Induction of TNF-like factor by murine macrophage-like cell line J774.1 on treatment with *Sarcophaga* lectin

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On stimulation with *Sarcophaga* lectin, the mouse macrophage-like cell line J774.1 secreted a factor like the tumor necrosis factor (TNF) into the culture medium. This factor was a protein with a molecular weight of 40000–45000, and was cytotoxic to L-929 cells, but not to normal embryonic fibroblasts. This factor was effective on both the ascites form and solid form of sarcoma 180 transplanted into ICR mice.

Tumor necrosis factor Sarcophaga *lectin* *Macrophage* *J774.1*

1. INTRODUCTION

Tumor necrosis factor (TNF) was first reported by Carswell et al. [1]. They found that it was induced in the serum of mice infected with bacillus Calmette–Guerin and subsequently injected with endotoxin. Subsequent studies showed that several human B-cell lines transformed by Epstein–Barr virus produced a similar factor and that its production was augmented by 4β-phorbol 12-β-myristate 13-α-acetate [2]. Since TNF has marked effects on solid tumors [1,3,4], it is worthwhile, from the clinical view point, to establish a procedure for reproducible production of TNF in large quantity.

This paper describes the production of a TNF-like factor by a murine macrophage-like cell line, J774.1 cells. These cells were stimulated with a lectin isolated from the hemolymph of *Sarcophaga peregrina* (flesh-fly) larvae, which among the various stimulants so far tested was the most effective for inducing production of this TNF-like factor. This lectin of *Sarcophaga* larvae is peculiar in that it appears in two different physiological states, injury of the body wall and pupation, in both of which phagocytic cells are supposed to be activated [5]. Pupation is a normal developmental process of holometabolous insects, in which larval tissues are extensively ingested by phagocytes, which suddenly start to recognize them as non-self for some unknown reason [6]. Therefore, lectins appearing in various animals at a specific developmental stage, in which elimination of unnecessary cells or tissues is progressing, may be useful for activating macrophages to produce a TNF-like factor.

2. MATERIALS AND METHODS

2.1. Induction of a TNF-like factor

Sarcophaga lectin was isolated as described before [5]. A murine macrophage-like cell line, J774.1, was cultured in RPMI-1640 medium supplemented with 10% fetal calf serum and antibiotics (100 units/ml of penicillin and 100 μg/ml of streptomycin). For induction of the TNF-like factor, the cells were grown in 6 cm tissue culture plates to a population of 4.2×10^6 cells/plate, and then the medium was changed to serum-free medium and 10 μg/ml of *Sarcophaga* lectin was added. After incubation for 24–48 h at 37°C under 5% CO_2 in air, the medium was collected for assay of cytotoxic activity.

2.2. Cytotoxicity assay

The cytotoxic activity of the TNF-like factor was assayed by measuring release of radioactivity from L-929 cells labeled with [^{3}H]thymidine. Wells contained 2.4×10^4 ^{3}H-labeled L-929 cells and 200 μl

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of MEM containing 10% fetal calf serum and serially diluted test samples. After incubation for 42 h at 37°C, percent lysis was calculated from the following equation:

$$\text{Percent lysis} = \frac{\text{Experimental count} - \text{Control count}}{\text{Total count} - \text{Control count}} \times 100$$

Total count: radioactivity in the supernatant after treating the cells with 0.5% sodium dodecyl sulfate. Control count: radioactivity released in the absence of test sample. One unit of cytotoxic activity was arbitrarily defined as the amount causing the release of 35% of the radioactivity under these conditions.

3. RESULTS

When the murine macrophage-like cell line J774.1 was treated with *Sarcophaga* lectin, significant cytotoxic activity was released into the medium. This cell line produced a certain basal level of cytotoxic activity without any stimulus, but the production was greatly enhanced in the presence of *Sarcophaga* lectin, as shown in fig.1. This activity was not detectable 3 h after addition of the lectin, but became detectable after 24 h and then increased almost linearly until at least 72 h.

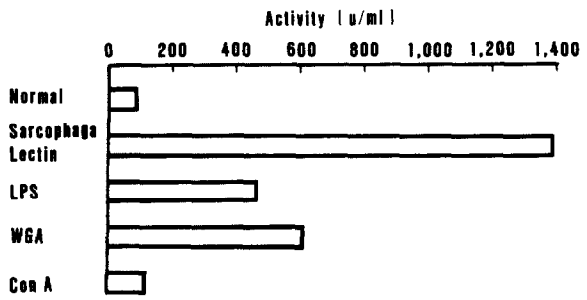

Fig.2. Effects of various stimulants on the production of TNF-like factor. J774.1 cells were cultured in the presence of various stimulants in medium containing 10% fetal calf serum. Each stimulant was added at the concentration causing maximum production of TNF-like factor (10 μg/ml). After 24 h incubation, the activity in the medium was determined.

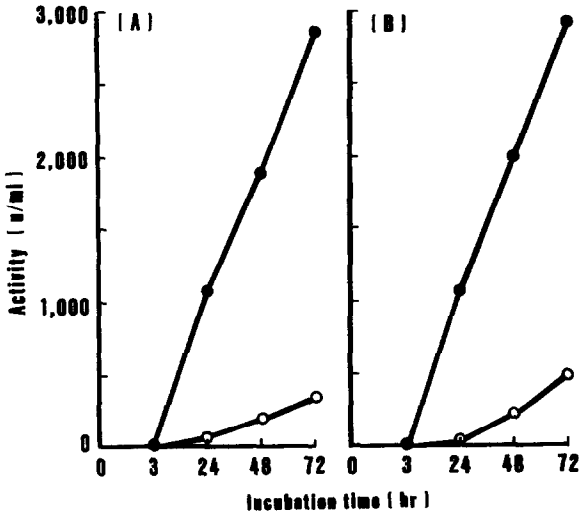

Fig.1. Time course of production of TNF-like factor. At time 0, 10 μg/ml of *Sarcophaga* lectin was added. Samples of medium were taken at intervals and their cytotoxic activity was compared with that of control medium containing no *Sarcophaga* lectin. (A) Production in serum-free medium; (B) production in medium containing 5% fetal calf serum. ●, with lectin; ○, without lectin.

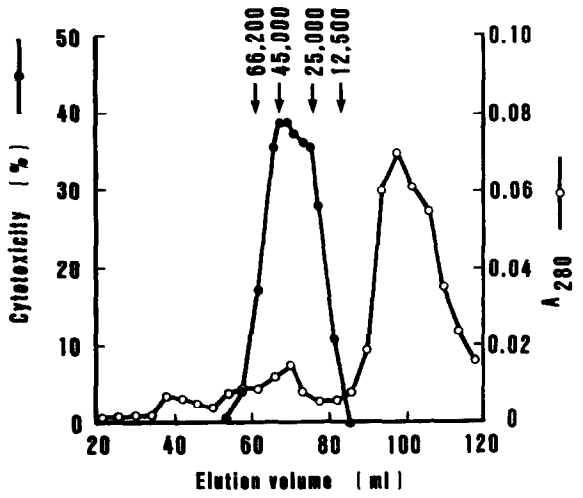

Fig.3. Elution profile of TNF-like factor from Sephadex G-200. Culture medium was dialysed against phosphate-buffered saline (PBS), lyophilized and applied to a column of Sephadex G-200 (1.6 × 60 cm) equilibrated with PBS. The column was calibrated with the following molecular weight markers: bovine serum albumin (66 200), ovalbumin (45 000), α-chymotrypsinogen (25 000) and cytochrome *c* (12 500).

Table 1

Effects of various treatments on the stability of the TNF-like factor

Treatment	Conditions[a]	Residual activity
Heat treatment	37°C	100%
	60°C	71
	95°C	0
Trypsin	control	100%
	0.1 mg/ml	20.3
	1.0 mg/ml	13.1

[a] Samples were heated for 30 min at the indicated temperatures, or treated with trypsin at the indicated concentrations for 30 min at 37°C

The effects of various stimulants on induction of TNF-like activity were examined. As evident from fig.2, LPS (lipopolysaccharide of *E.coli* 0111:B4) and WGA (wheat germ agglutinin) were effective, but their effects were much lower than that of *Sarcophaga* lectin. The induction by WGA was not detectable when serum-free medium was used, suggesting that in the absence of serum, WGA was toxic to J774.1 cells.

We partially purified this TNF-like factor by passing the lyophilized medium through a column of Sephadex G-200. Since the factor was induced

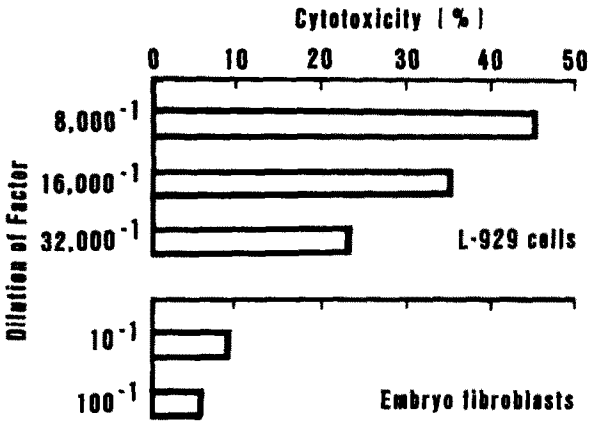

Fig.4. Specificity of cytotoxicity of the TNF-like factor. The preparation from the column of Sephadex G-200 was serially diluted and its effects on L-929 cells and embryonic fibroblasts prepared from ICR mouse embryo were examined.

Table 2

Effect of TNF-like factor on sarcoma 180 transplanted intraperitoneally into ICR mice

	Dose (units/mouse/day)[a]	Cured mice
PBS	–	0/9
TNF-like factor	60	1/6
	600	3/6

[a] Animals were treated with this dose of TNF-like factor three times on days 1, 2 and 3, and examined on day 30

in serum-free medium, the amount of contaminating protein in the medium was relatively small. As shown in fig.3, the molecular weight of the active fraction recovered from the column was estimated to be 40 000–45 000. The characters of the active fraction recovered from Sephadex G-200 were examined. As summarized in table 1, this factor is a protein, since its activity was lost on treatment with trypsin; moreover, 70% of its activity remained after heating at 60°C for 30 min, but all the activity was lost on heating at 95°C.

The cytotoxic effect of this factor on primary cultures of normal fibroblasts prepared from ICR mouse embryo was compared with that on L-929 cells. As shown in fig.4, normal fibroblasts were not sensitive to this factor, and the ratio of cytotoxicity on normal cells and transformed cells was more than 3000:1 when the same units of factor were applied to the same number of cells. Thus, it is conceivable that this factor is cytotoxic to transformed cells but not to normal cells. In this respect, this factor is quite similar to TNF [1,7].

Finally, we tested the effect of this substance on transplanted tumors. To test its effect on ascites

Table 3

Effect of TNF-like factor on solid sarcoma 180

	Cured mice	Tumor size (mean ± SD, mm)	Tumor weight[a] (mean ± SD, g)
Control	0/10	17.6 ± 7.0	2.51 ± 2.48
Treated	4/6	6.1 ± 8.7	0.60 ± 0.93

[a] Animals were treated with 526 units/mouse/day of TNF-like factor every other day from day 6 to day 20, and examined on day 30

tumor, we inoculated 1×10^5 sarcoma 180 cells into the abdominal cavity of ICR mice (male, 7 weeks old), and from the next day injected 60 or 600 units of TNF-like factor intraperitoneally once a day for 3 days. To test its effect on a solid tumor, we inoculated 2×10^5 sarcoma 180 cells intradermally into the right flank of ICR mice, and from 6 days later injected 526 units of TNF-like factor around the tumor every other days 8 times. Tumors were inspected 30 days after inoculation. As summarized in tables 2 and 3, this factor was effective on both the ascites and solid tumor. Significant necrosis of the solid tumor was detected the day after treatment with this factor.

4. DISCUSSION

In this work we found that *Sarcophaga* lectin greatly stimulated the production of a TNF-like factor by J774.1 cells. This finding is interesting from two points of view. One is the function of animal lectin induced humorally at a specific stage of development. Probably, such lectin generally stimulates phagocytic cells to scavenge unnecessary or unusual cells so that developmental processes proceed smoothly. The other is the function of this factor itself. Since this factor can be obtained in quantity, and our results suggested that it has significant antitumor activity, its characterization seems worthwhile from a therapeutic view point. Probably, a similar factor can be obtained when a certain subset of human macrophages is stimulated by *Sarcophaga* lectin.

REFERENCES

[1] Carswell, E.A., Old, L.J., Kassel, R.L., Green, S., Fiore, N. and Williamson, B. (1975) Proc. Natl. Acad. Sci. USA 72, 3666–3670.
[2] Williamson, B.D., Carswell, E.A., Rubin, B.Y., Prendergast, J.S. and Old, L.J. (1983) Proc. Natl. Acad. Sci. USA 80, 5397–5401.
[3] Kull, F.C. and Cuatrecasas, P. (1981) J. Immunol. 126, 1279–1283.
[4] Bloksma, N., Kuper, C.F., Hofhuis, F.M.A., Benaissa-Trouw, B. and Willers, J.M.N. (1983) Cancer Immunol. Immunother. 16, 35–39.
[5] Komano, H., Mizuno, D. and Natori, S. (1980) J. Biol. Chem. 255, 2919–2924.
[6] Richards, O.W. and Davis, R.G. (1977) in: IMMS' General Textbook of Entomology, 10th edn., vol. 1, pp. 234–247, John Willy and Sons, New York.
[7] Mannel, D.N., Meltzer, M.S. and Mergenhagen, S.E. (1980) Infect. Immun. 28, 204–211.