Immunologic studies of peripheral blood lymphocytes and lymph nodes in Hodgkin's disease

A preliminary report[1]

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Peripheral blood lymphocytes (PBL) and lymph nodes from patients with newly diagnosed and untreated Hodgkin's disease were studied to determine the distribution of T lymphocytes. Fluorescence-activated cell sorting of PBL was performed on 11 out of 12 patients using monoclonal antibodies to lymphocyte differentiation antigens. A reduction in T lymphocytes (anti-T_3) was found in 5 patients, a reduction of T_4 lymphocytes was found in 3 patients, and an increase in the PBL T_8 cells occurred in 1 patient. In three cases, the T_4:T_8 PBL ratio was decreased. Complete staging was performed on all 12 patients. No correlation between clinical stage and T-cell subpopulations was apparent. Evidence of sustained immune depletion was found in 1 patient at relapse. Efforts to monitor immune function in Hodgkin's disease should continue and may provide a data base from which immunopathologic staging can be developed.

Index terms: Hodgkin's disease, analysis • Lymphatic system, analysis • Lymphocytes, analysis

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The interference with normal immune homeostasis that occurs with the development of Hodgkin's disease seems to be centered on T-lymphocyte function.[1] Along with lymphocytopenia and impaired cell-mediated hypersensitivity, a depletion of T lymphocytes from peripheral blood occurs and can be detected at diagnosis,[2] become progressive with time,[3] and persist despite achievement of remission.[4] Analysis of distribution of subsets of T lymphocytes capable of accelerating or suppressing an immune response is now possible with the use of monoclonal antibodies.[2,5] It is hoped that study of the change in lymphocyte subset distribution may facilitate insight into how the host-disease interaction operates.

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In this study, we sought to prospectively characterize T-cell subsets in the peripheral blood and lymph nodes from patients with Hodgkin's disease using mouse monoclonal antibodies to lymphocyte antigens. In addition, clinicopathologic staging and treatment outcome were monitored longitudinally with the goal of providing a clinical correlation that could serve as a basis for designing an immunopathologic staging system.

Materials and methods

Twelve patients with newly diagnosed and untreated Hodgkin's disease, all of whom had been referred to the Cleveland Clinic's Department of Medical Oncology between May 1, 1981, and February 1, 1983, were entered into this study. The age range of these 4 men and 8 women was 20–71 years (median, 34.5 years old). Eleven patients were found to have nodular sclerosing Hodgkin's disease and 1 had the mixed cellularity variant.

All patients were subtyped using the Lukes-Butler classification.[6] Clinical and/or pathologic staging of disease using the Ann Arbor criteria[7] was performed based on a complete history and physical examination, complete blood count, blood chemistries, chest roentgenography, bone marrow biopsy, lymphangiography, liver biopsy, and (in five cases) staging laparotomy with splenectomy. Systemic symptoms in Hodgkin's disease ("B" symptoms) included weight loss of 10% or more during the last six months, night sweats, or inexplicable fever.

Consent to participate in this study was obtained in accordance with institutional and National Cancer Institute guidelines.

Lymphocytes were purified from heparinized peripheral blood by centrifugation on a Ficoll-Hypaque gradient, followed by incubation with carbonyl iron particles and centrifugation to remove the phagocytic cells. T lymphocytes were quantitated by their ability to form rosettes with neuraminidase-treated sheep red blood cells (E rosettes).

Peripheral blood T-helper and T-suppressor lymphocytes were quantitated using the fluorescence-activated cell sorter (FACS II, Becton-Dickinson, Sunnyvale, Calif.) and mouse monoclonal antibodies: OKT3 (total T cells), OKT4 (T helper-inducer), and OKT8 (T suppressor-cytotoxic) (Ortho Diagnostics, Raritan, N.J.). After initial purification, the lymphocyte preparations were incubated with the monoclonal antibodies and stained with fluorescein isothiocyanate-labeled goat anti-mouse IgG (Cappel Labs, West Chester, Pa.). As controls, lymphocytes were processed as just described, but omitting the incubation step with monoclonal antibodies. The FACS II was adjusted with glutaraldehyde-fixed red blood cells, and appropriate gating to exclude undesired contaminating cells was carried out. At least 10,000 cells from each preparation were counted, and the number of T helpers (T_4) and T suppressors (T_8) was calculated by electronically dividing the 100× fluorescence profile by the total scatter profile of the test sample and subtracting a similar profile of the control sample. The helper-to-suppressor T-cell ratio (T_4:T_8 ratio) was calculated by dividing the percentage of T_4-positive mononuclear blood cells by the percentage of T_8-positive mononuclear blood cells. Absolute T_4 and T_8 lymphocyte counts were calculated by multiplying the fraction of fluorescent mononuclear cells (detected with the appropriate monoclonal antibodies) by the absolute lymphocyte count (derived from an automated total white blood cell count [Coulter S+] and an automated differential lymphocyte determination [Hematrak] performed on peripheral blood specimens).

Immunohistologic evaluations were performed using monoclonal antibodies T_4 (inducer/helper), T lymphocytes (some macrophages), and T_8 (cytotoxic/suppressor T cells) in conjunction with the indirect immunoperoxidase and the avidin-biotinylated peroxidase techniques.[8] Frozen tissue sections were allowed to air-dry overnight, fixed in cold acetone for five minutes, and the acetone allowed to evaporate. Tissue sections were washed with phosphate-buffered saline (pH, 7.6) for 10 minutes and incubated with 10–20 mg/mL of mouse monoclonal antibody. Tissue sections were washed in phosphate-buffered saline and then incubated with either peroxidase-conjugated rabbit anti-mouse IgG (indirect procedure) or sequentially in biotinylated affinity-purified horse anti-mouse IgG and pre-formed avidin-biotinylated peroxidase complex. The color reaction product for the enzyme was developed with 3-amino-9-ethylcarbazole, slides counterstained with hematoxylin, and sections mounted with Aquamount. The immunostained sections were examined with the conventional light microscope.

Results

The *Table* outlines our results of studies on

Table. Immunologic and immunohistologic data

Case	Stage*	PBL Lymphocytes ($\times 10^3/mm^3$)	Lymphocyte Subpopulations				Nodule Formation	Intranodular T_4:T_8	Extranodular T_4:T_8
			E-Rosettes	T_3†	T_4†	T_8†	T_4:T_8 ratio			
1	IA	1.4	–	67 (0.9)	55 (0.7)	12 (0.1)	4.4	–	–	–
2	IA	2.5	75.2	79 (2.0)	49 (1.2)	29 (0.7)	1.7	–	–	–
3	IIA	2.5	78.0	91 (2.2)	67 (1.6)	18 (0.4)	3.5	1+	3:1	–
4	IIA	1.5	58.3	58 (0.8)	31 (0.4)	26 (0.3)	1.2	–	–	–
5	IIIA	1.8	43.0	82 (1.6)	70 (1.2)	22 (0.3)	3.2	–	–	–
6	IIIA	0.2	53.2	–	–	–	–	–	–	–
7	IIIB	1.4	–	66 (0.9)	41 (0.5)	25 (0.3)	1.6	–	–	–
8	IIIB	2.0	80.5	90 (1.8)	64 (1.2)	20 (0.4)	3.2	–	–	–
9	IIIB	1.4	69.4	73 (1.0)	49 (0.6)	24 (0.3)	2.0	–	–	–
10	IIIB	1.0	54.7	64 (0.6)	35 (0.3)	20 (0.2)	1.7	3+	2:1	2:1
								2+‡	10:1	3:1
11	IVB	0.9	–	72 (0.6)	58 (0.5)	13 (0.1)	4.4	–	–	–
12	IVB	1.8	57.8	49 (0.8)	16 (0.2)	30 (0.3)	0.5	3+	10:1	3:1
Normal values			75.2 ± 5.3	77.6 ± 7.5	49.5 ± 9.0	24.6 ± 5.3				

* Ann Arbor Classification for Staging of Hodgkin's Disease[7]
† Percent (absolute number × $10^3/mm^3$)
‡ At relapse

peripheral blood mononuclear cells. The mean lymphocyte count was $1,500/mm^3 \pm 0.6$, with 6 of 12 patients being lymphopenic ($\leq 1500/mm^3$) at the time of diagnosis. The mean PBL T_4:T_8 ratio was 2.4 ± 1.2; it was <2.0 in five cases and <1.0 in one at the time of diagnosis. The *Table* also outlines the results of immunohistochemical analysis of involved lymph nodes at diagnosis from 3 of the patients; in each instance, significant endogenous peroxidase activity, as assessed using the nonimmune mouse immunoglobulin control preparation, was present, principally as a consequence of eosinophil peroxidase. This endogenous peroxidase activity was acknowledged during interpretation of immunostained preparations with primary mouse monoclonal antibodies. Areas of lymph node involved by Hodgkin's disease demonstrated no immunostaining of the Reed-Sternberg cell population or atypical mononuclear variants. Small lymphocytes were predominantly T_4+, and the approximate tissue T_4:T_8 ratio ranged from 3:1 to 10:1. In the 2 patients with significant nodularity, the T_4:T_8 ratio was much higher in the nodular areas as opposed to the non-nodular architectural pattern. Cells that were T_4 positive were not simply restricted to small lymphocytes since some tissue macrophages with more abundant cytoplasm were also positive.

Patients were treated with radiotherapy and/or chemotherapy, and all 12 achieved complete remission. Median disease-free survival was 35+ weeks (average follow up, 64+ weeks), with 3 patients (37%) (Cases 8, 10, and 12) suffering a relapse.

Discussion

The appearance of Reed-Sternberg cells within lymph nodes of patients with Hodgkin's disease is associated with a number of T-lymphocyte-mediated events, including the proliferation of T cells; the production of lymphokines that attract eosinophils, fibroblasts and other lymphocytes; and the elaboration of endogenous pyrogen. This intensive T-lymphocyte-mediated reaction in turn leads to the replacement of normal nodal architecture by a seemingly neoplastic growth. As this process spreads to other lymph node groups, there is a corresponding loss of cell-mediated immune responsiveness characterized by loss of cutaneous cell-mediated hypersensitivity, lymphopenia, and impaired ability to defend against intracellular pathogens.

Our study demonstrates that lymphopenia is common in patients with newly diagnosed Hodgkin's disease, and that variable decreases in total T cells, as well as the T_4 and T_8 subsets, occur. Because of the small number of patients included

in this study, correlation of these data with stage, symptoms, and outcome is speculative. However, when data are grouped for disease stage (I and II vs. III and IV) or the presence or absence of symptoms (A vs. B), no significant differences or trends involving either total T cells or the various subsets are present. It appears that the lymphopenia present in stages III and IV and in patients with B symptoms accounts for any differences seen. This is the contrast to the report by Posner et al[2] in which significant reductions of relative and absolute numbers of total T cells were noted in patients with B symptoms.[2] Likewise, we observed no consistent alteration in T_4 (helper) and T_8 (suppressor) lymphocyte subpopulations. The inclusion of these data with staging did not appear to predict disease recurrence and/or treatment failures.

Immunohistologic data are available in only 3 patients and, in two instances (Cases 3 and 10), appear to correlate with peripheral blood findings. In the third patient, a marked discrepancy is present; the reasons for this are unclear.

The results obtained in this study are similar to those described previously, and no clinical utility for the immunologic studies performed can be demonstrated. Previous attempts to correlate immunologic reactivity with prognosis[9] have also been unsuccessful. The possibility that the addition of immunohistologic data may prove to be of more value does exist. The changes in T-cell and T-lymphocyte subpopulations alone do not account for the impaired lymphocyte function found in this disorder.

Analogous to the success achieved in treatment following accurate clinical staging, methods of gauging the presence of immune depletion and the rate at which it occurs in patients with Hodgkin's disease could lead to similar improvement. Further prospective longitudinal analysis needs to continue to form such a data base.

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