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DEMONSTRATION OF REAGIN OF A NEW SPECIFICITY IN SERA OF TREATED RAGWEED-SENSITIVE PERSONS

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IN 1911, Noon¹ demonstrated that patients suffering from hay fever showed clinical improvement after they had received a series of graded injections of pollen extract. This procedure of "desensitization" or "hypo-sensitization" has since been modified only slightly and constitutes the main treatment offered to clinically allergic persons. The mechanism by which protection is afforded by the injection of the pollen extract is still poorly understood. In 1935 Cooke and associates² demonstrated the presence of a factor in sera of treated allergic persons not found in sera of nontreated persons. This factor, which they referred to as "blocking antibody," was capable of combining with the allergen in vitro and of preventing it from reacting in vivo with the skin-sensitizing factor or reagin. The presence of blocking antibody in treated allergic persons was later confirmed by Loveless,³ Frank and Gelfand,⁴ Sherman,⁵ Cooke and colleagues,⁶ and more recently in this laboratory.¹² Although Loveless⁷ claims that this is the factor responsible for the clinical improvement shown in treated patients suffering from hay fever, other attempts at correlating blocking antibody titers with clinical results have not been very rewarding.^{5, 8-11}

The present study was undertaken to demonstrate differences which may exist between sera of treated† and nontreated allergic persons with respect to their reactivity to fractions of ragweed pollen extract.

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†All ragweed-sensitive persons who had undergone at least one series of injections of ragweed pollen extract are referred to as treated or desensitized. Sera obtained from such persons are referred to as treated sera as compared to untreated sera from untreated allergic persons.

METHODS AND RESULTS

Two allergen preparations of ragweed pollen (*Ambrosia artemisiifolia*) were used: (1) WSR, the whole water-soluble extract of defatted ragweed pollen and (2) dialysate, the fraction of WSR which dialyzed through Visking

TABLE I. REAGINIC ACTIVITY IN SERA AND SKIN OF UNTREATED ALLERGIC INDIVIDUALS TO DIALYSATE AND WSR

SERUM OR SKIN OF PATIENT TESTED	REACTION BY PASSIVE TRANSFER TO:		REACTION BY DIRECT SKIN TEST TO:	
	DIALYSATE	WSR	DIALYSATE	WSR
B. P.	0	5+	*	*
A. L.	0	5+	*	*
A. P.	0	5+	*	*
L. M.	0	2+	0	4+
M. G.	0	3+	0	6+
D. G.	0	4+	0	6+
S. R.	0	4+	0	4+
P. G.	0	4+	0	6+
A. W.	0	1+	0	3+
#9	0	3+	*	*
#10	0	3+	0	2+
#11	0	2+	0	2+
#18	0	5+	*	*
#22	0	2+	*	*
#23	0	3+	*	*
#48	0	2+	*	*
#49	0	1+	0	3+
#50	0	3+	*	*
#51	0	3+	0	3+
#53	0	4+	1+	4+
#54	0	4+	1+	5+
#57	0	4+	4+	5+
#58	0	4+	*	*
#59	0	4+	*	*
#62	0	4+	1+	4+
#64	0	2+	*	*
#65	0	2+	*	*
#66	0	2+	1+	3+
#68	0	4+	*	*
#69	0	3+	0	5+
H. L.	1+	5+	*	*
R. A.	2+	2+	*	*
#52	3+	3+	2+	4+
#61	2+	2+	*	*
#63	3+	2+	2 +	4+
#67	2+	4+	*	*
P. M.	*	*	0	5+
R. N.	*	*	1+	4+
R. S.	*	*	0	6+
R. K.	*	*	0	4+
F. J.	*	*	0	2+
#60	*	*	2+	4+
#71	*	*	6+	6+
#73	*	*	3+	6+
#74	*	*	0	2+

*Test not performed.

cellophane tubing in twenty-four hours at 4° C. Both allergen preparations were dissolved in saline and prepared in concentrations of 1,000 units per milliliter (1 unit = 10⁻⁵ mg. nitrogen).

Blood was drawn from treated and untreated ragweed-sensitive patients and the sera were separated by centrifugation and sterilized by Seitz

filtration. The undiluted serum (0.05 ml.) was injected into two sites on the back of a normal, nonallergic person; twenty-four hours later the sensitized sites were challenged with WSR and dialysate and the reactions were graded 0, 1+, 2+, 3+, etc., depending on the size of the wheal and the surrounding erythema.

As can be seen from Tables I and II, when the method of passive transfer was used, all sera reacted to WSR. On the other hand, only six of thirty-six sera from untreated and twenty-seven of thirty-two sera from treated subjects reacted to dialysate.

Intradermal skin tests were performed on untreated and treated persons with both WSR and dialysate. Only eleven of twenty-six untreated persons, as compared to all of the ten treated persons, reacted to dialysate. All patients reacted to WSR (Tables I and II).

TABLE II. REAGINIC ACTIVITY IN SERA OF TREATED ALLERGIC PERSONS TO DIALYSATE AND WSR

SERUM TESTED	REACTION BY PASSIVE TRANSFER TO:	
	DIALYSATE	WSR
B. P.	5+	5+
F. W.	4+	4+
A. S.	3+	3+
R. D.	5+	4+
#3	2+	2+
#4	1+	4+
#5	1+	3+
#7	3+	4+
#10	1+	4+
#11	1+	2+
#12	2+	3+
#14	4+	4+
#13	1+	1+
#25	4+	4+
#28	2+	3+
#29	2+	3+
#35	4+	4+
#36	2+	3+
#37	1+	2+
#38	4+	4+
#40	3+	5+
#41	2+	3+
#42	4+	3+
#43	4+	4+
#45	4+	4+
#55	3+	3+
#56	2+	4+
#22	0	3+
#24	0	3+
#33	0	3+
#44	0	2+
#46	0	4+

In addition, ten persons who had undergone treatment reacted without exception by direct skin test to dialysate and WSR. All reactions to dialysate were comparable to reactions evoked by the injection of WSR (3+ or greater).

In addition, sera were obtained from five allergic patients before and after they had undergone desensitization treatment. Both the pre- and post-treatment sera were tested by passive transfer. As can be seen from Table III, none of the five sera had reagins to dialysate before treatment. After treatment, however, the sera of four of these five persons possessed reagins to

dialysate demonstrable by passive transfer. The fifth patient became reactive to intradermal injection of dialysate, although no reagin directed toward dialysate could be detected in his serum by the passive transfer method. Sera obtained from one of the five patients (B.P.) one month and eighteen months after his last ragweed injection were equally reactive to dialysate (Table III).

TABLE III. REAGINIC ACTIVITY IN SERA OF SENSITIVE PERSONS BEFORE AND AFTER TREATMENT

SERUM TESTED	REACTION BY PASSIVE TRANSFER TO:	
	DIALYSATE	WSR
B. P. Pre*	0	5+
Post†	5+	5+
Post 2‡	5+	5+
#10 Pre	0	3+
Post	1+	4+
#11 Pre	0	2+
Post	1+	2+
#18 Pre	0	5+
Post	2+	4+
#22§ Pre	0	2+
Post	0	3+

*"Pre" refers to serum obtained before treatment.

†"Post" refers to serum obtained from the treated person after the ragweed season.

‡This serum was taken eighteen months after Patient B. P. received his last injection of ragweed pollen.

§Patient #22 reacted by skin test to dialysate (2+) after undergoing treatment, although his serum contained no detectable reagins by passive transfer.

Two sera containing blocking antibodies were tested for their capacity to inactivate the allergens in the dialysate by the method used in this laboratory.¹² Neither of these sera demonstrated any ability to inactivate dialysate in vitro although both, in serum dilutions as high as 1:32, were capable of blocking WSR.

DISCUSSION

Since 86 per cent of all treated sera and only 17 per cent of all untreated sera possessed reagins which reacted to dialysate, it appears that reagins of a new specificity were formed by ragweed-sensitive patients undergoing desensitization treatment with ragweed extracts. In fact, the presence of these reagins after treatment and their absence prior to treatment was demonstrated in the sera of four out of five patients (Table III).

The significance of the reagins detected only in the sera of treated patients is not clear at this time. It is possible that they may be responsible for the increased clinical sensitivity observed in some treated persons. Patients B. P. and B. W. both claimed to have had more pronounced clinical symptoms after treatment, although their sera had blocking antibody titers to WSR of 1:8 and 1:16, respectively. Both possessed reagins to dialysate in high titer in their sera. On the other hand, it is worth mentioning that we have detected reagins to WSR in the skin of many persons who have never

had symptoms of clinical allergy. We have also recently demonstrated¹⁴ that formation of reagins can be induced in a few nonallergic persons without invoking a condition of clinical allergy.

From the foregoing results, it would appear that the "true allergens" (those which induce clinical allergy in untreated persons) are present only in the nondialyzable fraction of WSR, which constitutes only 2 to 4 per cent (by weight) of the whole WSR. With a number of sera from untreated subjects the dialyzed residue of WSR* was substituted for whole WSR in the passive transfer test and results identical with those shown in Table I were obtained.

The mechanism by which both clinical sensitivity and skin-sensitizing activity are induced in susceptible subjects is not known. An "incubation" period of a year or two is generally necessary in the majority of patients coming to a ragweed-laden area for the first time. Presumably, the first exposure to ragweed induces the formation of reagin, and clinical sensitivity may or may not become manifest. In this connection, it is difficult to explain why most allergic persons possessed reagins to the allergens of the dialysate only after treatment. In further studies on this problem three normal, non-allergic volunteers were injected twice a week for six weeks with a large quantity of WSR (a total of 2,500,000 units each). No reagins could be demonstrated, either by direct intradermal test or by the method of passive transfer.

Another observation which requires explanation is why some untreated ragweed-sensitive persons possessed reagins to the allergens in the dialysate. It may be significant that all of these untreated persons who reacted by skin test to dialysate also reacted to some allergens other than ragweed (dust, horse dander, grass, etc.). Since it has been demonstrated previously that cross-reactivity exists between different allergens, it is possible that the reagins reacting with the allergens in the dialysate in these patients were formed on stimulation with other allergens. However, one cannot exclude the possibility that these persons were inherently capable of forming these reagins after exposure to all the allergens in ragweed pollen during the ragweed season.

As can be seen in Table I, five untreated persons gave positive reactions on intradermal skin tests with dialysate, although their sera possessed no reagins to this allergen preparation by passive transfer. Four of these five persons showed small reactions by direct skin test (+1). Therefore, one can conclude that reagins to dialysate were present in negligible quantities in their skins. Two possible explanations for this observation are: (1) The intradermal skin test is more sensitive than the passive transfer test for the detection of small quantities of reagin. This explanation alone, however, seems doubtful in this case, since reactions in these persons by direct skin tests with WSR were not significantly larger than the reactions obtained with their sera by passive transfer. (2) These persons may have formed reagins

*The fraction of WSR left inside the dialyzing sac after twenty-one days of dialysis against running tap water is referred to as the dialyzed residue of WSR.

in very low concentration to the allergens in the dialysate during the ragweed season. These reagins may have been "bound"* to the skin and remained there while their concentration in the blood diminished when further stimulation for their formation ceased.

CONCLUSIONS

1. It has been shown that the injection of whole ragweed pollen extract into ragweed-sensitive subjects leads to the formation of reagins not present before treatment.

2. These reagins have a different specificity as compared to the "normal" skin-sensitizing antibody formed in untreated allergic persons.

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*Loveless,¹³ in 1941, showed that reagin has a very marked affinity for the skin and is capable of being "bound" to it.