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IMMUNODEFICIENCY AND PROGNOSIS IN PATIENTS WITH NON-HODGKIN'S LYMPHOMAS

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Abstract

Monocyte depleted blood lymphocyte subpopulations, their functions and relation to prognosis were studied in 68 untreated adult patients with non-Hodgkin's lymphomas classified according to the Kiel nomenclature. The median observation time was 48 months (range 41–60). The mean total blood lymphocyte and T (SRBC-rosetting) cell counts were significantly decreased as compared with age-matched controls (n=57). Twenty-five per cent of the patients had a monoclonal blood B lymphocyte population. The spontaneous lymphocyte DNA synthesis, measured as incorporation of ^{14}C-thymidine, was increased and the response to mitogen and antigen stimulation was decreased. Blood lymphocyte counts and functions before treatment were not related to the rates of remission or survival.

Non-Hodgkin's lymphomas (NHL) constitute a group of tumours which is heterogeneous with respect to histopathology and prognosis. Recent progress in the characterization of cells belonging to the immune system has led to classifications combining immunologic and morphologic characteristics (20, 26). However, clinical predictors of prognosis including histopathology are not sufficiently reliable why further search for more apt prognostic factors to identify poor risk patients is warranted (2, 27).

Patients with tumours of the immune system often have immunologic defects which increase the susceptibility to infections or may influence the clinical course. Thus, a strong association between certain pretreatment lymphocyte functions and prognosis has been reported in patients with Hodgkin's disease (HD) (5, 6). Various immunologic aberrations have also been described in NHL (1, 3, 13, 18, 22–24). However, the relationship between pretreatment immunologic variables and prognosis has not been clearly defined in NHL. This report describes the preatretment immune status in relation to the clinical course and survival in a consecutive series of adult patients with 'non-leukemic' NHL.

Material and Methods

Patients. Sixty-eight previously untreated patients with non-leukemic NHL, 30 men and 38 women with a median age of 59 years (range 20–87 years) were studied. The Kiel nomenclature was used for histopathologic classification (20). Lymphomas referred to the group of low grade malignancy were lymphocytic lymphomas (n=0), immunocytomas (n=12), centroblastic/centrocytic (n=22) and centrocytic (n=4) lymphomas while centroblastic (n=11), immunoblastic (n=8) and lymphoblastic (n=1) lymphomas were included in the group of high grade malignancy. Ten patients had histopathologically unclassified NHL. Extensive clinical, radio-

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graphic and laboratory evaluations were performed to define the initial extent of the disease according to the Ann Arbor classification (7). Patients in clinical stages I and II received radiation therapy to involved regions. The target absorbed doses were 40 Gy fractionated during 4 weeks, administered by orthovoltage or megavoltage techniques. Clinical stage III and IV patients with 'low grade malignancy' received either COP (cyclophosphamide, vincristine, prednisone; n=24) (25) or prednimustine (chlorambucil ester of prednisolone; n=7) (16). Patients with 'high grade malignancy' were given CHOP (COP and adriamycin; n=4) (12) or MeV (methotrexate, cyclophosphamide, vincristine; n=4) (19). Patients with advanced stage who were not histopathologically subclassified were treated with prednimustine (n=2), COP (n=3) or CHOP (n=2). One stage IV patient with abdominal involvement refused chemotherapy and was given extended radiation therapy.

Complete remission was defined as disappearance of all clinically detectable disease, ascertained by repeating the staging procedure.

Controls. The age matched control group consisted mainly of hospital staff members and their relatives (n=57).

Delayed skin hypersensitivity. Purified protein derivate of tuberculin (PPD; State Serum Institute, Copenhagen, Denmark) was used. Two TU PPD (0.1 ml) was injected intradermally on the volar surface of the forearm. The skin reaction was evaluated after 48 hours. An induration with a mean diameter of 6 mm or more was considered positive. The same batch of PPD was used throughout the study. The collection of lymphocytes always preceded skin testing.

Purification of lymphocytes. Lymphocytes were enriched by gelatin sedimentation of defibrinated venous blood. After ingestion of iron powder, phagocytic cells were removed by a magnet. Cells to be tested for lymphocyte subpopulations were further purified by floatation on a Ficoll-Isopaque gradient to remove remaining red blood cells. The cell suspension contained ≥98% lymphocytes. For further details see HOLM et coll. (14).

Lymphocyte counts and subpopulations. The total number of lymphocytes in blood was determined. E^+ cells (T lymphocytes) were identified as lymphocytes forming non-immune rosettes with sheep red blood cells (SRBC) at 4°C (17). B lymphocytes ($smIg^+$) were identified by direct immunofluorescence technique using $F(ab')_2$ fragment of the specific 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 three times at 37°C to remove absorbed IgG (29).

Mitogens and antigens. Concanavalin A (ConA) (Pharmacia Fine Chemicals, Uppsala, Sweden), pokeweed mitogen (PWM) (Gibco, Berkeley, CA, USA) and purified protein derivate of tuberculin (PPD) (State Serum Institute, Copenhagen, Denmark) were used. The same batches of mitogens and antigen were used throughout the study.

DNA synthesis. The lymphocytes were washed in Hank's solution and resuspended in RPMI 1640 culture medium with 10 mmol/l Hepes (Biocult, Glasgow, Scotland) supplemented with 2 mmol/l glutamine, 100 IU penicillin, 100 μg streptomycin/ml and 15% heat inactivated (60 min at 56°C) normal human AB serum. To roundbottomed wells in microplates (Flow Laboratories, Edinburgh, Scotland) were added 10^5 lymphocytes with or without mitogen or antigen. The cells were incubated at 37°C in humid air with 5% CO_2 for 24 hours (spontaneous DNA synthesis) and for 72 hours (mitogen and antigen induced DNA synthesis). Thymidine-2-^{14}C 1.85 kBq (0.05 μCi; specific activity 185 Bq, Amersham International, England) was added 24 hours before the end of culture. The incubation was stopped by cooling the plates and the cells were harvested with an automatic device (Titertek, Flow Labs.). The radioactivity was determined in a Packard Liquid Scintillation counter. The results were expressed as log mean cpm of tripiclate runs.

Statistical methods. As the number of lymphocytes in blood was log-normally distributed total cell numbers were logarithmically transformed. For statistical evaluation two tailed non-parametric tests were used. Survival curves were generated according to PETO et coll. (28) and differences in survival were analysed by the log rank test taking censored data into account (28). The Cox' proportional hazards linear model was used to determine the relative importance of main prognostic factors (8).

Results

Pretreatment lymphocyte counts. Twenty-nine per cent (20/68 patients) had lymphocytopenia ($<1.0\times10^9$/l). The mean numbers of total lymphocytes and total E^+ cells were significantly lower in

Table 1

Pretreatment lymphocyte subpopulations and mitogen induced lymphocyte DNA synthesis in living and deceased patients (mean ± SE)

	Controls (n=57)	All patients[1] at diagnosis (n=68)	Living patients (n=31)	Deceased[2] patients (n=37)
Lymphocyte populations (log No.$\times 10^{-2}$ µl)				
Total	3.23±0.03	3.08±0.04**	3.13±0.05	3.06±0.05
E^+	3.05±0.03	2.84±0.04***	2.86±0.06	2.82±0.06
$smIg^+$	1.84±0.03	1.75±0.08	1.67±0.11	1.93±0.14
Lymphocyte DNA synthesis (log cpm)				
Spontaneous	1.63±0.03	1.83±0.04***	1.78±0.04	1.92±0.06*
ConA 20 µg/ml	3.15±0.05	2.77±0.06***	2.65±0.09	2.91±0.06
ConA 40 µg/ml	3.26±0.05	2.90±0.06***	2.85±0.09	2.96±0.06
PWM 1 µg/ml	2.57±0.07	2.36±0.06*	2.33±0.08	2.43±0.08
PWM 10 µg/ml	3.12±0.04	2.79±0.06***	2.69±0.01	2.84±0.08
PPD 2.5 µg/ml	2.51±0.07	2.22±0.06**	2.25±0.09	2.21±0.10

* =$p<0.05$; ** =$p<0.01$; *** =$p<0.001$.

[1] Comparison is made between patients and controls.

[2] Comparison is made between living and deceased patients.

Table 2

Clinical characteristics of patients with monoclonal B-lymphocytes in blood and lymph nodes

Histology	Tumour smIg phenotype	Clinical stage	Total number ($\times 10^{-9}$)	$smps^+$ (%)	$sm\kappa^+$ (%)	$sm\lambda^+$ (%)	$\kappa:\lambda$ ratio
M.l.Ic	µκ	IIIA	3.10	36.0	28.0	7.0	4.0
M.l.Ic	nd	IVA	0.85	35.0	2.5	35.0	0.1
M.l.Ic	µλ	IVA	5.85	4.0	1.0	3.0	0.3
M.l.Ic	nd	IVB	1.27	49.0	4.0	60.0	0.1
M.l.Ic	µκ	IVB	4.06	30.0	36.0	4.5	8.0
M.l.Ic	nd	IVB	1.44	7.0	3.0	4.0	0.8
M.l.Cb/Cc	nd	IIIA	1.47	48.0	49.0	1.0	49.0
M.l.Cb/Cc	µλ	IVB	0.58	43.0	5.5	37.0	0.2
NHL NOS	nd	IIIB	0.51	22.5	13.5	2.5	5.4
NHL NOS	nd	IVA	0.51	48.0	49.0	1.0	49.0
M.l.Lb (Burkitt type)	nd	IA	1.70	2.0	1.0	3.0	0.3
M.l.Ib	µλ	IIIA	1.49	2.0	0.5	1.0	0.5
M.l.Cb	µκ	IA	0.90	2.0	2.0	0.5	4.0
Healthy subjects (n=44)							
Mean ± SE			2.4±0.2	4.1±0.3	2.9±0.2	1.5±0.1	2.0±0.1
Range			(1.3–3.7)	(1.0–8.5)	(0.5–6.0)	(0.5–3.5)	(1.0–3.0)

nd = not done.

patients as compared with controls while $smIg^+$ cell counts did not differ (Table 1).

Total lymphocytes and total E^+ cell counts were not stage dependent (data not shown). $SmIg^+$ cell counts were higher in stage III and IV patients (1.95±0.14; mean log No.$\times 10^{-2}$ µl) than in stage I and II disease (1.54±0.06; $p<0.01$). However, when patients with monoclonal blood B cells were excluded the B lymphocyte counts showed no stage dependency. Twenty-five per cent (13/52 tested patients) had a $\kappa:\lambda$ ratio outside the normal range (Table 2). The relative $smIg^+$ cell counts were with-

in the normal range in 5 of the cases. The light chain phenotype of lymph node tumour cells studied in 6 of 13 patients was the same as that dominating on the blood lymphocytes in all cases.

Lymphocyte stimulation. The spontaneous lymphocyte DNA synthesis was higher in patients than in controls ($p<0.001$; Table 1) and most marked in patients with clinical stage III and IV disease ($p<0.05$) and with B symptoms ($p<0.01$) (data not shown). Lymphocytes of the patients were poorly stimulated by mitogens and PPD antigen (Table 1; Fig. 1). This difference did only reach statistical significance below 60 years due to the age dependent decrease in lymphocyte activations by mitogens in helthy controls (Fig. 1). In this age group the lymphocyte response to ConA and PWM stimulation showed a slight stage dependency when cases with monoclonal blood B lymphocytes were excluded ($p<0.05$).

No difference in mitogen and antigen induced lymphocyte DNA synthesis between patients in different histologic subgroups or between patients with 'low' and 'high' grade malignancy was observed as exemplified by the lymphocyte response to mitogen ConA (40 μg/ml); 2.83±0.03 for low grade patients and 2.91±0.10 for high grade malignancy patients (data not shown). Neither were any differences in mitogen responses found between patients with or without peripheral monoclonal B lymphocytes.

Delayed skin hypersensitivity. Seventeen of 33 patients were PPD anergic. No relation to age, stage or histopathology was shown. In vitro lymphocyte activation by the same antigen was low in the non-responders (1.93±0.14) and well preserved in the PPD positive patients (2.42±0.12) ($p<0.05$).

Prognosis. The median observation time at follow-up was 48 months (range 41–60 months). Forty-three per cent (29/68 patients) achieved complete remission. The proportions of responders were the same above and below the age of 60. Eleven of 14 patients with low grade malignancy in clinical stages I and II achieved complete remission as compared with 7 of 24 patients in advanced clinical stages ($p<0.01$). Seven of 12 patients with high grade malignancy in stages I and II entered complete remission compared with only 1 of 8 with advanced disease (NS). Lymphocyte counts and response to mitogens and PPD did not differ between responders and non-responders.

The median duration of first complete remission

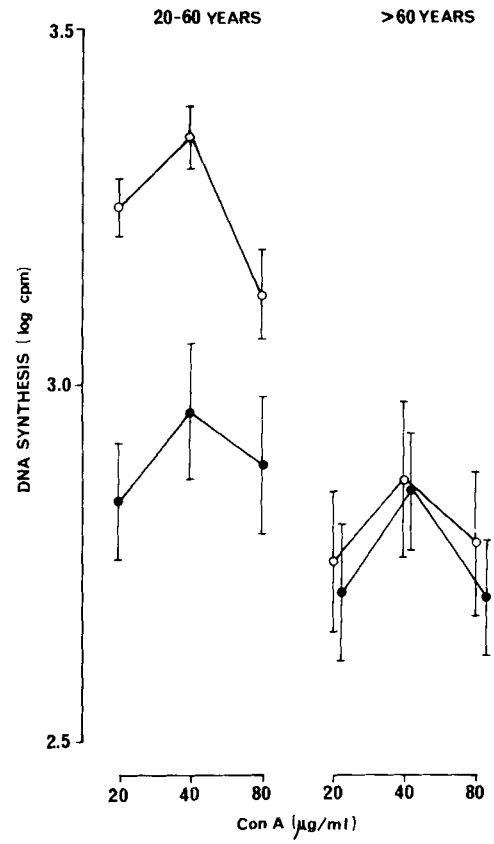

Fig. 1. Lymphocyte stimulation by ConA in relation to age (log mean cpm ± SE) in healthy controls (○) (n=57) and patients (●) (≤60 years n=29, >60 years n=26).

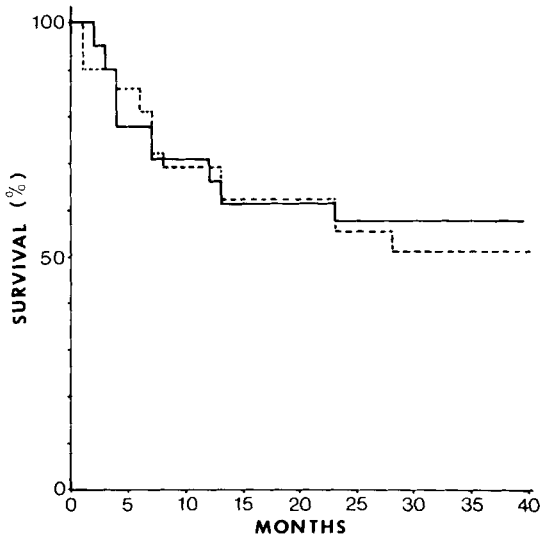

Fig. 2. Survival of patients with spontaneous lymphocyte DNA synthesis <1.8 mean log cpm (— n=26) or ≥1.8 mean log cpm (--- n=26) ($p<0.67$).

was 15.1 months (range 1.5–60.0 months). Sixteen of 29 complete responders relapsed. Remission duration was not related to blood lymphocyte subpopulations or functions (data not shown).

The median survival time was 36 months. Advanced clinical stage ($p<0.01$) and high grade malignancy histopathology ($p<0.01$) were associated with a poor survival. Lymphocyte counts and response to mitogens and PPD did not differ between deceased patients and survivors (Table 1).

In order to further study a possible relationship between the pretreatment immune status and survival patients with lymphocyte subpopulations and functions below the median value of the whole patient group were compared with those with values equal to or above the median. No relationship between lymphocyte functions and survival was found as exemplified by the spontaneous DNA synthesis in Fig. 2. Also when patients in various stages with low grade or high grade malignancy were analysed no differences in survival between the groups could be seen. Multivariate analysis according to Cox confirmed the strong prognostic influence of histopathology and stage. However, the immunologic variables under study did not contribute to the prognostic information (data not shown).

Discussion

Evaluation of the pretreatment immune status in 68 untreated non-leukemic NHL patients revealed lymphocytopenia, involving T cells. Lymphocyte DNA synthesis induced by ConA, PWM and PPD was impaired while spontaneous DNA synthesis was raised in the patient group. Cutaneous anergy to 2 TU PPD was noted in about 50 per cent of the patients tested. The results suggest a dysfunction mainly affecting T lymphocytes. T cell functions decrease with increasing age in healthy persons (32) (Fig. 1) and no difference was noted between patients and healthy persons older than 60 years. The results confirm and extend those of previous studies (3).

The mechanism of the lymphocyte dysfunction in NHL is unclear. Lymphocytopenia already observed by ROSENBERG et coll. (31) to be a common finding sometimes of prognostic importance might indicate that mitogen responsive T cells are selectively depleted from blood. However, the normalization of the mitogen induced DNA synthesis in complete remission despite continuous lymphocytopenia may contradict this assumption (22). By using monoclonal antibodies (30) different subsets of T lymphocytes can be identified and our own studies indicate reduced numbers of helper T cells ($OKT4^+$ cells) resulting in a relative increase in suppressor cytotoxic T cells ($OKT8^+$ cells) in peripheral blood of untreated NHL patients (24). The same relation between $OKT4^+/OKT8^+$ T cells was noted in lymph nodes as in peripheral blood of untreated NHL patients which may contradict an altered distribution of T lymphocyte subsets in active NHL. However, a redistribution between various lymphoid compartments could not be fully evaluated as bone marrow and spleen were not investigated.

In some respects the lymphocyte abnormalities in NHL are analogous to those seen in untreated HD (13, 15). The increased spontaneous DNA synthesis noted in untreated HD and NHL patients, believed to reflect an ongoing immune response or inflammatory process, is normalized during remission (5, 9, 22).

Both untreated HD and NHL patients exhibit an impaired response to mitogens and PPD antigen. However, in contrast to HD the stage dependent impairment of T cell functions is normalized in complete remission in NHL patients and reappears parallel to relapse of the disease. Consequently, the impaired T cell function seems to be associated with active disease. A similar relationship has been described in patients with solid tumours (10).

In the present study 25 per cent of untreated patients had an abnormal blood lymphocyte $\varkappa:\lambda$ ratio suggesting the presence of monoclonal B lymphocytes. The light chain isotype was the same as on the patients' lymph node tumour cells. Thus, an abnormal ratio in patients with normal leukocyte counts may suggest a 'leukemic' spread of the disease. Our results in these respects support those of other studies (4, 11, 21, 22).

The relationship between pretreatment lymphocyte abnormalities and prognosis was analysed and compared with well-known clinical prognostic factors. A slightly better prognosis for NHL patients with normal lymphocyte counts has been reported by ROSENBERG et coll. (31). ANDERSON et coll. (3) also showed that total lymphocyte counts, delayed skin hypersensitivity to recall antigens and lymphocyte mitogen responses were associated with histology and stage. In the present investigation a relationship between histology and immunologic varia-

bles could not be demonstrated. Another histopathologic classification, i.e. the Kiel nomenclature was however used and our patients were elderly. T lymphocytes and mitogen reactivity are known to decline with increasing age (32). The higher median age of the patients in the present study could probably partly contribute to the lack of correlation. This study started in 1977 when other resources for exploring the immune system in larger patient groups were available than today when monoclonal antibodies have offered new opportunities. Studies of other immune functions are in progress to give a more detailed picture of a possible relationship to prognosis in NHL. Thus, the presence of monoclonal blood B lymphocytes in these studies seems to be related to survival and monitoring patients during the course of the disease may give valuable clinical information. T lymphocyte subsets might also have impact on prognosis.

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