

CELL TRANSFER OF DELAYED HYPERSENSITIVITY TO RAGWEED FROM ATOPIC SUBJECTS TREATED WITH EMULSIFIED RAGWEED EXTRACTS

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IN 1959, in the course of studying the absorption of emulsified ragweed pollen antigen, it was observed that several nonatopic subjects developed delayed skin reactivity to ragweed extract after receiving an injection of the ragweed emulsion.¹ Subsequently, it was shown in a larger group of nonatopic subjects of a prison population that immediate as well as delayed hypersensitivity could be produced by injections of such emulsions.² The skin reactivity was successfully transferred to nonatopic recipients; the immediate reactivity by means of donors' serum,³ and the delayed reactivity by means of peripheral blood leukocytes. It was noted, however, that a large number of subjects who were naturally sensitive to ragweed did not develop delayed skin reactivity after receiving ragweed emulsion therapy.

In a further study it was shown that delayed skin reactivity to another antigen, timothy, could be induced by injection of timothy emulsion in nonatopic persons,⁴ and that development of immediate sensitivity was more frequent in an atopic group, whereas development of delayed sensitivity was more prominent in the nonatopic group. Several alternative explanations were offered for the failure of ragweed-sensitive subjects to develop delayed skin reactivity. By inducing delayed skin reactivity to ragweed in timothy-sensitive persons, it was demonstrated that the atopic individual does not lack the mechanism for developing delayed skin reactivity. The influence of the inflammation resulting from histamine release or the antigen-antibody reaction was not responsible for inhibiting the induction of delayed skin reactivity. This was demonstrated by the failure to prevent delayed skin reactivity to ragweed after an injection of an emulsion of histamine plus ragweed, or timothy plus ragweed, in timothy-sensitive individuals.

The possibility still existed that even though the presence of an immedi-

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ately reacting antigen did not interfere with *inducing* delayed sensitivity, it might interfere with the delayed *skin test reaction*. A state of true delayed hypersensitivity might be present even in the absence of the delayed skin reaction. The prevention of the delayed skin reaction might be due either to nonspecific inflammation which occurs as a result of the immediate reaction or to a binding or modification of the antigen in the immediate response that precludes its participation in or its availability for the delayed reaction. If this were the case, it would be expected that the peripheral blood leukocytes of such an individual could transfer delayed hypersensitivity to a nonatopic recipient.

PROCEDURES AND RESULTS

Transfer of Delayed Skin Reactivity in Ragweed-Sensitive Subjects Treated With Emulsified Ragweed Extracts.—Human donors and recipients for this experiment were inmates of a penal institution. Donors of leukocytes were 10 spontaneously ragweed-sensitive subjects who had received an injection of emulsified ragweed extract. None demonstrated delayed skin reactivity to ragweed.

The method of leukocyte separation was a modification of that described by Gold and Cole.⁵ Sterile siliconized glassware was used. One hundred milliliters of venous blood was mixed thoroughly with 50 ml. of 1.5 per cent disodium ethylenediaminetetraacetate in 0.7 per cent NaCl. The blood and EDTA mixture was then divided into 75 ml. portions which were layered on 125 ml. of 28.5 per cent polyvinylpyrrolidone in 0.9 per cent NaCl in a 250 ml. Erlenmeyer flask. In 5 minutes, rouleaux formation of erythrocytes occurred and large clumps of erythrocytes began to sink to the bottom of the flask while the leukocytes remained above. In about 45 minutes the separation was almost complete and the entire layer above the polyvinylpyrrolidone was pipetted off and placed in 50 ml. screw-top centrifuge tubes. Centrifugation was carried out at 2,000 r.p.m. for 10 minutes. The supernatant solution was discarded and the white and red blood cellular precipitate was washed twice with a solution consisting of 25 ml. 0.9 per cent NaCl, 1 ml. 1.15 per cent KCl, 2 ml. of phosphate buffer (17.8 Gm. $\text{Na}_2\text{HPO}_4 \cdot \text{H}_2\text{O}$ plus 20 ml. N HCl diluted to 1 liter) and 0.25 ml. 0.11 M EDTA $\text{Na}_2\text{H}_2\text{O}$. The cells were washed a third time in the buffered salt solution free of EDTA and suspended by agitation.

Equal volumes of the cell suspension were injected intradermally and subcutaneously into the deltoid areas of a nonatopic recipient. Intradermal challenge with the appropriate antigen was performed 6 days later at a distal site on the forearm. Twenty-four and 48 hours after challenge the sites were examined. Reactions were considered positive if definite erythema and induration of at least 5 mm. was seen. Negative reactions showed at most only minimal erythema.

Of the 10 spontaneously ragweed-sensitive subjects who received an injection of emulsified ragweed extract and who failed to show delayed skin reactivity, 4 were capable of transferring delayed reactivity by their peripheral leukocytes. Skin biopsies were performed on the positive reactions at 24 hours;

they showed marked perivascular infiltration with mononuclear cells, consistent with the typical reaction of delayed hypersensitivity⁶ (Fig. 1).

Peripheral blood leukocytes obtained from 20 ragweed-sensitive subjects, 6 of whom had been untreated and 14 of whom had prolonged treatment with aqueous extract, failed to transfer delayed hypersensitivity. Therefore, in a certain number of naturally ragweed-sensitive subjects who had received an injection of ragweed extract in emulsion, a state of delayed hypersensitivity existed, although the delayed skin reaction was absent. The delayed reactivity was probably of a fairly marked degree, since Lawrence⁷ and others have pointed out the requirement of this degree of sensitivity for successful transfer of delayed hypersensitivity.

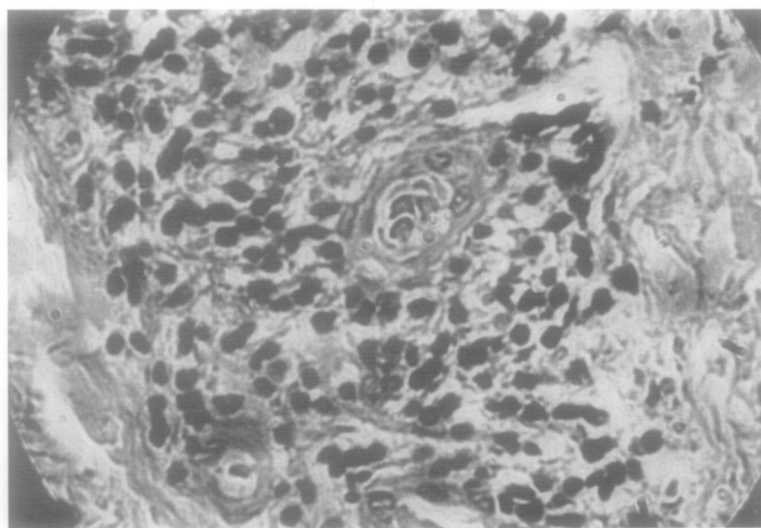


Fig. 1.—Photomicrograph of a skin biopsy, made 24 hours after intradermal injection of ragweed, shows perivascular infiltration with mononuclear cells.

Combination of Immediate and Delayed Reacting Antigens.—Six subjects were selected who had both natural immediate reactivity to ragweed and natural delayed reactivity to trichophytin. They were first tested with serial dilutions of trichophytin and then with comparable dilutions of trichophytin combined with ragweed. Tables I and II show the findings in one subject, which were representative of those in the others. It is apparent that the presence of an immediately reacting antigen does modify, but only moderately, the response to a delayed reacting antigen. Combination of trichophytin* with Compound 48/80,† a potent histamine releaser, resulted in a comparable slight diminution in the delayed response.

**Trichophyton rubrum* was grown in our laboratory in submerged culture on a shaking machine. The mold was drained, dried, and extracted with 5 per cent glycerosaline.

†Supplied by Burroughs Wellcome and Co., Lot 31862, Tuckahoe, N. Y.

The Effect of Sensitizing Skin With Ragweed Reagin on the Demonstration of Delayed Hypersensitivity to Ragweed.—Three nonatopic subjects were selected in whom a definite delayed skin reaction to ragweed had been induced. Potent ragweed reaginic serum was injected intradermally in the forearm. One day later, 0.02 ml. of 1:1,000 ragweed extract was injected in this same site and an adjacent site. Twenty-four hours later the two sites were examined. Ragweed extract injected in the untreated site showed definite erythema and induration, ranging from 10 to 25 mm. in diameter. The site previously injected with ragweed reaginic serum and then later with ragweed extract showed only minimal erythema (Fig. 2). Injection of sites with normal serum produced no effect on the delayed reaction to ragweed.

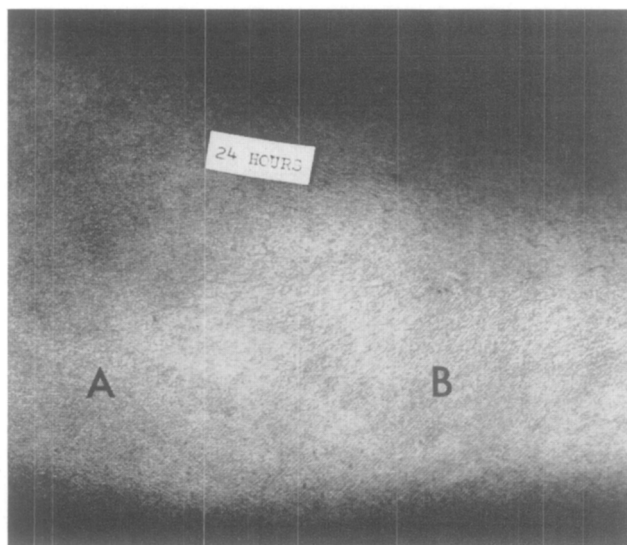


Fig. 2.—Site A, injected with ragweed extract, shows marked erythema and induration at 24 hours. Site B, previously injected with ragweed reaginic serum and then later with ragweed extract, shows only minimal erythema.

DISCUSSION

Our findings suggest some change in the antigen resulting from the immediate antigen-antibody reaction as an explanation for the failure to obtain a delayed skin reaction in spontaneously ragweed-sensitive subjects who had received ragweed emulsion. If the antigen is actually consumed and removed then the method of removal is not ascertained. While precipitating antibody and antigen complexes have been shown to be phagocytized and rapidly catabolized,^{8, 9, 10} the fate of nonprecipitating antibody and antigen complexes is not as well defined.

In addition to actual removal of the antigen, one might postulate an alteration in the antigen resulting from its combination with reagin. It has been suggested^{11, 12} that the immunological specificity of the delayed type of hyper-

TABLE I. EFFECT OF IMMEDIATE REACTIONS FROM VARYING CONCENTRATIONS OF RAGWEED EXTRACT ON THE DELAYED REACTIVITY TO 1:1,000 TRICHOPHYTIN*

CONCENTRATION OF RAGWEED EXTRACT ADDED TO TRICHOPHYTIN	DEGREE OF SKIN REACTION TO TRICHOPHYTIN AT 48 HOURS
None	+++
1:10,000,000	+++
1:1,000,000	+++
1:100,000	+++
1:10,000	++
1:1,000	+

**Legend:* 1+ = 5-10 mm. erythema and induration; 2+ = 10-15 mm. erythema and induration; 3+ = 15-20 mm. erythema and induration.

TABLE II. EFFECT OF AN IMMEDIATE REACTION FROM STANDARD CONCENTRATION OF RAGWEED EXTRACT ON THE DELAYED REACTIVITY TO VARYING CONCENTRATIONS OF TRICHOPHYTIN*

REACTIONS FROM TRICHOPHYTIN ALONE	REACTIONS FROM TRICHOPHYTIN AFTER ADDITION OF 1:10,000 RAGWEED EXTRACT
1:1,000 +++	++
1:3,000 +++	++
1:9,000 ++	+
1:27,000 +	±
1:81,000 ±	0

**Legend:* 1+ = 5-10 mm. erythema and induration; 2+ = 10-15 mm. erythema and induration; 3+ = 15-20 mm. erythema and induration.

sensitivity reaction involves larger areas of the antigenic molecule, in contrast to the smaller specificity requirement of the immediate type of hypersensitivity reaction. Altering some area of the antigenic molecule by complexing with reagin may be sufficient to change the immunological specificity for the delayed reaction.

It must be admitted that we are not totally satisfied with the above explanations. It cannot be overlooked that there are instances of both immediate and delayed sensitivity occurring in the same individual to the same crude antigen in such spontaneous sensitivities as insects and molds. It is possible that in such instances the antigenic component responsible for each type of reaction is different. It is possible also that there is a difference in the mechanism of the spontaneous and induced sensitivities.

SUMMARY

1. Four of 10 spontaneously ragweed-sensitive subjects who received an injection of emulsified ragweed extract and who failed to show delayed skin reactivity were capable of transferring delayed reactivity by their peripheral blood leukocytes.

2. Twenty ragweed-sensitive subjects, 6 of whom had never been treated and 14 who had had long-term conventional treatment with aqueous ragweed, failed to show a cellular transfer.

3. Nonspecific inflammation from a wheal and erythema reaction due to Compound 48/80 or an unrelated immediate antigen-antibody reaction resulted in only moderate diminution in the delayed response to a delayed reacting antigen.

4. Skin tests in nonatopic persons possessing delayed skin reactivity to ragweed, who were passively sensitized with ragweed reaginic serum and subsequently injected with ragweed antigen, failed completely to give a delayed reaction.

5. Although the findings suggest a mechanism of consumption or modification of the antigen by the immediate antigen-antibody reaction, thus removing its availability for the production of a delayed reaction, other observations suggest that such a mechanism may not be present under all circumstances.

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