INTERFERON INDUCTION BY AN ANTITUMOR ANTIBIOTIC, LYMPHOMYCIN

Sir:

Lymphomycin, an antitumor antibiotic produced by *Streptomyces* sp. S-66, is an acidic, black-colored protein with a molecular weight of 11,000. Chemical and biological characteristics of this antibiotic were described in our previous reports[1,2]. A marked antitumor activity of lymphomycin was demonstrated in a limited number of tumors of both ascites and solid forms in mice. Among solid tumors, lymphatic leukemia SN-36 was found to be very sensitive to this antibiotic, whereas EHRLICH and BASHFORD 63 carcinomas were relatively resistant. The antibiotic showed an apparent cytotoxic effect on BURKITT lymphoma cells (P_3HR-1), human lymphoblastoid cells (NC-37) and mouse peritoneal macrophages at the concentrations of 5~10 mcg/ml, whereas no such effect was found in HeLa and L cells in monolayer culture and mouse adenocarcinoma FM 3 A cells in suspension culture, even at the concentration of 100 mcg/ml. These results obtained both *in vivo* and *in vitro* suggested a selective activity of lymphomycin on lymphocytes or lymphoblastoid cells. Further study conducted recently revealed the interferon-inducing activity of the antibiotic both *in vivo* and *in vitro*, and the results obtained will be reported in this communication.

The *dd* strain of mice weighing 18~20 g and young adult white rabbits weighing 1.0~1.2 kg were used for interferon induction. Indiana strain of vesicular stomatitis virus (VSV), which was propagated in RK-13 cells, was employed as a challenge virus for interferon assay. Poly I : C[3] purchased from Miles Laboratories (U. S. A.), and double-stranded RNA isolated from mushroom spores[4] were used as standard inducers. For the interferon induction and assay, a minor modification of LAMPSON's procedure was followed[5]. Briefly, mice or rabbits were injected intravenously with lymphomycin and poly I : C, and 2~6 hours later serum specimens were taken for antiviral assay. Assays for antiviral activity of rabbit sera were carried out with RK-13 cells grown in EAGLE's MEM supplemented with 10% bovine serum at 37°C for 3 days. After overnight incubation with appropriately diluted serum specimens in a maintenance medium (EAGLE's MEM with 2% bovine serum), the cultures were washed with HANKS' balanced salt solution, and challenged with 100 TCD_{50} of VSV. Thymidinekinase-less mutant strain of L cells (L-1D) was used for the assay of mouse-induced interferon by the same procedures. Reciprocal of the serum dilution which induced a definite inhibition of the viral cytopathic effect was taken as an interferon titer.

In vitro induction of interferon was carried out as follows: Primary culture of embryonic rabbit kidney cells and RK-13 cells grown in the 10% calf serum-MEM medium for 3 days at 37°C were treated for 18 hours with inducers dissolved in a maintenance medium, and induction of cellular resistance to viral infection was measured as described above. For the tests of host-species specificity of induced interferon, primary chick embryo cells cultured in EARLE's balanced salt solution with 5% lactalbumin hydrolysate and 5% bovine serum (SLE medium) were used. At the time of interferon assay, the culture medium was replaced by HANKS' balanced salt solution with 5% lactalbumin (LH medium) containing 2% horse serum.

Interferon induction by lymphomycin was first studied in rabbits by injecting 16 mg/kg concentration intravenously and the antiviral

Fig. 1. Kinetics of serum interferon induction after single injection in rabbits.

Sixteen mg/kg of lymphomycin and 0.4 mg/kg of poly I : C were dissolved in 1/10 M phosphate buffered saline and injected intravenously. Interferon titer of rabbit sera was measured as described in text.

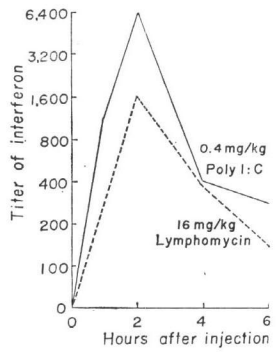

Fig. 2. Kinetics of serum interferon induction after single intravenous injection in mice.

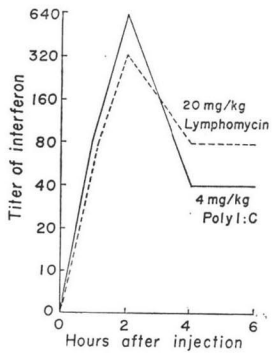

Table 1. Effect of heat, pH and trypsin on the activity of rabbit serum interferon[1]

Treatment		IF titer/ml	% Loss of activity
Temperature[2] (1 hr, pH 7.0)	22°C	1,600	
	37	1,600	0
	47	800	50
	56	200	88
	66	100	94
pH[3] (1 hr, 23°C)	1.0	< 100	>94
	2.1	1,600	0
	7.2	1,600	
	9.9	400	75
Trypsin[4] (3 hrs, 37°C)	0 mcg/ml	120	
	100	60	50
	1,000	<60	>50

1) Interferon samples were prepared as serum of rabbits which were injected with 16 mg/kg dose of lymphomycin.

2) Resistance to heat was determined by exposing sera to each temperature between 22°C and 66°C and for 1 hour at neutral pH.

3) Dialysis against 0.1 M KCl-HCl buffer (pH 1.0 and pH 2.1) and 0.1 M borax-NaOH buffer (pH 9.9), were conducted at 4°C for 24 hours, and were followed by dialysis against phosphate buffered saline (pH 7.2) for 24 hours after incubation for 1 hour at 23°C.

4) Serum samples containing each concentration of trypsin were incubated at 37°C for 3 hours.

activity of serum specimens was compared with that induced by 0.4 mg/kg of poly I : C. As shown in Fig. 1, on the administration of lymphomycin, 2-hour type interferon (so-called "preformed") was produced similar to poly I : C, although interferon titer obtained with lymphomycin was lower than that of poly I:C. Lymphomycin also induced interferon in mice (Fig. 2). Twenty mg/kg dose, which corresponds to one fourth of mouse LD_{50}, stimulated a production of the circulating interferon with the peak titer at 2 hours.

Rabbit serum specimens were tested for the biochemical properties of interferon, such as heat and pH stabilities, trypsin sensitivity, isoelectric point and molecular weight (Table 1). The antiviral activity was sensitive to the tryptic digestion and resistant to low pH (2.0, 1 hour) and heat (37°C, 1 hour). Isoelectric point determined by electro-focusing method was 6.8 and the molecular weight, between 65,000 and 120,000 by gel filtration. High degree of species specificity of the antiviral activity was also found (Table 2). These characteristics of lymphomycin-induced interferon were shared with the interferon induced by poly I:C.

In vitro induction studies were carried out using primary rabbit kidney and RK-13 cells

Table 2. Species specificity of serum interferons induced in animals by lymphomycin[1]

Specimens obtained from	Interferon titer[2]		
	RK-13	L-1D	Chick embryo
Rabbit	1,600	<10	<10
Mouse	<10	320	<10

1) Sixteen mg/kg (mouse) and 20 mg/kg (rabbit) dose of lymphomycin were injected intravenously.

2) Titer was measured by reciprocal of the serum dilution which gave a definite protection against a challenge of 100 TCD_{50} of VSV.

Table 3. Interferon induction in cell cultures with poly I : C, mushroom spore-RNA and lymphomycin

Inducers	Chemical nature	MIC[1] in mcg/ml	
		on RK-13	on PRKC[2]
Poly I:C	Double-stranded RNA	0.01	0.001
Spore-RNA	Double-stranded RNA	>100	0.03
Lymphomycin	Protein	>100	0.8

1) Minimum interference inducing concentration.

2) Primary culture of rabbit kidney cells.

(Table 3). In RK-13 cells, poly I : C at the concentration of 0.01 mcg/ml stimulated the production of interferon, whereas mushroom double-stranded RNA and lymphomycin failed to induce at 100 mcg/ml concentration. In the primary culture of rabbit kidney cells, where poly I : C and mushroom double-stranded RNA induced interferon at the concentrations of 0.001 mcg/ml and 0.03 mcg/ml, respectively, lymphomycin, induced the interferon at the dose of 0.8 mcg/ml.

In summary, an antitumor antibiotic, lymphomycin, was characterized not only by the selective inhibitory effect on the growth of lymphatic cells but also by interferon induction as reported here. Further chemical characterization is in progress in the light of polyanionic structure of known inducers[6].

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