Eur J Cancer Clin Oncol, Vol. 21, No. 7, pp. 775-783, 1985.
Printed in Great Britain.

0277-5379/85$3.00+0.00

An Assessment of Natural Cell-mediated Cytotoxicity in Patients with Malignant Lymphoma*

FRANK HEALY,†‡ ROBERT C. REES† and BARRY W. HANCOCK§
Departments of †Virology and §Medicine, University of Sheffield Medical School, Sheffield, U.K.

Abstract—*Fifty per cent of untreated malignant lymphoma patients were shown to have profoundly reduced levels of peripheral blood lymphocyte-natural cell mediated cytotoxicity (PBL-NCMC) when tested against the leukaemic cell-line K562; assessment of NCMC in unfractionated blood from a large number of these patients showed a comparable reduction in activity. High levels of NCMC were observed in some patients with stage III/IV non-Hodgkin's lymphoma (NHL), while Hodgkin's disease (HD) patients over* 40 yr *of age had normal levels. In both NHL and HD patients there was no correlation between NCMC and absolute lymphocyte count or lymphoma histology. Human interferon was seen to boost NCMC in the majority of NHL patients, but most HD patients were non-responders. Almost all patients tested had normal number of target binding lymphocytes, and most had numbers of HNK-1$^+$ cells within the control range. However, on exposure to IFN, the number of target binding lymphocytes increased in over half of the patients tested, with some patients showing an increase in HNK-1$^+$ cells, in the majority of cases without enhancement of NCMC.*

INTRODUCTION

PERIPHERAL blood lymphocytes (PBLs) are capable of killing *in vitro* a variety of tumour, virus-infected and fungal targets without deliberate prior immunization [1-4]. The subset of cells responsible for this unprimed or 'natural' cell-mediated cytotoxicity (NCMC), natural killer (NK) cells, seems to be heterogeneous with regard to surface membrane phenotype and target specificity [5, 6]. Although the relevance of NCMC to *in vivo* situations remains controversial, there is increasing evidence of its intrinsic importance in host defence [7-9].

Studies have shown that tumour-bearing animals [10] and patients with malignant disease [11, 12] have reduced levels of NCMC and children with Chediak-Higashi syndrome, who have a genetic deficiency of NCMC, have a concomitantly increased risk of developing a lymphoma [13, 14].

Accepted 15 January 1985.
*This work was supported by the Yorkshire Cancer Research Campaign.
‡To whom requests for reprints should be addressed at: Department of Virology, Medical School, Beech Hill Road, Sheffield S10 2RX, U.K.

Opportunistic infections are a common complication in malignant lymphoma and patients develop an increased susceptibility to viral, bacterial and fungal agents which in healthy individuals prove less pathogenic. Infections in lymphoma patients are a common cause of mortality [15, 16] and in theory a suppression of NCMC may critically impair host defences against infection in those patients.

Patients with a malignant lymphoma display a variety of immunological abnormalities [17]. These have been well documented and include anergic responses to recall skin test antigens [18], defective proliferative responses of lymphocytes to mitogens [19] and allogeneic cells [20], and delayed homograft and heterograft rejection [21, 22]. In this study spontaneous levels of NCMC in untreated patients with malignant lymphoma were examined, and the findings analysed for correlations with the patients' clinical status. In addition, the NCMC of whole blood samples from patients was assessed to determine (a) if the NCMC of whole blood was more closely related to clinical status; and (b) if the NCMC of whole blood from individual patients corresponded to the NCMC of their PBLs. These findings are

correlated with the expression of the HNK-1 lymphocyte surface marker [23], the ability to form conjugates with NCMC sensitive targets and susceptibility of cytotoxic effector cells from patients to the augmenting effect of interferon.

MATERIALS AND METHODS

Patients and controls

This study involved 88 patients with recently diagnosed malignant lymphoma: 26 with Hodgkin's disease (mean age 37 yr) and 62 with non-Hodgkin's lymphomas (mean age 53 yr). Apart from five patients who were assessed in relapse, none of the patients had undergone therapy. Staging of the disease was done according to the Ann Arbor criteria [24]; lymphomas were histologically defined by the Rye classification [25] for Hodgkin's disease and the British National Lymphoma Investigation [26] for non-Hodgkin's lymphomas. A control group of 35 individuals (mean age 46 yr) was also assessed.

Effector cells

Ten millilitres of peripheral blood were drawn into sterile vacutainer tubes containing heparin. One millilitre of blood was retained for use in the WB assay and lymphocyte-rich mononuclear cells (PBLs) were isolated from the remainder by centrifugation of Ficoll-Hypaque. The PBLs were washed (×3), counted and viability determined using Trypan-blue exclusion, and resuspended in RPMI-1640 supplemented with 10% newborn calf serum (RPMI-NBCS).

Target cells

The human leukaemic cell line K562 was used in natural cell-mediated cytotoxicity (NCMC) assays. It was maintained in stationary suspension culture in RPMI-NBCS supplemented with antibiotics.

Interferon

Freeze-dried preparations of human lymphoblastoid (NAMALVA) α-interferon (IFN) were kindly provided by Drs Fantes and Johnston (Wellcome). The IFN was aliquoted and stored at −80°C and diluted to 1000 units/ml in RPMI-NBCS prior to use in tests.

Pre-treatment with IFN

Effector cells were resuspended to a concentration of 2×10^6/ml and 100 units of IFN in 0.1 ml RPMI-NBCS were added. RPMI-NBCS (0.1 ml) was added to an equivalent number of control effectors. After overnight incubation at 37°C in a humidified atmosphere of 5% CO_2 and 95% air, the cells were washed (×3) and resuspended in RPMI-NBCS at 2×10^6/ml.

NCMC assay

K562 target cells in 0.2 ml RPMI-NBCS were labelled with Na_2 [^{51}Cr]0_4 (100 μCi, 3700 kBq) (Radiochemical Centre, Amersham, U.K.) for 1 hr at 37°C, at which time they were washed (×3), resuspended in 10 ml RPMI-NBCS and incubated for a further 1-hr period at 37°C. The cells were then washed again (×3), counted and resuspended at 1×10^5 cells/ml in RPMI-NBCS. WB diluted ½ and ¼, or effector cells at known concentrations, were prepared in RPMI-NBCS and 0.1 ml vols added, in triplicate, to round-bottomed microtest plate wells. K562 target cells were added to each well (1×10^4 in 0.1 ml) and the plates incubated for 4 hr at 37°C in a 5% CO_2/95% air atmosphere. Control wells containing 0.1 ml targets and 0.1 ml RPMI-NBCS were incubated at the same time. The plates were centrifuged at 200 *g* for 5 min and 0.1 ml supernatant removed into separate wells. They were then oven-dried, sealed with parafilm and the wells individually counted in a gamma-spectrophotometer.

The per cent ^{51}Cr release (test release) was determined using the formula:

$$\%\ ^{51}\text{Cr release} = \frac{(1/2\ \text{s/n}) \times 2}{(1/2\ \text{s/n}) + (\text{cell pellets} + 1/2\ \text{s/n})} \times 100,$$

where s/n = supernatant.

The per cent cytotoxicity was calculated as:

$$\%\ \text{cytotoxicity} = \frac{(\text{test release}) - \text{spontaneous release})}{100 - (\text{spontaneous release})} \times 100.$$

The spontaneous release from targets, i.e. in the absence of effectors, was between 5 and 10%.

Conjugate assay

K562 (0.1 ml, 10^6/ml) was added to 0.1 ml of PBLs (10^6/ml) in a 10-ml plastic centrifuge tube and left at room temperature for 5 min before being centrifuged at 120 *g* for 5 min. The cells were left pelleted for a further 5 min to maximize binding, then gently resuspended using a Gilson pipetman set at 100 μl. Conjugates of PBLS with target cells were enumerated by microscopic observation in a haemocytometer; at least 150 PBLs were counted in each test.

Indirect immunofluorescent staining cells

A maximum of 10^6 cells were placed in microcentrifuge tubes (Alpha labs), centrifuged for 5 min at 500 *g* and the supernatant removed. Anti-Leu 7 (Becton Dickinson; 100 μl) was added, the cells were resuspended and left to stand at room temperature for 20 min and then washed in PBS (×2); 0.1 ml of fluorescein-conjugated goat anti-mouse antiserum (Tago) was added to each test and they were then left to stand at room

temperature for a further 20 min, at which time the cells were washed again in PBS (×2). After the final wash the cells were retained as a pellet in the residual PBS (about 50 μl) and placed on ice. At least 200 lymphocytes were counted in each test and the percentage staining enumerated. Stock solutions of antisera were titrated against normal PBLs and diluted accordingly.

RESULTS

NCMC activity of lymphoma patients

Peripheral blood lymphocytes (PBLs) from a total of 88 untreated lymphoma patients and 35 normal (control) individuals were assessed for NCMC against K562 target cells in a 4-hr ^{51}Cr-release assay. Using the Wilcoxon two-sample rank test, compared with controls the NCMC of PBLs from patients with Hodgkin's disease (HD) was profoundly lower at effector to target (E:T) ratios of 20:1 ($P < 0.001$) and 10:1 ($P < 0.001$) (Fig. 1a). Similarly, the NCMC of PBLs from NHL patients was found to be greatly reduced compared with control PBLs at 20:1 ($P < 0.001$) and 10:1 ($P < 0.001$). In the absence of definitive criteria for normal NCMC, 15% specific cytotoxicity at an E:T ratio of 20:1 was chosen as a reference point. The reduced cytoxicity of lymphoma patient's PBLs is illustrated by the fact that while 80% of controls had an activity greater than 15%, 47% of HD patients and 53% of NHL patients had levels of NCMC below 15%.

In an attempt to gain an impression of *in vivo* NCMC, whole-blood (WB) samples from 50 lymphoma patients and 20 controls were assessed using a modification of the standard ^{51}Cr-release assay [27], and the results are shown in Fig. 1(b). As with PBLs, the NCMC of whole-blood samples from untreated lymphoma patients was found to be depressed when compared with control bloods at dilutions of ½ ($P < 0.01$ for both HD and NHL patients) and ¼ ($P < 0.01$ for HD and NHL patients). Again, choosing a reference point of 5% specific cytotoxicity at a ½ dilution of WB, it can be seen that while only 10% (2/20) of controls demonstrated NCMC below 5%, 45% (5/11) HD patients and 46% (18/39) NHL patients had a NCMC level below 5%. No difference was observed between the NCMC levels of HD and NHL patients with either PBLs or WB.

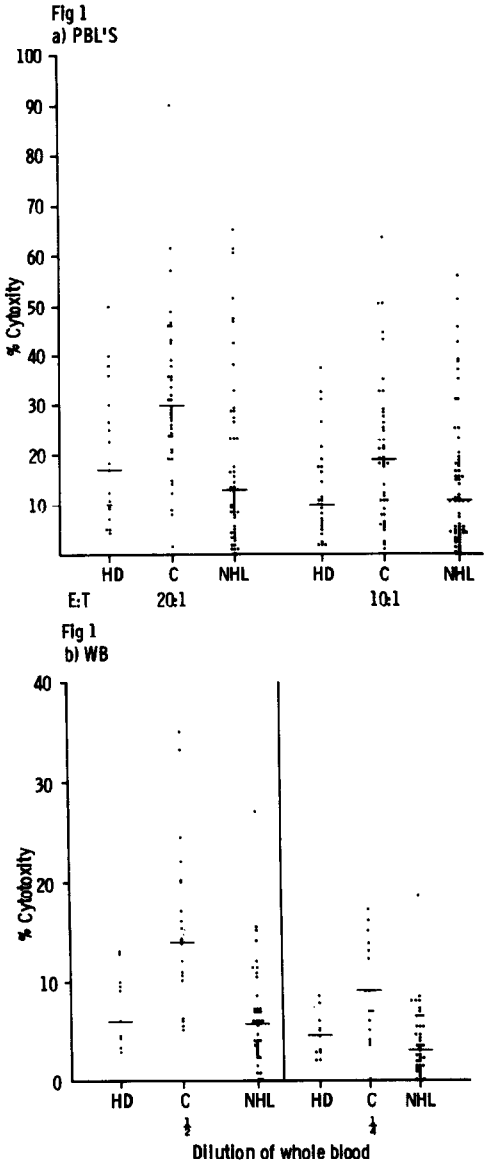

Fig. 1. A comparison between NCMC of lymphoma patients and controls. (a) PBL's from a total of 26 patients with Hodgkin's disease (HD), 62 with non-Hodgkin's lymphoma (NHL) and 35 controls were assessed for NCMC against K562 in a 4-hr ^{51}Cr-release assay at effector to target (E:T) ratios of 20:1 and 10:1 (bar lines indicate median value). (b) Whole blood (WB) from 39 NHL patients, 11 HD patients and 15 controls was assessed for NCMC against K562 in a 4-hr ^{51}Cr-release assay at dilutions of 1/2 and 1/4 (bar lines indicate median value).

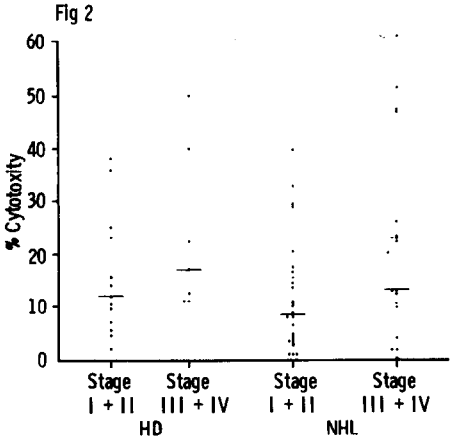

Fig. 2. NCMC in relation to clinical stage of lymphoma (bar lines indicate median value).

Relationship of NCMC to clinical status of patients

To determine if the NCMC of untreated lymphoma patients could be of value in

preliminary assessments, spontaneous levels of NCMC were compared with the clinical status of the patients. As shown in Fig. 2, no difference in PBL-NCMC was observed between HD patients with localized (stages 1 and 2) disease and those with disseminated disease (stages 3 and 4). On testing PBLs from NHL patients with disseminated lymphomas, a proportion of these had higher levels of NCMC than PBLs from other patients; this pattern was also observed when the NCMC of WB samples from the two groups of patients were compared. Furthermore, no correlation was observed between the levels of NCMC (WB or PBL) from lymphoma patients with either histological type of lymphoma ($r = 0.114$) or absolute lymphocyte count ($r = 0.018$). However, as shown in Fig. 3, we observed a positive

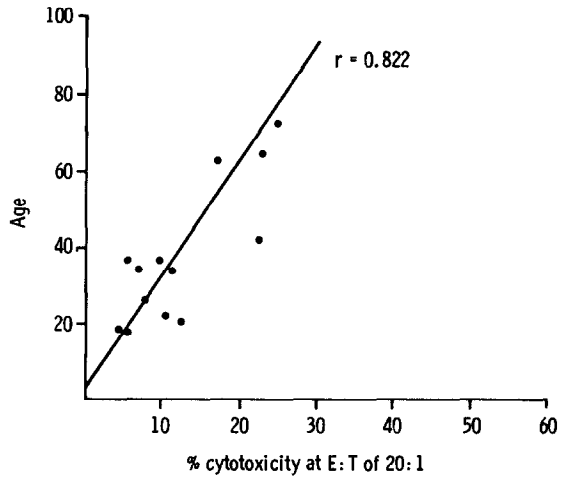

Fig. 3. Regression analysis of PBL-NCMC from HD patients against age.

*Table 1. Target-binding and HNK-1 staining lymphocytes in lymphoma patients and controls (*recurrent)*

(a) Target-binding PBLs

Patient	Diagnosis	% conjugates	Controls	% conjugates
TW	NHL	17.8	CP	15.1
RH	NHL	11.4	AB	20.3
AM	NHL	8.3	IB	19.2
MG	NHL	5.3	AG	14.1
ER	NHL	15.8	FH	16.3
HM	NHL	24.6	BA	11.0
DW	NHL	34.0	IS	9.8
DM	HD	13.0	AW	20.8
MS	NHL	20.3	RJ	9.0
SL	HD	9.1	AA	18.9
LH	NHL	22.2	RR	15.1
WL	NHL	14.4	RP	27.7
LN	HD	15.6	AC	19.6
SK	HD	27.0	TM	32.0
DE	HD	25.4	AJ	34.0
		mean = 17.6 ± 7.9		mean = 18.9 ± 7.5

(b) HNK-1 staining cells

Patient	Diagnosis	% HNK-1$^+$	Patient	Diagnosis	% HNK-1$^+$	Control	% HNK-1$^+$
PW	NHL	12.9	SL	HD	2.8	AS	11.5
LB	NHL*	13.3	WL	NHL	16.7	BA	29.9
DE	NHL	17.0	LW	HD	20.0	IS	16.2
AP	NHL	3.0	SK	HD	7.7	AW	5.3
GD	NHL	8.1	DE	HD	0.9	RJ	16.3
AC	NHL	3.0				AA	18.9
TWD	NHL	11.0		mean = 11.1 ± 6.4		RCR	8.5
JB	NHL	16.0				LS	12.0
WM	NHL	3.6				RP	19.0
BK	HD	23.5				AMB	2.2
IG	NHL	8.7				AC	15.0
HH	NHL	14.4				FH	20.0
JK	HD	4.4				CWP	26.1
AM	NHL	18.2				TM	21.1
MG	NHL	9.5				AJ	7.1
HM	NHL	12.5					
PW	NHL	17.8				mean = 15.3 ± 7.7	
MB	NHL	5.3					
DM	HD	7.8					
MS	NHL	18.5					

correlation between the NCMC of PBLs from patients with HD and the age of the patient ($r = 0.822$, $P < 0.001$). The NCMC of WB samples from these patients, however, did not correlate with age and no such correlation was observed with PBLs from either NHL patients or controls, or with WB from HD, NHL patients or controls.

HNK-1 expression on, and target binding by, PBLs from lymphoma patients

The monoclonal antibody Leu-7 was used in membrane fluoresence tests to identify the lymphocyte subset expressing the surface determinant HNK-1 in lymphoma patients and control subjects. This antibody also cross-reacts with a subset of Leu-2a (non-NK, T-suppressor/cytotoxic) cells; however, at the time of study Leu-7 was the best NK marker available. An average of 15.3% (±7.7 S.D.) of PBLs from controls expressed the HNK-1 antigen (Table 1b). Although this was higher than the average for lymphoma patients (11.1 ± 6.4 S.D.), the difference was not statistically significant. Values obtained from controls showed a significant correlation between HNK-1 expression and NCMC ($r = 0.5384$, $P < 0.05$), but no such correlation was found in the lymphoma group. Examination of conjugate formation of patients' PBLs with K562 targets failed to show a significant difference from values obtained from controls (17.6 ± 7.9 S.D. and 18.9 ± 7.5 S.D. respectively; Table 1a). Furthermore, no correlation was found between the relative numbers of target-binding lymphocytes and NCMC from either control or lymphoma patients PBLs, or the percentage of target-binding lymphocytes and expression of HNK-1 antigen with either control or patient PBLs.

Modulation of NCMC by IFN

Interferon (IFN) is capable of enhancing the NCMC of PBLs from normal individuals. In this study the effect of IFN on NCMC against K562 of PBLs from 25 lymphoma patients was assessed and compared with controls (Table 2). Of nine Hodgkin's patients tested, only three showed a significant increase in NCMC following overnight incubation with IFN. An initially low level of NCMC did not predispose a failure to respond. In contrast, 12 out of 16 non-Hodgkin's lymphoma patients tested showed significant enhancement in NCMC after incubation with IFN. Of the four who failed to respond three had very low levels of NCMC, although in many cases a normal level was not a prerequisite for enhancement. Nineteen control samples were assessed, of which four failed to show a significant augmentation. Three of these four controls had relatively high pretreatment levels, and this may account for their inability to show further significant enhancement on exposure to IFN.

The ability of PBLs (+ or - IFN) to form conjugates with K562 target cells was assessed for

Table 2. Effect of exogenous interferon on the NCMC of PBLs from lymphoma patients

Patient	Diagnosis	-IFN	+IFN	E:T	*P* value
DB	HD	4.9	10.8	8:1	<0.025
RJ	HD	30.9	66.5	10:1	<0.001
AB	HD	2.2	2.0	10:1	N.S.
KF	HD	10.4	4.2	5:1	<0.025
DN	HD	9.4	7.5	20:1	N.S.
SL	HD	10.9	13.0	20:1	N.S.
LN	HD	23.0	18.3	20:1	<0.01
SK	HD	11.7	16.8	20:1	<0.01
DE	HD	13.9	5.0	20:1	<0.01
JH	NHL	0.6	3.5	10:1	N.S.
RH	NHL	1.1	12.3	10:1	<0.005
MH	NHL	12.6	24.4	20:1	<0.001
WM	NHL	8.2	15.8	40:1	<0.001
CS	NHL	12.2	40.5	20:1	<0.001
HW	NHL	12.7	20.6	20:1	<0.001
DH	NHL	8.2	28.6	20:1	<0.001
AD	NHL	4.5	18.3	10:1	<0.001
HH	NHL	1.8	2.8	20:1	N.S.
ED	NHL	22.9	29.9	20:1	<0.01
GF	NHL	13.2	27.2	20:1	<0.001
RB	NHL	1.3	1.5	10:1	N.S.
AM	NHL	28.9	35.1	20:1	<0.01
MG	NHL	29.3	44.8	20:1	<0.001
LH	NHL	10.4	20.1	20:1	<0.001
WL	NHL	32.7	27.4	20:1	<0.01

Table 3. Effect of exogenous interferon on target binding by PBLs

Patient	% conjugates -IFN	% conjugates +IFN	% difference in cytotoxicity +IFN	E:T	Controls	% conjugates -IFN	% conjugates +IFN	% difference in cytotoxicity +IFN	E:T
1	17.8	26.6	+1.0	5:1	1	11.0	11.0	+4.3	10:1
2	11.4	17.4	+3.6	4:1	2	9.8	9.5	+8.6	10:1
3	8.3	4.9	+6.2	20:1	3	9.0	6.6	+9.8	10:1
4	5.3	14.1	+15.5	20:1	4	18.9	11.2	+20.3	10:1
5	24.6	25.8	-1.4	10:1	5	15.1	23.9	+1.1	20:1
6	34.0	28.4	-2.1	10:1	6	19.6	14.0	+14.2	10:1
7	13.0	27.0	-1.9	20:1	7	27.7	24.8	+21.0	10:1
8	20.3	30.5	-0.3	20:1	8	41.1	65.0	+27.5	10:1
9	9.1	5.3	+2.1	20:1	9	32.0	25.9	+8.9	10:1
10	22.2	19.7	+9.7	20:1	10	26.8	44.0	+14.3	10:1
11	14.4	22.1	-0.9	20:1	11	34.6	27.8	+11.4	10:1
12	15.6	18.9	+4.7	20:1					
13	27.4	30.5	+5.1	20:1					
14	25.4	36.7	-1.2	20:1	Mean:	22.3 ± 10.8		23.9 ± 17.4	
15	14.2	8.4	+2.5	20:1					
Mean:	17.5 ± 8.0		21.1 ± 9.6						

PBLs were incubated overnight with or without IFN, washed, assessed for their ability to bind to leukaemia cell target K562 and for NCMC in the 4-hr ^{51}Cr-release assay at the effector to target (E:T) ratios shown.

a group of 11 controls and 15 patients with malignant lymphoma (Table 3). IFN treatment did not affect target binding by control lymphocytes; 7/11 controls showed no change in number of bound lymphocytes, whereas most PBLs gave increased levels of NCMC against K562 following IFN treatment. The pattern with PBLs from lymphoma patients was less clear. Seven out of 15 showed no change in the percentage of target-binding lymphocytes, but in only four of these was the NCMC boosted. Seven out of 15 patients showed an increase in target-binding lymphocytes, in the majority of instances without a demonstrable increase in NCMC.

Expression of HNK-1 on PBLs after overnight incubation with IFN was tested in a group of 17 controls and 13 patients (Table 4). The numbers of PBLs expressing HNK-1 was unaffected by IFN in the majority (10/17) of controls. Of these ten, seven showed a marked increase in NCMC. Only 2/17 showed an increased HNK-1 expression, accompanied in both cases by an increase in NCMC. Of the remaining control PBLs tested the number of HNK-1$^+$ cells was reduced following

Table 4. Effect of exogenous interferon on the expression of the NHK-1 marker on PBLs

Patient	-IFN	+IFN	Controls	-IFN	+IFN
1	23.6	33.3	1	29.9	20.1
2	18.2	16.8	2	16.2	16.9
3	9.5	9.3	3	5.3	5.5
4	12.5	13.6	4	16.3	31.1
5	17.8	27.4	5	18.9	23.0
6	7.8	16.1	6	1.3	3.1
7	18.5	19.0	7	12.0	4.0
8	2.8	5.4	8	19.0	14.9
9	16.7	14.8	9	2.2	3.0
10	20.0	14.2	10	15.0	14.0
11	7.7	8.9	11	19.8	10.4
12	0.9	0.0	12	8.5	6.6
13	14.0	19.1	13	20.0	5.7
			14	26.1	27.6
Mean:	13.1 ± 6.9	15.2 ± 8.7	15	21.1	11.8
			16	17.2	24.7
			17	7.1	5.0
			Mean:	15.1 ± 8.0	13.4 ± 9.2

IFN-treatment, with three of the five showing augmented NCMC. With the lymphoma patients, IFN had a similar effect on HNK-1 expression. The majority (8/13) showed no alteration in the number of lymphocytes expressing HNK-1, although only three gave increased levels of NCMC. Four patients showed an increased number of HNK-1 lymphocytes with IFN pre-treatment, but a concomitant increase in NCMC was seen in only one sample.

DISCUSSION

The results presented here, in addition to an earlier report by Hawrylowicz *et al.* [28], clearly show that the NCMC of PBLs from the majority of untreated malignant lymphoma patients is severely depressed. In this study, in which a larger group of patients were compared with age-matched controls, we found that a depression in NCMC was equally common in HD and NHL patients. Reduced levels of cytotoxicity were observed using either patients' whole blood, where the presence of serum components and other cell types may influence cytotoxic activity, or separated PBLs. Interestingly, on comparing the NCMC of WB with an equivalent concentration of PBLs from 30 patients, we found that the activity of WB was demonstrably higher in ten, and in only three patients out of the 30 was the activity of PBLs greater than WB [Healy and Rees, unpublished observations]. Detailed study is currently being undertaken to determine the effects of patients' plasma on the NCMC of PBLs from normal individuals.

Among untreated patients with stage III/IV NHL were a group which had higher levels of NCMC than stage I/II NHL patients and controls in assays using patients PBLs or WB. Cells which suppress NCMC have been found in normal individuals [29] and cancer patients [30]. An increase in number or efficacy of these cells may be responsible for the reduction in NCMC seen in lymphoma and other cancer patients. However, in particular forms of lymphoma associated with more severe T-cell abnormalities [31, 32] a reduction of suppressor cell action may result in a corresponding increase in NCMC. At this stage an increased NCMC may be ineffective against a large tumour load, particularly without the support of specific cell-mediated immunity (CMI). No correlation was observed between increased cytotoxicity and histology, although the heterogeneity of lymphomas within the non-Hodgkin's class and the poorly understood nature of the malignant cells make interpretation in this respect difficult. We are currently investigating T-cell subsets and monocyte numbers in lymphoma patients and comparing these with clinical status.

The normal levels of NCMC in our sample of HD patients older than 45 yr may also be the result of a decreased specific or non-specific suppression, although it should be noted that our sample group was relatively small. A correlation between age and NCMC has been reported for normal individuals [33], although the authors indicated that this was less apparent in older individuals. We found no correlation with age for either NHL patients or controls.

Age (in NHL patients), sex, blood lymphocyte count, stage and histological type of disease appear to have little bearing on NCMC in lymphoma patients. Impaired mitogen responses have been demonstrated in healthy twin siblings of HD patients and in both consangiunous and non-consangiunous relatives [34] and, although information has not been documented on their NCMC status, it is possible that the low levels of NCMC seen in lymphoma patients may have a genetic basis. It is equally possible that a viral infection, acting directly or through suppressor cells, reduces NCMC, thus allowing the tumour to 'take'. In either case, environmental factors could have an important aetiological role.

Depressed NCMC cannot be explained by a reduction in the number of lymphocytes bearing 'receptors' for K562 since lymphoma patients had similar numbers of target-binding lymphocytes to controls. According to previous studies [35] only a proportion of target-binding lymphocytes are cytotoxic (NK cells); these have been shown to express the differentiation antigen, HNK-1 [23]. Although the number of circulating HNK-1$^+$ cells appeared not to be significantly lower in our patients compared with control subjects, the expression of HNK-1 did not correlate with cytotoxicity in patients, suggesting that the reduced NCMC may reflect a defect in the lytic mechanism of these cells.

The augmentation of NCMC by exogenous IFN has been widely reported [36, 37]. Previous studies have shown that the NCMC of patients with solid tumours is not affected by IFN treatment [38] and in a recent study PBLs from more than 50% of malignant lymphoma patients did not respond [28]. Our present results show that exogenous IFN did not affect the number of HNK-1$^+$ cells or, as previously reported by Targan and Dorey [39], the number of target-binding lymphocytes from controls. Its effect on patient's PBLs was similar, although in some patients IFN induced an increase in the number of target-binding lymphocytes, and in a few cases HNK-1$^+$ cells, without enhancing NCMC. This increase in non-specific binders may reflect a block in the full expression of lytic potential in some patients although this issue has not yet been

fully resolved in the present study. We have also shown that whereas PBLs from HD patients failed to respond to IFN pre-treatment, NHL PBLs showed an enhancement comparable with controls.

It is clear from this study that many NHL patient's PBLs can bind to NK-susceptible targets, show a normal level of staining with anti-leu-7 monoclonal antibody and, in particular, give enhanced NCMC from low spontaneous levels after IFN treatment. This is compatible with an increased 'threshold of activation' for cytotoxicity, a situation analogous to the 'lazy NK cell' seen in Chediak-Higashi syndrome [40]. The NK cytotoxic factors (NKCF) recently reported by Wright and Bonavida [41] may provide an additional explanation for the low NCMC in HD patients. Since IFN had no effect on the NCMC of PBLs from the majority of these patients, both in our study and that of Hawrylowicz *et al.* [28], the limited production or release of NKCF may help explain our observations.

Acknowledgements—We wish to thank Mrs D. Edey and Mr A. Clegg for their excellent technical assistance, the consultant medical staff of Weston Park Hospital for access to their patients, and Mrs S. Badger for typing the manuscript.

REFERENCES

1. Herberman RB, Holden HT. Natural cell-mediated immunity. *Adv Cancer Res* 1978, **27**, 305-337.
2. Ault KA, Weiner HL. Natural killing of measles infected cells by human lymphocytes. *J Immunol* 1979, **122**, 2611-2616.
3. Welsh RM, Hallenbeck LE. Effect of virus infections on target cell susceptibility to natural killer cell-mediated lysis. *J Immunol* 1980, **124**, 2491-2497.
4. Murphy JW, McDaniel DO. *In vitro* effects of natural killer (NK) cells on *Cryptococcus neoformans*. In: Herberman RB, ed. *NK Cells and Other Natural Effector Cells*. London, Academic Press, 1982, 1105-1112.
5. Fitzgerald PA, Kirkpatrick D, Lopez C. Studies on cell surface markers on NK cells: evidence for heterogeneity of human NK effector cells. In: Herberman RB, ed. *NK Cells and Other Natural Effector Cells*. London, Academic Press, 1982, 73-78.
6. Massucci G, Massucci MG, Bejarano MT, Klein E. Human blood lymphocyte subsets characterized by monoclonal reagents. In: Herberman RB, ed. *NK Cells and Other Natural Effector Cells*. London, Academic Press, 1982, 91-98.
7. Kasai M, Lederc JC, McVay-Beudrean L, Shen FW, Cantor H. Direct evidence that NK cells in non-immune spleen cell populations prevent tumour growth *in vivo*. *J Exp Med* 1979, **149**, 1260-1264.
8. Hanna N, Burton RC. Definitive evidence that natural killer (NK) cells inhibit experimental tumour metastasis *in vivo*. *J Immunol* 1981, **127**, 1754-1758.
9. Massucci MG, Bejarano MT, Vanky F, Klein E. Cytotoxic and cytostatic activity of human large granular lymphocytes against allogeneic tumour biopsy cells and autologous EBV-infected B-lymphcytes. In: Herberman RB, ed. *NK Cells and Other Natural Effector Cells*. London, Academic Press, 1982, 1047-1054.
10. Herberman RB, Nunn ME, Lavrin DH. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumours I. Distribution of reactivity and specificity. *Int J Cancer* 1976, **18**, 593-604.
11. Takasugi M, Ramseyer A, Takasugi J. Decline of natural non-selective cell-mediated cytotoxicity in patients with tumour progression. *Cancer Res* 1977, **37**, 413-418.
12. Ziegler HW, Kay NE, Zarling JM. Deficiency of natural killer cell activity in patients with chronic lymphatic leukaemia. *Int J Cancer* 1981, **27**, 321-327.
13. Klein M, Roder J, Haliotis T *et al.* Chediak-Higashi gene in humans II. The selectivity of the defect in natural killer and antibody dependent cell-mediated cytotoxicity function. *J Exp Med* 1980, **151**, 1049-1058.
14. Roder JC, Haliotis T, Klein M *et al.* A new immunodeficiency disorder in humans involving NK cells. *Nature* 1980, **284**, 553-555.
15. Gowing NFC. Opportunistic infections. In: Smithers DW, ed. *Hodgkin's Disease*. Edinburgh, Churchill Livingstone, 1973, 21-34.
16. Armstrong D. Infectious complications of lymphosarcoma. In: Mollander DW, ed. *Lymphoproliferative Disease*. Springfield, IL, Thomas, 1975, 94-109.
17. Hancock BW. Immunosuppression in cancer. In: Hancock BW, Ward AM, eds. *Immunological Aspects of Cancer*. The Hague, Martinus Nijhoff, 1984, 147-162.
18. Young RC, Carder MP, Haynes HA, Devita VT. Delayed hypersensitivity in Hodgkin's disease. *Am J Med* 1982, **52**, 63-72.
19. Hersh EM, Oppenheim JJ. Impaired *in vitro* lymphocyte transformation in Hodgkin's disease. *N Engl J Med* 1965, **273**, 1006-1012.

20. Aisenberg AC. Quantitative estimation of the reactivity of normal and Hodgkin's disease lymphocytes with thymidine 2-C-14. *Nature* 1965, **205,** 1233.
21. Kelly WD, Lamb DL, Varco RL, Good RA. An investigation of Hodgkin's disease with respect to the problem of homotransplantation. *Ann NY Acad Sci* 1960, **87,** 187-202.
22. Miller DG, Lizardo JG, Synderman RK. Homologous and heteroglogous skin transplantation in patients with lymphomatous disease. *JNCI* 1961, **26,** 569-583.
23. Abo T, Balch CM. A differentiation antigen of human NK and K cells identified by a monoclonal antibody (HNK-1). *J Immunol* 1981, **127,** 1024-1029.
24. Carbone PP, Kaplan HS, Mussoff K, Smithers DW, Turbiana M. Report of the staging committee on Hodgkin's disease. *Cancer Res* 1971, **31,** 1860-1861.
25. Lukes RJ, Craver LF, Hall TC, Rappaport H, Rubin T. Report of the nomenclature committee. *Cancer Res* 1966, **26,** 1311.
26. Bennett MH, Farrer-Brown G, Henry N, Jelliffe AM. Classification of non-Hodgkin's lymphomas. *Lancet* 1974, **ii,** 405-406.
27. Rees RC, Platts A. A modified short-term cytotoxicity test: assessment of natural cell-mediated cytotoxicity in whole blood. *J Immunol Methods* 1983, **62,** 79-85.
28. Hawrylowicz CM, Rees RC, Hancock BW, Potter CW. Depressed spontaneous natural killing and interferon augmentation in patients with malignant lymphoma. *Eur J Cancer Clin Oncol* 1982, **18,** 1081-1088.
29. Tarkkanen J, Saksela E, Paavolaineu M. Suppressor cells of natural killer activity in normal and tumor-bearing individuals, *Clin Immunol Immunopathol* 1983, **28,** 29-38.
30. Uchida A, Mickshe M. Suppressor cells for natural killer activity in carcinomatous pleural effusions of cancer patients. *Cancer Immunol Immunother* 1981, **11,** 255-263.
31. Wedelin C, Bjorkholm M, Holm G, Ogenstad S, Johansson B, Mallstetdt H. Lymphocyte function in untreated Hodgkin's disease: an important predictor of prognosis. *Br J Cancer* 1982, **45,** 70-79.
32. Anderson TC, Jones SE, Soehnlen BJ, Moon TE, Griffith K, Stanley P. Immunocompetence and malignant lymphoma: immunologic status before therapy. *Cancer* 1981, **48,** 2702-2709.
33. Abo T, Cooper MD, Balch CM. Postnatal expansion of the natural killer and killer cell populations in humans identified by the monoclonal HNK-1 antibody. *J Exp Med* 1982, **155,** 321-326.
34. Bjorkholm M, Holm G, Mellstedt H. Immunological family studies in Hodgkin's disease: is the immunodeficiency horizontally transmitted? *Scand J Haematol* 1978, **20,** 297-305.
35. Silva A, Bonavida B, Targan S. Mode of action of interferon-mediated modulation of natural killer cytotoxic activity: recruitment of pre-NK cells and enhanced kinetics of lysis. *J Immunol* 1980, **125,** 479-484.
36. Trinchieri G, Santoli D. Antiviral activity induced by culturing lymphocytes with tumour-derived or virus transformed cells. Enhancement of human natural killer activity by interferon and antagonistic inhibition of susceptibility of target cell to lysis. *J Exp Med* 1978, **147,** 1314-1333.
37. Moore M, Potter MR. Enhancement of human cell-mediated cytotoxicity by interferon. *Br J Cancer* 1980, **41,** 378-387.
38. Kadish AS, Doyle AT, Steinhaver EH, Ghossein NA. Natural cytotoxicity and interferon production in human cancer: deficient natural killer activity and normal interferon production in patients with advanced disease. *J Immunol* 1981, **127,** 1817-1822.
39. Targan S, Dorey F. Interferon activation of "pre-spontaneous killer" (Pre-SK) cells and alteration in kinetics of lysis of both "Pre-SK" and active SK cells. *J Immunol* 1980, **124,** 2157-2161.
40. Targan SR, Oseas R. The "lazy" NK cells of Chediak-Higashi syndrome. *J Immunol* 1983, **130,** 2671-2674.
41. Wright S, Weitzen ML, Kahle R, Grainger GA, Bonavida B. Studies on the mechanism of natural killer cytotoxicity II. Coculture of human PBL with NK-sensitive or resistant cell lines stimulates release of natural killer cytotoxic factors (NKCF) selectively cytotoxic to NK-sensitive target cells. *J Immunol* 1983, **130,** 2479-2483.