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Research Article

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Studies of Lymphocyte Transfer Reactions in Hodgkin's Disease *

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In Hodgkin's disease there is an early and consistent depression of delayed (cell-mediated) hypersensitivity (1–3). The mechanism of this immunological defect is unknown, and its elucidation may throw light on the process of cellular hypersensitivity and on the pathogenesis of Hodgkin's disease. Recently, Gray and Russell (4) have described a lymphocyte transfer technique for screening donor/recipient compatibility in human homotransplantation in which purified lymphocytes are transferred from the proposed recipient into the skin of a panel of proposed donors. The test is closely modeled after a similar technique employed by Brent and Medawar (5, 6) in guinea pigs. In essence it is assumed that the reaction of the transferred recipient lymphocytes against the proposed donor skin (a graft-versus-host phenomenon) is based on histocompatibility differences and that the intensity of this reaction predicts the severity of the ensuing host attack on the grafted organ. In the guinea pig this conclusion appears justified, but at this time critical evaluation of the human lymphocyte transfer reaction is incomplete.

Recognizing the shortcomings imposed by limited knowledge about the normal lymphocyte transfer reaction in man, we still felt that the Hodgkin's patient offered a unique situation for study of the phenomenon. This paper represents the conclusions drawn from 85 transfers of Hodgkin's and normal peripheral lymphocytes to Hodgkin's and non-Hodgkin's individuals. Although many of the questions raised by the present work remain unanswered, it has given additional insight into the mechanism of the human lymphocyte transfer reaction and has suggested a lymphocyte defect in Hodgkin's disease.

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Methods

Selection of patients. Hodgkin's disease patients selected as either donors or recipients, with one exception, were the same patients employed in an earlier study (7) where anergy had been established by failure to develop skin sensitivity following the application of dinitrochlorobenzene. The immunological status of the one exception, Hodgkin's recipient BC, was not established. Donors with a history of jaundice were not used. Patients with terminal disease were not studied, but four Hodgkin's patients had obviously far advanced disease when investigated (confirmed by their death within 3 months of testing) and are therefore considered in a separate table from the remainder of the Hodgkin's recipients.

Non-Hodgkin's recipients of Hodgkin's lymphocytes were volunteers with metastatic cancer of breast or kidney origin (specified in the tables). These recipients had proven metastatic neoplasm, but were in good general health, ambulatory, and without debility or weight loss. Other investigators have demonstrated (8, 9) that such cancer patients are not anergic inasmuch as they respond to a battery of delayed allergens (diptheria toxoid, streptokinase-streptodornase, mumps skin test antigen, *Trichophyton gypseum, Candida albicans,* and tuberculin PPD) in the same way as a control population. In the present work only three of the non-Hodgkin's recipients (VS, EA, and MT) were studied in this way, but all reacted strongly to one or more of these allergens.

Preparation of lymphocytes. Only minor modifications have been made in the technique of Gray and Russell (4) for the preparation of human lymphocytes. The initial volume of blood to be defibrinated was increased to 50 ml, but the defibrinated blood was diluted as these authors suggest in the ratio of 1 ml of 3.5% polyvinylpyrrolidone (PVP, mol wt 25,000) to 2.5 ml blood. The diluted blood was allowed to settle for 90 minutes at room temperature. The buffy coat obtained from this sedimentation was centrifuged (1,500 rpm for 5 minutes), resuspended, and recentrifuged (800 rpm for 5 minutes) without modification (4).

When employing blood from Hodgkin's patients, separation of lymphocytes and granulocytes on the final centrifugation is variable, and considerable judgment is re-

quired in selecting the appropriate part of the supernatant for the lymphocyte preparations. With some Hodgkin's samples lymphocyte preparations of excellent purity were obtained from this supernatant, whereas with others essentially all the lymphocytes were present in the upper part of the centrifuged layer and had to be recovered to obtain an adequate yield. At best, Hodgkin's disease blood with high and variable sedimentation rates, high polymorphonuclear and platelet counts, and low lymphocyte counts (10) is a poor source for lymphocyte preparations of high purity.

Reading of tests. In Tables I to V the induration in millimeters of the averaged longitudinal and transverse diameters of the skin reactions is entered, and erythema without induration or exceeding induration is entered as a number within parentheses. Where unspecified by

TABLE I

Effect of volume and cell number on the lymphocyte transfer reaction

No.	Recipient	Donor	Volume	No. of lympho-cytes	Lympho-cytes	Diameter* of test	
						48 hours	7 days
			ml	$\times 10^6$	%	*mm*	*mm*
1.	AA (Normal)	BW (Normal)	0.05	3.2	68	5	7
			0.10	6.5		6	8
			0.20	13.0		7	9
		AA (Normal)	0.20	13.4	76	0	0
2.	VS (Cancer)	AA (Normal)	0.02	2.0	51	0(5)	6
			0.05	5.0		?5	6
			0.10	10.0		?5	7
		BW (Normal)	0.02	2.5	65	5	5
			0.05	6.0		4	5
			0.10	12.5		7	7
		PM (Hodgkin's)	0.02	3.0	77	5	0 (4)
			0.05	7.2		6	0 (6)
			0.10	14.5		5	0 (5)
3.	EA (Cancer)	AA (Normal)	0.05	5.5	58	4	7
			0.10	11.0		5	8
		BW (Normal)	0.05	6.0	53	1	8
			0.10	12.0		2	8
		LS (Hodgkin's)	0.05	0.5	5	0	4
			0.10	1.0		3	5
			0.15	1.5		4	5
4.	CC (Cancer)	AA (Normal)	0.05	3.3	68	12 (16)	7
			0.15	9.9		11 (15)	8
		BW (Normal)	0.05	3.8	52	9	7
			0.15	11.4		11	8
		EM (Hodgkin's)	0.05	1.5	69	6	5
			0.15	4.5		7	7
		RS (Hodgkin's)	0.05	1.0	15	7 (10)	5
			0.15	3.0		7 (11)	6
5.	LS (Hodgkin's)	AA (Normal)	0.05	5.0	69	2	5
			0.10	10.0		2	4
			0.20	20.0		4	9
		BW (Normal)	0.05	7.5	75	4	8
			0.10	15.0		4	5
			0.20	30.0		6	11
		LS (Hodgkin's)	0.15	2.4	39	0	0
6.	PM (Hodgkin's)	AA (Normal)	0.05	4.4	82	10 (20)	6
			0.15	13.2		10 (22)	8
		BW (Normal)	0.05	5.6	64	8	10
			0.15	16.5		9	10
		PM (Hodgkin's)	0.20	4.8	72	0	0
7.	RS (Hodgkin's)	AA (Normal)	0.05	3.3	68	6	5
			0.15	9.9		9	8
		BW (Normal)	0.05	3.8	52	5	5
			0.15	11.4		6	5
		EM (Hodgkin's)	0.05	1.5	69	7	5
			0.15	4.5		7	6
		RS (Hodgkin's)	0.10	2.0	15	0 (?3)	0

* Average diameter of induration. When erythema exceeds induration, this is noted separately in parentheses; otherwise induration and erythema are coextensive. A question mark indicates barely perceptible induration or faint erythema.

TABLE II

Lymphocyte transfers to recipients with early and moderately advanced Hodgkin's disease

No.	Recipient	Donor	No. of lympho-cytes	Lympho-cytes	Interval*	Diameter† of test		
						48 hours	7 days	11–14 days
			$\times 10^6$	%	*weeks*	*mm*	*mm*	*mm*
1a.	LS	AA (Normal)	5.0	69		2	5	3
	LS	BW (Normal)	7.5	70		4	8	6
1b.	LS	AA (Normal)	4.2	100	10	5	8	6
	LS	BW (Normal)	3.6	100	10	3	10	7
	LS	PM (Hodgkin's)	4.5	98	10	5	9	0 (5)
1c.	LS	AA (Normal)	5.0	100	15	4	3	0
	LS	DG (Normal)	4.2	96	15	3	4	0
	LS	EM (Hodgkin's)	2.1	100	15	4	0 (?2)	0
	LS	SP (Hodgkin's)	3.2	62	15	5	0 (?2)	0
2.	RP	AA (Normal)	4.4	82		7	14	
	RP	BW (Normal)	5.6	64		5	10	
	RP	PM (Hodgkin's)	4.8	72		7	0 (2)	
3a.	PM	AA (Normal)	4.4	82		10 (20)	6	
	PM	BW (Normal)	5.6	64		8	10	
3b.	PM	AA (Normal)	3.4	90	22	6	8	6
	PM	DG (Normal)	1.7	100	22	5	5	?4
	PM	RP (Hodgkin's)	2.9	50	22	8	0 (5)	0 (?5)
	PM	SP (Hodgkin's)	2.1	64	22	4	0 (?3)	0 (?3)
3c.	PM	RW (Normal)	3.3	92	2	7	10	10‡
	PM	EA (Cancer)	2.1	82	2	5	4	3
4.	RS	AA (Normal)	3.3	68		6	5	
	RS	RW (Normal)	3.8	52		5	5	
	RS	EM (Hodgkin's)	4.5	69		7	6	
5.	EM	AA (Normal)	3.1	78		9 (20)	5 (7)	
	EM	BW (Normal)	3.6	92		7	4	
	EM	RP (Hodgkin's)	1.5	53		7	5	
	EM	HJ (Hodgkin's)	0.5	54		?4	0 (4)	
6.	BC	AA (Normal)	3.2	89		6 (15)	9	7
	BC	GF (Normal)	2.4	90		6	8	7
	BC	ML (Hodgkin's)	4.2	84		6 (14)	5	0 (5)
7a.	WD	AA (Normal)	2.2	89		9	6	0 (?4)
	WD	GF (Normal)	2.4	90		9	7	6
	WD	ML (Hodgkin's)	4.1	84		9	7	0 (?4)
	WD	HJ (Hodgkin's)	1.3	78		?2	0 (2)	0
7b.	WD	RW (Normal)	3.3	92	6	7	5	0 (?4)
	WD	EA (Cancer)	2.1	82	6	9	9	0 (?4)
8.	SP	AA (Normal)	5.1	98		8 (11)	9	9
	SP	BW (Normal)	4.6	96		5	8	6
	SP	RP (Hodgkin's)	2.4	90		3	0 (2)	0
	SP	RS (Hodgkin's)	3.2	92		3	0 (2)	0

* Time in weeks from the last previous lymphocyte transfer.
† See Table I.
‡ Induration undiminished at 21 days.

parentheses, induration and erythema are coextensive. Erythema without induration and induration 2 mm or less in diameter were not considered significant (11). Considerable preliminary exploration demonstrated that readings at 48 hours, at 7 days, and at 11 to 14 days were necessary for the purposes of the present investigation. In several of the early transfers the last reading was not made, with consequent difficulty in interpretation of these tests.

Results

Preliminary findings. The results of 85 lymphocyte transfers involving 12 Hodgkin's recipients (Tables II and III) and six non-Hodgkin's in-

dividuals (Table IV) are presented in detail in Tables I to IV and summarized in Table V. Although Table V presents an over-all summary of the work, it is only by examination of Tables II to IV that differences in reactivity between Hodgkin's and normal lymphocytes in the same recipient can be appreciated.

Table I contains the results of 18 homologous transfers in which two or more volumes and numbers of lymphocytes were injected into the same recipient. The data indicate that between the range of 2 to 20 million cells and .05 to .20 ml, cell number and volume have little influence on the magnitude of the transfer reaction. Gray and Russell have reported similar findings (4). The four autologous controls reported in Table I were also negative.

For reasons outlined in the previous section it was not possible to obtain lymphocyte preparations of as high purity from Hodgkin's patients as from normal individuals. Technique improved during the course of the present work, and the percentage of lymphocytes exceeded 80% in the later transfers. In looking over the results with this point in mind, i.e., possible false reactions due to excessive contamination of lymphocyte preparations, there appears to be little correlation between contamination and reactions at any time period.

Transfer reaction at 48 hours. In reviewing the transfer reactions at 48 hours (Table V), the difference between the behavior of Hodgkin's and normal lymphocytes is not impressive, although normal lymphocytes do tend to give a stronger reaction than Hodgkin's cells in Hodgkin's recipients. Thus 17 of 33 transfers of normal cells to Hodgkin's patients gave strong reactions, and only six failed to react, whereas only six of 22 transfers of Hodgkin's cells to Hodgkin's patients gave strong reactions and nine failed to react. However, the impression gained from a review of the 48-hour reactions (Tables II to IV) is that a particular recipient tends to have reactions of similar magnitude regardless of the lymphocyte source. Thus two transfers to non-Hodgkin's recipients (4 and 6 in Table IV) displayed strong reactions to the various donor lymphocytes, whereas other non-Hodgkin's recipients reacted less strongly. Among the Hodgkin's recipients (Tables II and III) there were also patients who displayed good 48-hour reactions to both Hodgkin's and normal lymphocytes and some who reacted poorly. Three of the poorest 48-hour

TABLE III

Lymphocyte transfers to recipients with far advanced Hodgkin's disease*

No.	Recipient	Donor	No. of lympho-cytes	Lympho-cytes	Inter-val†	Diameter† of test		
						48 hours	7 days	11–14 days
			$\times 10^6$	%	*weeks*	*mm*	*mm*	*mm*
1.	RB	AA (Normal)	4.0	88		2		
2.	AML	AA (Normal)	1.1	98		0	4	
	AML	BW (Normal)	7.0	99		2 (3)	8	
	AML	PM (Hodgkin's)	4.5	96		2 (3)	0	
	AML	RP (Hodgkin's)	1.8	90		0	0	
3.	HJ	AA (Normal)	1.6	86		2	0	6‡
	HJ	AA (Normal)	8.0	86		?4	?4	8‡
	HJ	WD (Hodgkin's)	3.5	86		?4	2	0
	HJ	WD (Hodgkin's)	7.0	86		?4	0 (3)	0
4a.	AW	AA (Normal)	3.7	98		0 (3)	6	6
	AW	BW (Normal)	4.1	93		2 (3)	7	8
	AW	PM (Hodgkin's)	2.7	86		5	0 (5)	0 (?5)
	AW	WD (Hodgkin's)	3.8	88		?4	0 (4)	0 (?4)
4b.	AW	RW (Normal)	4.3	100	1	6	7	8
	AW	LS (Hodgkin's)	1.5	100	1	0(3)	0 (?3)	0 (?4)
4c.	AW	AA (Normal)	3.3	90	2	7	5	
	AW	EM (Hodgkin's)	3.6	80	2	2 (4)	0 (4)	

* Died within 3 months of lymphocyte transfer.
† See Table II.
‡ Induration undiminished at 21 days.

TABLE IV

Lymphocyte transfers to non-Hodgkin's recipients*

No.	Recipient	Donor	No. of lympho-cytes	Lympho-cytes	Inter-val†	Diameter† of test		
						48 hours	7 days	11–14 days
			$\times 10^6$	%	*weeks*	*mm*	*mm*	*mm*
1.	VS	AA (Normal)	5.0	51		?5	6	
	VS	BW (Normal)	6.0	64		5	5	
	VS	PM (Hodgkin's)	7.2	77		5	0 (5)	
2a.	AA	BW (Normal)	6.4	68		5	7	
2b.	AA	BW (Normal)	5.4	100	24	5	9	0 (?5)
2c.	AA	BW (Normal)	4.5	94	2	5	0 (4)	0
2d.	AA	RW (Normal)	4.8	98	1	6	8	0
3a.	EA	AA (Normal)	5.5	58		4	7	
	EA	BW (Normal)	6.0	53		1	8	
3b.	EA	AA (Normal)	3.0	98	24	4	0 (?4)	0
	EA	RW (Normal)	4.8	96	24	5	6	?5
	EA	ML (Hodgkin's)	4.0	66	24	3	0 (?3)	0
	EA	EM (Hodgkin's)	1.1	100	24	5	6	0
4.	CC	AA (Normal)	3.3	68		12 (16)	7	
	CC	BW (Normal)	3.8	52		9	7	
	CC	EM (Hodgkin's)	4.5	69		6	6	
	CC	RS (Hodgkin's)	3.0	15		7 (10)	5	
5a.	MT	AA (Normal)	3.4	90		?3	5	0 (?5)
	MT	DG (Normal)	1.7	100		6	4	0
	MT	RP (Hodgkin's)	2.9	50		0 (5)	0 (4)	0
	MT	SP (Hodgkin's)	2.1	64		5	0 (?4)	0
5b.	MT	AA (Normal)	2.8	98	6	5	0	0
	MT	RW (Normal)	3.3	100	6	4	0 (4)	0
6a.	LR	AA (Normal)	3.6	84		9	8	7
	LR	RW (Normal)	4.6	96		7	8	6
	LR	LS (Hodgkin's)	1.1	84		8	0 (6)	0 (?5)
	LR	RS (Hodgkin's)	2.2	84		10	0 (8)	0 (?7)
6b.	LR	AA (Normal)	5.2	80	1	6	0 (4)	0
	LR	EM (Hodgkin's)	2.4	60	1	6	0 (5)	0
6c.	LR	BW (Normal)	5.0	100	1	7	5	0

* Recipients VS, EA, and MT had metastatic carcinoma of the breast; recipients BC and LR had metastatic hypernephroma. All were ambulatory and in good clinical condition.

† See Table II.

reactors were among the Hodgkin's recipients in very poor condition who died within 3 months of testing (recipients 1, 2, and 3 in Table III).

Transfer reactions in Hodgkin's recipients at 7 and 11 to 14 days. The transfer reactions at the two later time intervals (7 and 11 to 14 days) gave the results of most interest and can best be presented together. In the Hodgkin's recipients presented in Tables II and III and summarized in Table V, the greater intensity of the transfer reaction of normal lymphocytes (in comparison with Hodgkin's cells) that was suggested at 48 hours was quite marked at 7 days and even more conclusive at 11 to 14 days. Thus 16 of 21 transfers of Hodgkin's lymphocytes gave no induration at 7 days, whereas only one of 31 transfers of normal lymphocytes to Hodgkin's patients gave a negative reaction at this time. Similarly, the 14 Hodgkin's transfers to Hodgkin's recipients available at 11 to 14 days were all negative, but only six of 22 normal lymphocyte transfers to these recipients displayed no significant reaction at this time. As the individual transfers of Tables II and III demonstrated, this difference between Hodgkin's and normal lymphocytes was quite definite, since the transfers that gave the most equivocal results, No. 4 and 5 of Table II, were done before the importance of following the reaction to the end of the second week was realized. The difference in reaction between normal and

TABLE V

Summary of lymphocyte transfer reactions

Donor	Recipient	Time	No. of transfers*	Diameter of induration			
				0–2	3–5	>5	Average
				mm	*mm*	*mm*	*mm*
Normal	Non-Hodgkin's	48 hours	14	2	7	5	5.5
		7 days	14	1	3	10	5.1
		11–14 days	9	7	0	2	1.4
Normal	Hodgkin's	48 hours	33	6	10	17	5.0
		7 days	31	1	11	19	6.6
		11–14 days	22	6	2	14	4.6
Hodgkin's	Non-Hodgkin's	48 hours	10	1	4	5	5.5
		7 days	10	7	1	2	1.7
		11–14 days	7	7	0	0	0
Hodgkin's	Hodgkin's	48 hours	22	9	7	6	3.7
		7 days	21	16	2	3	1.6
		11–14 days	14	14	0	0	0

* Includes all transfers in Tables II to IV except repeat transfers from the same donor to the same recipient.

Hodgkin's lymphocytes is illustrated in Figure 1, a photograph of the 7-day reaction in Hodgkin's recipient 8 of Table II.

Transfer reactions in non-Hodgkin's recipients at 7 and 11 to 14 days. A difference between the behavior of normal and Hodgkin's lymphocytes at later time intervals (similar to the difference observed in Hodgkin's recipients) was also seen in non-Hodgkin's individuals (Tables IV and V). Thus seven of ten Hodgkin's transfers gave insignificant reactions at 7 days, and none of the seven transfers available at 11 to 14 days gave any reaction. Conversely, only one of 14 transfers of normal lymphocytes to normal (non-Hodgkin's) individuals was negative at 7 days.

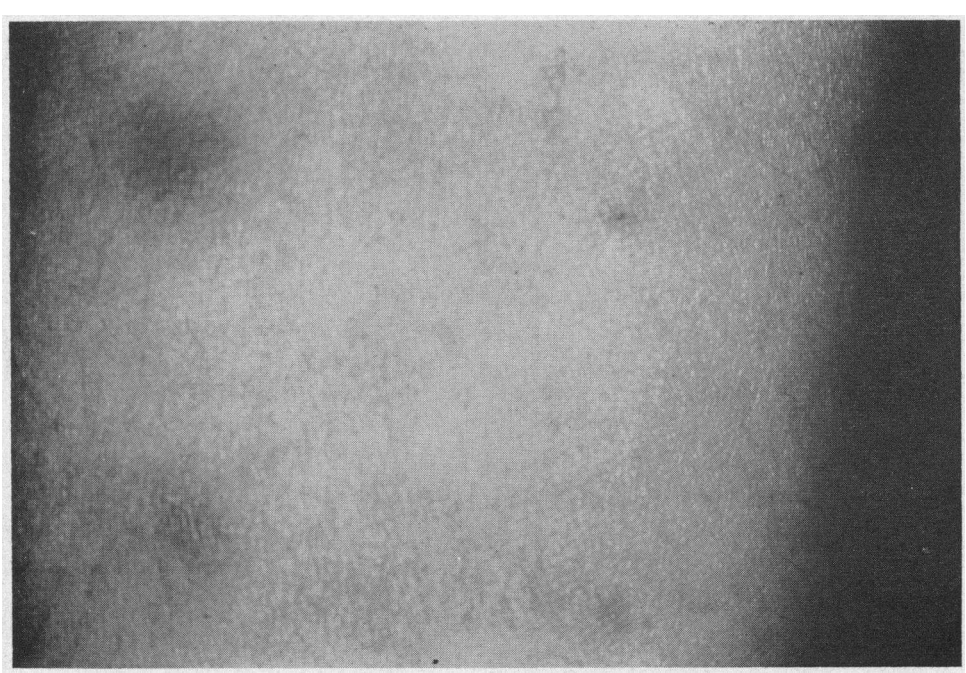

FIG. 1. THE 7-DAY SKIN REACTIONS IN HODGKIN'S RECIPIENT 8 (TABLE II). Reaction in upper left from donor AA (normal), lower left from BW (normal), upper right from RP (Hodgkin's disease), and lower right from RS (Hodgkin's disease).

Comparison of Hodgkin's and non-Hodgkin's recipients at 7 and 11 to 14 days. Another point of interest emerges when the results of transfers of normal lymphocytes to non-Hodgkin's and Hodgkin's recipients are compared. Fourteen of 22 normal lymphocyte transfers to Hodgkin's patients remained strongly positive at the 11- to 14-day observation, but only two of nine normal transfers to non-Hodgkin's recipients were positive at this time. Indeed, two of the normal lymphocyte transfers to Hodgkin's patients were still positive at the end of the third week (Table II, transfer 3c; Table III, transfer 3), a noteworthy occurrence since Gray and Russell (4) report that most normal reactions have disappeared by the eighth day. Thus the transfer reactions of normal lymphocytes persist for a longer time in the Hodgkin's patient than they do in the non-Hodgkin's. In non-Hodgkin's recipients, normal lymphocytes are much more reactive than Hodgkin's cells at 7 days, but at 11 to 14 days the reactions of both cells have regressed.

Repeated transfers to the same recipient. Although it was not the aim of the present work to study in detail the behavior of lymphocytes transferred from one normal individual to another, certain points did require clarification. For practical reasons (the desire to obtain a maximal amount of information from a limited number of patients) it was desirable to use recipients on more than one occasion. For this reason, it was necessary to obtain at least preliminary information on the results of repeated transfers to normal (non-Hodgkin's) individuals, since at the present time these data are not available in the literature. Repeated transfers of lymphocytes from the same normal individual to the same recipient never led to an increased reaction upon the second transfer, and on a number of occasions led to a markedly abbreviated reaction (Table IV, recipients 2, 3, 5, and 6). Repeat transfers involving a second donor and the same recipient were done only a few times, but in these few there was no clear evidence that the first reaction had influenced the second.

Discussion

Although the transfer of lymphocytes from one individual to another is operationally simple, on a theoretical level it represents a most complex phenomenon. Immunological reactivity in two directions is possible, either from grafted lymphocytes against host or from host against graft. Both of these reactions could be expected to be modified or determined by the respective histocompatibility antigens of host and graft, and in both, reactivity residing in donor and host lymphoid cells at the time of transfer and that developing after the delay required for sensitization in immunological systems must be considered. Finally, the strong possibility exists that at least part of the skin reaction seen after lymphocyte transfer is not immunological in nature.

Before proceeding with the major argument, a second point should be considered: the immunological status of the non-Hodgkin's recipient. The normal immunological status of the metastatic cancer patient in good general condition is assumed only on the basis of the reactivity to a battery of skin allergens which, in patients of this type, parallels that of a normal population. In point of fact only three of our five non-Hodgkin's recipients were evaluated in this way, a defect of the present experiments.

With this in mind it is significant that a difference in reactivity between lymphocytes of the anergic Hodgkin's patient and the normal could be observed. This difference was not clearly seen at 48 hours, but was quite evident at 7 and 11 to 14 days, when the reaction of the Hodgkin's lymphocyte was observed to fade out and the normal lymphocyte reaction persisted. The difference between the Hodgkin's and normal lymphocyte was more conclusive in the Hodgkin's than in the non-Hodgkin's recipient. This is probably related to the longer and more complete evolution of the reaction of transferred normal cells in the anergic Hodgkin's recipient; this permits a longer period after subsidence of the initial reaction of the Hodgkin's cells when the persisting reaction of the normal cells can be observed. If we posit an important role of lymphoid blood cells in the mediation of delayed hypersensitivity [for which there is considerable evidence (12–15)], the lymphocyte defect manifest in the abnormal transfer reaction of Hodgkin's cells presumably contributes to the depression of delayed hypersensitivity seen in active Hodgkin's disease. The depression of circulating

lymphocytes reported in Hodgkin's disease (10) may also contribute to the anergy, but does not influence the transfer reaction, since equal numbers of normal and Hodgkin's cells have been employed in the present experiments. [A preliminary report on lymphocyte transfer reactions in uremic subjects suggests a defect in uremic lymphocytes (16)].

A second abnormality emerges when the Hodgkin's patient is compared with the non-Hodgkin's as recipient rather than as lymphocyte donor. A group of Hodgkin's patients, three of the four with far advanced disease, displayed depressed 48-hour reactions to both normal and Hodgkin's lymphocytes. Finally, the reaction of the normal lymphocyte in the Hodgkin's individual was protracted beyond that of normal cells in normal or non-Hodgkin's recipients. This last is reasonably explained by the delayed rejection of the transferred normal lymphocyte in the anergic Hodgkin's recipient, an explanation consistent with the delayed rejection of skin homografts observed in Hodgkin's disease (17–19), and is closely parallel to the enhanced lymphocyte transfer reaction seen in irradiated guinea pig recipients (20). Our observations on the transfer reactions of normal cells in non-Hodgkin's recipients support the idea that sensitization of the host contributes to the termination of the transfer reaction and the corollary that the delay in homograft rejection seen in the Hodgkin's patient results in a protracted reaction of normal transferred lymphocytes, i.e., second transfers of the same donor lymphocytes to the same recipient result in an abbreviated reaction.

The abnormality that the Hodgkin's patient displays as recipient, discussed in the preceding paragraph, is less regular than the defect of the transferred Hodgkin's lymphocyte. This suggests that the two phenomena reflect separate immunological deficiencies or that the first-mentioned is a manifestation of a more advanced anergic state than the latter. It must be conceded that Hodgkin's disease is not an immunological entity, but a clinical condition with varying involvement of the reticuloendothelial system. Although a depression of delayed hypersensitivity is detectable early in the course of the illness before the disease is generalized, there is every reason to believe that the anergy is not complete at the outset and progresses with advancing disease. For this reason the four patients with far advanced Hodgkin's disease were separated from the remainder of the Hodgkin's patients for consideration in the tables. It is possible that the additional defect in the transfer reaction of these far advanced Hodgkin's recipients (depressed 48-hour reaction) reflects the les specific type of anergy seen in the debilitated cancer patient (9).

The present studies raise certain questions about the nature of the normal human lymphocyte transfer reaction. Brent and Medawar (6) consider the early reaction (24 to 48 hours) in the guinea pig to be graft-versus-host, whereas the later reaction (4 days) is assumed to be host-versus-graft. Recently, these workers (20) demonstrated that F_1 hybrid lymphocytes fail to give the early transfer reactions in inbred parental-strain recipients, whereas reactions were obtained with parental lymphocytes transferred to the F_1 hybrid, compelling evidence that in the guinea pig the early reaction is largely graft-versus-host in character (4).

On the basis of the similar behavior of different lymphocyte preparations (from both Hodgkin's and normal donors) in the same recipient (either Hodgkin's or non-Hodgkin's), the present experiments suggest that host rather than donor factors are important in the early lymphocyte transfer reaction. Clearly, we cannot exclude granulocyte contamination or residual immunological reactivity of the Hodgkin's patient from contributing to the early (48-hour) reactions of Hodgkin's lymphocytes. However, immunological reactivity as measured by the ability of the lymphocyte donor to develop sensitization with dinitrochlorobenzene certainly correlates much better with the late (7- and 11- to 14-day) reactions than with the early one. On the basis of the present work it would seem more likely that the early reaction reflects a more primitive type of immunological reactivity or perhaps is not even strictly immunological in nature. Nevertheless, since the early reaction is not seen in autologous transfers, it clearly does have something to do with individuality and by suitable control of host reactivity may still serve as an assay of graft-host histocompatibility (4). If the lymphocyte transfer reaction could be assumed to evolve much more slowly in man than in the guinea pig, much of

the conflict of interpretation between the present work and that of Brent and Medawar (6) could be resolved.

In a number of transfer reactions, among both normal and Hodgkin's recipients, a substantially greater reaction was found at 7 days and later than at 48 hours. This finding is consistent with the development of sensitization against the host within the grafted lymphocytes, although it is certainly open to other explanations.

Thus, in addition to suggesting a qualitative difference in the lymphocytes of patients with Hodgkin's disease, the present investigations are compatible with certain interpretations of the normal lymphocyte transfer reaction. The initial reaction at 48 hours appears to be largely host determined, whether on an immunological or non-immunological basis is not clear. At later time periods this host component subsides, and the 7-day and later reactions appear to be primarily graft-versus-host in nature (arguing from the failure of Hodgkin's disease lymphocytes to react at this time). Finally, sensitization of the host seems to terminate the reaction without any new visible event. It must be recognized that with the theoretical complexities of the lymphocyte transfer reaction, this formulation must remain tentative.

Summary

Eighty-five lymphocyte transfer reactions employing six non-Hodgkin's and 12 Hodgkin's recipients have been studied. The skin reaction of transferred Hodgkin's lymphocytes is only slightly impaired at 48 hours, but is markedly depressed at 7 days and at 11 to 14 days compared with normal lymphocytes transferred to the same recipient. The difference between Hodgkin's and normal lymphocytes is best observed in the Hodgkin's recipient, where the duration of the transfer reaction of normal lymphocytes is abnormally protracted. The defect of the Hodgkin's lymphocyte is presumed to contribute to the cutaneous anergy seen in Hodgkin's disease.

On the basis of the present studies, it is felt most likely that host factors are important in the 48-hour lymphocyte transfer reaction, whereas the 7-day reaction is a graft-versus-host phenomenon. The 48-hour reaction appears to reflect a different (more primitive) type of immunological response than the later reactions; only the later reactions correlate well with the anergy of the lymphocyte donor. Termination of the transfer reaction is believed to be caused by sensitization of the host with rejection of the transferred lymphocytes.

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References

1. Parker, F., Jr., H. Jackson, Jr., G. Fitz Hugh, and T. D. Spies. Studies of diseases of the lymphoid and myeloid tissues. IV. Skin reactions to human and avian tuberculin. J. Immunol. 1932, **22**, 277.
2. Schier, W. W., A. Roth, G. Ostroff, and M. H. Schrift. Hodgkin's disease and immunity. Amer. J. Med. 1956, **20**, 94.
3. Aisenberg, A. C. Hodgkin's disease—prognosis, treatment and etiologic and immunologic considerations. New Engl. J. Med. 1964, **270**, 617.
4. Gray, J. G., and P. S. Russell. Donor selection in human organ transplantation. A possible screening test. Lancet 1963, **2**, 863.
5. Brent, L., J. B. Brown, and P. B. Medawar. Quantitative studies on tissue transplantation immunity. VI. Hypersensitivity reactions associated with the rejection of homografts. Proc. roy. Soc. B 1962, **156**, 187.
6. Brent, L., and P. B. Medawar. Tissue transplantation: a new approach to the "typing" problem. Brit. med. J. 1963, **2**, 269.
7. Aisenberg, A. C. Studies on delayed hypersensitivity in Hodgkin's disease. J. clin. Invest. 1962, **41**, 1964.
8. Sokal, J. E., and N. Primikirios. The delayed skin test response in Hodgkin's disease and lymphosarcoma. Effect of disease activity. Cancer (Philad.) 1961, **14**, 597.
9. Lamb, D., F. Pilney, W. D. Kelly, and R. A. Good. A comparative study of the incidence of anergy in patients with carcinoma, leukemia, Hodgkin's disease and other lymphomas. J. Immunol. 1962, **89**, 555.
10. Wiseman, B. K. The blood pictures in the primary diseases of lymphatic system: their character and significance. J. Amer. med. Ass. 1936, **107**, 2016.
11. Rosenthal, S. R., and J. E. P. Libby. Simultaneous multiple tuberculin testing. Bull. Wld Hlth Org. 1960, **23**, 689.
12. Lawrence, H. S. The delayed type of allergic inflammatory response. Amer. J. Med. 1956, **20**, 428.

13. Waksman, B. H. Delayed hypersensitive reactions: a growing class of immunologic phenomena. J. Allergy 1960, **31**, 468.
14. Waksman, B. H., S. Arbouys, and B. G. Arnason. The use of specific "lymphocyte" antisera to inhibit hypersensitive reactions of the "delayed" type. J. exp. Med. 1961, **114**, 997.
15. Gowans, J. L., D. D. McGregor, D. M. Cowen, and C. E. Ford. Initiation of immune responses by small lymphocytes. Nature (Lond.) 1962, **196**, 651.
16. Bridges, J. M., S. D. Nelson, and M. G. McGeown. Evaluation of the lymphocyte transfer test in normal and uremic subjects. Lancet 1964, **1**, 581.
17. Green, I., M. Inkelas, and L. B. Allen. Hodgkin's disease: a maternal-to-fœtal lymphocyte chimæra? Lancet 1960, **1**, 30.
18. Kelley, W. D., D. L. Lamb, R. L. Varco, and R. A. Good. An investigation of Hodgkin's disease with respect to the problem of homotransplantation. Ann. N. Y. Acad. Sci. 1960, **87**, 187.
19. Miller, D. G. Patterns of immunological deficiency in lymphomas and leukemias. Ann. intern. Med. 1962, **57**, 703.
20. Brent, L., and P. B. Medawar. Nature of the normal lymphocyte transfer reaction. Nature (Lond.) 1964, **204**, 90.