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Short Communication

Growth Inhibition of an MC-Induced Mouse Sarcoma by TCGF (IL 2)-Containing Preparations

Preliminary Report

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Summary. *Supernatants from Con A-stimulated rat spleen cell cultures containing T cell growth factor inhibited growth of a transplantable 3-methylcholanthrene-induced sarcoma in syngeneic mice. The tumour-inhibitory effects were dependent on the concentration of T cell growth factor and repeated injections of the supernatants.*

Introduction

T cell growth factor (TCGF) or interleukin 2 (IL 2) [1] has been defined as a class of potent immunoregulatory molecules [7, 8, 12] that stimulate continuous proliferation of activated T lymphocytes in culture [12, 13]; it has been implicated in T cell-dependent immune responses and shown to be effective in restoring immunologic deficiencies in vivo [2, 15].

Since T cell-mediated and T cell-dependent tumour-specific defence mechanisms have been demonstrated in a variety of experimental and human neoplasms [3, 5, 9–11], it can be assumed that injections of TCGF into tumour-bearing individuals may produce therapeutic effects by both amplification and activation of the available defence effector components.

To investigate whether this hypothesis is correct and whether the local administration of TCGF in vivo might be of immunotherapeutic value in the treatment of cancer, TCGF-containing supernatants from Con A-stimulated rat spleen cell cultures [12] were injected repeatedly into mice inoculated previously with cells of a transplantable syngeneic MC-induced sarcoma.

Materials and Methods

Mouse fibrosarcoma MC11 was induced in C57BL10/ScSnPh (B10) mice with 3-methylcholanthrene (MC) and characterized as described earlier [4]. The tumour was shown to carry tumour-associated transplantation antigen (TATA) and to grow in 100% of the inoculated syngeneic mice from a minimum dose of 10^2 cells [4].

Groups of 3-month-old B10 males received doses of 5×10^4 MC11 tumour cells SC at day 0 and were then given repeated injections of TCGF-containing or control preparations in 0.5-ml amounts of medium at days 1, 3, 5, 7, 9, 11, 13, 15, 17, and 19. The injections were given SC around the site of the tumour inoculum.

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Supernatants of Con A-stimulated rat spleen cell cultures (48 h incubation; 5 μg Con A/ml; 5×10^6 spleen cells/ml) were produced as proposed by Kendall Smith [12, 13]. The activity of TCGF in the supernatants was assayed by ^{3}H-Tdr incorporation into CTLL cells and expressed after probit analysis in units per millilitre [12–14]. The CTLL cell line of Gillis and Smith [6] was kindly provided by Dr K.-O. Grönvik, University of Uppsala, with the permission of Dr Kendall Smith. A sample of standard TCGF preparation provided by Dr K.-O. Grönvik, University of Uppsala, was assigned a value of 1.0 U/ml. The activity of five other TCGF preparations used was compared with that of this standard preparation. As control preparations, tissue culture medium (RPMI 1640 supplemented with 10% fetal bovine serum) incubated at 37° C for 48 h with and without 5 μg Con A/ml and supernatants from rat spleen cell cultures incubated in Con A-free medium for 48 h were used.

During growth of the tumours, mean tumour diameters were determined in the experimental and control groups twice a week. The latency periods of tumours and survival of tumour-bearing mice were also recorded. Mice showing no palpable tumours 100 days after inoculation of tumour cells were considered tumour-free. The significance of differences between tumour takes in the experimental and control groups was estimated by the usual chi-square test. The differences between survival time in the experimental and control groups were evaluated by Student's *t*-test.

Table 1. Therapy of mice carrying transplanted syngeneic MC-induced sarcoma MC11 with TCGF-containing supernatants from rat spleen cell cultures[a]

Treatment of mice	No. of mice with tumours/ total no. of mice	Survival of tumour-bearing mice in days (mean ± SE)
Medium alone	24/24	54.4 ± 3.3
Medium with Con A[b]	24/24	50.7 ± 2.9
Medium from Con A-free spleen cultures	23/23	56.6 ± 3.7
TCGF-containing medium[c]	18/23	70.1 ± 4.2*

[a] Spleens from 3-month-old male rats of inbred Lewis strain; this experiment was repeated four times with similar results
[b] 5 μg Con A/ml
[c] 1.78 TCGF *U*/ml
* $P < 0.01$; compared with medium from Con A-free cultures

Table 2. Therapy of MC11 sarcoma-bearing mice with supernatants from rat spleen cultures differing in TCGF concentration

Experiment no.	TCGF (U/ml)	Treatment of mice	No. of mice with tumours/ total no. of mice	Survival of tumour-bearing mice in days (mean ± SE)
1	0.75	TCGF	9/9	65.0 ± 8.9
		O[a]	10/10	47.9 ± 2.4
2	0.88	TCGF	10/10	54.8 ± 4.6
		O	10/10	42.9 ± 4.6
3	2.68	TCGF	6/13*	81.8 ± 4.7**
		O	14/14	62.6 ± 3.2
4	3.91	TCGF	2/10***	–
		O	10/10	73.2 ± 9.3

[a] Tissue culture medium without TCGF; other controls (medium with Con A; medium from Con A-free spleen cultures) gave similar results (see Table 1)

* $P < 0.01$

** $P < 0.005$

*** $P < 0.001$; 1×10^3 MC11 tumor cells were inoculated in this experiment

Results

Pooled supernatants from Con A-stimulated spleen cell cultures, containing 1.78 TCGF U/ml and injected repeatedly into recipients of MC11 tumour transplants during the latency period of tumour growth, delayed the appearance of tumours, decreased the percentage of tumour takes, and prolonged the survival of tumour-inoculated mice (Table 1). A single injection of the same TCGF-containing supernatant was without effect.

Treatment of tumour-bearing mice with TCGF-containing preparations differing in the concentration of TCGF revealed that TCGF concentrations higher than 1 U/ml were necessary to produce a significant tumour-inhibitory effect (Tables 1 and 2).

Discussion

Local administration of TCGF-containing preparations around the site of the growing tumour inoculum was shown to have a significant tumour-inhibitory effect. This effect was due neither to the lectin itself nor to the products of non-stimulated spleen cells. The possibility is not excluded that other factors, such as IL 1, were also present in the supernatants of Con A-stimulated spleen cell cultures and may have contributed to the effects observed. To prove that the inhibition of tumour growth was due to the presence of TCGF in the supernatants, purified TCGF preparations should be used. However, the correlation between the concentration of TCGF and the in vivo effects of the supernatants from Con A-stimulated spleen cell cultures indicates that TCGF may play an important role in the tumour-inhibitory effects observed. Two supernatants containing a subthreshold TCGF concentration (less than 1 U/ml) gave no significant inhibition of tumour growth, whereas three other supernatants with more than 1 U/ml inhibited tumour growth. Since T cell-mediated effector mechanisms are known to be important in the immune reaction directed against TATA of MC-induced murine sarcomas [5, 11] it is possible that the injections of TCGF into the area of the tumour-draining lymph nodes operate by selectively increasing the pool of tumour-activated T cells participating in the TATA-directed rejection reaction. To maintain a sufficient local concentration of TCGF in the tumour-draining lymph nodes, which are primarily involved in the early defence reaction, both local administration and repeated administration of TCGF are of importance. If the observed tumour-inhibitory effects appear to be due to the TCGF and to have more general validity, a new immunotherapeutic approach to the treatment of cancer based on the utilization of the immunoregulatory effects of lymphokines can be proposed.

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