

Induction of human γ interferon by *Sarcophaga peregrina* humoral lectin

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Humoral lectin isolated from the hemolymph of injured *Sarcophaga peregrina* (flesh-fly) larvae was found to activate human peripheral blood cells to produce interferon activity. This interferon was inactivated by dialysis against a solution of pH 2.0 and by heat treatment at 56 °C for 30 min, indicating that it was a γ interferon. The role of this lectin in the defence mechanism is discussed from the viewpoint of comparative immunology.

Sarcophaga lectin γ Interferon Peripheral blood leukocyte T-cell Comparative immunology

1. INTRODUCTION

We reported the purification of a galactose-binding lectin from the hemolymph of *Sarcophaga peregrina* (flesh-fly) larvae [1]. A striking feature of this lectin is that it is induced under two different physiological conditions in which the defence system is supposed to be activated: namely, injury of the body wall and pupation. When the body wall of the larvae is injured with a hypodermic needle, the chance of invasion of pathogens should increase, while at pupation, most larval tissues are decomposed and the resulting tissue fragments are scavenged by phagocytes, which recognize them as non-self at this stage.

Since this lectin seems to participate in activation of the defence system of *S. peregrina*, we are interested in its effect on immune cells of higher organisms from the viewpoint of comparative immunology. Therefore, we examined its effect on mouse macrophages and polymorphonuclear leukocytes and found that it activated these cells to be cytotoxic to syngeneic tumor cells in vitro [2,3]. We found that it also activated the killing activity

of mouse bone marrow cells on *Candida parapsilosis* in vitro [4]. These results strongly suggested that this lectin has the unique ability to activate mammalian immune cells.

We describe the induction of γ interferon by *Sarcophaga* lectin using T-cells prepared from human peripheral whole blood. This seems to be the first case of stimulation of the production of γ interferon in vitro by a lectin of animal origin.

2. MATERIALS AND METHODS

2.1. *Sarcophaga lectin*

Sarcophaga lectin was isolated as described previously [1] from the hemolymph of third instar larvae of *S. peregrina* whose body wall had been pricked with a hypodermic needle. About 1 mg of pure lectin was routinely obtained from 120 ml hemolymph.

2.2. Cells

Peripheral blood leukocytes (PBL) were separated from human peripheral whole blood on a Ficoll-hypaque gradient as described in [5]. T-rich lymphocytes were prepared from PBL by passing the cells through a nylon wool column according to the method of [6]. FL cells were main-

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tained in minimum essential medium supplemented with 5% calf serum.

2.3. Interferon induction

PBL or T-rich lymphocytes were suspended at a final concentration of 5×10^6 cells/ml in RPMI-1640 medium containing 10% heat-inactivated fetal calf serum. They were then incubated with various amounts of *Sarcophaga* lectin at 37°C in a humidified atmosphere of 5% CO₂ in air for an appropriate period. The cells were then removed by centrifugation and the interferon activity of the resulting supernatant was measured.

2.4. Interferon assay

Interferon activity was titrated by a cytopathic inhibition microassay method using FL cells and Sindbis virus, as described in [7]. Standard human leukocyte interferon from NIH was used as a reference and titers were expressed in standard international reference units.

3. RESULTS

3.1. Induction of interferon by *Sarcophaga* lectin

Induction of interferon in the culture medium of human PBL by a final concentration of 0.1 µg/ml of *Sarcophaga* lectin was examined with time. As shown in fig.1, the effect of lectin on induction of interferon was not obvious until day 2, but the interferon titer was significantly higher than that in control culture medium on days 3 and 4, indicating that *Sarcophaga* lectin enhances the production of interferon at a relatively late stage. This is quite different from previous results on the induction of interferon by poly I:poly C showing that the interferon titer reached a saturation level in about 12 h when heated poly I:poly C was added to the same culture medium [8].

Next, the dose-dependence of the induction of interferon by the lectin was investigated by measuring the titers in culture media on day 4. As is evident from fig.2, the interferon titer increased with the amount of lectin up to 0.5 µg/ml. However, more than 1 µg/ml was inhibitory, probably because of its toxic effect. In all these experiments, significant levels of interferon activity were produced even in the absence of *Sarcophaga* lectin. This may reflect the activity of some mitogenic substance in fetal calf serum. Therefore,

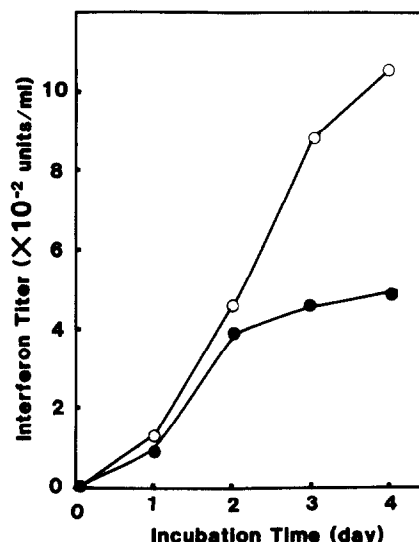


Fig.1. Induction of interferon by *Sarcophaga* lectin. PBL (5×10^6 cells/ml) were cultured in the presence or absence of 0.1 µg/ml of *Sarcophaga* lectin. Samples were taken at intervals and the interferon activity in the culture medium was measured. (○—○) Medium of lectin-treated cells; (●—●) control medium.

in order to assess the capacity of *Sarcophaga* lectin to induce interferon activity, we cultured PBL in serum-free medium. As shown in table 1, significant interferon activity was induced when PBL were cultured for 60 h in the presence of 0.1 µg/ml of *Sarcophaga* lectin. The viability of the cells in serum-free medium at this time was 46% and that in the presence of *Sarcophaga* lectin was 26%, as judged by the Trypan-blue exclusion test. Therefore, we concluded that *Sarcophaga* lectin has ability to induce interferon activity.

3.2. Identification of the induced interferon

The effect of *Sarcophaga* lectin on the induction of interferon activity depended upon the sample of PBL used. Only some of the cells were sensitive to the lectin. Therefore, we fixed the amount of lectin and the incubation conditions for induction of interferon at 0.1 µg/ml and 60 h at 37°C in the presence of 10% fetal calf serum, respectively, and examined the pH and temperature sensitivities of the interferon activity induced. Since it was difficult to obtain a constant background level, we simply subtracted the background activity in

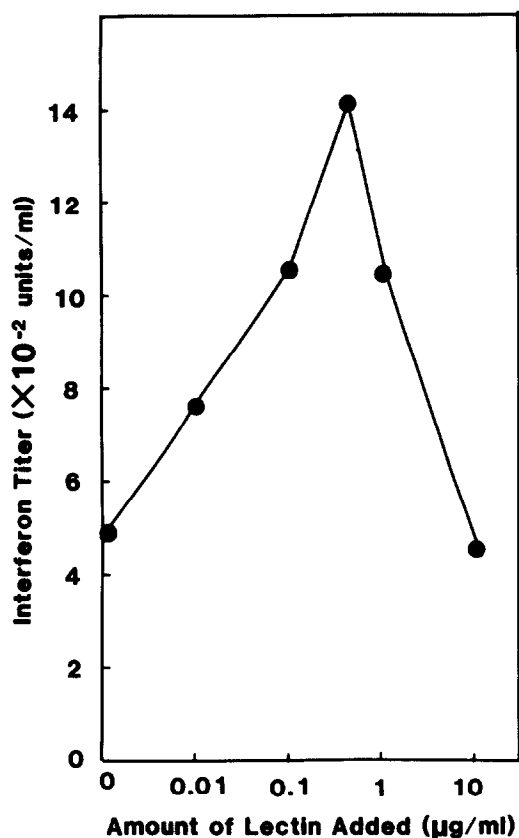


Fig.2. Dose-dependence of induction of interferon. *Sarcophaga* lectin was used at concentrations of 0 to 10 µg/ml, and the interferon activity in the culture medium was assayed on day 4.

calculating the net interferon activity induced by *Sarcophaga* lectin.

The interferon activity induced using three different blood samples was completely inactivated by dialysing the culture medium first against 0.1 M

Table 1

Interferon induction with three blood samples

Amount of lectin (µg/ml)	Interferon titer (units/ml)		
	A	B	C
0	<6	<6	<6
0.1	120	290	165

Interferon activity was induced by incubating the PBL for 60 h at 37°C in serum-free medium in the presence or absence of 0.1 µg/ml of *Sarcophaga* lectin

Table 2

Acid- and heat-sensitivities of induced interferon

Sample	Residual interferon activity (units/ml)			
	Control	Treatment:		
		pH 7.0	pH 2.0	56°C
D	550	450	<6	<6
E	320	190	<6	<6
F	790	550	<6	<6

Acid-sensitivity was tested by dialysing culture medium containing interferon activity first against 0.1 M KCl-HCl solution (pH 2) or phosphate-buffered saline (pH 7) for 24 h at 4°C, and then against minimum essential medium to adjust the pH to 7.4. Heat sensitivity was tested by heating the culture medium at 56°C for 30 min. After these treatments, residual activity was measured

KCl-HCl solution, pH 2.0, for 24 h at 4°C and then against RPMI-1640 medium to adjust the pH to 7.4, but it was stable on dialysis of the medium against phosphate-buffered saline, pH 7.0, as shown in table 2. The interferon activity was also inactivated when the culture medium was heated at 56°C for 30 min. These results strongly suggest that the interferon induced by *Sarcophaga* lectin was γ interferon.

It is known that γ interferon is synthesized by T-cells. Therefore, to confirm that T-cells actually responded to *Sarcophaga* lectin, we fractionated PBL on a nylon wool column and collected the T-rich lymphocyte fraction. The proportion of E-

Table 3

Effect of *Sarcophaga* lectin on T-rich lymphocytes

Lectin	Interferon activity (units/ml)		
	Blood sample:		
	G	H	I
-	630	310	890
+	1410	1120	2820

PBL were passed through a nylon wool column. The effect of *Sarcophaga* lectin on non-adherent cells was examined

rossette forming cells in the non-adherent cells was more than 90%, indicating that the non-adherent cells were enriched in T-cells. As shown in table 3, the T-rich lymphocytes clearly responded to *Sarcophaga* lectin and produced interferon activity, indicating that γ interferon was induced under these conditions.

4. DISCUSSION

We demonstrated that production of γ interferon by human peripheral blood cells was stimulated by a lectin isolated from the hemolymph of injured *S. peregrina* larvae. This lectin has been shown to participate in the removal of sheep red blood cells injected into the abdominal cavity of *S. peregrina* larvae (in preparation), and the same protein was found to activate murine immune cells in various ways [2-4]. Therefore, conceivably, there is a defence mechanism mediated by this kind of humoral lectin in both invertebrates and vertebrates. In vertebrates, it may be active only in the early stage of ontogeny and become dormant during development, being replaced by an immune network, whereas in invertebrates such as insects, it may be conserved throughout life. The fact that *Sarcophaga* lectin stimulated induction of human γ interferon indicates that human T-cells have a receptor for this lectin. Probably, the receptor remains active, even when production of such lectin has stopped.

This is the first report that an animal lectin

stimulates the production of human γ interferon. It is not certain whether *S. peregrina* larvae produce γ interferon, but it is possible that this lectin activates their hemocytes to produce an interferon-like substance to prevent infection when their body wall is injured.

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