[CANCER RESEARCH 42, 2063–2068, May 1982]
0008-5472/82/0042-0000$02.00

Natural Killer Activity in Spleens and Lymph Nodes from Patients with Hodgkin's Disease[1]

Luigi P. Ruco,[2] Antonio Procopio, Stefania Uccini, Enrico Marcorelli, and Carlo D. Baroni

IV Chair of Pathological Anatomy [L. P. R., A. P., S. U., C. D. B.] and Institute of Radiology [E. M.], School of Medicine, University of Rome, Rome, Italy

ABSTRACT

Natural killer (NK) activity against K-562 myeloid cell line was evaluated in 21 spleens and 14 lymph nodes from patients with Hodgkin's disease (HD). NK activity of eight HD-involved (HD^+) spleens [556 lytic units (LU)] was found 5-fold higher than that of 13 HD-uninvolved (HD^-) spleens (112 LU) ($p < 0.01$). Moreover, NK activity of HD^+ spleens was significantly different ($p < 0.05$) from that of three spleens involved by non-HD lymphoma (100 LU). NK activity of four spleens from nonneoplastic patients (250 LU) was intermediate between those of HD^+ and HD^- spleens. Lymph node cells were about 10-fold less cytotoxic. NK activity of seven HD^+ lymph nodes (43 LU) was 3-fold higher than that of three lymph nodes involved by non-HD lymphoma (8 LU). However, these differences were not statistically significant. Our data are compatible with increased NK activity in HD^+ tissues as well as with depressed NK activity in HD^- tissues. The observation that NK activity of peripheral blood leukocytes from nine HD patients (116 LU) ($p < 0.05$ only at 100:1 effector:target cell ratio) may support the latter interpretation. Partial characterization of effector cells in HD^+ and in HD^- spleens indicated that, in both instances, NK cells were nonadherent and almost equally distributed between the erythrocyte-negative and -positive cell fractions. Finally, NK activity of both HD^+ and HD^- spleen cells could be further potentiated *in vitro* by interferon.

INTRODUCTION

Several studies have demonstrated that human PBL[3] exert NK activity *in vitro* against neoplastic cell lines (9, 22). The large granular lymphocyte was indicated recently as the NK effector cell (25, 26). Presence of low-affinity erythrocyte receptors (29) and reactivity with anti-T-lymphocyte antisera (14) and with OKT-10 monoclonal antibodies (19) suggest that NK cells belong to the T-cell line. However, the evidence that NK cells react also with OKM-1 monoclonal antibodies (16) may indicate that the NK cell population is rather heterogeneous. HD is a human lymphoma often associated with defective cell-mediated immunity. Abnormal distributions of T_g and T_m in peripheral blood and in lymphoid tissues (8), increased numbers of T-lymphocytes in HD^+ tissues (15, 20), and decreased T-cell mitogen reactivity in HD^+ spleens and lymph nodes as compared to HD^- tissues (2, 30) were all observed in HD patients. These data suggest that HD is associated with marked alterations of number, distribution, and function of T-lymphocyte subpopulations.

In the present study, we have evaluated NK activity in peripheral blood, spleen, and lymph nodes obtained from HD patients. Our results indicate that tissue distribution of NK activity is also profoundly affected in HD. In fact, NK activity was found increased in HD involved tissues whereas it was found decreased in HD^- tissues. Furthermore, these alterations seem to be peculiar to HD since spleen or lymph node involvement by non-HD lymphoma was not found associated with increased NK activity.

MATERIALS AND METHODS

Patients. Peripheral blood, spleens, and lymph nodes were obtained from 27 untreated patients with histologically proven HD and from 6 untreated patients with non-HD lymphoma. All patients were hospitalized at the Radiology Institute of the University of Rome in the period September 1980 to June 1981. Age, sex, histology, and clinical stage of HD patients are listed in Table 1. Peripheral blood from HD patients was obtained on the day of staging laparotomy and splenectomy but prior to anesthesia. Spleens from nonneoplastic donors were obtained from 3 patients with traumatic rupture of the organ and from one patient with hypersplenism syndrome. Normal peripheral blood was obtained from 28 healthy donors (16 male and 12 female; mean age, 32 ± 10).

Cell Preparations. Fragments from spleens and lymph nodes were minced and passed through a stainless steel sieve. Cell suspensions in RPMI 1640 (Eurobio, Paris, France) were layered onto Lymphoprep (Nyegard, Oslo, Norway) and centrifuged at 400 × *g* for 40 min at room temperature. Heparinized peripheral blood was diluted 1:2 with RPMI 1640 and layered on Lymphoprep. The mononuclear cell layer was collected and washed twice with medium. The cell concentration was adjusted to 10×10^6 cells/ml in RPMI 1640 containing 10% FCS (Eurobio). Differential cell counts were made on Giemsa-stained cell smears prepared by cytocentrifugation (Cytospin centrifuge; Shandon Southern Instruments, Camberley, England).

Adherent cells were separated from nonadherent by 3 successive passages on plastic Petri dishes. Adherent cells were obtained by scraping the plastic surface with a rubber policeman.

Erythrocyte-positive and -negative cell fractions were prepared as follows. Equal volumes of cell suspensions containing 5×10^6 cells/ml and 1% neuraminidase-treated sheep RBC (Sclavo, Siena, Italy) were incubated at 37° for 15 min. This cell suspension was spun at 200 × *g* for 5 min and then incubated at 4° for 60 min (13). After incubation, the cell suspension was layered on Lymphoprep and centrifuged at 400 × *g* at room temperature for 30 min (21). Erythrocyte-negative cells were recovered from the interface; erythrocyte-positive cells were obtained from the pellet after lysis of the attached sheep RBC by Tris-buffered 0.83% ammonium chloride (pH 7.2).

NK Assay. NK activity was tested against human myeloid cell line K-562. Target cells were maintained in RPMI 1640 containing L-glutamin, gentamicin, and 10% FCS in 75-sq cm tissue culture flasks (3024 F; Falcon Plastics, Oxnard, Calif.). The cells were passed 2 to 3 times per week. Target cell labeling was performed by incubating 5×10^6 cells

[1] Supported by Consiglio Nazionale delle Richerche Contract 800147496, Progetto Finalizzato "Controllo della crescita neoplastica", sottoprogetto "Controllo Immunitario."

[2] To whom requests for reprints should be addressed, at IV Cattedra di Anatomia ed Istologia Patologica, Viale Regina Elena 324, Università di Roma, 00161 Roma, Italy.

[3] The abbreviations used are: PBL, peripheral blood leukocytes; NK, natural killer; HD, Hodgkin's disease; HD^+, Hodgkin's disease involved; HD^-, Hodgkin's disease uninvolved; RPMI 1640, Roswell Park Memorial Institute Tissue Culture Medium 1640; FCS, fetal calf serum; LU, lytic unit(s).

Received July 10, 1981; accepted January 7, 1982

Table 1
Age, sex, histology, and clinical stage of patients

Case	Age	Sex	Lymphoid organ	Involvement	Histology	Clinical stage	NK activity[a] 12:1[b]	25:1	50:1	100:1
1	45	F	Spleen	–	HD-MC[c]	III	0	2	2	4
4	51	M	Spleen	–	HD-MC	II	2	2	11	34
5	17	F	Spleen	–	HD-NS	II	1	0	4	10
6	16	F	Spleen	–	HD-NS	II	0	3	4	17
7	18	F	Spleen	–	HD-NS	II	26	51	68	73
12	31	M	Spleen	–	HD-NS	II	2	9	16	37
18	38	M	Spleen	–	HD-LP	II	1	2	5	7
20	19	M	Spleen	–	HD-NS	II	11	19	30	47
21	24	F	Spleen	–	HD-NS	II	2	8	19	49
26	27	F	Spleen	–	HD-NS	II	0	3	21	52
27	28	M	Spleen	–	HD-NS	II	0	4	12	27
28	46	F	Spleen	–	HD-NS	II	3	8	9	32
37	35	M	Spleen	–	HD-MC	II	10	17	34	51
						Mean ± SD	5 ± 7	10 ± 14	18 ± 18	34 ± 21
2	51	M	Spleen	+	HD-MC	IV	8	14	27	30
10	41	M	Spleen	+	HD-MC	III	47	58	68	66
24	46	M	Spleen	+	ND-NS	IV	31	48	63	69
29	24	F	Spleen	+	HD-NS	III	8	19	30	39
30	54	F	Spleen	+	HD-MC	IV	36	55	61	67
34	46	M	Spleen	+	HD-MC	III	11	24	43	60
39	30	M	Spleen	+	HD-MC	III	32	44	54	59
40	20	M	Spleen	+	HD-NS	III	19	30	48	61
						Mean ± SD	24 ± 15	37 ± 17	50 ± 15	56 ± 14
17	60	F	Spleen	+	Lymphoma		1	1	5	23
33	66	F	Spleen	+	Lymphoma		4	8	16	28
35	42	F	Spleen	+	Lymphoma		6	8	24	46
						Mean ± SD	4 ± 2	6 ± 4	15 ± 9	32 ± 12
3	70	F	Spleen		Hypersplenism		2	2	6	12
13	11	M	Spleen		Normal		13	23	32	41
31	45	F	Spleen		Normal		18	28	48	62
32	36	F	Spleen		Normal		18	39	57	60
						Mean ± SD	13 ± 7	23 ± 15	36 ± 22	44 ± 23
6	16	F	LN	–	HD-NS	II	0	0	0	3
7	18	F	LN	–	HD-NS	II	0	2	6	14
12	31	M	LN	–	HD-NS	II	0	0	0	3
20	19	M	LN	–	HD-NS	II	0	0	2	4
21	24	F	LN	–	HD-NS	II	0	0	2	6
26	27	F	LN	–	HD-NS	II	0	0	0	0
37	35	M	LN	–	HD-MC	II	0	0	0	0
						Mean ± SD	0 ± 0	0.3 ± .8	1 ± 2	4 ± 5
8	43	M	LN	+	HD-LP	II	1	2	6	13
10	41	M	LN	+	HD-MC	III	4	13	21	44
15	43	M	LN	+	HD-LP	II	2	4	8	11
18	38	M	LN	+	HD-LP	II	0	1	4	5
22	40	F	LN	+	HD-MC	II	0	2	6	13
25	26	F	LN	+	HD-NS	II	0	0	0	0
41	37	M	LN	+	HD-LD	III	1	1	3	8
						Mean ± SD	1 ± 2	3 ± 5	7 ± 7	13 ± 14
13	74	M	LN	+	Lymphoma		2	2	4	4
23	69	M	LN	+	Lymphoma		0	0	0	1
38	20	M	LN	+	Lymphoma		1	0	1	2
						Mean ± SD	0.3 ± .6	1 ± 2	2 ± 2	2 ± 2

[a] ^{51}Cr specific release.
[b] Effector:target cell ratio.
[c] MC, mixed cellularity; NS, nodular sclerosis; LP, lymphocyte predominance; LN, lymph node; LD, lymphocyte depletion.

with 150 μCi of sodium [^{51}Cr]chromate (Radiochemical Centre, Amersham, England) for 60 min at 37°; cells were then washed twice with medium and resuspended at the concentration of 10^5 cells/ml in 10% FCS RPMI 1640. One hundred μl of this cell suspension (10^4 ^{51}Cr-labeled K-562 cells) were added to each well of a round-bottomed microtiter plate (3040; Falcon Plastics). Cell suspensions were tested in triplicate at several effector:target cell ratios (100:1 to 12.5:1). Autologous controls (unlabeled target cells added to the labeled targets) were used to determine spontaneous release (SR) in all experiments. Microtiter plates were incubated for 4 hr at 37° in 5% CO_2 humidified air. Total counts were determined by direct measurement of cpm present in 10^4 ^{51}Cr-labeled K-562 cells. Specific release was calculated according to the formula:

$$\% \text{ of specific release} = \frac{\text{cpm experimental} - \text{cpm SR}}{\text{cpm total counts} - \text{cpm SR}} \times 100.$$

In all experiments reported in this paper, SR ranged from 5 to 13% of total counts.

In vitro treatment with interferon was made by incubating 5×10^6 spleen cells in 0.5 ml 10% FCS RPMI 1640 with or without 1000 units of partially purified fibroblast interferon (HEM Research, Rockville, Md.; 3×10^7 units/protein mg) for 3 hr at 37° in 5% CO_2 humidified air. Preliminary experiments indicated that 30 min of incubation with 1000 units interferon resulted in maximal expression of NK activity. A LU was defined as the number of effector cells required to produce 30% cytotoxicity. This value was selected because it generally fell in

the linear portion of the dose-response curve. LU/10^7 cells were calculated to provide an index of the relative cytotoxic reactivity of a given cell population.

RESULTS

NK Activity in Spleens and Lymph Nodes. In the present study, we have evaluated NK activity against the K-562 cell line of cells from 21 spleens (Table 1; Chart 1) and 14 lymph nodes (Table 1; Chart 2) obtained from HD patients. NK activity of HD^+ tissues was compared to equal numbers of cells from HD^- tissues. Results from these experiments indicated that cells from HD^+ tissues were more cytotoxic than cells from HD^- tissues. On the average, NK activity of cells from 8 HD^+ spleens (556 LU) was about 5-fold higher than that of cells from 13 HD^- spleens (112 LU). Lymph node cells were about 10-fold less cytotoxic than spleen cells against K-562 tumor cells. Nevertheless, the average NK activity of 7 HD^+ lymph nodes (43 LU) was about 3-fold higher than that of 7 HD^- lymph nodes (15 LU). Statistical evaluation of these results indicated that the difference between HD^- and HD^+ spleens was highly significant at all effector:target cell ratios tested. The difference between HD^- and HD^+ lymph nodes was not statistically significant.

An abnormal distribution of NK activity in lymphoid tissues of HD patients was also suggested by experiments in which PBL and spleen cells from the same patient were tested simultaneously (Chart 3). In fact, the ratio between NK activity of PBL and spleen cells was found to be approximately 1 in one nonneoplastic patient as well as in 2 patients whose spleen was not involved by the disease. This ratio was found to be higher than 1 (1.7, 3.7, and 7.3, respectively) in 3 patients with HD^+ spleens. In Charts 1 and 2, we have also reported NK activity of cells from 3 spleens (100 LU) and 3 lymph nodes (8 LU) involved by non-HD lymphoma. Cytotoxic activity of these cells was very similar to that of cells from HD^- tissues and was significantly different ($p < 0.05$) from that of cells from HD^+ spleens. Finally, since NK activity of 4 nonneoplastic spleens (250 LU) was found intermediate between those of HD^- and HD^+ spleens, we were unable to establish if NK activity was enhanced in HD^+ tissues or depressed in HD^- tissues. This latter interpretation seems to be supported by preliminary experiments indicating that the NK activity of PBL from 28 healthy donors (208 LU) was about 2-fold higher than that of PBL from 9 HD patients (116 LU) (Chart 4). This difference, however, was statistically significant ($p < 0.05$) only at the highest effector:target cell ratio tested.

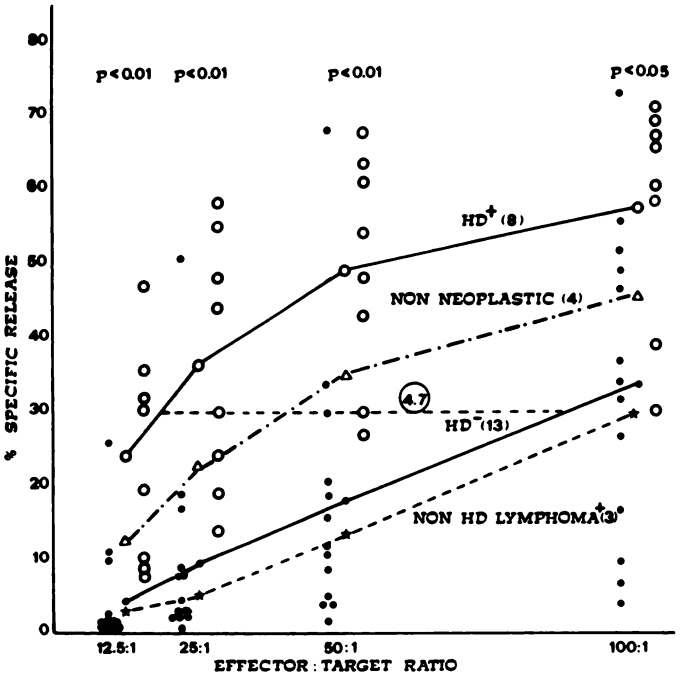

Chart 1. NK activity *in vitro* against K-562 target cells of cell suspension from: 13 HD^- spleens (●); 8 HD^+ spleens (○); 3 spleens involved by non-HD lymphoma (★); and 4 nonneoplastic spleens (△). NK activity was evaluated in a 4-hr ^{51}Cr release assay. Statistical analysis indicated that NK activity of HD^+ spleens was significantly different from that of HD^- spleens ($p < 0.01$ at 12.5:1, 25:1, and 50:1 effector:target ratio; $p < 0.05$ at 100:1 ratio) as well as that of spleens involved by non-Hodgkin's lymphoma ($p < 0.05$ at all effector:target ratios). All the other differences were not statistically significant (*NS*) (Student's *t* test).

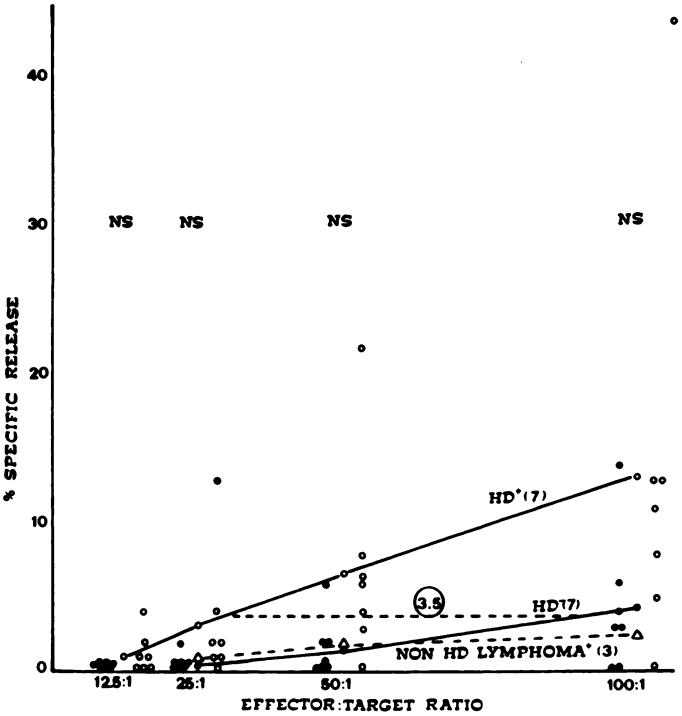

Chart 2. NK activity *in vitro* against K-562 target cells of cell suspensions from 7 HD^+ lymph nodes (○), 7 HD^- lymph nodes (●), and 3 lymph nodes involved by non-HD lymphoma (△). All the differences are not statistically significant (Student's *t* test).

Characterization of Splenic NK Cells. In human PBL, NK cells are Fc-positive nonadherent cells which can be detected in the erythrocyte-negative as well as the erythrocyte-positive cell fractions (29). In the experiments shown in Charts 5 and 6, we have evaluated the plastic adherence and the presence of erythrocyte receptors in NK cells obtained from HD^- and HD^+ spleens. Our results demonstrate that, in both instances, NK cells were found to be nonadherent and almost equally distributed between the erythrocyte-negative and -positive cell fractions.

***In Vitro* Activation by Interferon.** NK activity is increased by interferon (27) or by supernatant fluids from human lymphoma cell lines (3, 12). In the experiments reported in Chart 7, cells from 2 nonneoplastic spleens, 4 HD^- spleens, 5 HD^+ spleens, and 2 spleens involved by non-HD lymphoma were cultured in 10% FCS RPMI 1640 with or without 1000 units of partially

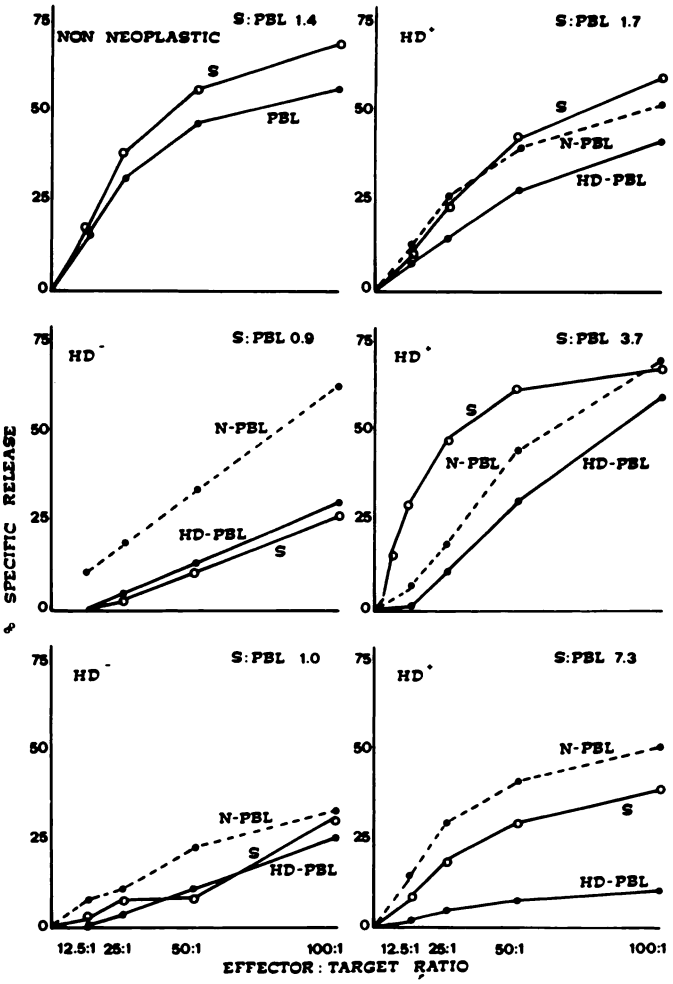

Chart 3. NK activity of spleen cells (*S*) and PBL obtained from the same patient. PBL and spleens (2 HD^- and 3 HD^+) were obtained from 5 HD patients and from one nonneoplastic patient (*N*). PBL from healthy donors are reported as a positive control. The spleen:PBL ratio is reported in the chart.

purified fibroblast interferon for 3 hr. Results from these experiments indicate that HD^- spleen cells were less cytotoxic than HD^+ spleen cells even after *in vitro* treatment with interferon. However, they indicate also that NK activity of HD^+ spleen cells could be potentiated by interferon almost at the same extent as that of HD^- spleen cells (HD^-, from 500 to 800 LU, ratio 1.6; HD^-, from 131 to 250 LU, ratio 1.9).

DISCUSSION

Human PBL from normal donors exert *in vitro* NK activity against different tumor cell lines (22). Distribution of NK activity in peripheral lymphoid tissues was poorly investigated in humans. NK activity was evaluated in cells from either normal (6) or tumor draining lymph nodes (6, 28) and from tonsils (6, 17). NK activity of all these lymphoid tissues was found to be much lower than that of PBL. In the present study, we have evaluated NK activity in 4 nonneoplastic spleens, 13 HD^- spleens, and 7 HD^- lymph nodes. Furthermore, in 6 patients, NK activity was measured simultaneously in peripheral blood and spleen. Our results clearly indicate that, in humans, PBL and spleen cells exert similar levels of NK activity; in contrast and as previously reported (28), lymph node cells are poorly cytotoxic. Thus, tissue distribution of NK activity in humans is similar to that described for rats (18) and for guinea pigs (1) and is slightly different from that observed in mice (10). In fact, in this last species, lymph node cells were found moderately cytotoxic.

Characterization of NK effector cells has not led to any definitive conclusion. Nonadherent lymphoid cells with intracytoplasmic Giemsa-positive granules, the large granular lymphocyte (25, 26), were indicated recently as NK effector cells. However, both T-cell markers and monocyte-myeloid markers were demonstrated on large granular lymphocytes (19). Fur-

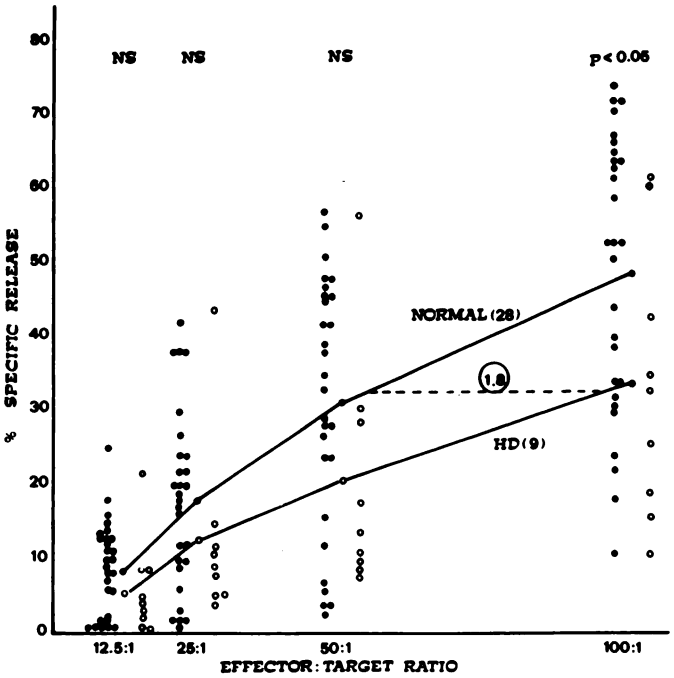

Chart 4. NK activity *in vitro* against K-562 target cells of PBL obtained from 28 healthy donors (●) and 9 HD patients (○). Differences were tested by Student's *t* test. *NS*, not significant.

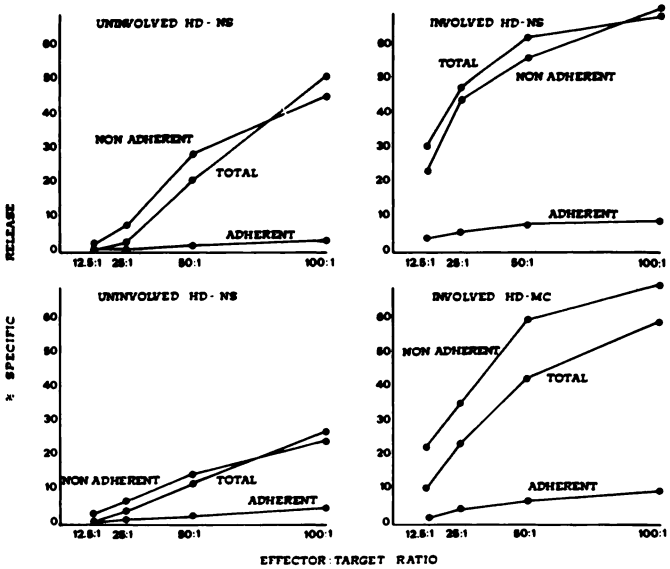

Chart 5. NK activity of adherent and nonadherent cells obtained from 2 HD^- and 2 HD^+ spleens. Nonadherent cells were obtained after 3 successive passages on plastic surfaces. Adherent cells were obtained by scraping the plastic surface with a rubber policeman after extensive washing of nonadherent cells. *NS*, nodular sclerosis; *MC*, mixed cellularity.

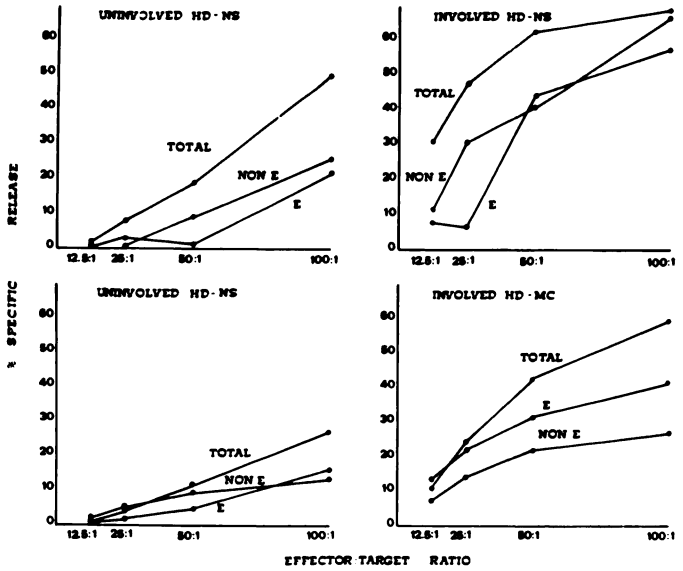

Chart 6. NK activity of erythrocyte-positive (*E*) and -negative (*NON E*) cell fractions obtained from 2 HD⁻ and 2 HD⁺ spleens. The phytohemagglutinin proliferative response of cell fractions, measured as [^{3}H]thymidine incorporation, was kept as a positive control of the separation procedure (Experiment 1: unfractioned, 25,610 cpm; erythrocyte positive, 21,771 cpm; erythrocyte negative, 4,650 cpm. Experiment 2: unfractioned, 63,160 cpm; erythrocyte positive, 80,100 cpm; erythrocyte negative, 5,160 cpm. Experiment 3: unfractioned, 10,830 cpm; erythrocyte positive, 18,460 cpm; erythrocyte negative, 950 cpm. Experiment 4: unfractioned, 122,010 cpm; erythrocyte positive, 143,190 cpm; erythrocyte negative, 5,160 cpm). *NS*, nodular sclerosis; *MC*, mixed cellularity.

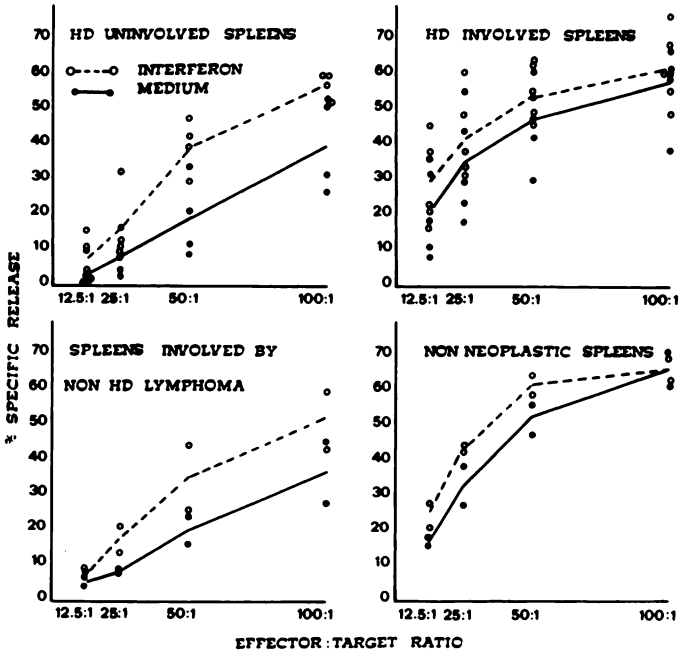

Chart 7. NK activity of spleen cells after treatment with interferon. Spleen cells from 4 HD⁻ spleens, 5 HD⁺ spleens, 2 spleens involved by non-HD lymphoma, and 2 nonneoplastic spleens were cultured in 10% FCS RPMI 1640 with (○) or without (●) 1000 units interferon for 3 hr prior to being added to K-562 target cells.

thermore, it was recently reported that purified human monocytes are cytotoxic against K-562 cells in a short-term ^{51}Cr release assay (7). Thus, since cells from different origin are able to exert cytotoxic activity *in vitro*, identification and quantification of NK effector cells in lymphoid tissues is highly difficult.

Our data indicate that cell suspensions from HD⁺ tissues are more cytotoxic than those from HD⁻ tissues. This finding may be explained either by increased levels of NK activity in HD⁺ tissues (higher frequency of effector cells and/or higher levels of activation of NK cells) or by depressed NK activity in HD⁻ tissues. The observation that NK activity of PBL from 9 HD patients was, on the average, 2-fold lower than that of PBL from 28 healthy donors ($p < 0.05$ only at 100:1 effector:target cell ratio) may indicate that NK activity is effectively suppressed in HD⁻ tissues. In this regard, it should be noted that suppressor monocytes acting through prostaglandin release were repeatedly described in PBL of HD patients (11, 23, 24) and that prostaglandins suppress NK activity of normal human PBL (5). Thus, a prostaglandin-mediated suppression of NK activity in PBL of HD patients does not seem to be an unlikely event. Finally, it cannot be excluded that the neoplastic cell present in HD lesion is *per se* cytotoxic.

Increased NK activity could not be demonstrated in spleens and lymph nodes involved by non-HD lymphoma. This observation, besides providing a further element to differentiate HD from other lymphoma, is strikingly similar to another observation made recently in SJL/J mice (4). In fact, in this strain of mice, spleen or lymph node involvement by transplantable reticulum cell neoplasms, a HD-like murine tumor, was found associated with increased levels of NK activity. In contrast, spleen or lymph node involvement by other types of tumors was never found associated with alterations of NK activity. Further characterization of this system indicated that the neoplastic cells were not *per se* cytotoxic and that the increased NK activity was due to activation of the host cells by the reticulum cell neoplasm cells.

ACKNOWLEDGMENTS

The authors thank Dr. Alberto Mantovani for kindly providing interferon preparations.

REFERENCES

1. Altman, A., and Rapp, H. J. Natural cell mediated cytotoxicity in guinea pigs: properties and specificity of natural killer cells. J. Immunol., *121:* 2244–2252, 1978.
2. Baroni, C. D., Ruco, L., Uccini, S., Foschi, A., Occhionero, M., and Marcorelli, E. Tissue T lymphocytes in untreated Hodgkin's disease. Morphological and functional correlations in spleens and lymph nodes. Cancer (Phila.), in press, 1982.
3. Biron, C. A., Hutt-Fletcher, L. M., Wertz, G. T., and Pagano, J. S. Interferon production and activation of nonspecific effector cells by stimulation with lymphoblastoid cell lines *in vitro*. Int. J. Cancer, *27:* 185–190, 1981.
4. Chang, K. S. S., and Log, T. Natural killer cell activity associated with reticulum cell neoplasms. Int. J. Cancer, *25:* 405–416, 1980.
5. Droller, M. J., Schneider, M. U., and Perlmann, P. A possible role of prostaglandins in the inhibition of natural and antibody-dependent cell-mediated cytotoxicity against tumor cells. Cell. Immunol., *39:* 165–177, 1978.
6. Eremin, O., Ashby, J., and Stephens, J. P. Human natural cytotoxicity in the blood and lymphoid organs of healthy donors and patients with malignant disease. Int. J. Cancer, *21:* 35–41, 1978.
7. Fischer, D. G., Hubbard, W. J., and Koren, H. S. Tumor cell killing by freshly isolated peripheral blood monocytes. Cell. Immunol., *58:* 426–435, 1981.
8. Gupta, S. Subpopulations of human T lymphocytes. XVI. Maldistribution of T cell subsets associated with abnormal locomotion of T cells in untreated adult patients with Hodgkin's disease. Clin. Exp. Immunol., *42:* 186–195, 1980.
9. Herberman, R. B., and Holden, H. T. Natural cell mediated immunity. Adv. Cancer Res., *27:* 305–377, 1978.
10. Herberman, R. B., Nunn, M. E., and Lavrin, D. H. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors distribution of reactivity and specificity. Int. J. Cancer, *16:* 216–229, 1975.
11. Hillinger, S. M., and Herzig, G. P. Impaired cell mediated immunity in

Hodgkin's disease is mediated by suppressor lymphocytes and monocytes. J. Clin. Invest., *61:* 1620–1627, 1978.
12. Hofman, F. M., and Spina, C. A. Enhancement of spontaneous killer cytotoxicity by soluble factors. Clin. Exp. Immunol., *42:* 589–596, 1980.
13. Jondal, M., Holm, G., and Wigzell, H. Surface markers on human T and B lymphocytes. I. A large population of lymphocytes forming nonimmune rosettes with sheep red blood cells. J. Exp. Med., *136:* 207–215, 1972.
14. Kaplan, J., and Callewaert, D. M. Expression of human T lymphocyte antigens by natural killer cells. J. Natl. Cancer Inst., *60:* 961–964, 1978.
15. Kaur, J., Spiers, A. S. D., Catovsky, D., and Galton, D. A. G. Increase of T lymphocytes in the spleen of Hodgkin's disease. Lancet, *2:* 800–802, 1974.
16. Kay, H. D., and Horwitz, D. A. Evidence by reactivity with hybridoma antibodies for a probable myeloid origin of peripheral blood cells active in natural cytotoxicity and antibody-dependent cell-mediated cytotoxicity. J. Clin. Invest., *66:* 847–851, 1980.
17. Nelson, D. B., Bundy, B. M., and Strober, W. Spontaneous cell-mediated cytotoxicity by human peripheral blood lymphocytes *in vitro*. J. Immunol., *119:* 1401–1405, 1977.
18. Oehler, J. R., Lindsay, L. R., Nunn, M. E., and Herberman, R. B. Natural cell-mediated cytotoxicity in rats. I. Tissue and strain distribution and demonstration of a membrane receptor for the Fc portion of IgG. Int. J. Cancer, *21:* 204–209, 1978.
19. Ortaldo, J. R., Sharrow, S. O., Timonen, T., and Herberman, R. B. Determination of surface antigens on highly purified human NK cells by flow cytometry with monoclonal antibodies. J. Immunol., *127:* 2401–2409, 1981.
20. Pinkus, G. S., Barbuto, D., Said, J. W., and Churchill, H. W. Lymphocyte subpopulations of lymph nodes and spleens in Hodgkin's disease. Cancer (Phila.), *42:* 1270–1279, 1978.
21. Potter, M. R., and Moore, M. PHA stimulation of separated human lymphocyte populations. Clin. Exp. Immunol., *21:* 456–467, 1975.
22. Rosenberg, E. B., McCoy, J. L., Green, S. S., Donnelly, F. C., Siwarsky, D. F., Levine, P. H., and Herberman, R. B. Destruction of human lymphoid tissue-culture cell lines by human peripheral lymphocytes in ^{51}Cr release cytotoxicity assay. J. Natl. Cancer Inst., *52:* 345–352, 1974.
23. Schechter, G. P., and Soehnlen, F. Monocyte-mediated inhibition of lymphocyte blastogenesis in Hodgkin's disease. Blood, *52:* 261–271, 1978.
24. Schechter, G. P., Wahl, L. M., and Oppenheim, J. J. Suppressor monocytes in human disease: a review. *In*: M. R. Escobar and H. Friedman (eds.), Advances in Experimental Medicine and Biology, Part B, pp. 283–298. New York: Plenum Publishing Corp., 1980.
25. Timonen, T., Ortaldo, J. R., and Herberman, R. B. Characteristics of human large granular lymphocytes and relationship to natural killer and K cells. J. Exp. Med., *153:* 569–582, 1981.
26. Timonen, T., Saksela, E., Ranky, A., and Häyry, P. Fractionation, morphological and functional characterization of effector cells responsible for human natural killer activity against cell line targets. Cell. Immunol., *48:* 133–148, 1979.
27. Trinchieri, G., and Santoli, D. Antiviral activity induced by culturing lymphocytes with tumor-derived or virus-transformed cells. Enhancement of human natural killer cell activity by interferon and antagonistic inhibition of susceptibility of target cell lysis. J. Exp. Med., *147:* 1314–1333, 1978.
28. Vose, B. M., Vanky, F., Argov, S., and Klein, E. Natural cytotoxicity in man: activity of lymph node and tumor-infiltrating lymphocytes. Eur. J. Immunol., *7:* 753–757, 1977.
29. West, W. H., Cannon, G. B., Kay, H. D., Bonnard, G. D., and Herberman, R. B. Natural cytotoxic reactivity of human lymphocytes against a myeloid cell line: characterization of effector cells. J. Immunol., *118:* 355–361, 1977.
30. Willson, J. K. V., Jr., Zaremba, J. L., and Pretlow, T. G. Functional characterization of cells separated from suspensions of Hodgkin's disease tumor cells in an isokinetic gradient. Blood, *5:* 783–797, 1977.

Natural Killer Activity in Spleens and Lymph Nodes from Patients with Hodgkin's Disease

Luigi P. Ruco, Antonio Procopio, Stefania Uccini, et al.

Cancer Res 1982;42:2063-2068.