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K Cell Activity in Ovarian Cancer Patients Given Chemotherapy*

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Abstract—*K cell activity was evaluated in 20 patients with advanced ovarian epithelial tumours, using ^{51}Cr-labelled murine lymphoma cells as targets. Cancer patients showed significantly lower K cell activity than 40 sex and age matched controls. Cyclophosphamide, given alone or in combination with adriamycin, did not significantly affect K cell activity per unit number of lymphoid cells, whereas lymphoproliferative responses to phytohaemagglutinin and pokeweed mitogen were markedly suppressed by these agents. Patients showing an objective response to chemotherapy had K cell activity values similar to non responders.*

INTRODUCTION

NON-IMMUNE lymphoid cells can express cytotoxicity *in vitro* on target cells in the presence of specific antibody [1, 2]. In human peripheral blood, effector cells involved in antibody-dependent cellular cytotoxicity (ADCC) do not show the typical markers of mature T and B cells [3–5]. Circulating human monocytes mediate ADCC on chicken and human erythrocytes [3, 4], but only mature macrophages show significant ADCC on nucleated mammalian cells [6]. The nature of human peripheral blood non phagocytic killer cells (K cells) lysing antibody-coated tumour target cells in short term ^{51}Cr-release assays remains to be positively defined [1–3].

There are indications in experimental models that ADCC might represent an effective mechanism in the control of tumour growth [7–11]. However little effort has been made to analyse modifications of K cell activity in tumour bearing animals and patients. In the spleen and lymphnodes of mice bearing experimental tumours activation of the effector cells of ADCC has been demonstrated [12, 13]. On the other hand in a population of patients with various types of tumours, some of them treated with chemotherapy or radiotherapy, impaired K cell activity has been reported [14, 15]. this paper reports an evaluation of K cell activity in patients with advanced ovarian cancer and an investigation of the effect of chemotherapy on this cellular effector mechanism.

MATERIALS AND METHODS

Patients

Seventeen patients with stage III (F.I.G.O.) epithelial cancer of the ovary and 3 patients with stage IIC (mean age 52, range (29–76) were examined in this study. None of them had previously received either chemotherapy or radiotherapy. In 2 patients K cell activity was measured both before and 10–15 days after surgical excision of the tumour. Since these two examinations gave similar results, only the 2nd test performed just before starting chemotherapy will be reported. The patients in this investigation were part of a group randomly allocated to receive either cyclophosphamide (CY, 100 mg *per os* per day, 10 patients) or the same regimen of CY in combination with adriamycin (ADM, 50 mg/m^2 i.v. every 28 days). The results of this clinical trial will be the subject of a subsequent report (Mangioni *et al.*, in preparation).

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Venous blood was obtained before chemotherapy and 28 (1st cycle) and 84 days (3rd cycle) after the beginning of the treatment. As controls, 40 age-matched (mean age 47, range 22–67) women, admitted to the same clinic with benign gynecological disorders, were studied concomitantly.

Target cells

The TLX9 lymphoma of C57 Bl/6 origin was obtained through the courtesy of Dr. R. Evans, Chester Beatty Research Institute, Sutton, Surrey, England. The tumour line used in these experiments was maintained in suspension culture in RPMI 1640 medium supplemented with 20% fetal bovine serum.

Anti-TLX9 serum

Rabbit anti-TLX9 serum was prepared by injecting rabbits subcutaneously with 2×10^8 cells in complete Freund's adjuvant. After 20 days the animals were inoculated with 10^8 lymphoma cells s.c. and, 10 days later, serum was collected, heat inactivated and stored at −80°C. At the optimal 1:20,000 dilution employed in these tests, the serum did not interfere with the viability and proliferative capacity of tumour cells *in vitro*.

Effector cells

Mononuclear cells were separated from heparinized venous blood by centrifugation at 400 ***g*** for 20 min on Ficoll–Hypaque. The cells were washed twice with MEM and resuspended in RPMI 1640 medium containing 10% fetal bovine serum and 50 μg/ml gentamycin (growth medium).

ADCC assay

Antibody-dependent lysis of TLX9 lymphoma cells was assessed basically as previously described [6]. Briefly, ^{51}Cr-labelled TLX9 tumour cells (2×10^4) were incubated for 20 hr with effector cells and 1:20,000 diluted rabbit anti-TLX9 serum in a final volume of 2 ml growth medium in plastic tubes (Cat 2098, Falcon). At least 3 attacker to target cell (A:T) ratios (30:1; 10:1, 3:1) were used in each experiment with 3 replicates per experimental group. The percentage of specific lysis was calculated as:

$$\frac{\text{release with antibody} - \text{release without antibody}}{\text{total releasable radioactivity per sample}} \times 100.$$

Releasable radioactivity assessed by osmotic lysis was 75% of total isotope incorporated. Spontaneous release in the absence of effector cells was 0.7–2%/hr of incubation. In the absence of specific antibody, effector cells occasionally showed significant natural cytotoxicity on tumour cells which however never exceeded 10% lysis and was observed only at the highest A:T ratio (30:1).

Lymphoproliferative responses

Mononuclear cells (6×10^4/well) were cultivated for 72 hr in microtiter plates (Sterilin, Cat M24 AR) in 0.12 ml RPMI 1640 medium containing 20 mM Hepes, 20% fetal bovine serum and 50 μg/ml gentamycin. Phyto haemagglutinin (PHA, Wellcome) and pokeweed mitogen (PWM, Difco) were added at the previously determined optimal concentration of 1%. Twenty hours before termination of the experiment, 0.5 μCi/well of ^{3}H-thymidine (2 Ci/mmole, Amersham) was added in a volume of 0.02 ml. Cultures were harvested on glass fiber filters using an automatic apparatus (Skatron, Norway) and were counted in Instafluor (Packard) using a Nuclear Chicago scintillation spectrometer. Three replicates were made per experimental group.

Statistical analysis

Controls and ovarian cancer patients were compared by the Mann–Whitney test. Changes in immune responsiveness during chemotherapy were compared to pretreatment values by the Wilcoxon Signed Rank Test.

RESULTS

The number of mononuclear cells in the peripheral blood of untreated patients with ovarian cancer and controls was similar (24 ± 2 and $26 \pm 2 \times 10^5$/ml respectively, mean ±S.E.). After 1 or 3 cycles of chemotherapy, mononuclear cell numbers were reduced to 14 ± 3 and $11 \pm 2 \times 10^5$/ml respectively ($P < 0.01$ vs prechemotherapy values). The percentages of monocytes in the mononuclear cell preparations assessed morphologically on Wright-stained smears was similar in all groups considered, values being 8.6, 9.8, 9.4 and 10.6% in controls, untreated patients, and patients after 1 or 3 cycles of chemotherapy, respectively. No differences were observed between the two chemotherapy protocols used in this study. K cell activity was significantly lower in untreated patients than in controls

(Fig. 1). At an A:T ratio of 30:1 controls showed a mean of 59% specific lysis as compared to 38% in cancer patients ($P<0.001$). At an A:T ratio of 10:1 the mean percentages of specific cytotoxicity were 50 and 33% in the control and tumour bearing population, respectively ($P<0.01$). At 3:1, the difference between control and cancer patients (29% as compared to 19%) was of borderline statistical significance ($P<0.05$). It should be noted that the sex and age matched population with benign gynecological disease employed as controls in this study had K cell activity values identical to those of a series of 15 normal donors from this laboratory tested concomitantly. K cell activity also was evaluated in 18 patients after 1 cycle and in 15 after 3 cycles of chemotherapy. As shown in Fig. 2, chemotherapy did not significantly modify K cell activity in the peripheral blood of tumour bearing patients, although a trend towards an increase in ADCC values was noted. No significant differences were detected between the CY and CY+ADM protocols (Fig. 2). In a limited number, 10, of these patients, lymphoproliferative responses to optimal concentrations of PHA and PWM were measured concomintantly before and after chemotherapy. As shown in Table 1, in untreated patients lymphoproliferative response values were similar to those of normal individuals but, in agreement with previous reports in non-tumourous conditions [16], after 28 days of treatment with CY alone or in combination with ADM a marked reduction in lymphoproliferative responses was observed. A similar suppression was also found in 6 patients after 3 cycles of chemotherapy (results not presented).

The objective response rate (defined as the percentage of individuals showing at least a 50% reduction in size of measurable lesions) in the group of patients tested in this study was 60% (12/20). No significant differences in pretreatment ADCC values were detected between responder and nonresponder ovarian cancer patients (Table 2) nor was objective response associated with significant changes in K cell activity during chemotherapy.

DISCUSSION

The results obtained using murine tumour cells as targets show that patients with advanced ovarian cancer have impaired K cell activity when compared to sex and age matched controls. These data are consistent with previous reports showing [14, 15] impaired

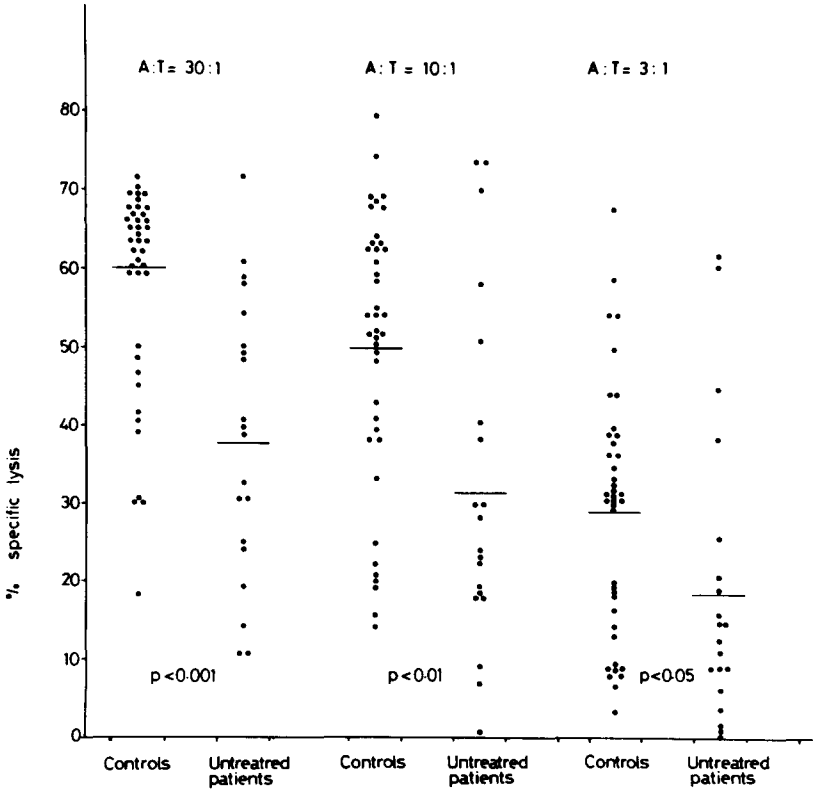


Fig. 1. K cell activity in untreated patients with ovarian cancer.

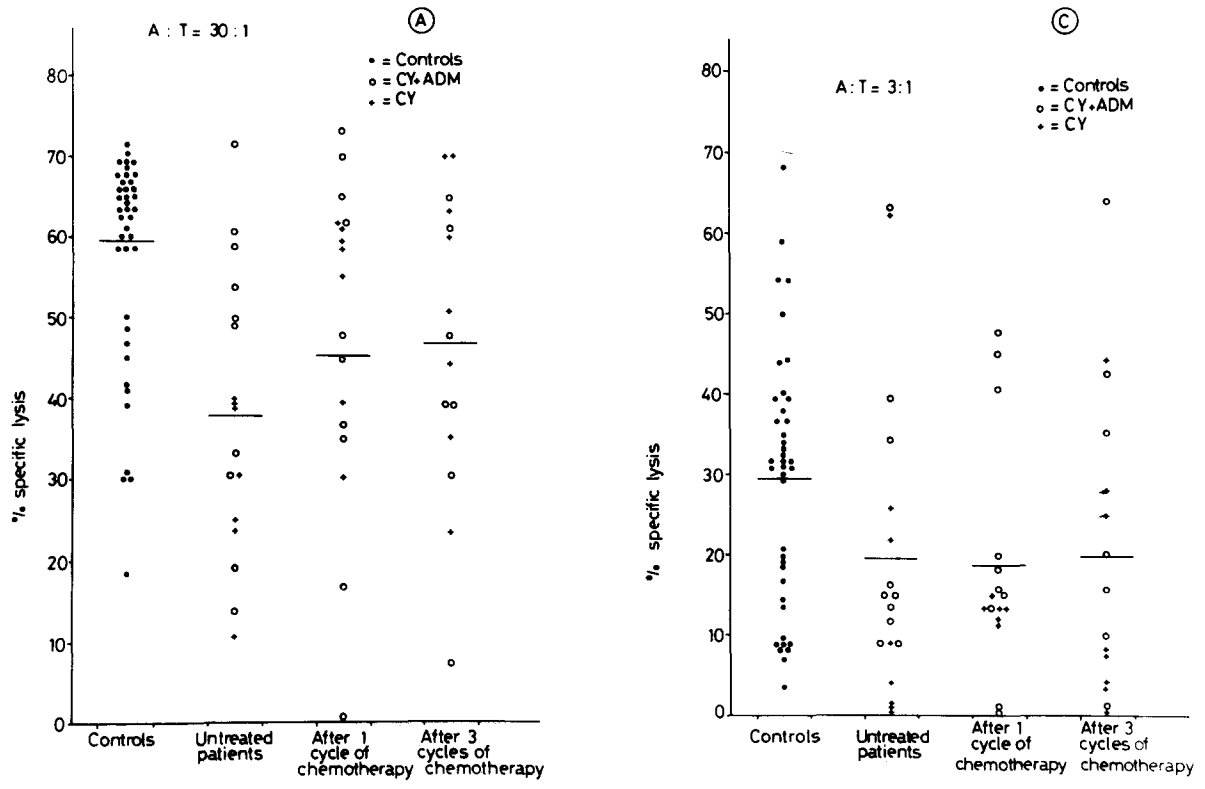
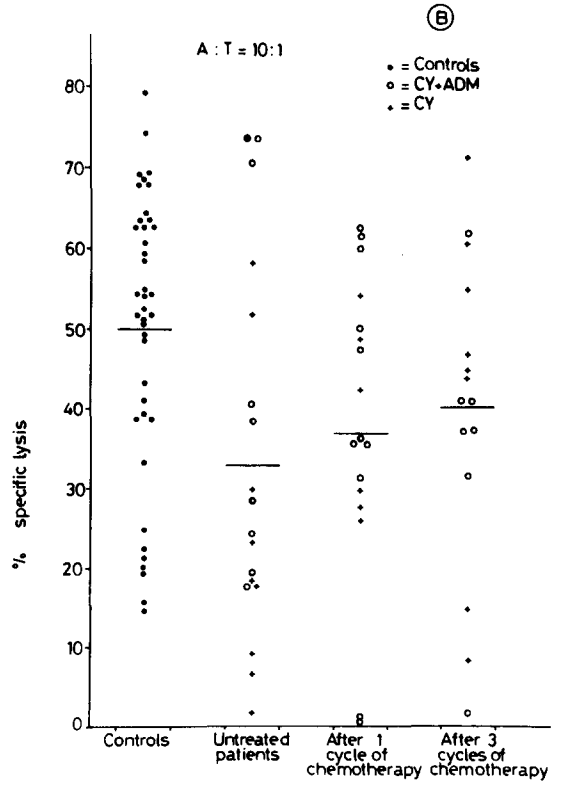


Fig. 2. Effect of chemotherapy on K cell activity in patients with ovarian cancer. Tests were done 28 (1 *cycle*) *or* 84 days (3 *cycles*) *after starting treatment with CY (X) or CY+ADM (0).*



ADCC effector function in a population of patients with various types of tumours, some treated with chemotherapy or radiotherapy. On the other hand, our findings are at variance with those obtained in experimental models: in the lymph nodes and spleen of tumour-bearing mice increased K cell activity was found, using as targets either chicken erythrocytes in a cytolysis test or tumour cells in a cytostasis assay [12, 13]. The different type of target cells or of cytotoxicity assays, possibly involving different subsets of effector cells, might at least partially account for the apparent discrepance between the existing human and animal data.

The mechanism(s) responsible for impaired K cell activity in ovarian cancer patients has not been elucidated. Circulating inhibitors of ADCC have been reported in sera of breast carcinoma patients [17] and, if confirmed in ovarian cancer, might account for the lower K cell activity observed in this study. However, we have so far been unable to demonstrate any ADCC inhibitory activity of serum in these ovarian cancer patients. In fact

Table 1. Effect of chemotherapy on lymphoproliferative responses to PHA and PWM in ovarian cancer patients

	PHA*	PWM*
Normal laboratory donors ($n=19$)	23,860 (33,540–18,930)	9663 (11,840–8230)
Controls ($n=17$)	18,023 (26,280–16,240)	8775 (10,990–6510)
Untreated patients ($n=10$)	26,879 (29,360–22,890)	9232 (11,360–6930)
Patients after 1 cycle of chemotherapy ($n=10$)	8232† (10,120–6140)	5219‡ (6890–2160)

*Results are presented as median cpm with range shown in parenthesis.
†$P<0.01$ vs untreated patients (Mann–Whitney).
‡$P<0.05$ vs untreated patients (Mann–Whitney).

Table 2. K cell activity in responder and non responder ovarian cancer patients

	% specific lysis (median)		
	30:1*	10:1*	3:1*
Controls ($n=40$)	63	54	31
Untreated patients ($n=20$)	39¶	28‖	16§
R† untreated ($n=12$)	35	28	15
NR‡ untreated ($n=8$)	42	29	19
R† after 1 cycle ($n=10$)	46	40	19
NR‡ after 1 cycle ($n=8$)	46	36	17

*A:T (attacker to target cell ratio).
†Responders.
‡Non responders.
§$P<0.05$ (Mann–Whitney) vs control.
‖$P<0.01$ (Mann–Whitney) vs control.
¶$P<0.001$ (Mann–Whitney) vs control.

serum from the patients tested in the present study had no significant effect on K cell activity when compared to serum of the control population.

CY, alone or in combination with ADM, did not significantly modify K cell activity although, as previously reported by others in non-tumourous conditions [16], it resulted in marked impairment of blastogenic responses to PHA and PWM. However, since mononuclear cell counts were markedly reduced after treatment with chemotherapy, total ADCC effector capacity per ml of blood was presumably decreased by the cytotoxic agents used in this study. Thus, in cancer patients the effect of CY on K cells and lymphoid cells responsive to mitogens appears similar to that of radiotherapy [18].

In principle the lack of inhibitory effect on K cell activity with the chemotherapy protocols used here might be due to their antitumoural efficacy, a reduction in tumour burden compensating for the toxicity of these drugs on ADCC effector cells. However, K cell activity was similar in responder and non responder patients. Moreover, in the mouse it has been shown that one of the drugs used in this study, ADM, did not affect spleen ADCC in terms of specific cytotoxicity [19].

These observations thus support the possibility that human peripheral blood K cell activity might be relatively resistant to the cytotoxic effect of CY, alone or in combination with ADM, compared to lymphoid populations responsive to PHA and PWM. It is of interest that on the other hand human peripheral blood K cell activity has been reported to be selectively suppressed by azathioprine with no concomitant significant impairment of the blastogenic response to PHA [20].

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