Association of an Interleukin Abnormality With the T Cell Defect in Hodgkin's Disease

By Richard J. Ford, Jerry Tsao, Nicola M. Kouttab, Chintaman G. Sahasrabuddhe, and Shashi R. Mehta

The cellular immune defect in untreated Hodgkin's disease (HD) has long been recognized. This defect appears to be responsible for at least some of the morbidity and ultimately the mortality associated with the disease. In recent years, many studies have shown that the T cell component of the immune response is the apparent site where the defect in HD exists and where the immunoregulatory abnormalities that may account for the deficit are observed. The discovery of the lymphokines and monokines, comprising the human interleukin system, has elucidated some aspects of the regulatory control of the functional pathways involved in T lymphocyte activation and proliferation. The interleukin system can therefore provide the framework to dissect immunodeficiency states, such as that seen in HD. The present study indicates that HD patients' interleukin 1 (IL1) response appears to be normal, as is their T cell proliferative response to exogenous IL2. Interleukin 2 production by HD patients' peripheral blood mononuclear cells, however, is decreased when compared with age/sex-matched controls. The inability to generate IL2 after appropriate stimulation may reflect either a primary cellular defect or a regulatory defect, such as excessive immunosuppression, giving rise to the characteristic T cell hyporesponsiveness seen in HD.

IN THE LAST 15 YEARS, there have been many studies that document the presence of a cellular immune defect in untreated Hodgkin's disease (HD),[1][4] although the initial discovery of the defect was actually made much earlier by Dorothy Reed in 1902.[5] Most of these studies have impugned the T cell as the apparent site of this defect, which is manifested by impaired delayed hypersensitivity,[6] reduced responses to T cell mitogens, such as phytohemagglutinin (PHA),[7] and decreased E rosette formation.[8] More recent studies have focused on immunoregulatory abnormalities mediated through suppressor mechanisms involving monocytes[9,10] or suppressor T cells,[11,12] as well as a variety of circulating soluble suppressor factors.[13,14] However, the relationship of these T cell abnormalities to the neoplastic disease process in HD remains obscure.

The discovery of T cell growth factor (TCGF; IL2) several years ago[15] and the subsequent delineation of the role of the interleukins (IL1, IL2) in the immunologic regulation of the human T cell system[16,17] have significantly improved our understanding of T cell function. These studies have begun to define the concatenation of events involving the recognition of antigen and cellular interactions in generating the biologically functional molecules. These molecules are called lymphokines and monokines and are required to stimulate proliferation and differentiation within the immune system.[18] Given the pivotal importance of the interleukin system in T cell-mediated cellular immunity, it should now be possible to identify at least the site(s) of functional abnormalities in a defective T cell system, such as that seen in HD. To explore this possibility, we report our studies on the interleukin system in 14 untreated patients with nodular sclerosing Hodgkin's disease (NSHD). The major defect observed in these patients, as compared with normal controls, was in the production of TCGF (IL2), whereas IL1 production and the ability of patients' T cells to respond to exogenous IL2 in vitro appeared to be unimpaired.

MATERIALS AND METHODS

Patient Population

The patient population consisted of 14 newly diagnosed, untreated patients with the pathologic diagnosis of NSHD.[19] The patients ranged in age from 17 to 34 years. One hundred twenty milliliters of peripheral blood was drawn from these patients at the time of initial presentation, before clinical staging procedures were instituted. After clinical staging, the patients ranged from IA to IVB.[20] Age- and sex-matched control volunteers had blood drawn at the same time, and the blood specimens were processed together for obtaining peripheral blood mononuclear cells (PBMC).

Cell Preparation

Peripheral blood specimens were diluted and layered onto Ficoll-Hypaque (F/H) gradients and centrifuged to obtain PBMC. Leukocyte differential counts were made to establish the ratio of lymphoid cells to monocytes in the PBMC. Monoclonal antibody analysis for T cells utilizing OKT11, OKT4, OKT8[21] (Ortho Pharmaceuticals, Raritan, NJ), B cells (B1; Coulter, Hialeah, Fla),[22] and monocytes (Mo2, Coulter)[23] were also performed on both patient and control PBMC preparations. The patient's WBC count was usually mildly lymphopenic, and the PBMC varied slightly in lymphocyte:monocyte ratios; however, the patients' white cell numbers were usually within 10% of the controls. There was also some variance in the T

cell subsets, but the T cell helper/suppressor ratio of the patients was similar to the controls in both peripheral blood and PBMC; T4 and T8 percentages were both ± 5% of control values. None of the patients exhibited a greatly aberrant T cell profile by monoclonal antibody analyses.

Mitogen Stimulation

Mitogen responses to phytohemagglutinin (PHA; Difco, Detroit. MI) were assayed on patient and control PBMC using both optimal (1% vol/vol) and suboptimal (0.25%, 0.50%, 0.75%) final mitogen concentrations. The assays were set up in triplicate in microwells and incubated for 72 hours at 37 °C in 5% CO_2. At 48 hours of culture, 1 μCi of ^{3}H-thymidine (^{3}H-Tdr) (6 Ci/mmol/L) was added to each well. The microwells were harvested 24 hours later on a microharvester, and ^{3}H-Tdr incorporation was determined using a liquid scintillation spectrometer.

Delayed-Type Hypersensitivity (DTH) Skin Tests

DTH skin testing was performed on the Hodgkin's patients at the time of presentation. A battery of six recall antigens, including dermatophytin, Candida, streptokinase-streptodornase (SK-SD), mumps, keyhole limpet hemocyanin (KLH), and PPD, were placed on the patients' forearm and evaluated at 24 and 48 hours, as previously described.[24]

Interleukin 1 Production

PBMC from F/H gradients were suspended in Hanks' balanced salt solution (HBSS) at 5×10^6/mL. Ten milliliters of this suspension was added to glass Petri dishes for one hour, and the nonadherent cells subsequently removed from the plates with Ca^{++}/Mg^{++}-free HBSS. The adherent cells were then removed with trypsin and gentle scraping with a rubber policeman. Viability was then determined by trypan blue exclusion, and nonspecific esterase staining (NSE)[26] was performed as an indicator for cells of the monocyte/macrophage lineage. NSE was found to be positive for 80% to 85% of the adherent cells. Monoclonal antibody characterization of the adherent cells with Mo2 (Coulter) or OKM1 (Ortho) by indirect immunofluorescence was positive for 75% to 90% of the adherent cells. One million viable cells in 1 mL of RPMI and 5% fetal calf serum (FCS) were cultured in tissue culture tubes (Falcon Plastics, Oxnard, Calif) and stimulated with 1 μg/mL lipopolysaccharide (LPS, Difco) for 24 hours at 37 °C. The resulting supernatants were then dialyzed against RPMI overnight.

Interleukin 1 Assay

Interleukin 1 assays were performed essentially according to the method of Mizel et al.[25] Thymocytes were obtained from C3H/HeJ mice six to eight weeks of age (Jackson Labs, Bar Harbor, Me). The adherent cell supernatants were then assayed at multiple dilutions with and without Con-A (1.25 μg/mL) in microwells at 10^6 thymocytes/well. The plates were incubated for 72 hours at 37 °C; 24 hours prior to harvest, 0.5 μCi of ^{3}H-Tdr (6 Ci/mmol) was added to each well. The cultures were harvested with a microharvester, and ^{3}H-Tdr incorporation was determined in a liquid scintillation counter.

Interleukin 2 Generation

Patient and control PBMC from F/H gradients were washed in RPMI, and 2 mL of 1×10^6 cells/mL suspension were transferred to tissue culture tubes (Falcon, 16 × 125 mm). These cells were stimulated with Con-A (Pharmacia) at 1.25 μg/mL final concentration, while control tubes received no Con-A. The tubes were then incubated for 48 hours in a CO_2 incubator. The supernatants were

then dialyzed against RPMI overnight at 4 °C. Con-A activity in the supernatants could be blocked with alpha-methyl mannoside, but this was found to be unnecessary, as the target cells (long-term cultured T cells) were not sensitive to Con-A stimulation.

Interleukin 2 Assay

Dialyzed supernatants from Con-A-stimulated cultures were then added to long-term (at least four to five weeks in vitro) T cell lines, which were generated from mixed lymphocyte cultures (MLC) and maintained on partially purified IL2 preparations. These cells were shown to be IL2-dependent by failure to proliferate and cell death within 24 to 72 hours after deletion of the growth factor-containing media. For the growth factor assay, the T cells were washed twice to eliminate residual IL2 and subsequently exposed to various dilutions of experimental and control supernatants for 72 hours in microtiter wells with 0.2×10^6 cells/well. Then, 0.5 μCi of ^{3}H-Tdr was added to the cultures for the final 24 hours in vitro. At 72 hours, the wells were harvested, and ^{3}H-Tdr incorporation was determined in a liquid scintillation spectrometer.

T Cell Reactivity to Exogenous IL2

In this assay, 20×10^6 PBMC from HD patients and controls were stimulated with 0.75% PHA in RPMI 1640 (Irvine Scientific, Santa Ana, Calif) and 10% fetal calf serum (Irvine) and incubated at 37 °C in 5% CO_2 for seven to ten days. The cells were washed in HBSS and rosetted with neuraminidase-treated sheep red blood cells (E_n) for 24 hours at 4 °C. The E_n rosettes were then pelleted on F/H gradients and the red cells subsequently lysed with Tris-NH_4Cl. The T cell blasts were then set up in microtiter wells at 0.2×10^6 blasts/well in RPMI and 10% FCS. A known standard DEAE-partially-purified IL2 preparation[27,46] was added at varying dilutions (5% to 40% vol/vol), and the wells were incubated for 72 hours at 37 °C. Twenty-four hours prior to harvest, 0.5 μCi of ^{3}H-Tdr (6 Ci/mmol/L) was added to each well. The wells were harvested on a microharvester and counted as before on a liquid scintillation spectrometer.

RESULTS

Patient Population and Clinical Data

The untreated patient population in this study was quite characteristic of the younger age group of Hodgkin's disease (HD) patients, showing an age range of 17 to 34 years of age and exhibiting the nodular sclerosis histopathology. Table 1 shows some clinical features of this patient group, including the staging data and skin test reactivity to a variety of recall antigens. It should also be noted that virtually all of the patients tested exhibited at least some degree of immunodeficiency (ID) to recall antigens, and that 3/11 HD patients tested showed severe ID. It can also be seen that these three patients with severe ID had disseminated (stage III or IV) rather than localized disease.

Mitogen Responsiveness to Phytohemagglutinin

Numerous previous studies, including our own, have shown that patients with untreated HD have a defect in the PHA responsiveness of their T lymphocytes, best demonstrated by suboptimal stimulation with the

Table 1. Nodular Sclerosis Hodgkin's Disease Patient Population

Patient*	Age (Years)	Skin Test Reaction†	Clinical Stage‡
P.M.	26	Moderate ID	IIA
A.O.	28	Severe ID	IIIA
J.M.	27	Moderate ID	IIA
J.L.	26	Severe ID	IVB
P.G.	17	Reactive (normal)	IIA
E.P.	34	ND	IIIB
K.G.	19	Moderate ID	IIB
M.M.	17	Moderate ID	IIA
E.M.	33	Severe ID	IIIB
C.W.	22	Moderate ID	IA
R.W.	29	Moderate ID	IIIA
B.A.	24	Moderate ID	IIIEB
C.R.	31	ND	IIB
M.C.	34	ND	IIIA

*Untreated patients with the pathologic diagnosis of nodular sclerosis Hodgkin's disease were studied at the time of presentation, prior to clinical work-up and staging procedures.

†Skin testing was performed in the standard manner for dermatophytin, Candida, streptokinase-streptodornase, mumps, keyhole limpet hemocyanin (KLH), and PPD. Severe immunodeficiency (ID) was defined as reactivity to two or less recall antigens. Moderate ID was greater than two but less than five. Normal was reactivity to five or more.

‡Clinical staging procedures included lymphangiogram, bone marrow, CAT scan, bone marrow biopsy, and staging laparotomy in lymphangiogram-negative patients.

mitogen.[28,29] When PHA responses were measured in our present group of patients and compared with age/sex-matched controls, we again observed a similar result. Figure 1 shows that mean PHA responses of the HD patients' mononuclear cells were lower than the controls (27,153 cpm ± 14,922 *v* 43,192 cpm ± 19,967, respectively), but that there was considerable overlap between the two groups with regard to individual responses to PHA.

IL1 Production by HD Patient Adherent Mononuclear Cells

The importance of IL1 in the scheme of human T cell activation has been previously demonstrated.[30] This monokine appears to function in the initiation of T cell activation by stimulating a T cell subset to secrete the lymphokine IL2 (TCGF). To ascertain if Hodgkin's patients possibly had a defect in this component of the interleukin system, we studied adherent cell populations from F/H-separated PBMC, which cell surface phenotypic analysis and histochemistry revealed to be mostly (> 80%) cells of the monocyte/macrophage lineage. As can be seen in Fig 2, however, the ability to generate IL1 in LPS-stimulated patient monocytes did not differ significantly from the response seen in age/sex-matched controls in the standard murine thymocyte comitogenic assay.
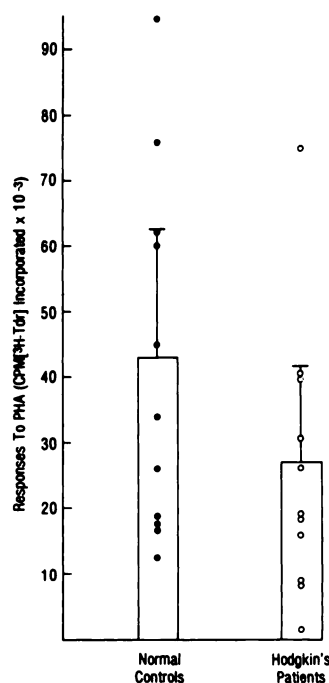

Fig 1. The response of untreated nodular sclerosing Hodgkin's disease patients' PBMC to PHA (0.75% vol/vol). PBMC from F/H gradients were stimulated with PHA for 72 hours; 0.5 µCi of ³H-Tdr was added to the cultures 24 hours prior to harvest.

Inability of Hodgkin's Patients' Mononuclear Lymphoid Cells to Generate Interleukin 2

IL2 production by human T cells allows for the proliferative expansion of activated immunocompetent T lymphocytes in the immune response.[31] To test whether this important immunologic function was
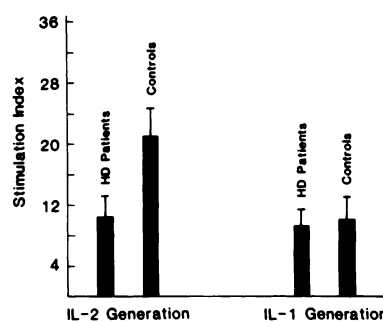

Fig 2. IL1 and IL2 responses of patients' peripheral blood leukocytes compared to age/sex-matched controls. IL1 activity was generated from LPS-stimulated (1 µg/mL) adherent PBMC after 24 hours in vitro and assayed on murine thymocytes in a comitogenic assay with Con-A. IL2 activity was generated by stimulating PBMC with Con-A (1.25 µg/mL) in vitro for 48 hours at 37°C. The resulting supernatants were dialyzed and added to long-term human T cell lines. The IL2 activity is assessed by adding 0.5 µCi of ³H-Tdr for the final 24 hours of a 72-hour assay. Both IL1 and IL2 responses of HD patient and control leukocytes are expressed by stimulation index (SI): cpm (cells + stimulus)/cpm (cells alone).

intact in HD patients, their PBMC were stimulated with Con-A for 72 hours, and the resulting culture supernatants were then tested for IL2 activity on long-term (> 30 days) in vitro propagated human T cell populations. Table 2 and Fig 2 show that the HD patients had a significant decrease in their ability to generate IL2 activity when compared to age-matched controls. This defect was observed in all but one member of the patient group studied, although the degree of impairment varied somewhat among the individual patients (Table 2). The severity of the IL2 generation decrement appeared to correspond generally to the degree of PHA hyporesponsiveness and with cutaneous anergy (Fig 3), although its relationship to clinical stage of disease is less certain.

In Vitro Response of Hodgkin's Patients' T Lymphocytes to Exogenous IL2

In addition to generation of IL1 and IL2 by HD patients' leukocytes, another possible site of functional impairment in T cell reactivity could be the inability of the patients' T cells to respond to exogenously supplied IL2 in vitro. To test this possibility, PHA-stimulated T cell blasts, maintained in vitro for seven to eight days, were assayed for their response to partially purified IL2 preparations. In Table 3, it can be seen that individual HD patients' T cells could respond to exogenous IL2 in a manner similar to controls and that the HD patients as a group responded similarly to controls (Table 3).

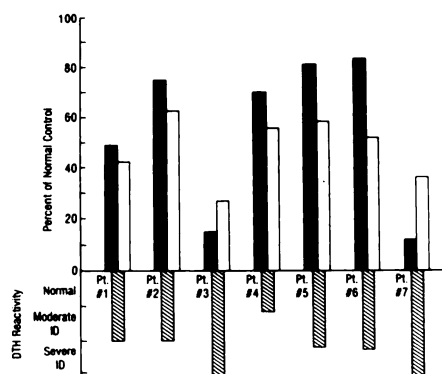Table 4 summarizes the results of the four parame-

Fig 3. The relationship of IL2 production to PHA response and DTH (skin test) reactivity is shown for a representative group of Hodgkin's disease patients. ■ = PHA response, □ = IL-2 response.

ters studied on the HD patients and presents the statistical analysis of the patient group as compared to age/sex-matched controls. The data indicate that the IL2 generation is the only cytokine parameter studied that was significantly decreased in the HD patients, although PHA responses were generally reduced and cutaneous anergy was observed in the patient population.

DISCUSSION

The T cell defect in HD remains a controversial conundrum, although few investigators now doubt its presence or importance in the pathophysiology of HD.[32] The exact cause of the T cell defect is still obscure, but the methodology for exploring the various facets of human T cell-mediated immunity are beginning to allow the dissection of the various stages in T cell activation from a functional perspective. Our findings using in vitro assays designed to assess specific functional activities suggest that the major site of T cell hyporesponsiveness in HD patients is the relative inability of their T lymphoid cells to generate IL2 (TCGF) activity after appropriate stimulation. These data identify the apparent site(s) of T cell hyporesponsiveness in our patients but not the underlying cause. The PBMC populations studied did not appear greatly altered in lymphoid and monocyte numbers, ratios, or subpopulations from the age/sex-matched controls, as our cellular phenotyping data indicate, but lower IL2 activity was consistently observed in the HD patients. Several possibilities for such a finding can be entertained, including a relative loss of IL2-producing cells, a refractoriness of the IL2-producing cell to its putative stimulator (ie, IL1), a suppressive mechanism mediated by suppressor cells of one type or another, or soluble products from such cells with suppressive activity. Unfortunately, our current understanding of the

Table 2. IL2 Generation in Hodgkin's Patients and Controls

Cell Source	Supernatant Concentration*		
	5%†	10%	20%
Pt. 1	8,402‡	11,268	12,820
Control	21,031	26,296	36,829
Std. control§	25,522	31,305	39,995
Pt. 2	1,280	3,856	3,640
Control	8,660	15,279	26,680
Std. control	12,680	18,980	36,620
Pt. 3	2,303	8,167	12,098
Control	8,006	15,083	26,728
Std. control	8,306	14,648	31,622
Pt. 4	15,686	25,065	32,273
Control	24,460	43,948	36,620
Std. control	19,893	38,040	44,612

*Supernatants were generated as described in Materials and Methods.

†Final concentration (vol/vol) of supernatant added to long-term T cell lines.

‡The cpm minus background of the means of triplicate assays.

§Standard control IL2 was prepared from DEAE-purified LyCM, as described in Meir and Gallo.[27]

Table 3. Effect of Exogenously Supplied IL2 on Activated Hodgkin's Disease T Cells

		^{3}H-Tdr (cpm)			
		Exp 1	Exp 2	Exp 3	Exp 4
Normal controls	Cells alone*	281	492	4,721	9,100
	Cells + IL2† (10%)	11,259	27,777	32,072	31,914
Hodgkin's disease	Cells alone	508	1,043	889	1,379
patients	Cells + IL2 (10%)	10,078	46,432	19,677	38,850

Representative data from four of the 14 HD patients and their sex/age-matched controls are shown. Data depict the mean counts per minute (cpm) of triplicate cultures.

*PBMC from F/H gradients were stimulated with 0.75% PHA in RPMI and 10% FCS for seven to ten days in 5% CO_2 at 37 °C. The cells were then washed, and the T cells were rosetted with SRBC and subsequently pelleted on F/H gradients.

†Partially purified IL2 was prepared from lymphocyte conditioned media, prepared according to the methods of Meir and Gallo,[27] taken through the DEAE column chromatography step. The IL2 preparations were shown to be active by both ^{3}H-Tdr incorporation assays and by stimulating cell growth on long-term human T cell lines.

precise T cell subsets involved in generating IL2 in normal individuals and the mechanism involved in the IL1-mediated stimulation of IL2 release are still incomplete. Although it has been shown that the helper T cell (T4; T_H) is the T cell subset that is primarily responsible for IL2 production after mitogenic or antigenic stimulation,[33] the T4 cells do not appear to be reduced proportionally in our patients or in the other series of untreated HD studied so far.[34,35] It is also likely, however, that the T4 subset is quite heterogeneous. Several laboratories have reported different functional activities in T cell subpopulations derived from T4 cells separated by other monoclonal antibodies that putatively can discriminate subsets of T4.[36,37] A selective loss in such a T4 subset responsible for IL2 production could account for the IL2 deficit seen. This paucity of information on normal IL2 generation makes it difficult to define, with any precision, abnormalities in the immunodeficient state, although similar defects in IL2 production have been reported in senescence[38] and, most recently, the acquired immune deficiency syndrome (AIDS).[39] This may mean that defective IL2 production is a fairly common site of T cell abnormalities in the immunodeficient state.

With regard to excessive suppressor mechanisms as a possible cause of the immune defect, both T cell and

Table 4. Summary of Growth Factor Responses of Hodgkin's Patients' PBMC as Compared to Age-Matched Controls

Assay	Percent of Age/Sex-Matched Controls*	P Value†
PHA responsiveness of PBMC	70.5 ± 11.6 (11)‡	P < .1
IL1 generation by macrophages	90.9 ± 11.1 (13)	P < .3
IL2 generation by PBMC	49.6 ± 8.3 (14)	P < .020
Response of T blasts to exogenous IL2	101.0 ± 24.0 (14)	P > .5

*(^{3}H-Tdr in patient cells/^{3}H-Tdr in age/sex-matched control) × 100 (± SEM).

†Derived from paired t test.

‡Number in parentheses represents the number of patients/controls studied.

monocyte-mediated suppressor abnormalities have been reported in HD,[9-12] and it is possible that such mechanisms could be acting on the cells that produce IL2 or their precursors. This possibility is suggested, as Fisher and his colleagues have shown that effector T cells from HD patients have increased sensitivity to the suppressive effects of both suppressor T cells and monocytes.[40]

Earlier studies have indicated that monocyte-mediated suppression, principally through prostaglandin E_2 (PGE_2) production and partially reversible by indomethacin, accounted for a considerable degree of the suppressive abnormalities seen in HD.[41] Our earlier studies, however, suggested that this might be due to a relative enrichment of the mononuclear cell populations of HD patients for monocytes.[29] More recently, it has been reported that monocytes from HD patients produce more PGE_2 than controls,[42] again suggesting that PGE_2, with its immunosuppressive properties, may play a role in the immunodeficiency of HD,[43] as PGE_2 has also been shown to suppress human IL2 production.[44] Whether this means that HD monocytes are, in general, hypersecretors of PGE, or possibly that only a subpopulation (eg, the oft referred to but poorly characterized "abnormal monocyte"[45]) of monocytes in HD patients is actually involved in actively suppressing the immune responses of these patients, remains to be delineated. The elucidation of the actual mechanism involved in the T cell defect in HD may open the possibility of immunorestorative procedures for correction of the defect, either through replacement therapy or possibly by other techniques of biologic response modification. This type of approach, if feasible, may significantly reduce the immunodeficiency-associated stigmata associated with Hodgkin's disease.

ACKNOWLEDGMENT

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