Eur J Cancer Clin Oncol, Vol. 19, No. 10, pp. 1405–1409, 1983.
Printed in Great Britain.

0277-5379/83$3.00+0.00

In Vitro Radiosensitivity of the Spontaneous Cytotoxicity of Blood Lymphocytes in Patients with Untreated Hodgkin's Disease

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Abstract—*The in vitro X-ray sensitivity of the natural killer (NK) cell activity and interferon (IFN)-inducible NK activity of blood lymphocytes obtained from 12 patients with untreated Hodgkin's disease (HD) and 12 healthy subjects was compared. It was observed that the NK activity against K562-cells decreased in a roughly linear fashion within the dose range of 4.0–16.0 Gy and there was no demonstrable difference between patients and controls. IFN treatment of the irradiated cells increased their NK activity to a higher relative extent than in unirradiated cells. On a relative basis, this increase became more pronounced as the X-ray dose was increased. The extent by which IFN could augment NK activity of lymphocytes, whether irradiated or not, did not differ between patients and controls. It is concluded that the radiosensitivity of NK activity and IFN-inducible NK activity of blood lymphocytes in HD is similar to that of healthy individuals, which is in contrast to T-cells and T-cell responses in this disease.*

INTRODUCTION

PATIENTS with untreated Hodgkin's disease (HD) frequently display defective T cell-mediated immune responses. This abnormality may be manifested *in vivo* by impaired delayed cutaneous responses to recall antigens [1] and *in vitro* by reduced mitogenic responses of the blood lymphocytes to polyclonal mitogens [2] and specific antigens [2, 3]. It was previously observed that the mitogenic responses of blood lymphocytes from untreated HD patients are frequently more sensitive to X-ray exposure *in vitro* than lymphocytes obtained from healthy subjects and patients with other tumors of lymphoid or non-lymphoid origin [4–7].

In this investigation we have extended our previous studies to examine if the *in vitro* radiosensitivity of the natural killer (NK) activity of HD patients lymphocytes differs from that of healthy subjects.

MATERIALS AND METHODS

Patients and controls

Twelve patients with HD were tested. There were five females and seven males, with an age range of 31–73 yr (mean 49 yr). The diagnostic procedures included a clinical staging according to the Ann Arbor classification [8] and a histopathological examination [9]. The clinical staging was based on complete blood cell counts, liver and renal function tests, chest X-rays, emission tomography of spleen and liver, abdominal CT-scan and bone marrow aspiration. In addition, patients below 50 yr of age underwent a lymphangiography. The histopathological types and clinical stages of the disease were as follows: one patient had lymphocytic predominance, eight nodular sclerosis and three mixed cellularity. Two patients had stage IA, eight stage IIA, one stage IIBE and one stage IIIA. The tests reported in this article were performed within 3 weeks after lymph node biopsy but before lymphangiography, splenectomy or any treatment.

Twelve healthy controls, matched for age and sex with the patients, were examined in parallel.

Preparation of lymphocytes

Venous blood was drawn in heparinized glass tubes and lymphoid cells were separated by centrifugation on a layer of Ficoll-Isopaque [10]. Phagocytic cells were removed magnetically after addition of carbonyl iron powder. Approximately

Accepted 10 March 1983.

95% of the cells were classified as lymphocytes and viability always exceeded 90% as assessed by trypan blue staining.

Irradiation of lymphocytes

This technique has been described in detail before [4]. In short, lymphocytes suspended in Eagle's Minimal Essential Medium supplemented with Earle's salts (MEM) were poured into Petri dishes and exposed to varying doses of ionizing radiation using a Siemen's X-ray machine operated at 140 kV and 20 mA. The doses delivered were 4.0, 8.0 and 16.0 Gy (1 Gy = 100 rad). Control cells were treated identically except that they were not irradiated.

Interferon (IFN) preparation

Partially purified IFN-α (PIF) was obtained from human leukocytes exposed to Sendai virus as described by Mogensen and Cantell [11]. The preparations had a specific activity of approximately 3×10^6 IU of IFN per mg of protein. It was kindly supplied by Dr. Cantell, Helsinki, Finland.

IFN treatment of lymphocytes

Within 1 hr following X-ray exposure IFN was added to half of the irradiated and unirradiated dishes at a final concentration of 1000 units per ml. The cell preparations, with and without IFN, were then incubated in a humidified 5% CO_2–air atmosphere at 37°C for 20 hr. They were then washed twice by centrifugation in MEM and examined for NK activity (see below).

Isotope labelling of target cells

K562 cells derived from a human myeloid leukemia served as target cells in the cytotoxicity tests. They were labelled with ^{51}Cr as described [12].

NK cell assay

The cytotoxic assays were performed in triplicate in round-bottomed wells moulded in a plastic plate (Falcon products). Each plate contained 96 wells. Five thousand ^{51}Cr-labelled target cells together with varying numbers of viable lymphocytes were added to each well containing 0.2 ml of RPMI 1640 medium supplemented with 10% heat-inactivated AB+ serum. The lymphocyte:target cell ratios were 50:1, 25:1 and 12.5:1. As controls 5×10^3 ^{51}Cr-labelled target cells in medium was used to measure spontaneous release. For measuring maximum release 5×10^3 ^{51}Cr-labelled cells were suspended in Triton (phenoxypolyethoxyethanol) solution diluted 1:25 in water. The cells were brought in close contact by low speed centrifugation and the plates were incubated in a humidified 5% CO_2–air atmosphere for 4 hr. The supernatants were then harvested without disrupting the cell pellet using a Titertek supernatant collection system. The released isotope and the pellet were counted in a gamma counter and expressed as counts/min. (Variability within the triplicates did not exceed 10%.) A cytotoxic index (CI) was calculated according to the following formula:

$$CI = \frac{\%\text{ isotope release with lymphocytes} - \%\text{ spontaneous release}}{\%\text{ maximum isotope release} - \%\text{ spontaneous release}}.$$

Data processing and statistical evaluation

The CI of irradiated cells which were not IFN-treated were related to that of unirradiated, non-IFN-treated cells within each experiment. The latter was put as 100%. The values of irradiated IFN-treated cells were processed in an analogous way. Mean values and SE of different experiments were calculated on an arithmetic rather than a geometric basis since in some tests cytotoxicity was extremely low or absent. Statistical significance between groups of observations was tested using Student's t test.

RESULTS

NK activity of unirradiated lymphocytes

NK activity of lymphocytes from HD patients and healthy controls was compared. Table 1 shows that cytotoxicity of the patients lymphocytes was somewhat higher than that of the controls. This difference was statistically significant at an effector:target cell ratio of 12.5:1 ($P < 0.01$). Treatment of the lymphocytes with IFN significantly increased cytotoxicity of both patients and controls. The extent by which IFN augmented NK activity did not differ between the two groups of individuals.

Impact of irradiation on the NK activity of lymphocytes

Figure 1 shows the relative NK activities of lymphocytes exposed to varying X-ray doses *in vitro*. At all three lymphocyte:target cell ratios cytotoxicity decreased in a fairly linear fashion by increasing the X-ray dose. There were no significant differences between patients and controls. IFN treatment increased NK activity to a higher relative extent in irradiated than in unirradiated lymphocytes. This effect also appeared to be radiation-dose-dependent, e.g. IFN augmented cytotoxicity to a relatively higher extent in lymphocytes exposed to 16 than 4 Gy. The extent by which IFN augmented cytotoxicity of irradiated lymphocytes did not differ between patients and controls.

Table 1. NK activities, expressed as CI of unirradiated blood lymphocytes obtained from HD patients and healthy controls

Lymphocyte:target cell ratio	Untreated lymphocytes	IFN-treated lymphocytes	Difference	*P* value
		Patients ($n = 12$)		
50:1	0.44 ±0.06	0.63 ±0.04	0.19 ±0.05	<0.005
25:1	0.36 ± 0.05	0.58 ± 0.04	0.21 ± 0.05	<0.005
12.5:1	0.26 ± 0.04	0.48 ± 0.04	0.22 ± 0.04	<0.001
		Controls ($n = 12$)		
50:1	0.33 ± 0.04	0.54 ± 0.04	0.22 ± 0.04	<0.001
25:1	0.23 ± 0.03	0.47 ± 0.06	0.22 ± 0.05	<0.005
12.5:1	0.14 ± 0.02	0.39 ± 0.06	0.21 ± 0.04	<0.001

In each test half of the lymphocytes were treated with IFN (see Materials and Methods). The lymphocytes were tested at three different effector:target cell ratios. Mean values ± S.E. are presented. *P* values denote the statistical significance of the difference between untreated and IFN treated cells.

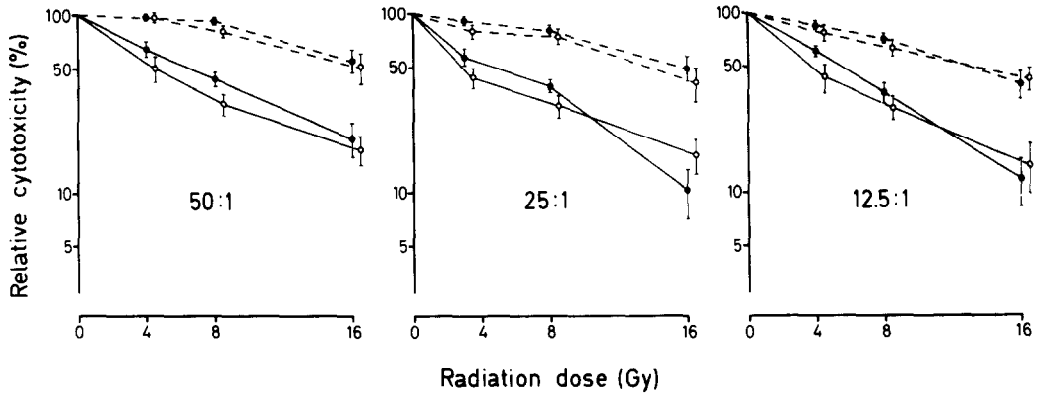

Fig. 1. Effect of X-ray exposure on the relative NK activity of blood lymphocytes from patients with HD ● and healthy subjects ○. In half of the tests the irradiated and unirradiated lymphocytes were treated with IFN ____ and the others were processed without IFN ——. Three different lymphocyte:target cell ratios are presented. Symbols represent mean ± S.E. The absolute values, expressed as CI, of the unirradiated cells are presented in Table 1.

DISCUSSION

It has previously been shown that the mitogenic responses of human blood lymphocytes obtained from healthy individuals display a biphasic sensitivity pattern to varying doses of X-rays *in vitro* [4–7]. This indicates that there are two subpopulations of mitogen-responsive lymphocytes which differ with respect to radiation sensitivity: one which is relatively 'radiosensitive' (approximately 80%) and one which is relatively 'resistant' (approximately 20%). In patients with untreated HD, however, the latter subpopulation is frequently smaller or seems to be missing [6, 7]. Such a radiosensitivity pattern has not been observed in patients with other tumors [5, 7] or autoimmune disease (unpublished results).

The aim of the present investigation was to examine if another type of lymphocyte response, namely NK activity, also exhibits an increased radiosensitivity in HD compared to healthy subjects. Our results have shown that the NK activity of HD peripheral blood lymphocytes against K562-cells is somewhat higher than that of healthy controls (Table 1), which is in slight discordance with other reports [13]. This is probably not due to an activation of cytotoxic cells *in vivo* by IFN or other agents capable of augmenting NK activity [12, 14] since IFN treatment of lymphocytes *in vitro* augmented cytotoxicity to the same extent in patients and controls (Table 1). Exposure of lymphocytes to IFN *in vivo* is known to reduce the extent by which IFN treatment *in vitro* can augment NK-activity [15].

A more likely explanation is that the frequency of cells which may display NK activity is increased in the blood of HD patients. This thesis is supported by the finding that the frequency of T lymphocytes expressing Fc-receptors for IgG, which may be a phenotypic characteristic of suppressor T cells [16], is frequently abnormally high in HD [17, 18], and there is evidence that at least some suppressor cells may function as NK cells [19].

Exposure of lymphocytes to varying X-ray doses reduced the NK activity in a fairly linear fashion, and there was no detectable difference between patients and controls (Fig. 1). This indicates that the lymphocyte population exerting NK activity or this cellular function does not exhibit an increased radiosensitivity in HD, which is in contrast to mitogen responses of lymphocytes (see above). It is thus possible that the lymphocyte defect in HD is mainly confined to cells within the T cell lineage. This possibility is further supported by the finding that lymphocytes forming rosettes with sheep red cells in HD are frequently more sensitive to *in vitro* X-irradiation than in healthy subjects [20].

Treatment of irradiated lymphocytes with IFN augmented their NK activity to a higher relative extent than in unirradiated cells (Fig. 1). There may be several explanations for this finding. One interpretation could be that the decline of NK activity following irradiation is not due to a killing of cytotoxic cells but mainly an inhibition of their function. This inhibition may be reverted by IFN. Another, probably less likely, explanation could be that IFN enhances the repair of X-ray damaged lymphocytes. Still a third possibility could be that pre-NK cells, known to be boosted by IFN [21], are more radioresistant than mature NK cells. Such an explanation has been posed by others showing that the reduction of NK activity following whole-body exposure of rats to ionizing radiation can be reverted by treatment with polyinosinic-polycytidylic acid, which is known to be a potent IFN inducer [22]. A fourth explanation could be that X-ray therapy primarily interferes with spontaneous IFN production.

Other investigators have observed that exposure of human blood lymphocytes to doses below 10 Gy may enhance NK activity whereas there is a reduction at higher doses [23, 24]. Such an augmented NK activity, however, was not observed in the present investigation. This disagreement may be due to the fact that different experimental conditions were used. For instance, in our study the NK assay was performed 20 hr after X-ray exposure whereas the other investigators tested their cells within a few hours.

In conclusion, our results seem to indicate that the *in vitro* radiosensitivity of NK activity and IFN-inducible NK activity in HD do not differ from those of healthy subjects.

Acknowledgements—The authors wish to thank Mrs Lena Virving for excellent technical assistance. This work was supported by grants from the Cancer Society in Stockholm and the Swedish Cancer Society.

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