

THE SPECIFICITY OF BLOCKING ANTIBODY INDUCED BY GRASS POLLEN EXTRACTS*†

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IN 1916 Cooke and Vander Veer¹ reported a study of twenty cases of grass-sensitive hay fever patients, in whom skin tests with extracts of the pollens of six grasses were positive. Variations in degree of the positive reactions were only slight. Treatment with one grass pollen extract produced clinical relief of symptoms from exposure to any of the six grasses. They postulated the antigenic identity of the various grass pollens.

Since that time many reports have established the close relationship between all grass pollen proteins. Coca and Grove² demonstrated complete cross neutralization between various grasses, employing the in vivo neutralization test. Chobot,³ Piness and Miller,⁴ and Cooke and associates⁵ demonstrated similar results using the in vitro neutralization technique. Reciprocal cross neutralization between two or more grasses was shown to exist.

Hampton, Stull, and Cooke,⁶ in 1939, showed the relationship between timothy, orchard, and June grass in studies with the Dale test in experimentally sensitized guinea pigs. The specific antigen always neutralized the uterine horn to test with related antigens, whereas related antigens neutralized to test with specific antigens in some cases, but failed to do so in others. This gave further evidence that extracts of grass pollens are closely related, but indicated they are not identical.

The present study consists of observations on the development of blocking antibody in sensitive and nonsensitive persons after injections of timothy, orchard, and June grass pollen extracts, and on the specificity of such antibodies with respect to extracts of pollen of these three grasses. Previous experiments with blocking antibody induced by injections of ragweed pollen extract have indicated that blocking antibodies are more specific in their reaction than the corresponding skin sensitizing antibodies⁷ and, therefore, might be expected to give a better indication of the relationship between various related antigens.

SELECTION OF CASES

Group I.—Clinically sensitive grass hay fever patients were selected who showed a marked reaction on skin test with 100 protein nitrogen units of June, timothy, and orchard grasses, and whose serum transferred this sensitivity to normal skin on test with grass pollen extracts containing one protein nitrogen unit per cubic centimeter. Four patients were treated with timothy pollen extract, similar numbers with June and orchard grasses.

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Group II.—Six individuals without clinical history of pollen sensitivity, but having other stigmas of allergic disease, were given massive doses of pollen extract. Three received timothy, one received June, and two received orchard grass pollen extracts. The total doses averaged 400,000 to 500,000 protein nitrogen units, given in six to eight weeks.

Group III.—One volunteer with a negative clinical and family history of allergy, no evidence of allergic disease, and with negative skin tests was given 1,000,000 units of June grass extract in three weeks. The administration of large doses of antigen had previously¹⁶ been shown in this clinic to stimulate production of blocking antibody in a nonsensitive individual.

METHOD OF STUDY

Sera obtained before treatment were designated as "A" sera, those obtained after treatment as "P" sera.

Blocking antibody was demonstrated by a method employed in previous studies at this clinic.⁸ This method is semiquantitative and subject to certain errors, but gives useful information if the necessary controls are used.*

Sensitized sites were made in the skin of the back of a normal subject by injecting 0.1 c.c. of an exquisitely grass-sensitive serum (OP), diluted 1:10, into each site. At the same time, one site each was made with the heated A and P serum being tested.

The sera being examined were heated at 56° C. for four hours, and the skin-sensitizing antibody thus destroyed.⁹ Mixtures were then made as follows: heated A or P serum, 0.1 c.c.; 0.8 per cent sodium chloride solution, 0.8 c.c.; and grass pollen extract (100 protein nitrogen units per c.c.), 0.1 cubic centimeter.

Six such mixtures were made containing A and P sera with timothy, June, and orchard grass pollen extracts. These mixtures were then serially diluted with saline so that tubes contained 3, 1, and 0.3 protein nitrogen units of pollen extract per cubic centimeter, respectively, with comparable dilutions of the serum. Each site was tested by the injection of 0.025 c.c. of one of the mixtures of pollen extract and heated serum. The mixtures made with A serum served as controls for those made with P serum. The sites made with heated A and P serum were tested with timothy pollen extract, 10 protein nitrogen units per cubic centimeter, to establish the complete inactivation of the skin-sensitizing antibody in the heated sera.

EXPERIMENTAL RESULTS

Group I. Clinically Sensitive Grass-Hay Fever Patients.—Of twelve cases, four treated with each of the three grass pollen extracts and all enjoying satisfactory relief of clinical symptoms, seven developed blocking antibody reacting with all three pollens. The degree of blocking varied but was similar with all three pollens and not related directly to the amount of treatment given. The

*Results obtained by this method cannot be compared quantitatively with those obtained by the neutralization method of Loveless.⁹

blocking antibody was uniformly less marked than in ragweed hay fever patients receiving comparable treatment. Five patients did not develop demonstrable blocking antibody, although having good protection against hay fever. In two of these patients the total dosage was less than 20,000 units.

TABLE I. BLOCKING ANTIBODY TEST WITH SERUM OF GRASS-SENSITIVE PATIENT (K) TREATED WITH TIMOTHY POLLEN (TOTAL DOSAGE, 21,600 PROTEIN NITROGEN UNITS)

ANTIGEN CONCENTRATION (PROTEIN NITROGEN UNITS PER ML.)	REACTION OF PASSIVE TRANSFER SITES SENSITIZED WITH GRASS-SENSITIVE SERUM OP, 1:10; TESTED 48 HOURS LATER WITH:					
	K SERUM A + TIMOTHY	K SERUM A + ORCHARD	K SERUM A + JUNE	K SERUM P + TIMOTHY	K SERUM P + ORCHARD	K SERUM P + JUNE
10*	+++	+++	+++	+	±	++
3	+++	+++	+++	+	±	+
1	++	+++	++	±	0	±
1/3	++	++	+	0	0	0

K Serum A, taken before treatment, and K Serum P, taken after treatment, both heated four hours at 56° C. and shown by control tests on the same subject not to sensitize skin after heating.

*Mixtures made: 0.1 ml. heated K Serum (A or P); 0.1 ml. grass pollen, 100 protein nitrogen units per milliliter; 0.8 ml. saline. Serial dilutions to 3, 1, and 1/3 units made with saline.

TABLE II. BLOCKING ANTIBODY TEST WITH SERUM OF GRASS-SENSITIVE PATIENT (C) TREATED WITH JUNE GRASS POLLEN (TOTAL DOSAGE, 35,685 PROTEIN NITROGEN UNITS)

ANTIGEN CONCENTRATION (PROTEIN NITROGEN UNITS PER ML.)	REACTION OF PASSIVE TRANSFER SITES SENSITIZED WITH GRASS-SENSITIVE SERUM OP, 1:10; TESTED 48 HOURS LATER WITH:					
	C SERUM A + TIMOTHY	C SERUM A + ORCHARD	C SERUM A + JUNE	C SERUM P + TIMOTHY	C SERUM P + ORCHARD	C SERUM P + JUNE
10*	+++	+++	+++	+++	+	+
3	+++	+++	+++	++	+	+
1	+++	++	++	+	+	±
1/3	+++	++	++	0	0	0

C Serum A, taken before treatment, and C Serum P, taken after treatment, both heated four hours at 56° C. and shown by control tests on the same subject not to sensitize skin after heating.

*Mixtures made: 0.1 ml. heated C Serum (A or P); 0.1 ml. grass pollen, 100 protein nitrogen units per milliliter; 0.8 ml. saline. Serial dilutions to 3, 1, and 1/3 units made with saline.

TABLE III. BLOCKING ANTIBODY TEST WITH SERUM OF GRASS-SENSITIVE PATIENT (J) TREATED WITH ORCHARD GRASS POLLEN (TOTAL DOSAGE, 7,720 PROTEIN NITROGEN UNITS)

ANTIGEN CONCENTRATION (PROTEIN NITROGEN UNITS PER ML.)	REACTION OF PASSIVE TRANSFER SITES SENSITIZED WITH GRASS-SENSITIVE SERUM OP, 1:10; TESTED 48 HOURS LATER WITH:					
	J SERUM A + TIMOTHY	J SERUM A + ORCHARD	J SERUM A + JUNE	J SERUM P + TIMOTHY	J SERUM P + ORCHARD	J SERUM P + JUNE
10*	++	+++	++	++	++	++
3	++	++	++	+	++	+
1	+	++	++	±	+	±
1/3	±	+	+	0	0	0

J Serum A, taken before treatment, and J Serum P, taken after treatment, both heated four hours at 56° C. and shown by control tests on the same subject not to sensitize skin after heating.

*Mixtures made: 0.1 ml. heated J Serum (A or P); 0.1 ml. grass pollen, 100 protein nitrogen units per milliliter; 0.8 ml. saline. Serial dilutions to 3, 1, and 1/3 units made with saline.

Table I demonstrates the presence of antibody blocking timothy, orchard, and June grass in the serum of a patient treated with timothy grass. Tables II and III illustrate essentially similar results in patients treated with June and orchard grass, respectively.

Group II. Patients With Associated Allergies But No Pollen Sensitivity.—Of the six patients in this group, four developed blocking antibody reacting with all three pollen extracts. The degree of reaction with the three pollens was not always equal but was not consistently greater than the pollen which had been used for treatment. The sera of two other patients, treated with orchard and June grass, respectively, showed no blocking antibody.

An interesting observation in three of the four patients with blocking antibody was the development of a slight positive skin reaction to grass pollen following the massive doses of pollen antigen. All of these patients had negative skin tests with timothy, 10,000 protein nitrogen units per cubic centimeter prior to the injections. After injections of 400,000 to 620,000 protein nitrogen units of timothy pollen extract during a period of twelve to fifteen weeks, these patients showed skin reactions with wheals 5 mm. in diameter on test with timothy, 100 protein nitrogen units per cubic centimeter. These skin reactions persisted six weeks after the course of pollen extract injections was discontinued. During the following pollen season, none of these patients had any symptoms attributable to grass pollen.

Group III. Nonallergic Volunteer.—This one nonallergic volunteer was given 1,000,000 protein nitrogen units of June grass in three weeks and developed blocking antibody to the three grass pollen antigens. This subject did not develop any skin reaction to grass pollens as a result of the injections.

DISCUSSION

Each of the three grass pollen extracts used, timothy, June, and orchard grass satisfactorily protected grass pollen-sensitive patients against hay fever (in the New York area). All of the pollen extracts appeared equally active in inducing the formation of blocking antibody, and the antibody found blocked all three pollens. The degree of blocking demonstrated was not uniform for all three grasses in every case, and did not show any constant pattern with any one antigen. The variations were probably within the limits of error of the method used. The results tend to confirm the existence of a close relationship between grass pollen antigens of different genera.

The development of a positive skin test to timothy pollen extract (100 protein nitrogen units per cubic centimeter) in persons with associated allergic disease, but previously showing negative reactions to 10,000 protein nitrogen units per cubic centimeter following injections of grass pollen extract, was of interest and contrary to the prevailing opinion. However, the reactions were only slight (5 mm. wheal) and not accompanied by clinical grass hay fever.

The possibility of active sensitization by injections of pollen extract was investigated by Lamson and Miller¹⁰ with negative results. Brunner¹¹ reported active sensitization in three of five persons injected with *Ascaris* extract (which

has since been repeatedly demonstrated), but only in one of eight persons injected with orris and in no instance with egg albumin or rabbit epithelium. He did not use pollens in his study. More recently, Wodehouse¹² has described development of positive skin reactions to ragweed pollen without hay fever symptoms, apparently indicating active sensitization of four test subjects used in passive transfer studies. All of these persons had past histories of urticaria, rhinitis, or dermatitis but had previously shown negative reactions to tests with ragweed pollen extract. It seems that injections of pollen extract into individuals with a past or family history of allergic disease may occasionally give rise to a mild active sensitization manifested by positive skin reactions but not by clinical symptoms.

The fact that five of twelve grass hay fever patients had satisfactory relief by pollen injections without developing demonstrable blocking antibody might be taken as evidence that some other factor was involved in protection, but in view of the small number of cases involved, this cannot be considered a significant contribution to the controversy concerning the role of blocking antibody in the clinical response to injection treatment of allergic disease.¹³⁻¹⁵

SUMMARY

1. Clinically sensitive grass hay fever patients, treated with either timothy, June, or orchard grass, developed adequate clinical protection.
2. Blocking antibody was demonstrated against all three grasses, irrespective of the grass pollen used in treatment, in seven out of twelve cases.
3. The degree of blocking demonstrated was not constant for all three grasses and did not show any constant pattern with any one antigen. This tends to confirm the existence of a close relationship between the grass pollen antigens.
4. Blocking antibody was developed in a normal person after injections of massive doses of grass pollen extract. The blocking occurred against related grasses, as well as the one used for stimulation of the antibodies. The skin test remained negative.
5. In patients with other forms of allergy and previously showing negative skin tests to grass pollens, a positive skin test was induced by pollen injections without the development of any clinical symptoms. Blocking antibody was induced concomitantly.

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