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B and T lymphocytes in Hodgkin's disease

ANALYSIS AT DIAGNOSIS AND FOLLOWING THERAPY

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SUMMARY

B and T lymphocytes were studied in the blood and lymph nodes of fifty patients with Hodgkin's disease. At diagnosis, most patients (77%) had normal percentages of circulating B and T lymphocytes. Most patients (60%) also showed normal percentages of B and T lymphocytes in involved lymph nodes. Splenectomy had no effect on circulating B and T lymphocytes in four of five patients studied 2 weeks postoperatively. Seventeen patients were studied before and after treatment to determine the effect of therapy. Very high percentages of B lymphocytes were found in patients studied following radiotherapy. It appears that the known defects in cell-mediated immunity in Hodgkin's disease are not expressed as significant abnormalities in B-or T-lymphocyte ratios in blood or lymphoid tissues at diagnosis. The proliferation of B lymphocytes following radiotherapy could represent a compensatory mechanism to cell-mediated deficiency or a loss of normal suppressor activity of T lymphocytes.

INTRODUCTION

Patients with Hodgkin's disease have been shown to have defects in cell-mediated immunity (Schier *et al.*, 1956; Good, Kelly & Gabrielsen, 1962). They demonstrate delayed homograft rejection (Kelly *et al.*, 1960) diminished delayed hypersensitivity (Sokal & Primikirios, 1961; Aisenberg, 1962; Chase, 1966; Eltringham & Kaplan, 1973; Young *et al.*, 1973) and impaired lymphocyte function (Hersh & Oppenheim, 1965; Han & Sokal, 1970; Corder *et al.*, 1972; Matchett, Huang & Kremer, 1973; Levy & Kaplan, 1974). In other diseases associated with defects in cell-mediated immunity, abnormalities in the ratio of B and T lymphocytes in the blood have been documented (Gajl-Peczalska *et al.*, 1972; Papamichail *et al.*, 1972; Gajl-Peczalska *et al.*, 1973a). Studies of B and T cells in Hodgkin's disease have given conflicting results, some investigators reporting normal percentages of B and T lymphocytes and other low percentages of T lymphocytes (Cohnen *et al.*, 1973a,b: Froland, 1972; Falleta *et al.*, 1973; Gajl-Peczalska *et al.*, 1973b; Aiuti & Wigzell, 1973; Andersen, 1974; Michlmayr *et al.*, 1974; Ramot *et al.*, 1973; Gallmeier *et al.*, 1973; Kaplan *et al.*, 1974; Kaur *et al.*, 1974). However, quantification of B and T lymphocytes on the same specimen of blood in untreated patients with Hodgkin's disease has rarely been reported. T lymphocytes have generally been reported to be increased in the few lymph nodes from patients with Hodgkin's disease so far studied (Braylan, Jaffe & Berard, 1974; Aisenberg & Long, 1975).

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We have studied B and T lymphocytes in the blood and lymph nodes of thirty-five untreated and fifteen treated patients with Hodgkin's disease. Circulating B and T lymphocytes were studied serially, before and after splenectomy and before and after treatment in order to determine the effect of splenectomy, radiotherapy and chemotherapy. Delayed hypersensitivity and serum immunoglobulin levels were simultaneously studied and correlated with B and T lymphocyte numbers.

MATERIAL AND METHODS

Patients. Fifty patients with Hodgkin's disease, seen by the Medical Oncology Service at the University of Minnesota Hospitals between June 1972 and February 1975, were studied. Lymph nodes from all patients were histologically subclassified according to the method of Lukes, Butler & Hicks (1966). Thirty-seven of the patients were classified as nodular sclerosis, nine as mixed cellularity, one as lymphocyte predominance, two as lymphocyte depletion, and one was unclassifiable. Patients were staged according to the recommendations of the 1971 Ann Arbor Conference on staging in Hodgkin's disease (Carbone *et al.*, 1971). All previously untreated patients underwent staging laparotomy unless they had pathologically documented stage IV disease.

Percentages and absolute numbers of circulating B and T lymphocytes were evaluated in thirty-five untreated patients; seventeen of these patients had follow-up post-treatment studies. In five untreated patients circulating B and T lymphocytes were studied before and after splenectomy. B and T lymphocytes were quantified in the lymph nodes of nineteen patients.

Lymphocyes. Blood from the patients was collected in heparin and incubated with latex particles and iron-containing lymphocyte separating reagent (Technicon Instruments Corporation, Tarrytown, New York) for 30 min at 30° C on a rocker platform. Lymphocytes were separated on Ficoll–Hypaque gradient as previously described (Gajl-Peczalska *et al.*, 1973c). These lymphocytes were evaluated for B-cell markers by immunofluorescence and for T-cell markers by spontaneous rosette formation with sheep red blood cells (SRBC). The white blood cell total and differential counts were simultaneously determined to permit the calculation of the absolute number of circulating B and T lymphocytes.

Immunofluorescence. Monospecific antisera directed against the major heavy chain classes (α, μ, γ) and anti-κ light chain were obtained from goats and conjugated with fluorescein isothiocyanate and methyltetrarhodamine isothiocyanate as previously described (Gajl-Peczalska *et al.*, 1973c). Antisera directed against λ light chain were purchased initially from Meloy Laboratories, Inc. (Springfield, Virginia) and later from Behring Diagnostics. All antisera were checked for specificity by immunoelectrophoresis, gel double diffusion, absorption with appropriate antigens and staining of tissues including myeloma cells of known specificity. Immunofluorescence staining was carried out as previously described (Gajl-Peczalska *et al.*, 1973c). In all cases the evaluation was done with all five monospecific antisera. Two hundred cells were evaluated on every slide using the Zeiss ultra-violet microscope equipped with Ploem vertical illumination and phase contrast.

Increased percentages of B lymphocytes in the blood were defined as greater than 34% B cells, and decreased percentages as less than 11%. Increased numbers of B lymphocytes per mm^3 were defined as greater than 790 and decreased numbers as less than 151. Ninety per cent of the eighty-seven normal controls fell within these limits. We have defined as a monoclonal proliferation of B cells those cases where the percentage of B lymphocytes was increased and 70% or more of these B cells carried on their surface one type of heavy and one type of light chain.

T-(sheep red blood cell) rosette assay. Lymphocytes were mixed with an equal volume of unsensitized washed SRBC (0·5% suspension), incubated for 1 hr at 4°C and the rosettes counted in a haemocytometer as previously described (Kersey *et al.*, 1973). Lymphocytes were considered to be positive when three or more SRBC were bound to the surface of latex-negative cells. Increased percentages of T lymphocytes in the blood were defined as greater than 76% T cells and decreased percentages as less than 44%. Decreased absolute numbers of T cells were defined as less than $800/mm^3$. Percentages of T lymphocytes less than 44% are more than 2 s.d. below the norm.

Tissue study. A portion of lymphoid tissue, obtained at surgery for diagnostic purposes, was gently minced in culture medium and aspirated with a syringe through diminishing gauge needles. The cell suspensions were washed three times and stained with fluoresceinated antisera and evaluated by the T-rosette assay, as described above. The viability of cells was always 90% or more as determined by the exclusion of trypan blue. We have studied twenty histologically normal lymph nodes from patients without Hodgkin's disease. In these, the range of the B lymphocytes was from 12 to 46% (mean 30) and the range of T lymphocytes from 27 to 58% (mean 47).

In sixteen of the patients a portion of the lymphoid tissue was rapidly frozen in isopentane (precooled in liquid nitrogen) and cut in a cryostat. Sections were fixed for 10 min in acetone and stained directly with the same fluoresceinated antisera.

Immunoglobulin determination. Serum immunoglobulins were quantified by single radial immunodiffusion using commercial plates (Hyland Division, Travenol Laboratories, Costa Mesa, California).

Skin tests. Skin tests used for evaluation of T-lymphocyte function were as follows: PPD; mumps; trichophyton; *Candida*; streptokinase–streptodornase. Response (erythema and induration of 5 mm or more at 24–48 hr) to at least one of the antigens was considered a positive result.

RESULTS

Immunological studies in untreated patients

Blood. At diagnosis, twenty-nine out of thirty-five (83%) patients had normal percentages of B lymphocytes in the blood (Table 1). Six out of thirty-five (17%) untreated

TABLE 1. Circulating B and T lymphocytes in untreated patients with Hodgkin's disease

Pt no.	Age Sex	Stage	Histology*	Lymphocytes per mm³	B lymphocytes (%	no./mm³)	T lymphocytes (%	no./mm³)	Skin tests
1	18F	IIIA	NS	2412	25	603	50	1206	+
2	18M	IIA	NS	1787	11	196	56	1001	+
3	12F	IIA	NS	1122	13	145	41	460	−
4	19M	IIIA	NS	1470	13	191	62	911	+
5	27M	IIIA	NS	2548	18	459	n.d.	—	−
6	20M	IIIA	NS	1001	17	170	50	500	+
7	34M	IIIA	NS	696	8	56	60	418	+
8	22F	IIIA	NS	2142	24	514	52	1114	+
9	30F	IIB	NS	2651	21	557	n.d.	—	+
10	26F	II_EA	NS	945	17	161	46	435	n.d.
11	24F	IIB	NS	1512	17	257	55	832	+
12	19F	IIIB	NS	2366	14	331	65	1538	+
13	56M	IIIA	NS	3496	14	489	n.d.	—	+
14	18F	IVB	NS	345	11	40	70	241	+
15	25M	IIB	NS	270	34	92	n.d.	—	+
16	16F	IIIA	NS	2700	17	459	46	1242	+
17	17M	IIA	NS	1683	18	303	47	791	+
18	26M	IIB	NS	1935	22	426	63	1219	+
19	44F	IIA	NS	1550	6	93	61	945	n.d.
20	14F	II_EA	NS	1314	28	368	n.d.	—	+
21	25F	IIIA	NS	4410	31	1367	44	1940	+
22	51M	IVB	NS	973	19	185	59	574	+
23	79F	IIB	NS	1377	25	344	53	730	+
24	40M	IB	NS	3354	15	503	n.d.	—	+
25	19F	IIA	NS	1564	5	78	63	985	+
26	75M	IA	LP	1898	20	380	47	893	+
27	44M	IIA	MC	1690	16	270	60	1014	n.d.
28	17M	IA	MC	1910	24	459	63	1203	+
29	42M	IVA	MC	1802	6	108	n.d.	—	−
30	61M	IIB	MC	1750	4	47	n.d.	—	+
31	58F	IIB	LD	1620	3	49	56	907	−
32	63F	IIIB	Unk	1115	24	267	52	579	+
33	27M	IA	NS	1127	25	282	40	451	+
34	59M	IIIA	MC	2604	12	312	44	1146	−
35	51M	IVB	LD	275	21	58	n.d.	—	−
Controls (mean ± s.d.)					22 ± 8	411 ± 216	60 ± 8	1700 ± 450	

n.d. = Not determined.

* NS = nodular sclerosis; LP = lymphocyte predominance; MC = mixed cellularity; LD = lymphocyte depletion; Unk = unclassifiable.

patients showed decreased percentages of B lymphocytes in the blood. Low percentages of B lymphocytes observed in these patients did not correlate with the stage of disease, histologic diagnosis or the age of the patient. Two out of twenty-six (8%) untreated patients showed decreased percentages of T lymphocytes in the blood.

Decreased absolute numbers of B lymphocytes were found in ten patients; in five (50%) a low percentage of B lymphocytes accounted for this decrease. Low absolute numbers of T lymphocytes/mm^3 were present in ten out of twenty-six patients (38%) and usually related to lymphopenia. In no patient was evidence of a monoclonal proliferation of B cells found. The cells bearing heavy and light chains were usually present in normal proportions.

Lymph nodes. Of fourteen lymph nodes studied at diagnosis eleven showed evidence of Hodgkin's disease (Table 2). In two out of ten (20%) involved lymph nodes studied, the percentage of T lymphocytes was increased. In the other patients the percentage of T lymphocytes fell within the normal range. In two out of ten (20%) the percentage of B lymphocytes was decreased, and in one the percentage of B lymphocytes was increased. In seven out of ten patients with involved lymph nodes studied the percentage of B lymphocytes fell within the normal range. In the two patients where the percentage of B lymphocytes was very low, plasma cells were abundant.

TABLE 2. B- and T-cell evaluation of lymph nodes in patients with Hodgkin's disease

Tissue studied pretreatment			Tissue studied at relapse		
Patient number	Lymph nodes		Patient number	Lymph nodes	
	%B	%T		%B	%T
	Involved node*			Involved node	
1	22	36	36	48	43
2	18	78	37	17	n.d.
5	4	50	38	42	47
6	43	53	39	26	n.d.
7	n.d.	60			
8	26	38			
12	38	n.d.			
21	54	32			
22	8	44			
31	42	56			
35	25	35			
	Uninvolved node			Uninvolved node	
25	24	55	13	96	8
27	49	57			
33	37	n.d.			
Normals	n = 20	n = 12			
Mean	30	47			
Range	12–46	27–58			

n.d. = Not determined.

* Involved node = Hodgkin's disease histologically present in node studied for surface markers.

Serum immunoglobulins and skin testing. Serum immunoglobulin levels were normal in all patients. Twenty-six of thirty-two (81%) patients showed evidence of reactivity to one or more skin testing antigens. No correlation was found between skin test reactivity and other parameters such as lymphopenia, stage of the disease or number of T lymphocytes.

TABLE 3. Circulating B and T lymphocytes in untreated patients before and 2 weeks after splenectomy

Patient number	Splenectomy status	B lymphocytes		T lymphocytes	
		%	no./mm³	%	no./mm³
5	Pre	18	459	n.d.	—
	Post	23	n.d.	60	n.d.
8	Pre	24	514	52	1114
	Post	24	164	56	383
11	Pre	17	257	55	832
	Post	41	1343	41	1343
32	Pre	24	267	52	572
	Post	17	146	54	463
33	Pre	25	282	40	451
	Post	27	400	54	800
Controls (mean ± s.d.)		22 ± 8	411 ± 216	60 ± 8	1700 ± 450

n.d. = Not determined.

TABLE 4. Circulating B and T lymphocytes in Hodgkin's disease patients treated with radiotherapy

Patient number	Time since end of therapy (months)	Disease status*	B lymphocytes		T lymphocytes	
			%	no./mm³	%	no./mm³
1	Pre	IIIA	25	603	50	1206
	1	CR	52	n.d.	44	n.d.
	11	CR	61	1684	29	800
2	Pre	IIA	11	196	56	1001
	9	CR	60	n.d.	40	n.d.
3	Pre	IIA	13	145	41	460
	12	CR	39	1189	54	1646
4	Pre	IIIA	13	191	62	911
	2	CR	29	n.d.	47	n.d.
5	Pre	IIIA	18	459	n.d.	—
	7	R	53	n.d.	50	n.d.
6	Pre	IIIA	17	170	50	500
	1	CR	16	190	42	499
7	Pre	IIIA	8	56	60	418
	1	CR	6	183	60	1830
20	Pre	II_EA	28	368	n.d.	—
	13	CR	63	1739	40	1104
25	Pre	IIA	5	78	63	985
	7	CR	43	n.d.	21	n.d.
36	20	R	63	2118	34	1143
37	13	R	36	653	n.d.	—
38	24	R	69	1755	53	1348
40	20	R	66	438	57	378
Controls (mean ± s.d.)			22 ± 8	411 ± 216	60 ± 8	1700 ± 450

n.d. = Not determined.

* CR = complete remission; R = relapse.

Circulating B and T lymphocytes before and after splenectomy

The effect of splenectomy on circulating B- and T-lymphocyte percentages and numbers was studied in five patients (Table 3). All patients were studied pre-splenectomy and about 2 weeks post-splenectomy, prior to the initiation of treatment. In only one patient was there any striking change in the percentages of B or T lymphocytes. This patient showed an increased percentage of B lymphocytes when compared with the value obtained prior to splenectomy.

B- and T-lymphocyte studies in treated patients

Serial studies. Before and after treatment. Nine patients were studied prior to and 1–13 months after termination of radiotherapy (Table 4). Radiotherapy consisted of standard mantle, periaortic or inverted Y ports. Seven of the nine patients showed a marked increase in percentage of B lymphocytes following radiotherapy. This increased percentage of B lymphocytes was associated with a trend towards decreased percentages of T lymphocytes. Absolute numbers of B and T lymphocytes in patients following radiotherapy were usually normal and the same or higher than pretreatment values.

TABLE 5. Circulating B and T lymphocytes in Hodgkin's disease patients treated with chemotherapy

Patient number	Months on chemotherapy	Time since last chemo-therapy (months)	Disease status*	B lymphocytes		T lymphocytes	
				%	no./mm³	%	no./mm³
8	0	Pre	IIIA	24	514	52	1114
	8	1	CR	17	376	46	1018
9	0	Pre	IIB	21	557	n.d.	—
	9	1	CR	32	557	57	993
10	0	Pre	II_EA	17	161	46	435
	27	2	CR	24	n.d.	54	n.d.
21	0	Pre	IIIA	31	1367	44	1940
	3	1	PR	27	946	59	2067
	5	1	CR	35	n.d.	47	n.d.
22	0	Pre	IVB	19	185	59	574
	3	1/2	PR	21	66	50	156
23	0	Pre	IIB	25	344	53	730
	25	On treatment	R	17	57	62	208
29	0	Pre	IVA	6	108	n.d.	—
	27	2	CR	12	365	59	1794
32	0	Pre	IIIB	17	189	54	601
	8	1	CR	30	924	51	1571
38	15	2	CR	33	653	15	297
41	120	1	CR	22	137	48	298
	126	3	CR	49	922	31	583
42	56	8	CR	18	n.d.	61	n.d.
	56	16	CR	34	292	n.d.	—
43	24	17	R	33	262	n.d.	—
44	27	On treatment	CR	19	301	51	808
45	24	3	R	54	591	50	547
46	46	2	CR	26	128	50	247
47	3	1	CR	14	201	33	475
48	18	3	R	19	313	24	396
49	44	2	CR	41	764	57	1062
50	50	2	CR	17	209	31	382
Controls (mean ± s.d.)				22 ± 8	411 ± 216	60 ± 8	1700 ± 450

n.d. = Not determined.

* CR = complete remission; PR = partial remission; R = relapse.

Eight patients were studied at diagnosis and subsequent to aggressive four drug combination chemotherapy (Table 5). Six showed a slight to moderate increase in the percentage of B lymphocytes above pretreatment values, but in all but one patient the percentages of B lymphocytes remained in the normal range. The percentages of T lymphocytes changed very little with treatment, being in the normal range prior and subsequent to treatment. In some patients the absolute numbers of both T and B lymphocytes were decreased following chemotherapy due to lymphopenia.

Previously treated patients. Fourteen patients were already treated at the time of first study. Four of these patients were first evaluated at the time of relapse, 13–24 months following the termination of radiotherapy (Table 4). All four showed high percentages of B lymphocytes. In one patient this was associated with a low percentage of T lymphocytes. Only one patient had lymphopenia. Eleven patients who had been treated with chemotherapy were studied (Table 5). Three showed high percentages of B lymphocytes. These patients were all at least 2 months since their last cycle of chemotherapy. Five out of eleven (45%) patients showed decreased percentages of T lymphocytes.

Lymph nodes. In five previously treated patients lymph nodes were biopsied 6–24 months following the termination of therapy because of suspected recurrent disease (Table 2). Four of these lymph nodes were involved with Hodgkin's disease. One of these lymph nodes showed a slight increase in the percentage of B cells but otherwise the percentages of B and T lymphocytes were normal. The biopsied lymph node in patient 13 showed atypical hyperplasia. In this node 96% of cells were immunoglobulin bearing and only 8% were T lymphocytes. This patient has been followed off of all treatment for 9 months since the biopsy without evidence of recurrent Hodgkin's disease.

DISCUSSION

In the present group of thirty-five untreated patients with Hodgkin's disease the percentages of circulating B and T lymphocytes were usually normal at diagnosis. In only 17% of patients were B lymphocytes decreased, and low percentages of T lymphocytes were noted in only 8%. The present results confirm our earlier observations and those of others on B lymphocytes in untreated patients with Hodgkin's disease (Cohnen *et al.*, 1973a; Falleta *et al.*, 1973; Gajl-Peczalska *et al.*, 1973b; Gallmeier *et al.*, 1973). Other studies of T lymphocytes only indicated normal percentages of T lymphocytes (Froland, 1972; Michlmayr *et al.*, 1974) or decreased percentages of T lymphocytes (Cohnen *et al.*, 1973b; Aiuti & Wigzell, 1973; Andersen, 1974) in the peripheral blood. Simultaneous quantitations of B and T lymphocytes in untreated patients with Hodgkin's disease have been rarely reported. The largest study we know of (ten patients) also found normal percentages of B and T lymphocytes (Kaur *et al.*, 1974).

We know of no report of patients serially studied prior to and following therapy. High percentages of B lymphocytes have been reported in a few patients treated by radiotherapy (Falleta *et al.*, 1973; Engeset *et al.*, 1973). In the present study we found that B lymphocytes increased in all except two patients following radiotherapy. The two patients who failed to show elevated B lymphocytes in the blood were studied only 1 month after termination of irradiation. Our increase in B lymphocytes was sustained up to 13 months after treatment; no patients have been studied longer than 13 months post-treatment. These results contrast with the observations of Falleta *et al.* (1973) who reported a return to normal levels of B lymphocytes within 6–12 months following therapy in patients with stage I and II Hodgkin's disease.

In contrast to the patients treated with radiotherapy, the percentage of B lymphocytes was not elevated in most of our patients treated with chemotherapy. However, the time lapse between the last treatment and date of study was shorter for most patients on chemotherapy

relative to those treated with irradiation. All of the patients on chemotherapy who had elevated percentages of B lymphocytes were off therapy for at least 2 months.

The mechanism of disturbed B- and T-lymphocyte ratios in treated patients is unknown. An impairment of cell-mediated immunity in Hodgkin's disease has been well documented. One can speculate that the proliferation of B lymphocytes may either represent a compensatory mechanism to cell-mediated deficiency or loss of normal suppressor activity of T lymphocytes. Either mechanism could account for the increased *in vitro* synthesis of IgG by splenic cells from patients with Hodgkin's disease (Longmire *et al.*, 1973). We previously observed that patients with Di George syndrome and lepromatous leprosy who have cell-mediated immune defects have elevated numbers of B lymphocytes (Gajl-Peczalska *et al.*, 1972, 1973a).

The role of splenectomy must also be considered. Though only one of our five patients studied shortly after splenectomy and prior to treatment showed a rise in the percentage and absolute number of B lymphocytes, we have found that patients with idiopathic thrombocytopenia treated only with splenectomy tend to have elevated B lymphocytes in the blood (unpublished observations). It is possible that removal of the spleen can affect maturation of B lymphocytes into plasma cells. It is also possible that splenectomy results in a removal of lymphocytes which normally function as suppressor cells.

Braylan *et al.* (1974) studied two lymph nodes involved by Hodgkin's disease; the percentage of T lymphocytes was increased in both. We have studied involved lymph nodes from eleven untreated patients; two showed an increase in T lymphocytes. Aisenberg & Long (1975) recently reported a high percentage of T lymphocytes in the lymph nodes of patient with Hodgkin's disease. However, such high values were obtained mainly by anti-human thymocyte antiserum in immunofluorescent assay, not by T-rosette formation. Differences in the techniques used may account for the differences in results obtained by Aisenberg and us. In two of our involved lymph nodes the percentage of B lymphocytes was low. These lymph nodes showed many plasma cells; it is possible that enhanced transformation of B lymphocytes into mature plasma cells might be responsible for the low percentage of B cells.

Reed–Sternberg cells have been postulated to be of B cell (Leech, 1973; Halie *et al.*, 1974; Taylor, 1974), T cell (Order & Hellman, 1972; Biniaminov & Ramot, 1974) and monocytic-histiocytic origin (Kadin *et al.*, 1974). Regardless of their origin the present study indicates that the percentages of B and T lymphocytes are usually not significantly disturbed in untreated patients with Hodgkin's disease. The well-described functional deficiencies of T lymphocytes do not appear to be expressed as significant abnormalities of T or B lymphocytes in the blood or lymphoid tissues.

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