

Studies of perennial ragweed immunotherapy

Associated changes in cellular responsiveness, total serum antigen-binding capacity, and specific IgE antibody concentrations

John S. Irons, M.D.,* Jacob J. Pruzansky, Ph.D., Roy Patterson, M.D.,
and C. Raymond Zeiss, M.D. Chicago, Ill., and El Paso, Texas

Perennial ragweed immunotherapy was studied in 24 patients with ragweed pollenosis. Cellular responsiveness was determined by measuring the cellular reactivity and sensitivity to ragweed antigen E (RW-AgE) by RW-AgE-induced leukocyte histamine release. Serum blocking antibody content was determined by measuring the serum RW-AgE binding capacity by ammonium sulfate coprecipitation of bound RW-AgE. Specific IgE (anti-RW-AgE) concentration was determined by polystyrene tube radioimmunoassay. Cellular responsiveness decreased with continuing immunotherapy, as did the specific IgE concentrations. The serum RW-AgE binding capacity, in contrast, increased as treatment continued. The absence of demonstrable correlations between RW-AgE binding capacity and cellular reactivity, cellular sensitivity, and specific IgE concentrations contrasted impressively with the demonstration of multiple significant correlations between the change in the RW-AgE binding capacity and the other parameters studied. The degree of increase in RW-AgE binding capacity correlated significantly with the degree of decrease in both the specific IgE concentration ($p < 0.04$) and the cellular sensitivity to RW-AgE ($p < 0.003$). These findings suggest that the active process of blocking antibody production, rather than the passive presence of blocking antibody, is related to the process which decreases the specific IgE concentration and the degree of cellular responsiveness and therefore results in clinical improvement.

Since its initial description by Noon in 1911,¹ immunotherapy has repeatedly been reported to be an effective treatment of allergic diseases.²⁻¹⁸ Many studies have been done exploring the cellular and immunologic changes associated with immunotherapy. It has been hoped that such research

would help define the mechanism by which such therapy results in clinical improvement.

Decreased cellular responsiveness (reactivity or sensitivity), as determined by antigen-induced leukocyte histamine release,^{9, 13, 15-26} increased serum blocking antibody (IgG) content,^{13, 18, 21, 27, 28} and decreased serum reagin content,^{19, 29, 30} have previously been reported in some patients with immunotherapy-associated clinical improvement. It has seemed probable that all of these phenomena are related to the mechanism of clinical improvement, but their exact contribution to this yet unknown mechanism remains unclear.

One repeated problem has been the inability to correlate, in a statistically significant manner, any one parameter with both the extent of treatment and the degree of clinical improvement.³¹ The purpose of this investigation was to further explore the relationships between the extent of treatment, the degree of cellular

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Reprint requests to: Dr. Roy Patterson, 303 E. Chicago Ave., Chicago, Ill. 60611.

*During the majority of this study, Dr. Irons was a Fellow supported by United States Public Health Service Grant No. AI 11403 and the Ernest S. Bazley Grant; Present address: Oklahoma Allergy Clinic, Inc., P.O. Box 26827, Oklahoma City, Okla. 73126.

responsiveness, blocking antibody content, and the serum reagin or specific IgE concentrations in patients receiving perennial ragweed (RW) immunotherapy.

MATERIALS AND METHODS

Patients

Twenty-four patients 16 to 56 years of age were studied. Each had RW pollenosis manifested by rhinitis, asthma, or both. The diagnosis in each case was established by standard clinical evaluations including history, physical examination, and cutaneous tests.³² All 24 patients received immunotherapy with RW extract; other inhalant allergen extracts were included when indicated. Each patient was studied both prior to the initiation of immunotherapy and periodically during the course of the treatment. A total of 63 treatment samples were available from the 24 patients for measurement of cellular responsiveness (reactivity and sensitivity) and blocking antibody content. Fifty-two treatment samples from 21 of the 24 patients were available for measurement of the concentration of specific IgE directed against RW-AgE.

Immunotherapy

Perennial RW immunotherapy generally began with the subcutaneous injection of 0.05 cc of 1:5,000 w/v extract. The dosage was advanced as tolerated, usually by 0.05 cc/wk to a maximum dose of 0.50 cc of 1:50 w/v extract. Because of the difficulty in establishing the biologic activity of an allergenic extract, the cumulative treatment dose was defined as: the cumulative number of protein nitrogen units (PNU) of RW antigen administered, assuming 1:50 w/v RW extract = 10,000 PNU/ml.³³ The duration of treatment was defined as the number of weeks elapsed since the first RW immunotherapy injection.

Measurement of cellular responsiveness by leukocyte histamine release

RW-AgE-induced leukocyte histamine release dose-response curves were established as described previously.³⁴

Cellular reactivity (R) was defined as the maximum percent of the cellular histamine released, regardless of the effecting RW-AgE concentration.

Cellular sensitivity (S) was defined as minus the log of the microgram of RW-AgE per milliliter required to release 50% of the cellular histamine. The "minus" was included in this definition so that a decrease in the numerical representation of S would indicate that the cells had become less sensitive to the challenging antigen.

Measurement of blocking antibody content by antigen-binding capacity

Total serum RW-AgE binding capacity was determined using a modification of the binding technique initially described by Lidd and Farr,³⁵ who used radiolabeled crude RW. In these studies, a similar technique was used except that RW-AgE was labeled with ¹²⁵I. The blocking antibody content as measured by RW-AgE binding capacity (B) was

defined as the log of the nanograms of AgE bound by one milliliter of serum at the point where 50% of the trace-labeled antigen was bound. The antibody was precipitated in 40% saturated (NH₄)₂SO₄.

Measurement of specific IgE (anti-RW-AgE) concentration by polystyrene tube radioimmunoassay

The concentration of serum IgE directed against RW-AgE was determined using the polystyrene tube radioimmunoassay technique initially described by Zeiss and co-workers.³⁶ The specific (anti-RW-AgE) concentration (E) was defined as the log of the nanograms of specific IgE present in one milliliter of serum.

Designation of sample groups

Samples collected prior to the initiation of immunotherapy were designated pretreatment (PT) samples. Those collected during the course of therapy were designated treatment (T) samples. For purposes of comparison, the T samples were subgrouped into early treatment (ET) and later treatment (LT) groups. The ET samples were those collected from patients after they had received a cumulative treatment dose less than 3,000 PNU of RW antigen. The LT samples corresponded to cumulative treatment doses between 3,000 and 80,000 PNU of RW antigen. These designations were applied as subscripts to the parameters studied.

Computation of parameter changes

The change from pretreatment (Δ) was calculated to equal the treatment value minus the pretreatment value. For illustration purposes, let X indicate R, S, B, or E, then $\Delta X_T = X_T - X_{PT}$, $\Delta X_{ET} = X_{ET} - X_{PT}$, and $\Delta X_{LT} = X_{LT} - X_{PT}$. Applying the definitions cited above, ΔR was calculated as the difference of percent values and ΔS , ΔB , and ΔE were calculated as the differences of log values. A negative Δ value indicated that the measured parameter had decreased with treatment.

Statistical analysis

Among the alternative methods of expressing the results of the *in vitro* studies, S, B, and E were expressed as log values. One of the earliest problems encountered in the handling of these data was the very wide individual variation in the absolute magnitude of these three parameters. It seemed appropriate to accumulate data in the form of "log X." When this is done, Δ values, when calculated as $(\log X)_T - (\log X)_{PT}$, represent proportionate changes in these parameters, regardless of the absolute magnitude of the PT values.

Changes from pretreatment were analyzed for significance using Student's t test for paired observations. Group-to-group differences were analyzed by comparison of means (Student's t test on unpaired observations) or contingency analysis (χ^2). The Standard Correlation Coefficient (r) was calculated to identify significant correlations.³⁷ A preprogrammed Monroe Model 1930 calculator was used for all analyses and probability determinations.

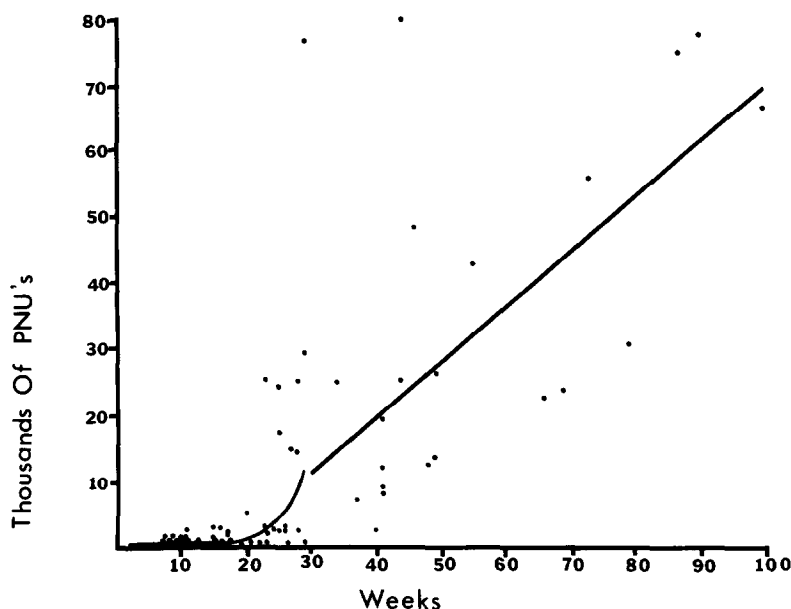


FIG. 1. Perennial RW immunotherapy. Correlation of cumulative treatment dose (ϵ PNU) with duration of treatment (weeks). At weeks < 30 : $r = 0.76$, $p < 0.001$, ϵ PNU = antilog $(0.095 \text{ weeks} + 1.321)$. At weeks > 30 : $r = 0.66$, $p < 0.002$, ϵ PNU = $825 \text{ weeks} - 13,500$.

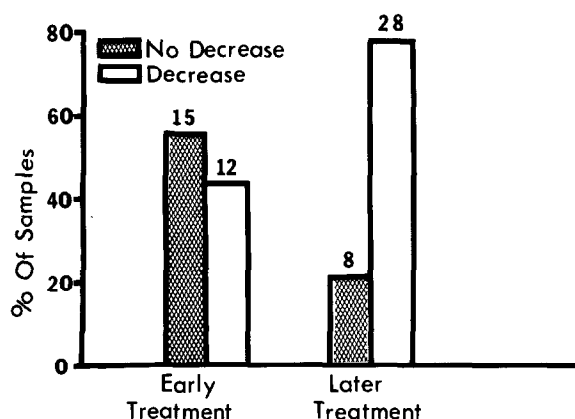


FIG. 2. Immunotherapy-associated changes in cellular responsiveness to RW-AgE. Frequency of decrease from pretreatment ($\Delta R < 0$ or $\Delta S < 0$, as per text) in early (ϵ PNU $< 3,000$) and later ($3,000 < \epsilon$ PNU $< 80,000$) treatment periods. The greater frequency noted in the later phase of treatment is significant ($\chi^2 = 7.7143$, $p < 0.006$).

RESULTS

Correlation between cumulative treatment dose and duration of treatment

The cumulative treatment dose (ϵ PNU) correlated significantly with the duration of treatment (weeks) ($r = 0.75$, $p < 0.001$, ϵ PNU = $749 \text{ weeks} - 7,890$). Based upon this regression equation, the group of patients studied, when considered over their entire course of treatment, increased their cumulative treatment dose by approximately 750 PNU per week.

During the initial stages of immunotherapy, as the dose is being increased weekly to a maintenance dose, the cumulative treatment dose could be expected to increase logarithmically. In fact, at less than 30 weeks, the correlation between PNU and weeks was greater as a semilog function. As maintenance dosage is reached, and the interval between injections is increased, the cumulative treatment dose could be expected to increase linearly. At greater than 30 weeks, the correlation between PNU and weeks was greater as a linear function. These relationships are depicted in Fig. 1.

Relationships with cumulative treatment dose and duration of treatment

Cellular responsiveness. Decreased cellular responsiveness (R or S) was noted more frequently in the later (28/36), than in the early (12/27), phase of treatment. ($\chi^2 = 7.7143$, $p < 0.006$, Fig. 2).

As is demonstrated in Fig. 3, R decreased as treatment continued: the decrease from pretreatment reached statistical significance ($p < 0.015$) during the later phase of treatment (mean $\Delta R_{LT} = -5.598$).

Fig. 3 illustrates that initially S increased: the early increase from pretreatment (mean $\Delta S_{ET} = +0.332$) was significant ($p < 0.05$). As treatment continued, S decreased: the later treatment levels (mean $S_{LT} = 2.971$) were significantly less ($p < 0.05$) than the early treatment levels (mean $S_{ET} = 3.339$).

Despite the above, no statistically significant corre-

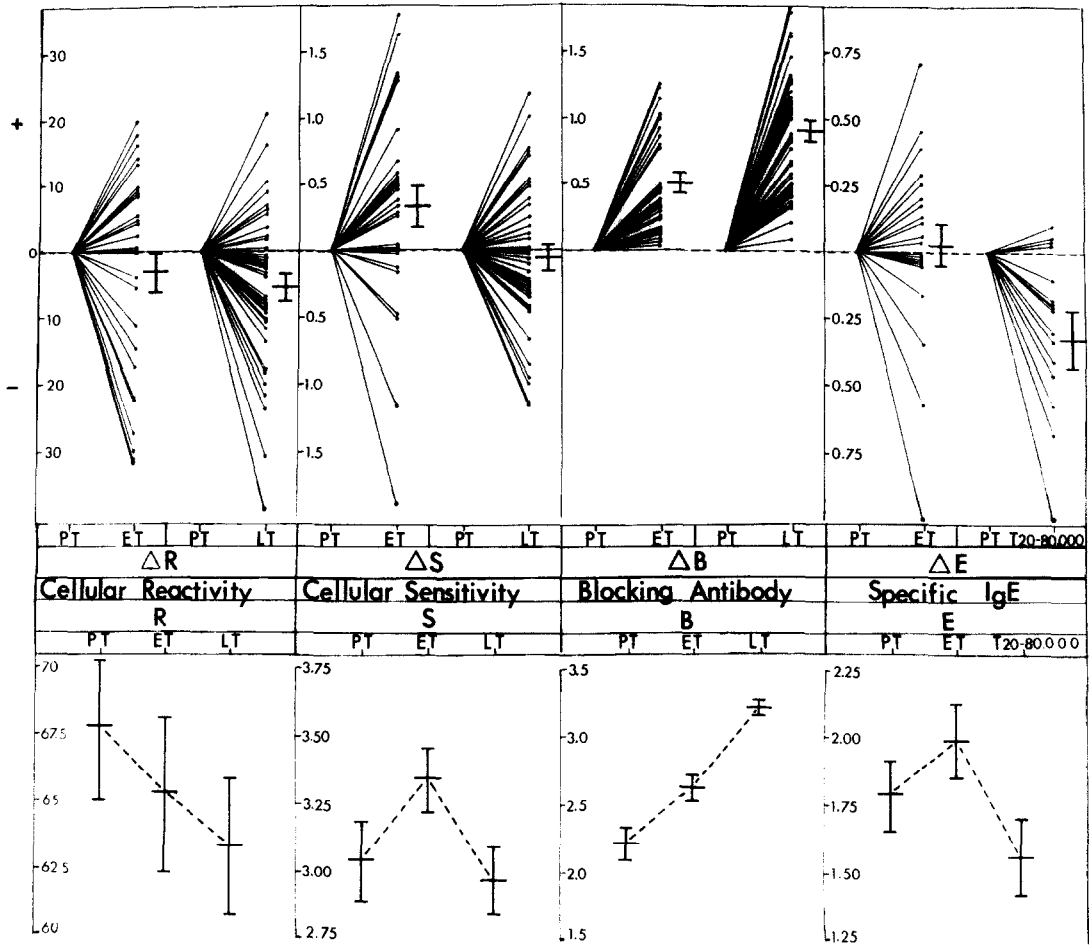


FIG. 3. Changes noted during continuing perennial RW immunotherapy. Treatment periods (PT, ET, LT, and T_{20,000-80,000}) are as defined in the text and in the list of specific abbreviations. Horizontal lines indicate sample mean ($\pm$ SEM). Δ values, upper curves; parameter means, lower curves. *Cellular reactivity to RW-AgE:* Significantly decreased ($p < 0.015$) from pretreatment in the later phase of treatment (mean, $\Delta R_{LT} = -5.598$). *Cellular sensitivity to RW-AgE:* Significantly increased ($p < 0.05$) from pretreatment in the early phase of treatment (mean $\Delta S_{ET} = +0.332$) and significantly lower ($p < 0.05$) in the later (mean $S_{LT} = 2.971$) than in the early (mean $S_{ET} = 3.339$) phase of treatment. *RW-AgE binding capacity (blocking antibody content):* Significantly increased ($p < 0.001$) from pretreatment in both the early (mean $\Delta B_{ET} = 0.509$) and the later (mean $\Delta B_{LT} = +0.909$) phases of treatment and significantly greater ($p < 0.001$) in the later (mean $B_{LT} = 3.220$) than in the early (mean $B_{ET} = 2.632$) phase of treatment. *Specific-IgE concentration:* Significantly decreased ($p < 0.01$) from pretreatment in the latest (20,000 to 80,000 PNU) phase of treatment (mean, $\Delta E_{20,000-80,000} = -0.327$) and significantly lower ($p < 0.05$) in the latest (mean $E_{20,000-80,000} = 1.562$) than in the early (mean $E_{ET} = 1.981$) phase of treatment.

lations were found between any of the parameters of cellular responsiveness (R, S, ΔR , or ΔS) and either the cumulative treatment dose or the duration of treatment.

Blocking antibody content. From Fig. 3 it can be noted that B increased as treatment continued: the increase from pretreatment was highly significant ($p < 0.001$) during both the early (mean $\Delta B_{ET} = +0.509$) and the later (mean $\Delta B_{LT} = +0.909$) phases of treatment. Furthermore, the increase continued with continuing therapy. The later treatment levels (mean $B_{LT} = 3.220$) were significantly greater

($p < 0.001$) than the early treatment levels (mean $B_{ET} = 2.632$).

B correlated significantly with both the cumulative treatment dose ($p < 0.001$) and the duration of treatment ($p < 0.001$). ΔB also correlated significantly with these two measurements of the extent of therapy ($p < 0.001$ and $p < 0.04$, respectively).

Examining the correlation between the RW-AgE binding capacity and the cumulative treatment dose further, it was noted that the correlation retained its significance if considered separately in the early phase of treatment ($r = 0.61$, $p < 0.001$, $B_{ET} =$

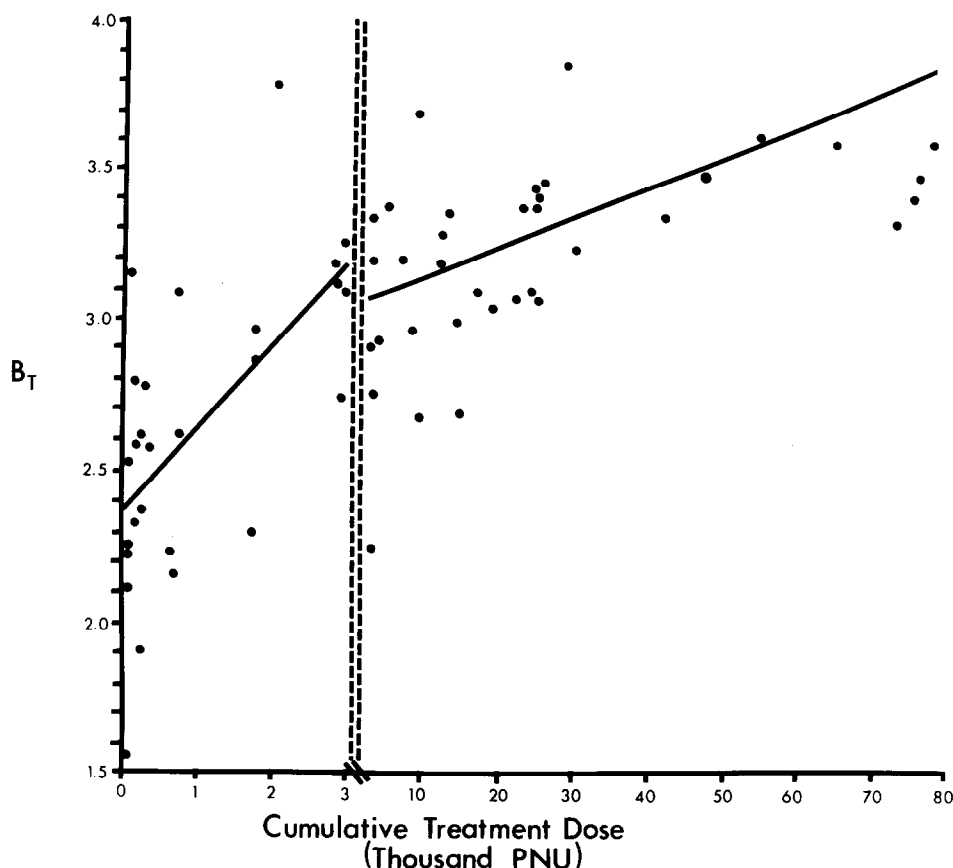


FIG. 4. Perennial RW immunotherapy: Correlation of RW-AgE binding capacity (blocking antibody content) (B_T) with cumulative treatment dose (ϵ PNU). During the early phase of treatment: $r = 0.61$, $p < 0.001$, $B_{ET} = 0.00027 \epsilon\text{PNU} + 2.370$. During the later phase of treatment: $r = 0.53$, $p < 0.001$, $B_{LT} = 0.001 \epsilon\text{PNU} + 3.031$.

$0.00027 \epsilon\text{PNU} + 2.370$) and in the later phase of treatment ($r = 0.53$, $p < 0.001$, $B_{LT} = 0.00001 \epsilon\text{PNU} + 3.031$) (Fig. 4); i.e., over the range of PNU given, the RW-AgE binding capacity increased at an exponential rate: during the early phase of treatment, at the rate of 0.27 log for every 1,000 PNU given, and during the later phase of treatment, at the rate of 0.01 log for every 1,000 PNU given.

Specific IgE concentration. Fig. 3 illustrates that E decreased at a high cumulative treatment dose: the decrease from pretreatment reached statistical significance ($p < 0.01$) after patients had received between 20,000 and 80,000 PNU of RW antigen (mean, $\Delta E_{20,000-80,000} = -0.327$). Furthermore, the levels in this latest phase of treatment (mean $E_{20,000-80,000} = 1.562$) were significantly lower ($p < 0.05$) than those in the early phase of treatment (mean $E_{ET} = 1.981$).

Despite the above, no statistically significant correlation was found between E and either the cumulative treatment dose or the duration of treatment. ΔE , however, did correlate significantly and inversely with these two measurements of the extent of therapy ($p < 0.01$ and $p < 0.03$, respectively).

Looking at the significant changes in relation to the treatment phase during which they were noted, it can be seen that during early treatment S and B increased from pretreatment values, while R and E remained unchanged. During later treatment, S fell to below early treatment levels, B continued to increase, and R fell to below pretreatment values. At the latest phase of treatment studied (20,000-80,000 PNU), E fell to below both pretreatment and early treatment levels.

Correlations between cellular reactivity and sensitivity

R correlated significantly with S prior to treatment ($r = 0.67$, $p < 0.001$, $S_{PT} = 0.035 R_{PT} + 0.650$).

Of the 63 treatment samples, 58 were found to have changes from pretreatment R and S. Concordant variation of these two measurements of cellular responsiveness was the rule: in 21 of the 58, both parameters increased, and in 23 of the 58, both parameters decreased ($\chi^2 = 16.4837$, $p < 0.001$).

During treatment, R and S correlated significantly one with the other ($r = 0.78$, $p < 0.001$, $S_T = 0.039 R_T + 0.643$), and each with its corresponding pretreatment value ($R_{PT}:R_T$, $r = 0.50$, $p <$

0.001; $S_{PT}:S_T$, $r = 0.50$, $p < 0.001$). These latter two correlations were even more striking during the later phase of treatment ($r = 0.60$, $p < 0.001$, $R_{LT} = 0.703 R_{PT} + 14.873$) ($r = 0.67$, $p < 0.001$, $S_{LT} = 0.795 S_{PT} + 0.559$).

Thus, concordant variation in the direction of change from pretreatment R and S was the rule. And those with greater cellular responsiveness prior to the initiation of immunotherapy would tend to have the greater, though generally decreased, cellular responsiveness during treatment.

Correlations between RW-AgE binding capacity and specific IgE concentrations

The treatment values of both B and E correlated significantly with their corresponding pretreatment values ($r = 0.68$, $p < 0.001$, $B_T = 0.568 B_{PT} + 1.701$) ($r = 0.78$, $p < 0.001$, $E_T = 0.907 E_{PT} + 0.109$). Thus, while B uniformly increased with continuing immunotherapy, those which began with the higher pretreatment RW-AgE binding capacities tended to achieve the higher levels with treatment. Those with higher specific IgE concentrations prior to the initiation of immunotherapy similarly tended to have the higher, though in this case generally decreased, concentrations during treatment.

However, no correlation could be found between B and E either prior to ($p > 0.10$) or during treatment ($p > 0.50$).

Correlations between cellular parameters (R and S) and antibody measurements (B and E)

Neither R nor S could be found to correlate with B, either prior to ($p > 0.40$ and $p > 0.50$, respectively) or during treatment ($p > 0.35$ and $p > 0.30$, respectively). In contrast, R correlated significantly with E both prior to ($p < 0.04$) and during the later phase of treatment ($p < 0.01$). Likewise, S correlated with E during the later phase of treatment ($p < 0.05$). The degree of cellular responsiveness thus correlated with the specific IgE concentration but not with the RW-AgE binding capacity.

Changes in RW-AgE binding capacity associated with a decrease in cellular responsiveness

Fig. 5 shows the ΔB values for four groups of samples by treatment period (early and later) and by the absence or presence of a corresponding decrease in cellular responsiveness. During the later phase of treatment, the change in RW-AgE binding capacity was significantly greater ($p < 0.02$) in the group showing a decrease in cellular responsiveness (mean, $\Delta B_{LT} = 1.019$) than that in the group showing no decrease in cellular responsiveness ($\Delta B_{LT} = 0.523$).

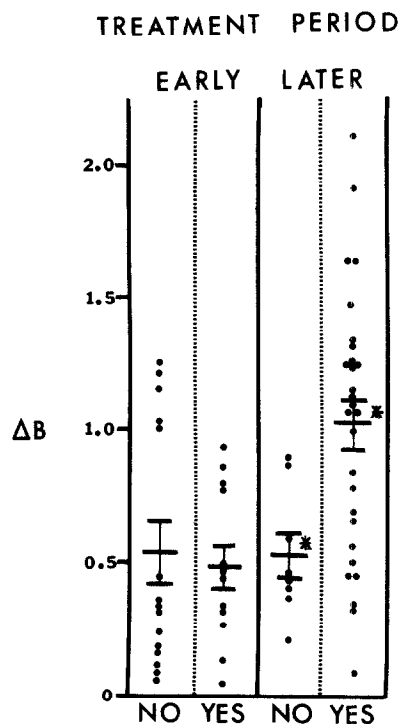


FIG. 5. Immunotherapy-associated changes in RW-AgE binding capacity (ΔB) associated with a decrease in cellular responsiveness to RW-AgE. $\Delta B = B_T - B_{PT}$; other definitions as per Fig. 2. Horizontal lines indicate sample mean ($\pm$ SEM). *Significantly different ($p < 0.02$) by Student's *t* test on unpaired observations.

This difference was not noted during the early phase of treatment (mean $\Delta B_{ET} = 0.484$ compared to 0.529 , $p > 0.75$).

Correlations with the change in RW-AgE binding capacity noted during the later phase of treatment

Correlations with achieved treatment values. The change in RW-AgE binding capacity (ΔB_{LT}) correlated significantly and inversely with R_{LT} ($p < 0.003$) and E_{LT} ($p < 0.001$), but not with S_{LT} ($p > 0.10$).

It is pertinent to note that B_{LT} failed to correlate significantly with these three parameters ($B_{LT}:R_{LT}$, $p > 0.99$; $B_{LT}:S_{LT}$, $p > 0.45$; and $B_{LT}:E_{LT}$, $p > 0.45$).

Correlations with the change from pretreatment values. The change in RW-AgE binding capacity (ΔB_{LT}) correlated significantly and inversely with ΔS_{LT} ($p < 0.003$) and ΔE_{LT} ($p < 0.04$), but not with ΔR_{LT} ($p > 0.10$).

Again, B_{LT} failed to correlate significantly with these parameters ($B_{LT}:\Delta R_{LT}$, $p > 0.85$; $B_{LT}:\Delta S_{LT}$, $p > 0.65$; and $B_{LT}:\Delta E_{LT}$, $p > 0.30$).

Thus, ΔB_{LT} has been found to be significantly correlated with several of the parameters studied. Of special note is the fact that ΔB_{LT} correlated sig-

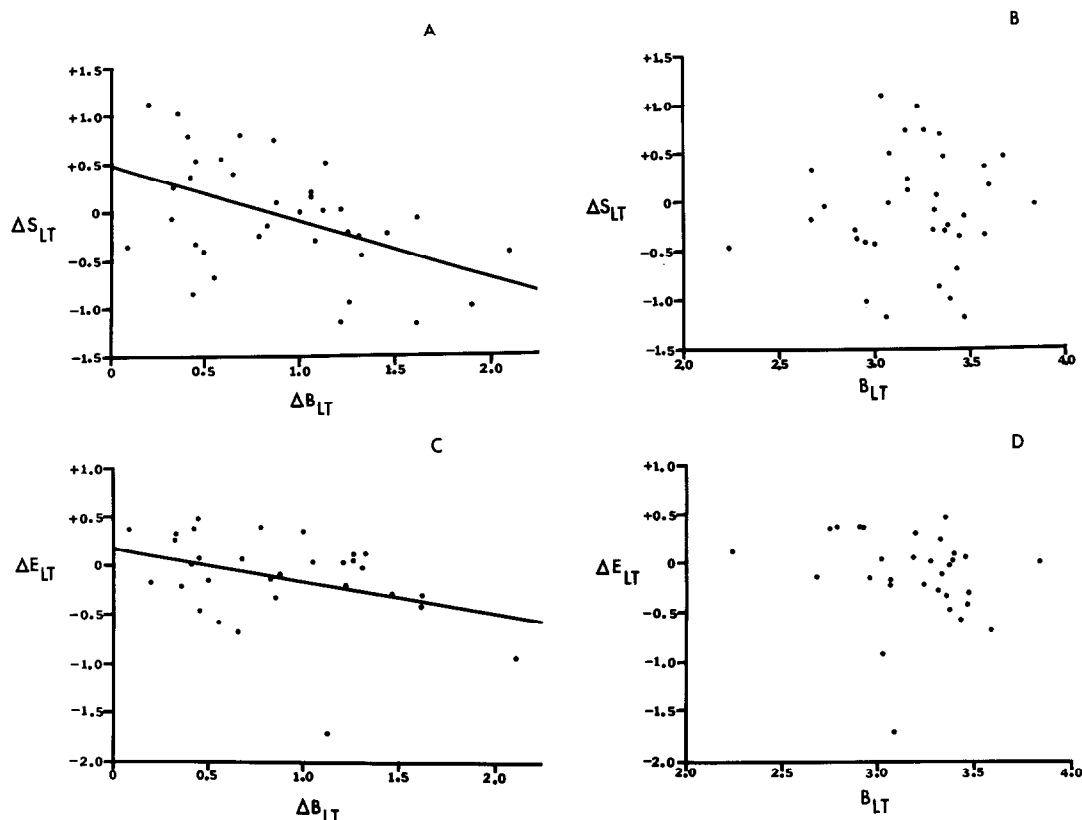


FIG. 6. Relationships noted during the later phase of treatment between the immunotherapy-associated changes in both the cellular sensitivity to RW-AgE (ΔS_{LT}) and the specific IgE concentration (ΔE_{LT}) and both the immunotherapy-associated change in RW-AgE binding capacity (ΔB_{LT}) and the actual RW-AgE binding capacity achieved with treatment (B_{LT}). *A*, ΔS_{LT} : ΔB_{LT} , $r = -0.49$, $p < 0.003$, $\Delta S_{LT} = -0.588 \Delta B_{LT} + 0.470$. *B*, ΔS_{LT} : B_{LT} , $p > 0.65$. *C*, ΔE_{LT} : ΔB_{LT} , $r = -0.37$, $p < 0.04$, $\Delta E_{LT} = -0.338 \Delta B_{LT} + 0.175$. *D*, ΔE_{LT} : B_{LT} , $p > 0.30$.

nificantly and inversely with both ΔS_{LT} and ΔE_{LT} ; i.e., during the later phase of treatment, the log increase from pretreatment RW-AgE binding capacity correlated significantly with both the log decrease from pretreatment cellular sensitivity to RW-AgE and the log decrease from pretreatment specific IgE concentration. B_{LT} failed to correlate significantly with these parameters (Fig. 6).

DISCUSSION

Numerous investigators have demonstrated that the serum blocking antibody content correlates with the extent of therapy, i.e., with the total cumulative treatment dose^{12, 17, 19, 38} and with the duration of treatment.^{20, 39} In contrast, the blocking antibody content has been roughly associated only with the degree of clinical improvement^{12, 13, 16, 17, 19, 20, 25, 40, 41}; i.e., the patients who develop the higher levels of blocking antibody have been demonstrated to have the greater degree of clinical improvement. While this group-to-group association exists, significant correlation (r) between the level of blocking antibody and the degree of clinical improvement has not been reported.

Conversely, the change in cellular responsiveness

(reactivity or sensitivity) has been demonstrated to correlate, statistically, with the degree of clinical improvement¹⁶ but not with the extent of therapy. The serum reagin content has not been found to correlate, in a statistically significant manner, either with the extent of therapy or with the degree of clinical improvement.

It is hoped that further study of the interrelationships and correlations between these cellular and immunologic parameters may shed some light on our understanding of the mechanism by which immunotherapy results in clinical improvement.

Cellular responsiveness

Cellular reactivity and sensitivity have previously been shown to be significantly correlated in pretreatment atopic serum.⁴² The present study demonstrates a significant correlation between these two parameters of cellular responsiveness in both pretreatment and treatment samples.

It has also been demonstrated^{15, 18, 21, 25} that there is a greater tendency for decreased cellular responsiveness with larger doses of therapy. This association has been supported by this study. A decrease from the

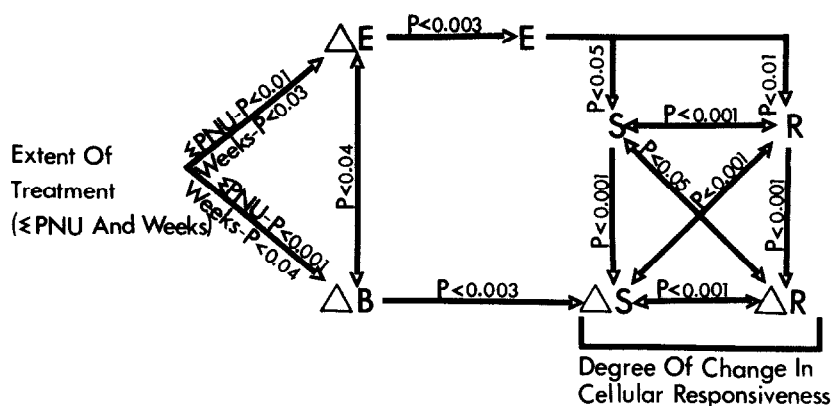


FIG. 7. Chain of relationships, based upon demonstrably significant correlations, leading from the extent of treatment to the degree of change in cellular responsiveness. P = probability that the two examined parameters are not correlated given the calculated *r* value.

pretreatment level of cellular responsiveness was noted more frequently in the later than in the early phase of treatment. The cellular reactivity to RW-AgE noted during the later phase of treatment was significantly decreased from pretreatment levels; and while the cellular sensitivity to RW-AgE was noted to increase during the early phase of treatment, by the later phase of treatment it had significantly decreased from this initial elevation.

Despite these associations, we have been unable to demonstrate significant correlations (*r*) between either the cumulative treatment dose or the duration of treatment and the degree of cellular responsiveness (*R* or *S*) or the degree of decrease occurring with treatment (ΔR or ΔS).

Blocking antibody content

In this study, the serum blocking (nonreaginic) antibody content was measured by RW-AgE binding capacity. If antigen binding capacity is to be equated with blocking antibody content, one key theoretical objection must be overcome. It could always be argued that the ammonium sulfate coprecipitation technique measures all specific immunoglobulins, i.e., not only nonreaginic but also reaginic antibodies. It is generally accepted that the nonreaginic antibody concentration is significantly greater than the specific IgE antibody concentration, especially in immunotherapy-treated patients, and therefore it could be reasoned that antigen binding capacity primarily reflects the nonreaginic antibody content. The lack of correlation between the RW-AgE binding capacity and specific IgE concentration, plus the finding that the RW-AgE binding capacity increased while the specific IgE concentration decreased, convinces us that antigen binding capacity is a legitimate representation of serum blocking antibody content.

The level of circulating blocking antibody was found to steadily rise during immunotherapy. The

level noted during the later phase of treatment was significantly greater than that noted during the early phase of treatment, with both being significantly greater than pretreatment levels. In contrast to the quality of the association noted with cellular responsiveness, the association between the blocking antibody content and the extent of therapy has been found to be a statistically significant correlation. The blocking antibody content correlated significantly with both the cumulative treatment dose and the duration of treatment. The rate of blocking antibody production (i.e., the slope of the PNU:B regression line, Fig. 4) was greatest during the early phase of treatment.

Specific IgE concentration

Like cellular responsiveness, the specific IgE concentration was found to decrease with continued immunotherapy; this finding has also been reported using other measurements of reagin content.^{19, 29, 30} The polystyrene tube radioimmunoassay used in this study to measure specific IgE antibody cannot be interfered with by antibody of other classes directed against AgE in the same serum.³⁶ It will, therefore, give a true measure of IgE antibody against AgE even in the presence of significant amounts of blocking antibody. The decrease from pretreatment specific IgE concentration was found to be significant only during the latest phase of treatment studied, i.e., at a cumulative treatment dose between 20,000 and 80,000 PNU. The specific IgE concentrations corresponding to this phase of treatment were significantly lower than both the pretreatment and the early treatment values.

While the above indicates that the extent of therapy is an important factor associated with the lower specific IgE concentrations, no significant correlation (*r*) could be found between either the cumulative treatment dose or the duration of treatment and the specific IgE concentration.

Inferences based upon observed correlations

On the basis of significant correlations (r) noted in this study, Fig. 7 diagrams a chain of relationships leading from the extent of treatment to change in cellular responsiveness which has previously been correlated with the change in the severity of symptoms.¹⁶

The extent of treatment (both the cumulative treatment dose and the duration of treatment) has been found to correlate significantly, over the entire course of treatment, with both the *log-increase* in blocking antibody content (ΔB_T) and the *log-decrease* in specific IgE concentration (ΔE_T). These two changes from pretreatment levels correlated significantly with each other during the later phase of treatment only (ΔB_{LT} with ΔE_{LT}).

Also noted during the later phase of treatment was that the log-change in specific IgE concentration (ΔE_{LT}) correlated significantly with the log specific IgE concentration (E_{LT}), which in turn correlated significantly with the cellular reactivity (R_{LT}) and the log cellular sensitivity (S_{LT}). Finally, both of these measurements of the degree of cellular responsiveness correlated with the degree of change in cellular responsiveness (ΔR_{LT} and ΔS_{LT}). Also, the *log-increase* in blocking antibody content (ΔB_{LT}) correlated with the *log-decrease* in cellular sensitivity (ΔS_{LT}).

Levy and co-workers¹⁶ hypothesized that a possible mechanism associating decreasing cellular responsiveness and blocking antibody could be a suppression of IgE antibody production due to the presence of high blocking antibody concentrations. Tada and Okumura⁴³ suggested that blocking antibody (IgG) could inhibit IgE production via a feedback regulatory mechanism. Lichtenstein and co-workers¹⁹ reported a significant rank correlation between the blocking antibody titer and the drop in specific IgE concentration, but they questioned the existence of a feedback mechanism.

While we have been able to demonstrate a significant degree of correlation between the specific IgE concentration and the degree of cellular responsiveness, we have been unable to demonstrate a significant correlation between the level of blocking antibody and either the specific IgE concentration or the degree of cellular responsiveness. The presence of statistically significant correlations, during the later phase of treatment, between the *log-increase* in blocking antibody content and both the *log-decrease* in specific IgE concentration and the *log-decrease* in cellular sensitivity may suggest that a common mechanism is simultaneously responsible for these changes. These correlations would suggest that, during the later phase of treatment, the active process of

blocking antibody production, rather than the passive presence of blocking antibody, is related to the process which decreases the specific IgE concentration and the degree of cellular responsiveness and presumably, therefore, results in clinical improvement.

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