

Antibody response following immunotherapy in ragweed hay fever: Allpyral vs. whole ragweed extract

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The antigenicity of commercially available ragweed pollen extracted with pyridine and precipitated with alum (Allpyral) was compared with that of aqueous ragweed extract. The antibody response was measured by the inhibition of histamine release from sensitive human leukocytes challenged with either ragweed antigen E or whole aqueous extract. A group of patients treated with aqueous extract had a significantly greater antibody response than a similar group treated with Allpyral, even though the latter received 30 times as much antigen. Another comparison showed that patients who had received high dosage Allpyral therapy (average: 0.80 mg. of protein N. or 80,000 PNU) had antibody levels which did not differ significantly from those of untreated control patients.

Although debate continues on the merits of therapeutic immunization (desensitization) in clinical pollinosis, both proponents and critics agree that there is room for improvement in the currently used regimens.¹⁻³ Recent efforts have centered on purifying the allergenic materials used for immunization,⁴ or on the use of adjuvants to increase the antigenic potency and to decrease the local and systemic side effects caused by the administration of allergens.^{5, 6} These side effects are a cause of concern to patient and doctor alike and severely limit the quantity of allergenic material which can be administered to sensitive patients. On the basis of extensive work in animals it seemed likely that the incorporation of the allergenic materials in adjuvants would lead to a decreased rate of release of injected antigen, thus minimizing side effects and leading to an increased antigenic stimulus.⁷ Two such ad-

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juvant-allergen preparations have had extensive clinical use; in one the allergens are incorporated into an oil and water emulsion ("repository therapy"),⁵ and in the other the allergens are precipitated with alum.⁶

This paper will consider Allpyral,* a commercial preparation made by pyridine extraction of the allergenic material, followed by alum precipitation. The advantage claimed for this preparation has been a decreased number of local and systemic reactions following its administration.⁸ Surprisingly, however, there has been little work evaluating the antigenic stimulus provided by Allpyral preparations. Recently, two studies have been published on experience obtained with Allpyral ragweed. One suggests that the alum-precipitated material was adequate to stimulate antibody responses in a significant number of subjects,⁹ but the other did not find this to be the case.¹⁰ While the exact relationship between the circulating antibody induced by immunotherapy and the clinical effectiveness of this regimen is not clear, most workers would not question the contention that the preparation used for immunotherapy must be antigenic.¹¹

We have previously reported a technique which enables us to measure quantitatively those antibodies which can inhibit an *in vitro* allergic response.¹² The assay system is based on the antigenically induced release of histamine from the isolated leukocytes of sensitive human donors.¹³ Antibody is measured by its ability to combine with antigen in the fluid phase, thus preventing it from triggering the cellular response. In the present report this technique is used to compare the antibody response induced with pyridine-extracted, alum-precipitated ragweed antigens (Allpyral) with the usual form of therapy using aqueous ragweed extracts. Since all patients, treated or untreated, have a measurable level of antibody,¹⁴ these two groups have been contrasted with a group of allergic individuals who received placebo injections.

MATERIALS AND METHODS

Antigen preparations

Allpyral was purchased from Dome Chemicals, Inc. Aqueous ragweed extract was prepared by Hollister-Stier Laboratories for the National Institutes of Health Committee on Standardization of Allergens. Antigen E was prepared and supplied by Dr. T. P. King.^{4, 15}

Antibody assay

Details of leukocyte preparation, the histamine release reaction, and the fluorometric assay of histamine have been previously reported.^{13, 14, 16} In the assays reported below a constant quantity of ragweed antigen E or aqueous ragweed pollen extract was mixed with a fixed dilution of normal (NHS) or the various allergic sera (AHS) to be tested. The antigen concentration was adjusted so that 70 to 80 per cent release occurred in the presence of normal serum. The antigen-serum mixtures were added to aliquots of a leukocyte suspension from a single allergic donor and the per cent histamine release

*Dome Chemicals, Inc., 125 West End Ave., New York, N. Y. 10023.

(H.R.) was determined. Per cent inhibition was calculated from the formula:

$$\frac{\text{H. R. in NHS} - \text{H. R. in test AHS}}{\text{H. R. in NHS}}$$

Serum samples

Sera from normal or allergic donors were obtained within 3 to 4 hours after venipuncture and stored without preservative at -20°C . All sera from allergic donors were obtained in early September, 2 to 3 weeks after their final immunization.

Patients and treatment

Sera from 15 patients treated with Allpyral ragweed extract were studied. These patients had been under treatment for either 3 or 4 seasons with Allpyral and had not previously been treated with whole ragweed extract. The diagnosis of ragweed hay fever had been made clinically and confirmed by skin test as previously described.¹⁴ In 1966 each patient received 15 weekly injections, starting at a dose of 50 to 500 PNU and doubling the amount each week until a maximum dose of 20,000 PNU was achieved, unless dosage was limited by local or systemic reaction. Four patients were unable to achieve the projected maximum dose.

Sera from 26 patients treated with whole aqueous extract in 1965 and 1966 were also examined. These patients had also been under treatment for 2 to 4 years by 1966. They received 15 weekly preseasonal injections, with an initial dose based on their previous reactivity. The dose was doubled each week unless limited by local or systemic reaction. None of the patients was able to achieve the projected maximum dose. Table I shows the total preseasonal doses achieved by these patients.

Sera from 15 patients given placebo injections in 1966 were also examined. These patients had been under observation for a period similar to the other two groups but had received preseasonal injections of a buffered diluent with enough histamine to mimic a mild local reaction.

The three groups of patients were alike in the criteria for acceptance to the study, but the Allpyral-treated patients were not matched by cell sensitivity for inclusion in the combined laboratory clinical study.¹⁴ Subsequent to this original grouping we have come to believe that cell-sensitivity matching is of importance for clinical evaluation, and so we are unable to report

Table I. *Treatment data on the three groups studied*

Group	Pollen N. administered (mcg.)			% Treated in previous year
	Average	Median	Range	
<i>Aqueous ragweed</i>				
1965	27	21	1.7-125	79
1966	123	81	19-309	100
<i>Allpyral ragweed</i>				
1966	803	820	47-1,056	100

on clinical efficacy. There is no reason, however, to believe that the Allpyral group differed from the general population of allergic individuals in terms of their ability to make antibody.

Statistical methods

A statistical approach was suggested and carried out by Mr. Frank Adkinson. The results were analyzed in a single factor analysis of variance format (fixed-constants model) as formulated by Weiner.¹⁷ The per cent inhibition data were first subjected to an antilogarithmic transformation in order to achieve direct proportionality between measurements of inhibition and antibody titer. Nonorthogonal comparisons were computed to test for differences among the various treatment means.

RESULTS

Relationship between serum concentration and per cent inhibition of histamine release

The degree of inhibition of the in vitro allergic reaction caused by an allergic serum is a function of the serum concentration. Fig. 1 shows the results of an experiment in which a constant amount of antigen was mixed with dilutions of a serum from a treated allergic patient and added to aliquots of a cell population obtained from a single donor. The serum concentration was kept constant at 10 per cent through the use of normal serum, and controls were run with normal serum alone. Two points are pertinent to the assays described below: (1) as the serum concentration is reduced about eightfold, the per cent inhibition decreases from about 80 to 15; and (2) below 15 and above 85 per cent inhibition, the curve sometimes becomes asymptotic and

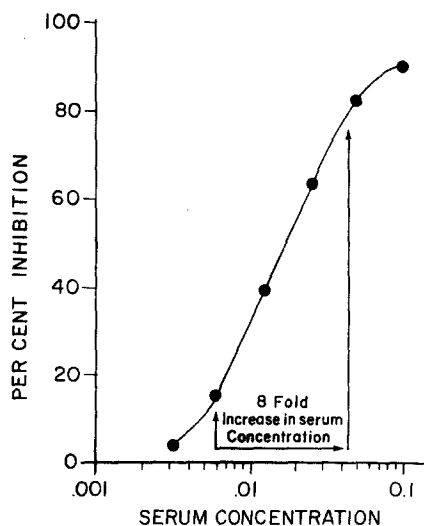


Fig. 1

The effect of concentration on the ability of serum from a treated allergic donor to inhibit in vitro histamine release induced with ragweed antigen E.

relatively insensitive to serum concentration. Thus in the measurements which follow it is not possible to compare sera which cause inhibition falling at the extremes of the curve with those whose inhibition falls on the linear portion. It should be emphasized that, while this is a typical serum, it does not follow that the slopes of the inhibition curves in all sera are exactly similar.

Inhibition of histamine release by sera from patients treated with aqueous and Allpyral ragweed extracts

Fifteen sera from patients treated with Allpyral ragweed and 26 sera from patients treated with aqueous ragweed were tested for their ability to inhibit the histamine release induced by ragweed antigen E. The sera from patients treated with aqueous extract were obtained in 1965 and 1966 and are considered separately because the antigen dosage administered was higher in 1966. The average preseasonal dosage of aqueous ragweed extract administered was 27 mcg. of protein nitrogen (2,700 PNU) in 1965 and 123 mcg. (12,300 PNU) in 1966. The Allpyral group received 803 mcg. (80,300 PNU). Complete details of the immunotherapeutic regimen in each group are given in Table I.

In this assay a 1:40 serum dilution from each patient was mixed with a concentration of antigen which in the presence of normal serum caused 80 per cent histamine release. The per cent inhibition of each serum relative to control is shown in Fig. 2. The Allpyral-treated group averaged 10.7 ± 3.2 per cent inhibition, and the aqueous-extract-treated groups inhibited 31.9 ± 5.2 per cent and 65.6 ± 5.5 per cent for 1965 and 1966, respectively. Each of the groups differed from each other significantly ($p < 0.01$). Thus, the group treated with aqueous ragweed extract in 1965 had a greater antibody response than the Allpyral group, even though these patients received only 3 per cent of the antigenic mass administered to the Allpyral group.

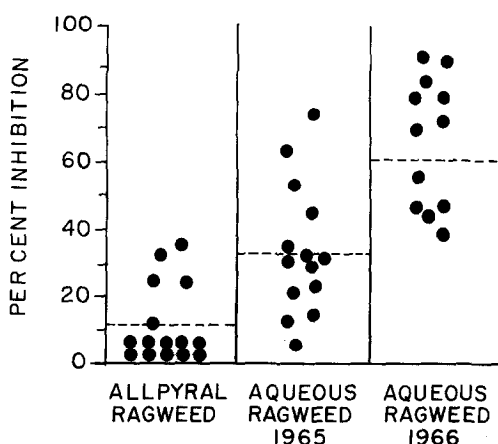


Fig. 2

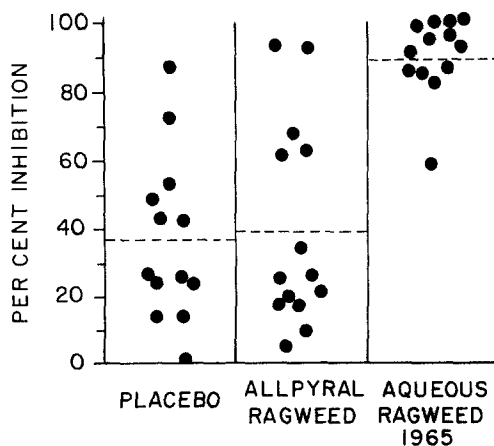
Inhibition of antigen E-mediated histamine release by the sera of allergic patients treated as indicated. The final serum concentration in each case was 1:40. Each group differed significantly from the other two ($p < 0.01$).

Inhibition of histamine release by sera from patients receiving specific immunotherapy contrasted with those receiving placebo therapy

In view of the poor antibody response in patients treated with Allpyral, it was of interest to compare their sera with those of patients who had not received specific immunotherapy. In studying this question, the final serum concentration was increased to 1:10 and an experiment similar to that just described was carried out (Fig. 3). The average inhibition of histamine release was 35.5 ± 6.7 , 38.0 ± 8.0 , and 89.2 ± 3.2 for the placebo, Allpyral, and aqueous extract (1965) treated groups, respectively. There was no significant difference between the control and Allpyral groups, while the difference between these two groups and the group treated with aqueous ragweed extract was again significant ($p < 0.01$).

Inhibition of histamine release induced by crude ragweed pollen

Although antigen E has been shown to be the most potent allergen in ragweed pollen,^{4, 15} the results reported above may be challenged on the ground that we are studying a response to only one of the antigens in ragweed pollen. It is conceivable that in the preparation of Allpyral ragweed antigen E is lost, while others are retained, although this is unlikely inasmuch as it appears that antigen E shares groups with other major ragweed pollen antigens.¹⁸ In order to look for responses to antigens other than E, histamine release was induced with an aqueous extract of ragweed pollen, and the inhibition caused by sera from the three groups studied above was determined (Fig. 4). The results were essentially the same. The placebo group averaged 14 per cent inhibition and was not significantly different from the Allpyral group, which averaged 25 per cent inhibition. Once again the group treated

**Fig. 3**

Inhibition of antigen E-mediated histamine release by sera from placebo-treated patients contrasted with that by sera from patients treated with aqueous and Allpyral ragweed extracts. The final serum concentration was 1:10. The placebo- and Allpyral-treated groups do not differ, but the average inhibitory potency of each was significantly less than that of the ragweed-treated group ($p < 0.01$).

with aqueous ragweed extract, which averaged 51 per cent inhibition, showed a significantly greater antibody response ($p < 0.01$).

DISCUSSION

Antibody is measured in this study by its ability to inhibit an in vitro allergic response which has been found to quantitatively correlate with the clinical severity of allergic symptomatology.^{14, 19} This antibody is of the IgG class²⁰ and is presumably the same species as is measured by blocking titrations in the skin.²¹ The in vitro assay procedure is, however, at least ten times as precise as skin titrations for blocking antibody.¹⁹ It has not yet been demonstrated that the titer of this or any other type of antibody is related to the clinical relief which can, in some instances, be obtained by immunotherapy.^{14, 21, 22} We have, however, shown that immunotherapy with a low dosage of ragweed pollen leads to a relatively small increase in antibody levels and is clinically ineffective, while higher doses cause both significant symptom relief and increased antibody levels.^{14, 19} It was also found that, by and large, those patients with higher antibody levels had fewer symptoms than those with less antibody.¹⁹ This correlation is not sufficiently precise, however, to conclude that there is a causal relationship.

In spite of the lack of a clear-cut relationship between the blocking antibody level and clinical relief, it is generally assumed that the effects of immunotherapy are a result of the antigenicity of the materials administered.¹¹ Indeed, it is difficult to conceive of an alternative to this assumption. On the basis of the previous data it appears that the antigenicity of pyridine-extracted, alum-precipitated ragweed preparations is extremely limited. Even after the administration of extremely large doses of the Allpyral ragweed mixture, it was impossible to detect a significant increase in antibody level relative to controls. Even assuming that a few patients do respond, their

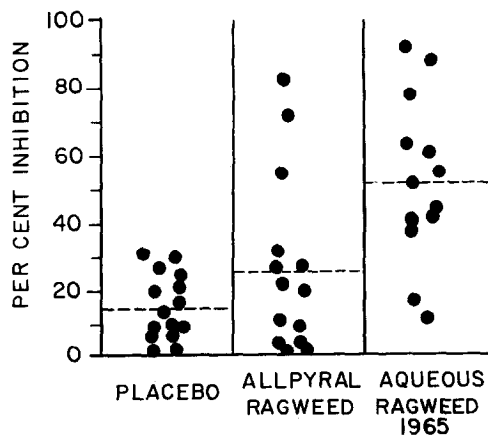


Fig. 4

Inhibition of histamine release caused by an aqueous extract of whole ragweed pollen by the sera of allergic patients treated as indicated. The final serum concentration was 1:10. The placebo- and Allpyral-treated groups do not differ but the average inhibitory potency of each was significantly less than that of the ragweed-treated group ($p < 0.01$).

response is significantly less than can be obtained with 3 per cent as much aqueous extract. Not included in this paper are the sera of 12 patients who in 1964 underwent immunotherapy with an average of about 9 mcg. protein N. of aqueous extract. These patients had a significant antibody response with 1 per cent the antigenic mass that the Allpyral-treated patients received.¹⁴

This data tend to confirm the results of Reisman and Arbesman,¹⁰ who, in studying hemagglutination titers in the sera of 12 patients treated with Allpyral ragweed extract, found that in 7 there was no change in titer, while in 3 the titer decreased and in 2 it increased. Our data lead to conclusions which stand in contrast to those of Spiegelman and colleagues,⁹ who studied the hemagglutination titers of a much larger group of patients. Their data (Fig. 2) show that 61 per cent of the patients had no change in titer, while 29 per cent had a two- to fourfold increase. Only 4 per cent had an eightfold increase, while in 6 per cent the hemagglutination titer fell. There is no statistical analysis of the significance of these results and no comparison with the results obtained with untreated donors. It is not clear, therefore, that Spiegelman and colleagues⁹ demonstrated a significant rise in titer. It should be added that our results were obtained with Allpyral ragweed dosage levels considerably greater than those used by Reisman and Arbesman¹⁰ or Spiegelman and colleagues.⁹ The only other evidence relating to the antigenicity of Allpyral ragweed preparations is that of Gordon and Mansmann,²³ who reported that Allpyral ragweed is effective in inducing Arthus reactions in guinea pigs.

The Allpyral ragweed preparation is reported to cause relatively few local or systemic reactions.^{6, 8} There are two possible explanations for this. Holzman and Norman²⁴ have shown in animal studies that a considerable proportion of antigens precipitated by the high concentration of alum used in Allpyral are absorbed very slowly. Secondary antibody responses to antigens so retained were, however, at least as good as the responses to antigens dissolved in saline. The second explanation for the observed lack of local or systemic reactions to Allpyral is that it contains little active antigen. It is possible that the alkaline extraction of pollen with pyridine has considerably reduced the antigenicity of those proteins which are immunogenic in man. The lack of any increase of antibody in humans immunized with large doses of this material supports the latter possibility.

A number of studies have suggested that Allpyral ragweed is as clinically effective as aqueous ragweed extract.^{6, 8, 25, 26} None of these studies was controlled by the inclusion of a placebo-treated group. It is evident, from our work as well as that of others, that the placebo effect in immunotherapy is large.^{1, 3, 11, 14} We have shown, for example, that immunotherapy with doses of aqueous ragweed extract averaging 8.8 mcg. of protein N. (88 PNU) and ranging from 0.3 to 29 mcg. of protein N. (30 to 2,900 PNU) has an effect no greater than placebo in spite of the fact that these doses have been used for preseasonal immunotherapy at our institution and have resulted in satisfaction to doctor and patient alike.¹⁴ In most of the studies, the effect of Allpyral has been compared to that of groups receiving aqueous therapy with dosage schedules in this range. An explanation compatible with the data presently available is that the placebo

effect obtained with Allpyral is of about the same order of magnitude as that obtained with low dose aqueous extract.

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