

THERAPY OF RAGWEED POLLENOSIS WITH BLOCKING ANTIBODY

A Preliminary Report

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TWENTY-SEVEN years ago, in 1935, Cooke¹ and his associates first described the development of a blocking antibody in the sera of hay fever subjects undergoing desensitization therapy. The clinical significance attributed to the blocking antibody and the resulting divergence of views are recorded in our previous publication.²

In 1957, Sherman³ reviewed the important subject of "Reagin and Blocking Antibodies." The following quotations from his paper are as pertinent as they are challenging: "The most important question about the blocking antibody is the one that is most difficult to answer. Does it protect the patient against hay fever? . . . You all recall many conflicting reports in previous meetings . . . as to the relationship of blocking antibody titers to clinical protection. I think it is safe to say that there has been no significant correlation between blocking antibody titers and clinical results. If the blocking antibody is the protective factor—and it may well be—the fact remains to be proved."

Our investigation has been designed to answer the question whether or not blocking antibody has the capacity to protect a patient against ragweed pollenosis. An adequate supply of blocking antibody was of paramount importance to determine its therapeutic efficacy. This was readily accomplished by immunizing nonatopic individuals with an extract of short ragweed pollen—a method first described by Cooke, Loveless, and Stull.⁴ The details of this phase of our investigation extending over a period of 2 years have been fully reported in a previous communication.⁵

METHODS AND MATERIAL

The inmates of one of the penal institutions of the District of Columbia were made available for this study. After thorough screening, a group of 35

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volunteers was finally selected for the course of immunization. Each volunteer received eighty-three subcutaneous injections of short ragweed pollen extract, containing a total of 3.3 million Noon units or 2 million protein nitrogen units.

Three blood collections were made from the group during the first year—one just prior to immunization; the second—6 months later, and the third one at the end of the year. During the second year, bloods were collected at intervals of 3 months. Approximately one pint of blood from each donor was received in citrate solution. Each pool of the collected bloods was processed into four fractions: Fraction II, gamma globulin; Fraction III, beta globulin; Fraction IV, alpha globulin; and Fraction V, albumin. The blood, however, collected from the seventh and final bleeding was not fractionated.

The gamma globulin fraction of the fifth bleeding contained the maximum amount of blocking antibody as determined by the "in vivo method." We were accordingly provided with an adequate supply of blocking antibody fractions and of whole sera from the fifth and subsequent bleedings; and we were enabled to apply the therapeutic test to our ragweed-sensitive patients during the 1960 and 1961 seasons.

Our approach to the treatment of patients during the 1960 ragweed season was of necessity exploratory in character. Sera or the gamma globulin fractions containing blocking antibody were administered to 45 patients on a preseasonal or co-seasonal basis, or both. In 28 cases, such administration was given concurrently with ragweed pollen extract. Seventeen patients, however, received blocking antibody fractions on a co-seasonal basis only. Judgment of the effectiveness of therapy was based upon the report of the patient and evaluation of physical signs. Moreover, each patient was provided with a chart for the daily recording of symptoms and of medication used.

For purpose of control, 5 patients were treated with blood fractions free of blocking antibody. No change was effected in the course of their severe symptoms. The results of treatment with blocking antibody in the case of our 45 patients were as follows: good, 16; fair, 12; and poor, 17.

Findings similar to our own were obtained through the cooperation of four allergists. Dr. William B. Sherman of New York, Dr. Max Samter of Chicago, Colonel Harold E. Ratcliffe of Walter Reed Army Hospital, and Dr. Richard Todd of the Children's Hospital of Washington, D. C., were each supplied with an adequate amount of blood fractions, from the pre- and post-immunization bleedings. A code number was attached to each vial to fulfill the requirements of a "double blind study."

A total of 38 patients were treated by the four collaborators. The fractions were administered during the season. A few patients had preseasonal injections of pollen extract. The results are summarized in Table I.

With an equal number of cases in both groups, there were more failures among the patients to whom blocking antibody fractions had been administered.

We are now confronted with the causes of failure in 17 cases of a total of 45 in our series and in 15 cases of a total of 19 in the "double blind" series. Two possibilities must be considered—either the blocking antibody is inadequate in function or inadequate in amount to function effectively.

In the "double blind" series, the amount administered varied from a single dose of 2.5 c.c. to a maximum dose of 15 c.c. in divided doses. We must repeat that at the outset of our investigation there was no precedent to follow. We were interested in the reaction of our patients to the administration of blocking antibody, with and without the addition of pollen extract. A survey of our techniques revealed that the number of doses varied from one to ten, that the interval between doses ranged from 3 days to 7 days, and that the minimum volume of blocking antibody fraction was 1 c.c. and the maximum 30 c.c. in divided doses. All these variables were attributable to conditions of our clinic patients over whom we had no control.

TABLE I. DOUBLE BLIND STUDY (1960 SEASON)

RESULTS	CONTROL GROUP TREATED	GROUP TREATED WITH BLOCKING ANTIBODY
	WITHOUT BLOCKING ANTIBODY	
Good	4	2
Fair	4	2
Poor	11	15
Totals	19	19

The experience of 2 patients in our series who had failed to derive any benefit from co-seasonal therapy with blocking antibody proved enlightening. The duration of their disease was 20 and 23 years, respectively. The symptoms were disabling. Larger doses were purposely administered to them. They each received a total of 20.5 c.c. and 55 c.c. of gamma globulin. At the end of the season, these 2 highly sensitive patients reported that they had recovered from their disability more quickly than they ever had before in 20 years. Their observation suggested the possibility that the protective antibody might have been impaired in the chemical fractionation of the serum. Accordingly, when the seventh and final bleeding of our immunized group was conducted on Dec. 16, 1960, fractionation was omitted and the whole serum was reserved for the treatment of patients during the 1961 season. In determining the blocking antibody content of this antiserum by the "in vivo method," it was shown that 0.01 represented the highest concentration of ragweed antigen, measured in milligrams of nitrogen per milliliter which the serum was capable of neutralizing.

During the 1961 season, a total of 52 ragweed-sensitive patients were studied. They were divided into four groups, which we shall now describe.

Group I, our control group, consisted of 7 patients. They were all inmates of the penal institution. The only medication dispensed was a placebo. The albumin and beta globulin fractions, void of blocking antibody, were administered to them. Patient C. C2 of this group experienced severe symptoms which were allayed for 2 days by the injection of 20 c.c. of antiserum. He was transferred to another institution; when interviewed later, he reported that he had had a "bad season." The same report was given by 5 other inmates. One of this group had a fair season.

TABLE II. GROUP I. (CONTROL GROUP WITH CO-SEASONAL THERAPY, 1961)

PATIENT	AGE (YR.)	SEX	NO. OF TREAT- MENTS	MATERIAL	TOTAL VOLUME (C.C.)	REMARKS
C. G1	63	M	4	Albumin fraction	7.5	Fair
C. C2	34	M	1	Albumin fraction	10	Anti-ragweed serum adminis- tered to note effects on severe symptoms
			1	Anti-ragweed serum	20	Bad season
C. B3	37	M	3	Albumin fraction	40	Bad season
C. A4	33	M	5	Albumin fraction Beta globulin	100	Bad season
C. K5	39	M	5 1	Albumin fraction Beta globulin	55	Severe symptoms until mid-Sep- tember
C. H6	57	M	1 1	Albumin fraction Beta globulin	20	Bad season
C. N7	49	M	2 1	Beta globulin Albumin fraction	30	Asthma lessened, but continued in severe form after September 28

Group II comprised 8 patients who had received antiserum, varying from 1.15 c.c. to 37.3 c.c. in volume, in addition to the regular course of desensitization with pollen extract. Seven patients reported good results and one patient fair results. It must be stated that 6 of this group had received courses of desensitization in prior years. The remaining 2 patients had their introduction to specific therapy in 1961. The administration of antiserum was preseasonal in 4 cases, co-seasonal in 2, and both preseasonal and co-seasonal in the remaining 2.

TABLE III. GROUP II. (PATIENTS RECEIVING COURSE OF DESENSITIZATION WITH POLLEN EXTRACT SUPPLEMENTED WITH ANTISERUM, 1961)

PATIENT	AGE (YR.)	SEX	NO. OF TREATMENTS WITH ANTI- SERUM	TIME OF ADMINIS- TRATION OF ANTISERUM	VOLUME OF ANTISERUM (C.C.)	RESULTS
A 1.	62	M	11	Preseasonal	22.0	Good
C 2.	61	M	8	Preseasonal	5.8	Fair
F 3.	54	M	20	Preseasonal	37.3	Good
K 4.	36	M	5	Preseasonal	6.6	Good
S 5.	25	M	3	Co-seasonal	15.0	Good
N 6.	52	M	2	Co-seasonal	12.0	Good
H 7.	41	F	10 3	Preseasonal Co-seasonal	7.8 6.0	Good
S 8.	34	F	6 5	Preseasonal Co-seasonal	1.15 5.0	Good

The patients in Group III, unlike those of Group I and II, had received only the whole serum on a preseasonal and co-seasonal schedule. Of the 14 patients in this group, the results of therapy were good in 5, fair in 5, and poor in 4 of them. The experience of Patient P. PS. J1 was one of unusual interest and significance. When treated in 1960, he received a total of 20.5 c.c. of gamma globulin (fifth bleeding) from August 24 to September 16 without relief of symptoms. In 1961, he received 20 c.c. of serum preseasonally and 55 c.c. of serum during the season. He regarded the results as "phenomenal." For the first time in 10 years, he was not confined to bed in early September, and lost no time from his work. His co-seasonal dosage in 1961 was two and one half times greater than in 1960. "The larger the dose, the greater the protection," was indeed suggested thereby.

TABLE IV. GROUP III. (PRESEASONAL AND CO-SEASONAL SCHEDULE WITH ANTIRAGWEED SERUM ONLY, 1961)

PATIENT	AGE (YR.)	SEX	NO. OF PRE- SEASONAL TREATMENTS	VOLUME OF PRESEASONAL TREATMENTS (C.C.)	NO. OF CO- SEASONAL TREATMENTS	VOLUME OF CO-SEASONAL TREATMENTS (C.C.)	TOTAL VOLUME (C.C.)
<i>Good Results</i>							
P. PS. J1	41	M	12	20	7	55	75
Cl. PS. B2	57	F	2	10	4	20	30
Cl. PS. H3	18	F	7	27	4	20	47
Cl. PS. M4	39	F	15	46	7	50	96
Cl. PS. F5	35	M	10	36	3	25	41
<i>Fair Results</i>							
Cl. PS. G 1a	63	F	13	42	4	25	67
Cl. PS. D 2a	33	F	13	42	7	35	77
Cl. PS. M 3a	42	M	14	44	6	40	84
Cl. PS. M 4a	21	F	5	19	5	30	49
Cl. PS. H 5a	50	F	6	16	3	15	41
<i>Poor Results</i>							
Cl. PS. D 1b	53	F	10	50	4	23	73
Cl. PS. D 2b	22	F	7	23	3	25	48
Cl. PS. B 3b	65	M	12	42	7	54	96
Cl. PS. H 4b	38	F	14	41	8	50	91

It must also be emphasized that 13 of the 14 patients in Group III were clinic patients. The majority presented a diversity of complications, other than allergic; some unfortunates had neither the ability nor the desire to follow instructions.

Happily, Group IV, the largest in our series with 23 patients, foreshadows the treatment of choice. The antiserum was administered after the onset of symptoms. Good results were secured in 17 patients, fair results in 5, and poor results in one. The latter attributed his distress to the fact that he was constantly exposed in his quarters to the spraying with insecticides.

Thirteen of this group were prison inmates and formed a captive clientele. They were exposed to the pollen of the air of open country. Housing and food were alike for all. Medication was in the form of a placebo. The variables, encountered in private and clinic practice, were reduced to a minimum. We are thus afforded adequate criteria for the evaluation of our treatment. The average dose of antiserum for the members of this group was 64 c.c.

TABLE V. GROUP IV. (SEASONAL SCHEDULE WITH ANTISERUM ONLY, 1961)

PATIENT	AGE (YR.)	SEX	NO. OF TREATMENTS	TOTAL VOLUME (C.C.)
<i>Good Results</i>				
P. A. 1	32	F	4	25
P. B. 2	15	M	4	40
P. L. 3	16	M	8	40
P. R. 4	47	F	9	53
P. W. 5	32	M	4	40
P. Y. 6	43	M	3	60
R. A. 7	38	F	7	130
R. A. 8	39	M	7	100
R. R. 9	45	M	8	100
R. W. 10	35	M	7	120
R. W. 11	46	M	5	60
R. W. 12	42	M	4	60
R. W. 13	44	M	1	10
R. W. 14	40	M	9	90
Cl. L. 15	20	F	4	25
Cl. D. 16	34	F	3	30
Cl. H. 17	3	M	3	15
<i>Fair Results</i>				
R. B. 1a	54	M	4	60
R. H. 2a	40	M	6	60
R. M. 3a	36	M	3	50
R. W. 4a	37	M	5	60
Cl. F. 5a	13	M	6	60
<i>Poor Results</i>				
R. K. 16	55	M	8	100

The following summary of our experimental procedure with the use of blocking antibody during the ragweed season of 1961 records an advance in the control of the disease (Table VI).

TABLE VI. SUMMARY (PATIENTS TREATED—RAGWEED SEASON, 1961)

GROUP	RESULTS			
	GOOD	FAIR	POOR	TOTAL
I. Control	0	1	6	7
II. Pollen extract and antiserum	7	1	0	8
III. Antiserum only, pre-seasonal and co-seasonal	5	5	4	14
IV. Antiserum only, co-seasonal	17	5	1	23
			Total	52

In a previous communication,² we have indicated the limitations of the immunologic significance of the blocking antibody content of the sera of treated ragweed-sensitive patients. Accordingly, no attempt was made to control our dosage of antiserum by measuring the blocking antibody content of the blood of the recipients. This view was confirmed by a study, made in November, 1960, of a nonatopic male, 51 years of age, measuring 5 feet 6 inches in height, and weighing 155 pounds. Fifty cubic centimeters of gamma globulin, derived from our sixth bleeding, was injected subcutaneously—20 c.c. in each buttock and

5 c.c. in each upper arm. A blood sample was collected immediately before the injection of the blocking antibody fraction. Additional samples were taken one, 2, 3, and 24 hours after the injection. Our tests did not reveal the presence of blocking antibody in the five blood specimens examined. Moreover, no induration and no signs of irritation were present at the four sites of the injection. Two explanations have been offered for the failure to demonstrate blocking antibody in treated hay fever subjects, (1) the fixation of the blocking substance in the shock organs, (2) the problem of dilution. In the case of our experimental subject, who was nonatopic, there was an absence of both the allergen and sensitizing antibody; hence no fixation was likely to occur. It may be, however, that our method of detection was not sensitive enough.

DISCUSSION

As previously noted, Cooke and his associates have made a notable contribution in describing the formation of the blocking antibody in hay fever patients. It develops in the course of desensitization or immunization with pollen extract. The next logical step for them was to apply their concept to the clinical test.¹ Accordingly, 20 hay fever subjects were transfused with blood from ragweed-sensitive patients who had been treated for at least one year. The recipients of this theoretically immune blood received no other treatment. Satisfactory results were obtained in 16 of the 20 patients thus treated. Ninety-six other patients received repeated subcutaneous injections of 10 c.c. doses of supposedly immune serum. There was clinical improvement in 60 per cent. However, there was no statement made concerning the actual number of injections or of the time interval between injections.

Three years later, a confirmatory clinical test was reported by Langner and Kern.⁶ Two of their ragweed-treated patients were bled 7 to 10 days after their last pollen injection. Two hundred cubic centimeters of blood was obtained from each donor. The pooled sera were dried according to the technique of Flossodorf and Mudd. Nine ragweed subjects each received one injection only of the lyophilized serum. The dose administered represented 50 c.c. of the ragweed serum. Of the 9 ragweed cases, there were 2 in which there was no benefit from the treatment. It is noteworthy that all the recipients of the lyophilized serum experienced local and general reactions, some quite marked.

Swineford and Bray,⁷ in their use of serum from treated patients, attempted to minimize the reactions reported by Langner and Kern. The former heated the sera to 60° C. for one hour to destroy the reagin or sensitizing antibody. In sharp contrast, in our investigation, we encountered one case of hives among the 52 patients treated with the immune serum which was obtained by immunization of nonatopic individuals. By the injection of heated post-treatment sera, Swineford and Bray⁷ afforded "lasting relief" to 3 patients. Of these, 2 had been unsuccessfully treated previously and one was untreated. They afforded no relief, however, to 3 other patients who had been unsuccessfully treated by desensitization. We are fully in accord with Swineford's statement⁸ that the use of immune serum offers a therapeutic approach which is ripe for exploration.

It is obvious that the dosage of the immune serum, administered by the several investigators and by ourselves, has been quite arbitrary, and that the preseasonal administration serves no useful purpose. The important change in our technique has been to increase the dosage during the 1961 season. It is essential to apply the principle gained from experimental work in the laboratory. It has been observed that hypersensitive animals can be made refractory by the injection of an excess of circulating antibody in order to neutralize the antigen before the latter reacts with fixed antibody. The results achieved in 1961, as indicated in the summary, justify that principle in great measure. This is especially true of Group IV, in which 17 of 23 patients have derived great benefit from co-seasonal therapy.

Since the days of Noon,⁹ the basic treatment of pollen-sensitive subjects has been one of active immunization whereby blocking antibodies are formed in individuals. Efforts have been made, successful in part, to confer a passive immunity by the administration of immune sera, derived from hay fever subjects who have undergone desensitizing treatments for at least one year. No prior attempt, however, has been made to utilize sera from immunized nonatopic individuals—a course which is more practical.

We are encouraged in our investigation by an analogous procedure described by Kraut¹⁰ in the control of tetanus infection. In order to eliminate possible allergic reactions from the use of equine serum or bovine serum antitoxin, Kraut recommends the administration of homologous antitoxin as follows: (1) in the form of transfusion of actively immunized human blood, (2) by plasma obtained from this type of blood, and (3) by using the gamma globulin fraction filtrated from the hyperimmunized human blood.

At this time, a warning note must be sounded lest a similar analogy be drawn between our homologous immune serum and the Pollantin of Dunbar and the Graminol of Weichardt.

SUMMARY AND CONCLUSION

As we review the therapeutic trials of the past 2 years, the results obtained during the ragweed season of 1961 stand out in marked contrast with those of the 1960 season. In 1960, the successfully and unsuccessfully treated patients were almost equal in number. The doses of immune serum or of the blocking antibody fraction were much smaller in volume. The concurrent administration of ragweed pollen extract with the blocking antibody fraction or the preseasonal administration of the latter serves no useful purpose. On the contrary, the blocking antibody serves its useful purpose in the presence of allergen and reagin. This can only prevail when patients inhale the air-borne allergen. We are, therefore, encouraged in this preliminary study by the therapeutic results achieved with the patients in Group IV. The requisite conditions were present at the time. Of 23 ragweed-sensitive patients, treated co-seasonally with our larger doses of immune serum, 7 secured good results, 5 fair, and one poor results. The last named may have fared otherwise if exposure to insecticide spray were avoided.

We now return to the question propounded by Sherman—does the blocking antibody protect the patient against hay fever? Our judgment, based upon the most critical phase of our study, is that blocking antibody does afford protection. We believe that definite advance has been made toward the passive immunization of the pollen-sensitive patient. However, more encouragement and more study needs be given in order to attain the objective—overdue and overlooked.

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