including puromycin, none of the compounds reviewed except L-asparaginase led to a significant increase in malformations, but rather caused generalized toxic responses such as fetal resorption or weight reduction. Although human exposure to puromycin would not be expected to be significant, puromycin may serve as a model compound for microbial toxins that do pose environmental hazards to humans. Many mycotoxins are reported to be embryotoxic[1], and many of these are known to act by inhibiting protein synthesis[2].

Total embryonic phospholipid content was reduced by puromycin in a way that did not appear to be independent of overall growth inhibition. However, cholesterol content did not decrease significantly. Cytotoxic agents can interfere with the feedback control of cellular cholesterol synthesis with a subsequent intracellular accumulation of cholesterol and its esters[16]. It is possible, then, that increases in cholesterol caused by increased cytotoxicity partially offset decreases in cholesterol caused by reduced embryo size. Measurable changes in individual phospholipid species in embryos exposed to a teratogenic insult have been reported[6]. However, the total embryo phospholipid content used in the present study may not be as sensitive a parameter as ratios of individual phospholipid species for detecting embryotoxic-related changes. Schmid et al.[17], in comparing the effects of cyclophosphamide and acrolein on cultured rat embryos, were able to differentiate the teratogenic effects of the former compound and embryolethal effects of the latter compound. The present study reinforces the utility of rodent embryo culture in making this distinction, and it demonstrates the potential of this system for evaluating some of the underlying mechanisms of teratogenesis.

Acknowledgments. The authors thank Mr James Rorie for care and breeding of the animals and Ms Norma Yess for editorial assistance.

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0014-4754/87/040433-03$1.50 + 0.20/0

Interferon-β can induce the production of plasminogen activator by cultured human cancer cells

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Summary. Three cultured human cell lines, renal cancer cells (ACHN), bladder cancer cells (EJ), and fibroblasts transformed in culture by Co-60 gamma rays (KMST-6), when treated with interferon-β, produced 1.5 to 4 times as much plasminogen activator as the untreated control cultures. This enhanced production of PA was inhibited by cycloheximide or actinomycin D.
Key words. Plasminogen activator; interferon; cultured human cancer cells.

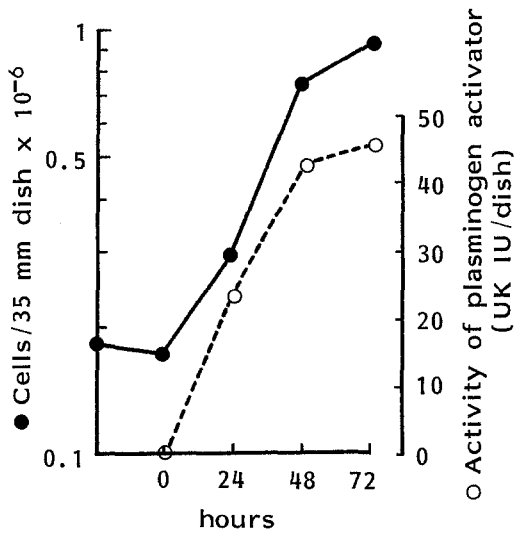
As an antiviral agent, interferon (IFN) was discovered by Isaacs and Lindenmann[1]. Since then interferons have been found to have various biological activities other than antiviral action, such as enhancement of host immune response[2], induction of the major histocompatibility complex class I[3] and class II antigen[4], induction of cell differentiation[5], inhibition of cell growth[6], and antiproliferative activity against tumor cells[7]. In addition, IFNs induce the cellular production of some biologically active substances such as prostaglandin E in fibroblasts[8], colony-stimulating factors in macrophages[9], and fibronectin, also in macrophages[10]. Although various mechanisms have been proposed for IFN's activities as reviewed by Sen[11], the precise mechanisms of the various actions of IFNs are not yet clearly understood. In this communication, we describe the effects of IFNs on the production of plasminogen activator (PA) by cultured human cancer cells.

Materials and methods. We used the following human cells: a renal carcinoma cell line (ACHN)[12], a urinary bladder tumor cell line (EJ)[13], and human fibroblasts transformed in culture by Co-60 gamma rays (KMST-6)[14]. These cell lines were maintained in Eagle's minimum essential medium (MEM, Nissui Seiyaku, Tokyo) supplemented with 10% FBS. When PA was assayed, we used a serum-free defined medium described by Namba et al.[15], which consisted of a 1:1 mixture of Dulbecco's modified MEM (Nissui) and Ham's F12 (Nissui) supplemented with 0.1% bovine serum albumin fraction V (Sigma, St. Louis, MO), 10 μg/ml of transferrin (Collaborative Research, Waltham, MA), 1 μg/ml of insulin (Sigma), and 5 μg/ml of oleic acid (Sigma). These additives were used in place of FBS in order to avoid complications due to possible activators and/or inhibitors of PA in serum. We detected neither activators nor inhibitors of PA in this defined medium. The cells could grow well in this medium.

We used three types of human IFNs: IFN-β produced in fibroblasts (Mochida Pharmaceutical Co., Tokyo), recombinant IFN-β, (rIFN-β, Kyowa Hakko Co. Ltd, Tokyo), and recombinant IFN-γ (Japan Roche Co., Tokyo).

For assay of PA produced by cells into culture medium, cells were seeded into plastic dishes (35 mm in diameter) in 2 ml of MEM with 10% FBS at a density of 10^5 cells/dish. Twenty-four hours later, this medium was removed, the dishes were rinsed twice with PBS, and 2 ml of the defined medium was added to cultures with IFN, whose concentrations are indicated in Results. We harvested the assay media at 72 h after the start of the culture and centrifuged them to remove cells or cell debris. The assay of PA was carried out using a chromogenic substrate

Experientia 43 (1987), Birkhäuser Verlag, CH-4010 Basel/Switzerland



Time course of PA production by human renal carcinoma cells (ACHN).

(S-2251, Kabi, Stockholm), as described by Friberger and Knös[16]. The values for each point represent an average of two determinations. Urokinase (1,200 IU/vial) used as a reference for PA activity and plasminogen (700 CU/vial) were obtained from Green Cross, Co., Osaka.

For measurement of cell growth, cells were detached from dishes with 0.2% trypsin solution, stained with 1% crystal violet in 0.1 M citric acid, and counted by means of a hemocytometer.

Results. First, we examined the relationship between cell growth and PA production. On day 1 we replaced the medium containing 10% FBS by the defined one. Thereafter we maintained the cultures by refeeding them with fresh, defined medium and examined the cell number and the PA in the spent medium every 3 days. Although the data are not presented here, the production of PA was higher during the exponential stage of cell growth compared with that of confluent cultures. Consequently, our present studies on the production of PA were carried out using exponentially growing cells.

Then we investigated the time course of PA release from the cells into the culture medium over a period of 72 h. The medium was collected at 24, 48, and 72 h after initiation of cultures and the cells were counted for comparison of cell number and PA activity in the medium. As shown in the figure, the activity of PA was highest in the culture medium from 72-h cultures. Therefore we examined the PA activity in the media of IFN-treated and untreated control cultures after a 72-h incubation. As shown in table 1, cultures of the three human cancer cell lines treated with IFN-β revealed a 1.5- to 4-fold increase in the production of PA over the respective untreated control values. In addition, the enhancement of PA by the IFN showed a dose-response relationship. Although the data are not shown, there was no difference in the induction activity between IFN-β and rIFN-β. In contrast to IFN-β, IFN-γ did not cause increased production of PA, although the concentrations of IFN-γ used were chosen to give about the same degree of growth inhibition as those of IFN-β.

In order to test whether the enhanced production of PA depends on de novo protein synthesis, we added cycloheximide or actinomycin D to IFN-β-treated cultures. As shown in table 2, both drugs drastically reduced the amount of PA detected in the medium. We also investigated the intracellular level of PA in cells treated with IFN-β and/or the inhibitors by determining the PA content of cell lysates. As shown in table 2, the inhibitors strikingly blocked the activity of PA.

Discussion. We have demonstrated that IFN-β enhanced the production of PA by the human cancer cells examined. IFNs are generally known to inhibit protein synthesis via their enhancement of the activity of 2′-5′-synthetase or protein kinase, and this mechanism has been used to explain the antiviral or antiproliferative effects of IFNs. On the other hand, there have been several reports that IFNs induce certain types of cellular proteins, such as colony-stimulating factors[9], fibronectin[11], HLA-DR[17,18], immunoglobulins[19], and surface tumor-associated antigens[20]. Thus, some activities of IFNs may be related to the regulation of differentiated functions of cells. In fact, when human promyelocytic cells (HL-60) were treated with IFN, they differentiated into mature granulocytes[5]. Our present study showed that IFN-β enhanced the production of PA. The production of PA may also be classified as one of the differentiated functions of cells. Although the mechanism by which IFNs occasionally increase certain types of cellular proteins remains unexplained as yet, several interpretations are considered as explanations of these phenomena, including transcriptional or post-translational regulation and possible decreases in catabolism of specific proteins. Our data are as yet far from proving these hypotheses. However, it is intriguing that IFNs have various effects on cell functions.

IFN-β enhanced the production of PA, while IFN-γ did not. These findings indicate that IFN-β and IFN-γ somewhat differ from each other in biological activity. Therefore, if there is a possibility that enhanced PA activity could influence malignant phenotypes of cancer cells such as morphological transformation, anchorage-independent growth, tumorigenicity, invasive growth, or metastasis[21-26], IFN-γ may be more suitable than IFN-β for clinical use. In any event, since IFNs have various effects on cells, some of which have not been clearly elucidated, and since cancer cells have the potential to develop aberrant functions, IFNs should be used cautiously in the treatment of patients with cancers.

Table 1. Effects of interferons on production of PA

Cell line	Interferon (type, units/ml)		Cell growth (% of control)	PA activity* (% of control)
ACHN		0	100	100
	β	5,000	53.8	136
	β	10,000	56.2	133
	β	20,000	46.0	163
ACHN		0	100	100
	γ	500	55.8	102
	γ	1,000	55.0	110
	γ	2,000	50.0	107
EJ		0	100	100
	β	5,000	77.7	158
	β	10,000	80.9	168
	β	20,000	56.8	400
KMST-6		0	100	100
	β	5,000	50.4	186
	β	10,000	52.6	192
	β	20,000	42.4	232

* PA activity was determined as the amount of PA produced/10^5 cells.

Table 2. Effects of cycloheximide and actinomycin D on PA production by human renal carcinoma cells (ACHN) treated with IFN-β*

	Inhibitors	PA activity/ dish**	PA activity/ 10^6 cells**
Medium	–	100	100
	CHX (0.1 μg/ml)	30.4	30.1
	ACD (1 μg/ml)	10.3	18.5
Cell lysate***	–	100	100
	ACD (1 μg/ml)	2.2	3.5

* The cells were exposed to inhibitors for 48 h in the presence of 10,000 U/ml of IFN-β. ** The PA activity of cultures treated with inhibitors was expressed as a percent of that activity found in controls treated with 10,000 U/ml of IFN-β alone. *** Cell lysates were prepared by the procedure described by Jones et al.[22].

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0014-4754/87/040435-03$1.50 + 0.20/0

Prevention of experimental liver metastases by D-galactose

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22 July 1986

Summary. The metastasis of malignant tumors from a primary site to near and distant secondary sites is probably the most important event in the pathogenesis of cancer and it accounts for most cancer deaths[1]. Whereas advances in the treatment of primary cancer have led to increased patient survival, metastatic cancers are still the most difficult group of diseases to treat successfully[2]. As organ-characteristic lectins play an important role in the organ manifestation of metastatic islets[3,4], it might be possible (e.g. during surgical operations on malignant tumors) to block those organ-characteristic lectins with the appropriate receptor-bearing glycoconjugates in order to inhibit the metastatic spread. Recent experiments have demonstrated that neuraminidase treatment of tumor cells (mouse sarcoma-1) alters in vivo (Balb/c-mice) the organotropic distribution of metastases; instead of being found exclusively in the lung, they are found both in lung and liver. However, pre-injection and regular application of D-galactose – the same holds for arabinogalactan[5,6,13] – prevents the settling of metastases in the liver but does not influence the metastatic process to the lung, whereas mannan – as a galactose-free control substance – does not alter the initial pattern of metastasis to lung and liver.
Key words. Liver lectins; metastases; D-galactase.

In the course of our studies on the role of lectins as tools for tumor marking, we postulated in 1979 that the process of metastasis is analogous to that of bacterial infection and that the organotropy of metastasis, like that of infection, is mediated either by lectins in the invaded organs or by lectins on the invading tumor cells or bacteria[7,13,15]. After the discovery of vertebrate lectins by Ashwell and his group[10] we advanced the hypothesis that especially those organ-characteristic lectins may act as acceptors of malignant tumor cells in the metastatic process by interacting with cryptic precursor carbohydrate structures on the surfaces of metastatic tumor cells[6,7]. Because of the galactose specificity of those vertebrate lectins (e.g. of the Hepatic-Binding-Protein, HBP) we suggested that blockage with competitive receptor-bearing glycoconjugates may inhibit metastatic spread into the liver [4–6]. In the meantime we have obtained experimental evidence that certain metastasizing animal tumors treated in this way show no spreading of metastases into the liver. Recently, a similar inhibition model for infection proved that the lectin mediated organotropy of bacterial infections may also be inhibited by blocking the bacterial lectins with appropriate receptor-specific carbohydrates[14]. Our results concerning the inhibition of liver metastasis by the blocking of hepatocyte lectins with D-galactose can be summarized as follows.

In vitro, rosette formation between freshly isolated human organ cells (hepatocytes) and metastasizing tumor cells can be completely inhibited by low concentrations (5 mg/ml) of single sugars (D-galactose) or glycoconjugates (arabinogalactan) in the growth medium, demonstrating the participation of mitogenic lectins (e.g. the galactose specific HBP) in the adhesion phenomenon. Addition of glucose, mannose or galactose-free glycoconjugates (pullulan) does not inhibit this rosette formation. Neuraminidase treatment of the tumor cells, which removes the terminal sialic acid and exposes penultimate galactose residues, leads to a highly increased adhesion[8].

In vivo, the capacity of D-galactose to block the liver lectin (HBP) was tested by intravenous administration of tritiated α_1-acid-(asialo)glycoprotein to Balb/c-mice (100 µg solubilized in 0.1 ml PBS). In accordance with the results of Ashwell and Morell[10] this glycoprotein was rapidly cleared (within 15 min) from the circulation and taken up by the liver. Pre-injection of D-galactose (15 min before glycoprotein injection) caused a markedly delayed elimination of asialoglycoprotein from the serum. After 30 min an increase of radioactivity of more than 90% was present in the serum (105 dpm/µl serum after α_1-acid-(asialo)glycoprotein injection without receptor blocking compared to 200 dpm/µl after receptor blocking by pre-injection of