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Augmentation of *In Vivo* Antitumour Activity of Xenogeneic Antiserum by Autotransplanted Normal Spleen Cells in Mice

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Abstract—*Inbred CFW/L1 mice were injected i.p. with* 10^5 *or* 10^6 *Ehrlich ascites carcinoma (EAC) cells and* 24 hr *later were given i.p. injection of rabbit anti-EAC serum.* In vivo *tumour growth was suppressed with the use of antiserum in* 90% *of mice injected with* 10^5 *and in* 40% *of mice injected with* 10^6 *EAC cells. Intraperitoneal injection of normal spleen cells into syngeneic or autologous recipients* 0.5 hr *after inoculation of* 10^6 *EAC cells augmented the antitumour effect of antiserum and this combined treatment suppressed tumour growth in* 90–100% *of mice. Syngeneic or autologous spleen cells, when transplanted alone, demonstrated weak but significant antitumour effect in mice inoculated with* 10^5 *EAC cells. Normal spleen cells from CFW/L1 donors showed natural as well as significantly higher antibody-dependent cellular cytotoxicity (ADCC) against EAC cells* in vitro. *In our study we demonstrated the augmentation of* in vivo *antitumour activity of xenogeneic antiserum by transplantation of normal autologous lymphoid cells.*

INTRODUCTION

THERE is increasing evidence that antiserum against a tumour could be used to suppress tumour growth *in vivo* [1–6]. It was further observed that this antitumour effect could be strengthened by the injection of normal syngeneic [7] or allogeneic [8] lymphoid cells. Syngeneic lymphocytes are, unfortunately, rarely available, at least as far as human cancer immunotherapy is concerned. On the other hand, transplantation of allogeneic lymphocytes may induce development of the graft-vs-host reaction even with the use of an HLA matched donor [9, 10].

Therefore, we decided to check whether antitumour activity of xenogeneic antiserum could be potentiated by transplantation of autologous spleen cells.

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MATERIALS AND METHODS

Mice

Inbred CFW/L1 mice, 6–10-weeks old, were provided by the National Institute of Hygiene, Warsaw. They were used for the experiments and the routine maintenance of the tumour.

Tumour

The Ehrlich ascites carcinoma obtained from the Institute of Oncology, Warsaw, was maintained in our laboratory by weekly i.p. passage of tumour cell suspensions in CFW/L1 mice.

Tumour cell suspensions

Tumour cell suspensions were prepared as described previously [11], and 10^5 or 10^6 EAC cells suspended in 0.1 ml MEM were injected i.p. into recipient mice.

Transplantation of normal syngeneic spleen cells

Spleen cells from untreated donor mice were isolated mechanically as described pre-

viously [11]. They were washed twice in MEM and filtered through a 200-mesh stainless steel wire sieve. Cell viability, as estimated by trypan blue exclusion test, ranged between 90 and 94%. Cell suspension was adjusted to 3×10^8 cells/ml and 0.2 ml of the cell suspension was injected i.p. into the recipient mice 0.5 hr after EAC cell inoculation. This dose of spleen cells (6×10^7) was used in the previous study [11]. For each experimental group spleen cells from 10–15 mice were pooled together.

Autotransplantation of normal spleen cells

Mice were anaesthetized with chloral hydrate. Under aseptic conditions the vessels were ligated and the spleens were removed. Spleen cells were isolated mechanically. The abdominal cavity was closed with a single suture through all layers. Sham-operated mice underwent the same procedure, except that their spleens were not ligated or removed. Immediately after operation 10^5 or 10^6 EAC cells were given i.p. in 0.1 ml MEM and 0.5 hr later autologous spleen cells were injected i.p. in 0.2 ml MEM. At the same time sham-operated animals were given i.p. injection of 0.2 ml MEM.

Preparation of rabbit anti-EAC serum

Rabbits were immunized 5 times at 1-week intervals with washed EAC cells. The first 3 doses contained 10^8 cells and the last 2 doses contained 10^7 cells. Each dose was divided into 4 equal parts and injected s.c. and i.m. without adjuvant. The rabbits were bled 7 days after the last injection. The sera obtained from 3 rabbits were pooled and heat-inactivated. Antiserum was absorbed twice (1 and 12 hr) at 4°C with 20% v/v CFW/L1 spleen and kidney cells and stored at −20°C. Agglutination titer of this antiserum against EAC cells was 1:128. More than 50% of tumour cells were agglutinated at this dilution of serum within 1 hr [12]. Normal rabbit serum obtained from the intact rabbit was treated and stored in the same way as antiserum. Rabbit anti-EAC serum or normal rabbit serum was given i.p. in a volume of 0.3 ml 24 hr following the injection of EAC cells.

In vitro *test for natural and antibody-dependent cellular cytotoxicity.*

This was performed as previously described [11]. The ^{51}Cr labeled EAC cells were washed three times and incubated in 0.25 ml MEM + 10% FCS with effector spleen cells at 100:1 effector: target cell ratio for 6 hr in a 37°C, 5% CO_2 incubator. Effector spleen cells were isolated and pooled from 4 untreated mice. Anti-EAC or normal rabbit serum was added at a 10^2-fold dilution. Each test was performed with six replicates. ^{51}Cr release was measured in the supernatants and percentage cytotoxic activity calculated according to the formula:

$$\%\ ^{51}Cr\ \text{release} = \frac{\text{test release} - \text{background release}}{\text{maximal release} - \text{background release}} \times 100.$$

Background release was determined by incubating target cells with unlabeled EAC cells (7–15% of the total label) and maximal release by treating target cells with distilled water overnight (75–86% of the total label).

RESULTS

In the present study normal spleen cells showed low but significant antitumour activity in syngeneic and in autologous recipients against EAC cells injected in a dose of 10^5 cells per mouse (Table 1).

In vivo tumour growth was suppressed with the use of antiserum in 90% of mice injected with 10^5 and in 40% of mice injected with 10^6 EAC cells, within 50 days of observation (Table 1). Mean survival time (±S.E.) for mice in group 1 (control) injected with 10^5 or 10^6 EAC cells alone was 28.0±0.6 and 25.5 ±0.5 days, respectively.

Injection of normal spleen cells into syngeneic or autologous recipients of 10^6 EAC cells augmented the antitumour effect of antiserum and this combined treatment suppressed tumour growth in 90–100% of mice (Table 1).

In vitro experiments demonstrated natural cytotoxicity of normal spleen cells against EAC (Table 2). Analogously to the *in vivo* experiments, there was an increase in antitumour effect when normal spleen cells and anti-EAC serum were used simultaneously (Table 2).

DISCUSSION

EAC was chosen for our experiments because of its availability in all laboratories and its consistent growth characteristics. Despite strain nonspecificity, EAC may be used for studies on tumour immunotherapy [13] as immunity against EAC does not seem to be directed against H-2 antigens [14].

In vivo and *in vitro* antitumour activity of normal mouse spleen cells against EAC has

Table 1. Augmentation of in vivo *antitumour activity of rabbit antiserum by normal syngeneic or autotransplanted spleen cells*

Treatment of EAC recipients	Mice challenged with 10^5 EAC cells No. of mice dead of tumour growth/No. of mice tested*	Mice challenged with 10^6 EAC cells No. of mice dead of tumour growth/No. of mice tested*
	Different from group 1	Different from group 1
1. Medium only	20/20	20/20
2. Normal rabbit serum	12/12	Not tested
3. Syngeneic spleen cells	10/15 at $P<0.01$†	14/14
4. Anti-EAC serum	1/10 at $P<0.001$†	6/10 at $P<0.01$†
5. Syngeneic spleen cells +anti-EAC serum	0/12 at $P<0.001$†	0/12 at $P<0.001$† and from group 4 at $P<0.05$†
6. Autologous spleen cells	11/14 at $P<0.05$†	9/10
7. Autologous spleen cells +anti-EAC serum	1/10 at $P<0.001$†	1/11 at $P<0.001$† and from group 4 at $P<0.05$†
8. Sham operation	15/15	12/12

*All mice were inspected daily for 30 days.
†As determined by the chi-square test.

Table 2. Natural and antibody-dependent cellular cytotoxicity of mouse spleen against EAC cells in vitro

Target cells mixed with:	Percentage of ^{51}Cr release (±S.E.)
Spleen cells	7.4±1.8*
Anti-EAC serum	2.2±1.5
Spleen cells+ anti-EAC serum	23.1±1.6†
Spleen cells+ normal rabbit serum	7.3±2.1*

*Different from the background at $P<0.05$ as determined by Student's *t*-test.
†Different from natural cytotoxicity mediated by spleen cells alone at $P<0.001$ as determined by Student's *t*-test.

already been reported in our previous publication [11], in which the possible participation of natural killer cells (NKC) in the observed *in vivo* antitumour activity of normal spleen cells was discussed. Recently, direct evidence was obtained that NKC could prevent *in vivo* tumour growth in mice [15].

Suppression of tumour growth in an animal with the use of the antiserum against a tumour was observed by numerous investigators [1–6]. Recently, anti-EAC serum was reported to induce lysis of tumour cells *in vivo* within 30 min of antiserum injection [16].

In our present study we demonstrated the augmentation of *in vivo* activity of xenogeneic antiserum by transplantation of normal syngeneic lymphoid cells (Table 1). Our *in vitro* experiments suggest that ADCC may be a possible mechanism for the increased antitumour effect in mice treated with spleen cells in addition to anti-EAC serum (Table 2).

We proved that this strengthened antitumour effect could also be achieved with the use of autologous lymphoid cells (Table 1). The latter observation may extend application of this method in cancer immunotherapy. Nevertheless, before definite conclusions can be drawn from the above experiments, some questions need elucidation. Successful immunotherapy of ascites tumour may not necessarily apply to other types of tumours. In our experiments spleen cells for autotransplantation were removed from normal mice, although for better simulating cancer immunotherapy they should be removed from tumour-bearing animals. It should also be remembered that autotransplanted spleen cells may not resume their activity compared to that of normal spleen, and this may be the functional equivalent of splenectomy. In rats, however, splenic tissue autotransplanted in multiple fragments i.p. or s.c. demonstrated immune responses comparable to those of normal spleen [17].

Using antitumour antibodies it must not be forgotten that, if not successful, they may in certain situations enhance tumour growth [18, 19].

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