

Transfer of immediate hypersensitivity by lymphocytes from blood of ragweed-sensitive patients

Samuel E. Rynes, M.D., Charles F. Milon, M.D., and
Howard C. Leopold, M.D., Philadelphia, Pa.

Studies were made to determine whether intradermal implants of viable lymphocytes would transfer the immediate type of hypersensitivity to ragweed. Suspensions of lymphocytes were prepared from tuberculin-positive ragweed hay fever patients by drawing 150 ml. of blood. Yields of approximately 1 ml. containing about 90,000 to 100,000 lymphocytes per cubic millimeter were obtained. Lymphocytic cellular-transfer sites were established in 6 nonallergic tuberculin-negative recipients by injecting, intradermally, 0.1 ml. of the lymphocyte suspension within 5 hours. Control sites of 0.1 ml. of the last saline washings obtained in preparing the lymphocyte suspension and P-K transfer sites with the donors' sera were also injected. Lymphocyte cell, P-K, and control sites were tested with ragweed and read within 15 minutes for immediate reactions. A lymphocyte cell-transfer site and a remote skin area were tested with PPD and read in 48 hours. Immediate whealing reactions to ragweed at the lymphocyte cell sites were shown in 3 of 6 recipients; in 2, the reaction was equivocal and in one, negative. Positive tuberculin reactions developed at the lymphocyte cell sites in 3 of 6 recipients, and 2 of these 3 also reacted to tuberculin at remote areas. These studies indicate that the immediate type of hypersensitivity may be transferred by intradermal lymphocyte cell transfer, as well as the delayed type.

The transfer of immediate type sensitivity by leukocytes, with utilization of human donors and recipients, has been studied by Walzer and his co-workers¹⁻⁴ and by Freedman, Fisher, and Cooke⁵ with contradictory results. Walzer and

From the Allergy Division, Department of Medicine, Jefferson Medical College and Hospital, Philadelphia, Pa.

Supported by Grant GSR27 from the National Institutes of Health.

Read at the Twenty-second Annual Meeting of the American Academy of Allergy, New York, N. Y., Feb. 23, 1966.

Received for publication March 28, 1966.

his co-workers reported the transfer of small positive immediate type reactions to ragweed pollen extract when suspensions of both viable leukocytes and extracts of fragmented white blood cells were used to transfer sensitivity.^{1, 2} Subsequently, they noted the transfer of immediate reactions to timothy pollen at leukocytic transfer sites and related the presence or absence of positive whealing reactions at these sites to the clinical immunologic status of the donor.³ Walzer and Bowman were also able to demonstrate the transfer of the immediate type of sensitivity by the use of leukocytes from a human donor who had been artificially sensitized to *Ascaris lumbricoides* worms without the transfer of a reaction to the serum of the donor.⁴ Freedman, Fisher, and Cooke⁵ did not obtain immediate transfer of ragweed reactions at sites prepared with leukocytes disrupted mechanically, or by freezing and thawing, or with viable leukocytes. In their study, leukocytic suspensions or extracts from patients sensitive to penicillin and horse serum failed to produce positive immediate reactions to these substances.⁵ The transfer of delayed hypersensitivity by lymphocytes from nonatopic human subjects sensitized by water-in-oil emulsions of ragweed or timothy antigen was reported by Slavin, Garvin, and Feinberg.⁶ More recently, tissue culture studies of lymphocytes from allergic patients by Rose and his co-workers⁷ have shown evidence of the presence of an antibody in the tissue culture fluid capable of passively sensitizing monkey ileum to ragweed antigen.

The present study was made in an effort to determine whether intradermal implants of viable lymphocytes, which usually mediate the delayed type of sensitivity,^{8, 9} would also transfer the immediate type of hypersensitivity to local skin areas.

METHOD

Lymphocytes were obtained from 3 tuberculin-positive ragweed-sensitive donors who exhibited pronounced hay fever symptoms. The technique of differential sedimentation described by Brent and Medawar¹⁰ was followed in preparing the lymphocytic suspensions. Approximately 150 ml. of venous blood was drawn, divided into 3 large sterile tubes, and defibrinated mechanically by twirling the blood with a glass rod bent into zig-zag angles. After defibrination, an amount of 3 per cent dextran (clinical 100—molecular weight 124,000) in physiologic salt solution, equal to the volume of the defibrinated blood, was added to each tube. The tubes were then incubated at 37° C. for about 90 minutes, until the mixture separated into a supernatant layer containing most of the lymphocytes and a compact sediment, chiefly composed of red blood cells. The supernatant was pipetted off and the first residue, which contained the red blood cells, discarded. The supernatant was then centrifuged for 10 minutes at 1,500 r.p.m. at room temperature, leaving a residue chiefly of lymphocytes but with some red blood cells still present. After this centrifugation, the supernatant was removed and the sediment, rich in lymphocytes, was resuspended with several milliliters of the supernatant. This was again incubated at 37° C. for an hour to allow excess red blood cells to settle to the bottom. The supernatant, containing lymphocytes, was pipetted off and was again centrifuged at 1,500 r.p.m. for 10 minutes, leaving a sediment of highly concentrated lymphocytes.

In order to remove any adherent serum reagin, these cells were washed with 40 ml. of sterile normal saline and the tube was centrifuged, again discarding the supernatant. The sediment was now washed a second time by adding another 40 ml. of normal saline, and this was then centrifuged. Removal of the second 40 ml. of saline (part of which was preserved for control tests) left a residue of approximately 1 ml. of packed cells, predominantly lymphocytes. In the process of mechanical defibrination and during the course of incubation, a large proportion of the polymorphonuclear leukocytes was removed by adherence to the glass rods and to the walls of the glass tubes, and in the sediments containing the red blood cells. Viability of the lymphocytes in the final residue of packed cells was ascertained by studying wet slide preparations of the cells stained with 0.2 per cent trypan blue (4 parts diluted with 1 part concentrated, 4.25 per cent saline) according to the technique described by Boyse, Old, and Thomas.¹¹ At least 90 per cent of the lymphocytes were found to be viable by this method.

In all, four such suspensions were prepared from 3 different donors. Cell counts, using Wright stain, showed the lymphocytic content to be as follows:

Donor V. S.—total white blood cells = 155,000 per cubic millimeter, lymphocytes 58 per cent or 89,900 per cubic millimeter.

Donor J. Z.—total white blood cells = 178,000 per cubic millimeter, lymphocytes 60 per cent or 106,800 per cubic millimeter.

Donor M. V. (I)—total white blood cells = 177,000 per cubic millimeter, lymphocytes 62 per cent or 109,740 per cubic millimeter.

Donor M. V. (II)—total white blood cells = 182,000 per cubic millimeter, lymphocytes 55 per cent or 100,100 per cubic millimeter.

These cell suspensions definitely contained a much larger percentage of lymphocytes than the normal lymphocyte cell distribution in blood. Although it is unlikely, this study does not completely exclude the possibility that other cells in the white blood series might have participated in the reactions.

The recipients for the passive transfer study consisted of 6 healthy non-allergic young men who were previously tuberculin negative when tested with tuberculin, purified protein derivative, second test strength (PPD, Parke-Davis). Lymphocytic cellular transfer sites were established by injecting, intradermally, 0.1 ml. of the lymphocytic cell suspension within 5 hours of the time the blood was drawn from the donors. The number of lymphocytes injected into each of the cellular transfer sites was approximately as follows:

Recipient M. Z. (from Donor V. S.) 8,900,000 cells

Recipient E. N. (from Donor J. Z.) 10,600,000 cells

Recipient K. H. (from Donor J. Z.) 10,600,000 cells

Recipient R. T. (from Donor M. V.—I) 10,900,000 cells

Recipient R. G. (from Donor M. V.—II) 10,000,000 cells

Recipient J. P. (from Donor M. V.—II) 10,000,000 cells.

To rule out the possibility of reactions from serum reagin adherent to the cells, a control site of 0.1 ml. of the last saline washings obtained in preparing the suspensions was also injected. Ordinary P-K transfer sites with serum from the donors were prepared in the opposite arm of the recipients. These pre-

liminary P-K transfer tests were made before the preparation and injection of the cell suspensions. The sera gave positive passive transfer reactions on testing with an extract of equal parts of low and giant ragweed in dilutions of 100 or 200 protein nitrogen units (PNU) per milliliter up to concentrations of 10,000 PNU per milliliter. This extract was prepared and standardized in the allergy laboratory of the Jefferson Medical College Hospital. The serum sites for P-K transfer were tested with ragweed pollen extract to insure that the donors possessed circulating antibodies (reagin).

The transfer cell sites were considered ready for testing as soon as the induration from the injection of the cell suspension subsided. This required from 3 to 7 days, but 9 days was necessary in one recipient. Cell-transfer sites in all 6 recipients were tested with the same extract of ragweed pollen in concentrations of 5,000, 10,000, or 34,000 PNU per milliliter. Other cell-transfer sites were tested with PPD, second test strength. The control sites with the final saline washings were tested with ragweed pollen extract (10,000 PNU per milliliter).

Control tests for the cellular and serum transfer sites were made with corresponding concentrations of pollen extract in normal nonsensitized skin areas of the recipients. Additional control tests in the cellular sites and adjacent normal skin areas were made with buffered saline solution. In 3 recipients, remote normal skin areas (on the opposite arm) were also tested with PPD, second test strength. The volume of pollen extract injected for testing in all areas was 0.01 ml., intradermally, and the volume of PPD injected was 0.1 ml., intradermally.

RESULTS

The tests with ragweed extract were read in 15 minutes by recording the size of the wheal produced. No test was considered positive unless the size of the wheal in the lymphocyte-cell site significantly exceeded that produced in the control tests made in normal skin and the sites containing the final saline washings. In 3 recipients, definite wheals were observed at the site of the cell transfer when these were tested with ragweed pollen extract. In these cell sites, the excess of the diameter of the wheal over that produced in normal skin control sites ranged from 3 to 7 mm. In 2 of these 3 recipients, the control tests made at the final saline-washings sites showed no variation from the normal skin and the other showed a wheal only 1 mm. larger than the normal skin. These findings would seem to rule out the possibility that adhered serum reagin might be responsible for the wheals obtained at the lymphocyte-cell sites.

Two of the positive ragweed reactions were found at the cell sites from the blood of one donor, M. V., who furnished blood twice. In the first trial, one recipient, R. T., was tested and developed a small wheal (3 mm.) at a cell site tested with ragweed 10,000 PNU per milliliter. In the second experiment with cells derived from the blood of the same donor, M. V., 2 recipients were tested with ragweed. In one of these, R. G., a positive reaction to ragweed 34,000 PNU per milliliter with a wheal of 5 mm. was shown, although a cell site tested with ragweed 10,000 PNU per milliliter had reacted negatively (Fig. 1). The second recipient, J. P., failed to show a positive response to ragweed at the cell site even with the 34,000 PNU per milliliter ragweed testing strength. The cells

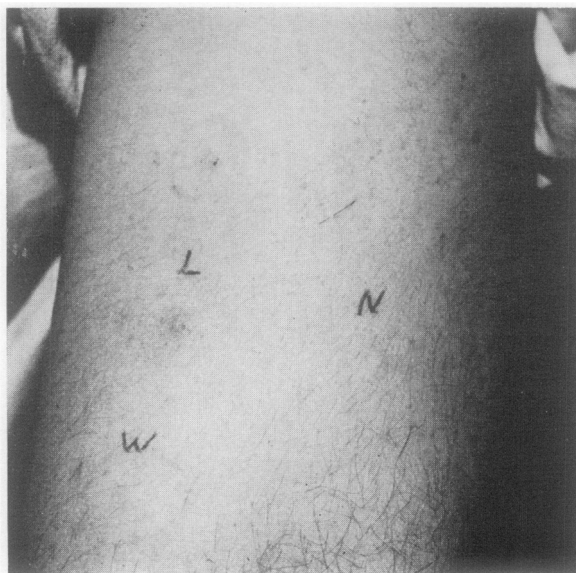


Fig. 1

Development of wheal within 15 minutes at the lymphocyte cell-transfer site (donor M. V. [II], recipient R. G.) by challenge with ragweed extract, 34,000 PNU per cubic centimeter is shown under *L*. Negative response to same challenge resulted at control sites with final washings under *W* and in normal skin under *N*.

from another donor, J. Z., were tested with ragweed 10,000 PNU per milliliter in 2 recipients with a positive 7 mm. reaction appearing in one, K. H., and an equivocal reaction of 1.5 mm. in the other, E. N. A cell site from the third donor, V. S., was tested in one recipient, M. Z., and showed only an equivocal reaction of 1 mm.

Positive transfer reactions of the immediate wheal type to ragweed were, therefore, shown to the cells from 2 donors in 3 recipients.

Positive tuberculin reactions appeared at the cell-transfer sites in 3 recipients and these ranged in size from 6 to 15 mm. Two of these reactions were shown to the cells of donor M. V. In one recipient, R. T., sensitized with M. V. cells, positive reactions to both ragweed and tuberculin developed at sensitized cell sites. The second recipient, R. G., sensitized with M. V. cells reacted to ragweed but not to tuberculin, while the third recipient, J. P., showed no reaction to ragweed but did develop a tuberculin reaction. The remaining positive tuberculin reaction appeared in recipient E. N., who had shown only an equivocal reaction to ragweed with the cells of donor J. Z. In the recipient of J. Z. cells who had reacted to ragweed, K. H., no tuberculin reaction was obtained.

In 2 of the 3 recipients who reacted to tuberculin at the cell-transfer sites, additional tuberculin tests were made in the normal skin of the opposite arm and both showed positive tuberculin reactions, 11 and 12 mm. in diameter, in these remote areas (Fig. 2). This would indicate that the localized intradermal transfer of lymphocytes produced a generalized delayed hypersensitivity to tuberculin in these recipients.

The P-K tests made with serum from all 3 donors were positive in all 6 recipients, indicating that reagins to ragweed were present in all donors and that the recipients were capable of being sensitized to reagin.

The results of these experiments are summarized in the accompanying tables (Tables I and II).

DISCUSSION

These findings indicate that it may be possible to transfer the immediate whealing type of sensitivity into normal skin areas passively sensitized by the intradermal injection of cellular suspensions containing a high percentage of

Table I. *Serum passive transfer (P-K) tests*

Donor	Recipient	Serum sites			Normal skin control (diameter)
		2Rs* 100 PNU (diameter)†	2Rs 200 PNU (diameter)†	2Rs 10,000 PNU (diameter)	
V. S.	M. Z.	NT‡	NT	4 mm.	1 mm.
J. Z.	E. N.	NT	NT	12 mm.	0
J. Z.	K. H.	NT	NT	10 mm.	0
M. V. (I)	R. T.	NT	NT	2 mm.	0
M. V. (II)	R. G.	NT	8 mm.	10 mm.	2 mm.
M. V. (II)	J. P.	8 mm.	NT	12 mm.	0

*2Rs, extract of equal parts of low and giant ragweed prepared in the Allergy Laboratory of Jefferson Medical College Hospital and standardized in protein nitrogen units.

† Diameter of wheal formed.

‡NT, not tested.

Table II

Donor (Date blood drawn)	Recipient (Date of test)	Lymphocyte cell transfer tests								
		2Rs* 5,000 PNU			2Rs 10,000 PNU			2Rs 34,000 PNU		
		Site†	Control†	Result	Site	Control	Result	Site	Control	Result
V. S. 8-18-66	M. Z. 8-25-65	3 mm.	1.5 mm.	±	2.5 mm.	1.5 mm.	±	NT‡		
J. Z. 8-27-65	E. N. 8-30-65	NT‡			5 mm.	3.5 mm.	±	NT		
J. Z. 8-27-65	K. H. 9-3-65	NT			8 mm.	1 mm.	+	NT		
M. V. (I) 9-10-65	R. T. 9-13-65	NT			3 mm.	0	+	9 mm.	2 mm.	+
M. V. (II) 10-22-66	R. G. 10-29-65	NT			2 mm.	2 mm.	-	7.5 mm.	2.5 mm.	+
M. V. (II) 10-22-65	J. P. 11-1-65	NT			1.5 mm.	2.5 mm.	-	3.5 mm.	3.5 mm.	-

*2Rs, extract of equal parts of low and giant ragweed prepared in the Allergy Laboratory of

†Diameter of wheal formed.

‡NT, not tested.

washed viable lymphocytes. Individual variations in the sensitizing potential of the donor cells or in the capacity of the recipient to become sensitized may account for the fact that each donor's cells did not sensitize every recipient in whom these cells were injected.

In these experiments, cells from one donor, M. V., transferred an immediate

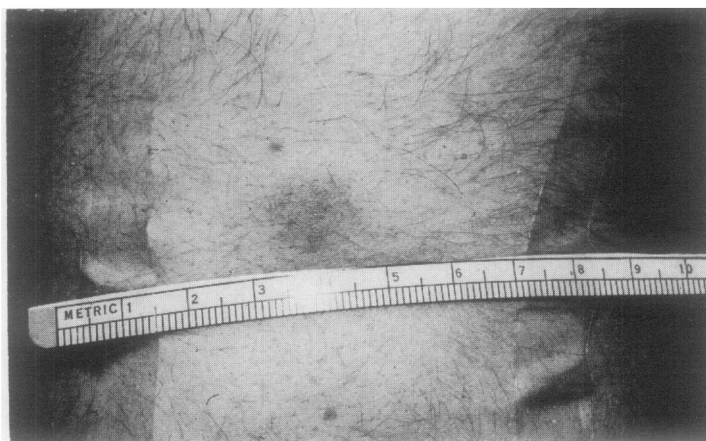


Fig. 2

Appearance of positive tuberculin reactions in remote normal skin area 5 days after transfer of lymphocyte cells from donor M. V. (II) to recipient J. P. and 48 hours after challenge with PPD, second testing strength. A similar reaction appeared at the lymphocyte cell-transfer site.

<i>Buffered saline</i>		<i>Saline washings tests</i>			<i>Tuberculin tests</i> <i>PPD, second test strength</i>		
					<i>Normal skin</i> <i>(prior to</i> <i>lymphocyte</i> <i>transfer)</i>	<i>Lymphocyte</i> <i>cell site</i>	<i>Normal skin</i> <i>(after lympho-</i> <i>cyte transfer)</i>
<i>Site</i>	<i>Control</i>	<i>Site</i>	<i>Control</i>	<i>Result</i>			
0	0	NT			—	—	NT
	NT	1.5 mm.	1.5 mm.	—	—	10 mm.	NT
	NT	0	0	—	—	—	NT
2 mm.	0	0	0	—	—	15 mm.	12 mm.
	NT	2.5 mm.	1.5 mm.	±	—	—	—
1.5 mm.	1.5 mm.	1 mm.	1 mm.	—	—	6 mm.	11 mm.

Jefferson Medical College Hospital and standardized in protein nitrogen units.

reaction to ragweed in 2 of 3 recipients; cells from another, J. Z., transferred the immediate reaction to ragweed in 1 of 2 recipients, while cells from the third donor, V. S., failed to transfer the ragweed reaction in a single recipient. Although the transfer of delayed hypersensitivity to tuberculin occurred in 3 recipients, this did not parallel the transfer of the immediate hypersensitivity to ragweed. Only one of the recipients who had acquired sensitivity to ragweed, R. T., developed delayed hypersensitivity to tuberculin. The variation in the transfer of immediate and delayed hypersensitivity may be the result of immunologic processes that still remain unelucidated.

Lymphocytes have been recognized as capable of transferring delayed type hypersensitivity but are not usually considered to be involved in the transfer of immediate type hypersensitivity. Humoral reagins are the classic means of transferring the immediate type of hypersensitivity. In our study, white blood cell suspensions, containing a high percentage of lymphocytes, successfully transferred an immediate type of hypersensitivity. It is still a matter for conjecture whether the cells formed an antiragweed antibody, released contained antibody, or whether reagin attached to the surface of the cells was released during the interval between the time the cells were injected and the time the local skin areas were tested with ragweed antigen. The possibility that some reagin may have remained attached to the lymphocyte cells cannot be positively excluded. However, the negative tests to ragweed at the cell sites in recipient J. P., who showed a strongly positive P-K reaction to ragweed, would tend to indicate that attached reagin was not present in the cellular test site. The amount of ragweed antibody produced or deposited in the normal skin areas locally sensitized by lymphocytes may be extremely small and difficult to detect.

Rose and his co-workers⁷ demonstrated that tissue culture fluid of lymphocytes from ragweed-sensitive patients was able to passively sensitize monkey ileum *in vitro*, and the addition of specific allergen produced a positive reaction. Heating the tissue culture fluid to 56° C. for 4 hours destroyed their ability to sensitize. Proper controls failed to sensitize the monkey ileum. They interpreted this data to suggest that an antibody is either produced or liberated from stimulated lymphocytes in tissue culture. Evidence from this source, from the findings of Walzer and Bowman,⁴ and our own findings would indicate that the lymphocyte may play a role in the immediate as well as the delayed type of hypersensitivity and that the lymphocyte may be a more important factor in the immediate type of sensitivity than has been formerly recognized.

SUMMARY

An attempt was made to transfer the immediate reaction to ragweed antigen by means of highly concentrated suspensions of viable lymphocytes obtained from 3 donors with severe hay fever symptoms and circulating reaginic antibodies to ragweed. Positive immediate wheal reactions to ragweed antigen appeared in 3 of the 6 recipients whose skin was locally sensitized with these cell suspensions. Two recipients showed equivocal reactions to ragweed, and the other failed to react to ragweed at the cell-transfer site. No significant wheals to ragweed tests appeared at sites made with the last saline washings obtained from

the preparation of the cell suspensions, indicating that a reaction to reagin adherent to the cells was unlikely, although this possibility cannot be excluded.

The transfer of tuberculin reactions occurred in 3 recipients, indicating that the technic employed was adequate to transfer delayed type hypersensitivity.

The technical assistance of Miss Viola Lynch is gratefully acknowledged.

REFERENCES

1. Walzer, M., and Glazer, I.: Passive Transfer of Atopic Hypersensitiveness in Man by Means of Leukocytes, *Proc. Soc. Exper. Biol. & Med.* **74**: 872, 1950.
2. Walzer, M., Bowman, K. L., and Strayman, S.: Leukocytic Transfer of the Immediate Type of Allergic Reaction in Man, *J. ALLERGY* **28**: 206, 1957.
3. Walzer, M., and Bowman, K. L.: Leukocytic Transfer of the Immediate Types of Allergic Reaction in Man. II. The Correlation of Leukocytic Transfer With Other Sensitivity Factors, *J. ALLERGY* **31**: 46, 1960.
4. Walzer, M., and Bowman, K. L.: Leukocytic Transfer of Immediate-Type Hypersensitiveness in Man. V. Transfer of Experimental Sensitivity to *Ascaris* Antigen, *Proc. Soc. Exper. Biol. & Med.* **105**: 246, 1960.
5. Freedman, S. O., Fisher, J. P., and Cooke, R. A.: A Study of Leukocytic Antibodies in Allergic Patients, *J. ALLERGY* **28**: 501, 1957.
6. Slavin, R. G., Garvin, J. E., and Feinberg, S. M.: Transfer of Delayed Hypersensitivity in Man by Lymphocyte Preparation of Peripheral Blood, Presented at the Twenty-first Annual Meeting of the American Academy of Allergy, Bal Harbour, Fla., Feb. 15, 1965.
7. Rose, N. R., Girard, J. P., Kunz, M. L., Kobayashi, S., Dolovich, J., and Arbesman, C. E.: Tissue Culture of Lymphocytes From Allergic Patients, Presented at the Twenty-second Annual Meeting of the American Academy of Allergy, New York, N. Y., Feb. 22, 1966.
8. Chase, M. W.: The Cellular Transfer of Cutaneous Hypersensitivity to Tuberculin, *Proc. Soc. Exper. Biol. & Med.* **59**: 134, 1945.
9. Samter, M., editor: *Immunologic Diseases*, Boston, 1965, Little, Brown & Company, p. 268.
10. Brent, L., and Medawar, P. B.: Tissue Transplantation: A New Approach to the Typing Problem, *Brit. M. J.* **2**: 269, 1963.
11. Boyse, E. A., Old, L. J., and Thomas, G.: A Report on Some Observations with a Simplified Cytotoxic Test, *Transpl. Bull.* **29**: 63, 1962.

Erratum

In the article by Samuel J. Prigal, "The ferric chloride spot test for the evaluation and standardization of emulsions of allergens," in the January, 1967, issue of the *JOURNAL*, page 1, the "Discussion" preceded the "Methods and results" section. This order should have been reversed.