Eur J Cancer Clin Oncol, Vol. 19, No. 6, pp. 747–755, 1983.
Printed in Great Britain.

0277-5379/83$3.00+0.00

Longitudinal Studies of Blood Lymphocyte Functions in Non-Hodgkin's Lymphoma*

CHRISTINA SIMONSSON-LINDEMALM,†‡ PETER BIBERFELD,§ MAGNUS BJÖRKHOLM,¶ GÖRAN HOLM,** BO JOHANSSON,† HÅKAN MELLSTEDT,† BO NILSSON|| and ÅKE ÖST§

†*Radiumhemmet and the Department of Radiobiology,* §*Department of Pathology and* ||*Department of Cancer Epidemiology, Karolinska Hospital, Stockholm, Sweden,* ¶*Department of Medicine, Danderyd's Hospital, Stockholm, Sweden and* *******Department of Clinical Immunology, Huddinge Hospital, Stockholm, Sweden*

Abstract—*The study comprises 26 patients with non-Hodgkin's lymphoma who entered complete remission. Lymphocyte subsets and spontaneous and mitogen (ConA, PWM)-induced DNA synthesis were recorded in purified blood lymphocytes harvested before treatment, in remission and in relapse. The T and B lymphocytopenia noted in untreated patients persisted in complete remission. At diagnosis the spontaneous DNA synthesis was increased and the response to mitogen was decreased as compared to controls. Both functions became normalised during complete remission in most patients under 60 yr. The lymphocyte abnormalities reappeared during relapse. A low response to mitogen (ConA) in complete remission seemed to predict early relapse in patients younger than 60 yr. It is concluded that functional abnormalities of blood lymphocytes in non-Hodgkin's lymphoma are closely related to the presence of active disease.*

INTRODUCTION

NON-HODGKIN'S lymphoma (NHL) of the B cell type may be associated with abnormal serum immunoglobulin levels and antibody deficiency as well as the presence of monoclonal serum immunoglobulins [1, 2]. In addition, monoclonal B cells may often appear in blood during active disease [3, 4]. Impairment of delayed hypersensitivity and abnormalities of blood T lymphocyte functions are also often seen [5–9]. Similar T cell defects have been described in patients with Hodgkin's disease (HD), while deficient humoral immunity is a late finding in these patients [6, 10, 11]. Moreover, lymphocyte abnormalities in HD are strongly associated with poor prognosis and often partly persist for many years in apparently cured patients [12, 13]. The variation of the lymphocyte capacity in patients with NHL during different phases of the disease and its clinical significance is less clearly defined.

The aims of the present study were to evaluate blood lymphocyte subsets and lymphocyte functions before treatment, during remission and in relapse and the prognostic information yielded by the lymphocyte abnormalities.

MATERIALS AND METHODS

The material comprises 26 patients with NHL admitted to Radiumhemmet and the Seraphimer Hospital between November 1977 and April 1979 who had entered complete remission (CR). The patients were tested at the time of diagnosis [9] and in complete remission. Of 31 patients who entered CR 26 were retested and included in the present study. There were 19 women and 7 men, with a mean age of 55 yr (range 20–79 yr) (Fig. 1). The initial evaluation of the patients included a detailed history, physical examination, routine blood analysis, serum electrophoresis and liver enzyme, bone marrow and lymph node biopsies, chest radiograms, liver and spleen scans and computerised tomography of the abdomen. The patients were clinically staged according to the Ann Arbor recommendations [14]. The Kiel nomenclature was used for histopathological classification [15].

Accepted 2 November 1982.
*This study was supported by grants from the Swedish Cancer Society, the Cancer Society in Stockholm, the Karolinska Institute Foundation, the Felix Mindus Research Foundation, Axel and Margaret AX:son Johnson Foundation and the Seraphimer Hospital Foundation.
‡To whom requests for reprints should be addressed at: Radiumhemmet, Karolinska Hospital, S-104 01 Stockholm, Sweden.

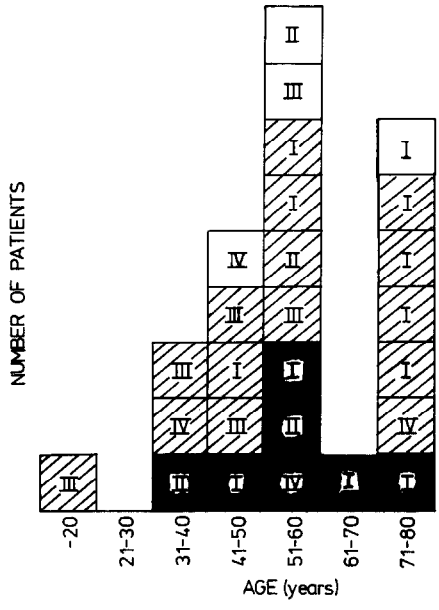

Fig. 1. Distribution according to age and stage of patients achieving complete remission. One square represents one patient. ▨ Patients with low grade malignancy according to the Kiel classification; ■ patients with high grade malignancy according to the Kiel classification; □ patients with unclassifiable lymphomas.

Clinical stage I and II patients received involved field irradiation (RT) to a target dose of approximately 40 Gy fractionated over 4 weeks. Clinical stage III and IV patients were given multiple drug chemotherapy (CT). All but one patient received the CVP combination (cyclophosphamide, vincristine, prednisone) [16]. One CB lymphoma received the CHOP combination (cyclophosphamide, adriamycin, vincristine, prednisone) [17]. CR was defined as total disappearance of tumor and symptoms and normalization of laboratory tests. Two patients, one CC of the ileum and one LBL (Burkitt type) in the neck region, refused other treatment than surgical excision and are not included in Table 1.

Patients given RT were tested 15–18 months after cessation of therapy and patients given CT were tested 8 weeks after the latest course. The reason for the different intervals was the observation that in RT patients the response to mitogens was decreased shortly after RT but returned to a constant level approximately 12 months after cessation of RT. Chemotherapy, on the other hand, was found to have a less-pronounced and more short-lasting effect on the lymphocyte stimulation. Patients in relapse were retested when the relapse was diagnosed and before reinstitution of treatment.

Control subjects

Fifty-seven age-matched healthy persons mainly from the hospital staff and their relatives were used as controls. There were 22 men and 35 women, with a mean age of 44 yr (range 20–91 yr). All were free of drugs. One control was included in each lymphocyte experiment.

Lymphocyte count

White blood cells were counted after staining in Türk's solution. The percentage of lymphocytes was determined by a differential count of blood smears stained with May–Grünwald and Giemsa. The total number of lymphocytes was calculated.

Purification of lymphocytes

Lymphocytes were purified by gelatin sedimentation of defibrinated venous blood [18]. After ingestion of iron powder phagocytic cells were removed with a magnet [19]. Lymphocytes to be tested with cell surface markers were purified by floatation on a Ficoll-Isopaque gradient to remove remaining red blood cells. For further details of purification procedure see Holm *et al.* [20].

E-binding (E^+) lymphocytes

E^+ lymphocytes were identified as lymphocytes forming non-immune rosettes with sheep red blood cells (SRBC) at 4°C. Two hundred lymphocytes were counted and cells forming rosettes with 3 or more SRBC were considered positive [20].

IgG Fc receptor-bearing (EA^+) lymphocytes

Lymphocytes with receptors for the Fc part of IgG were determined by a modification of the rosette technique described by Hallberg *et al.* [21]. The percentage of lymphocytes binding 3 or more ox red cells coated with rabbit IgG anti-ox antibodies was determined by counting 200 cells.

Surface membrane immunoglobulin ($smIg^+$)-bearing lymphocytes

$smIg^+$ lymphocytes were identified by direct immunofluorescence with $F(ab')_2$-fragments of polyspecific fluorescein-conjugated rabbit anti-human Ig serum and rabbit antisera against free κ and λ light chains (Dako, Copenhagen, Denmark). Before staining the lymphocytes were incubated in serum-free medium for 30 min at 37°C and washed 3 times at 37°C to remove absorbed Ig [22]. Membrane fluorescence was examined in a Zeiss fluorescence standard RA microscope in transmitted ultraviolet light at ×1000 magnification. A KP-500 filter for emission of fluorescence was used. Two to four hundred cells were counted on each slide.

Mitogens

Concanavallin A (ConA) was purchased from Pharmacia Fine Chemicals AB, Uppsala, Sweden.

Pokeweed mitogen (PWM) was obtained from Gibco, Berkeley, CA, U.S.A. One batch of each mitogen was used throughout the study.

DNA synthesis

Lymphocytes were suspended in RPMI 1640 with 10 mM HEPES (Biocult Laboratories Ltd, Glasgow, U.K.), 2 mM glutamine, 100 IU penicillin and 100 μg streptomycin/ml supplemented with 15% heat-inactivated (60 min at 56°C) human AB serum. One hundred thousand lymphocytes were transferred to round-bottomed wells in microplates (Flow Laboratories, Edinburgh, U.K.) and mitogen was added. The total volume in each well was 150 μl. The cells were incubated at 37°C in humid air with 5% CO_2 for 72 hr. Thymidine-2-[^{14}C] (0.05 μCi, specific activity 60 mCi/mM; Radiochemical Centre, Amersham, U.K.) was added 24 hr before the end of culture. The incubation was stopped by cooling the plates and the cells were harvested with an automatic device (Titertek, Flow Laboratories). The radioactivity was determined in a Packard Liquid Scintillation Counter. The results are expressed as the log mean counts/min of triplicate incubations. The spontaneous DNA synthesis was measured as thymidine incorporation during the first 24 hr of culture.

Statistics

As the number of lymphocytes can be regarded as approximately log-normally distributed, total cell numbers were logarithmically transformed [23]. For statistical evaluation non-parametric tests were used [24, 25].

RESULTS

Pre-treatment immune status

The mean percentages of E^+, EA^+ and $smIg^+$ lymphocytes did not differ significantly from controls (Table 1). As the total lymphocyte counts were low in patients, their total E^+, EA^+ and $smIg^+$ lymphocyte counts were reduced in comparison with healthy controls (Table 1). The mitogen-induced lymphocyte activation was decreased while the spontaneous lymphocyte DNA synthesis was increased (Table 1). Individual data for patients below and above the age of 60 yr are presented in Tables 2 and 3. Seven of 16 young patients and 3 of 10 aged patients were in advanced clinical stage. All histological groups were represented. Analysis of this small material did not reveal age, clinical and histological dependence of lymphocyte subpopulations and functions.

Immune status during CR

The percentage of E^+ lymphocytes increased in most cases, while the percentage of EA^+ lymphocytes decreased during CR as compared to pre-treatment values ($P<0.05$; Tables 1 and 2). The percentage of $smIg^+$ lymphocytes remained unchanged. The pre-treatment lymphocytopenia persisted during CR.

The spontaneous DNA synthesis decreased ($P<0.05$) and the ConA response increased significantly ($P<0.05$) during CR as compared to pre-treatment levels (Tables 1 and 2). The alterations were most pronounced in patients below 60 yr.

Table 1. *Lymphocyte subpopulations and* in vitro *lymphocyte DNA synthesis in patients and controls*

	Radiotherapy (n = 14)		Chemotherapy (n = 10)		Controls
	Before treatment	In remission	Before treatment	In remission	(n = 57)
Lymphocyte counts (mean log No./mm³ ± S.E.)					
Total	2.92 ± 0.10***	2.90 ± 0.06***	3.06 ± 0.12***	2.99 ± 0.09***	3.23 ± 0.03
E^+	2.78 ± 0.07***	2.73 ± 0.09**	2.69 ± 0.12*	2.82 ± 0.08*	3.05 ± 0.03
EA^+	2.36 ± 0.06***	2.36 ± 0.15*	2.36 ± 0.16***	2.03 ± 0.13***	2.69 ± 0.03
$smIg^+$	1.43 ± 0.05***	1.49 ± 0.13**	1.58 ± 0.35	1.51 ± 0.14*	1.84 ± 0.03
(mean % ± S.E.)					
E^+	61.8 ± 3.6	65.8 ± 2.5	57.2 ± 5.9	64.5 ± 2.4	65.0 ± 0.6
EA^+	26.4 ± 2.4	22.2 ± 3.9	21.0 ± 3.3	15.1 ± 2.3***	29.0 ± 1.4
$smIg^+$	3.5 ± 0.5	3.1 ± 0.9	3.3 ± 0.8	4.1 ± 1.3	4.1 ± 0.3
DNA synthesis (mean log cpm ± S.E.)					
ConA 20 μg/ml	2.81 ± 0.11***	2.95 ± 0.16	2.84 ± 0.17*	3.07 ± 0.14	3.15 ± 0.05
ConA 40 μg/ml	2.95 ± 0.11***	3.04 ± 0.14	2.97 ± 0.16**	3.19 ± 0.14	3.26 ± 0.05
ConA 80 μg/ml	2.82 ± 0.14*	2.86 ± 0.16	3.01 ± 0.11	3.03 ± 0.13	3.06 ± 0.06
PWM 1 μg/ml	2.47 ± 0.14	2.47 ± 0.15	2.40 ± 0.16	2.64 ± 0.09	2.57 + 0.07
PWM 10 μg/ml	2.83 ± 0.15**	3.06 ± 0.11	2.82 ± 0.19**	2.93 ± 0.09	3.12 ± 0.04
Spontaneous	1.84 ± 0.05***	1.74 ± 0.08**	1.87 + 0.07*	1.76 ± 0.11	1.63 ± 0.03

Significances of differences are calculated between patients and controls by the Wilcoxon-Mann-Whitney test.
* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

Table 2. Immunological variables at diagnosis and in complete remission of NHL patients younger than 60 yr

	At diagnosis							In complete remission						
		Lymphocytes				DNA synthesis			Lymphocytes				DNA synthesis		
Histology/ clinical stage	Total (log No./ mm³)	SmIg⁺ (%)	SmIg⁺ κ:λ	E⁺ (%)	EA⁺ (%)	Spontaneous (mean log counts/min)	ConA 20 μg/ml (mean log counts/min)	Total (log No./ mm³)	SmIg⁺ (%)	SmIg⁺ κ:λ	E⁺ (%)	EA⁺ (%)	Spontaneous (mean log counts/min)	ConA 20 μg/ml (mean log counts/min)	Time to relapse (months)
IC															
IA	3.06	2.0	2.0	57	36	1.87	3.49	3.18	5.5	1.7	68	35	1.87	3.70	CR 40
IA	2.80	6.0	nd	nd	40	1.76	3.25	2.71	5.5	2.1	68	nd	1.86	3.80	1
CB/CC															
IA	3.13	nd	nd	67	21	1.69	2.62	2.91	5.4	nd	64	nd	nd	2.54	2
IIA	3.10	1.0	2.0	35	24	1.71	2.80	2.92	5.5	0.9	80	10	1.46	2.88	12
IIIA	3.21	3.5	2.3	65	20	nd	nd	3.43	2.0	1.0	74	16	1.26	2.38	12
IIIA	3.06	nd	nd	66	nd	1.61	2.11	3.17	2.0	1.6	68	4	1.52	3.63	21
IIIA	2.49	2.0	3.0	59	nd	nd	nd	2.76	1.0	nd	61	14	1.76	3.06	7
IIIA	3.06	2.5	1.5	65	27	2.03	3.22	nd	nd	nd	nd	26	1.79	3.00	10
IVA	3.20	nd	nd	65	8	1.86	3.05	2.59	1.2	nd	66	nd	nd	3.69	20
CB															
IA	2.95	2.0	4.0	75	11	1.67	1.88	3.04	2.8	1.5	73	nd	nd	2.29	4
IA	3.17	3.0	2.0	72	24	1.85	2.86	2.42	3.0	1.6	66	24	1.81	3.22	CR 42
IIA	1.85	7.0	2.0	41	18	1.85	3.15	2.79	14.0	3.0	61	7	1.65	3.12	12
IBL															
IIA	2.89	nd	nd	68	25	nd	2.84	nd	9.5	2.9	nd	nd	nd	3.09	6
NOS															
IIA	3.06	3.0	2.0	56	14	1.85	3.32	nd	nd	nd	nd	nd	nd	nd	3
IIIB	2.70	5.0	2.5	60	32	2.01	2.98	2.80	13.5	5.5	63	12	1.62	3.14	6
IVA	3.17	2.0	3.5	60	nd	2.32	2.59	3.11	7.0	1.0	76	26	1.36	3.37	CR 50
Total	2.93	3.3	2.4	60.7	23.0	1.85	2.86	2.91	5.6	2.1	68.3	17.4	1.63	3.13	
Mean ± S.E.	0.08	0.4	0.2	2.7	2.6	0.05	0.12	0.07	1.1	0.4	1.6	3.1	±0.06	±0.1	

nd = not done; CR = complete remission.

Table 3. Immunological variables at diagnosis and in complete remission of NHL patients older than 60 yr

Histology/ clinical stage	At diagnosis: Lymphocytes Total (log No./ mm³)	SmIg⁺ (%)	SmIg⁺ κ:λ	E⁺ (%)	EA⁺ (%)	DNA synthesis: Spontaneous (mean log counts/min)	ConA 20 μg/ml (mean log counts/min)	In complete remission: Lymphocytes Total (log No./ mm³)	SmIg⁺ (%)	SmIg⁺ κ:λ	E⁺ (%)	EA⁺ (%)	DNA synthesis: Spontaneous (mean log counts/min)	ConA 20 μg/ml (mean log counts/min)	Time to relapse (months)
IC															
IA	2.95	3.0	2.0	67	34	1.83	2.59	2.98	8.5	1.5	73	42	1.41	2.07	12
IVA	3.93	35.0	0.1	16	nd	nd	nd	3.28	1.5	1.5	72	17	1.75	2.82	CR
CB/CC															
IA	2.80	5.0	1.5	67	31	1.71	2.82	3.15	5.5	nd	64	24	1.71	2.62	5
IA	3.49	nd	1.0	84	21	1.96	2.39	2.79	nd	nd	nd	nd	nd	3.08	CR
IIIA	2.76	4.5	3.0	52	nd	nd	nd	2.93	nd	1.5	70	20	2.11	3.08	CR
CC															
IA	3.16	1.0	2.0	70	38	1.57	1.58	2.97	9.0	0.8	75	12	1.64	1.79	12
CB															
IVA	3.08	nd	nd	77	18	2.00	3.04	3.05	2.5	nd	60	12	2.25	3.39	2
IBL															
IA	2.79	3.0	1.5	61	32	1.67	2.36	3.01	5.0	1.0	62	10	1.75	2.69	CR
LBL															
IA	3.32	2.0	0.3	35	47	1.85	3.52	3.11	3.0	1.3	77	nd	1.63	3.18	CR
NOS															
IA	2.92	4.0	nd	61	33	1.94	3.01	2.85	4.0	3.5	57	22	1.73	2.50	CR
Total	3.12	7.1	1.4	59.0	31.8	1.82	2.66	3.01	4.9	1.59	67.7	19.8	1.78	2.72	
Mean ± S.E.	0.11	4.0	0.3	6.4	3.2	0.05	0.21	0.04	0.9	0.33	2.4	3.6	0.08	0.15	

nd = not done; CR = complete remission.

Except for a minor reduction of IgA during CR, no significant changes of serum immunoglobulins were noted (data not shown).

Immune status during relapse

Ten patients, all but one below 60 yr, were studied during their first relapse. Eight cases had reduced percentages of E^+ lymphocytes during relapse (mean ± S.E.: 49.5 ± 9.0) as compared to CR (69.0 ± 2.0). However, the difference was not statistically significant. Total lymphocyte counts remained essentially unchanged.

The spontaneous DNA synthesis was significantly augmented ($P < 0.05$; Fig. 2), while the ConA-induced lymphocyte activation was reduced (Fig. 3). The response of lymphocytes to PWM decreased, but the difference was not statistically significant (data not shown).

The serum immunoglobulin concentrations did not change significantly during relapse as compared to CR.

The ratio between κ-bearing and λ-bearing blood lymphocytes in healthy persons is 2:1 (range 1.0–3.0). One IC clinical stage IVA lymphoma had a high level of $smIg^+$ cells at diagnosis. This patient and another woman with an LBL clinical stage IA had abnormal ratios at diagnosis which normalized during remission (Table 3).

One CB/CC lymphoma clinical stage IIA, one NHL NOS clinical stage IIIB and one CC lymphoma clinical stage IA had normal κ:λ ratios at diagnosis but became abnormal in CR. Six to twelve months later these three patients had clinical signs of relapse.

$smIg^+$ cells were studied during relapse in 4 patients. Two exhibited abnormal κ:λ ratios (0.8, 9.5) and 2 were normal (2.0, 2.9).

Lymphocyte function during CR in relation to prognosis

The 15 patients younger than 60 yr were grouped according to their lymphocyte response to ConA (20 μg/ml) during CR (Table 4). The mean response of lymphocytes from healthy controls younger than 60 yr was chosen as a limit value between high and low responders. The distribution of age and clinical stage was similar

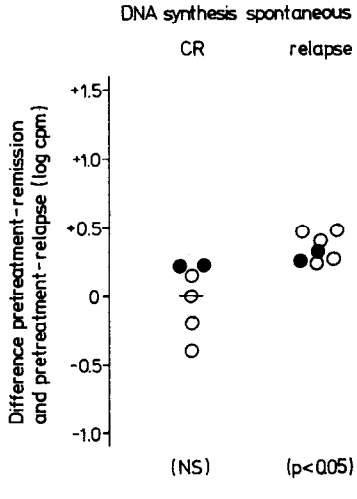

Fig. 2. Spontaneous lymphocyte DNA synthesis. The difference between pre-treatment and remission log counts/min and between pretreatment and relapse log counts/min are shown for patients given CT (open symbols) or RT (filled symbols). Patients older than 60 yr *are indicated by* ⊖.

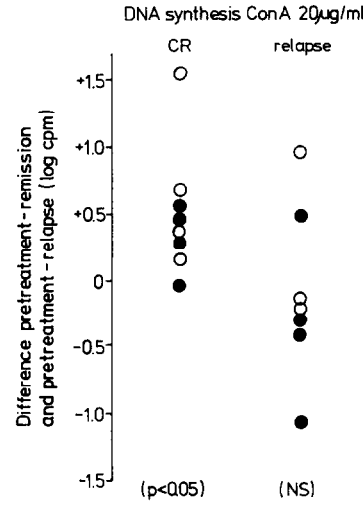

Fig. 3. Lymphocyte DNA synthesis after stimulation with ConA, 20 μg/ml. *The difference between pre-treatment and remission log counts/min and between pre-treatment and relapse log counts/min are shown. For explanation of symbols see legend to Fig.* 2.

Table 4. *The prognostic significance of ConA-induced* (20 μg/ml) [^{14}C]*-thymidine incorporation into remission lymphocytes from patients below the age of* 60 yr

Clinical course after the test	No. of patients Lymphocyte stimulation (log counts/min) ≤ 3.2	> 3.2	Total
Complete remission	0	3	3
Relapse	9	3	12
Total	9	6	15

Chi^2 test ($P < 0.05$).

in the 2 groups. The number of patients who relapsed was lower in the high response group than in the low responders ($P < 0.05$). Three patients who had good lymphocyte response (Table 4) relapsed. The time from the test to first relapse was 1, 20 and 21 months respectively (Table 2). None of the low responder cases experienced first remission duration longer than 12 months.

DISCUSSION

In vitro lymphocyte tests were performed before and after treatment in 26 NHL patients who achieved CR. Of the total of 68 patients 31 (46%) entered CR. Five were not available for remission tests. This rather low number of first CR is probably due to the high median age of the material and may also reflect the referral patterns of the hospitals. Moreover, leukemic patients were excluded from the study.

The method for lymphocyte purification includes a step of adhesion resulting in less than 1% contaminating monocytes. This is one factor leading to good reproducibility with minor interpersonal variations at repeated testing [20]. It is therefore possible to compare consecutive results of tests at prolonged intervals in the same patient.

In the patients tested a T lymphocytopenia and a reduced lymphocyte stimulation by ConA and PWM which was noticed at diagnosis were partly normalised during CR despite a persisting lymphocytopenia. The lymphocyte dysfunction reappeared during relapse. The blood T lymphocyte abnormalities in active NHL are similar to those described in active HD [11]. However, in HD the T cell abnormalities evaluated by mitogen stimulation remained essentially unchanged during complete and unmaintained remission many years after cessation of therapy [12]. This may indicate a persistent T cell deficiency in HD [12]. The relation of cellular immune functions in NHL during CR and its deterioration during recurrence of the disease is similar to that observed in some non-lymphoid malignancies [26–29]. It may therefore be concluded that the immunodeficiency in NHL as in some solid tumours is related to and possible caused by tumour or tumour-associated factors.

The improvement of *in vitro* mitogen proliferative response in CR confirms the findings by others [30]. However, King *et al.* demonstrated decreased ConA- and PHA-induced DNA synthesis in 5 stage III and IV NHL patients in CR [31]. However, three of these patients had received combined RT and CT.

A relationship between the pre-treatment immunodeficiency and prognosis has been a matter of concern in many malignancies. Thus the T cell abnormalities are related to poor prognosis in HD [13]. This does not seem to be the case in NHL [9]. These observations further underline the fundamental differences between HD on the one hand and NHL and some solid tumors on the other regarding the mechanisms and clinical implications of the T lymphocyte defects.

In this small sample impairment of the T lymphocyte activation by ConA during CR seemed to predict relapse in NHL patients below the age of 60 yr. The reason for this is not known. However, as lymphocyte stimulation was depressed in cases with detectable tumour, some patients considered to be in CR may have had undetectable tumor at the time of testing. These preliminary data may therefore suggest that lymphocyte function tests could be useful for predicting relapse. A prospective study to eludicate this point is in progress.

Most NHL lymphomas are of B lymphocyte type with monoclonal B cells in tumour-involved tissues [15, 32]. Monoclonal B lymphocytes may disseminate into the blood stream, leading to an abnormal distribution of κ-bearing and λ-bearing lymphocytes. Abnormal κ:λ lymphocyte ratios have been noticed in 20–60% of NHL patients [3, 4, 32–34]. Using the direct immunofluorescence technique with $F(ab')_2$ fragments of the antibodies monoclonal blood lymphocytes were found in 13 of 68 patients with untreated NHL [9]. Only 2 of these patients achieved CR. In 3 cases the κ:λ ratio was normal before treatment and became abnormal during CR. These patients eventually relapsed. However, 7 of 14 patients with normal κ:λ ratio in CR relapsed. These findings may indicate that repeated analysis of monoclonal blood cells during CR may be useful to predict relapse in B-type NHL which should be confirmed in a larger material.

Acknowledgements—We thank Margret Wahlström and Eva Gurden for skilful technical assistance and Eva-Britt Carlsson for typing the manuscript.

REFERENCES

1. ALEXANIAN R. Monoclonal gammopathy in lymphoma. *Arch Intern Med* 1975, **135**, 62–72.
2. MURRAY JL, HURTUBISE PE, YOUNG DC, BALCERZAK SP, LOBUGLIO AF. Correlation of

prognostic factors and blood lymphocyte subtypes in non-Hodgkin's lymphoma. *Cancer* 1980, **46**, 1817–1824.

3. AULT K. Detection of small numbers of monoclonal B lymphocytes in the blood of patients with lymphoma. *N Engl J Med* 1979, **300**, 1401–1405.
4. GARRETT JV, SCARFFE JH, NEWTON RK. Abnormal peripheral blood lymphocytes and bone marrow infiltration in non-Hodgkin's lymphoma. *Br J Haematol* 1979, **42**, 41–50.
5. JONES SE, GRIFFITH K, DOMBROWSKI P, GAINES JA. Immunodeficiency in patients with non-Hodgkin lymphomas. *Blood* 1977, **49**, 335–344.
6. HEIER HE, KLEPP R, GUNDERSEN S, GODAL T, NORMANN T. Blood B and T lymphocytes and *in vitro* cellular immune reactivity in untreated human malignant lymphomas and other malignant tumors. *Scand J Haematol* 1977, **18**, 137–148.
7. HANCOCK BW, BRUCE L, SUGDEN P, WARD M, RICHMOND I. Immune status in untreated patients with lymphoreticular malignancy—a multifactorial study. *Clin Oncol* 1977, **3**, 57–63.
8. ADVANI SH, DINSHAW KA, NAIR CN *et al.* Immune dysfunction in non-Hodgkin lymphoma. *Cancer* 1980, **45**, 2843–2848.
9. LINDEMALM C, BIBERFELD P, BJÖRKHOLM M *et al.* Immunodeficiency and prognosis in patients with non Hodgkin lymphomas. Submitted.
10. ELTRINGHAM JR, KAPLAN HS. Impaired delayed hypersensitivity responses in 154 patients with untreated Hodgkin's disease. *Natl Cancer Inst Monogr* 1973, **36**, 107–115.
11. HOLM G, MELLSTEDT H, BJÖRKHOLM M *et al.* Lymphocyte abnormalities in untreated patients with Hodgkin's disease. *Cancer* 1976, **37**, 751–762.
12. BJÖRKHOLM M, WEDELIN C, HOLM G, JOHANSSON B, MELLSTEDT H. Longitudinal studies of blood lymphocyte capacity in Hodgkin's disease. *Cancer* 1981, **48**, 2010–2015.
13. WEDELIN C, BJÖRKHOLM M, HOLM G, OGENSTAD S, JOHANSSON B, MELLSTEDT H. Lymphocyte function in untreated Hodgkin's disease. An important predictor of prognosis. *Br J Cancer* 1982, **45**, 70–79.
14. CARBONE PP, KAPLAN HS, MUSSHOFF K, SMITHERS DW, TUBIANA M. Report of the committee on Hodgkin's disease staging classification. *Cancer Res* 1971, **31**, 1860–1861.
15. LENNERT K, MOHRI N, STEIN H, KAISERLING E. MÜLLER HERMELINK HK. Malignant lymphomas other than Hodgkin's disease. In: *Handbuch der speziellen patologischen Anatomie und Histologie.* Berlin, Springer, 1978, I Band Teil 3B.
16. LUCE JK, GAMBLE JF, WILSON HE *et al.* Combined cyclophosphamide, vincristine and prednisone therapy of malignant lymphoma. *Cancer* 1971, **28**, 306–316.
17. GOTTLIEB JA, GUTTERMAN JU, MCCREDIE KB *et al.* Chemotherapy of malignant lymphoma with adriamycin. *Cancer Res* 1973, **33**, 3024–3228.
18. COULSON AS, CHALMERS DG. Separation of viable lymphocytes from human blood. *Lancet* 1964, **i**, 468–469.
19. LUNDGREN G, ZUKOSKI CH F, MÖLLER G. Differential effects of human granulocytes and lymphocytes on human fibroblasts *in vitro*. *Clin Exp Immunol* 1968, **3**, 817–836.
20. HOLM G, PETTERSSON D, MELLSTEDT H, HEDFORS E, BLOTH B. Lymphocyte subpopulations in peripheral blood of healthy persons. *Clin Exp Immunol* 1975, **20**, 443–447.
21. HALLBERG T, GURNER BW, COOMBS RR. Opsonic adherence of sensitized ox red cells to human lymphocytes as measured by rosette formation. *Int Arch Allergy Appl Immunol* 1973, **44**, 500–513.
22. PETTERSSON D, MELLSTEDT H, HOLM G. IgG on human blood lymphocytes studied by immunofluorescence. *Scand J Immunol* 1978, **8**, 535–542.
23. LUTZ W. Statistical methods as applied to immunological data. In: WEIR DM, ed. *Handbook of Experimental Immunology.* Oxford, Blackwell Scientific Publications, 1967, 1163–1206.
24. SIEGEL S. The Wilcoxon matched-pairs signed-ranks test. In: *Non Parametric Statistics for the Behavioral Sciences.* Tokyo, McGraw-Hill Kogakusha, International Student Edition, 1956, 75–86.
25. SIEGEL S. Mann-Whitney *U* test. In: *Non Parametric Statistics for the Behavioral Sciences.* Tokyo, McGraw-Hill Kogakusha, International Student Edition, 1956, 114–127.
26. EILBER FR, NIZZE JA, MORTON DL. Sequential evaluation of general immune competence in cancer patients: correlation with clinical course. *Cancer* 1975, **35**, 660–665.
27. MILLER JJ, GAFFNEY PR, REES JA, SYMES MO. Lymphocyte reactivity in patients with carcinoma of the breast and large bowel. *Br J Cancer* 1975, **32**, 16–20.

28. RAFLA S, YANG SJ, MELEKA F. Changes in cell-mediated immunity in patients undergoing radiotherapy. *Cancer* 1978, **41**, 1076–1086.
29. ANDERSON MJ, CAMPBELL JB, WOOD SE, BOYD JE, KELLY F. Lymphocyte subpopulations in mammary cancer after radiotherapy. *Clin Oncol* 1975, **1**, 201–206.
30. YONKOSKY DM, FELDMAN MI, CATHCART ES, KIM S *et al.* Improvement of *in vitro* mitogen proliferative responses in non-Hodgkin's lymphoma patients exposed to fractionated total body irradiation. *Cancer* 1978, **42**, 1204–1210.
31. KING GW, YANES B, HURTUBISE PE, BALCERZAK SP, LOBUGLIO AF. Immune function of successfully treated lymphoma patients. *J Clin Invest* 1976, **57**, 1451–1460.
32. GAJL-PECZALSKA KJ, BLOOMFIELD CD, COCCIA PF, SOSIN H, BRUNNING RD, KERSEY JH. B and T cell lymphomas. Analysis of blood and lymph nodes in 87 patients. *Am J Med* 1975, **59**, 674–685.
33. LIGLER FS, SMITH RG, KETTMAN JR *et al.* Detection of tumor cells in peripheral blood of non leukemic patients with B cell lymphoma: analysis of clonal excess. *Blood* 1980, **55**, 792–801.
34. KRAJEWSKI AJ, DEWAR AE. Studies on blood lymphocytes of patients with nodular poorly differentiated lymphocytic lymphoma. *J Clin Pathol* 1981, **34**, 896–901.