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Multiple Defects of T Helper Cell Function in Newly Diagnosed Patients with Hodgkin's Disease

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T helper cell (TH) function, as assessed by interleukin-2 (IL-2) production and [^{3}H]thymidine incorporation, was studied in 47 newly diagnosed untreated patients with Hodgkin's disease (HD) and 34 healthy controls. Three different stimuli were used to stimulate *in vitro* peripheral blood mononuclear cells (PBMC): influenza A vaccine (FLU), HLA alloantigens (ALLO) and phytohaemagglutinin (PHA). Four different patterns of TH function were observed in HD patients: (1) IL-2 production in response to all of the stimuli (40%); (2) IL-2 production in response to ALLO and PHA but not to FLU (26%); (3) IL-2 production in response to PHA alone (19%); and (4) failure to respond by IL-2 production to any of the three of the stimuli (15%). Thus, defective *in vitro* TH function was detected in the majority of these patients (60%). Defective TH function was observed in none of the 34 controls. Severely compromised TH function (patterns 3 and 4) tended to be associated with more advanced clinical presentation and more compromised haematological parameters ($P < 0.05$). The IL-2 production assay was more sensitive than the proliferative assay as only 30% of the HD patients failed to proliferate in response to FLU, and none failed to proliferate in response to either ALLO or PHA; this assay can detect subtle, multiple patterns of immune dysregulation in untreated HD patients. Our results suggest that HD is associated with a fundamental dysregulation in TH function, illustrate the complexity of such dysregulation, and raise the possibility that HD progression will be associated with a type-1-type-2 switch in immunoregulatory cytokine production.

Key words: Hodgkin's lymphoma, immunology, T helper, interleukin-2
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INTRODUCTION

A VARIETY OF defects in cell-mediated immunity has been reported in patients with Hodgkin's disease (HD), including negative delayed-type hypersensitivity reactions, delayed allograft rejection and alterations in graft versus host reactivity *in vivo*, as well as defective proliferative responses in antigen-stimulated cultures and mixed lymphocytic reaction, and mitogen-stimulated interleukin-2 (IL-2) production *in vitro* [1–9]. However, a more complete analysis of the depth and the complexity of the defect in T helper cell (TH) function has not been performed in HD patients. We have developed a sensitive *in vitro* IL-2 production assay for the analysis of defects detected in TH function, in which peripheral blood mononuclear cells (PBMC) stimulated with antigens activate the immune response through different T-helper-antigen presenting cell (APC) pathways [10]. These TH-APC interactions can be divided into three distinct categories based on the T cell subsets and source of APC utilised. Thus, TH responses to recall antigens such as influenza A vaccine (FLU) are dependent on $CD4^+$ T cells and autologous APC. In contrast, responses to HLA alloantigens (ALLO) can be generated by three different pathways of help: (1) $CD4^+$ T cells and autologous APC (similar to the requirement for recall antigens); (2) $CD4^+$ T cells and allogeneic APC; and (3) $CD8^+$ T cells and allogeneic APC [10]. The first of these pathways requires antigen processing and is referred to as the indirect pathway, whereas pathways (2) and (3) involve direct recognition of HLA antigens on the allogeneic APC, and constitute the direct pathways of antigen recognition.

We have used this approach to detect subtle, multiple defects in TH function in: (a) individuals who are seropositive for the human immunodeficiency virus (HIV^+) [11]; (b) kidney allograft patients on immunosuppressive protocols [12, 13]; and (c) patients who have systemic lupus erythematosus [14]. The study of these TH-APC pathways has permitted us to subdivide asymptomatic, HIV+ individuals on the basis of *in vitro* immune function into categories that are indistinguishable by clinical parameters [11]. In fact, the sequential and progressive loss of IL-2 production in response to recall antigens, then to ALLO, and finally to phytohaemagglutinin (PHA) is predictive for three clinically relevant AIDS parameters: (1) reduction in the number of $CD4^+$ T cells [15]; (2) time to AIDS diagnosis; and (3) time to death [16]. Furthermore, *in vitro* analysis of renal recipients by the indirect and the direct TH-APC pathways has been used

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to diagnose patients who are undergoing acute and chronic allograft rejection at the time of analysis [12], and is also predictive of those patients who will lose their kidney grafts within a 3-year period [13]. Thus, *in vitro* analysis of these TH-APC pathways by stimulation with recall antigens, allogeneic PBMC or PHA has recently been shown to be a valuable predictor for clinically relevant events involving two unrelated immunological disorders — progression to AIDS and allograft rejection. Although several previous reports have demonstrated *in vitro* immunological defects to various stimuli in HD patients, none has used the approach outlined above to compare, in the same study, the responses of PBMC of untreated HD patients. The present report is the first attempt to make such a comparative analysis in HD patients prior to therapy, and to correlate the patterns of immune dysfunction seen with clinical staging. If successful, such a study might be not only of diagnostic value, but could also have mechanistic implications concerning immune dysregulation in cancer.

In the present study, we assessed *in vitro* TH function by IL-2 production in response to FLU, ALLO, and PHA by PBMC from 47 newly diagnosed, untreated HD patients, as well as from 34 age- and sex-matched healthy controls. We report here the identification of three distinct patterns of TH dysfunction in HD patients, and that defective TH function can be detected in the majority (60%) of these patients.

PATIENTS AND METHODS

Patients and clinical evaluation

The case series consisted of 34 healthy controls and 47 patients with histological diagnosis of Hodgkin's disease, consecutively entered in controlled clinical trials. All patients with HD were previously untreated with chemotherapy: 45 were newly diagnosed and T helper cell function was evaluated before therapy. 2 patients were in first recurrence after radiation therapy; in these 2 patients TH function was tested 10–28 months after completion of radiotherapy and before starting chemotherapy. Clinical stage was determined according to the Ann Arbor classification [17], modified at the Cotswold meeting [18]. Therefore, pretreatment evaluation consisted of detailed history, complete physical examination, complete blood cell count, erythrocyte sedimentation rate, serum copper level, biochemical analysis of liver, bone and renal function, and two needle bone marrow core biopsies from bilateral iliac crest. Radiological investigation included chest X-ray, computed tomography and/or magnetic resonance imaging of the thorax and abdomen, bipedal lymphangiography and any additional imaging study necessary to clarify the significance of symptoms and/or physical signs. The palpable or the abdominal lymph nodes were defined as bulky if the largest dimension of the node or the nodal mass was 10 cm or greater, whereas a mediastinal mass was defined as bulky when the maximum width was equal to or greater than one third of the transverse diameter of the thorax at the level of T5–T6. The therapeutic programme consisted of extended radiotherapy alone for patients with favourable stage IA, and combined chemoradiotherapy for all other patients.

Processing of blood

Whole blood from HD patients and healthy control donors was drawn in Vacutainer tubes containing preservative-free heparin (Becton-Dickinson, Rutherford, New Jersey, U.S.A.). PBMC were separated on lymphocyte separation medium (LSM; Organon Teknika Corp., Durham, North Carolina, U.S.A.), washed twice in phosphate-buffered saline (PBS), and resuspended at 3×10^6/ml in RPMI 1640 (Gibco Laboratories, Grand Island, New York, U.S.A.) containing 0.5% penicillin, 0.5% streptomycin, 1% glutamine and 10 mM HEPES (MEDIA). Counting of viable cells was performed by Trypan blue exclusion.

In vitro *assays for T helper function*

For IL-2 production and proliferation assays, 3×10^5 PBMC were placed in flat-bottom wells of microtitre culture plate (Costar, Cambridge, Massachusetts, U.S.A.) in a final volume of 0.2 ml (MEDIA) along with: (a) no stimulation (medium background); (b) influenza virus vaccine prepared with a mixture of A/Taiwan, A/Shanghai and B/Victoria, 24 μg/l (FLU; final dilution 1:1000); (c) a pool of irradiated (50 Gy) allogeneic PBMC (1×10^5 cells per well) from two or more unrelated healthy control volunteers (ALLO); or (d) PHA (M form, Gibco) at a final concentration of 1:100. Three replicate cultures were performed for each stimulation. Pooled human plasma was added to each well (1:20 final) 1 h after sensitisation of the PBMC. In the IL-2 assay, the anti-IL-2 receptor antibody, humanised monoclonal anti-Tac (Becton-Dickinson) was added to the cell cultures on the first day at a final concentration of 1 μg/ml to inhibit IL-2 consumption. To assess IL-2 production, culture supernatants were harvested after 7 days, and total IL-2 produced throughout the culture period was determined by testing each supernatant for the ability to stimulate the proliferation of an IL-2-dependent mouse continuous T lymphocyte line (CTLL). Five successive 2-fold dilutions in triplicate were set up to test the supernatants for ability to stimulate the proliferation of 8×10^3 CTLL/well in 96-well microtitre plates. After 24 h, the CTLL cultures were pulsed with 1 μCi of [^{3}H]thymidine, and harvested 18 h later. The method used to calculate IL-2 units has been described previously [12]. Briefly, the units were calculated as a constant multiplied by the reciprocal supernatant dilution corresponding to half-maximal CTLL proliferation. This dilution was computed by extrapolation of the line generated by linear regression analysis of the counts per minute (CPM) for CTLL proliferation as a function of the supernatant dilution, using off-plateau values [12]. For the proliferative assay, cultures were pulsed with 1 μCi of [^{3}H]thymidine 6 days after antigenic stimulation and harvested 18 h later. In the IL-2 assay, patients were defined as positive to a given antigen if the units of IL-2 in the stimulated culture were >3 S.D. above the mean units of IL-2 of the unstimulated cultures of the healthy controls donors. The mean IL-2 units of unstimulated cultures was 0.87 for the healthy controls and 0.95 for the HD patients. In the proliferative assay, patients were defined as being responsive to a given antigen if the mean cpm of the stimulated cultures was >3-fold above the mean unstimulated cpm of the healthy controls. The cutoff values were 4.9 units for the IL-2 assay and 6233 CPM for the proliferation assay.

To analyse the alloantigen presenting/stimulating ability, the PBMC from HD patients were used as allogeneic stimulators for PBMC from healthy controls (responder PBMC). Responder PBMC were either undepleted or were depleted of their own antigen presenting cells (APC). Depletion of APC was achieved by a two-step procedure involving sequential plastic and nylon fiber-adhesive procedures described in detail previously [19].

RESULTS

PBMC from 34 healthy control donors and 47 newly diagnosed untreated HD patients were tested for *in vitro* IL-2 production after stimulation with FLU, ALLO or PHA. Complete IL-2

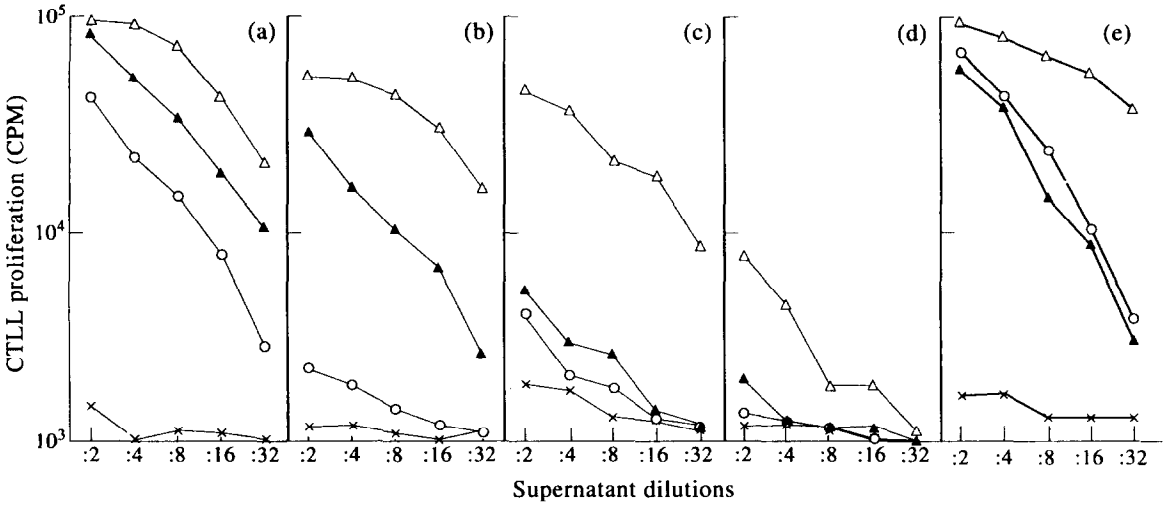

Figure 1. Whole IL-2 titration curves produced in response to stimulation with FLU (○), ALLO (▲) and PHA (△) or no stimulation (+) for a healthy control and 4 HD patients in different TH functional categories (see text for details). (a) +/+/+ HD patient; (b) −/+/+ HD patient; (c) −/−/+ HD patient; (d) −/−/− HD patient; (e) healthy control.

titration curves are shown in Figure 1 for one representative healthy control and 4 HD patients. PBMC from all 34 control donors (100%) produced IL-2 in response to FLU, ALLO and PHA and were similar to that of panel E. One of the four functional patterns illustrated in panels a–d was observed for each of the 47 HD patients. The first pattern was characterised by positive response to all of the stimuli (similar to the controls) and was observed in 19/47 (40%) patients (Figure 1a); in the second pattern (Figure 1b), observed in 12/47 (26%) patients, the response to FLU was absent, and IL-2 production in response to ALLO and PHA was positive but reduced compared to controls. In the third pattern, observed in 9/47 (19%) patients (Figure 1c), PBMC responded to PHA but failed to respond to FLU and ALLO. Finally, PBMC from 7/47 patients (15%) (Figure 1d) failed to respond to any of the stimuli. Flow cytometric analysis of PBMC indicated that none of these patients showed a reduction in the number of $CD3^+$ and $CD4^+$ T lymphocytes (data not shown). Our results demonstrate that four distinct patterns of TH function can be identified in HD patients, and indicate that defective IL-2 TH function is present in the majority (60%) of newly diagnosed untreated patients with HD.

PBMC from 34 of the control donors and from all 47 of the HD patients were also tested in the same experiments for proliferative responses to the same panel of stimuli. PBMC from all of the 34 control donors (100%) proliferated in response to FLU, ALLO and PHA. PBMC from 14/47 (30%) of the patients failed to proliferative in response to FLU, but did proliferate in response to ALLO or PHA. PBMC from none of the 47 patients (0%) showed defective proliferation in response to either ALLO or PHA (see Table 1). With two exceptions, a concordance between defective proliferation and defective IL-2 production in response to FLU was observed. In both of the exceptions, the proliferative response to FLU was positive, whereas IL-2 production was negative. For simplicity we will refer to those patients who, by IL-2 production, responded to FLU, ALLO and PHA as +/+/+; to those who failed to respond to FLU, but not to ALLO or PHA as −/+/+; to those who only responded to PHA as −/−/+; and to those who did not respond to any of the stimuli as −/−/−. No patients were found (by either IL-2 production or proliferative assay) who responded to FLU but not to ALLO or PHA, or who responded to FLU and ALLO but not to PHA.

Figure 2 shows the mean and individual values for IL-2 production (units of IL-2) by PBMC from each of the 34 controls and of the 47 HD patients in response to FLU, ALLO and PHA. The IL-2 produced is expressed in units of IL-2. Defective IL-2 production in response to FLU was observed in 28/47 (60%) HD patients (Figure 2a), but in none of the 34 controls (Figure 2b). The corresponding mean units of IL-2 produced by FLU-stimulated PBMC from the patients was 4.9; the mean for the controls was 19.4. IL-2 production by ALLO-stimulated PBMC was defective in 16/47 (34%) patients (Figure 2c), but in none of the controls (Figure 2d). The mean units of ALLO-stimulated IL-2 produced was 18.1 for the HD patients and 29.8 for the controls. Finally, PBMC from 7 HD patients, but none of the controls (compare Figure 2e with 2f), showed defective IL-2 production, even when stimulated with PHA. The mean units of IL-2 produced was 16.8 for the HD patients and 73.5 for the controls.

The data on *in vitro* generation of IL-2 by PBMC of the 47 HD patients are summarised in Table 2. This table also presents the clinical and haematological parameters, as well as age and sex of the 47 HD patients. The clinical parameters considered were the Rye clinical stage (I–IV) of Hodgkin's disease at diagnosis, the histological classification [nodular sclerosis (NS), mixed cellularity (MC), lymphocytes depletion (LD) and lymphocyte predominance (LP)], the presence of a bulky retrosternal mass and the absence (A) or presence of symptoms (B). As

Table 1. Fraction and percentage of unresponsive patients to FLU, ALLO and PHA

Donor status	Tested by IL-2 production			Tested by proliferation		
	FLU	ALLO	PHA	FLU	ALLO	PHA
Hodgkin's lymphoma	28/47 (60%)	16/47 (34%)	7/47 (15%)	14/47 (30%)	0/47 (0%)	0/47 (0%)
Controls	0/34 (0%)	0/34 (0%)	0/34 (0%)	0/34 (0%)	0/34 (0%)	0/34 (0%)

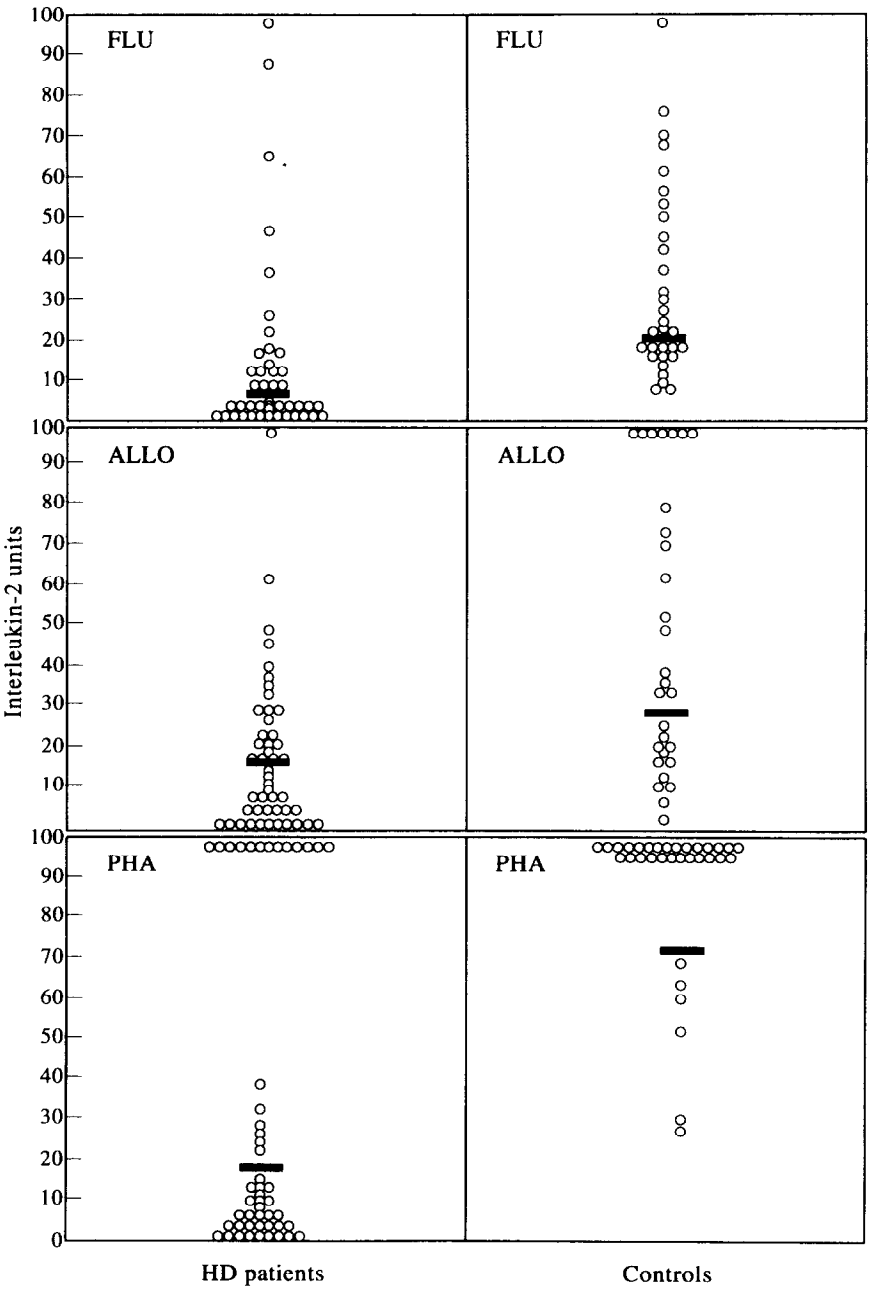

Figure 2. Relative IL-2 units produced by 47 HD patients and 34 healthy controls in response to stimulation with FLU, ALLO or PHA. The mean value is indicated in each panel by a black bar.

shown in Table 2, the initial clinical presentation of the −/−/− patients was more often severe (stage III or IV), symptomatic (B), and associated with a bulky retrosternal mass and a histological LD profile. Because of the relatively small number of patients in each group, however, none of the differences reached statistical significance. Six haematologic parameters were considered: erythrocyte sedimentation rate (ESR), concentration of copper (Cu^{2+}), concentration of calcium (Ca^{2+}), haemoglobin (Hb), concentration of alkaline phosphatase and of fibrinogen. For each parameter, the overall median and the median value for each TH functional group is shown. The median values for ESR, Cu^{2+}, alkaline phospatase and fibrinogen were higher in the more defective TH patients (−/−/+ and −/−/−); the trend was statistically significant ($P < 0.05$) between +/+/+ and −/−/− (Jonckheere test for trend) for Cu^{2+}. The trend was also statistically significant ($P < 0.05$) when the values for the combined +/+/+ and −/+/+ groups were compared with the −/−/+ and the −/−/− groups (Wilcoxon rank sum test). The trends for ESR, alkaline phosphatase and fibrinogen were positively correlated with each other and with the trend for Cu^{2+} ($P < 0.001$), as well as negatively correlated with Hb ($P < 0.001$) (Spearman rank correlation method). Table 2 also illustrates that no correlation was observed that involved age or sex, and any TH functional subgroup.

Finally, PBMC from 11HD patients in all four of the TH functional categories were used as stimulators in a mixed lymphocyte reaction, as previously reported for AIDS patients [19]. PBMC from healthy control donors were used as responder cells, either before or after depletion of their own antigen-presenting cells. No defects in the allogeneic stimulatory capacity were

Table 2. Summary of haematological, histological and immunological parameters for 47 newly diagnosed patients with Hodgkin's lymphoma

	Hodgkin's lymphoma patients					
	Overall median	Overall range	+/+/+ (n = 19)	−/+/+ (n = 12)	−/−/+ (n = 9)	−/−/− (n = 7)
Haematological parameters						
ESR (1 h)	26.5	5–121	21	26	45.5	35.5
Cu^{2+} (μg/ml)	163	10–332	150	142	200	209
Hb (g/l)	13.1	8.4–17.7	13.8	12.9	12.1	12.5
Fibrinogen (mg/ml)	461	146–990	428	341	582	660
Ca^{2+} (mg/ml)	9.4	8.2–10	9.3	9.5	9.2	9.4
Alk. phosp. (U/l)	193	52–316	186.5	158	243	208
Clinical parameters						
Stage I	6		3 (16%)	2 (17%)	0 (0%)	1 (14%)
Stage II	31		13 (68%)	9 (75%)	7 (78%)	2 (29%)
Stage III	5		2 (11%)	0 (0%)	1 (11%)	2 (29%)
Stage IV	5		1 (5%)	1 (8%)	1 (11%)	2 (29%)
A	23		12 (63%)	5 (42%)	3 (33%)	3 (43%)
B	24		7 (37%)	7 (58%)	6 (67%)	4 (57%)
Bulky	17		5 (26%)	4 (33%)	4 (44%)	4 (57%)
Histological parameters						
NS	8		1 (5%)	4 (33%)	2 (22%)	1 (14%)
MC	7		4 (21%)	2 (17%)	1 (11%)	0 (0%)
NS/CM	21		9 (47%)	5 (42%)	5 (56%)	2 (29%)
LP*	3		2 (11%)	0 (0%)	0 (0%)	1 (14%)
LD†	6		1 (5%)	1 (8%)	1 (11%)	3 (43%)
Not classified	2		2 (11%)			
Age (years)	28.7		29.3 (19–49)	40.8 (25–61)	32.5 (16–48)	23.1 (19–29)
Sex (male/female)	23/24		10/9	4/8	5/4	4/3
Immunological parameters						
IL-2 units FLU	4.9		15.8	2.2	2.9	4.7
IL-2 units ALLO	18.1		27.8	19.4	2.7	4.1
IL-2 units PHA	16.8		29.9	11.3	34.3	3.1

*2 cases LP; 1 case SN variety LP. †2 cases LD; 6 cases SN variety LD. ESR, erythrocyte sedimentation rate; Cu^{2+}, copper concentration; Hb, haemoglobin; Ca^{2+}, calcium concentration; Alk. phosp., alkaline phosphatase; NS, nodular sclerosis; MC, mixed cellularity; LD, lymphocyte depletion; LP, lymphocyte predominance. A, Absence of symptoms; B, Presence of symptoms; Bulky, Presence of bulky restrostenal mass.

detected by IL-2 production either when HD patients PBMC were used as stimulators of whole PBMC from healthy controls, or when HD patients PBMC were used as stimulators of APC-depleted PBMC from health controls (data not shown). Similar results were obtained using the proliferative assay.

DISCUSSION

We analysed the TH function of 47 newly diagnosed untreated patients with HD and of 34 healthy controls by antigen and mitogen-stimulated IL-2 production and proliferation. Intact IL-2 production in response to FLU, ALLO and PHA was observed in all of the controls (100%). A complex pattern of TH dysfunction was observed in the HD patients such that only 40% produced IL-2 in response to FLU, ALLO and PHA; 26% were incapable of generating IL-2 in response to the recall antigen FLU, but responded to ALLO and PHA; 19% responded to PHA only, and 15% could not generate IL-2 in response to either FLU, ALLO or PHA. As has been the case in analysis of TH function in HIV infection [11], the IL-2 assay was more sensitive than the proliferative assay, as the latter was able to detect a defect in only recall antigen-specific TH function, and for only 14/47 (30%) HD patients. The mechanism(s) responsible for the alteration in TH function of HD patients is not known, but could involve immunoregulatory cytokines as was reported for HIV^+ individuals (see below). Soluble inhibitory factors (SIF), partially identifiable as soluble IL-2 receptors (sIL-2R), have been demonstrated in the sera of HD patients [19–22]; sIL-2R have also been shown to be present in the culture supernatants of activated PBMC from HD patients [23]. We were unable to detect a defect in APC function in the HD patients by using their PBMC as allostimulators in a mixed lymphocyte reaction in which the responding T cells were from healthy control donors.

Other studies have reported defects in cell mediated immunity, and that *in vitro* antigen- and mitogen-stimulated IL-2 production may be impaired in HD [1–9]. In the present study, we make the novel observation of the presence of multiple, complex TH defects in HD, and their association with impaired

haematological and histopathological parameters. Thus, HD patients with the most impaired TH function (−/−/+ and −/−/−) tend to present with the more severe forms of HD (stage III or IV, bulky mediastinal involvement, lymphocyte depletion histology, B type symptoms). These same patterns of TH dysfunctional patients are also statistically correlated with the presence of compromised haematological parameters at presentation.

Using a similar approach, in which we analysed *in vitro* antigen- and mitogen-stimulated IL-2 production, we reported a similar pattern of TH dysfunction in asymptomatic, HIV+ individuals. In these individuals, we detected a sequential and progressive loss in TH function in the direction +/+/+ to −/+/+ to −/−/+ and finally to −/−/− that was predictive of clinically relevant AIDS-related events. For example, the extent of TH dysfunction is predictive of the reduction in the number of $CD4^+$ lymphocytes [15] and progression to AIDS [16]. In paediatric AIDS, the severity of TH functional defects is statistically correlated with augmented incidence of recurrent bacterial and opportunistic infections [24]. Thus, we have been able to stage HIV+ symptomatic individuals by TH immune dysfunction. We have recently correlated such TH dysfunction with a change from type-1 immunoregulatory cytokines profile (high IL-2 and IFN-γ with low IL-4 and IL-10 production) to a predominant type-2 profile (low IL-2 and IFN-γ with high IL-4 and IL-10 production) [11, 25, 26]. From this perspective, it may be mechanistically relevant that: (1) AIDS-related malignancies, such as Kaposi sarcoma [27–30], non-Hodgkin's lymphoma [31–34] and cervical carcinoma [35–37], are diagnosed in more than 40% of HIV-infected individuals (reviewed in [38, 39]); and (2) increases in constitutive type-2 cytokines, such as IL-6 and IL-10, that downregulate T cell proliferation and antigen-stimulated IL-2 production, are seen in these patients [40–43]. Thus, it is possible that a switch from a type-1 to a type-2 cytokine profile occurs in HD, and that progression in the disease will be reflected by the extent of TH immune dysregulation detected by type-1 versus type-2 cytokine analysis. Taken together, the findings of the present study suggest a potential value for including the analysis of TH function in the initial staging of HD. All of the 47 HD patients in this study are currently enrolled in a follow-up study to determine: (a) whether chemoradiotherapy will result in changes in the IL-2 production profiles, for example from −/−/+ to −/+/+ or +/+/+, as was observed in AIDS patients receiving zidovudine or dideoxynosine [24,44]; and (b) whether a −/−/+ or a −/−/− pattern of TH dysfunction will be predictive for recurrence of the tumour.

Finally, the differences in TH function that we detected in these patients may have mechanistic implications for the preferential role of the immune system in surveillance against cancer. Individuals with other types of malignancies should be tested for possible changes in their type-1/type-2 immunoregulatory cytokine profile.

1. Young RC, Corder MP, Haynes HA, DeVita VT. Delayed hypersensitivity in Hodgkin's disease. *Am J Med* 1972, **52**, 63–71.
2. Case CM, Good RA. Comparison of *in vitro* and *in vivo* multiple parameters in untreated patients with Hodgkin's disease. *Cancer* 1976, **38**, 181–197.
3. Moghe MV, Advani SH, Gangal SG. Demonstration of inhibitory factors affecting cell-mediated immunity in patients with Hodgkin's disease. *Eur J Cancer Clin Oncol* 1980, **16**, 937–941.
4. Ford RJ, Tsao J, Kouttab NM, *et al.* Association of an interleukin abnormality with the T cell defect in Hodgkin's disease. *Blood* 1984, **64**, 386–392.
5. Soillou JP, Douillard JY, Vie H, *et al.* Defect in lectin-induced IL-2 production by peripheral blood lymphocytes of patients with Hodgkin's disease. *Eur J Cancer Clin Oncol* 1985, **21**, 935–942.
6. Romagnani S, Rossi Ferrini PL, Ricci M. The immune derangement in Hodgkin's disease. *Sem Hematol* 1985, **22**, 41–55.
7. Zamkoff KW, Reeves WG, Paolozzi FP, *et al.* Impaired interleukin regulation of the PHA response in Hodgkin's disease. *Clin Immunol Immunopathol* 1985, **35**, 111–124.
8. Damle RN, Tatake RJ, Adavani SH, Gangal SG. Affinity of IL-2 receptors and proliferation of mitogen-activated lymphocytes in Hodgkin's disease. *Br J Cancer* 1990, **61**, 404–406.
9. Damle RN, Adavani SH, Gangal SG. Impairment in proliferation, lymphokine production and frequency distribution of mitogen-responsive and interleukin-2 producing cells in Hodgkin's disease. *Cancer Immunol Immunother* 1991, **34**, 205–210.
10. Via CS, Tsokos GC, Stocks NI, *et al.* Human *in vitro* allogeneic responses: demonstration of three pathways of T helper cell activation. *J Immunol* 1990, **44**, 2524–2229.
11. Clerici M, Stock NI, Zajac RA, *et al.* Detection of three distinct patterns of T helper cell dysfunction in asymptomatic, human immunodeficiency virus-seropositive patients. Independence of CD4+ cell numbers and clinical staging. *J Clin Invest* 1989, **84**, 1892–1899.
12. Muluk SC, Clerici M, Via CS, *et al.* Correlation of in vitro CD4+ T helper cell function with clinical graft status in immunosuppressed kidney transplant patients. *Transplant* 1991, **52**, 284–291.
13. Shulik RD, Muluk SC, Clerici M, *et al.* Value of in vitro CD4+ T helper cell function test for predicting long term loss of human renal allografts. *Transplantation*, in press.
14. Via CS, Tsokos GC, Bermas B, *et al.* T cell-antigen presenting cell interaction in human systemic lupus erithematosus: evidence for heterogeneous expression of multiple defects. *J Immunol* 1993, **151**, 3914–3922.
15. Lucey DR, Melcher G, Hendrix CW, *et al.* The U.S. Air Force HIV study 1985–1990: immunological analyses, seroconversion and the potential utility of a T-helper functional assay to predict change in CD4+ T-cell counts during early stage of HIV infection. *J Infect Dis* 1992, **164**, 631–640.
16. Dolan MJ, Melcher GP, Clerici M, Shearer GM. A T cell functional assay combined with measurement of CD4+/CD29 bright cells is predictive of death in HIV infection. IX International Conference on AIDS. Berlin, June 6–11, 1993. Abstract WS-B02-6, p. 94.
17. Carbone PP, Kaplan HS, Musshof K, *et al.* Report of a committee on Hodgkin's disease staging classification. *Cancer Res* 1971, **31**, 1860–1861.
18. Hister TA, Crowther D, Sutcliff SB, *et al.* Report of a committee convened to discuss the evaluation and staging of patients with Hodgkin's disease. *J Clin Oncol* 1989, 7, 1630–1636.
19. Clerici M, Landay AL, Kessler HA, *et al.* Multiple patterns of alloantigen presenting/stimulating cell dysfunction in HIV infected individuals. *J Immunol* 1991, **146**, 2207–2213.
20. Golding B, Golding H, Lommityer R. Production of leukocytes inhibitory factor (LIF) in Hodgkin's disease: spontaneous production of an inhibitor of normal lymphocyte transformation. *Clin Immunol Immunopathol* 1977, 7, 114–122.
21. Pizzolo G, Chilosi M, Vinante F, *et al.* Soluble interleukin-2 receptors in the serum of patients with Hodgkin's disease. *Br J Cancer* 1987, 55, 427–428.
22. Damle RN, Advani SH, Gangal SG. Analysis of regulation of T cell responses by soluble inhibitory factors from the sera of patients with Hodgkin's disease. *Int J Cancer* 1990, **50**, 192–196.
23. Chopra RK, Powers DC, Kendig NE, *et al.* Soluble interleukin-2 receptors released from mitogen-stimulated human peripheral blood lymphocytes bind interleukin-2 and inhibit IL-2 dependent cell proliferation. *Immunol Invest* 1989, **18**, 961–973.
24. Clerici M, Roilides E, Butler KM, *et al.* Changes in T helper cell function in human immunodeficiency virus infected children during dideoxynosine therapy as a measure of antiretroviral activity. *Blood* 1992, **80**, 2196–2202.
25. Clerici M, Hakim FT, Venzon DJ, *et al.* Changes in interleukin 2 and interleukin 4 production in asymptomatic, HIV-seropositive individuals. *J Clin Invest* 1993, **91**, 759–765.
26. Clerici M, Wynn TA, Berzofsky JA, *et al.* Role of interleukin-10 (IL-10) in T helper cell dysfunction in asymptomatic individuals infected with the human immunodeficiency virus (HIV-1). *J Clin Invest* 1994, **93**, 768–775.
27. Jaffe HW, Choi K, Thomas PA, *et al.* National case-control

study of Kaposi's sarcoma and *Pneumocystis carinii* pneumonia in homosexual men: Part 1. Epidemiologic results. *Ann Intern Med* 1983, **99**, 145–151.
28. Beral V, Bull D, Darby S, *et al.* Risk of Kaposi's sarcoma and sexual practices associated with faecal contact in homosexual or bisexual person with AIDS. *Lancet* 1992, **339**, 632–635.
29. Beral V. Epidemiology of Kaposi's sarcoma. In Veral V, Jaffe HW, Weiss RA, eds. *Cancer, HIV and AIDS*. New York, Cold Spring Harbor Laboratory, 1991, 5–22.
30. Huang YQ, Li JJ, Kim KS, *et al.* HIV-1 infection and modulation of cytokine and growth factor expression in Kaposi's sarcoma-derived cells *in vitro*. *AIDS* 1993, 7, 317–322.
31. Pluda JM, Yarchoan R, Jaffe ES, *et al.* Development of non-Hodgkin's lymphoma in a cohort of patients with severe human immunodeficiency virus (HIV) infection on long term antiretroviral therapy. *Ann Intern Med* 1990, **113**, 276–282.
32. Gail MH, Pluda JM, Rabkin CS, *et al.* Projection of the incidence of non-Hodgkin's lymphoma related to acquired immunodeficiency syndrome. *J Natl Cancer Inst* 1991, **83**, 695–701.
33. Beral V, Peterman T, Berkelman R, *et al.* AIDS-associated non-Hodgkin lymphoma. *Lancet* 1991, **337**, 805–809.
34. Roithmann S, Tourani JM, Andreu JM. AIDS-associated non-Hodgkin lymphoma. *Lancet* 1991, **338**, 884–885.
35. Giorda G, Vaccher E, Volpe R, *et al.* An unusual presentation of vulvar carcinoma in an HIV patient. *Gynecol Oncol* 1992, **44**, 191–194.
36. Maiman M, Fruchter RG, Serur E, *et al.* Human immunodeficiency virus and cervical neoplasia. *Gynecol Oncol* 1990, **38**, 337–382.
37. Matorras R, Ariceta JM, Corral J, *et al.* Human immunodeficiency virus-induced immunosuppression: a risk factor for human papilloma virus infection. *Am J Obstet Gynecol* 1991, **164**, 42–44.
38. Biggar RJ. Cancer in acquired immunodeficiency syndrome: An epidemiological assessment. *Semin Oncol* 1990, **17**, 251–260.
39. Levine AM. AIDS-related malignancies: the emerging epidemic. *J Natl Cancer Inst* 1993, **85**, 1382–1397.
40. Breen EL, Rezal AR, Nakajima K, *et al.* Infection with HIV is associated with elevated IL-6 levels and production. *J Immunol* 1990, **144**, 480–484.
41. Gotlieb WH, Abrams JS, Watson JM, *et al.* Presence of interleukin 10 (IL-10) in the ascites of patients with ovarian and other intra-abdominal cancers. *Cytokine* 1990, **4**, 385–390.
42. Emilie D, Toitou R, Raphael M, *et al.* *In vivo* production of IL-10 by malignant cells in AIDS lymphoma. *Eur J Immunol* 1992, **22**, 2937–2942.
43. Benjamin D, Knobloch TJ, Abrams J, *et al.* Human B cell IL-10: B cell lines derived from patients with AIDS and Burkitt's lymphoma constitutively secrete large quantities of IL-10. *Blood* 1992, **80**, 1289–1299.
44. Clerici M, Landay AL, Kessler H, *et al.* Reconstitution of T helper cell function following zidovudine therapy in HIV infected patients. *J Infect Dis* 1992, **166**, 723–730.

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A Randomised Study of Cisplatin Versus Thiotepa as Induction Chemotherapy in Advanced Ovarian Carcinoma

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Between 1980 and 1984, a total of 171 patients with advanced epithelial ovarian carcinoma and residual tumour after surgery were randomly assigned to treatment groups receiving either cisplatin or thiotepa. The objective of the study was to evaluate the regimes with regard to response and survival. The two groups were well balanced with respect to age, FIGO stage, histology, grade and residual tumour after surgery. In the cisplatin group, 66% responsed to treatment compared to 38% in the thiotepa group ($P < 0.00005$). The median progression-free survival was 10.5 months and 6.3 months, respectively. The corrected survival was somewhat, but non-significantly, higher in the cisplatin group than in the thiotepa group, with an 8-year corrected survival of 10.6 and 7.4%, respectively. In a multivariate analysis, based on progression-free survival with FIGO stage, residual tumour after surgery, histological type and grade as covariables, treatment with thiotepa had a relative risk of 1.64 compared to cisplatin (95% confidence interval 1.17–2.30, $P = 0.004$).

Key words: ovarian cancer, cisplatin, thiotepa
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INTRODUCTION

SINCE CISPLATIN was introduced as single-agent treatment in patients with advanced epithelial ovarian cancer, several studies [1] have demonstrated improved survival using this drug. Later, there were high expectations for combination chemotherapy, that is cisplatin with doxorubicin, hexamethylmelamine, cyclophosphamide and various other cytostatics, but results on prolonged long-term survival were disappointing [2–4]. Some have argued that single-agent cisplatin is as effective as platinum-based combinations when it comes to overall survival, and the