IMMUNOLOGIC COMPETENCE OF THORACIC DUCT CELLS

I. Delayed Hypersensitivity*

By JOHN E. COE,‡ M.D., JOSEPH D. FELDMAN, M.D., and SUN LEE, M.D.

(From the Division of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, California)

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The pathogenesis of the lesions of delayed hypersensitivity is obscure, in part because the cell type or types which can mediate the reaction are unknown. Lymphoid cells are thought to be carriers of this type of immunologic information, although recently conventional antibody of high affinity has been proposed as the mediator (1) and additional reports have supported the proposal (2, 3). The small lymphocyte has been advocated as the immunologically competent cell, capable of antibody formation and homograft destruction (4, 5). Since thoracic duct lymph contains a high proportion of small lymphocytes, a number of recent investigations has used the lymph for immunologic studies. Wesslén described the transfer of delayed hypersensitivity by thoracic duct cells but he did not determine whether the small lymphocyte or other cell types were the responsible agents (6). Gowans and his colleagues transferred relatively "pure" populations of thoracic duct small lymphocytes to destroy long standing homografts, a process considered to be similar to that of delayed hypersensitivity (4).

In this report, cells of thoracic duct lymph, harvested from Lewis rats sensitized to purified protein antigens, were found to be capable of transferring delayed hypersensitivity to isogenic recipients. The results indicated that conventional circulating antibody did not mediate the lesion of delayed hypersensitivity, but the cell type or types transmitting this immunological state could not be precisely defined.

Materials and Methods

Antigens.—Hen egg albumin (HEA)[1] and rabbit gamma globulin (RGG)[2] were used for

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‡ Postdoctoral Research Fellow, supported by United States Public Health Service Training Grant 5T1GM683. Present address: National Institutes of Health, National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratory, Hamilton, Montana.

[1] K & K Laboratories, Inc., Jamaica, New York.

[2] Pentex, Inc., Kankakee, Illinois.

sensitization and testing. I^{131}-labeled[3] RGG was prepared according to the method of McConahey and Dixon (7) utilizing chloramine T.

Immunization.—Inbred Lewis rats[4] were used as donors, averaging 300 g in weight, and as recipients, ranging from 100 to 200 g in weight. Donor animals were immunized by injection into the 4 foot-pads of 5 μg of protein emulsified in 0.5 ml of complete Freund's adjuvant containing 2 mg of tuberculin.[5] Delayed hypersensitivity could be detected as early as 6 days after sensitization with RGG and 8 days after HEA, and maximal response was reached 10 and 12 days, respectively, after injection of the antigens.

Thoracic Duct Cells.—Thoracic ducts of donor Lewis rats were cannulated 6 to 12 days after initiation of immunization. The operated animals were placed in modified Bollmann cages, fed a pellet diet, and received ad lib. drinking water consisting of 0.4% NaCl and 2.5% dextrose. Lymph was collected in 250 ml sterile flasks holding 5 ml of Earle's solution, the latter containing 100 units of penicillin and of streptomycin and 30 units of heparin/ml. At 24-hr intervals the collected lymph was centrifuged at 600 RPM for 10 min. The button of cells was resuspended in a known volume of a mixture of Earle's solution and 15% (v/v) Lewis rat serum. Total cell number was determined by counting in a hemocytometer. The cells were again centrifuged lightly and resuspended in Earle's solution-Lewis serum mixture to yield a cell concentration of 1×10^8/ml. Brush and spread smears were stained with Wright's stain and 1000 to 2000 cells were recorded for each differential examination. Cells were classified as small lymphocytes (SL) and large cells (LC); the latter category included chiefly large and medium-sized lymphocytes, and other types such as blast, plasma, and unidentified cells. The thoracic duct cells, adjusted to desired concentrations, were injected intravenously into recipient Lewis rats.

Skin Tests.—Recipient rats were skin tested either promptly after or at varying intervals following transfer of thoracic duct cells. The skin test was performed by injecting 5 μg of antigen in 0.05 ml of saline intradermally into flank skin. 24 to 30 hr later the area of induration was measured and the skin test site removed. After fixation in 10% formalin, 3 to 4 slices across the central part of the excised tissue were processed for histology. Some donor rats were similarly tested at varying times following establishment of the thoracic duct fistula.

All skin test sites were examined histologically (hematoxylin and eosin stain) and the reaction was considered positive only if all the following were observed: (*a*) at least 3 perivascular and/or perineural clusters of mononuclear cells in one cross-section of skin; (*b*) dispersed mononuclear cells in the dermis, without obvious pattern; (*c*) a small number of polymorphonuclear leukocytes, less than 5% of the infiltrating elements; and (*d*) the absence of smudging or necrosis of the walls of small vessels.

Antibody Determination.—Antibody to RGG was determined by whole-body counting in a scintillation counter after intravenous injection of 3 to 10 μg of labeled antigen. The disappearance rates were graphed by plotting daily the amount of radioactivity remaining in the rat (which ingested KI in its drinking water). The appearance of antibody was signaled by the sudden decrease of whole-body radioactivity and its loss to less than 5% of the original dose. Disappearance curves of RGG-I^{131} in untreated control rats were obtained whenever antibody formation was determined in experimental animals. The method, under the conditions used in these experiments, can detect as little as 0.008 μg of antibody N/ml (8).

Anti-RGG and anti-HEA antibodies were also demonstrated by the Preer technique (9) and in addition, in some instances, anti-HEA antibodies were detected by Farr's antigen-binding capacity method (10).

X-Irradiation.—Some recipient rats were given 550 R total body irradiation, 30 min before transfer of thoracic duct cells. A leucite animal container was placed midway between 2 op-

[3] Iso/Serve, Cambridge 39, Massachusetts.

[4] Microbiological Associates, Bethesda, Maryland.

[5] Tuberculin, Parke, Davis & Co., Detroit, Michigan, Lot No. 078539.

posing 220 kv Picker X-ray machines, 160 cm apart. The machines were operated at 210 kv, 15 ma, with an inherent filtration of 0.25 mm Cu plus 1 mm Al, and together they delivered 55 R/min at either surface and uniformly throughout a paraffin phantom placed in the animal container.

Controls—Thoracic duct cells from immunized donors were pooled and divided into aliquots containing 100 to 350 $\times$ 10^6 cells. The suspensions were frozen and thawed until the cells were destroyed and then were administered intravenously to 7 recipients which were promptly skin tested. Thoracic duct cells from the same donors were collected either the day before or the day after the above procedure and were injected in doses of 75 to 150 $\times$ 10^6 cells into 11 recipient rats which were then skin tested. Lesions of delayed hypersensitivity were not elicited in any of the 7 rats given frozen-thawed material and were present in all 11 receiving intact cell suspensions.

RESULTS

General Considerations

Thoracic ducts of Lewis rats were cannulated 6 to 10 days after sensitization with RGG and 8 to 12 days after sensitization with HEA. At these times delayed hypersensitivity was manifested in donor rats by typical gross and microscopic lesions, and small amounts of circulating anti-RGG and anti-HEA antibody were detectable by immune elimination of RGG-I^{131} and by the antigen-binding capacity technique, respectively.

During the first 2 days 75 to 300 $\times$ 10^6 cells were collected each 24 hr from single donors. Subsequently the number fell rather rapidly and was maintained at 25 to 50 $\times$ 10^6 cells/24 hr for several weeks. In the first 24 hr collection small lymphocytes constituted 90 to 95% of the total output. The percentage of small lymphocytes gradually fell, reached a level of 55 to 70% by the 3rd day of drainage, and was maintained at this level throughout the period of observation.

The Relationship of Cell Number and Type to Passive Transfer of Delayed Hypersensitivity

Successful passive transfer of delayed hypersensitivity was accomplished in over 125 recipients. The number of cells needed to achieve this varied from donor to donor and was inversely related to the length of time of thoracic duct drainage. From lymph collected during the first 24 hr after cannulation, more than 75 $\times$ 10^6 cells were needed to effect consistently a passive transfer of delayed hypersensitivity. From lymph collections beyond the 1st day, the number of cells needed for successful transfer diminished and as few as 7 $\times$ 10^6 cells were sufficient to achieve this on one occasion. In Table I data are shown for a series of cell transfers. When cell numbers from the 1st day drainage exceeded 75 $\times$ 10^6, passive transfer of delayed hypersensitivity was successful in 14 of 16 recipients; with 75 $\times$ 10^6 and less, passive transfer of delayed hypersensitivity was effected in 2 of 10 recipients. By contrast, with collections of thoracic duct cells from the 2nd day of drainage and beyond, 75 $\times$ 10^6 cells

TABLE I

Relationship of Thoracic Duct Cell Numbers, Duration of Drainage, and Skin Tests in Recipients

1st day lymph						
Donor	Cells transferred × 10^6	Skin test micro	Donor	Cells transferred × 10^6	Skin test micro	Time of collection after cannulation
						day
P32	10	−	P82	14	+	9
P32	20	−	P82	17	−	10
P32*	40	−	P92	22	−	6
D14	40	−	P82	23	+	8
D27	40	+	D27	24	+	4
D30	50	+	D30	25	−	2
P90	50	−	P84	26	−	3
P111	50	−	D27	27	+	5
P50	75	−	P28	29	+	2
P50	75	−	D11	30	−	4
		2/10	P90	34	−	6
P32	80	+	P82	34	+	7
P84	98	+	P84	35	+	2
P61	100	+	P39*	37	+	6
P93	100	+	P90	37	−	5
D82	129	+	D12	39	+	2
D13	129	+	P32*	40	+	5
D12	137	+	P61	40	+	3
P90	139	+	P84	49	+	3
P50*	150	−	P84	57	−	8
P39*	150	−	P32	57	−	4
D27	150	+	P61	60	+	3
D29	160	+	D14	72	+	3
P111	166	+	P92	75	+	5
P93	200	+				15/24
D11	219	+	P93	88	+	6
P28	266	+	P90	89	+	8
		14/16	D13	100	+	4
			P111	112	+	6
			P111	125	−	5
			D29	125	+	3
			D11	141	+	3
			P50*	150	+	2
						7/8

Micro, microscopic here and in following tables.

* Did not transfer delayed hypersensitivity with cells collected during the 1st day's drainage but did with as many or fewer cells from a subsequent collection.

and less were capable of transferring delayed hypersensitivity in 15 out of 24 recipients.

There were, in addition, three instances in which delayed hypersensitivity

was not transferred by cells of the 1st day's drainage, but subsequently after 2 to 6 days of drainage the same or a lesser number of cells was capable of effecting passive transfer (footnote to Table I).

An experiment was designed, therefore, to compare the number of thoracic duct cells of a 1st day's drainage needed to transfer delayed hypersensitivity with the number needed of a 6th day's collection from the same donor. Graded doses of cells from 24-hr lymph collections were given to individual recipients so that the threshold number of cells required for successful passive transfer could be determined on the 1st and 6th day of drainage. As shown in Table

TABLE II

Effect of Prolonged Thoracic Duct Drainage on the Number of Cells Needed to Transfer Delayed Hypersensitivity

R 151 No. of cells transferred × 10^6				R 152 No. of cells transferred × 10^6				R 154 No. of cells transferred × 10^6				R 159 No. of cells transferred × 10^6			
Total	SL*	LC‡	Skin test	Total	SL	LC	Skin test	Total	SL	LC	Skin test	Total	SL	LC	Skin test
1st day drainage															
75	68.3	6.7	+	80	72.8	7.2	+	100	92.0	8.0	+	125	113.8	11.2	+
53	48.2	4.8	−	60	54.6	5.4	−	75	69.0	6.0	−	100	91.0	9.0	−
25	22.8	2.2	−	40	36.4	3.6	−	50	46.0	4.0	−	75	68.3	6.7	−
				20	18.2	1.8	−	25	23.0	2.0	−	50	45.5	4.5	−
6th day drainage															
28	18.2	9.8	−	37	27.4	9.6	+	14	9.3	4.7	−	37	24.7	12.3	+
23	15.0	8.0	+	20	14.8	5.2	−					25	16.7	8.3	−

* Small lymphocytes.

‡ Large cells, chiefly large and medium sized lymphocytes.

II, 75 to 125 × 10^6 cells were required to effect a positive passive transfer with cells collected during the first 24 hr. The 6th day's cell collections from the same donors were capable of achieving a successful transfer of delayed hypersensitivity in 3 instances, with ⅓ to ½ the number needed earlier. At this latter time the small lymphocyte population had decreased, on the average, from 91 to 68%, while the large cell population had increased from 9 to 32%. In one instance the number of cells collected during the 6th day were 14 × 10^6 and was apparently insufficient to transfer delayed hypersensitivity. There was no apparent relationship between the absolute number of small lymphocytes transferred in the 1st and 6th days' drainage and the development of a positive skin test. Rather, the absolute number of large cells and positive skin reactions were better correlated. It seemed that about 7 × 10^6 large cells was the lower threshold for transferring delayed hypersensitivity.

To control the temporal aspect of the experiment, donor rats were first cannulated 15, 16, 17, and 22 days after beginning sensitization with 5 μg of HEA in complete Freund's adjuvant. From collections at this later time cell numbers needed to transfer delayed hypersensitivity ranged between 80 and 120 $\times 10^6$ and were no more efficacious than cells collected during the 1st day at earlier times after immunization.

TABLE III

Persistence of Active Delayed Hypersensitivity in Draining Donors Sensitized with RGG or HEA

Donor	Antigen	Time of drainage	Skin test	
			Gross	Micro
		day	*mm*	
36	RGG	4	0/10 × 10*	−
38	RGG	4	0/17 × 19	+
42	RGG	5	0/11 × 11	+
39	RGG	6	0/0	−
62	RGG	7	13 × 13/12 × 12	Arthus
64	RGG	7	0/12 × 12	Arthus
65	RGG	7	16 × 16/15 × 15	Arthus
71	RGG	9	0/0	−
97	RGG	17	0/0	+
98	RGG	18	0/0	−
131	RGG	28	0/0	−
10	HEA	5	9 × 10/32 × 25	+
86	HEA	9	0/20 × 20	+
90	HEA	11	0/15 × 15	+
67	HEA	18	0/8 × 8	+
134	HEA	19	0/15 × 15	+

* Immediate (4 hr)/delayed (24 to 30 hr).

Persistence of Delayed Hypersensitivity in Draining Donors

It was of interest to learn whether the continuing loss of thoracic duct cells affected delayed hypersensitivity in draining donors. Sensitized donor rats with thoracic duct fistulas of varying duration were removed from the restraining cage and skin tested (Table III). In addition, the presence of delayed hypersensitivity was checked by skin testing recipients of thoracic duct cells from immunized donors which had been draining for 9 to 21 days (Table IV). Actively sensitized donors exhibited positive skin test reactions up to 19 days after a thoracic duct fistula had been made, and were capable of transferring delayed hypersensitivity with 24-hr cell collections up to 20 days of drainage.

HEA appeared to be a better antigen than RGG for the production of delayed hypersensitivity. Positive HEA skin reactions were frequently elicited

in donors beyond 10 days of thoracic duct drainage and their gross measurements were usually larger than those induced by RGG. Positive RGG skin reactions were infrequently observed beyond 10 days of fistula (Table III). Similarly, in passively transferred recipients, HEA hypersensitivity was readily demonstrated when cells were collected between 2 and 3 wk after can-

TABLE IV

Duration of Thoracic Duct Drainage in Immunized Donors and Passive Transfer of Delayed Hypersensitivity

Day of 24 hr TD collection	RGG donors 97 and 98		HEA donor 67		HEA donor 78	
	No. cells transferred $\times 10^6$	Skin test micro	No. cells transferred $\times 10^6$	Skin test micro	No. cells transferred $\times 10^6$	Skin test micro
9	96	+				
10	71	+				
11	51	+				
12	48	−				
13			25*	+	44	+
			20	−		
14	43	+	25*	+	15	+
			14	+		
15	44	−	27			
16			28*	+		
			7	−		
17					60	+
18					30	+
19					18	−
20					23	+
21					32	−

* The total number of cells in brackets collected in 1 day and divided as shown between 2 recipients.

nulation, while RGG hypersensitivity was infrequently detected with cells collected during this time (Table IV). Microscopically, HEA-positive skin test sites were more heavily infiltrated with mononuclear cells than were RGG-positive skin test sites.

Persistence of Delayed Hypersensitivity in Lewis Recipients

In most of the studies on passive transfer of delayed hypersensitivity, the manifestations of delayed hypersensitivity have been short-lived, probably because the cell infusions have been made into homologous recipients. In inbred

guinea pigs Bauer and Stone (11) reported prolonged duration of the hypersensitivity state after passive transfer.

Twelve Lewis rats received varying numbers of thoracic duct cells from RGG- and HEA-sensitized donors. The recipients were skin tested only once at different intervals of time following transfer. Positive skin tests were elicited up to 25 days following cell infusion (Table V).

TABLE V

Persistence of Passively Transferred Delayed Hypersensitivity

Donor and antigen	Recipient	No. of cells transferred × 10^6	Days after transfer	Skin test micro
RGG (61 b)	1	60	5	+
RGG (93 b)	2	180	10	+
RGG (61)	4	100	10	+
RGG (82 a)	7	108	15	+
RGG (122 j)	12	178	25	+
HEA (133 a)	8	689	18	+
HEA (78 b)	9	100	20	+
HEA (78 a)	10	88	21	+
HEA (79 a)	11	142	21	+

TABLE VI

Enhancement of Delayed Hypersensitivity after Transfer of Sensitized Cells

Donor and antigen	No. of cells transferred × 10^6	1st Skin test		2nd Skin test	
		Time after transfer	Micro	Time after transfer	Micro
		day		*day*	
RGG (Pool 32)	80	0	+	20	+
	40	0	−	20	+
	20	0	−	20	+
	10	0	−	20	−
RGG (Pool 32 a)	150	0	−	19	+
	75	0	−	19	+
	25	0	−	19	+
HEA (D50)	150	0	−	20	−
	75	0	−	20	+
	75	0	−	20	+

Not only did delayed hypersensitivity persist in the recipient animal, but the degree of sensitivity apparently increased with time. Ten rats received graded numbers of thoracic duct cells from pools of RGG- and HEA-sensitized

donors. The recipients were skin tested promptly after transfer, and 24 hr later the injection sites were found to be negative grossly and microscopically in 9 of the 10 (Table VI). 19 and 20 days after cell infusion the same recipients were skin tested a second time and on this occasion the test sites were positive in 7 of 9 originally negative (Table VI). One animal which had been transfused with 10 x 10^6 thoracic duct cells showed no evidence of delayed hypersensitivity

TABLE VII

Effect of X-Irradiation on Passively Transferred Delayed Hypersensitivity

Donor and antigen	X-Ray*	Recipients				
		No. of cells transferred $\times 10^6$	Time tested after transfer	Skin test micro	Time tested (2nd time)	Skin test micro
	R		*day*		*day*	
A						
RGG (66 c)	0	130	0	+		
	550	130	0	—		
RGG (Pool)	0	200	0	+		
	550	200	0	—		
RGG (Pool 39 a)	0	100	1	+		
	0	60	1	+		
	550	100	1	—		
	550	100	1	—		
	550	100	1	—		
B						
RGG (Pool 32)	550	75	0	—	19‡	+
	550	25	0	—	19‡	+
	550	80	0	—	20‡	+
	550	40	0	—	20‡	+
	550	20	0	—	20‡	+
	550	10	0	—	20	—

* Given 30 min before transfer of cells.

‡ Antibody present in animal by immune elimination of labeled antigen and by Preer technique.

either on the first or second testing. It should be emphasized that the skin test dose of 5μg of antigen in saline did not induce a state of delayed hypersensitivity in control animals.

Effect of Radiation on the Passive Transfer of Delayed Hypersensitivity

There is evidence that the pathogenesis of the cutaneous lesion of delayed hypersensitivity is dependent upon a trigger mechanism initiated by the interaction of antigen and a small number of specifically sensitized cells (12-14). The host responds to the initial event with an infiltration of many lymphoid cells and

at 24 hr the lesion is composed predominantly of host cells. It was hypothesized that total body irradiation of the recipient might depress or eliminate the host response and thereby prevent the appearance of a lesion in a test site.

Thoracic duct cells were pooled from immunized donors and divided among 9 recipients as shown in part A, Table VII. Four of the recipients, without irradiation, were skin tested directly after cell transfer and all 4 exhibited positive lesions 24 hr later. Five of the recipients were given 550 R, total body irradiation, 30 min later were transfused with aliquots of the same pool, and were then skin tested. All 5 did not develop a positive lesion 24 hr later.

TABLE VIII

Delayed Hypersensitivity in Recipients with Circulating Specific Antigen

Recipient	No. of cells transferred	IV RGG -I^{131}			RGG skin test	
		Time after cell transfer	Amount	Onset of immune elimination	Time after RGG-I^{131}	Results
		day	*μg*	*hr*	*hr*	
1 (R27 c)	24	0	10	None	6	+
2 (R28 a)	29	0	10	None	6	+
3 (R122 d)	60	1	3	98	6	+
4 (R122 e)	108	0	3	98	6	+
5 (R122 f1)	228	0	3	73	9	+
6 (R122 f2)	228	0	3	93	9	+
7 (R122 g1)	283	0	3	69	10	+
8 (R122 g2)	283	0*	3	69	10	+
9 (R122 h)	297	1	4	67	8	+
10 (R122 i)	180	0‡	4	67	8	+

* RGG-I^{131} added to cells in vitro 20 min before injection.

‡ RGG-I^{131} added to cells in vitro 40 min before injection.

To learn whether a state of hypersensitivity would return after recovery from irradiation effects, 6 irradiated rats were transfused with graded numbers of thoracic duct cells from 2 pools of sensitized donors (part B, Table VII). Directly after transfer all were skin tested and found negative 24 hr later. 19 and 20 days after transfer, the 6 recipients were skin tested a second time. Five of the 6 developed positive skin lesions.

Delayed Hypersensitivity and High Affinity Antibody

It has been proposed that the pathology of delayed hypersensitivity is mediated by small amounts of circulating antibody with a high binding affinity (1-3). In this study, microgram quantities of RGG-I^{131}, administered to recipient rats at the time of passive transfer, were not eliminated at an accelerated rate until 3 to 4 days after injection. However, small amounts of antibody might not

be detected by this technique during the first few days after cell transfer because they might form soluble complexes in antigen excess (15). If very small quantities of circulating antibody of high binding affinity were responsible for the development of delayed hypersensitivity lesions 24 hr after passage of sensitized cells, then small quantities of antigen, injected intravenously before the skin test, should combine with the putative high affinity antibody and desensitize the animal.

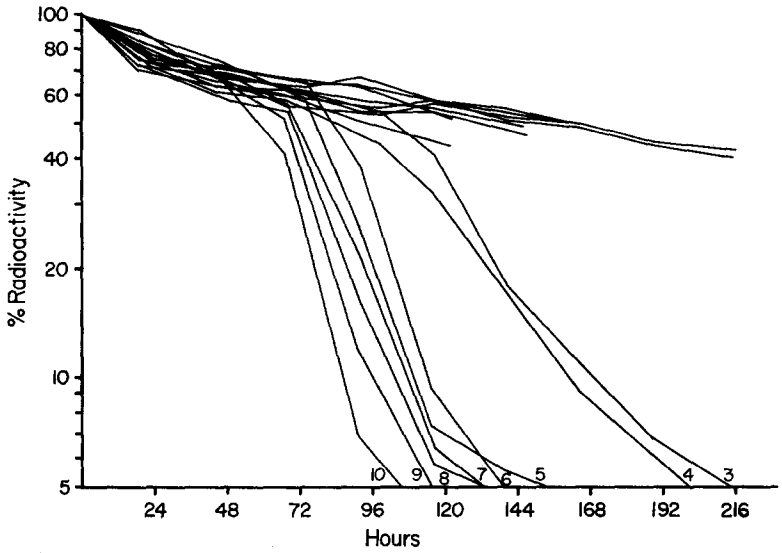

FIG. 1. Immune elimination of labeled antigen (curves 3 to 10, representing recipients 3 to 10 in Table VIII) in rats transfused with sensitized thoracic duct cells. Remaining curves show no immune elimination of labeled antigen in 6 controls and rats 1 and 2 of Table VIII.

Ten recipients were transfused with thoracic duct cells from immunized donors (Table VIII). Directly after or coincident with cell transfer the recipients were given intravenously 3 to 10 μg of labeled antigen (RGG-I^{131}). 6 to 10 hr after administration of antigen, the recipients were skin tested with RGG. All 10 recipient rats exhibited positive skin lesions 24 hr after skin testing. Immune elimination of labeled antigen did not occur in 2 and was observed to appear in the remaining 8 from 67 to 98 hr after injection of the labeled RGG (Table VIII and Fig. 1).

DISCUSSION

The present studies were undertaken to determine the competence of the small lymphocyte to transfer delayed hypersensitivity. Wesslén has already reported that thoracic duct cells removed from immunized guinea pigs were

capable of such transfer but his investigation was not designed to show which of the cell types present in thoracic duct lymph carried specific hypersensitivity (6). More recently Gowans and his colleagues have used relatively "pure" populations of small lymphocytes derived from rat thoracic duct lymph to destroy homografts, a process which they considered to be a manifestation of delayed hypersensitivity (4, 5). The role of the thoracic duct small lymphocyte in a system of delayed hypersensitivity utilizing purified proteins has not been examined. In our work we have been able to confirm that rat thoracic duct cells were competent to transfer this type of immunity in a model employing HEA and RGG.

Direct evidence that the small lymphocyte was the carrier of this type of immunologic competence was not obtained. Indeed, the results suggested that cells other than small lymphocytes might be the agents of transmission. Thoracic duct lymph collected during the 1st day after cannulation contained approximately 90 % small lymphocytes and was capable of transferring delayed hypersensitivity when cell numbers were greater than 75×10^6. Below this level it was difficult and infrequent to achieve transfer. In contrast, lymph collected after 2 or more days of drainage transferred delayed hypersensitivity when cell numbers ranged between 25 to 50×10^6, and the small lymphocyte population was about 70 % of the total cell population. When graded doses of cells were used and differential counts of donor lymph were made, calculations showed no relationship between the absolute number of small lymphocytes and the elicitation of positive skin reactions. Indeed, there was a correlation between the absolute number of large cells, mostly large and medium sized lymphocytes, and the appearance of positive skin tests. 7×10^6 large cells seemed to be the lower limit for successful transfer of hypersensitivity, whether 1st or 6th day collections were used.

Nonetheless, the small lymphocyte could not be excluded as a competent agent of transfer. Several studies have shown that the number of cells with specific sensitivity which could trigger the lesion of delayed hypersensitivity was quite small, a few per cent or less of the total lymphoid cells which were transfused (12-14). If a minor proportion of the small lymphocyte population was hypersensitive and if this minor group expanded during lymph drainage as a result of proliferation of precursors, then passive transfer could be successful despite a diminution of total numbers of small lymphocytes. Indeed, some of our data have indicated that an increase of passaged cells did occur, but they did not disclose the cell type or types involved. In Table VI, 9 recipients received varying numbers of thoracic duct cells, were promptly skin tested, and developed no positive reaction 24 hr later. Seven of the 9 were skin tested 19 to 20 days later and all developed positive reactions. 5 μg of skin test antigen, by itself, was unable to induce delayed hypersensitivity in normal control rats. However, such a dose might have enhanced the state of delayed hypersensitivity to detectable levels in rats which had received sensitized cells and which had

originally negative skin tests. Similarly, there were three instances in which thoracic duct cells of 1st day drainage from immunized donors did not transfer delayed hypersensitivity; the same number, or less, did transfer hypersensitivity after several days of donor drainage. These observations have persuaded us to think that the cells with delayed hypersensitivity proliferated either in a recipient or a donor, and these cells could have been small lymphocytes or any other type.

The state of delayed hypersensitivity was durable in both actively and passively immunized rats. Some donor rats were permitted to drain 25 days and lost 1 to 2 x 10^9 cells during this period. Despite the cellular loss positive skin reactions were elicited and by gross measurements were comparable with lesions induced after 1 or 2 days of duct fistula. (We have not tried to induce delayed hypersensitivity in rats with chronic lymph drainage.) In passively transferred recipients, with or without whole body irradiation, positive skin reactions were demonstrable up to 3 wk after cell transfusion. Bauer and Stone have also described the persistence of hypersensitivity in inbred guinea pigs transfused with sensitized isologous cells (11). However, in their recipients positive skin reactions did not appear until 1 to 2 wk after cell transfer, while many others have observed that positive skin tests were induced within the first 24 to 48 hr after transfer of homologous sensitized cells. The peculiarity of a lag period before hypersensitivity could be detected in Bauer and Stone's isologous guinea pigs was attributed to the use of a small number of cells, of lymphoid cells taken from guinea pigs not fully sensitized, and of the peritoneal route for cell transfer.

X-Irradiation of recipient rats before transfer of sensitized cells provided a demonstration that the delayed skin lesion seen after testing was predominantly due to host response. Total body irradiation of 550 R was sufficient to damage or kill enough host lymphoid cells so that infiltration of mononuclear cells at the injection site did not occur. A similar depression of passively transferred delayed hypersensitivity by X-irradiation of guinea pigs was described by Cummings et al. (16). If the delayed lesion is triggered by an interaction of antigen and a small number of cells with hypersensitivity it should be possible to find significant numbers of labeled sensitized cells in a test site of irradiated recipients without the corresponding heavy infiltrate of host elements. Alternatively, recovery of hypersensitivity in irradiated recipients might have been due to replacement of recipient lymphoid tissues by donor elements.

Throughout this paper we have used the phrase "cells with hypersensitivity", suggesting that cells were the mediators of delayed hypersensitivity. Recently Karush and Eisen (1), and Levine (2, 3) have proposed that small amounts of circulating antibody with high affinity were responsible for the pathology of this type of immunologic reaction. The data of Table VIII have indicated that there was no evidence for the presence of circulating high affinity antibody capable of eliminating specific antigen. Recipients were transfused with sensitized cells.

Immediately thereafter, or 1 day later, 3 to 10 μg of I^{131}-labeled specific antigen was injected intravenously. 6 to 10 hr following the administration of antigen the recipient rats were skin tested. All developed delayed lesions, and at this time labeled antigen was circulating and there was no evidence of immune elimination. In 8 of the 10 animals circulating antibody (i.e., immune elimination) appeared from 2 to 3 days after the skin lesion had developed and the remaining 2 did not eliminate the RGG-I^{131} during the period of observation.

Under the above conditions small amounts of circulating high affinity antibody might have been present, but undetected, in the form of complexes in antigen excess and there would be no immune elimination of antigen. In the 6 to 10 hr during which excess RGG-I^{131} circulated before skin testing was performed, most of the putative high affinity antibody should have combined with its antigen to form soluble complexes. There was no reason to believe that these complexes would accumulate preferentially at the skin test site nor that high affinity antibody would dissociate from its circulating antigen and associate with its skin test antigen during the development of the lesion. On the other hand, the demonstration of delayed hypersensitivity by a positive skin reaction in the presence of circulating antigen might have indicated that the amount of RGG-I^{131} given intravenously was insufficient to desensitize the host. Under these circumstances it would be unlikely that antigen was present in excess and, if so, high affinity antibody should have caused the detectable elimination of the RGG-I^{131}.

SUMMARY

Delayed hypersensitivity was produced in donor Lewis rats by sensitization with soluble protein antigens emulsified in complete Freund's adjuvant. Cells of their thoracic duct lymph were collected for varying periods of time and transferred intravenously to isogenic Lewis recipients. With this model the following conclusions were reached:

1. Delayed hypersensitivity was transferred by thoracic duct cells.

2. The longer the drainage of the thoracic duct, the fewer cells were needed to achieve a successful transfer. With continuing drainage the proportion of small lymphocytes decreased and large cells increased. There was, therefore, a better correlation between successful transfer of delayed hypersensitivity and the number of large cells transfused than between positive skin reactions and transfer of small lymphocytes.

3. Prolonged fistula of the thoracic duct did not diminish the skin reaction of sensitized donors to specific antigen.

4. Delayed hypersensitivity was elicited in recipients 3 wk after transfer of sensitized cells. There was evidence that delayed hypersensitivity was enhanced in recipients, possibly because of prior skin testing.

5. Total body X-irradiation abolished the lesions of passively transferred

delayed hypersensitivity. Recovery of positive skin tests was observed 19 to 20 days later.

6. The lesions of delayed hypersensitivity were probably mediated by cells. There was no evidence that a circulating high affinity antibody played a role in this type of immunologic reaction.

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