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PRODUCTION OF INTERFERON AND OTHER LYMPHOKINES DURING MURINE TUMOUR GROWTH. II. INDUCTION OF AN INTERFERON BY EHRLICH ASCITES CELLS IN OUTBRED MICE

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Summary. — A viral inhibitor produced in the peritoneal cavity of outbred Swiss mice inoculated with allogeneic Ehrlich ascites cells was identified as an interferon. It was antigenically related to viral interferons of type 1 and seems to be produced by macrophages. The interferon activity in the peritoneum reached maximal levels at 24 hr after tumour challenge. Interferon induction by Ehrlich ascites cells was not inhibited in lipopolysaccharide-hyporeactive mice. In addition, cell growth inhibition, enhanced interferon production after priming with small doses of this interferon, and increased susceptibility of cells treated with this interferon to the toxic effect of double-stranded RNA was demonstrated.

Key words: *allogeneic cells*; *interferon induction*; *interferon properties*

Introduction

The appearance of antiviral activity in response to tumour cells was demonstrated in different cell systems both in vitro and in vivo. Mouse L cells induce the production of an interferon-like virus inhibitor in sensitized mouse peritoneal cells (Lackovič and Borecký, 1970). Trinchieri *et al.* (1977) found viral inhibitors in the supernates of mixed cultures of lymphocytes and certain tumour-derived or virus-transformed cell cultures. Recently, they identified these inhibitors as interferons and characterized some of their antiviral and anticellular activities (Trinchieri *et al.*, 1978). Production of interferon in response to strictly syngeneic tumour cells in vivo was reported by Svet-Moldavsky *et al.*, (1974). Skurkovich *et al.* (personal communication, 1978) demonstrated induction of interferon by leukaemic cells in mice. But Gresser (personal communication, 1978) could not confirm the results of Svet-Moldavsky and only low titres of interferon-like activity were found in rats inoculated by Zajdela ascites cells (Zschiesche *et al.*, 1980).

Lackovič and Borecký (1979) demonstrated different kinetics of the antiviral and migration inhibitory activities appearing in the peritoneal cavity of mice after intraperitoneal inoculation with Ehrlich ascites cells. In the present study, the antiviral activity found in exudates of mice was characterized as an interferon.

Materials and Methods

Animals. Outbred Swiss mice weighing 16—20 g were obtained from the breeding farm Dobrá Voda.

Cells. Ehrlich ascites carcinoma cells (EAC) were obtained from Dr. V. Ujházy, Institute of Experimental Oncology, Slovak Academy of Sciences, Bratislava. They were maintained by passaging in the peritoneum of allogeneic mice at 8—10 days intervals. Monolayer cultures of mouse L-929 cells, human diploid lung (HD) cells from the Institute of Sera and Vaccines, Prague, and a long-term guinea pig tongue (GPT) cell line (Svobodová *et al.*, 1977) were grown in basal Eagle's medium (BEM) containing 5 % heat-inactivated calf serum (ICS) and antibiotics. Mouse peritoneal (MP) cells were obtained from non-irritated mice by washing their peritoneal cavities as described (Lackovič and Borecký, 1965). The washing fluids were collected, centrifuged, and the cells suspended (2.5×10^6 cells per ml) in BEM with 10 % heated calf serum and antibiotics.

Viruses. Encephalomyocarditis (EMC), vesicular stomatitis (VSV; Indiana strain) and vaccinia viruses were propagated in L cells. The stock virus titres were about $10^{7.5}$ (EMC), $10^{7.5}$ (VSV) and $10^{5.7}$ (vaccinia) $TCID_{50}$/ml. Newcastle disease virus (NDV, B_1 strain) was propagated in 11-day-old embryonated eggs. The virus stock contained 512 haemagglutinating units (HAU) per ml.

Chemicals. Double-stranded RNA (ds RNA) was prepared in Dr. J. Doskočil's laboratory (Institute of Molecular Genetics, Czechoslovak Academy of Sciences, Prague) by extraction of the replicative form of an amber mutant of f2 phage propagated in non-permissive *Escherichia coli* cells (Doskočil *et al.*, 1971). Dextran sulphate 500 was purchased from Pharmacia, Upsala, Sweden; lipopolysaccharide B from *E. coli* Olll:B_4 (LPS) from Difco Laboratories, Detroit, U.S.A.; and actinomycin D, trypsin, and trypsin inhibitor from Calbiochem, Los Angeles, U.S.A.

Interferon induction. Mouse exudate interferon was produced by peritoneal inoculation of various numbers of EAC cells per ml into mice. After 24 hr the peritoneal cavities of mice were each washed out with 1 ml of BEM. The fluids were collected and cell-free supernatants assayed for interferon. In some experiments, interferon induction by EAC cocultivated with mouse peritoneal macrophages was also tested. Mouse "viral" interferon was prepared as described (Lackovič and Borecký, 1965); this preparation contained 256 units/ml. Guinea pig interferon was prepared in guinea pig peritoneal cells with NDV-B_1 (Lackovič *et al.*, 1979); this preparation contained 128 units/ml. Human leukocyte interferon was prepared and purified by Dr. N. Fuchsberger (Institute of Virology, Bratislava) basically according to Cantell *et al.* (1974); this preparation contained 25000 units/ml. Other interferons employed were used without prior purification or concentration. The anti-interferon serum used was prepared by immunization of sheep with mouse L-cell interferon (Dr. V. Hajnická). One ml of this antiserum contained 4000 anti-interferon units (Fuchsberger *et al.*, 1976).

Assays of antiviral activity were done by the standard cytopathic effect (CPE) inhibition method using L cells and EMC virus with mouse interferons (Lackovič and Borecký, 1965), HD cells and VSV with human leukocyte interferon (Fuchsberger and Borecký, 1978), and GPT cells and VSV with guinea pig interferon (Lackovič *et al.*, 1979).

The cell-growth inhibitory (CGI) effect was tested in L cells as described by Borecký *et al.* (1973).

The toxicity of ds RNA was determined as described by Lackovič and Borecký (1976).

Separation of adherent and non-adherent cells. Pertitoneal exudate cells were separated into adherent and non-adherent fractions by a modification of Moisier's technique (McCombs *et al.*, 1974).

The cytotoxic activity of exudate (EAC) interferon was measured according to Klimpel *et al.* (1975). The units of cytotoxic activity were expressed as reciprocals of the dilution causing a 50 % reduction in the number of viable L cells as compared to controls.

Table 1. Development of antiviral activity in the peritoenal cavities of mice inoculated with EAC

No. of EAC per mouse	Antiviral activity (units per ml) at indicated hr after inoculation of EAC						
	6	12	24	48	72	96	120
10^6	<2	4	8	2	<2	<2	<2
10^7	4	16	32	8	2	<2	<2

Exudate pools from 6 mice were used to determine antiviral activities.

Results

Appearance of antiviral activity in the peritoneal cavities of mice inoculated with EAC

To determine the kinetics of antiviral activity in the peritoneal cavities of mice, groups of 6 mice were inoculated intraperitoneally with tumour cells (10^6 and 10^7 cells/ml respectively) and the exudates collected at intervals. As seen in Table 1, the highest antiviral activity in exudates of recipient mice was noted 24 hr after inoculation of 10^7 EAC per mouse. The antiviral activity ceased 2 days after the tumour challenge.

Characterization of cells responsible for interferon production

Exudate cells were obtained from the peritoneal cavities of mice at intervals after inoculation of 10^7 EAC per mouse and separated into adherent and non-adherent fractions. Unseparated, adherent, and non-adherent cell populations were then incubated at 37 °C for 24 hr. The cell-free supernatants were tested for antiviral activity (Table 2). The supernatants from the population of adherent cells that were withdrawn from the peritoneal cavities 3 or 6 hr after inoculation of mice with EAC and incubated in vitro exerted an

Table 2. Ability of peritoneal cell subpopulations to produce exudate interferon

Time after inoculation of EAC	Interferon titre (units) per ml			
	Cell-free exudate[1])	Incubation of cells at 37 °C for 24 hr		
		Whole[2])	Adherent[3])	Non-adherent[4])
30 min	<2	NT	<2	<2
3 hr	<2	4	8	<2
6 hr	<2	8	8	<2
24 hr	4	2	2	<2
Control mice	<2	<2	<2	<2

[1]) Exudates pooled from 8 mice. [2]) 1 ml contained 5×10^6 unfractionated cells. [3]) 1 ml contained 2.5×10^6 "plastic"-adherent cells. [4]) 1 ml contained 5×10^6 cells after depletion of the adherent cells.
NT = not tested.

Table 3. Comparison of the properties of exudate interferon with "classiocal" and "immunological" interferons

Properties	Interferon		
	Type I*	Type II*	EAE-IF
Physical-chemical			
Inactivated by trypsin	+	+	+
Stable at pH 2	+	0	+
Stable at 56 °C	0	+	0
Molecular weight	$30\text{-}100 \times 10^3$	$45\text{-}80 \times 10^3$	ND
Biological			
Action inhibited by actinomycin D	+	+	+
Inhibits RNA and DNA viruses	+	+	+
Does not neutralize viruses	+	+	+
Inhibits virus in:			
human diploid cells	0	0	0
guinea pig cells	+	0	+
Immunological			
Neutralized by antibody against L-cell (NDV) interferon	+	0	+

*According Youngner and Salvin (1973).
ND — Not done.

antiviral activity while non-adherent cell populations were unable to produce interferon.

Physico-chemical and biological properties of exudate interferon (EAE-IF)

The physico-chemical characteristics of EAE-IF showed a similarity with the "classical" viral interferons in the following parameters: the antiviral activity was destroyed by treatment with trypsin; it was heat-labile (rapid loss of activity at 56 °C-; and stable at pH 2 for 48 hr. Dialysis of the active exudate against phosphate buffered saline for 48 hr at 4 °C resulted in only a 2-fold decrease in antiviral activity (Table 3).

Biological characterization. When EAE-IF was removed from the indicator cells and the latter were carefully washed before challenge with EMC virus (i. e. after 24 hr of incubation), no inhibition of virus replication was detected. However, a significant antiviral effect was found when the exudate (16 units per ml) was left in contact with the virus-challenged cells. This finding made it necessary to reexamine our EAE-IF preparation according to other criteria established by Lockart (1973) for acceptance of a substance as an interferon.

For comparison, 4 units/ml of a "classical" viral NDV-B_1 interferon were estimated in parallel. EAE-IF still inhibited the CPE of 100 LD_{50} of EMC virus when added to the cells up to 60 min after challenge with virus while NDV interferon had no effect at this interval. EAE-IF prevented the infection of L cells also with other challenge viruses (EMC, VSV or vaccinia). No significant loss of virus infectivity was detected when the ability of

EAE-IF to neutralize the virus was tested in the virus neutralization test by the standard procudure.

Effect of actinomycin D. Taylor (1964) found that actinomycin D, which inhibits DNA-dependent RNA synthesis, prevented the development of resistance in response to interferon. To test this effect, L-cell monolayers

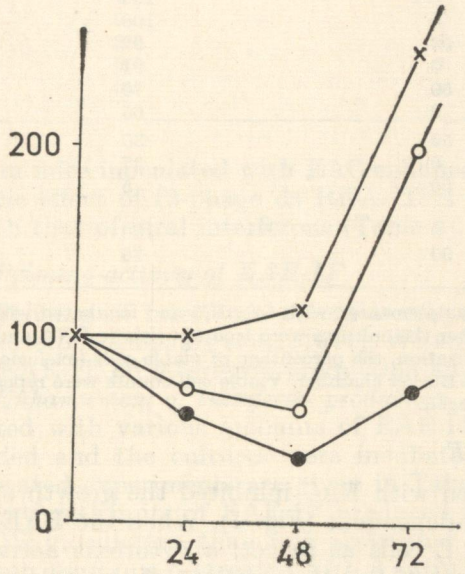

Fig. 1.
Inhibition of the growth of L cells by exudate interferon
× — Control, ○ — EAE-IF (2 units per ml), ● — EAE-IF (4 units per ml). Ordinate: number of cells per ml; abscissa: hr of incubation

were treated with EAE-IF 2 hr after addition of actinomycin D (0.1 μg/ml) and the cells were challenged with EMC 4 hr after interferon treatment. Controls were treated either with actinomycin D or with EAE-IF only. The results of this experiment supported the view that EAE-IF does not inactivate the virus directly but acts through the cell (Table 3).

Species specificity. EAE-IF was tested for species specificity in HD and GPT cells. The exudates were left in contact with cells until the CPE of challenge VSV was complete. A sample of homologous (i. e. either guinea pig or human leukocyte) interferon was included as a positive interferon control in each test. The mouse EAE-IF samples showed no antiviral activity in HD cells when as much as 16 units (calculated on the basis of CPE inhibition titration in mouse L cells) were used for treatment of the cells. But treatment of GPT cells with EAE-IF showed a clear antiviral activity in this system (Table 3).

Immunological properties of EAE-IF. In the test for neutralization of antiviral activity of EAE-IF, 10 antiviral units of interferon and different dilutions of the sheep anti-interferon serum were used. The antiviral activity of EAE-IF was effectively neutralized by the sheep anti-mouse interferon antibody. These results suggest that EAE-IF is antigenically similar to virus-induced interferon (Table 3).

Table 4. Toxicity of ds RNA for L cells treated with exudate and NDV interferon

Interferon treatment (units/ml)	ds RNA (μg/ml)	No. of viable cells in per cent of control
EAE-IF		
	0	100
0	0	100
0	50	92
4	0	91
4	50	70
8	0	65
8	50	30
16	0	37
16	50	5
NDV-IF		
16	0	100
16	50	36

L cells (10^5 cells per ml) were seeded in BEM supplemented with 5 % ICS and incubated with or without interferon for 18 hr at 37 °C. Thereafter the cultures were treated with ds RNA and incubated for an additional 48 hr. After trypsinization, the percentage of viable cells (exclusion of erythrosin B) was determined by counting in a Bürker chamber. Viable cell counts were repeated 2 times each from triplicate cultures and averaged.

Inhibition of cell growth by EAE-IF

The exudates from mice inoculated with EAC inhibited the growth of L cells. The inhibitory effect was dose-dependent (Fig. 1). When the EAE-IF was assayed for cytotoxicity using L cells as target, a cytotoxic activity could be detected in dilutions up to 1 : 4. But the meaning of this finding is weakened by the fact that also control washing fluids from normal mice exerted a weak (2 units) cytotoxic activity.

Ability od EAE-IF to increase the susceptibility of cells to ds RNA toxicity

Cells treated with interferon are more susceptible to the toxic effect of ds RNA than untreated cells (Stewart *et al.*, 1972). We found that exudates

Table 5. Enhancement of interferon production in mouse peritoneal cells primed with exudate interferon

EAE-IF (units/ml)	Stimulus (NDV)*	Interferon titre per ml Exp. 1	Exp. 2	Exp. 3
0	0	<4		
0	+	512	256	512
2	0	2	2	2
2	+	2048	1024	1024
8	0	8	8	8
8	+	1024	1024	1024

*Approximately 50 HAU per ml of cell suspension (2.5×10^6 cells per ml) were used for interferon induction.

Table 6. Priming of L cells pretreated with exudate interferon

Interferon treatmnet		Interferon titre after pretreatment (units/ml)
EAE-IF (units/ml)	NDV-IF (units/ml)	
0	256	256
2	256	2048
0	8	8
2	8	128
0	8	8
0.5	8	16

from mice inoculated with EAC enhanced the susceptibiiity of L cells to the toxic effect of f2-phage ds RNA. This activity appeared to be comparable with that of viral interferons (Table 4).

Priming activity of EAE-IF

Priming of interferon action is one of the many non-antiviral effects of interferons (Stewart *et al.*, 1971). We studied, therefore, the priming activity of EAE-IF on interferon production and action.

Enhancement of interferon production. Mouse peritoneal cells were preincubated with various amounts of EAE-IF for 3 hr at 37 °C. Then NDV was added and the cultures were incubated for another 48 hr. The results of repeated experiments are given in Table 5. Peritoneal cells pretreated with 2 antiviral units of EAE-IF produced 2 times more mouse interferon after NDV inoculation than non-pretreated cultures. The enhancing effect was lower in groups pretreated with 8 antiviral units of EAE-IF.

Priming of interferon action. L-cell monolayers were preincubated with 2 or 0.5 unit of EAE-IF for 3 hr at 37 °C. Then the cultures were exposed to

Table 7. Effect of dextran sulphate and lipopolysaccharide pretreatment on production of exudate interferon

Pretreatment	Stimulus	Antiviral activity		
		Exp. 1	Exp. 2	Exp. 3
Control mice	EAC	8	16	16
Dextran sulphate	EAC	<4	<4	<4
Dextran sulphate	LPS	4	ND	ND
LPS	EAC	16	16	ND
LPS	LPS	<4	<4	ND
Control mice	LPS	32	ND	ND

Groups of 6 mice were pretreated intraperitoneally with LPS (20 μg per mouse) or dextran sulphate 500 (200 μg per kg body weight). After 24 hr, the mice were given EAC (10^7 cells per mouse) or LPS (20 μg per mouse). Control mice were given PBS. Peritoneal cavities of the mice were washed out with 1 ml of BEM 24 hr after inoculation of EAC or 2 hr after LPS injection. Cell-free exudates were used for interferon assays.

ND — Not done.

various amounts of peritoneal cell (NDV) interferon and after 18 hr incubation challenged with EMC virus (100—1000 $TCID_{50}$). Table 6 demonstrates enhanced levels of protection in cultures primed with 2 units of EAE-IF.

Effect of dextran sulphate or endotoxin on the appearance of antiviral activity in mice inoculated with EAC

Peritoneal cells respond with interferon production when exposed to various inducers (Lackovič and Borecký, 1974). It was of interest to examine the relatedness of EAC- and endotoxin-induced interferons using mice made hyporeactive to endotoxin administration. We also examined the role of macrophages in the production of interferon in mice treated with dextran sulphate which is capable of eliminating macrophages (Hahn and Bierther, 1974). The results summarized in Table 7 indicate that dextran sulphate 500 was capable of suppressing the appearance of antiviral activity in the peritoneum of EAC-treated mice. The applied dose of LPS elicited a hyporeactivity confirmed by a disappearance of interferon response after a second dose of LPS 24 hr after the first one. Inoculation of LPS-hyporeactive mice with EAC did not lead to a reduction of EAE-IF. Rather, the levels of EAE-IF were increased in LPS-hyporeactive mice. These results suggest a difference between LPS- and EAC-induced interferon producer cells.

Discussion

The present study demonstrated that the antiviral activity of exudates of EAC-inoculated mice has several properties characteristic of interferons (Lockart, 1973). Some physico-chemical characteristics [heat-lability at 56 °C, stability at pH 2, neutralization by sheep anti-mouse (NDV) interferon antibody] suggest that this interferon might be of the type 1 (Younger and Salvin, 1973).

The highest antiviral activity in the mouse exudates was noted 24 hr after intraperitoneal inoculation of EAC (10^7 cells per mouse) and it ceased 48 hr after the tumour challenge. These data support those published by Svet-Moldavsky *et al.* (1974). We were unable to demonstrate interferon activity in extracts from EAC. On the other hand, adherent exudate cells from inoculated mice produced interferon when transferred into plastic dishes. This suggests that the interferon detected in the EAC exudate represents a product of peritoneal cells, not of EAC. The production of interferon in the mixture of peritoneal cells cocultivated with EAC was also demonstrated in vitro. Studies in which dextran sulphate 500 was used to eliminate macrophages (Hahn and Bierther, 1974) suggest that the production of EAE-IF is macrophage-dependent. But the producer cell was not identified.

Other results of our study suggest, however, that the appearance of antiviral activity in exudates of EAC-inoculated mice was not inhibited by pretreatment of mice with LPS. This finding does not support the view that macrophages play the main role in interferon stimulation by EAC. Further study is needed in this respect.

It is well established that potent interferon preparations, besides inducing an antiviral activity, exert also many non-viral effects, such as inhibition of the growth of homologous malignant and normal cells in culture, increase of the susceptibility of cells to the toxic effects of ds RNA and stimulation of interferon production by priming (Stewart *et al.*, 1971, 1972; Borecký *et al.*, 1973). The present results demonstrated that EAE-IF exerts similar effects. EAE-IF inhibited the growth of L cells and enhanced their susceptibility to the toxic effect of f2-phage ds RNA. These activities appear to be quantitatively comparable with those of viral interferon. Taken together, our results indicate that. among other cells products, an interferon-like substance is released during the interaction of tumour cells with the cells of the reticuloendothelial system in the peritoneal cavity. Its role in tumour cell growth regulation remains unclear.

References

Borecký, L., Fuchsberger, N., Hajnická, V., and Lackovič, V. (1973): Antiviral and cell-growth inhibitory effect of interferon. *Biomedicine* **19**, 281—286.

Cantell, K., Hirvonen, S., Mogensen, K. E., and Pyhälä, L. (1974): Human leukocyte interferon: production, purification, stability and animal experiments, pp. 35—38. In C. Waymouth (Ed.): *The production and use of interferon for the treatment and prevention of human virus infections. In Vitro Monograph* No. 3, The Tissue Culture Association, Rockville.

Doskočil, J., Fuchsberger, N., Vetrák, J., Lackovič, V., and Borecký, L. (1971): Double stranded f_2 phage RNA as interferon inducer. *Acta virol.* **15**, 523.

Fuchsberger, N., and Borecký, L. (1978): Quantitative immunoelectrophoresis of human interferon. A new apporoach to characterization of interferon preparations. *Acta virol.* **22**, 238—240.

Fuchsberger, N., Styk, B., Borecký, L., and Hajnická, V. (1976): Some biological activities of rabbit anti-interferon serum. *Acta virol.* **20**, 107—113.

Hahn, H., and Bierther, M. (1974): Effect of dextran sulphate 500 on cell-mediated antibacterial immunity in mice, pp. 249-259. In W.-H. Wagner and H. Hahn (Eds): *Activation of Macrophages.* Excerpta Medica, Amsterdam.

Klimpel, G. R., Day, K. D., and Lucas, D. O. (1975): Differential production of interferon and lymphotoxin by human tonsil lymphocytes. *Cell. Immunol.* **20**. 187—196.

Lackovič, V., and Borecký, L. (1965): The reticuloendothelial system and virus infection. II. Production of interferon and antibody-like substances in mouse peritoneal cells infected with myxoviruses in vivo. *Arch. ges. Virusforsch.* **17**, 619—630.

Lackovič, V., and Borecký L., (1970): Release of an interferon-like virus inhibitor during contact of mouse leukocytes with target cells. *Acta virol.* **14**, 178.

Lackovič, V., and Borecký, L. (1974): Peritoneal leukocytes central role in interferon production. *IRCS* (*Research in Hematology, Immunology and Allergy, Microbiology and Infectious Diseases*) **2**, 1080.

Lackovič, V., and Borecký, L. (1976): Increased resistance of an interferon-resistant cell subline to the toxic effect of double-stranded RNA. *Acta virol.* **20**, 347—1348.

Lackovič, V., and Borecký, L. (1979): Production of antiviral and migration inhibitory activities in the peritoneum of mice after inoculation with allogenic Ehrlich ascites cells. *Acta biol. med. germ.*, in press.

Lackovič, V., Kontsek, P., Svobodová, J., and Borecký, L. (1979): Long-term culture of guinea pig tongue cells: a suitable interferon system. *Acta virol.* **23**, 162—164.

Lockart, R. Z. (1973): Criteria for acceptance of a viral inhibitor as an interferon and a general description of the biological properties of known interferons, pp. 11—17. In N. B. Finter (Ed.): *Interferons and Interferon Inducers.* North Holland Co., Amsterdam.

McCombs, C., Hom, J., Talal, N., and Mishell, R. I. (1974): Decreased response of cultured New Zealand mouse spleen cells to sheep erythrocytes. *J. Immunol.* **112**, 326—332.

Stewart II. W. E., De Clercq, E., Billiau, A., Desmyter, S., and De Somer, D. (1972): Increased susceptibility of cells treated with interferon to the toxicity of polyriboinosinic, polyribocytidylic acid. *Proc. nat. Acad. Sci.* (*USA*) **59**, 1851—1854.

Stewart II. W. E., Gosser, L., and Lockart, R. Z. (1971): Priming a non-antiviral function of interferon. *J. Virol.* **7**, 792—801.

Svet-Moldavsky, G. J., Nemirovskaya, M. B., Osipova, T. V., Havina E. G., Zinsar, S. N., Karmanova, N. V., and Morozova, L. F. (1974): Interferonogenicity of antigens and "early" cytotoxicity of lymphocytes. *Folia biol.* (*Praha* **20**, 225—230.

Svobodová, J., Blaškovič, D., and Stančeková, M. (1977): Long-term cultivation of cells derived from guinea pig tongue. *Acta virol.* **21**, 349.

Taylor, J. (1964): Inhibition of interferon action by actinomycin. *Biochem, biophys. Res. Commun.* **14**, 447—451.

Trinchieri, G., Santoli, D., and Knowles, B. B. (1977): Tumor cell lines induce interferon in human lymphocytes. *Nature* (*Lond.*) **270**, 5638.

Trinchieri, G., Santoli, D., Dee, R. R., and Knowles, B. B. (1978): Anti-viral activity induced by culturing lymphocytes with tumor-derived on virus-transformed cells. Identification of the anti-viral activity on interferon and characterization of the human effector lymphocyte subpopulation. *J. exp. Med.* **147**, 1299—1313.

Youngner, J. S., and Salvin, S. B. (1973): Production and properties of migration inhibitory factor and interferon in the circulation of mice with delayed hypersensitivity. *J. Immunol.* **111**, 1914—1922.

Zschiesche, W., Fahlbusch, B., Schumann, I., Lackovič, V., and Borecký, L. (1980): Production of interferon and other lymphokines during murine tumour growth. I. Lymphokines in cell-free fluid of rat Zajdela ascites hepatoma. *Acta virol.* **24**, 37—44.