

Dose response of IgE and IgG antibodies during ragweed immunotherapy

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We studied the detailed dose-response relationship for ragweed (RW) antibody responses in 51 patients who received maximal-dose immunotherapy with crude RW extract. Serum RW-IgG and RW-IgE levels were determined by solid-phase radioimmunoassay at frequent intervals during initiation and maintenance of immunotherapy. Pretreatment RW-IgE ranged from 0.94 to 974 ng/ml (median 105); 45/51 patients had insignificant levels (<250 ng/ml) of RW-IgG. The maximal doses given ranged from 0.19 to 93.5 µg of RW antigen E per injection. All patients produced a significant IgG response (median peak 3462 ng/ml, range 689 to 24,395), and 46/51 had significant increases in IgE antibody (median peak 231 ng/ml, range 12 to 1528). A threshold dose was defined for each patient's IgG and IgE response as that dose level which initiated a persistent increment in immunoglobulin to $\geq 25\%$ of pretreatment levels. The median threshold dose for IgE was 0.13 µg of antigen E, which was achieved in a median time of 42 days. The threshold dose for IgG was significantly higher (median 0.56 µg of antigen E; $p = 0.001$) and occurred significantly later (median 79 days; $p = 0.003$). Despite variability over 3 orders of magnitude, the thresholds for IgE and IgG responses were significantly correlated for individual patients ($r = 0.487$; $p = 0.002$). The maximum RW-IgE response occurred in a median of 107 days, after which IgE antibodies declined in 46 of 49 patients. The maximal IgG response occurred significantly later (median 245 days; $p < 0.001$) and then plateaued or declined modestly. The doses required to achieve maximal IgE and IgG responses were significantly correlated ($r = 0.638$; $p < 0.001$). The maximum IgG response was positively correlated with the maximal dose of RW antigen E received ($r = 0.592$; $p < 0.001$). In 28 of the 51 patients, the incremental rise in total serum IgE was more than twice that observed for RW-IgE at the time of the maximum response, suggesting a nonspecific effect of RW immunotherapy on total serum IgE levels. This discrepancy could not be accounted for by environmental stimulation from other known allergens, as assessed by skin testing, or by pretreatment levels of RW-IgE or total IgE. These observations indicate that the human IgE antibody response during high-dose RW immunotherapy is more sensitive to both stimulation and suppression by continuous allergen administration than is the IgG response. This finding may have implication both for our understanding of the in vivo regulation of immune response during allergen immunotherapy and for future attempts to modify specific IgE antibody responses in man. (J ALLERGY CLIN IMMUNOL 73:94-104, 1984.)

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Previous studies of allergen immunotherapy have shown that significant relief of symptoms is accompanied by measurable immunologic changes.¹ These include a marked rise in serum IgG antibody, alteration in lymphocyte responsiveness to allergen in vitro, and increased IgG and IgA antibody in nasal secretions. Although first noted years ago by passive transfer,^{2,3} the initial increase in allergen-specific IgE that occurs early in immunotherapy is less well appreciated.

We have embarked on a longitudinal study of IgG and IgE antibody fluctuations during long-term immunotherapy with high doses of RW pollen extract.

Abbreviations used

RW:	Ragweed
RW-IgG:	Ragweed-specific IgG antibody in serum
RW-IgE:	Ragweed-specific IgE antibody in serum
E _{Th} :	Threshold dose for IgE response
G _{Th} :	Threshold dose for IgG response
E _{Max} :	Observations at maximum IgE response
G _{Max} :	Observations at maximum IgG response
RAST:	Radioallergosorbent test

In the first phase of this effort we have examined with frequent serologic assays the RW-IgG and RW-IgE antibody levels in 51 patients who have been initiated on RW immunotherapy under various but similar protocols during the period 1978-1981. This paper describes the dose response and kinetics for IgG and IgE antibody responses and their interrelationships.

MATERIALS AND METHODS

Patients

Fifty-four RW-sensitive patients entered one of four study groups that received high-dose RW pollen extract under slightly differing regimens. Each patient had a history of seasonal rhinitis in late August and September for at least 2 previous years and a positive skin test to RW pollen extract at a dilution of 1:312,500, w/v, or greater and to RW antigen E at a concentration of 0.01 µg/ml or less. Three patients dropped out of the studies for various reasons prior to the achievement of a maximal dose and have been omitted from the analysis reported here. The 51 remaining patients were treated according to previously published protocols, which are briefly summarized in Tables I and II. All patients began taking immunotherapy between late January and early March. All doses were advanced according to schedule until systemic reactions occurred (24 of 51 patients) or until a maintenance level dose (median 9.35 µg of RW antigen E) was achieved (cf. legend to Fig. 6). These maximal doses were continued indefinitely thereafter at intervals of about 2 wk.

Because all patients were not recruited concurrently and because in one group (group IV, Table I) the immunotherapy was a modified "cluster" regimen, we examined the principal conclusions of the study for their dependency on the inclusion of group IV patients and, conversely, for the similarity of group IV patients to the remaining three groups. In all statistical tests performed, it was not possible to distinguish the cluster-treated patients from the remainder of the study groups. For this reason, the final statistical analyses were performed on the combined total subject population and are presented as such in the Results.

Reagents

Short RW pollen extract (extracted at 1:10, w/v, and freeze-dried) was provided by Hollister-Stier Laboratories, Spokane, Wash. The same lot was used for diagnosis and treatment in all patients. Serial dilutions were performed in isotonic saline containing 0.4% phenol in 1978-1979 and in

normal saline with 0.03% human serum albumin and 0.4% phenol thereafter. A 1:20, w/v, dilution of this extract contained 187 µg/ml RW antigen E. All doses administered are reported in terms of antigen E content.

Observations

Serum samples were obtained before treatment, every 3 wk during the first 8 mo of immunotherapy, and at least four times a year (October, February, May, and July) after maximal doses were achieved.

RW-IgG was determined in all sera by the *Staphylococcus* protein A solid-phase radioimmunoassay previously described.⁴ The arbitrary unit used to quantitate RW-IgG in previous studies of this subject population⁵⁻⁷ has now been determined by saturation analysis to represent 0.25 ng of RW antibody. RW-IgE was determined by RAST⁸ using the identical solid-phase allergosorbent employed for the IgG measurements. Standardization of RW-IgE in ng/ml terms was achieved by a depletion of total IgE content with excess RW-agarose as previously described by Gleich et al.⁹ and Gleich and Jacobs.¹⁰ In all sera with appreciable IgG titers, multiple serum dilutions were evaluated for RW-IgE in order to detect and correct for potential influence of IgG on the quantitation of RW-IgE.^{11, 12} This is achieved by estimating RW-IgE content at progressive serum dilutions until the estimate corrected for dilutions is constant, indicating that the allergosorbent is in sufficient excess to bind most of the RW antibodies of both isotypes. Total serum IgE was determined by solid-phase radioimmunoassay as previously described⁸ on pretreatment sera and at least four times per year thereafter.

For analysis the following designations and definitions were employed. *Entry* refers to measurements made immediately prior to initiation of immunotherapy. *Threshold dose* (Th) was defined as that single dose of RW extract (in terms of antigen E content) which subsequently initiated a significant (≥25%) and persistent rise in serum levels of RW-IgG or RW-IgE antibodies. *Maximum response* (Max) refers to measurements made at the time when the peak serum levels of either RW-IgG or RW-IgE were observed. *Last* refers to the final measurements made (to date) on all patients who achieved a maximum antibody response.

Analysis of data

Data were analyzed by nonparametric statistical methods unless otherwise indicated in the text. For group comparisons, the Wilcoxon signed rank test was employed for paired data, and the Wilcoxon rank-sum test for non-paired data. Pearson correlation coefficients were employed throughout. For discrete variables, contingency table analyses by chi-square technique was utilized. These analyses were performed with the CLINFO computer system for clinical investigation.

RESULTS

Profile of a typical patient

Fig. 1 displays the dose regimen and serial antibody responses of a typical study patient. The threshold and maximal doses and antibody responses

TABLE I. Origin of study subjects and summary of immunotherapy schedule

Group	No. of subjects	Entry date	Immunotherapy schedule	Dose increment	Maintenance interval	Reference
I	8	2/79	Single inj. weekly (0.002-93.5 μ g of AgE over 24 wk)	1.5-2.0 $\times$	1 wk until 10/80, then 2 wk	5
II	18	2/80	2-3 inj. 20 min apart every 3 wk (0.03-18.7 μ g of AgE over 19 wk)	1.1-5.0 $\times$	3 wk until 10/80, then 2 wk	6
III	16	2/80	Single inj. weekly (0.002-18.7 μ g of AgE over 20 wk)	1.5-2.0 $\times$	1 wk until 10/80, then 2 wk	6
IV	9	3/81	2-3 inj. 20 min apart weekly (0.03-18.7 μ g of AgE over 9 wk)	1.1-5.0 $\times$	2 wk	7

inj = injection; AgE = RW antigen E.

TABLE II. Patient data at time of study entry

Parameter	No. of subjects	Median	Mean	Range
Sex				
Male	37			
Female	14			
Age (yr)				
20-29	14			
30-39	20			
40-49	17			
Prior RW IT				
Never	39			
>7 yr ago	12			
<7 yr ago	0			
Entry immunologic data				
Intradermal skin tests		1:200,000,000	—	1:10 ⁹ -1:312,500
Dil. RW extract, w/v, for end pt*		1.9×10^{-5}	—	3.8×10^{-6} - 1.0×10^{-2}
RWE (μ g/ml AgE) for end pt.				
Histamine release				
None	2			
<50%	3			
>50%	46			
RWE (μ g/ml AgE) for 50% release	46	1.5×10^{-5}	1.5×10^{-4}	5×10^{-7} - 6×10^{-3}
Serum RW-IgE (ng/ml)		105	168	0.94-974
Serum RW-IgG (ng/ml)				
Undetectable (<40)	40			
Insignificant (40-250)	5			
>250	6	435	723	268-1895
Total serum IgE (ng/ml)	51	412	776	19-2958

IT = immunotherapy; RWE = ragweed extract; AgE = RW antigen E.

*Skin test end points determined with saline diluent during 1979-1980 were converted to the comparable end point for human serum albumin diluent (used after 1980) by employing a 125-fold correction as previously shown to be appropriate.⁷

are indicated. Several findings depicted in Fig. 1 are representative of the entire study group (see below). First, the dose of RW pollen extract that subsequently initiated a significant increase in serum antibody was less for the RW-IgE response ($E_{Th} = 0.04 \mu$ g of RW antigen E) than for the RW-IgG ($G_{Th} = 0.66 \mu$ g of

RW antigen E). Second, this threshold response was achieved earlier for RW-IgE (42 days) than for RW-IgG (84 days). Third, the cumulative dose required for maximum antibody response was less for RW-IgE ($E_{Max} = 271 \mu$ g of RW antigen E) than for RW-IgG ($G_{Max} = 327 \mu$ g of RW antigen E). Fourth,

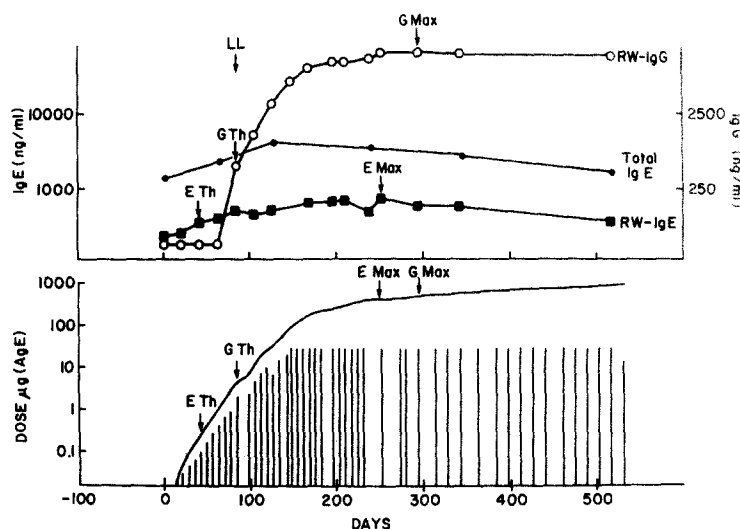


FIG. 1. Profile of a typical patient illustrating the dose regimens for immunotherapy and the IgE and IgG antibody responses. Plotted are the relationships between time expressed in days, individual and cumulative doses of RW pollen extract expressed in nanograms of antigen E (AgE), and serum levels of RW-IgE, RW-IgG, and total IgE. *Top panel*, RW-IgE, RW-IgG, total IgE data. LL, large local reaction. *Bottom panel*, Progressive cumulative dose (line) over respective single doses (bars) over time in days.

the maximum RW-IgE response occurred earlier (252 days) than the maximal IgG response (294 days). After achieving its peak value, RW-IgE declined significantly (51% over 265 days), whereas RW-IgG stabilized at >92% of the peak value (12,961 ng/ml) over the same interval. Finally, total serum IgE increased from 1080 to 2767 ng/ml over an initial interval of 252 days, during which RW-IgE increased from 199 to 635 ng/ml. This discrepancy between total and RW-IgE responses was observed in 28/51 patients, as discussed further below.

Profile of RW antibody levels

Fig. 2 displays the distribution of RW-IgE and RW-IgG levels at entry, the levels at the threshold dose for the first significant antibody response, the maximum levels achieved, and the last observation available. For RW-IgE (*left panel*), the entry RW-IgE levels were dispersed over 3 orders of magnitude (range 0.9 to 974 ng/ml). The median entry level of 105 ng/ml was significantly increased at E_{Th} (196 ng/ml; $p < 0.001$); a further significant increment to 267 ng/ml ($p < 0.001$) was observed at E_{Max} . Subsequent to the peak response, a significant decline in RW-IgE occurred, with antibody levels falling on average over $45\% \pm 3$ during observation periods ranging from 14 to 727 days (median 317).

In contrast, the RW-IgG distributions were less dispersed at every observation point than those for

RW-IgE. At entry, 40 of the 51 patients had undetectable RW-IgG (<40 ng/ml). The median peak RW-IgG response was 3462 ng/ml but ranged from 689 to 24,398 ng/ml. This peak response declined only minimally to a median of 2667 ng/ml (range 121 to 15,893) over a median of 262 days of observation (range 14 to 349). Although 35 of the 51 patients declined between maximum and last RW-IgG observations, the percent fall averaged only $31\% \pm 4.5$ (S.E.M.), compared with an average fall of $45\% \pm 3.3$ for RW-IgE over the same intervals.

Time required for threshold and maximum antibody responses

The time in days required for threshold and maximum antibody responses is shown in Fig. 3. As might be expected from the variability in treatment regimens, the range of times to threshold responses was considerable (from 14 to 259 days for RW-IgE and 21 to 231 days for RW-IgG). In 47 of 51 patients, the IgE began to rise before IgG. For the group, the E_{Th} occurred significantly earlier (median 42 days) than the G_{Th} (79 days; $p = 0.003$ by Wilcoxon paired sample test). Similarly, the maximal RW-IgE response occurred significantly earlier (107 days) than the maximum RW-IgG response (median 245 days; $p < 0.001$ by Wilcoxon test). The maximum IgE response occurred after the maximum IgG response in only four of the 51 patients.

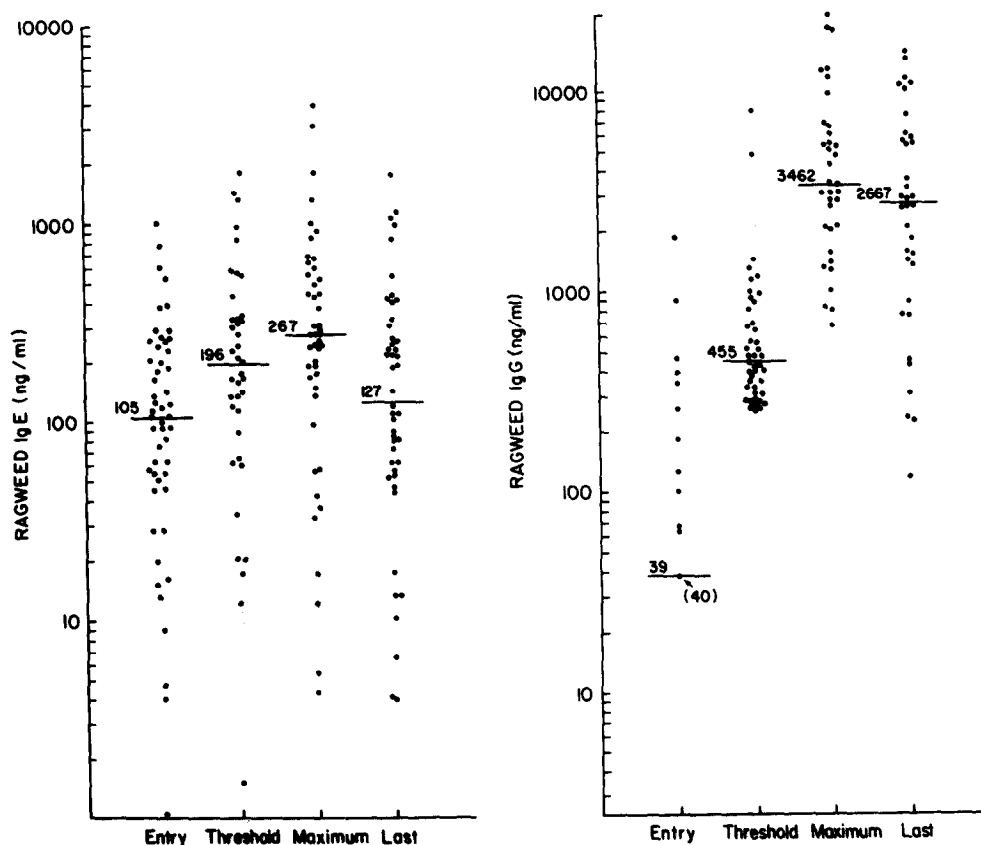


FIG. 2. Left panel, Serum RW-IgE levels during RW immunotherapy. RW-IgE levels were plotted for 51 patients prior to immunotherapy at entry, 39 patients at threshold (i.e., with a $\geq 25\%$ rise in RW-IgE levels during immunotherapy), and 46 patients at maximum and last. (Two patients had their entry antibody level as their maximal level and three patients had their maximal antibody level as their last value obtained, and therefore we were unable to be sure that we had determined the peak antibody response.) Horizontal lines, Median levels. Right panel, Serum ragweed IgG levels during RW immunotherapy. RW-IgG levels were plotted for 51 patients at entry, 43 patients at threshold ($\geq 25\%$ rise in RW-IgG levels during immunotherapy), and 35 patients at maximum and last. (Sixteen patients were excluded because maximum value was last value, and we were unable to be sure that we had determined the peak antibody response.) Horizontal lines, Median levels. Values < 39 ng are considered equal to 39.

Doses of RW extract required for threshold and maximum antibody responses

The single doses of RW extract that subsequently initiated a significant and persistent rise in RW antibody levels are shown in Fig. 4. The E_{Th} ranged from 0.01 to 10 μ g of antigen E in the 39 of 51 patients who were observed to have a significant boost in RW-IgE during therapy. The dose range for G_{Th} was equally expansive (range 0.03 to 18.7). The median G_{Th} (0.56 μ g) was significantly greater than the E_{Th} (0.13 μ g of antigen E; $p < 0.001$). Similarly the E_{Max} dose was significantly less than the G_{Max} dose (1.8 μ g vs. 4.7; $p < 0.001$). Similar dose relationships were found for the cumulative RW doses required for threshold and maximum antibody responses.

For individual patients, the log dose required for E_{Th} was significantly correlated with the dose for G_{Th} (Fig. 5, upper panel). A significant positive correlation also exists for E_{Max} and G_{Max} doses (Fig. 5, lower panel). Comparable correlation coefficients were found when cumulative doses for threshold and maximum were compared ($r = 0.412$, $p = 0.011$ for Th; $r = 0.551$, $p = 0.001$ for Max).

Correlation of maximal dose and RW-IgG response

Thirty-five patients achieved a stable G_{Max} on maximal dose therapy. There was a significant positive correlation ($r = 0.59$; $p < 0.001$) between the maximal dose of RW extract and the G_{Max} response observed (Fig. 6). Those 18 patients who achieved max-

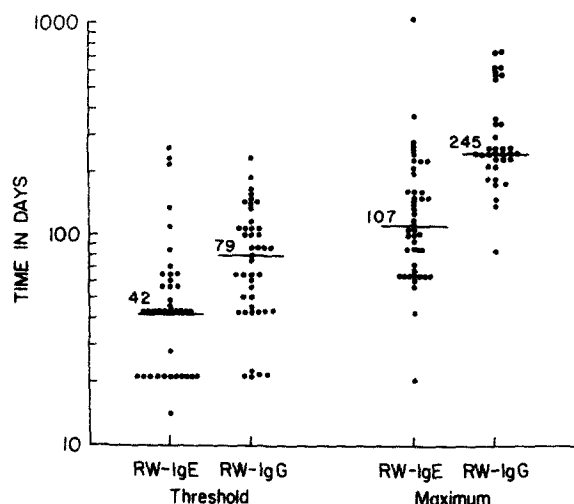


FIG. 3. Time required to achieve threshold and maximal antibody responses of RW-IgE and RW-IgG. *Horizontal lines*, Median values. The patient groups were defined as described in the legend for Fig. 2. Threshold RW-IgE occurred earlier (median time 42 days) than threshold RW-IgG (median time 79 days; $p = 0.003$). Maximum RW-IgE also occurred earlier (median time 107 days) than maximum RW-IgG (median 245 days; $p < 0.001$).

imal tolerated dose, defined as that RW dose above which unacceptable systemic reactions were observed, were similar in pattern to the remaining 17 patients who did not experience systemic reactions at or near their maintenance dose.

Increases in total serum IgE

In 40 of the 51 patients, total serum IgE rose concomitantly with RW-IgE during early immunotherapy. Because both RW-IgE and total IgE protein were calibrated in weight per volume terms (ng/ml), it was possible to examine how much of the observed increment in total IgE could be accounted for by RW-IgE. For this purpose, each patient's total IgE was determined in that serum sample corresponding to the peak RW-IgE response. In 24 patients where the serum of interest was exhausted, the closest available blood sample was evaluated instead. The increment in total IgE and RW-IgE over pretreatment values was then calculated. Allowing for errors in standardization and measurement in both assays, any patient whose RW-IgE accounted for 50% or more of the increment in total IgE was considered to have "concordant" results. That is, it was deemed probable that the increment in total IgE was, in large part, accounted for by the increase in RW-IgE stimulated by immunotherapy. Patients whose increment in total IgE was greater than twice that observed for RW-IgE were conversely identified as "discordant." By this classification scheme, 23 concordant and 28 discordant

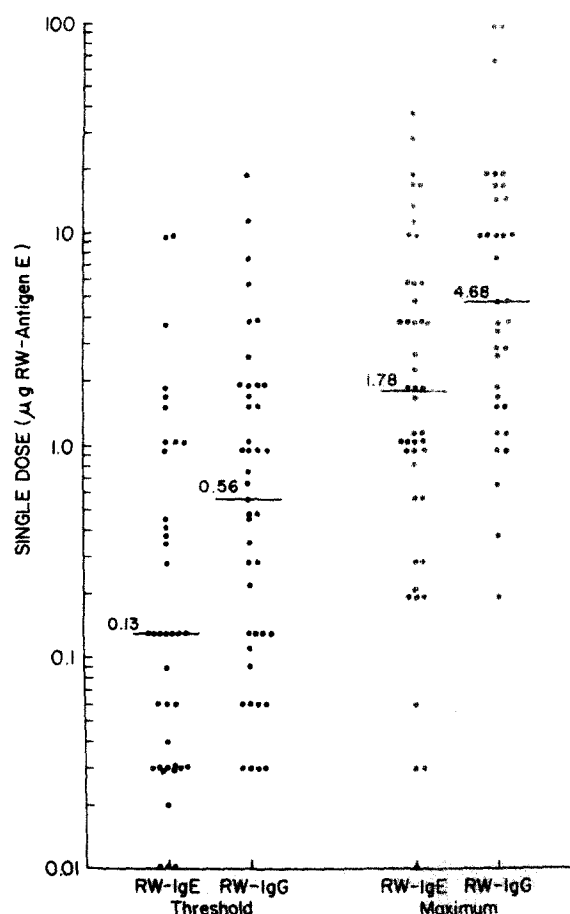


FIG. 4. Dose of RW extract required for threshold and maximum antibody response of RW-IgE and RW-IgG. *Horizontal lines*, Median values. Values for certain patients were excluded for reasons noted in Fig. 2. The single doses that immediately preceded attainment of threshold RW-IgE levels (median dose 0.13 μ g) were significantly less ($p < 0.001$) than those which preceded threshold RW-IgG levels (median dose 0.56 μ g). The single doses that immediately preceded attainment of maximum RW-IgE levels (median dose 1.78 μ g) were significantly less than those which preceded maximum RW-IgG (median dose 4.68 μ g; $p < 0.001$).

dant cases were identified. Table III examines the characteristics of these two subpopulations of patients. The two groups did not differ in distribution of age or gender. The entry levels of RW-IgE and total IgE did not differ between the two groups. Likewise the threshold doses for E and G antibody responses were similar. Interestingly, the increments in total IgE were comparable in the two groups, whereas there was a trend for the RW-IgE increases to be less in the discordant group (Table III).

Since relevant seasonal exposure to airborne allergens has been shown to boost IgE antibody synthesis, we examined the question of whether the disproportionate rise in total serum IgE could be accounted for

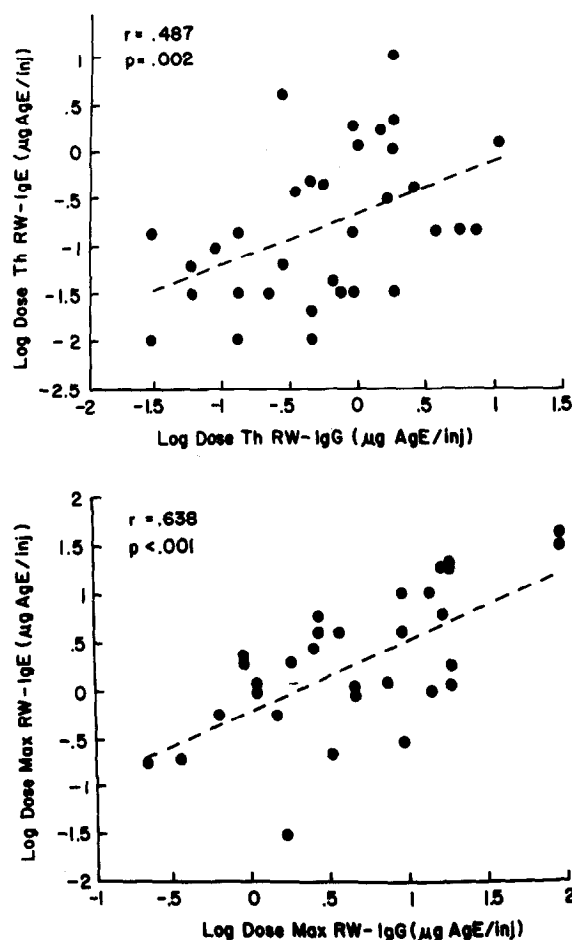


FIG. 5. Correlation of the doses of RW antigen E (*AgE*) producing the threshold (*Th*) (upper panel) and maximum (*Max*) (lower panel) responses for RW-IgE and RW-IgG. In both cases the doses required for RW-IgE and RW-IgG were significantly correlated. *inj*, Injection.

by environmental exposure to other allergens to which the patients were sensitive. This was examined in two ways. First, the frequency of positive skin tests to mixtures of extracts of grasses, trees, and molds were compared. As shown in Table III, no differences in the prevalence of positive skin tests to grasses and trees were observed between the two groups. For molds, the frequency of positive skin test were significantly *less* in the discordant group ($p = 0.05$) than in the concordant group. As a second index of seasonal exposure, we examined the time of the year in which the maximum IgE responses occurred. Again the two groups did not differ with regard to the prevalence of peak responses during three seasonal intervals from April through November. Thus neither of these statistical approaches was able to implicate seasonal exposure to other known allergens as a cause of the disproportionate increase in total serum IgE observed in 28 of the 51 patients.

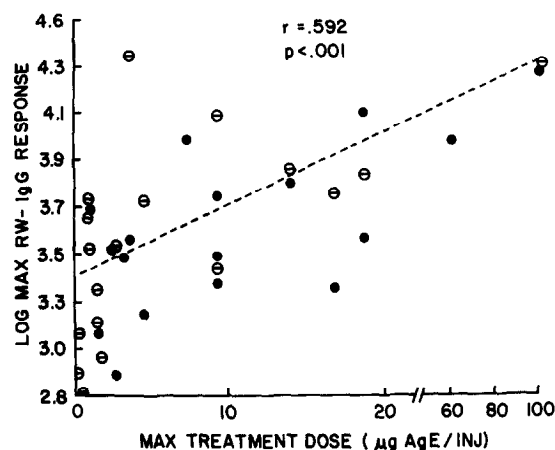


FIG. 6. Correlation of maximum single doses of RW pollen extract [antigen E (*AgE*)] required to achieve maximum RW-IgG. Higher treatment doses were associated with production of larger amounts of RW-IgG antibody ($r = 0.592$; $p < 0.001$). Open circles with bars, Patients whose maximal dose was established by a systemic reaction at that dose; closed circles, patients who did not sustain a systemic reaction at their maximum dose. Some of these patients nevertheless had had their dose escalations modified because of earlier systemic reactions. There is no discernible difference between the distribution of the two types of maximal-dose patients. Six patients achieved maximum doses as established by protocol ($\geq 18.1 \mu\text{g}$) without experiencing any systemic reactions. *INJ*, Injection.

DISCUSSION

The study was undertaken to examine the precise dose-response relationship for IgG and IgE antibody responses to standard RW immunotherapy. Fifty-one patients receiving variations of high-dose protocols were studied. When the RW-IgG and RW-IgE responses were normalized for each patient's respective peak antibody response, a composite dose response and kinetic course can be described for the group as a whole. Such a composite result is depicted in Fig. 7. The figure serves to recapitulate the principal findings of this study regarding the dose-response relationships for IgE and IgG antibodies during immunotherapy. (1) Significant rises in RW antibodies occur during immunotherapy in virtually all patients (49 of 51 patients had RW-IgE increases; all patients had RW-IgG increments). (2) The RW-IgE appears to occur earlier than the RW-IgG antibody response and at a lower dose of allergen, both in terms of single injection dose and cumulative dose. (3) The maximum RW-IgG response occurs later and at a higher allergen dose than the peak RW-IgE response. (4) After maximum response, RW-IgE declined significantly in 46 of 49 patients but the RW-IgG was either maintained at the

TABLE III. Characteristics of patients with concordant and discordant total IgE responses

	Concordant ($\frac{\text{tot IgE}}{\text{RW-IgE}} \leq 2$)	Discordant ($\frac{\text{tot IgE}}{\text{RW IgE}} > 2$)	p value
No. of subjects	23	28	0.47
Sex	19 M; 4 F	18 M; 10 F	0.13
Age			
20-29	6	8	
30-39	6	14	0.10
40-49	11	6	
Entry RW-IGE (ng/ml)			
Mean	176	163	
Median	116	101	0.719
Range	0.94-974	4-771	
Entry total E (ng/ml)			
Mean	704	723	
Median	353	488	0.265
Range	83-2958	19-2900	
Dose E _{Th} (μg of RW AgE)			
Median	0.13	0.13	0.402
Dose G _{Th} (μg of RW AgE)			
Median	0.28	0.94	0.342
RW-IgE (ng/ml)			
Median	176	103	0.099
Mean % change	200	87	0.013
Total E (ng/ml)			
Median	322	436	0.477
Mean % change	96	85	0.936
Positive non-RW skin tests			
Grass mix	18/22	23/28	0.63
Tree mix	18/22	20/28	0.30
Mold mix	12/22	8/28	0.05
Time of E _{Max} response			
April-May	9	8	0.15
June-August	11	11	
Sept.-Nov.	2	9	
Feb.	1		

peak level (16 patients) or declined modestly (35 patients).

Since the sensitivity of the assay for RW-IgG is considerably less (~40 ng/ml) than that for RW-IgE (~0.5 ng/ml), it is quite possible that the G_{Th} dose has been overestimated. It therefore remains a possibility that the G_{Th} would be similar or identical to the E_{Th} if the assay for RW-IgG could be made more sensitive. We believe that this possibility is unlikely for several reasons. First, the composite dose response curves for RW-IgG and RW-IgE are roughly parallel when normalized for peak values (Fig. 7). Second, the maximum RW-IgG response is clearly later and requires a higher dosage than that for E_{Max}. These observations suggest that since the maximum and ascending portions of the dose-response curve are shifted to the right for IgG, the threshold may also be

expected to be higher. Finally, experimental studies of reagenic antibody responses in mice indicate that with low-dose treatments, IgE antibody responses may be stimulated without a concomitant IgG isotype response.¹³ We therefore consider it reasonable to suppose that the entire dose-response curve is shifted to the right for the human RW-IgG response as compared with the RW-IgE response.

The fact that threshold doses for RW-IgE and RW-IgG are significantly correlated (Fig. 5) indicates that the ε and γ isotype responses are somehow linked to common environmental and/or genetic factors. These factors may predominate, since the threshold doses vary over 3 orders of magnitude among the patients studied. The nature of the factors that control the threshold for RW antibody responses remains obscure; threshold doses could not be correlated with

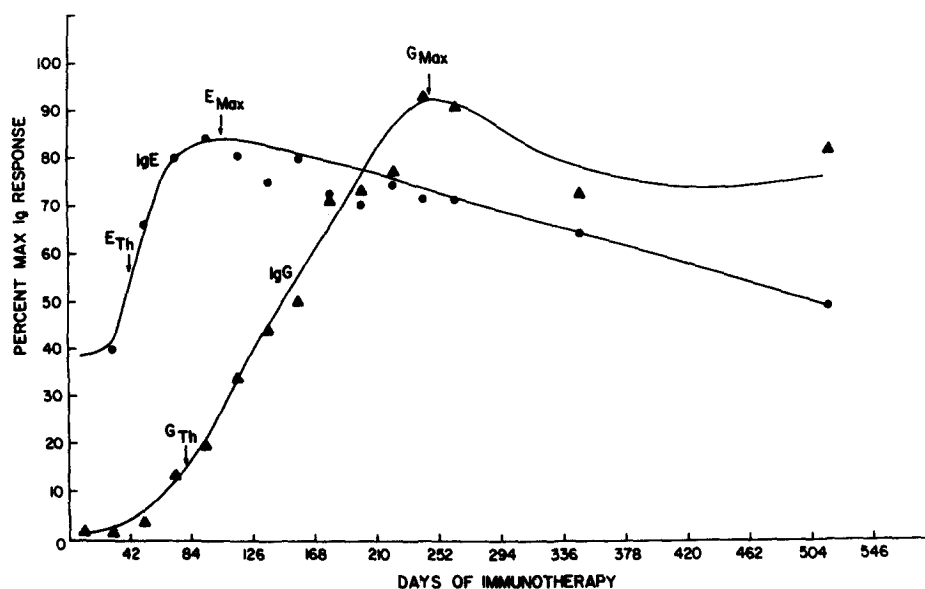


FIG. 7. Kinetics of RW-IgE and RW-IgG antibody responses in 51 patients undergoing RW immunotherapy. Each point is the median antibody response expressed as percent of maximum response for all patients who were treated during progressive 21-day intervals of treatment. Median threshold and maximal antibody responses are indicated for IgE and IgG. The kinetic drawing demonstrates that RW-IgE threshold and maximum levels occurred earlier than the corresponding RW-IgG threshold and maximum levels. After maximum, the RW-IgG curve plateaued and the RW-IgE fell (45%) to baseline.

any of a number of immunologic variates, including in the case of IgE, entry levels of RW-IgE or total IgE. Less surprising in view of the parallel dose-response curves (Fig. 7) is the finding of a significant correlation between the doses required to give maximum RW-IgG and RW-IgE responses.

Gleich et al.⁹ and Yunginger and Gleich¹⁴ previously demonstrated a positive correlation between IgG and IgE antibody levels over a single season of RW therapy ($n = 25$; $r_s = 0.73$; $p < 0.001$) and subsequently during perennial immunotherapy averaging 6 yr [$n = 63$; $r_s = 0.67$; $p < 0.001$]. In more recent studies utilizing a different antibody assay, the same group found a significant correlation between IgG and IgE antibody to antigen E (but not to RW extract) in July and October sera for each of the first 3 yr of immunotherapy.¹⁵ Our results, however, did not show a significant correlation of G_{Max} and E_{Max} ($r_s = 0.25$; $p = 0.09$) or of the maximum increments in RW-IgG and RW-IgE ($r_s = 0.14$; $p = 0.21$), whether expressed as amounts of antibody or as percent change. This difference may reflect the fact that our frequent measurements allowed determination of maximum antibody response and maximum incremental changes whereas the previous studies compared IgG and IgE antibodies drawn at arbitrary time points (before season and after season). Our data taken together suggest

that although the RW-IgG and RW-IgE responses are significantly linked in their dose-response relationships, the peak antibody levels achieved for RW-IgG are unrelated to those for RW-IgE.

We have demonstrated here, as in previous studies, a positive correlation between the dose of RW extract administered during immunotherapy and the subsequent peak RW-IgG antibody response mounted by a patient.^{16, 17} Since the doses achieved during human immunotherapy for reagenic diseases is limited by systemic reactions, it has not been possible to define precisely the top portion of the dose-response curve for RW-IgG. The problem is further compounded by the fact that dose-response curves for individual patients vary over 300-fold. It is therefore difficult to determine a single optimal effective dose that would be maximally stimulatory for most patients. This extensive variability of maximal antibody responses among patients suggests that serial determinations of IgG antibody is the only way to maximize an individual patient's IgG antibody response.

Sherman² and Sherman et al.¹⁸ demonstrated in 1940-1941 an increase in the amount of skin sensitizing antibody in the serum as an early response to pollen-extract injections. Lichtenstein et al.¹⁹ in 1973 utilized the RAST technique to demonstrate an early rise in serum RW-IgE levels in three of six patients

treated with RW extract. Gleich et al.¹⁵ recently demonstrated an abrupt increase in serum RW-IgE in 23 patients receiving initial RW immunotherapy between May and July. This present study provides the first detailed kinetic study of such a phenomenon by using the RAST technique. Forty-nine of 51 patients in our study had a significant increase in RW-IgE in early immunotherapy. These data suggest that such IgE increases may influence adverse reactions, which are most frequent in early immunotherapy. Data from these patients bearing on this topic will be presented elsewhere.

We and others have previously noted a clear-cut suppression of the usual season-induced rise of RW-IgE in treated patients.^{5, 6, 7, 14, 19, 20} Yunginger and Gleich¹⁴ have also observed a significant inverse correlation between entry IgE and the percent fall in IgE. We do not see this relationship in these patients ($r_s = -0.01$). Perhaps this discrepancy reflects the effects of long-term immunotherapy suppression (range 119 to 888 days) in the patients, as compared with observations across a single season as in most previous studies. The issue is of potential interest because any variable that could be measured before therapy, which would assist in predicting the likelihood of immunosuppression of IGE antibody with long-term therapy, might be of some clinical utility.

We are in agreement with Gleich and Yunginger on the lack of correlation between the peak IgG and the percent fall in IgE ($r_s = -0.08$). We and our colleagues²¹ also failed to observe such a relationship among patients treated long-term with RW allergoid. An earlier study from our division noted a significant inverse relationship between these variables. This may have reflected attenuation of the measurement of IgE by large quantities of RW-IgG, as has been observed with RAST.²² Our more recent findings and those of Gleich and Yunginger argue against IgG antibody acting to suppress the formation of IgE antibody.

The fact that 28 of 51 individuals had increases in total IgE greater than could be accounted for by the measured rise in RW-IgE remains unexplained. At least two explanations are possible. First, the excess IgE observed at peak RW-IgE response during immunotherapy could be due to concomitant environmental exposure to other allergens to which the subjects may be sensitive. This hypothesis was not supported by a comparison of the prevalence of positive skin tests or the season for peak IgE response between the 28 patients whose total IgE response was discordant and the 23 patients for whom total IgE increases were largely accounted for by RW-IgE responses. Nonetheless, the

hypothesis remains viable because specificities of IgE not tested for may be contributory.

An alternative explanation for the sometimes huge rises in total IgE compared to that for RW-IgE is that RW immunotherapy stimulates in a nonspecific manner the synthesis of IgE protein. Both the clinical response to treatment²³⁻²⁵ and the IgG antibody response²⁴ have been shown to be highly specific for the immunizing allergens. These well established observations do not preclude, however, the possibility that there is a nonspecific induction of IgE synthesis during intensive immunotherapy. Such nonspecific augmentation of IgE production is well established for certain rodent models of parasitic infections.^{26, 27} In addition, soluble factors that regulate IgE isotype responses have been described.²⁸ Further studies will be necessary to determine definitely whether nonspecific IgE synthesis is stimulated during intensive treatment.

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