

Clinical and immunologic studies of ragweed immunotherapy

H. Melam, J. Pruzansky, R. Patterson, and S. Singer *Chicago, Ill.*

A group of patients with ragweed (RW) pollinosis was treated with aqueous RW extract. An increase in IgG antigen-binding activity was the first immunologic change observed. This coincided with symptomatic improvement. After further therapy with sufficient antigen, cellular sensitivity, as measured by histamine release from washed leukocytes, and reaginic antibody titers decreased. The later changes are probably related to the long-lasting relief from allergic symptoms achieved with immunotherapy.

Injection therapy for allergic diseases due to inhalant antigens has been shown to be effective in various double-blind studies.^{1, 2} Immunologic and cellular changes associated with this type of treatment have been described. These include the induction of blocking antibody,^{3, 4} decrease in reaginic antibody,⁵ and decline in leukocyte sensitivity to antigen.⁶⁻⁹ As a working hypothesis, we considered that one or more of these factors might play a significant role in the reduction of clinical symptoms which parallel prolonged and intensive injection therapy. In this study, the sequence of changes in serum antigen-binding capacity, serum reaginic antibody levels, and peripheral blood leukocyte sensitivity was studied in individual ragweed-sensitive patients and correlated with clinical improvement of symptoms due to ragweed allergy.

MATERIALS AND METHODS

Subjects

Seven patients from the Allergy Clinics at the McGaw Medical Center with moderate to severe ragweed rhinitis with or without asthma were studied (Table I). The patients were selected if they were sufficiently sensitive that 5 ml. of washed leukocytes released 30 per cent or more of the histamine when incubated with 10^{-3} μ g of antigen. The patients were treated with injections of aqueous extracts of ragweed (RW) pollen in accordance with standard clinical practice.¹⁰ The rate of increase in dose of extract in each patient was dependent on the absence of systemic or large local reactions. Because of this, certain patients reached higher doses than did others during the same period of treatment. Each patient experienced at least one ragweed season while on treatment. Clinical evaluation of patients was made by grading daily symptoms and medication diaries on a scale of 0 to 3. Zero score means there were no symptoms and no medication was used on that day.

Antigen and isotope labeling

Purified ragweed fraction, A-IV-C, was prepared by the method of King and Norman¹¹ and labeled with ¹³¹I as previously described.¹²

From the Allergy-Immunology Section of the Department of Medicine, Northwestern University School of Medicine-McGaw Medical Center.

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TABLE I. Status of patients

Subject	Age (years)	Sex	Clinical symptoms	Duration of symptoms (years)
1	37	M	Ragweed rhinitis and asthma	7
2	41	F	Ragweed rhinitis	6
3	25	M	Ragweed rhinitis	3
4	24	M	Ragweed rhinitis	5
5	16	F	Ragweed rhinitis	2
6	31	M	Ragweed rhinitis	8
7	34	M	Ragweed rhinitis and asthma	10

Binding of ^{131}I -labeled RW antigen

Increasing amounts of ^{131}I RW were added to aliquots of serum. The ^{131}I RW bound to human gamma globulin was coprecipitated by addition of an excess of rabbit antiserum against human Cohn fraction II. Radioactivity was determined in the precipitates and supernatants.⁷ Curves were constructed by plotting the percentage of antigen in the precipitate against the quantity of antigen added. The quantity of antigen bound by 1 ml. of each serum in slight antibody excess was determined. Reproducibility of this assay has been shown to be at least ± 20 per cent. At the levels of antigen used, no specific binding of antigen could be detected in sera of nonatopic individuals. All sera were stored at -20°C . and assayed at the end of the study.

Gel diffusion autoradiography

The subjects' sera were reacted against goat antisera specific for α , γ , and μ chains in double gel-diffusion plates. After the preparations were thoroughly washed, the wells were charged with ^{131}I -labeled RW and incubated for 48 hours at 4°C . The slides were washed, dried and placed in contact with Kodak Redi-Pak No Screen x-ray film which was developed after appropriate periods of time. The slides were then stained with thiazine red.

Histamine release from leukocytes

Leukocytes were obtained from the peripheral blood of subjects and washed. The reactivity of peripheral leukocytes to purified RW antigen, as measured by histamine release, was determined with the use of the technique previously reported.⁷ The smallest quantity of antigen causing a 30 per cent release was defined as the cellular sensitivity.¹³ These determinations were highly reproducible, and a tenfold change in sensitivity was minimal but significant. It had been shown previously that continued treatment at high doses in each of 9 patients resulted in insignificant leukocyte histamine release at any level of antigen tested.

Monkey Prausnitz-Küstner (P-K) tests

Skin of rhesus monkeys was sensitized with 0.1 ml. of serial twofold dilutions of sera. Twenty-four hours later, 2 ml. of 0.5 per cent Evans blue dye and 1 ml. of 1:50 ragweed extract were injected intravenously.¹⁴ Reactivity was determined by the highest dilution of serum which produced a bluing reaction. Serial serum samples obtained from the same patient collected at different stages of the treatment course were all tested at the same time in the same recipient to avoid extraneous variations in response. All studies were done on a monkey who had received no prior testing. Titers were performed in duplicate and found to be reproducible. A fourfold or greater difference in titer was considered highly significant.

RESULTS

Histamine release

Reactivity of each patient's leukocytes to ragweed at different intervals during the treatment course is shown in Fig. 1. Before treatment, leukocytes of all 7

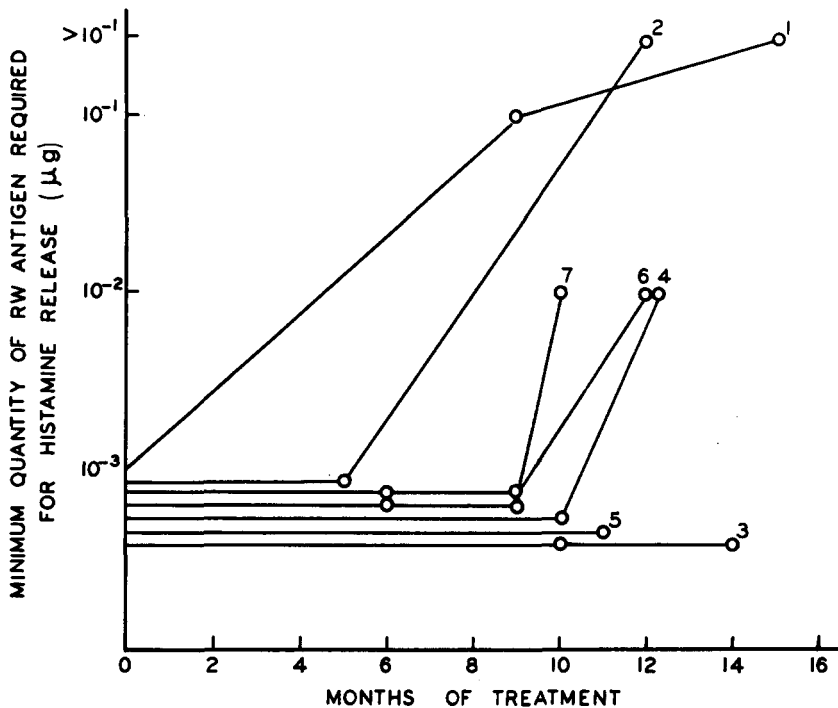


FIG. 1. Minimum quantity of antigen required for significant release of histamine at different stages in the treatment course of the subjects.

subjects reacted with a significant release of histamine to 10^{-3} μ g of RW. A decrease in leukocyte sensitivity occurred in one patient after 9 months of treatment, in one patient after 10 months of treatment, and in 3 patients after 12 months of treatment. Two patients had no change in leukocyte sensitivity, as measured by histamine release to antigen challenge, after 11 and 14 months of treatment, respectively.

The correlation between dose of ragweed extract, as measured in protein nitrogen units (PNU), and leukocyte reactivity is shown in Fig. 2. Leukocytes of 2 patients who were treated with over 100,000 PNU of crude ragweed extract no longer released histamine to challenge with 10^{-1} μ g of RW. Leukocytes from 3 patients decreased tenfold in sensitivity after these subjects were treated with 10,000 to 20,000 PNU of ragweed extract. The leukocyte sensitivity of 2 patients did not change after one subject was treated with 19,000 PNU of crude ragweed extract and the other with 21,000 PNU of crude ragweed extract.

Serum binding of RW

All 7 patients showed an increase in serum RW-binding activity during their courses of treatment (Fig. 3). Marked differences between the serum antigen-binding activities of the subjects were noted when they were compared at similar intervals during their treatment. Patients 1 and 2 had the greatest rate of increase in serum RW binding, Patients 3 to 6 the slowest rate of increase, while Patient 7 was intermediate.

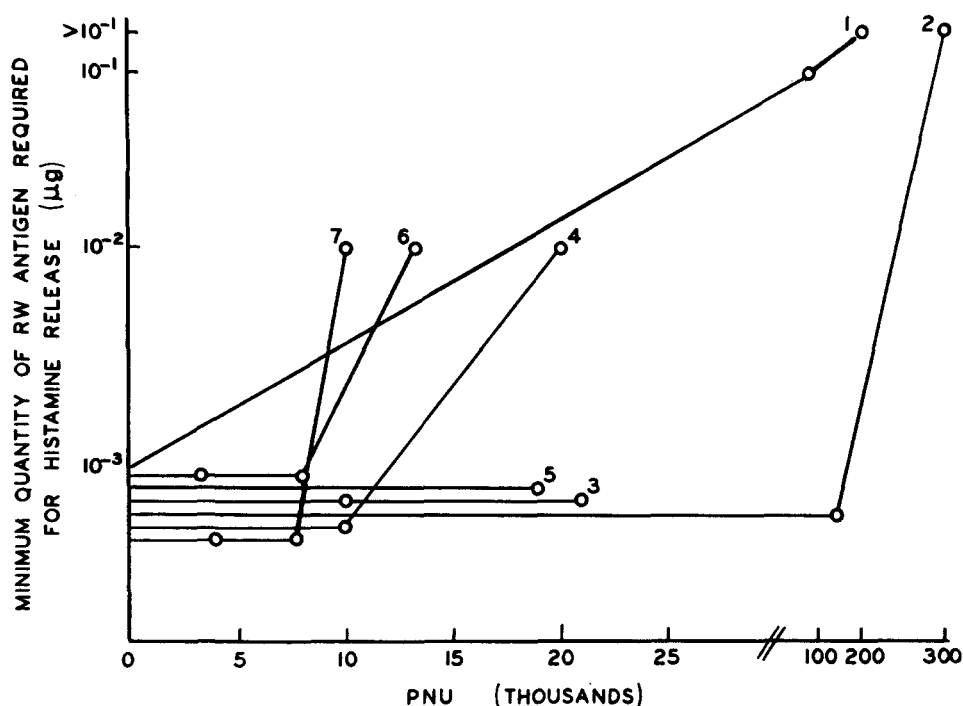


FIG. 2. Minimum quantity of antigen required for significant release of histamine at different treatment doses of ragweed extract (PNU) of the subjects.

TABLE II. P-K titers of pretreatment and treatment sera

Patient	Serum	Highest dilution of serum giving positive P-K	Months of treatment	PNU or RW extract received during treatment
1	Pretreatment	1:320	15	205,000
	Treatment	1:80		
2	Pretreatment	1:160	12	318,000
	Treatment	1:40		
3	Pretreatment	1:320	14	21,000
	Treatment	1:320		
4	Pretreatment	1:320	12	20,000
	Treatment	1:320		
5	Pretreatment	1:160	11	19,000
	Treatment	1:160		
6	Pretreatment	1:80	12	12,500
	Treatment	1:80		
7	Pretreatment	1:160	10	10,000
	Treatment	1:160		

Increases in serum RW binding of the subjects and the PNU of crude ragweed extract received during treatment are compared in Fig. 4. The variability in the increase of serum binding activity noted with duration of treatment is again seen. Although Patients 3 to 6 had similar pretreatment antigen-binding levels and the slopes appear to be fairly similar, the serum antigen-binding levels after they had each received 10,000 PNU of crude ragweed extract

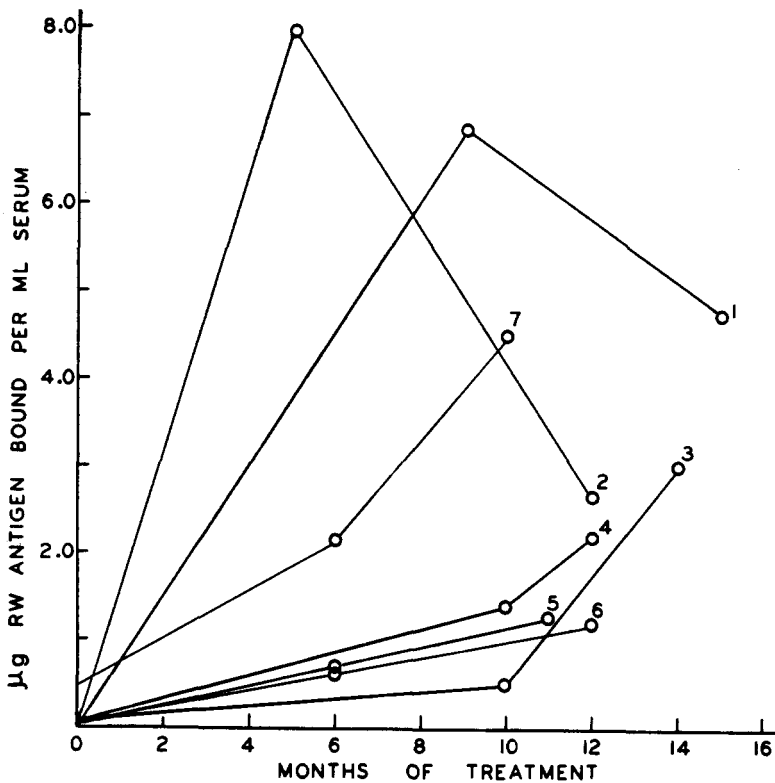


FIG. 3. Micrograms of RW antigen bound per milliliter of serum at different stages in the treatment course of the subjects.

showed a threefold range. Patient 7 had the highest pretreatment serum antigen-binding level and exhibited the greatest increase of serum RW-binding activity. Patients 1 and 2 reached high levels of serum antigen-binding activity which then decreased despite further treatment.

Gel diffusion autoradiography

Double gel diffusion autoradiographic preparations of pretreatment and treatment sera of three of the subjects against rabbit anti- γ chain serum are shown in Fig. 5. Similar results were obtained with the remaining patients. Although both pretreatment and treatment sera showed binding of ^{131}I RW, the marked increase of binding activity in the treatment sera is evident. No binding activity was found in either pretreatment or treatment sera from these subjects when the double gel diffusion autoradiographic preparations were made with goat anti- α chain or rabbit anti- μ chain sera.

Prausnitz-Küstner titrations

Results of P-K tests of pretreatment and treatment sera from each subject are shown in Table II. P-K titers of Patients 3 to 7 did not change. These

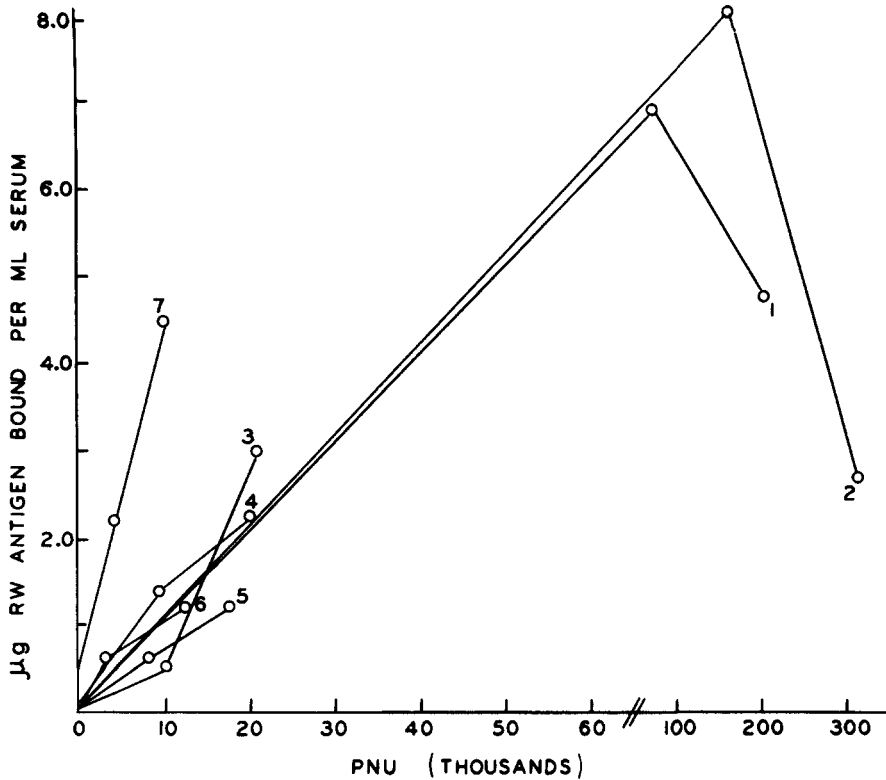


FIG. 4. Micrograms of RW antigen bound per milliliter of serum at different treatment doses of ragweed extract (PNU) of the subjects.

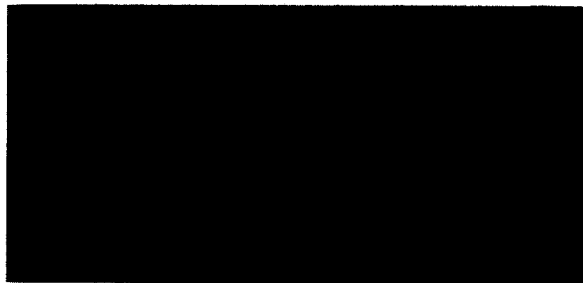


FIG. 5. Double gel diffusion autoradiographic preparations of Patients 1, 3, and 6. Center well contains rabbit anti- γ chain serum. Pretreatment sera are in **a** wells and treatment sera are in **b** wells.

subjects were treated with 10,000 to 21,000 PNU of crude ragweed extract. Patients 1 and 2 showed a fourfold decrease in reaginic antibody titers.

Patients' individual clinical and immunologic responses

All 7 patients became nearly asymptomatic during treatment. No patient required the use of any medications in the ragweed seasons during treatment.

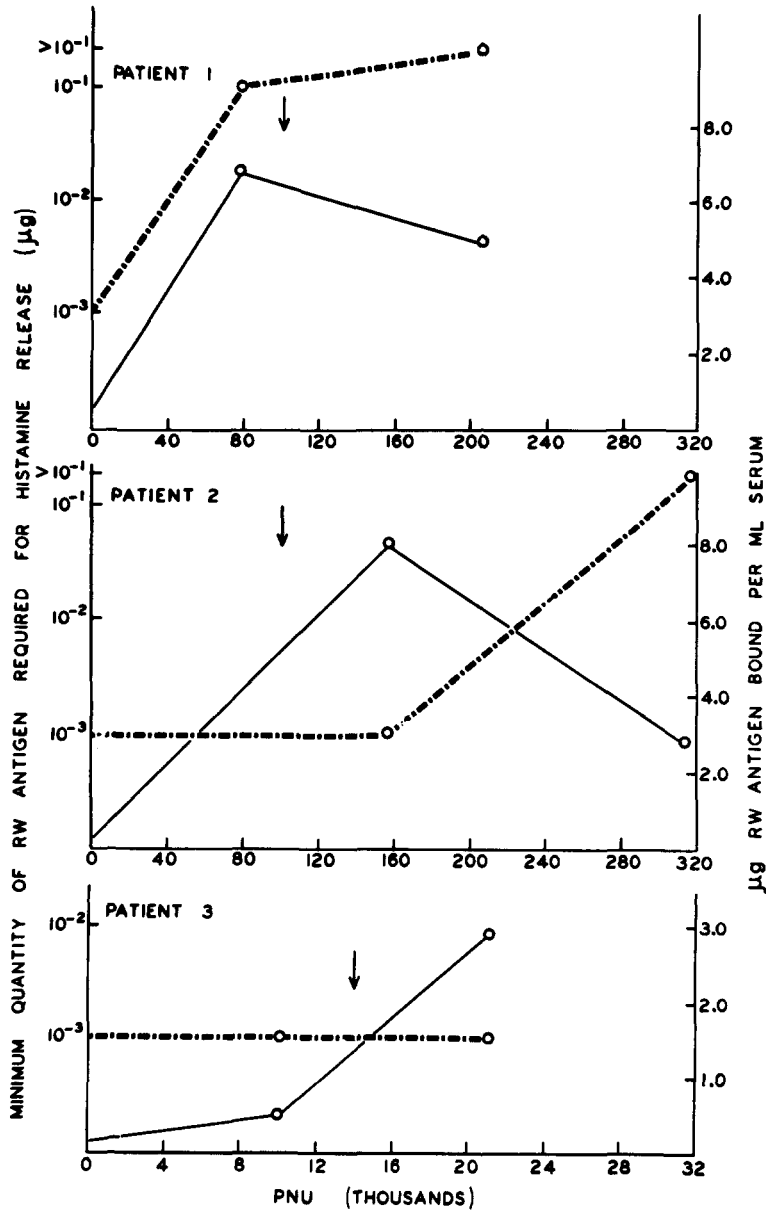


FIG. 6. Graphic representation of Patients 1-3 showing minimum quantity of antigen required for histamine release and serum antigen binding at different treatment levels and during the ragweed season. ↓, ragweed season; ○- - - - ○, histamine release; ○—○, RW binding.

Relationships between serum antigen-binding activity and leukocyte sensitivity at different phases of treatment for each patient are shown in Figs. 6 and 7. All 7 patients had an increase in serum antigen-binding activity with treatment. In these subjects this increase preceded the improvement in symp-

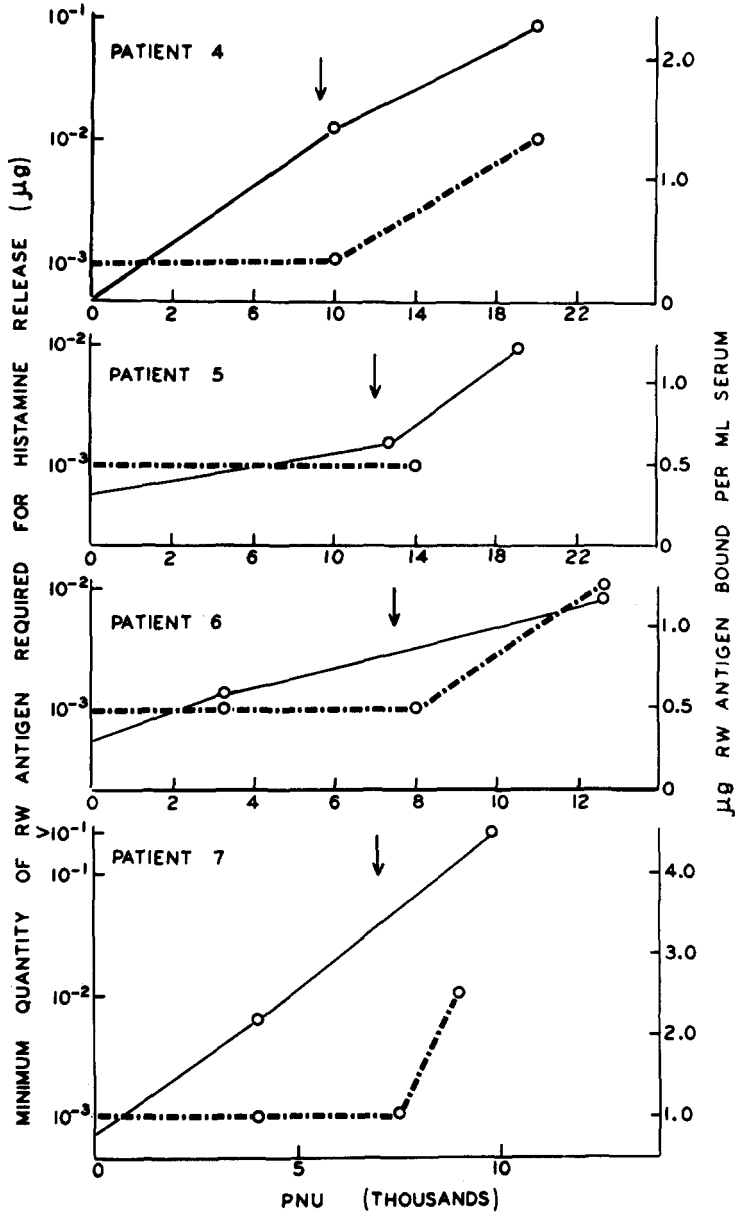


FIG. 7. Graphic representation of Patients 4 to 7 showing minimum quantity of antigen required for histamine release and serum antigen binding at different treatment levels and during the ragweed season. Legend same as that for Fig. 6.

toms experienced during their first ragweed season after treatment was started. Only Patient 1 (Fig. 6) showed a decrease in leukocyte sensitivity before the asymptomatic ragweed season. In 2 patients (Figs. 6 and 7) there was no change in leukocyte sensitivity even though these patients became

asymptomatic. In the other 4 patients the decrease in leukocyte sensitivity followed the asymptomatic ragweed season.

DISCUSSION

Study of this and previous groups of patients shows that changes in histamine release occur in relationship to the quantity of antigen administered and the duration of treatment. There are, however, no absolute correlations between the dose or length of treatment and the decline in cellular sensitivity. One individual showed a significant decrease in histamine release after 9 months of therapy, while another subject had no change in leukocyte sensitivity after 14 months of treatment. Similarly, one patient had a significant decrease in leukocyte reactivity after 10,000 PNU of ragweed extract, whereas a second patient showed no change in leukocyte sensitivity after receiving 21,000 PNU of extract.

However, the leukocytes of both patients who had received over 100,000 PNU of ragweed lost their sensitivity to even 10^{-1} μ g of RW as measured by histamine release. The leukocytes of all 11 patients studied in this laboratory who have received over 100,000 PNU of ragweed extract have lost sensitivity to the highest concentration of antigen tested.

The current group of patients also showed individual variability as to their levels of serum blocking activity when they were compared at similar doses of treatment extract or time intervals of treatment.

Only two patients had a decrease in serum reaginic antibody during their treatment. Both these patients received over 100,000 PNU of crude ragweed extract. The three patients who had a tenfold decrease in leukocyte sensitivity did not show any change in P-K titer.

The results of this study may be summarized as follows:

1. The first measurable immunologic change with hyposensitization treatment is an increase in serum antigen-binding activity. Serum immunoglobulin G is responsible for this activity in these patients.

2. Improvement in clinical symptoms occurs following this increase in serum antigen-binding activity and before cellular and other immunologic changes can be detected by the methods used.

3. After a certain level of serum antigen-binding activity has been reached, there may be a plateau or decrease in activity even with continued immunotherapy.

4. With continued immunotherapy there is a decrease in cellular sensitivity as measured by leukocyte histamine release to antigen challenge. We believe that all seven subjects in this study will lose cellular sensitivity to RW after receiving higher doses of antigen.

5. Reaginic antibody levels, as measured by P-K titers, eventually decrease with further therapy.

6. On the basis of a previous study¹⁵ it would seem that long-lasting relief from allergic symptoms caused by ragweed pollen is better correlated with decreased reaginic antibody levels and cellular sensitivity than with serum antigen-binding activity.

7. No absolute minimum dose of antigen or absolute period of treatment has been found that will cause the above changes in all subjects studied. Although the sequence of events appear constant in all of these patients, there is individual variability as to the treatment dose necessary to cause changes in the rate of increase of serum antigen-binding activity, decrease in cellular sensitivity, and decrease in reaginic antibody level.

Based on these results the following mechanisms might be operative in the relief of symptoms experienced in immunotherapy. The increase in IgG binding activity could result in the initial improvement which follows injections of antigen. This antibody is presumed to combine with environmental ragweed pollen in or on the respiratory mucosa. The IgG antibody, by combining with the RW determinants, prevents the allergic (reaginic) antibody on cell surfaces from reacting with the RW, which would result in the subsequent release of chemical mediators. This may cause the initial clinical improvement of symptoms that occurs despite both persistent cellular sensitivity, as demonstrated by histamine release from washed leukocytes, and unaltered levels of reaginic antibody. Lack of absolute correlation between symptom scores and blocking antibody concentration does not disprove the efficacy of blocking antibody. Differing environmental exposures and the degree of tolerance of symptoms of each individual may obscure a cause and effect relationship. No study has yet adequately evaluated the role of blocking antibody. The IgG antibody also permits larger doses of RW extract to be administered.

Following continued long-term therapy there is a loss of cellular sensitivity and a decrease in specific reaginic antibody available to sensitize mediator-releasing cells. The mechanism by which reaginic antibody is reduced following long-term high-dose therapy in certain patients is not understood. Specific induction of tolerance of cells producing IgE against RW by RW, IgE-RW, or IgG-RW complexes might occur.

Other mechanisms that may also be important in immunotherapy include reduction in the total numbers of mast cells, receptor sites, or receptor activity. These factors are as yet undefined.

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