	

NS = not significant.

*The slopes of the July IgE antibody to AgE were determined by regression analysis. For certain patients sera were available from 1970.

†The Wilcoxon signed rank test was used to compare slopes during different time periods.

‡The Mann-Whitney rank sum test was used to compare the slopes of the treated and control groups.

both groups showed seasonal rises and falls in the IgE antibody levels to AgE and SRW. The levels rose abruptly from July to October and declined more gradually from October to the following July. Table II shows that the magnitudes of the seasonal rises in 1973 and 1974 were related to the IgE antibody levels in July. Similarly, the magnitudes of the postseasonal falls in these years were related to the levels of IgE antibody in October. Nonimmunized patients continued to show these changes through 1979. Analysis of the IgE antibody levels to AgE in the untreated group showed a gradual reduction over the 7 yr of the study from a geometric mean of 62 U/ml in July 1974 to 41 U/ml in July 1979 ($p < 0.01$) and from 109 U/ml in October 1973 to 59 U/ml in October 1979 ($p < 0.001$). Changes in IgE antibody levels to SRW (Table II) generally paralleled those to AgE except that the relationships between seasonal changes and the levels before the changes were not as intense. In the untreated patients IgE antibody levels to SRW fell from a geometric mean of 110 ng/ml in July 1974 to 67 ng/ml in July 1979 ($p < 0.001$) and from 196 ng/ml in October 1974 to 105 ng/ml in October 1979 ($p < 0.001$). Table III shows that regression analysis of July and October IgE antibody levels to AgE and SRW for individual patients gave negative slopes. The IgE antibody halftimes calculated from these regressions had mean values of 7.9 yr for AgE and 4.5 yr for SRW.

Before treatment, the immunized patients showed rises and falls of IgE antibody to AgE and SRW that did not differ significantly from those in the untreated group. After immunization, the treated group showed an abrupt rise in the levels of IgE antibodies to AgE and SRW from May to July ($p < 0.001$ for both allergens). The magnitudes of the rises from May 1976

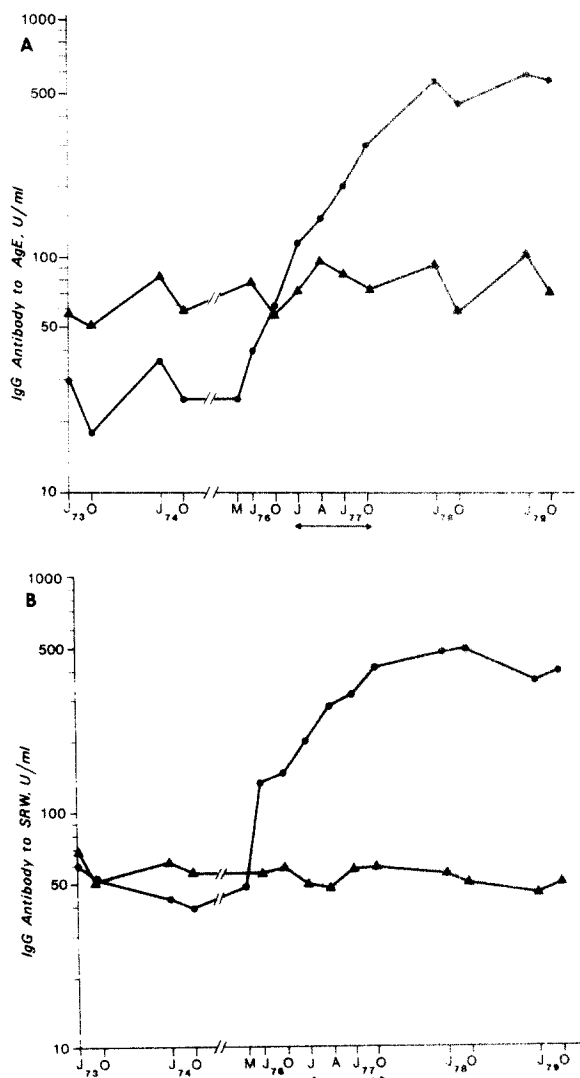
TABLE VI. Relationship between IgE and IgG antibody in treated patients

Time		AgE		SRW	
Year	Month	r_s	p value	r_s	p value
1976	July	-0.699	<0.001	<0.124	NS
	October	-0.793	<0.001	-0.110	NS
1977	July	-0.639	<0.005	-0.126	NS
	October	-0.647	<0.005	0.083	NS
1978	July	-0.504	<0.005	0.165	NS
	October	-0.580	<0.01	0.204	NS
1979	July	-0.432	NS	0.281	NS
	October	-0.463	NS	0.291	NS

NS = not significant.

to July 1976 approximated the seasonal rises and were positively correlated with the IgE antibody to AgE and SRW in May (AgE: $r_s = +0.561$, $p < 0.01$; SRW: $r_s = +0.697$, $p < 0.001$). As shown in Figs. 1 to 3 and Table IV, the expected seasonal rises of IgE antibodies to AgE and SRW were suppressed after treatment in comparison with the control patients. In addition, the magnitudes of the seasonal rises from July to October were no longer related to the levels of IgE antibody to AgE and SRW in July (Table II). In contrast, the magnitudes of the postseasonal falls in IgE antibody to AgE and SRW remained related to the October antibody levels. The postseasonal falls in the two groups did not differ before or during immunotherapy (Table IV), except for AgE in 1979. As a consequence of the suppression of IgE antibody rises, the levels in the immunized patients dropped strikingly so that by October 1978 and 1979 they were comparable in the two groups. The levels of IgE antibody to AgE dropped from a geometric mean of 164 U/ml in July 1974 to 53 U/ml in July 1979 ($p < 0.001$) and from 256 U/ml in October 1974 to 58 U/ml in October 1979 ($p < 0.001$). Similarly, the levels of IgE antibody to SRW dropped from a geometric mean of 139 ng/ml in July 1974 to 76 ng/ml in July 1979 ($p < 0.001$) and from 399 ng/ml in October 1974 to 84 ng/ml in October 1979 ($p < 0.001$). The reductions of IgE antibody to AgE and SRW from May 1976 to July 1979 were positively correlated with the levels of IgE antibody in May 1976 (AgE: $r_s = +0.744$, $p < 0.001$; SRW: $r_s = +0.655$, $p < 0.005$).

The effects of immunotherapy were also tested by regression analysis. Table V shows that regression analysis of the levels of IgE antibody to AgE in July in the treated and untreated groups did not differ prior to therapy (1973 and 1974), but after therapy (1976 to 1979) the slopes of these regression lines did differ.

**FIG. 4.** Geometric mean levels of IgG blocking antibody to SRW and AgE measured by RAST interference in untreated (Δ) and treated patients ($\bullet$). During 1977 blood samples were obtained at 3 mo intervals starting in January. A, AgE; B, SRW.

No difference in the slopes of the regression lines was seen in the untreated group before and after 1976, whereas in the treated group the slopes before and after initiation of immunotherapy in 1976 differed. Regression analyses of the results for October showed similar results.

Fig. 4 shows the levels of IgG blocking antibody to AgE and SRW. As expected, the levels of blocking antibody to both AgE and SRW rose abruptly in the immunized group from May to July 1976 ($p < 0.01$ and $p < 0.001$, respectively) and continued to rise until reaching a plateau in July 1978, 2 yr after initiation of treatment. The rises in IgG blocking antibody to AgE and SRW from May to July 1976 were not

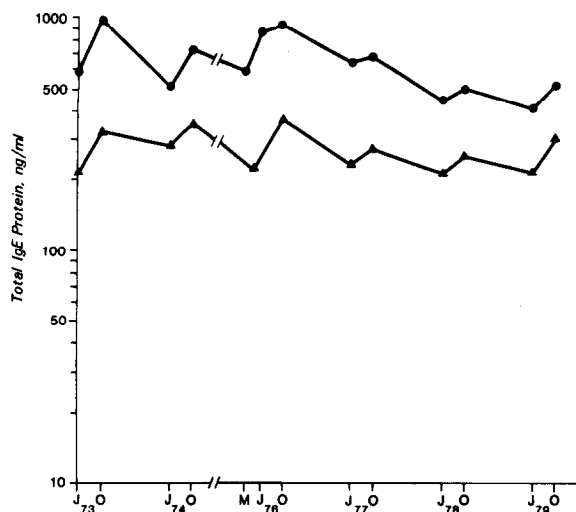


FIG. 5. Geometric mean levels of IgE serum protein in untreated (Δ) and treated ($\bullet$) patients.

related to levels of IgE antibody in May 1976 but were related to the dose of SRW extract given during this period (AgE, $r_s = +0.503$, $p < 0.05$; SRW, $r_s = +0.445$, $p < 0.05$). The levels of blocking antibody to AgE in the untreated patients, although low, showed a consistent pattern of increases from October to July followed by decreases from July to October ($p < 0.025$ in 1976, 1978, and 1979). In 1977 serum was obtained at 3 mo intervals and showed a peak level of blocking activity in April followed by a decline until October. The relationships of IgE and IgG antibodies in treated patients are shown in Table VI. In the case of AgE the levels of IgE and IgG are inversely related, whereas with SRW they are not significantly correlated. The rise in IgG antibody to AgE from May 1976 to July 1979 was not related to the fall of IgE antibody to AgE for the same period ($r_s = -0.242$, not significant). In contrast, the rise of IgG antibody to AgE from May 1976 to July 1976 was inversely related to the rise of IgE antibody to AgE for the same period ($r_s = -0.733$, $p < 0.001$).

The geometric mean levels of IgE protein (Fig. 5) show seasonal rises from July to October and postseasonal falls from October to July. The seasonal rises of IgE protein were not consistently correlated with the IgE levels in July (Table VII). In contrast, the post-seasonal falls were related to the October levels save for 1978 and 1979. In the untreated patients (Table III) the levels of IgE protein fell with time ($p < 0.003$) for both July and October values from 1973 to 1979 with mean halftime of 7.7 yr. The levels of IgE protein dropped from a geometric mean of 275 ng/ml in July 1974 to 166 ng/ml in July 1979 ($p < 0.001$) and from 337 ng/ml in October 1974 to 259

TABLE VII. Seasonal changes in IgE protein

	Group	r_s	p value
Seasonal rise vs July IgE protein level			
1973	T + C	0.3675	NS
1974	T + C	0.2364	NS
1976	C	0.3217	NS
	T	0.1986	NS
1977	C	0.4404	<0.05
	T	0.4410	<0.05
1978	C	0.2501	NS
	T	-0.0884	NS
1979	C	0.0147	NS
	T	0.0763	NS
Postseasonal fall vs October IgE protein level			
1973-1974	T + C	0.6757	<0.001
1974-1975	T + C	0.6569	<0.01
1976-1977	C	0.6135	<0.005
	T	0.6576	<0.001
1977-1978	C	0.6802	<0.002
	T	0.6519	<0.002
1978-1979	C	0.3249	NS
	T	-0.0060	NS

T = treated patients; C = control patients; NS = not significant.

ng/ml in October 1979 ($p < 0.002$). Comparison of the levels of IgE protein with IgE antibodies to AgE and SRW revealed positive correlations with r_s values ranging from +0.741 to +0.916 ($p < 0.001$ for all comparisons).

In the treated patients, the levels of IgE protein fell from 1976 through 1979 ($p < 0.002$ for both July and October values) from a geometric mean of 416 ng/ml in July 1974 (before immunotherapy) to 377 ng/ml in July 1979 ($p < 0.03$) and from 719 ng/ml in October 1974 to 439 ng/ml in October 1979 ($p < 0.001$). After immunotherapy, IgE protein rose ($p < 0.001$), and the rises were related to the May 1976 levels ($r_s = +0.633$, $p < 0.002$). Moreover, the rises of IgE protein from May to July 1976 were positively correlated with the rises of IgE antibody from May to July 1976 (AgE: $r_s = +0.818$, $p < 0.001$; SRW: $r_s = +0.864$, $p < 0.001$). Comparison of IgE protein in the patient groups showed a suppression of the seasonal rise in 1976 ($p < 0.01$) but not thereafter. Comparison of postseasonal falls of IgE protein did not reveal a difference between the patient groups at any time.

The cumulative doses of SRW extract received from May 1976 through August 1979 by the immunized patients ranged from 5.17×10^4 PNU (111.7 μ g of AgE) to 7.55×10^5 PNU (1631 μ g of AgE), with a mean cumulative dose of 4.81×10^5 PNU (1039 μ g of AgE). All patients received a maximum

dose of at least 1000 PNU per injection, and 15 of 19 patients who remained in the study through 1979 tolerated the maximum dose of 10,000 PNU per injection ($21.8 \mu\text{g}$ of AgE). The average maximal dose per injection was 8.82×10^3 PNU ($19 \mu\text{g}$ of AgE).

DISCUSSION

This study highlights several aspects of IgE antibody formation in ragweed hay fever. First, untreated patients show a regular rise and fall of IgE antibodies to AgE and SRW and of IgE protein, and these responses continued throughout the 6 yr of the study. This feature of the IgE immune response is clearly related to inhalation of pollen, and the finding that it continues over many years confirms and extends prior observations of ourselves¹² and others.⁹⁻¹¹ The seasonal and postseasonal changes in IgE antibodies and IgE protein are related to the levels before the change (Tables II and VII). Table II indicates that the postseasonal falls are more intensely correlated with the levels before the change (October) than is the case for the seasonal rises. The difference may be explained by variations in individual pollen exposure. The seasonal rises in IgE antibody occur shortly after the peak of ragweed pollination and are maximal about mid-October for patients in the southern Minnesota area.¹⁷ During this time, mononuclear cells capable of spontaneously producing IgE protein and IgE antibody to SRW appear in the bloodstream²³; by November such cells can no longer be detected.²⁴ The October to July postseasonal falls of IgE protein and IgE antibody lower the levels by about 50%. However, this fall is less than expected if IgE antibody production ceased after the season. Based on a $T_{1/2}$ of 2.7 days,²⁵ IgE antibody levels should decrease to vanishingly low levels over a period of 9 mo (corresponding to a total of $270/2.7 = 100$ half-lives). Thus the continuing presence of IgE antibody indicates that synthesis persists during the postseasonal fall. Our prior studies have shown that the postseasonal falls are exponential and the decay curves do not change over the 9 mo postseasonal period.¹² Finally, certain untreated patients failed to show regular seasonal changes of IgE antibody (see Fig. 1, a).

A second finding is the gradual fall of IgE antibodies in the untreated patients over the 7 yr period. The mean halftime of IgE antibodies to AgE and SRW calculated from the regression analyses was 6.2 yr for the untreated subjects. Based on another study of IgE protein levels, it seems likely that the peak of IgE production occurs during late childhood and adolescence, ages 6 to 15 yr, and gradually wanes thereafter.²⁶ The mechanisms controlling IgE antibody production with age are unknown; we do not believe

that change of exposure to pollen is an explanation for the falls with age because many patients actually increased their outdoor activities during the study period and ragweed pollen counts during the study period did not vary more than threefold. Interestingly, in May 1976 the levels of IgE antibodies in the untreated patients were inversely correlated with age ($r_s = -0.46$, $p < 0.05$). Finally, the results showing a gradual drop in IgE antibody may explain in part the clinical observation that patients often lose sensitivity to ragweed pollen during midlife.

A third finding concerns the magnitude of the effect of immunotherapy on IgE antibody levels and the timing of that effect. Prior studies showed partial suppression of the seasonal rises,⁹⁻¹² but the long-term effect on IgE antibody levels was not determined. We found that immunotherapy suppressed the seasonal rise in IgE antibodies during the 4 yr studied and reduced the level of IgE antibodies by a mean of 73% over the period from 1974 to 1979. However, Fig. 3 reveals that the rate of decrease of IgE antibodies was changing. During the first year of immunotherapy, the levels of IgE antibody dropped rapidly (July 1976 to July 1977). The rate of decrease is clearly less in each succeeding year as judged by comparison of the July to July values for 1977 through 1979. Thus the suppressive effect of immunotherapy on the seasonal rises, though still significant (Table IV), has diminished. In contrast, immunotherapy had no effect on postseasonal falls of IgE antibodies during the first 3 yr of the study, and only in 1978 and 1979 was an effect on the postseasonal fall of IgE antibody to AgE seen. Thus, immunotherapy suppresses the seasonal rise of IgE antibody stimulated by pollen inhalation, while failing to affect IgE production during the postseasonal period. Therefore the suppressor cell activity shown by Rocklin et al.²⁷ presumably affects cells stimulated recently by inhaled allergen while not altering IgE production during the postseasonal period. Surprisingly, our finding using quantitative radioimmunoassay methods of a 73% fall in IgE antibody in 4 yr agrees remarkably well with the approximately 80% reduction in IgE antibody to SRW over 4 yr of immunotherapy found by Connell and Sherman²⁸ using passive transfer.

Initiation of immunotherapy produced an abrupt increase in IgE antibodies to both AgE and SRW, and the magnitudes of these rises were related to the IgE antibody levels prior to immunotherapy and not to the quantity of pollen extract administered. Blocking antibodies rose and the initial rise was correlated with the extract dose injected and not with the IgE antibody levels prior to immunotherapy. In July 1978, after 2 yr of immunotherapy, blocking antibodies reached a

plateau value and remained at approximately that same level. Neither the plateau levels of blocking antibodies nor the rise of blocking antibody from May 1976 to July 1979 were related to the total dose of ragweed extract. Analysis of the relationship of IgE and IgG antibodies to AgE and SRW in immunized patients shows a significant inverse correlation for AgE and no significant correlation for SRW. This inverse relationship for IgE and IgG antibodies to AgE agrees with the finding by Lichtenstein et al.¹⁰ of a correlation between the increase in blocking antibody and the decrease in IgE antibody to AgE and points to a role for IgG antibodies in the effects of immunotherapy. This inverse relationship cannot be attributed to IgG blocking antibodies interfering in the RAST because we used quantities of solid-phase allergen sufficient to bind all the IgE antibody in the test sera. Earlier, we found positive correlations between the levels of IgE and IgG antibodies to AgE and suggested that a form of partial immunologic tolerance existed in these patients.^{12, 29} Although the patients in the present study have not been treated as long as the patients in the prior study, no decrease in the IgG antibody levels has appeared. The observations of Sherman et al.³⁰ suggest that levels of skin-sensitizing (IgE) and blocking (IgG) antibodies decrease as patients are treated for longer than 5 yr. Fig. 3 shows that the rate of decrease of IgE antibody is changing and it will be of interest to study these patients again after further treatment.

Finally, these results must be kept in mind as new therapies for IgE-mediated allergies are tested. IgE antibodies consistently showed seasonal rises and post-seasonal falls over the 6 yr period. During the post-seasonal falls, IgE antibody levels decreased only by about 50%, suggesting that synthesis of IgE antibody continues postseasonally. Immunotherapy clearly affected the seasonal rises, but it failed to affect the seasonal falls save for IgE antibodies to AgE in 1979. These findings suggest the existence of two phases of IgE antibody formation, and possibly two states of IgE-producing cells, one affected by immunotherapy and the other not.

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