

Induction of immediate and delayed skin reactivity with emulsion of purified ragweed antigen

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The induction of immediate or delayed skin reactivity in man following the injection of an emulsified whole ragweed pollen extract raised the question of the dependence of this activity on a nonantigenic component as an adjuvant and the possibility that a particular component might be responsible for a particular type of reactivity. Although we could not investigate all fractions of ragweed pollen, the availability of ragweed antigen E of King made possible a comparison of it with the extract of whole short ragweed pollen. Thirty-one nonatopic subjects were injected with an emulsion of 10,000 pollen units of the crude extract of short ragweed pollen, while 32 were given an injection of an emulsion of ragweed antigen E prepared from the same batch of pollen, the dose being comparable to the amount of E present in the crude extract. At approximately weekly intervals, intradermal tests were made with 0.05 ml. of 1:1,000 whole pollen extract and equivalent concentrations of antigen E. Several subjects who had immediate and delayed reactions were subjected to skin titrations with whole extract and antigen E, using serial dilutions of the material. The results indicate that emulsified antigen E is capable of inducing immediate or delayed sensitivity, and that the incidence and degree of this reactivity are not significantly different from that produced by whole extract emulsions. Antigen E and whole ragweed extract are equally capable of eliciting either an immediate or delayed reaction, regardless of the preparation employed in the emulsion. Exceptions to this generalization occur in individuals, and this may depend on other antigens than E in the crude preparation and/or trace heterologous antigens in the preparation of antigen E.

We have demonstrated previously that immediate or delayed hypersensitivity to ragweed pollen and similar antigens may be produced in man by the

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injection of antigen emulsified in incomplete Freund's adjuvant.^{1, 2} The immediate sensitivity manifests itself in immediate whealing skin reactions and in passive local transfer with the serum.³ The delayed type is evidenced by a delayed maculopapular skin reaction, transfer of this reactivity by peripheral blood leukocytes,^{2, 4} a tissue response in biopsied skin sections compatible with those found in delayed reactivity, and, after the nose is packed with the antigen, a nasal edema delayed in onset and showing a tissue reaction typical of the delayed type.⁵ This production of hypersensitivity has not been demonstrated with the injection of aqueous pollen extracts.

In connection with the antigen inducing and giving the skin reaction, several questions are at issue. The induction of hypersensitivity by the whole ragweed extract might be dependent on the presence of an antigenic substance other than that found in a purified fraction, such as ragweed antigen E.⁶ It might also depend on a nonantigenic component acting as an adjuvant. The immediate and delayed sensitivities might be due to different components of the whole extract. We felt that the answers to these considerations might give us further information on some basic questions in the mechanism of induced allergy, on the use of emulsions of antigens in the treatment of allergy, and on the use of antigen E in the treatment of allergy.

METHODS

Volunteers for these investigations were selected from inmates at Illinois State Penitentiary, Joliet, Illinois, on the basis of absence of a personal and family history of atopic disease and a negative intradermal skin test to whole short ragweed extract, 1:1,000.

Initially 28 subjects were injected. Fourteen were given an intramuscular injection into the arm of extract of short ragweed, prepared by us from ragweed pollen furnished by the Committee on Standardization of the Division of Biologic Standards of the Food and Drug Administration; the extract contained 10,000 pollen units (6,500 protein nitrogen units) emulsified in 1 part specially treated Arlacel A, 9 parts Drakeol 6-VR, and 5 parts aqueous extract to a total of 1 ml. by passing the mixture manually from one syringe to another through a double-hub needle. The other 14 subjects were given purified ragweed antigen E similarly emulsified and obtained from the same batch of short ragweed. The delay in injecting the latter group was due to the fact that antigen E was unavailable at the time the first group was injected. The dose of antigen E was 22 μ g, which was the amount of this antigen considered to be present in the quantity of extract of whole short ragweed used in the first group, as determined by the Committee on Standardization.

Skin tests were done at approximately weekly intervals, with short ragweed extract intradermally; immediate reactions and delayed reactions in 24 hours were observed. Only whole extract of short ragweed was used for testing at this time, because antigen E was not available during the first few weeks. When antigen E became available, selected reactive subjects were injected with several dilutions of antigen E and whole short ragweed and observed for both delayed and immediate reactions.

To make the series more statistically significant, an additional group of subjects was injected later and tested similarly, with the exception that all of them were tested both with whole short ragweed extract and with antigen E. Seventeen additional subjects were given emulsions of whole short ragweed and 18 were given antigen E, the aqueous preparations of these being supplied by the Committee on Standardization.

RESULTS

Reactions

The results of intradermal tests with 0.05 ml. of 1:1,000 extract are shown in Table I. Of the 31 subjects injected with whole short ragweed extract in emulsion, 14 were tested with the whole ragweed extract only. Three of these gave immediate reactions and 4, delayed. The group of 17 tested with both preparations showed 6 immediate and 3 delayed reactions to both. Of the 32 subjects injected with emulsified antigen E, 14 were tested with short ragweed only. Three gave immediate reactions and 4, delayed. Of the 18 tested with both preparations, whole ragweed extract showed 4 immediate reactions and 6 delayed, while antigen E showed 3 immediate and 5 delayed.

Titration

Several of the reactive subjects were compared by intradermal threefold serial tests with whole extract and with antigen E. The usual dilutions employed were 1:1,000 to 1:100,000; where indicated, 1:300 was also used. The end point varied from 1:1,000 to 1:100,000. A two or more place difference was regarded as significant. The results are shown in Table II. Of those individuals whose sensitivity was induced with emulsions of whole ragweed extract, 5 gave immediate reactions of approximately equal end point to whole ragweed and antigen E, and one reacted to whole ragweed extract 1:3,000 but not to antigen E 1:1,000. Of 4 subjects sensitized with whole ragweed, delayed reactions were equal in 1, were better to whole ragweed in 2, and in 1 a reaction was present to whole ragweed extract 1:3,000 but not to antigen E 1:300.

Of those sensitized with antigen E, 6 had immediate reactions. Two subjects reacted equally to both preparations, 1 reacted better to antigen E, 1 reacted to antigen E 1:1,000 but not to whole ragweed extract 1:1,000, and 2 reacted to whole ragweed but not to antigen E. Of the latter 2, one reacted to whole ragweed extract 1:1,000 and the other to 1:30,000; neither reacted to antigen E 1:300. Of the 6 individuals with titrated delayed reactions, 1 showed equal reactions to both preparations, 1 showed a better reaction to whole ragweed extract, 2 showed better reactions to antigen E, and 2 reacted to whole ragweed extract only (one to 1:3,000 and the other to 1:1,000; neither of them reacted to antigen E 1:300).

DISCUSSION

From the above findings some impressions emerge. First, it is obvious that emulsified antigen E is capable of producing skin sensitivity of the immediate and delayed type similar to that obtained with emulsified whole ragweed extract.

Table I. *Results of intradermal tests after injections of ragweed emulsion*

		<i>Tested with whole ragweed extract</i>		<i>Tested with antigen E</i>	
		<i>Immediate</i>	<i>Delayed</i>	<i>Immediate</i>	<i>Delayed</i>
Induced with whole ragweed extract	14	3	4	not tested	
	17	6	3	6	3
Induced with antigen E	14	3	4	not tested	
	18	4	6	3	5

Table II. *Comparison of titrations by intradermal tests with threefold serial dilutions of ragweed after skin sensitivity was induced with a ragweed emulsion*

<i>Reactions</i>	<i>Induced with whole ragweed extract</i>		<i>Induced with antigen E</i>	
	<i>Immediate</i>	<i>Delayed</i>	<i>Immediate</i>	<i>Delayed</i>
Antigen E only			1	
Antigen E better			1	2
Both equal	5	1	2	1
Whole ragweed extract better		2		1
Whole ragweed extract only	1	1	2	2
Total	6	4	6	6

The findings suggest further that in some subjects antigens other than antigen E, present in whole short ragweed, may either play a part in the production of hypersensitivity or may be the only antigens concerned with this production. On the other hand, the findings also suggest that antigen E may be somewhat altered in its preparation so that it may act differently in some subjects than the same fraction of the antigen in the mixture of whole ragweed. One might also speculate that whole extract may contain an inhibiting substance. We must also consider the fact that occasionally the small amount of antigen contaminant present in the purified antigen E may act as a major antigen, so that these subjects react to the whole short ragweed but not to antigen E. Antigen E appears to be as capable as whole short ragweed of producing delayed, as well as immediate, reactions. It is suggested, but not concluded, that the factor which determines whether a delayed or immediate reactivity will be induced or be shown on skin test is based on considerations (dosages, subject, and so forth) other than the particular antigenic components studied.

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