Induction of Interferon in Perfused Whole Rat-Lung Cultures[1] (37612)

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Inducers of interferon can be divided into two general groups. One group, which includes both the viruses and Poly I·Poly C, will induce interferon either in cell culture or in the intact animal (1, 2). The other group includes bacterial endotoxin and the synthetic polyanion, pyran copolymer, and induces interferon efficiently *in vivo* but with rare exception fails to induce interferon in cell culture (3). Since pretreatment of an animal with either actinomycin D or an inhibitor of protein synthesis will generally prevent the induction of interferon by inducers of the first group but usually not of the second group, it is believed by some that the latter act by effecting the release of preformed interferon while the former acts by inducing *de novo* synthesis of interferon.

With the availability of continuously perfused organ cultures (4) it becomes of interest to determine whether organs maintained in such a manner will 1) produce interferon; 2) respond to interferon inducers of both groups; and 3) lend themselves to the study of differences in mechanism of action of the two groups.

Materials and Methods. Organ perfusion system. The left lungs of 200-g white male rats (Charles River Laboratories, Wilmington, Massachusetts) which had been cesarean delivered and barrier sustained, were removed and the respective pulmonary arteries cannulated (4). Each cannulated lung was inserted into an organ perfusion chamber which was maintained in series with a pulsatile flow pump and a coil oxygenator supplied with a gas mixture of 5% CO_2 and 95% air. The organs were incubated at 35° and, until the interferon induction experiment on the following day, maintained on pulsatile perfusion with Eagle's basal medium supplemented with 2% fetal calf serum and potassium penicillin G, 100 units/ml (supplemented Eagle's medium).

Inducers of interferon. Poly I·Poly C (Microbiological Associates, Bethesda, Maryland) was used in concentrations of 10 μg/ml or 100 μg/ml in PBS with and without diethylaminoethyl–dextran (DEAE–D, mol wt ca. 2,000,000, Pharmacia, Uppsala, Sweden) 100 μg/ml. Sindbis virus, strain Ar-339 obtained from T. E. Frothingham, was used in a concentration of 1.6×10^8 PFU/0.5 ml. Pyran copolymer (lot XA-124-177, Hercules Incorporated, Wilmington, Delaware) was administered in concentrations of 20,000 and 500 μg/ml. Endotoxin (aqueous phenol extract of Salmonella typhosa 0901 prepared by Jon Rudbach) was used in concentrations of 25 and 2500 μg/ml.

Induction of interferon. The organ chamber was disconnected from the coil oxygenator and perfused with 30 ml of phosphate buffered saline (PBS) containing 0.01% calcium chloride and 0.005% magnesium chloride for 30 min. Then the organ was either perfused with 30 ml of a solution of a non-virus interferon inducer for 30 min or inoculated with Sindbis virus and the perfusion pump shut off for 30 min. The lungs were then perfused for 30 min with supplemented

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Eagle's medium and the organ chamber drained. The organ was perfused thereafter with oxygenated supplemented Eagle's medium. Samples for interferon assay were routinely collected after intervals of 6 and 24 hr. During induction by virus, samples were also collected at 3 hr and daily for 6 days.

Interferon assay. Plaque reduction assay was carried out on 72 hr confluent monolayers of rat-lung fibroblasts (3rd–10th passage of primary fibroblasts trypsinized from adult rat lungs). Monolayers were exposed to test samples diluted in Leibovitz medium supplemented with 20% fetal calf serum, 0.03% glutamine, 0.09% arginine, 0.01% glucose, 150 μg/ml of potassium penicillin G and 250 μg/ml of streptomycin sulfate for 18 hr. At that time the cells were washed twice, challenged with 100 PFU of vesicular stomatitis virus (5) and handled in the usual way for plaque assay (6). Plaques were counted at 48 hr and one interferon unit was defined as the amount which reduced the number of plaques by 50% when compared to the count in untreated monolayers.

Samples to be assayed for virus-induced interferon were dialysed for 24 hr *vs.* 0.2 *M* KCl at pH 2.0 and then for 24 hr *vs.* 100 vol of Geys balanced salt solution at pH 7.4.

Virus titration. Sindbis virus titrations were carried out on monolayers of primary chick-embryo fibroblasts by the plaque technique (7).

Results. Perfusates of rat lung organ cultures exposed to Poly I·Poly C in concentrations of 2.0, 20.0, or 40.0 μg/ml had no detectable interferon at 6 and 24 hr. On the other hand, when perfused lungs were exposed to 10 μg/ml of Poly I·Poly C in the presence of 100 μg/ml of DEAE–D, interferon was detected with titers of 28 and 39 units/2.0 ml at 6 and 24 hr, respectively (Table I). When the organ was exposed to Poly I·Poly C, 100 μg/ml, and DEAE–D, 100 μg/ml, the interferon titer was only 4 units/2 ml at 6 hr and 13 units/2 ml at 24 hr.

In order to determine whether the interferon was produced *de novo* or merely released, perfused rat lungs were treated with Poly I·Poly C, 10 μg/ml, and DEAE–D, 100 μg/ml, after a 30 min exposure to PBS or a solution of actinomycin D, 2 μg/ml, followed by a 30 min wash with PBS. The resulting interferon titers of the perfusate at 6 and 24 hr were, respectively, 30 and 34 units/2 ml following PBS and 18 and 16 units/2 ml following actinomycin D (Table I).

In the perfusates of rat lungs infected with Sindbis virus, interferon was not detected at 24 hr, when the peak virus titer was reached (Fig. 1). However, over the following 24 hr, the titer of interferon rose to 89 units/2 ml.

Treatment of perfused rat lungs with inducers usually effective only *in vivo* was not associated with significant interferon production. No interferon was detectable in perfusates of organs exposed to endotoxin, 25 or 2500 μg/ml, or 20 μg pyran copolymer/ml and only 3 units interferon/2 ml was detected at 6 and 24 hr after the exhibition of 100 μg pyran copolymer/ml.

Discussion. These experiments demonstrate

TABLE I. Induction of Interferon by Poly I • Poly C in Perfused Rat Lungs.

Pretreatment	Final treatment		Interferon	
	Poly I • Poly C (μg/ml)	DEAE–D (μg/ml)	6 hr	24 hr
None	10.	0	0[a]	0
None	10.	100.	28	39
None	100.	100.	4	13
PBS	10.	100.	30	34
Actinomycin D 2 μg/ml	10.	100.	18	16

[a] Units per 2 ml.

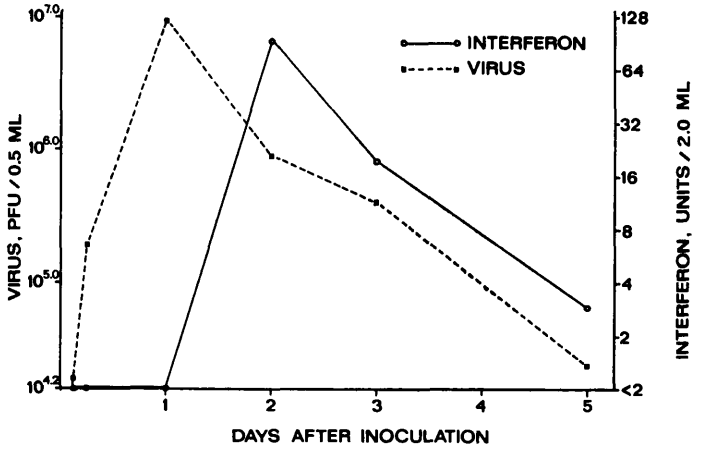

FIG. 1. Titer of interferon and virus in perfusion fluid following a 30 min exposure of rat lung to Sindbis virus and the subsequent removal of unadsorbed virus.

the production of interferon by perfused lungs which have been exposed to a variety of stimuli. This phenomenon may be exploited as a marker of viral growth in perfused organs or as an index of the viability of perfused organs. It may also be utilized to explore interferon responses of an intact isolated organ to a variety of stimuli.

The mechanisms of action of inducers of interferon effective in both cell cultures and the intact animal and of those inducers which act only *in vivo* are difficult to establish in the living animal. Differences in mechanism of interferon induction might be better characterized if both types of inducer were effective in the controlled conditions of the continually perfused organ culture. The latter partially simulates the anatomic relationships, blood gas pressures, pulsatile flow of nutrients, and continuous removal of wastes manifested by tissues *in vivo* (4).

In perfused whole rat lung cultures, interferon was induced by Sindbis virus and Poly I·Poly C combined with DEAE–dextran, both of which are known to be effective in cell culture and *in vivo*. Pyran copolymer and endotoxin, which usually are effective only in the intact animal, failed to induce interferon in organ cultures. The induction of interferon with Poly I·Poly C was only partially inhibited by pre-treatment of the organ culture with actinomycin D, 2 μg/ml. A similar finding has been reported in cell cultures of human skin fibroblasts using actinomycin D in a concentration of 1 μg/ml (3), and suggests the possibility of a significant difference between Poly I·Poly C and virus with respect to the induction of interferon.

Under our experimental conditions the responses of rat lungs to inducers of interferon differs from the response of intact animals. However, there is evidence that the lung can release interferon in response to endotoxin under conditions in which the animals were inoculated and the lungs then removed and incubated *in vitro* (personal communication from M. Ho, Y. H. Ke, and J. A. Armstrong, August, 1972). Thus, it appears that in the present study the *in vitro* conditions may have been inadequate to demonstrate an *in vivo* function of the lung or that some permissive role played by the intact animal for interferon release after endotoxin stimulation was lacking under our experimental conditions.

Poly I·Poly C induced interferon in perfused rat-lung cultures only in the presence of polycation. Inability to induce interferon in the absence of polycation has occasionally been found for Poly I·Poly C in cell culture and enhancement of the biological function by a polycation has been described (8). It is of interest that, as has also been found in cell culture, the concentration ratio of polycation to Poly I·Poly C was important (J. G. Tilles, unpublished). Thus, I:C at 10 μg/ml, with a concentration ratio of DEAE–D to I:C of 10, was more effective

in the induction of interferon than I:C at a concentration of 100 μg/ml with a ratio of DEAE–D to I:C of 1. Although the significance of this observation is not completely understood, it appears that a loss of effectiveness of Poly I·Poly C may occur through particle formation when the concentration ratio approaches 1.

Summary. Perfused rat-lung cultures responded to inducers of interferon in a manner similar to that of cell cultures. Interferon was not detected in perfusion fluid after the exhibition of endotoxin and pyran co-polymer, but high titers of interferon were found after inoculation with Sindbis virus. Poly I·Poly C combined with DEAE–dextran stimulated interferon production which was inhibited partially by actinomycin D pre-treatment.

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