7. Thames HD, Henry JH. Fractionation in radiotherapy. London: Taylor & Francis, 1987: pp 72, 74, 76, 77, 88, 94.
8. Slavin S. Elimination of GvHD by lymphocyte depletion ex vivo using Campath-1. In: Bone marrow transplantation for leukaemia, p. 22. International workship. England, London: Leukaemia Research Fund, 1984.
9. O' Donnoghue JA, Wheldon TE, Gregor A. The implications of in-vitro radiation-survival curves for the optimal scheduling of total-body irradiation with bone marrow rescue in the treatment of leukaemia. Br J Radiol 1987; 60: 279.
10. Tucker SL, Withers HR, Mason KA, Thames HD. A dose-surviving fraction curve for mouse colonic mucosa. Eur J Clin Oncol 1983; 19: 433.
11. Denekamp J. Cell kinetics and radiation biology. Int J Radiat Biol 1986; 49: 357.
12. Moulder JE, Fischer JJ. Radiation reaction of rat skin: the role of number of fractions and overall treatment time. Cancer 1976; 37: 2762.

IMMUNOLOGIC IMPAIRMENT AFTER LONG-TERM REMISSION IN SPLENECTOMIZED PATIENTS WITH HODGKIN'S DISEASE

Immunologic impairment has been demonstrated in patients with untreated Hodgkin's disease (1, 2). The immune status of patients apparently cured from Hodgkin's disease (HD) is therefore of interest and this concerns especially patients who have undergone staging splenectomy and therefore have increased risk of fulminant infections after heavy treatment for HD (3). However, the relevant studies done so far may not be conclusive since they were generally performed after relatively short follow-up (1–4 years), included only limited spectrum of tests (4–8), or were done prior to the availability of T-cell subset typing (9–10). We therefore decided to study the immunologic status of a group of patients in long and complete remission after staging splenectomy and treatment for HD.

Patients. Ten HD patients, who had been surgically staged including splenectomy prior to treatment, were immunologically studied after a median of 10 years (range 8.5–12.3 years) from their last treatment. Their data are summarized in Table 1.

They were histologically classified according to the Rye conference, and staged according to the Ann Arbor classification. Radiotherapy consisted of 40 Gy to extended fields, except for 2 patients who received total node irradiation and one who was not irradiated at all. Chemotherapy consisted of MOPP. Healthy individuals with comparable age and sex served as controls.

Immunological tests. Percentage of total peripheral blood lymphocytes (PBLs) and monocytes were determined by routine Giemsa staining. Mononuclear cells were isolated on a Ficoll-hypaque gradient. B-cells were identified with fluorescent anti-Ig (FAB fragment) antibodies after substraction of 'L' cells (Labile surface Ig). Total T-cells and their subsets were identified by monoclonal antibodies (OKT-3, OKT-4, OKT-8). Lymphocyte transformation was performed in triplicate and dose–response curves were established with 70, 17.5, 4.4 and 1.1 μg/well of phytohemagglutinin (PHA-P Difco). The cultures were harvested after 72 h. Serum IgG, IgA and IgM concentrations were determined by nephelometry and expressed in g/l. Skin tests were performed with 4 recall antigens, candida, PPD, tricophyton, and SK/SD. An induration of 5 mm or more after 48 h was considered positive. Patients with a positive response to at least one recall antigen were classified as responders. In addition, the response in each subgroup of patients was quantified by a skin test index (STI) which expressed the mean number of positive skin responses in patients in that subgroup. DNCB (Eastman 1-chloro-2, 4-dinitrobenzene) in freshly prepared acetone solution was used as a neoantigen for active sensitization at a dose of 1000 μg. Two weeks after primary sensitization, patients were challenged with four concentrations of DNCB (200, 50, 25 and 10 μg). Skin reaction to the challenging dose appearing within 48 h or even 4 to 10 days later, was considered positive if it included both an erythematous plaque and induration. Appearance of additional papular, vesicular or bullous eruptions indicated increased degrees of response.

Table 1

Patients' characteristics

Age/Sex	Histologic type*/Surgical stage	Treatment			Years between splenectomy (or end treatment) and immun. evaluation
		Radiotherapy		Chemotherapy	
		Mantle field	Paraaortic		
Early stage					
32/M	LP/IA	+	−	−	8.6 (8.5)
34/F	NS/IIA	+	+	−	10.7 (10.5)
37/F	NS/IIA	+	−	−	9.0 (8.8)
29/F	MC/IIA	+	−	−	12.7 (12.3)
Advanced stage					
42/M	NS + MC/IIB	+	−	+	10.0 (9.5)
21/M	MC/IIB	+	+	−	12.0 (11.7)
24/F	NS/IIEB	+	+	+	11.0 (9.3)
13/F	NS + MC/IIISA	+	+**	−	9.3 (9.0)
25/F	NS + MC/IIISB	−	−	+	11.9 (10.5)
16/M	MC/IIISB	+	+**	+	10.3 (9.3)

* LP = lymphocytic predominance; NS = nodular sclerosis; MC = mixed cellularity.
** Patients Nos. 8 and 10 received total nodal irradiation.

Statistical methods. All data were stored in a data base (Lotus 1-2-3 Development Corporation, Cambridge MA, 1993). Statistical evaluation of differences between means was determined by Student's t-test and the differences between ratios were evaluated by the χ^2-test including Yates' correction. The results of each test were summarized for the whole series and were compared to data obtained from our control groups. In addition, results from the subgroup of 4 patients in early stages (I-IIA) were compared to those of the 6 patients on more advanced stages (IIB-IIISB).

Results

Data for subsets of mononuclear cells are presented in Table 2. The total white blood cell count was significantly elevated in our patients ($p < 0.02$). This included a normal absolute number of lymphocytes although their percentage was reduced ($p < 0.01$). The patients showed B-cell lymphocytosis ($p < 0.05$). The percentage of T-cells was significantly reduced ($p < 0.01$) but not their absolute number (1 240 versus 1 400/mm^3).

T-helpers (OKT-4) were significantly fewer among HD patients ($p < 0.001$) while the T-suppressors (OKT-8) did not show a significant deviation from normal controls. As a result the helper/suppressor ratio showed a significant reduction among HD patients ($p < 0.05$). These changes in subpopulations of T-cells showed no dependency on stage.

Lymphoblastoid transformation response, elicited by PHA was significantly reduced in patients with HD, regardless of stage (Figure). This impairment was dose-dependent and inversely proportional to doses of mitogen. It became apparent at a dose of 17.5 μg/well but achieved statistical significance ($p < 0.02$) only at lower doses (4.4 and 1.1 μg/well).

The degree of spontaneous blastic transformation (BLANK) was higher in HD patients than among normal controls although no statistical significance was found. However, when evaluated by stage of disease, a statistically significant increment ($p < 0.05$) was found in advanced stages.

Both serum IgG (15.5 ± 2-4 versus 10.4 ± 2.4 g/l, $p < 0.01$) and serum IgM (1.65 ± 0.79 versus 1.01 ± 0.31 g/l, $p < 0.02$) were significantly higher than normal while IgA remained within the normal range.

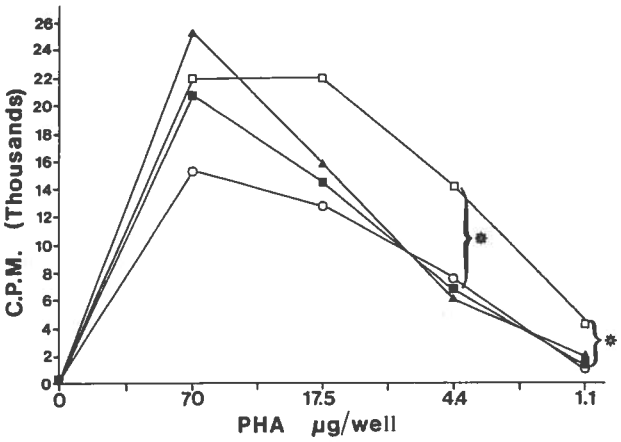

Figure. Lymphocytic stimulation: dose response curves. □ = normal control; ■ = Hodgkin's disease total group; ○ = Hodgkin's disease early stage; ▲ = Hodgkin's disease advanced stage.

*The difference between HD (total group) and normal controls in statistically significant ($p < 0.02$).

Evaluation of DHR by recall antigens (Ags), revealed only one anergic patient who did not respond to any of the 4 test Ags. The STI, however, showed a significant reduction of response to recall antigens among patients with advanced disease as compared to those in early stages of the disease (1.4 versus 3.25, $p < 0.02$). Also the capacity to become sensitized to a new antigen—DNCB—was severely impaired since only 37% of patients became sensitized to DNCB as compared to 87% of normal controls. This last impairment characterized all stages of the disease.

Table 2

Mononuclear cells and their subclasses in peripheral blood of patients with HD in remission: quantitative comparison with normal controls

Type of cells /mean ± SD	Patients	Control (Normal)
Total leukocytes (No./mm^3)	9 030 ± 2 721	6 560 ± 1 370
	$p < 0.02$	
% lymphocytes	26 ± 6.8	33.5 ± 5.47
	$p < 0.01$	
% monocytes	6.1 ± 3.6	6.9 ± 2
Total lymphocytes	2 348 ± 614	2 203 ± 635
% B cells	12.96 ± 4.8	8.73 ± 3.9
	$p < 0.05$	
% T cells (OKT4)	52.8 ± 6.87	63.53 ± 7.89
	$p < 0.01$	
Total T cells	1 240 ± 159	1 400 ± 403
% helper cells (OKT4)	34 ± 8.1	46.13 ± 6.13
	$p < 0.001$	
% suppressor cells (OKT8)	24.5 ± 5.72	25.12 ± 5.23
Ratio OKT4/OKT8	1.45 ± 0.43	1.94 ± 0.59
	$p < 0.05$	

Discussion

This study describes the complex immunological status of patients who were free of disease 10 years after splenectomy and treatment for HD. These apparently cured patients had impaired function of their T-lymphocytes as shown in vitro by reduced response to low concentrations of PHA and in vivo by a reduced capacity to develop sensitization to a new Ag (DNCB). These functional defects are in accordance with other series studied after shorter follow-up and seem to reflect the persistence of a condition originally detected in HD patients already at diagnosis (1, 2,11). In spite of an apparently normal count of total lymphocytes in our patients, there was a quantitative impairment of subpopulations of these cells with a reduced proportion of T-cells (OKT-3), due to a selective depletion of the helper (OKT-4) fraction resulting in a decreased helper/suppressor ratio.

Some previously published observations suggest that the depletion of helper cells was induced by previous irradiation (7). B-cell parameters were not depleted, as shown both quantitatively, by their increased proportion among normal lymphocyte count, and functionally by the increased production of IgM and IgG in vivo. It is noteworthy that this late overproduction differed from the normal (6, 11) or even reduced production (5, 8) reported after shorter follow-up.

Our study, in accordance with some observations made after shorter follow-up, suggests that splenectomy does not have an adverse effect on the studied immune parameters (4, 6, 8,12).

The present study is far from being conclusive due to its small patient material, lack of a control group of untreated HD patients, and lack of longitudinal studies of the different patients. We therefore present these results merely as a short communication and suggest that further studies be done on larger populations.

Key words: Hodgkin's disease, splenectomy, remission, immunologic status or deficiency.

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REFERENCES

1. Twomey JJ, Rice L. Impact of Hodgkin's disease upon the immune system. Semin Oncol 1980; 7: 114–25.
2. Kaplan HS. Hodgkin's disease: biology, treatment, prognosis. Blood 1981; 57: 813–22.
3. Weitzman S, Aisenberg AC. Fulminant sepsis after the successful treatment of Hodgkin's disease. Am J Med 1977; 62: 47–50.
4. Wagener Theo DJ, Geestman E, Borgonjen A, Haanen C. The influence of splenectomy on cellular immunologic parameters in Hodgkin's disease. Cancer 1976; 37: 2212–9.
5. Hancock BW, Lesley B, Milford-Ward A, Richmond J. Changes in immune status in patients undergoing splenectomy for the staging of Hodgkin's disease. Br Med J 1976; 1: 313–5.
6. Steele R, Han T. Effect of radiochemotherapy and splenectomy on cellular immunity in long-term survivors of Hodgkin's disease and non-Hodgkin's lymphoma. Cancer 1978; 42: 133–9.
7. Posner MR, Reinherz E, Lane H, Mauch P, Hellman S, Schlossman SF. Circulating lymphocyte populations in Hodgkin's disease after mantle and paraaortic irraditation. Blood 1983; 61: 705–8.
8. Björkholm M, Wedelin Ch, Holm G, Johansson B, Mellstedt H. Longitudinal studies on blood lymphocyte capacity in Hodgkin's disease. Cancer 1981; 48: 2010–5.
9. Kun LE, Johnson RE. Hematologic and immunologic status in Hodgkin's disease 5 years after radical radiotherapy. Cancer 1975; 36: 1912–6.
10. Fuks Z, Strober S, Bobrove AM, Sasazuki I, McMichael A, Kaplan HS. Long-term effects of radiation on T and B lymphocytes in peripheral blood of patients with Hodgkin's disease. J Clin Invest 1976; 58: 803–14.
11. Fisher RI, DeVita VT, Bostick F, et al. Persistent immunologic abnormalities in long-term survivors of advanced Hodgkin's disease. Ann Intern Med 1980; 92: 595–9.
12. Posner MR, Reinherz EL, Breard J, Nadler LM, Rosenthal DS, Schlossman SF. Lymphoid subpopulations of peripheral blood and spleen in untreated Hodgkin's disease. Cancer 1981; 48: 1170–6.

EFFECTS OF MELATONIN ON THE RELEASE AND PRODUCTION OF BLOOD LEUKOCYTES AFTER ADMINISTRATION OF A NOVEL TAURINE-BASED NITROSOUREA CYTOTOXIC AGENT

Human total blood leukocyte (WBC) concentrations fluctuate with a circadian rhythm and have a maximum at 24.00 h (1). This is a composite of rhythms due to different leukocyte subpopulations. Lymphocytes exhibit an acrophase at 24.00–04.00 h, for eosinophils this occurs during a dark period and for monocytes and polymorphonuclear neutrophils (PMN) a maximum occurs at about 20:00 h. In nocturnal rodents, the circadian rhythms in the number of circulating WBCs and different subclasses exhibit peak values at times of relative inactivity which are approximately 180° out of phase with diurnally active Man. Since the production of melatonin (MT) in most species, including Man, is also circadian with a peak occurring during the dark phase (2) it is possible that the production or release of WBCs may be synchronised to a MT related mechanism.

There is high risk of chronic infection as a result of myelosuppression which can be severe and prolonged following anticancer chemotherapy and quite often delayed bone marrow toxicity determines the limiting dose of anticancer agent which can be tolerated (3).

A very potent, novel anticancer agent based on taurine, 1-(2-chloroethyl) 3-[2-(dimethylaminosulphonyl)ethyl]-1-nitrosourea (TCNU) unfortunately also has marked haematopoietic toxicity (4–6). Recently, it has been demonstrated that MT may inhibit the immunosuppression induced by corticosterone (7, 8) and the anticancer agent, cyclophosphamide (9). Thus, MT may also be able to ameliorate the haematopoietic toxicity of TCNU.

The aim of this study was to determine whether exogenously administered MT could influence either the release or production of blood leukocytes, and/or reduce the severity of haematopoietic toxicity to TCNU in experimental animals.

Drugs. Melatonin was purchased from Sigma Chemical Co, Pool, Dorset, UK. TCNU was supplied by Dr M Biddy (Clinical Oncology Unit, University of Bradford, Bradford, UK) as a kind gift from Leo Laboratories, Helsingborg, Sweden. All other chemical reagents were Analar grade supplied by BDH Chemicals Ltd, Pool, Dorset, UK.

Animals. Male guinea pigs (Dunkin-Hartley, 802–1 012 g) and female rats (Sprague-Dawley CD strain, 228–374 g) were fed a standard laboratory diet (Labsure CRM) and water or water plus MT/vehicle ad libitum. Water consumption was monitored to determine melatonin dosage per animal.

Haematological measurements. Blood samples were collected into lithium heparinised tubes. Blood cell counts and cell size distributions of WBCs were obtained by semiautomated methods using a Coulter ZM cell counter and C1000 Channeliser (Coulter Electronics Ltd, Luton, Beds. UK). Differential WBC counts were determined on May-Grünwald-Giemsa stained blood films from counts of at least 200 cells. Haematocrit (PCV), RBC counts and haemoglobin (Hb) concentrations were determined by standard methods.

Statistics. All data are presented as the mean + SEM. Significant differences between MT treatment and vehicle upon leukocyte release were determined by unpaired Student's t-test. Comparisons between treatments and changes occurring with time in animals sampled on more than one occasion were tested for signficance by multiple analysis of variance (MANOVA). All statistical analyses were carried out on a Cyber 830 Main Frame computer using the statistical package for the social sciences (SPSS-X). Statistically significant differences were accepted if $p < 0.05$ and trends were indicated if $0.05 < p < 0.1$.

Study 1: Effect of MT on leukocyte release. Treated animals ($n = 6$) received MT in drinking water (40 mg/l) and controls