Immunologic Parameters in Childhood Hodgkin's Disease II. T and B Lymphocytes in the Peripheral Blood of Normal Children and in the Spleen and Peripheral Blood of Children with Hodgkin's Disease*

M. DE SOUSA,† C. T. C. TAN, F. P. SIEGAL, D. A. FILIPPA, R. TAN, AND R. A. GOOD

Memorial Sloan-Kettering Cancer Center New York, New York, USA

Summary

A study of peripheral blood lymphocyte populations in 27 children with Hodgkin's disease (HD) and 13 age-matched control subjects is presented. The absolute numbers and percentages of T and B lymphocytes identified by their surface marker characteristics were determined. In addition, in 13 HD children the percentages of T and B lymphocytes were estimated in the spleens removed at staging laparotomy. No differences were observed between the total peripheral blood lymphocyte counts of HD and control children, and we found no evidence of progressive lymphopenia with advancing stages of the disease. No decrease in the numbers of peripheral blood T lymphocytes was seen in this group of HD children. In contrast, the proportions and absolute numbers of B lymphocytes tended to be significantly lower in the children with HD than in the control subjects. In 9 of the 13 spleens studied high percentages of T lymphocytes were seen; low percentages of B lymphocytes were found in all spleens examined.

Speculation

The results are discussed in the light of the hypothesis that in HD an anomaly of the spleen environment is present at the early stages of the disease which results in sequestration of circulating T lymphocytes, and that this anomaly is closely related to the basic pathogenesis of the disease.

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† Requests for reprints should be addressed to: M. de Sousa, M.D., Ph.D., Cell Ecology Laboratory, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021.

INTRODUCTION

The existence of an abnormality of T cell function in Hodgkin's disease is well documented in adult patients (19). The actual mechanism of development of this abnormality, however, is not clear. Some authors have observed a low number of T cells in the peripheral blood (2,3,7), but this finding has not been confirmed (5,6). Progressive overall absolute T and B lymphopenias have however been observed in adult patients with advancing stages of the disease (5,6, 13). In a separate paper we reported the finding of an abnormality of the PHA responses of the peripheral blood lymphocytes in children with HD, irrespective of histopathology or stage of disease, similar to that described for adults (26). The simultaneous finding of normal PHA responses of the splenic lymphocytes was interpreted as indicating that in HD there is an abnormality of lymphocyte ecotaxis (8,26). In the present paper we explore further the question of lymphocyte distribution in HD by comparing the absolute numbers of T and B lymphocytes in the peripheral blood of normal and HD children and defining the proportion of the two major lymphocyte populations in the spleen of the children with HD.

MATERIALS AND METHODS

Peripheral Blood

Control Group (Table I): A total of 13 normal children, 6 males and 7 females, were used as controls for this study. Details of their ages, ranging from 5 to 16 years, and absolute lymphocyte counts are summarized in Table I. Characterization of the surface markers on the peripheral blood lymphocytes was done in all of these control children. Informed consent was obtained for all children.

TABLE I

AGE, SEX, AND TOTAL PERIPHERAL BLOOD LYMPHOCYTE COUNTS IN 13 CONTROL CHILDREN

Control Child	Sex	Age	Total Lymphocyte Count/mm^3
Q.R.	M	5	2975
R.J.	M	6	4515
M.P.	M	10	2490
M.J.	M	13	1775
D.J.	M	15	1914
R.A.	M	16	1452
F.G.	F	6	1224
F.N.	F	8	2146
M.W.	F	9	1320
M.N.	F	10	1392
M.P.	F	11	1440
M.S.	F	12	1911
M.E.	F	14	2580
		Mean ± 1 SD	2087 ± 907

Hodgkin's Disease Group (Table II): A total of 63 children with HD, treated at Memorial Sloan-Kettering Cancer Center between 1970 and 1976, were studied. There were 39 males and 24 females, between the ages of 4 years and 1 month and 16 years and 3 months. Total peripheral blood lymphocyte counts were performed at the time of staging laparotomy prior to treatment. The peripheral blood lymphocyte subpopulations were characterized for surface markers in 27 patients, 15 males and 12 females. Details of stage of disease and histopathology of the children included in this study are summarized in Table II. The distribution of histological types differs between the two sexes. Twenty-one of the 24 females (83%) were nodular sclerosing and no lymphocyte predominant or lymphocyte depleted types were found. Of the 39 males studied, 8 (21%) were diagnosed as lymphocyte predominant (LP), 18 (46%) were nodular sclerosing (NS), and 12 (31%) were mixed cellularity.

TABLE II

DISTRIBUTION OF HD CHILDREN ACCORDING TO DISEASE STAGE AND HISTOLOGY

	Male					Female			
Stage	LP	NS	MC	LD	Total	NS	MC	U	Total
IA	6(2)*	4	2(2)		12(4)	3(1)			3(1)
IB		2(1)			2(1)	2(1)	1		3(1)
IIA	1	2	1(1)		4(1)	5(3)	1		6(3)
IIB		3(1)			3(1)	4(3)		1(1)	5(4)
IIIA	1	4(2)	1(1)		6(3)				0
IIIB		3(2)	4(3)		7(5)	2(2)			2(2)
IVB			4	1	5(0)	5(1)			5(1)
Total	8(2)	18(6)	12(7)	1	39(15)	21(11)	2	1(1)	24(12)

* () numbers had analysis of cell surface markers.

LP = lymphocyte predominant; NS = nodular sclerosing; MC = mixed cellularity; LD = lymphocyte depleted; U = unclassified.

Spleen

Control Group (Table III): No childhood control spleens were available during the course of this study. Control values used throughout the study have therefore been derived from the published control values of studies in which spleens from traumatic cases were analysed (14,18). A summary of the control data is found in Table III.

TABLE III

CONTROL VALUES FOR LYMPHOCYTE SUBPOPULATIONS IN HUMAN SPLEENS

Author(s)	T	Ig Bearing
Greaves et al, 1974 (16)	33%	
Habeshaw and Stuart, 1974 (18)	36.5%	45%

Hodgkin's Disease Group (Table IV): Concomitant analysis of cell surface markers in the peripheral blood and spleen was done in nine children with HD at the time of staging laparotomy. In an additional four patients the spleen, but not the blood, was studied at the time of staging laparotomy. Disease stage and sex of the children included in this portion of the study are summarized in Table IV.

TABLE IV

DISEASE STAGE OF 13 PATIENTS STUDIED FOR THE CONCOMITANT ANALYSIS OF LYMPHOCYTE MARKERS IN PERIPHERAL BLOOD AND SPLEEN

Stage	Males	Females	Total
IA	2(1)*	1	3
IB	2	0	2
IIA	0	1	1
IIB	1	0	1
IIIA	1(1)	0	1
IIIB	2	2(1)	4
IVB	1(1)	0	2
Total	9(3)	4(1)	13(4)

* () = spleen only

Preparation of Lymphoid Cell Suspensions

Suspensions of lymphoid cells were obtained from uninvolved portions of the spleen by teasing pieces gently into RPMI 1640 under sterile conditions. After preparing the suspension the spleen cells were layered on a Ficoll-Hypaque density gradient (d:1.077). Peripheral blood lymphocytes were also obtained by separating 25 ml of heparinized blood (diluted in Hank's Balanced Salt Solution (HBSS, 1:1), on a Ficoll-Hypaque density gradient. Cells removed from the interface were washed three times in HBSS and then cell viability was checked by trypan blue exclusion. Only cell suspensions of more than 95% viability were analysed.

The suspensions were then divided into two separate samples, one to be used for rosette formation and membrane immunofluorescence, and the other for mitogen stimulation. To the former, 100n of 1% latex particles (0.86n diameter, Dow Chemical) were added, the cells were suspended in RPMI 1640 containing 20% foetal calf serum, and then incubated for 30-60 minutes at 37°C. After incubation with the latex particles, the cells were washed twice in RPMI.

Cell Surface Markers

Sheep erythrocyte rosettes were performed according to Bentwich et al (4); mouse erythrocyte rosettes by the method detailed by Gupta et al (17); cells with Fc receptors were detected by their ability to bind aggregated IgG (method detailed in 17) and form Ripley rosettes; i.e. form rosettes with human ORh positive erythrocytes sensitized with IgG anti-Rh of the Ripley type (16).

RESULTS

Lymphocyte Populations in Peripheral Blood of Normal and HD Children (Table V, Figures 1-3)

No differences were observed between the total lymphocyte counts of HD and control children. Moreover, we found no evidence of progressive lymphopenia with advancing stages of the disease. To the contrary, a slight increase in the total peripheral blood lymphocyte count was observed with progressively advanced disease (Table V, Figure 1). This increase in the total peripheral blood lymphocyte count reflected a comparable increase in the total T lymphocyte count (Table V, Figure 2). Cell surface marker analysis was done only in one untreated patient with stage IV disease, who had a total T lymphocyte count of 3471/mm^3, markedly higher than the mean control values.

In contrast, the proportion and absolute numbers of cells which bore surface Ig or were detected by the aggregate binding assay tended to be lower in the children with HD than the controls ($P < 0.05$ for stage I; $P < 0.001$ for stage II, and $P < 0.005$ for stage III, as compared to the controls), the cells detected as carrying Ig on their surface in the system used in the present study include both cells with intrinsic surface Ig (mostly IgM or IgD), the B cells, and cells which carry high affinity receptors for IgGFc. The latter cells are functionally heterogeneous. Cells of the B line were detected by two markers, the spontaneous mouse erythrocyte rosette and the presence of surface IgM. No significant differences from the values seen in normal children were found with the mouse rosette test in patients with HD. Cells having IgM on their surface were depressed in stages I and II ($P < 0.05$) and in III, but not in the single case with stage IV disease.

On the other hand, the cells reactive with human IgG coated erythrocytes (the Ripley rosettes) did not show depression. Thus, most of the decrease in Ig bearing cells appears to be consequent to a specific decrease in B cells which do not form mouse erythrocyte rosettes, but which carry surface IgM.

In HD patients there was a somewhat higher proportion of cells with no detectable markers than in normals. This was, however, not because of decreased proportions of T cells, but because of decreased proportions of Ig-bearing cells, both μ-bearing and those carrying other classes. The present findings were equally true whether expressed as absolute lymphocyte counts or as percentages (Table V).

TABLE V

PERCENTAGE DISTRIBUTION OF LYMPHOCYTE SURFACE MARKERS IN NORMAL AND HD CHILDREN

	Control	Hodgkin's Disease Stage I	Stage II	Stage III	Stage IV
Sheep R	74.4±31†	70.4±24 (6)*	76.2±15.3 (8)	78 ±10.4 (9)	89 (1)
SIg	22±5	10.5±5 (6)	13.75±7.4 (8)	11.5±4.5 (10)	12.5 (1)
SIgM	9.7±3.9	6.1±3.7 (5)	6.4±2.5 (4)	6 (1)	6 (1)
IgG	24±4.7	12.6±5.8 (4)	18.7±6.7 (4)	10.5±0.7 (2)	13 (1)
Ripley R	10.3±3.6	7.63±1.3 (4)	13.8±7 (4)	5.2±1 (2)	10.5 (1)
Mouse R	3.5±1.5	2±1.4 (2)	2.1±0.8 (4)	4.25±3 (2)	2.5 (1)

† = mean ± 1 SD. * = number of patients.

Concomitant Analysis of Peripheral Blood and Spleen Lymphocyte Populations (Table VI)

Concomitant analysis of peripheral blood and spleen lymphocyte populations was done in nine HD patients. In four additional patients, the spleen, but not the blood, was studied. All spleen cell suspensions studied were from histologically uninvolved sections of the spleen.

The finding of low percentages of T cells in the blood in three cases (23.5%, 45.5%, and 62.5%) was not accompanied by a simultaneous decrease in the percentage of T cells found in the spleen (61%, 51%, and 83%, respectively). On the contrary, in eight of the thirteen spleens of HD patients examined, percentages of T cells higher than those reported for normal human spleens (Tables III and VI) were found (range 56-75%). Low percentages of splenic T cells were observed in patients S.M., V.G., R.P. and L.F. In three of these four patients, disease involvement was observed in extra-lymphoid sites, namely bone (V.G. and L.F.) and lung (R.P.).

On the whole, low percentages of B lymphocytes (7-17%) were found in HD patients who had low percentages of T cells. The finding of low percentages of B cells in the spleen paralleled the concomitant B cell depletion of the blood (Table V). One striking finding was that in only five of eleven cases did the percentages of T and B lymphocytes account for the majority of splenic lymphoid cells.

TABLE VI

PERCENTAGE DISTRIBUTION OF T AND B LYMPHOCYTES IN PERIPHERAL BLOOD AND SPLEENS IN HD CHILDREN

			T		B (SIg)		B (IgM)	
Stage	Patient	Histology	Blood	Spleen	Blood	Spleen	Blood	Spleen
IA	G.E.	LP	23.5	61	14	-	-	-
	N.M.	LP	-	56	-	34	-	15
	S.M.	NS	84.5	35	3.5	11	2.5	6
IEB	M.B.(lung)*	NS	76	61	15	14	2	11
	J.V.	MC	65.5	76	11.5	17	9.5	3
IIA	J.S.	NS	45.5	51	-	-	-	-
IIEB	V.G.(bone)*	NS	80	45	3.5	12	4	8
IIIA	G.Z.	NS	-	51	-	25	-	-
IIIB	A.S.	MC	83.5	53	18	33	8	20
	C.D.	NS	62.5	83	9	16	3	10
	J.A.	NS	-	69	-	7	-	-
IIIEB	R.P.(lung)*	NS	64.5	19	10	36	6	23
IVB	L.F.(bone)*	MC	-	45	-	49	15	36
Mean ± 1 SD			65 ±20	54.2 ±16.9	10.4 ±5.2	23 ±13.2	6.5 ±4.4	14.6 ±10.3

* site of extra-lymphoid involvement by disease.

DISCUSSION

A number of explanations based on different ovservations have been used for the association of abnormal T cell function with HD in adult patients. Several of these different observations are: a) low numbers of peripheral blood lymphocytes (1) and T lymphocytes (2,3,7); b) the finding of functionally deficient populations of T cells at all stages of HD (5,6,7,21,22,26); c) the finding of autoantibodies against T lymphocytes (15); and d) the presence of serum factors capable of inhibiting E-rosette formation (12,23).

In our present series of studies of immunological function in children with HD (8,26), we have been investigating the additional possibility that the known anomalies of lymphocyte number and function in the peripheral blood reflect a maldistribution of T lymphocytes in the body rather than an absolute T cell deficit. Evidence for a "maldistribution hypothesis" derives from: a) the observation of a dichotomy of the PHA response in peripheral blood and spleen (8,20,22,26); b) finding of increased percentages of T lymphocytes in spleens from HD patients uninvolved by the disease (8,24); and c) failure of lymphocytes from splenectomized patients to respond to inhibition of E-rosette formation by HD serum (12,25).

The present results show that in children with HD there is neither lymphopenia nor a diminished number of T cells in the blood. Failure to observe depressed E-rosette formation in these patients may be the result of technical differences between laboratories or may be related to the fact that our patients are children. In the majority of spleens studied (nine of thirteen), higher percentages of T lymphocytes were present (range 51-83%) in the HD cases than in the literature controls. This finding, together with our previous report of normal PHA response of splenic lymphocytes in the same children (26), supports the interpretation that a sequestration of the PHA responsive T lymphocytes has occurred in the spleen with the concomitant appearance in the peripheral blood of a non-responsive thymus-derived E-rosette population. Further evidence for this view derived from the finding that after splenectomy the pattern of the PHA response in the blood returns to normal in children who remain clinically free of the disease (9).

It was of interest to find that, of the three patients with low T cell numbers in the spleen, two had disease in extra-lymphoid sites. We interpret these findings tentatively to reflect migration of lymphocytes to the disease site.

More intriguing was the finding of a real deficit of B lymphocytes both in spleen and blood. Low absolute counts of B lymphocytes have been reported also in the peripheral blood of adult patients (5,15), but no simultaneous blood-spleen determinations have previously been reported.

These findings illustrate well the value of multicompartmental analysis in discriminating between "real" and "apparent" lymphocyte deficits or deficiencies. In the case of HD, how the real B cell deficit and the T cell maldistribution relate to the pathogenesis of the disease remains unclear.

The knowledge that in HD excessive amounts of ferric iron (10) and of ferritin (11) are found in tissues and the recent observation of Moroz et al (23) demonstrating the presence of ferritin on the surface of T lymphocytes in HD patients, raise the possibility that abnormalities of lymphocyte distribution in HD may be associated with anomalies of iron handling and distribution of iron binding proteins in tissues. We are presently further investigating this possibility.

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General legends for Figures 1, 2, and 3:

Stages:

- ● - IA
- ○ - IB
- ■ - IIA
- □ - IIB
- ▲ - IIIA
- △ - IIIB
- ✣ - IVB

Shaded area represents control range (mean ± 1 SD)

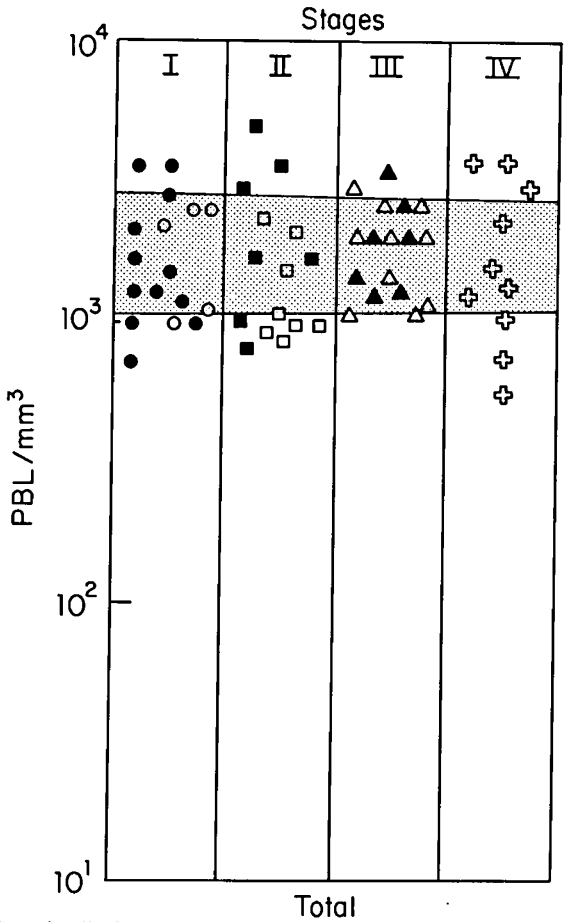

Figure 1: Absolute peripheral blood lymphocyte counts in HD children.

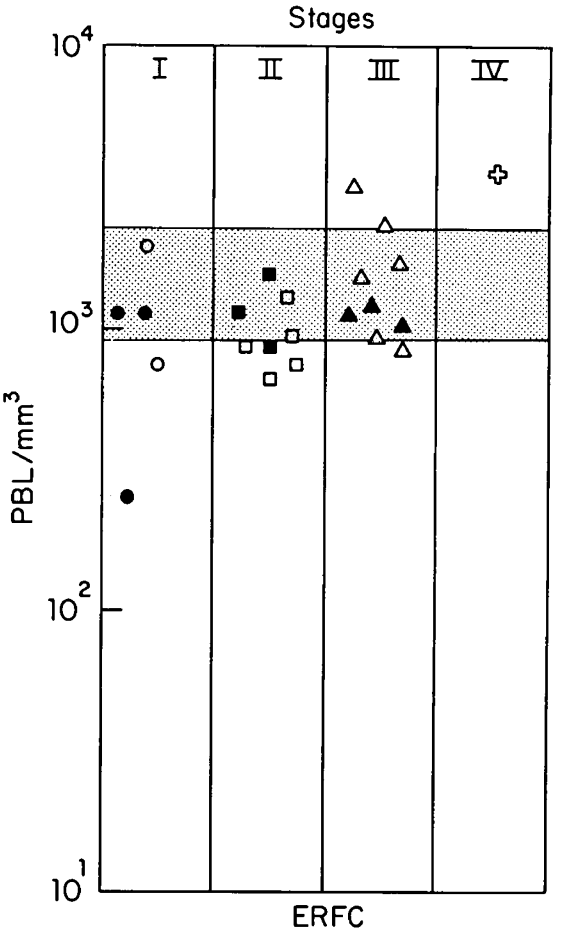

Figure 2: Absolute peripheral blood T lymphocyte counts in HD children, determined by spontaneous rosette formation with sheep erythrocytes (ERFC).

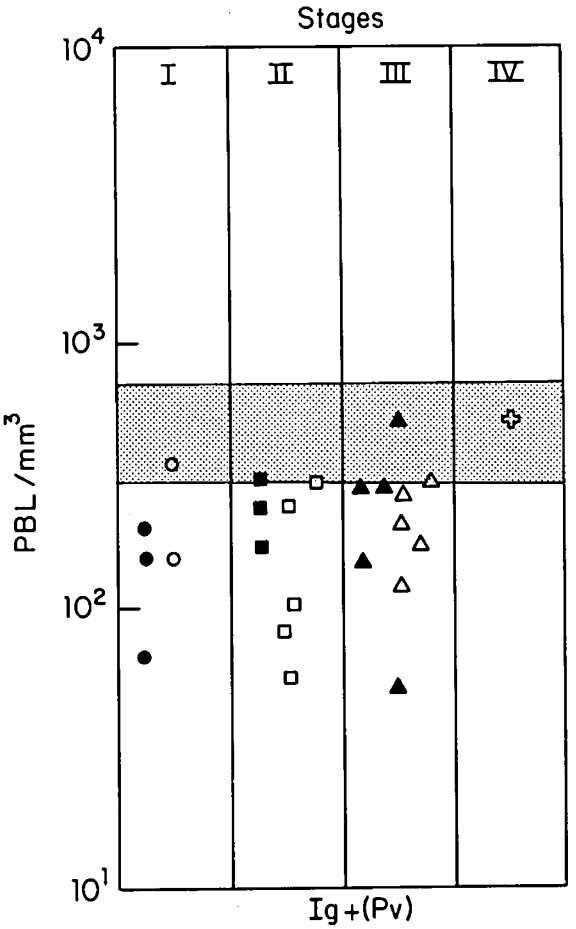

Figure 3: Absolute peripheral blood B lymphocyte counts in HD children, determined by the presence of surface immunoglobulin (Pv = polivalent anti-IgG antiserum).

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