

DELAYED AND IMMEDIATE SKIN REACTIVITY IN MAN AFTER THE INJECTION OF ANTIGEN IN EMULSION

Cell Transfer of the Delayed Sensitivity

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IN 1959 in the course of investigations on absorption and immunologic aspects of ragweed extract injected in Arlcel A-Drakeol emulsions it was found¹ that five out of six nonatopic subjects developed a delayed skin reactivity to ragweed antigen. The subject who had the strongest skin reactivity had marked inflammation of his arm at the site of the repository injection which later fluctuated and drained as a sterile abscess. In an effort to confirm these findings and to extend these observations, fifteen nonatopic volunteer subjects from a penal institution were selected for further study in 1960.

PROCEDURE

The subjects selected gave a negative personal and family history of atopic manifestations, as well as negative intradermal tests to mixed giant and short ragweed 1:1,000 (1,000 pollen units or 390 protein nitrogen units per milliliter). On July 26, 1960, each subject received an intramuscular injection of 0.5 ml. in each arm of an emulsion containing a mixture of 10,000 pollen units of giant and short ragweed extracts (3,900 protein nitrogen units per milliliter of emulsion). The emulsion mixture was prepared by adding 1 part of an equal mixture of giant and short ragweed extract containing 30,000 pollen units to 2 parts of an Arlcel A-Drakeol solution (1:9). The mixture was emulsified by passing through a double hub 18-gauge needle attached to two 10 ml. Luer-Lok syringes. The ragweed extract used in the emulsion was extracted with Coca-merthiolate solution and was the same as that used for the preliminary and subsequent skin tests, and supplied for the nationwide collaborative ragweed repository study by the Committee on Specific Therapy of the American Academy of Allergy.²

Beginning four weeks after the emulsion injection, intradermal tests were made with pollen extracts containing 10, 100, and 1,000 pollen units per milliliter. These tests were repeated at intervals for several months. Thirteen out

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Received for publication Feb. 16, 1961.

of the fifteen subjects showed a skin reactivity on the first test. In nine, the induced reactivity was of the delayed type and consisted of a papule with erythema beginning six to twenty-four hours after the injection, persisting to some extent for days and which, in the more extreme instance, resulted in vesiculation and a superficial necrotic lesion (Fig. 1). The specificity of the reactions was indicated by the inability of this ragweed preparation to react in other nonallergic individuals. In seven, the reaction was of the immediate wheal and erythema type, associated with itching and disappearance in one to several hours. Three of the nine delayed reactors had this immediate type reaction.

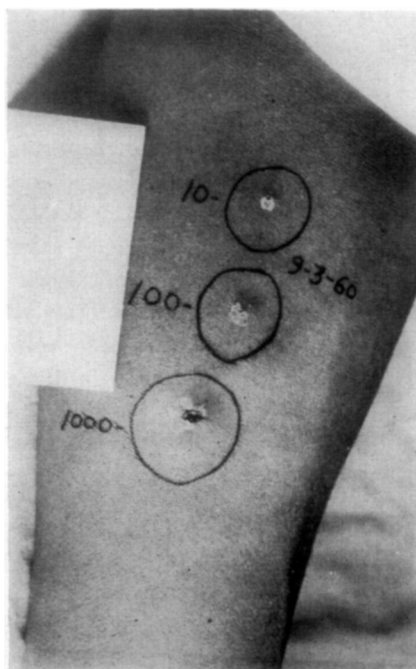


Fig. 1.—Vesiculation and superficial necrotic lesions in the Subject J. O. twenty-four hours after an intradermal injection of ragweed.

At the time of this writing, six months after the repository injection and five months after the original testing, one subject changed from an immediate into a delayed type reactor, one added a delayed reactivity to his immediate type, and a third added an immediate reactivity to his delayed type. Two of the original nonatopic individuals who received ragweed emulsion and did not develop delayed or immediate sensitivity were given a cell transfer and converted to a positive delayed reaction when tested six days later.*

Those subjects in whom a delayed hypersensitivity type of skin test was found had been observed to develop swollen and tender arms at the site of the repository injection, beginning on the average about ten days after the injection. Three of these developed distinct tense masses at the sites of injection and one

*One of the nonatopic subjects of the series described a year ago, who had developed a strong delayed skin reactivity to ragweed and a sterile abscess after a repository injection, was retested eighteen months after his initial sensitivity, and still showed a marked skin reactivity to ragweed 1:10,000.

appeared to be fluctuating, but did not drain. The latter subject complained of malaise and weakness for eight weeks after the injection and had limitation of movement of his arms for several weeks. The muscle swelling and limitation in motion slowly decreased and by the end of seven months only a firm 3 cm. mass remains. None of the subjects developed clinical signs of symptoms referable to pollenosis during the ragweed season.

Conjunctival tests were made with a small speck of whole ragweed pollen, using other pollen as a control test in the other eye and in the eyes of non-allergic individuals. Of the six subjects with immediate skin reactivity in whom conjunctival pollen tests were done, three gave a typically positive reaction. Passive transfer tests were done with the sera of six subjects with immediate type skin reactivity. Only one gave a definitely positive PK reaction.

At the same penal institution, twenty-seven patients with ragweed hay fever and with positive skin tests to ragweed extract received repository ragweed therapy in doses varying from 100 to 10,000 pollen units. None of these developed a delayed skin reaction to pollen extract and none of them experienced a delayed inflammation at the site of the repository injection. Eleven patients received the emulsion without the ragweed extract as part of a double-blind therapy study. None of these developed any type of delayed inflammatory response.

In the fifteen experimental subjects, the post-treatment sera failed to show precipitin reactions with ragweed extracts on gel diffusion, and were negative in attempts to produce passive cutaneous anaphylaxis in the guinea pig. Tanned cell hemagglutination studies* on the sera showed the following: The subject (J. O.), previously described as having had the most exquisite delayed hypersensitivity, showed a titer of 1:2,560. Twelve had titers ranging from 1:20 to 1:80. Two were negative, as were control sera. Aside from the one who had the very high titer, there was no obvious relationship between the titer, or absence or presence of hemagglutination, and the absence or presence of immediate or delayed skin reactivity.

The findings just described are summarized in Table I.

CELL TRANSFER

As shown by Landsteiner³ and Chase⁴ in animals and Lawrence and his associates⁵⁻⁸ in man, delayed hypersensitivity of certain types can be transferred by leukocytes either obtained from inflammatory exudates or from peripheral blood. Because the genesis of the type of delayed appearing hypersensitivity described by us was different from the conventional (microbial), it was important to determine whether it possessed the same characteristic of cell transfer. Each of three subjects with delayed hypersensitivity to ragweed contributed 250 ml. of blood. The white cells were harvested according to a modified method of Gold and Cole,⁹ using polyvinylpyrrolidone and heparin. The washed leukocytes were injected intradermally and subcutaneously into 2 sites.

Tests were made at the sites of the intradermal transfer and at distant sites with ragweed extract six days after the transfer. There was no immediate

*Done by Dr. Carl E. Arbesman, Buffalo, N. Y.

TABLE I. RESULTS OF INJECTION OF 10,000 POLLEN UNITS OF RAGWEED EMULSION INTO NONATOPIC SUBJECTS ON JULY 26, 1960

SUBJECT	FIRST POST-REPOSITORY TESTS (AUG. 26, 1960)				FINAL TESTS (JAN. 18, 1961)		INTRADERMAL TESTS WITH RAGWEED 1,000 μ /ML. AFTER SERUM TRANSFER
	DELAYED WEAKEST DILUTION POSI- TIVE POLLEN (μ /ML.)	IMMEDIATE WEAKEST DILUTION POSI- TIVE POLLEN (μ /ML.)	CONJUNCTIVAL TEST WITH WHOLE POLLEN (OCT. 18, 1960)	HEMAGGLU- TINATION TITER	DELAYED WEAKEST DILUTION POSI- TIVE POLLEN (μ /ML.)	IMMEDIATE WEAKEST DILUTION POSI- TIVE POLLEN (μ /ML.)	
1. H. F.	Neg.	1,000	Neg.	1:20	1,000	1,000	Neg.
2. R. K.	Neg.	1,000	Neg.	1:80	1,000	Neg.	Neg.
3. C. B.	Neg.	1,000	Neg.	1:80	—	—	Pos.
4. J. A.	10	1,000	Pos.	1:40	1,000	100	—
5. S. V.	1,000	100	Pos.	0	1,000	1,000	Neg.
6. J. O.	10	Neg.	—	1:2,560	10	100	Neg.
7. S. D.	Neg.	1,000	Pos.	1:80	Neg.	1,000	Neg.
8. R. H.	1,000	1,000	—	1:80	—	—	Neg.
9. L. B.	1,000	Neg.	—	1:80	1,000	Neg.	—
10. J. H.	1,000	Neg.	—	0	1,000	Neg.	—
11. D. M.	1,000	Neg.	—	1:40	1,000	Neg.	—
12. H. K.	1,000	Neg.	—	1:80	1,000	Neg.	—
13. G. W.	1,000	Neg.	—	1:40	1,000	Neg.	—
14. C. Mc.	Neg.	Neg.	—	1:80	1,000*	1,000	—
15. F. Z.	Neg.	Neg.	—	1:20	1,000†	Neg.	—

*WBC transfer from J. O. with conversion of skin test local and general when tested six days later.

†WBC transfer from S. V. with conversion of skin test local and general when tested six days later.

reaction, but beginning about six hours later a delayed type skin reaction occurred which was comparable in appearance to that previously obtained in the donor. Four positive cell transfers have been made from the three donors. Two of the recipients (C. Mc. and F. Z.) were in the original group of fifteen non-atopic subjects. They were tested and found to be negative prior to cell transfer, but gave a positive reaction when tested six days later. The other two recipients were nonatopic individuals and not sensitive to ragweed when tested prior to cell transfer. Transfer of the serum from the donor did not transfer delayed hypersensitivity.

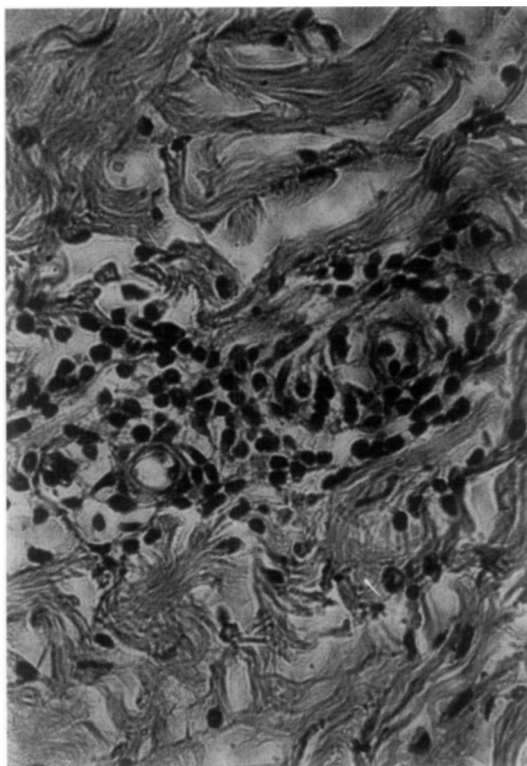


Fig. 2.—Photomicrograph of a skin punch biopsy made twenty-four hours after the intradermal injection of ragweed which shows perivascular infiltration with round cells. (January, 1961.)

A punch biopsy made twenty-four hours after the intradermal injection of the antigen at a site distant from the site of the transferred leukocytes showed a perivascular infiltration with round cells (Fig. 2). This finding is compatible with, but not diagnostic of, a delayed type of inflammatory response.

DISCUSSION

The clinical implications of the findings described here may be of importance. It is obvious that at least in nonatopic individuals, who are not allergic to ragweed, hypersensitivity is produced to ragweed antigen injected in Arlcel A-Drakeol emulsion. Thus far the immediate reacting antibodies

have been of moderate degree and unlikely to produce clinical pollenosis. Although the immediate reactivity has not disappeared after several months, neither has it increased. It is possible also that, in those who are allergic to ragweed, the injection of repository ragweed might actually increase the sensitizing antibody, although increased blocking antibody or other protective mechanisms might sufficiently neutralize and exceed the effect. If an increase in immediate reacting antibody in the amounts we have found is obtained in the allergic person, it would probably be insufficient to be titrated as a change in the already large titer of reagin. It is difficult to understand why in the series of induced delayed ragweed sensitivity last year we did not encounter any subjects who developed immediate reactivity.

The production of delayed reactivity may be of greater clinical importance. In the first place, as noted in this series and in those of a year ago, it is prone to manifest itself in severe prolonged inflammation and abscess formation at the site of injection. The development of delayed reactivity may also imply the production of a delayed type of inflammation on natural exposure to the antigen (for example, eye inflammation during the ragweed season). Thus far there has been no evidence of that. It is conceivable also, but even more highly speculative, that the repository therapy may in some way set off other types of allergic responses in viscera, perhaps unrelated to the original antigen. Until more extended investigations are made the inference should be that an antigen should not be injected in emulsion form unless the physician is certain that the patient is allergic to it. This implies the necessity for more careful appraisal of what is injected. A questionable dust reaction (which could be nonspecific), the use of mixtures of molds to some of which the patient may not be allergic, or the use of a variety of tree pollen extracts in a "tree" mixture could lead to trouble. There is reason to think that at least in some of the cases in which sterile abscess has occurred in the past the presence of a previous allergy to that antigen was questionable. Since any extract (ragweed pollen, for example) has a number of antigens and the patient may not be allergic to some of them, the likelihood of inducing new allergies must even be considered as a possibility in the average case. It is possible that the persistence of delayed sensitivity is to some extent influenced by the repeated intradermal tests.

The basic, and perhaps the practical, question is: Why does the non-allergic person develop a sensitivity from ragweed repository injection, while the person allergic to ragweed does not? Is this on a genetic or constitutional basis? Or does the lack of an immediate antigen-antibody reaction in some way favor the production of delayed sensitivity? Is this a peculiar property of the ragweed antigen? It has been also suggested that Arlacel A might be responsible while another emulsifying agent might not produce such effects. Investigations are under way in an attempt to answer these and other pertinent questions relative to this phenomenon of induced sensitivity.

This finding has also presented us with a tool for studying delayed sensitivity, the long-debated question of conversion of one type of sensitivity into the other, and a number of other aspects of sensitivity, particularly of the delayed type.

SUMMARY

In fifteen nonallergic subjects receiving ragweed in repository form, seven developed immediate skin reactivity, nine delayed reactivity, and three both. After six months all still react.

In those developing delayed skin reactivity there was an inflammatory swelling of varying degree at the site of the repository injection, beginning several days later and lasting for several weeks.

The delayed reactivity to ragweed was transferred locally and systemically by the injection of peripheral leukocytes from three subjects in whom delayed sensitivity had been induced by an injection of ragweed emulsion.

Punch biopsy from the delayed reaction produced in the recipient showed histologic changes characteristic (but not pathognomonic) for a delayed hypersensitivity response.

The findings point to the advisability of confining repository treatment to antigens to which the patient is known to be sensitive.

This investigative model offers an opportunity for basic studies on delayed hypersensitivity and the relationship between the delayed and immediate type.

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