# Participation of tumor killing factor in the antitumor effect of *Sarcophaga* lectin

Akira Itoh, Fukuichi Ohsawa, Yoshiaki Ohkuma and Shunji Natori

*Faculty of Pharmaceutical Sciences, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan*

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Antibody against tumor-killing factor inhibited cytotoxic activities produced in vitro by mouse peritoneal macrophages and in vivo in the serum of tumor-bearing mice in response to *Sarcophaga* lectin. These results suggest that tumor-killing factor participates in the antitumor effect of *Sarcophaga* lectin.

(Sarcophaga, *J774.1 cell*) *Lectin* *Tumor-killing factor* *Macrophage*

## 1. INTRODUCTION

*Sarcophaga* lectin is a galactose-binding lectin purified from the hemolymph of *Sarcophaga peregrina* (flesh-fly) larvae [1]. Although this lectin itself has no cytotoxic activity, it showed a marked antitumor effect when administered to tumor-bearing mice [2]. Conceivably macrophages activated by this lectin and cytokines produced by immune cells in response to this lectin cooperatively construct a systemic defence mechanism that is effective for regression of tumors. In fact, we have shown that *Sarcophaga* lectin activates macrophages and has significant cytotoxicity on both syngeneic and allogeneic murine tumor cells in the presence of macrophages [3,4]. We have also shown that cytotoxic activity transiently appears in the culture medium of murine peritoneal macrophages after addition of *Sarcophaga* lectin [2]. Furthermore, we have observed that cytotoxic activity transiently appears in the serum of tumor-bearing mice, but not normal mice, after injecting *Sarcophaga* lectin [2].

On the other hand, we have described the induction of a tumor-specific cytotoxic protein by a murine macrophage-like cell line J774.1 on treatment with *Sarcophaga* lectin, and the purification of this protein, termed tumor-killing factor (TKF), to homogeneity [5,6].

This paper demonstrates that antibody against TKF inhibits the cytotoxic activities produced in vitro and in vivo in response to the *Sarcophaga* lectin, suggesting that TKF is one of the major cytokines participating in the antitumor effect of *Sarcophaga* lectin.

## 2. MATERIALS AND METHODS

### 2.1. Sarcophaga *lectin and TKF*

*Sarcophaga* lectin was purified as described before from the hemolymph of *S. peregrina* third instar larvae whose body wall had been pricked by a hypodermic needle 24 h previously [1]. TKF was purified from the culture medium of J774.1 cells treated with 5 $\mu$g/ml of *Sarcophaga* lectin for 48 h as described [6].

### 2.2. *Antibody against TKF*

Purified TKF (75 $\mu$g) was thoroughly mixed with 1 ml complete Freund's adjuvant and injected subcutaneously into a male albino rabbit. A booster injection of 75 $\mu$g of TKF in incomplete Freund's adjuvant was given two weeks later. The animal was bled one week after the booster injection, and the titer of the serum obtained was checked by immunoprecipitation of radioiodinated TKF.

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2.3. *Cytotoxicity assay*

Cytotoxic activity in the sample was assayed essentially as described by Drysdale et al. [7], with L-929 cells as targets. After treatment of target cells with the sample containing cytotoxic activity, viable cells were stained with crystal violet, and the percentage cytotoxicity was calculated from the amount of dye adsorbed to the remaining cells. Neutralization of cytotoxic activity by antibody against TKF was determined by incubating the sample with antiserum for 6 h at 4°C, and then measuring cytotoxic activity.

2.4. Sarcophaga *lectin-dependent macrophage-mediated cytotoxic reaction*

The procedure used was as described [4]. Peritoneal macrophages of ICR mice and $^{51}Cr$-labeled Ehrlich ascites tumor cells were incubated in the presence of 20 $\mu$g/ml of *Sarcophaga* lectin for 17 h at 37°C, and then the $^{51}Cr$ released from the target cells was measured. The effect of antibody against TKF on this reaction was examined by adding antiserum to the incubation mixture 30 min before the target cells.

## 3. RESULTS

Since antibody against TKF is now available, we examined the effect of this antibody on various cytotoxic activities that are produced in vitro and in vivo in response to the stimulus of *Sarcophaga* lectin and are supposed to participate in the antitumor effect of *Sarcophaga* lectin.

3.1. *Inhibition of cytotoxic activity produced by peritoneal macrophages*

As reported before, we found that mouse peritoneal macrophages produce cytotoxic activity when they are cultured in the presence of *Sarcophaga* lectin [2]. However, the patterns of production of cytotoxic activity by peritoneal macrophages and J774.1 cells are different. Namely, when J774.1 cells are stimulated by *Sarcophaga* lectin, the cytotoxic activity in the medium increases linearly for at least 72 h, whereas when peritoneal macrophages are stimulated, the cytotoxic activity increases to a maximum 1.5 h after addition of the lectin, and then rapidly decreases. We examined the cytotoxic activity produced by peritoneal macrophages by testing whether the antibody inhibited the activity in the culture medium of peritoneal macrophages.

As shown in fig.1, the cytotoxic activity in the culture medium of macrophages was almost completely inhibited by the antibody against TKF, whereas normal serum had no effect, suggesting that the cytotoxic activity produced by peritoneal macrophages in the presence of *Sarcophaga* lectin is due to the same protein as TKF.

3.2. *Inhibition of cytotoxic activity induced in the serum of tumor-bearing mice*

*Sarcophaga* lectin itself has been shown to have a significant therapeutic effect when administered to tumor-bearing mice [2]. We demonstrated that when sarcoma 180 cells were inoculated into the abdominal cavity 24 h before intraperitoneal injection of *Sarcophaga* lectin, significant cytotoxic activity transiently appeared in the serum 1.5 h after injection of *Sarcophaga* lectin, but that this activity was not detectable in the serum of control animals after injection of *Sarcophaga* lectin [2]. This activity was found to be inhibited by the antibody against TKF, as shown in fig.2. Thus, it is clear that TKF is transiently induced in the serum of tumor-bearing mice soon after the injection of *Sarcophaga* lectin.

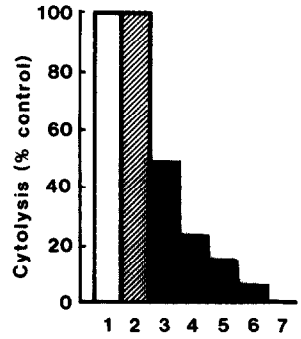


Fig.1. Inhibition by antibody against TKF of cytotoxic activity in the culture medium of peritoneal macrophages. Increasing amounts of antiserum were added to the culture medium of peritoneal macrophages containing 3.1 units of cytotoxic activity, and the remaining activity was assayed with L-929 cells as targets. Lanes: 1, control (without serum); 2, normal serum (3.2 $\mu$l); 3–7, 0.4, 0.8, 1.6, 3.2 and 6 $\mu$l antiserum, respectively.

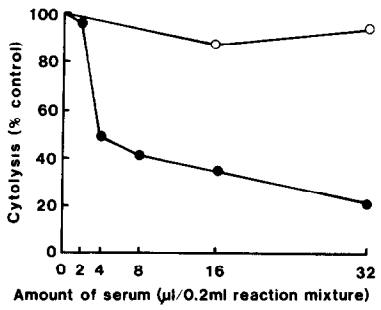


Fig.2. Inhibition by antibody against TKF of cytotoxic activity induced in the serum of tumor-bearing mice. Increasing amounts of antiserum were added to the serum of tumor-bearing mice treated with *Sarcophaga* lectin. The original cytotoxic activity of the serum was 14.2 units. Lanes: 1, control (without serum); 2, normal serum (3.2 μl); 3–7, 0.4, 0.8, 1.6, 3.2 and 6 μl antiserum, respectively.

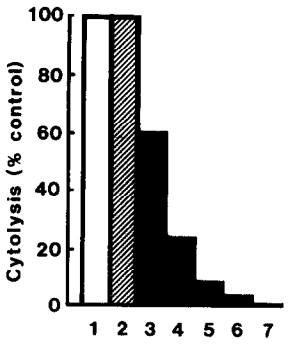


Fig.3. Effect of antibody against TKF on the *Sarcophaga* lectin-dependent macrophage-mediated cytotoxic reaction. Macrophages ($^{51}$Cr-labeled Ehrlich ascites tumor cells), *Sarcophaga* lectin, and various amounts of antiserum were incubated at 37°C for 17 h in RPMI 1640 medium, and then cytolysis of the tumor cells was measured. (○) Control serum, (●) antiserum.

### 3.3. *Participation of TKF in the* Sarcophaga *lectin-dependent macrophage-mediated cytotoxic reaction*

We have shown that *Sarcophaga* lectin exhibits macrophage-dependent cytotoxicity to both syngeneic and allogeneic tumors [3,4]. Namely, when either syngeneic MM46 tumor cells and C3H/He mouse macrophages, or Ehrlich ascites tumor cells and ICR mouse macrophages were incubated in the presence of *Sarcophaga* lectin, the target cells were lysed with time.

We tested the effect of antibody against TKF on the lysis of Ehrlich ascites tumor cells in this reaction. As shown in fig.3, antibody against TKF significantly and dose-dependently inhibited the lysis of Ehrlich ascites tumor cells, whereas normal serum had no effect on their lysis. Thus we conclude that TKF produced by peritoneal macrophages in culture in the presence of *Sarcophaga* lectin mainly participates in the lysis of target cells.

## 4. DISCUSSION

From the results obtained with antibody against TKF, we conclude that the cytotoxic activities detected in vitro and in vivo when peritoneal macrophages or tumor-bearing mice are treated with *Sarcophaga* lectin are due to immunologically the same protein as TKF, which is a cytotoxic protein produced by J774.1 cells in response to *Sarcophaga* lectin. Therefore, one of the major cytokines participating in the antitumor effect of *Sarcophaga* lectin is TKF. Since TKF has a similar amino acid composition to that of tumor-necrosis factor (TNF-α), TKF may be TNF-α itself or a related protein.

Since *Sarcophaga* lectin alone is sufficient to induce TKF, it may be important to investigate how the stimulus from this lectin is transmitted to the macrophage nucleus to activate the TKF gene selectively. Recently, we identified three proteins with affinities to *Sarcophaga* lectin in the macrophage membrane; these proteins have molecular masses of 170, 110 and 55 kDa, respectively [4]. Probably, some signal that is essential for subsequent activation of the TKF gene is created in situ when *Sarcophaga* lectin binds to one of these proteins. As this is a relatively simple system, it may be possible to use it to analyze the mechanism of selective activation of the TKF gene by *Sarcophaga* lectin.

We demonstrated that most of the *Sarcophaga* lectin-dependent macrophage-mediated cytotoxicity is due to TKF. However, there also seems to be another unknown cytotoxic mechanism, since about 20% of the cytotoxicity was not inhibited by the antibody against TKF even at higher doses, as shown in fig.3.

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