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Leukocytic Transfer of Immediate-Type Hypersensitiveness in Man. V. Transfer of Experimental Sensitivity to Ascaris Antigen. (26070)

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Leukocytic transfer with peripheral blood leukocytes in an immediate type of hypersensitiveness was first demonstrated in man in the atopic form of sensitivity(1). The transfer reactions to pollens and danders in the recipients were specific but consistently small, never exceeding a slight, or one-plus (+), positive reaction. In more recent studies on experimental human sensitization with *Ascaris lumbricoides* antigen, marked transfer reactions of an immediate type were obtained in 2 noteworthy instances. These occurred in separate studies on donors who had been sensitized by repeated intracutaneous injections of *Ascaris* antigen and who exhibited, at time of transfer, delayed and immediate forms of hypersensitiveness to this antigen. In the first case, to be presented by Walzer, Bowman, and Coleman in another communication, both types of sensitivity were simultaneously and decisively transferred to a normal recipient with peripheral blood leukocytes or with extracts of these cells. In the second case described below, only the immediate type was carried over to a normal recipient by leukocytic transfer. The findings in this study represent the first instance of marked immediate-type transfer reactions elicited in man at sites prepared with peripheral blood leukocytes where only an immediate type of hypersensitiveness was transferred.

Materials and methods. The method of preparation and standardization of extracts of *Ascaris lumbricoides* worms removed from infested pigs has been described(5). Extract 11 prepared in 1954 and extract 12 prepared in 1959 were used in this study.

Donors, selected from members of the hospital medical staff, were young adults with a negative history of parasitic infestation. They were screened by an intracutaneous test with an *Ascaris* extract containing 0.01 mg N/ml and by tests with a few common allergens to rule out the presence of pronounced sensitivities. A positive family or personal history of atopy was not considered a basis for rejection, but individuals with active clinical symptoms of allergy were excluded.

The recipients in these experiments were young female student nurses and laboratory technicians with negative histories of parasitic infestation. They likewise failed to react to the screening test with *Ascaris* extract. Some were atopic, but none had active symptoms at time of this study.

To study the development of hypersensitiveness in the donor, intracutaneous tests were performed weekly with *Ascaris* extracts 11 and 12, using approximately 0.01 ml of the 0.01 mg N/ml dilutions for each test. Results were read in 15 minutes and graded according to accepted standards(6). At completion of these tests performed on the donor's right arm, a sensitizing injection of a mixture containing equal concentrations of extracts 11 and 12 was administered intracutaneously on his left arm. The delayed reaction produced by this injection was read in 24 hours. The horizontal and vertical diameters of the reaction were measured, and amount of induration was estimated by palpation and recorded in terms of 0 to ++++ (Table I).

In the successful experiment, the immediate-type sensitivity of Donor WT was transferred to Recipient BE. Donor WT is a physician, 26 years of age, who gave a posi-

TABLE I. Experimental Sensitization of Donor WT to *Ascaris* Extract and Results of Leukocytic Transfer Tests.

Day after screening test	Sensitizing injections				Delayed-type reactions (24 hr)		Immediate-type reactions *Ascaris* extract			Results of transfer tests		
	Inj. No.	Amt, ml	Dilution, mg N/ml	Total dose, mg N	Size, mm	Induration	Test No.	No. 11	No. 12	Recipients	WBC sites	Serum sites
1	1	.05	.01	.0005	0	0	1	0	0			
10	2	.1	.05	.005	0	0	2	0	0			
17	3	.1	.1	.01	25 × 20	±	3	0±	±			
24	4	.1	.1	.01	20 × 20	+	4	±	±			
31	5	.1	.1	.01	15 × 20	+	5	0±	0±			
38	6	.1	.1	.01	30 × 30	++	6	+	±			
45	7	.1	.1	.01	50 × 50	++	7	+	+	DS	0	0
										BE	+++	0
										IL	0	0
52							8	+	+			

Scale of immediate-type skin reactions: + = wheal 0.5 cm in diameter with faint areola; ±, slightly smaller reaction; 0±, barely perceptible reaction; 0, negative reaction.
WBC = leukocyte sediment suspension (1:10).
Day 0, Feb. 7, 1960, day of screening test.

tive family and personal history of atopy and had no knowledge of any parasitic infestation. He failed to respond to screening tests with *Ascaris,* but showed weakly positive reactions to pollens and house dust. Recipient BE was a 17-year-old American-born female with a negative personal history of allergy, but her brother has hay fever. Her reactions to screening tests with *Ascaris* and with common allergens were negative, and she had no history of parasitic infestation.

The sensitization program carried out on Donor WT is presented in Table I. Delayed sensitivity was detected during the third week, soon followed by onset of immediate hypersensitiveness. Both forms developed slowly. By the seventh week, the immediate reaction to testing was no greater than a one-plus (+), and the delayed reaction following sensitizing injection was of moderate intensity.

A blood sample was drawn from Donor WT on day 45 before the skin tests for that day were performed. A sterile 1:10 suspension of leukocytic sediment in Tyrode's solution was prepared according to the previously described method(2) and was kept overnight in the refrigerator. On the following day, 0.05 ml of cell suspension was injected into each of 3 sites on the left arms of 3 recipients. On the right arm of each subject, 2 sites were prepared with Donor WT's serum derived from the same blood sample which provided the cells. After an interval of 11 days, the transfer sites were tested with 0.02 ml of *Ascaris* extract. Readings were taken in 20 minutes. Because leukocytic transfer reactions at this stage so closely resembled those regularly obtained at serum-sensitized passive transfer sites, the usual standards for reading the latter type of reaction(6) were also applied to the former.

Results. The 3 recipients were first tested on day 11 following transfer with a 0.01 mg N/ml dilution of *Ascaris* extract 12 and with a 0.05 mg N/ml dilution of extract 11. These tests were performed on 2 of the 3 leukocyte-injected sites on the left arm of each recipient, diluting fluid being used on the third site. In 2 recipients, results were completely negative (Table I). In Recipient BE, extract 12 injected into site 4 produced a moderate to marked positive (++±) reaction and extract 11 at site 5 elicited a marked positive (+++) reaction (Table II). The control test with diluting fluid on site 3 produced no reaction. When this site was retested 3 days later with an extract of ragweed pollen, an unrelated antigen, a negative reaction was obtained. A third test at site 3 on day 26 with *Ascaris* extract 12 elicited a positive (+) reaction. On the right arm, tests on day 11 at serum site 1 and at a control site with a 0.01 dilution of *Ascaris* extract 12 gave negative reactions.

On day 18, all points on both arms which

TABLE II. Results of Leukocytic Transfer Tests on Recipient BE.

Day after transfer	Test material	mg N/ml	Right arm: Site 1 serum	Right arm: Site 2 serum	Right arm: Control	Left arm: Site 3 WBC	Left arm: Site 4 WBC	Left arm: Site 5 WBC	Left arm: Control
11	*Ascaris* 12	.01	0		0		++±		0
	" 11	.05						+++	0
	Diluting fluid			0		0			
14	Ragweed	.01				0			
26	*Ascaris* 12	.01				+			0

Blood drawn on donor day 45, as in Table I.
Transfer made on donor day 46, being considered recipient day 0.
WBC = leukocyte sediment suspension (1:10).
Scale of positive reactions: slight +; moderate ++; marked with pseudopods +++; excessively marked ++++.

had been injected with *Ascaris* extract developed faint nonpruritic, nonindurated erythematous spots from 2 to 5 mm in diameter. Sensitized and control sites on both arms reacted equally. The *Ascaris* tests performed on day 26 were followed on day 27 by similar reactions of no greater intensity. These reactions bore no resemblance to the severe delayed reactions seen in a previous experiment in which the delayed type of sensitivity was carried over with the immediate type. The faint reactions seen here probably represented a delayed type of hypersensitiveness originating in Recipient BE as a result of the previous tests with *Ascaris* done on day 11.

In this series of experiments, 2 other subjects were sensitized with *Ascaris* to serve as donors. One developed immediate and delayed sensitivities comparable in degree to those of WT, but a leukocytic transfer trial on a normal recipient proved unsuccessful. In the second subject, both types of sensitivity were only weakly positive and no transfer tests were attempted with his cells.

Discussion. In these studies, the immediate whealing type of specific response was confined to the leukocyte-injected cutaneous areas on the recipient and closely resembled in appearance the reagin-mediated reactions of serum-sensitized passive transfer sites(6). In comparison to the latter, however, leukocytic transfer reactions tended to develop more gradually, to be more intensively and persistently pruritic, and to manifest a more pronounced and prolonged edema.

The positive reactions elicited by testing the leukocytic transfer sites definitely exceeded those obtained at the serum-transfer sites as well as the donor's own responses to the same tests. This is a reversal of the relationhip observed in passive transfer phenomena produced with reagin-bearing sera.

In delayed-type hypersensitiveness, Lawrence found the degree of donor sensitivity to be a decisive factor in the success of leukocytic transfer tests(4). Pronounced sensitivity in the donor did not appear to be a prerequisite for leukocytic transfer of the experimental immediate-type hypersensitiveness to *Ascaris* seen in these studies.

The negative results obtained in Recipients DS and IL (Table I) who received experimental treatment similar to that afforded Recipient BE are indicative of the importance of still unidentified recipient factors in leukocytic transfer tests. In *Ascaris* sensitization studies, there is a particular need to keep in mind the possible influence of a previous, unrecognized parasitic infestation on subsequent immunologic responses of some recipients and donors.

Slight (+) leukocytic transfer reactions have been repeatedly elicited in the naturally acquired (atopic) form of immediate-type sensitivity(1,3). Marked (+++) leukocytic transfer reactions have thus far been obtained in 2 cases of immediate-type hypersensitiveness, the second of which has been described here. The cell donors in both instances had been experimentally sensitized to *Ascaris* and exhibited immediate and delayed reactions to this antigen at time of transfer. In both cases only one of several recipients

tested showed positive transfer reactions. These findings, despite their limitations, render untenable the prevailing concept that only delayed forms of hypersensitiveness in man are transferrable by peripheral blood leukocytes.

Summary. From a human donor in whom immediate and delayed forms of sensitivity to *Ascaris lumbricoides* antigen had been experimentally induced, only the immediate type of sensitivity was transferred to one of 3 recipients by use of peripheral blood leukocytes. Markedly positive transfer reactions were obtained.

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Spontaneous Mammary Cancer Developing in Rats Born and Nursed by Mothers Treated with 3-Methylcholanthrene.* (26071)

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In the study of mammary carcinogenesis induced by 3-methylcholanthrene, one aspect of the investigation in this laboratory is concerned with the mechanism of chemical carcinogenesis. Development of mammary adenocarcinoma in rats following oral administration of 3-methylcholanthrene has been reported(1,2,3). These observations have indicated that the neoplasms induced by 3-methylcholanthrene were predominantly mammary cancers. The selective response of mammary tissue to orally administered 3-methylcholanthrene cannot be explained fully, since the mechanism of carcinogenesis is not known. However, Dao *et al.*(4) demonstrated selective concentration of administered polycyclic carcinogens in breast and fat and confirmed the earlier report by Shay *et al.*(5) of excretion of 3-methylcholanthrene into the milk of lactating female rats. These results raised certain questions: (a) If the chemical carcinogen can be excreted in the milk, could mammary cancer be induced in offspring suckled by mothers treated with 3-methylcholanthrene? (b) If 3-methylcholanthrene is administered to pregnant female rats, could it pass through the placental barrier, and induce, by its effect on the early embryo, mammary cancer in the offspring? (c) Can 3-methylcholanthrene induce a "product" which may be transmitted through the milk to the offspring, thus promoting tumor development?

The present article is concerned with some preliminary results of experiments undertaken in an attempt to answer these questions.

Materials and methods. In all experiments, 55- to 60-day-old mature female Sprague-Dawley albino rats were used. The animals were considered sexually mature when opening of the vagina and estrous cycles were established. Four groups of experiments were carried out: 1. 3-Methylcholanthrene was fed to the mature female rats at a dose level of 10 mg/ml sesame oil daily through a stomach tube for 6 days in one group and 10 days in the other. Each group consisted of 20 rats. Vaginal smears were taken daily after completion of feeding for 4 days to determine optimal time for mating. The female rats treated with 3-methylcholanthrene were then mated with normal males for a period of

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