Eur J Cancer Clin Oncol, Vol. 23, No. 11, pp. 1607–1613, 1987.
Printed in Great Britain

0277–5379/87$3.00+0.00

Induction of 2′,5′-Oligoadenylate Synthetase in Freshly Separated Malignant Cells from Solid Tumors. Variability in the Susceptibility of Interferon

STEFAN EINHORN,* FARKAS VÁNKY,*† DAN GRANDÉR* and JAN S. WILLEMS‡

Radiumhemmet, Karolinska Hospital, †Department of Tumor Biology, Karolinska Institute and ‡Department of Tumor Pathology, Karolinska Hospital, S-104 01 Stockholm, Sweden

Abstract—*The capacity of interferon (IFN) to induce the enzyme 2′,5′-oligoadenylate synthetase (2′,5′-A synthetase) in malignant cells from freshly explanted solid tumors was investigated. The malignant cells were separated from non-malignant cells by using velocity and density gradient sedimentation as well as adherence on plastic. During the test the cells were usually maintained on an extracellular matrix (ECM) in the wells of a microplate for* 24 h. *The tumor cells varied in their sensitivity to IFN-induced enhancement of 2′,5′-A synthetase. In five out of* 28 *tumor samples natural α-IFN induced no major enhancement in the intracellular levels of 2′,5′-A synthetase. The effect was dose-dependent and as little as* 0.5 *units of α-IFN/*ml *was sufficient to cause an increase in the intracellular levels of this enzyme. Some tumors differed in their susceptibility to α-, β- and γ-IFN, showing resistance to one IFN-type and sensitivity to the other two. As little as* 6×10^3 *cells/well were required for measuring the induction of 2′,5′-A synthetase. Measurement of induction of 2′,5′-A synthetase by IFN* in vitro *could have clinical relevance for pre-treatment testing of the susceptibility of primary tumor cells to IFN.*

INTRODUCTION

INTERFERON (IFN) preparations have been shown to induce remissions in patients with a variety of malignant diseases [1]. The mechanism behind the antitumor effects is not known and it has been suggested that IFN may act directly by inhibiting cell multiplication [2, 3] or by inducing differentiation [4]. Another possibility is that IFN acts by indirect mechanisms, by for instance regulating functions of the immune system [5]. The reason why some tumors respond to IFN treatment whereas others do not is not known and one possibility is that cells differ in the susceptibility to IFN. This has been shown to be the case for the cell multiplication inhibitory effects of IFN [6–8].

The 2′,5′-A oligoadenylate synthetase (2′,5′-A synthetase) system has been implicated in the antiviral and cell multiplication inhibitory effects of IFN [9]. 2′,5′-A synthetase polymerizes ATP into 2′,5′-oligoadenylates, which activates a latent endoribonuclease capable of degrading mRNA and rRNA [10]. In some cell systems, 2′,5′-A synthetase is induced in cells sensitive to the antiviral and/or cell growth inhibitory effects of IFN, whereas this enzyme is not induced in resistant cells [11–13]. In other cells, however, resistance to the antiviral or cell multiplication inhibitory effects of IFN is not related to the induction of 2′,5′-A synthetase [14, 15]. Almost all work on 2′,5′-A synthetase has been done in established cell lines or in normal cells. Only a few studies have been performed in which the 2′,5′-A system in freshly obtained primary tumor cells has been investigated and to our knowledge there have been no studies on the induction of 2′,5′-A synthetase in primary malignant cells from solid tumors. Since the 2′,5′-A system may be of importance in the antitumor action of IFN we have studied the induction of 2′,5′-A synthetase in freshly explanted solid tumor cells by IFN.

Accepted 6 May 1987.

MATERIALS AND METHODS

Patients

Malignant cells were obtained from surgical specimens of 28 patients, 13 with lung carcinoma (five with squamous cell carcinoma, four with adenocarcinoma, three with small cell carcinoma and

one with large cell carcinoma), nine with ovarian carcinoma [seven with seröpapillary, one with mucinous (patient 21) and one with endometroid (patient 22)], one with clear sarcoma, one with fibrosarcoma, one with osteogenic sarcoma, one with renal adenocarcinoma, one with malignant melanoma and one with extranodal lymphoma (growing as a solid tumor in the lung).

IFN preparations

The natural α-IFN was prepared from Sendai virus-induced human Namalwa cells and purified by an anti-IFN-antibody affinity system [16]. The specific activity of this preparation was 2×10^8 units/mg of protein and purity approx. 90%. Recombinant α_2-IFN (from Ernst-Boehringer-Institut für Arznemittel Forschung) was derived from *E. coli*. The specific activity of this preparation was 3.2×10^8 units/mg of protein and the purity was > 99%. *E. coli*-derived recombinant human γ-IFN (from Ernst-Boehringer-Institut für Arznemittel Forschung) had a specific activity of 2×10^7 units/mg of protein. Human β-IFN was produced from fibroblasts by induction with poly(I) poly(C). The specific activity of the partially purified preparation was 1.6×10^6 units/mg of protein [17]. The antiviral activities of the preparations was determined by assaying inhibition of the cytopathogenic effect of vesicular stomatitis virus in human fibroblasts as previously described [18]. The antiviral activities are expressed in international units by comparison with international reference preparations.

Separation of cells

Tumor cells were separated from surgical specimens. The details of this procedure have been described elsewhere [19, 20]. Briefly, cell suspensions were prepared by treatment of the minced tumor tissue with collagenase (3 mg/ml) and DNase (0.2 mg/ml). Separation of various cell components was performed by combination of 1 ***g*** velocity and density gradient sedimentations and by exploitation of the selective adherence properties of certain cell types. The products of each step were inspected after addition of trypan blue. Experiments were only performed with cultures which had at least 90% viability as measured after trypan blue staining.

Lymphoid cells were separated from venous blood by centrifugation on Ficoll-Hypaque [21] followed by washing. As determined after crystal violet staining, approx. 90% of the cells were lymphocytes, the remaining being mainly monocytes.

During separation and subsequent culture the cells were maintained in RPMI-1640 medium (Flow Laboratories Ltd, Ivine, Ayrshire, U.K.) with L-flutamine (200 mM solution, 1% by volume), benzylpenicillin (100 IU/ml), streptomycin sulfate (100 μg/ml), hepes buffer (01 mM/ml) and 10% of heat-inactivated human serum.

Morphological evaluation

Tumor cells (10^4) in 0.2 ml medium were deposited on slides with a cytocentrifuge (35 ***g*** for 5 min). The preparations were immediately fixed with Spray-cyte water-soluble fixative (Clay Adams Division, Becton Dickinson & Co., Parsippany, NJ) and stained according to Papanicolaou. Evaluation was performed by scoring 400–800 cells to determine the percentage of neoplastic cells related to the number of lymphocytes plasma cells, macrophages and other non-malignant cells. In three cases the number of tumor cells was not evaluated by a cytopathologist and the estimation of the proportion of malignant cells was therefore an approximation. Less than 10% contamination with non-malignant cells was regarded as a limit for successful separation. In four cases the contamination with non-malignant cells exceeded 10% (12, 19, 20 and 39%, respectively). The absolute majority of contaminating non-malignant cells were lymphoid. Since lymphoid cells were found not to influence the total levels of 2′,5′-A synthetase to any major extent (Table 4) the data from these cases have been included.

Production of ECM-coated wells

The method for production of ECM is a modification of the method described by Vlodavsky *et al.* [22]. Briefly, cultures of corneal endothelial cells were established from steer eyes as described [23]. Three $\times 10^3$ cells in medium (DMEM with 10% bovine calf serum, 5% fetal calf serum, gentamycin and fungizone) with 5% dextran T-40 were added to the wells of 96-hole microplates and cultured at 37°C in a 5 CO_2 humidified incubator until confluence. Every other day 100 ng/ml of fibroblast growth factor was added (a gift from Dr I. Vlodavsky). The cells were then maintained for an additional 6–8 days under the same conditions, but without the addition of fibroblast growth factor. The cultures were then washed and exposed to Triton X-100. Remaining nuclei and cytoskeletons were removed by exposure to NH_4OH followed by washing, leaving the underlying ECM intact and attached to the plastic.

Culture conditions

If not otherwise stated, 6×10^4 cells were transferred immediately after separation to the wells of 96-well flat-bottomed microplates, that were coated with the ECM. The wells were cultured for 24 h in the absence or presence of IFN at 37°C in a humidified 5% CO_2 incubator. In some wells the

proportion of viable cells was determined after trypsination and staining by trypan blue.

Assay for 2′,5′-A synthetase

The cytoplasmic levels of 2′,5′-A synthetase were determined as previously described [24]. Briefly, the cells were lysed by addition of 50 μl of NP40 to the wells. The plates were then centrifuged for 6 min at 3000 ***g*** and the supernatants collected and frozen at −80°C. For the enzyme assay 10 μl of the extract was added to poly(rI) poly(rC) agarose beads and the mixture incubated for 15 min at 30°C. The beads were then washed and the reaction mixture, containing 10 mM hepes buffer pH 7.5, 5 mM $MgCl_2$, 7 mM dithiothreitol, 10% glycerol, 2.5 mM [α-^{32}P]ATP (0.1–0.3 Ci/mmole), 3 mg/ml creatinkinase, 10 mM creatine phosphate and 40 μg/ml poly(rI) poly(rC), was added and the samples incubated for 16 h at 30°C. One unit of bacterial alkaline phosphatase in 140 mM Tris-base was then added. After 1 h at 37°C 20 μl of water was added after which the beads were removed by centrifugation. The samples were then run through 0.3 ml alumina columns equilibrated in 1 M glycine–HCl buffer pH 2 and collected in scintillation vials which were counted in the ^{3}H-channel of a scintillation counter (Packard). Two to four wells were used for each determination. A standard prepared from normal lymphocytes as well as a blank containing NP40 only was always included in the assay. Nmol ATP incorporated per 10^3–10^4 cells was calculated.

Relative 2′,5′-A synthetase induction was calculated as nmol ATP per 10^3–10^4 cells with IFN/nmol ATP per 10^3–10^4 cells without IFN. A doubling of the 2′,5′-A synthetase levels following treatment with IFN was arbitrarily chosen as a limit for IFN induced enhancement of 2′,5′-A synthetase.

Statistical analyses

Statistical significances were evaluated by Student's *t*-test, and by linear regression analyses.

RESULTS

There was a large variation between tumors in their sensitivity to IFN-induced enhancement of 2′,5′-A synthetase (× 0.7 to × 27.1). In 23 out of 28 tumors α-IFN induced a signifiant enhancement of 2′,5′-A synthetase, as defined by at least a doubling of the intracellular levels of this enzyme (Table 1). There was a large variation in the baseline levels of 2′,5′-A synthetase between different tumors (ranging from 3 to 2642 nmol ATP per 10^4 cells). There was, however, no significant correlation between baseline levels of 2′,5′-A synthetase and relative induction by IFN as tested by linear regression analysis ($r = 0.25$). It may be noted however, that no increase in 2′,5′-A synthetase was observed in the tumor sample with the highest baseline level of 2′,5′-A synthetase activity (patient 2). In four patients we tested whether the 24 h incubation period (in the absence of IFN) had any influence on the 2′,5′-A synthetase levels of the cells. During the 24 h culture period the mean levels of 2′,5′-A synthetase decreased by approx. 40%. There seemed to be no major difference between different tumor types with regard to baseline levels of 2′,5′-A synthetase and relative induction of 2′,5′-A synthetase.

The lack of induction of 2′,5′-A synthetase activity in some tumor cells could theoretically be explained by cell death in these samples. Cell viability after 24 h of incubation *in vitro* varied between 41 and 100% for the different tumors (mean 82%). However, there was no correlation between induction of 2′,5′-A synthetase and cell viability after the *in vitro* incubation as tested by linear regression analysis ($r = 0.21$). Furthermore, the viability in the samples showing no increase in 2′,5′-A synthetase activity was always above 80%.

To test the number of tumor cells required to measure changes in the levels of 2′,5′-A synthetase, 6×10^3, 2×10^4 or 6×10^4 tumor cells/well were incubated in the absence or presence of IFN for 24 h. As can be seen in Table 2, as little as 6×10^3 cells were sufficient for measuring changes in 2′,5′-A synthetase levels. The relative increase in 2′,5′-A synthetase following treatment with IFN was similar for the three cell concentrations used (Table 2).

In the experiments described above, the cells were cultured on an ECM. The ECM has been shown to facilitate cell growth and functions [25, 26]. To test whether the ECM was required for the induction of 2′,5′-A synthetase, 17 tumors were cultured either on ECM or on plastic. There was no major difference in the baseline levels of 2′,5′-A synthetase between tumor cells cultured on ECM or on plastic (Table 3). The relative induction of 2′,5′-A synthetase following incubation with IFN was higher in cells cultured on ECM, although the difference was not statistically significant ($P = 0.08$). There were statistically significant correlations in induction of 2′,5′-A synthetase between cells from the same tumor cultured on plastic and on the ECM ($P < 0.001$).

With few exceptions, the proportion of non-malignant cells was less than 5% after separation of the tumors (Table 1). Almost all these cells were lymphoid (lymphocytes or monocytes). To investigate the possiblity that these cells were responsible for the enhanced levels of 2′,5′-A synthetase, varying amounts of blood lymphoid cells from five tumor patients were tested for induction of 2′,5′-A synthetase in parallel with the tumor cells (Table 4). In 6×10^4 untreated tumor cells the mean levels of 2′,5′-A synthetase was 169 nmol ATP, whereas the mean levels in IFN-treated cells was 834 nmol ATP.

Table 1. *2′,5′-A synthetase levels of tumor cells from* 28 *patients after incubation for* 24 h *with or without* 5000 units/ml *natural α-IFN*

Patient	Diagnosis*	% tumor cells	No IFN (nmol ATP/10^4 cells)	IFN†
1	SCC	99	72	7.1
2	SCC	92	504	4.3
3	SCC	98	16	24.8
4	SCC	98	44	2.2
5	SCC	98	50	14.1
6	ACL	100	157	6.8
7	ACL	99	102	4.0
8	ACL	98	74	6.9
9	ACL	81	614	2.8
10	SCLC	99	16	1.9
11	SCLC	97	8	3.8
12	SCLC	100	9	3.2
13	LCLC	88	6	1.0
14	OC	78	413	3.2
15	OC	98	2642	0.7
16	OC	>95‡	112	3.6
17	OC	>95‡	81	4.9
18	OC	95	238	1.3
19	OC	95	25	2.9
20	OC	>95‡	44	11.8
21	OC	98	9	27.1
22	OC	98	926	2.6
23	OS	99	190	5.2
24	FC	98	78	2.3
25	MM	97	100	11.5
26	CCS	61	12	3.3
27	KC	96	296	1.9
28	KL	95	3	4.3
	Mean ± S.E.		244 ± 99	6.1 ± 1.2

*OC—ovarian carcinoma, SCC—squamous cell carcinoma, ACL—adenocarcinoma of the lung, OS—osteogenic sarcoma, SCLC—small cell lung cancer, MM—malignant melanoma, CCS—clear cell sarcoma, KC—kidney cancer, Ly—lymphoma, LCLC—large cell lung cancer.
†Relative 2′,5′-A synthetase levels (nmol ATP per 10^4 cells with IFN/nmol ATP per 10^4cells without IFN).
‡Not evaluated by cytopathologist.

Table 2. *2′,5′-A synthetase levels of various amounts of tumor cells after incubation for* 24 h *with or without* 5000 units/ml *of natural α-IFN. Means ± S.E. from nine patients*

	Cells/well		
	6×10^3	2×10^4	6×10^4
No IFN nmol ATP/10^4 cells	25 ± 9	46 ± 22	149 ± 62
IFN*	3.1 ± 0.7	2.7 ± 0.5	3.1 ± 0.4

*Relative 2′,5′-A synthetase levels.

Table 3. *2′,5′-A synthetase levels of tumor cells cultured on plastic or on ECM in the absence or presence of* 5000 units/ml *of natural α-IFN. Mean ± S.E. from* 17 *patients*

	Plastic	ECM
No IFN nmol ATP/10^4 cells	352 ± 165	292 ± 153
IFN*	4.8 ± 1.1	6.7 ± 1.9

*Relative 2′,5′-A synthetase levels (nmol ATP per 10^4 cells with IFN/nmol ATP per 10^4 cells without IFN).

The corresponding mean levels for the patients lymphocytes was 27 nmol ATP for untreated cells and 50 nmol for IFN treated cells. In all five cases tested, the 2′,5′-A synthetase levels of the lymphoid cells was substantially lower as compared to the malignant cells. It is thus likely that contamination of the tumor cell preparations with lymphoid cells only contributes minimally to the levels of 2′,5′-A synthetase observed following incubation in the absence or presence of IFN *in vitro*.

The levels of 2′,5′-A synthetase in primary tumor cells increased in a dose-dependent manner following incubation with varying concentrations of IFN (Fig. 1). An IFN concentration of 0.5 units/ml was sufficient to cause a statistically significant increase in the levels of 2′,5′-A synthetase. Maximal stimu-

Table 4. 2',5'-A synthetase levels of tumor cells and peripheral blood lymphoid cells from five patients after incubation for 24 h with or without α-IFN (natural, 5000 units/ml). Means ± S.E. for nmol ATP/10^4 cells are presented

	Lymphoid cells	Tumor cells
No IFN	27 ± 17	169 ± 87
IFN	50 ± 19	834 ± 370

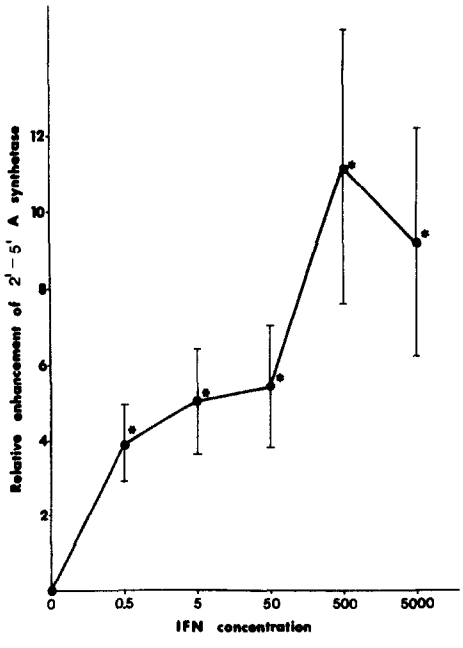

*Fig. 1. Relative enhancement of 2',5'-A synthetase levels following incubation with increasing doses of natural α-IFN for 24 h. Means ± S.E. from 10 patients. *P < 0.05.*

lation seemed to occur at doses of 500–5000 units/ml.

The capacity of various IFN preparations to induce 2',5'-A synthetase in primary tumor cells was tested in tumor samples obtained from 10 patients. Recombinant α-, β- as well as γ-IFN induced increases in the 2',5'-A levels in four tumor samples (patients 22, 24, 25, 27; Table 5). In three cases (patients 20, 23, 28), the cells responded to α-IFN and β-IFN, but not to γ-IFN and in one case the cells responded to β- and γ-IFN, but not to α-IFN (patient 29; Table 5).

DISCUSSION

In this work we show that malignant cells from solid tumors vary in their susceptibility to IFN as measured by induction of the enzyme 2',5'-A synthetase. In a majority of the tumors, 2',5'-A synthetase was induced by α-IFN to a varying extent, whereas in five tumor samples no major induction of 2',5'-A synthetase could be observed (Table 1). The variability in the extent to which IFN induced 2',5'-A synthetase could neither be related to cell death, nor to different baseline levels of 2',5'-A synthetase.

An interesting observation is that three tumors responded to α- and β-IFN, but not to γ-IFN, whereas another tumor responded to β- and γ-IFN, but not to α-IFN. This shows that different tumors vary in their susceptibility to different IFNs, and is in agreement with previous findings on the cell multiplication inhibitory effect of different IFNs in cell lines [27] and in primary ovarian carcinoma cells [8]. Whether or not this has relevance for the clinical situation is as yet not known.

The importance of the 2',5'-A synthetase pathway in the antiviral action of IFN is well established. However, we have little knowledge on the role this pathway may play in the antitumor action of IFN. One reason for this is our basic lack of understanding of the mechanism behind the antitumor effects of IFN. In a previous study we have shown that there is a strong correlation between induction of 2',5'-A synthetase and blast transformation in chronic lymphocytic leukemia cells treated with IFN *in vitro* [28]. Thus, blast transformation occurred only in malignant cells in which 2',5'-A synthetase was induced, whereas no blast transformation occured in cells not responding to IFN by induction of 2',5'-A synthetase, indicating that the 2',5'-A synthetase pathway is of importance for IFN-induced blast transformation.

The ECM has been shown to facilitate the attachment, growth and functions of cells *in vitro* [22, 25, 26]. In our study we found that in most tumors a large proportion of the malignant cells attached and adopted a flattened morphology after seeding on the ECM (data not shown). In contrast, the cells cultured on plastic were loosely attached and usually maintained a rounded morphology. Culture on ECM as compared to plastic had no major effect on the levels of 2',5'-A synthetase in cells not exposed to IFN, whereas induction of 2',5'-A synthetase seemed to be more pronounced after incubation on ECM as compared to plastic (Table 3). This indicates tht although culture of primary tumor cells on ECM probably is preferable, it is not a necessity when testing for induction of 2',5'-A synthetase. This is further supported by the finding that for individual patients, there was a close correlation in the relative induction on 2',5'-A synthetase for cells cultured on ECM as compared to plastic ($P < 0.001$).

In our study the tumor cells were separated from non-malignant cells by methods including Ficoll-Hypaque separation and Percoll density gradient separation [19, 20]. The resulting preparations usually contained < 5% non-malignant cells, mainly lymphocytes and monocytes (Table 1). In all cases tested, the levels of 2',5'-A synthetase in lymphoid cells following incubation in the absence

Table 5. 2′,5′-*A synthetase levels of tumor cells incubated for* 24 h *in the absence of IFN or in the presence of* 500 units/ml *of natural* α-, *natural* β-, *recombinant* α- *or recombinant* γ-*IFN*

Patient	No IFN* (nmol ATP/10^4 cells)	Natural α†	Recombinant α†	Natural β†	Recombinant γ†
12	9	3.2	2.8	3.4	0.9
22	926	2.6	1.8	1.3	1.2
25	100	11.5	10.1	16.8	6.5
26	12	3.3	3.7	3.0	1.0
3	16	24.8	29.9	36.0	24.9
20	44	11.8	17.0	19.2	4.2
27	296	1.9	1.9	1.8	1.6
21	9	27.1	5.3	24.9	22.9
28	3	4.3	4.0	4.4	1.6
13	6	1.0	1.0	28.7	9.4
Means ± S.E.	142 ± 92	9.2 ± 3.2	7.8 ± 2.9	14.0 ± 4.1	7.4 ± 2.9

*nmol ATP per 10^4 cells.
†Relative 2′,5′-A synthetase levels (nmol ATP per 10^4 cells with IFN-nmol ATP per 10^4 cells without IFN).

or presence of IFN was found to be considerably lower as compared to cells from malignant tumors (Table 4). This indicates that contaminating non-malignant cells have no major influence on the levels of 2′,5′-A synthetase observed after incubation of the tumor cell preparations with or without IFN. Due to this fact, data from four less successful separations (up to 39% non-malignant cells) have also been included in this paper. Preparations of cells from solid tumors, separated by Ficoll-Isopaque only, usually contain > 50% tumor cells, the rest being mainly lymphoid cells. The results obtained with these preparations do not differ to any major extent from the results obtained with purified tumor cell preparations (data not shown), indicating that more simple methods for obtaining fresh tumor cells, than the ones employed by us, can be used.

In this work we show that freshly obtained malignant cells from patients with solid tumors vary in their susceptibility to IFN, as measured by induction of 2′,5′-A synthetase. Two methods that are frequently being used for testing the sensitivity of freshly explanted tumor cells to IFN and other agents are the agar colony-assay described by Hamburger and Salmon [29] and the nude mice model [30]. In contrast to these methods, that of testing induction of 2′,5′-A synthetase is rapid, requires relatively few tumor cells and does not have the disadvantage of 'lack of growth'. The relevance of induction of 2′,5′-A synthetase to the antitumor effects of IFN is as yet not known. Studies relating the results from *in vitro* tests of 2′,5′-A synthetase induction to clinical effects of IFN treatment are now being initiated.

Acknowledgements—This work was supported by The Swedish Cancer Society, The Cancer Society of Stockholm and by PHS grant No. 5R01 CA25184–05 awarded by the National Cancer Institute DHHS.

The excellent technical assistance of Miss Ing-Marie Anjegård, Mrs Eva Oldmark, Mrs Birgitta Wester and Mr Dimitrios Papadimas is acknowledged. We thank Drs Ernest Knight Jr, Otto Adolf and David Secher for providing the IFN preparations used and Dr Israel Vlodavsky for providing the preparations of fibroblast growth factor. Drs K. Bäck, N. Einhorn, S. Ivert, U. Nilsonne and K. Sjöwall, are acknowledged for help in obtaining tumor samples. We thank Malin Wåhlin for careful typing of the manuscript.

REFERENCES

1. Strander H. Interferon treatment of human neoplasia. *Adv Cancer Res* 1986, **46**, 1–245.
2. Paucker G, Cantell K, Henle W. Quantitative studies on viral interference in suspended L cells. III. Effect of interfering viruses and interferon on the growth rate of cells. *Virology* 1962, **17**, 324–334.
3. Einhorn S, Strander H. Interferon therapy for neoplastic diseases in man *in vitro* and *in vivo* studies. In: 'Human Interferon. Production and Clinical Use', A Symposium Workshop, W. Alton Jones Cell Science Center, Lake Placid, U.S.A., 19–20 May 1977, 159–174.
4. Robèrt K-H, Einhorn S, Ostlund L, Juliusson G, Biberfeld P. Interferon induces differentiation in primary chronic lymphocytic leukemia cells. *Clin Exp Immunol* 1985, **62**, 530–534.
5. Einhorn S, Blomgren H, Jarstrand C, Strander H, Wasserman J. Influence of interferon-α

therapy on functions of the human immune system. In: *The Biology of the Interferon System.* Amsterdam, Elsevier Biomedical Press, 1983, 347–352.
6. Ernberg I, Einhorn S, Strander H, Klein G. Proliferation inhibitory effect of interferon on primary explants of Burkitt lymphoma: inverse relationship to patient survival. *Biomedicine* 1981, **35**, 190–193.
7. Epstein LB, Marcus S. Review of experience with interferon and drug sensitivity testing of ovarian carcinoma in semi-solid agar culture. *Cancer Chemother Pharmacol* 1981, **6**, 273–277.
8. Ling P, Einhorn S, Anjegård IM, Brenning G, Einhorn N, Strander H. Influence of interferon on the growth of primary ovarian carcinoma cells in a semi-solid agar system. *Med Oncol Tumor Pharmacother.* 1987, in press.
9. Lengyel P. Mechanisms of interferon action: the (2′,5′) $(A)_n$ synthetase–RNase L pathway. In: Gresser I, *Interferon.* ed. London, Academic Press, 1981, 78–99.
10. Kerr IM, Brown RE. PppA2′ p5′ A2′ −5A: an inhibitor of protein synthesis synthesized with an enzyme fraction from interferon-treated cells. *Proc Natl Acad Sci USA* 1978, **75**, 256.
11. Affabris E, Romeo G, Belardelli F *et al.* 2,5-A synthetase activity does not increase in interferon-resistant Friend leukemia cell variants treated with α/β interferon despite the presence of high-affinity interferon receptor sites. *Virology* 1983, **125**, 508–512.
12. Marti J, Vandebussche P, Silhol M *et al.* Effects on interferon on sensitive and resistant L 1210 cell lines. *J Interferon Res* 1981, **1**, 287–295.
13. Salzberg S, Wreschner DH, Oberman F, Panet A, Bakhanashvili M. Isolation and characterization of an interferon-resistant cell line deficient in the induction of (2′,5′) oligoadenylate synthetase activity. *Molec Cell Biol* 1983, **3**, 1759–1765.
14. Silverman RH, Krause D, Jacobsen J, Leisy SA, Barlow DP, Friedman RM. 2,5-A-dependent RNase levels vary with interferon treatment, growth rate and cell differentiation. In: *The Biology of the Interferon System.* DeMaeyer E, Schellekens H, eds. Elsevier, Amsterdam, 1983, 189–200.
15. Tovey MG, Dron M, Mogensen KE, Lebleu B, Mechti N, Begon-Lours-Guymarho J. Isolation of Daudi cells with reduced sensitivity to interferon. II. On the mechanisms of resistance. *J Gen Virol* 1983, **64**, 2649–2653.
16. Finter NB, Fantes KH. The purity and safety of interferons prepared for clinical use. The case for lymphoblastoid interferon. In: Gresser I, ed. *Interferon.* New York, Academic Press, 1980, 65–80.
17. Obert HJ. Clinical trials and pilot studies with beta-interferon in Germany. In: *UCLA Symposium on Molecular and Cellular Biology.* 1982, Vol. XXV, 426–432.
18. Einhorn L, Einhorn S, Wahren B. Interferon induction in human leukocytes after *in vitro* exposure to cytomegalovirus or Epstein–Barr virus. Influence of interferon on the expression of viral antigens. *Intervirology* 1985, **23**, 10–149.
19. Klein E, Vánky F, Galili U, Vose BM, Fopp M. Separation and characteristics of tumor infiltrating lymphocytes in man. In: Hanna MG Jr, Witz IP, eds. *Contemporary Topics in Immunobiology.* New York, Plenum, 1980, 79–107.
20. Vánky F, Klein E. Specificity of auto-tumor cytotoxicity exerted by fresh activated and propagated human T-lymphocytes. *Int J Cancer* 1982, **29**, 547–553.
21. Böyum A. Separation of leukocytes from blood and bone marrow. *Scand J Clin Lab Invest* 1968, **21**, Suppl. 97, 1–109.
22. Vlodavsky I, Ariav Y, Atzmon R, Fuks Z. Tumor cell attachment to the vascular endothelium and subsequent degradation of the subendothelial extracellular matrix. *Exp Cell Res* 1982, **140**, 149–159.
23. Gospodarowicz D, Mescher AR, Birdwell CR. Stimulation of corneal endothelial cell proliferation *in vitro* by fibroblast and epidermal growth factor. *Exp Eye Res* 1977, **25**, 75–89.
24. Revel M, Wallach D, Merlin G *et al.* Interferon-induced enzymes: microassays and their application; purification and assay of (2′,5′)-oligoadenylate synthetase and assay of 2′-phospho-diesterase. *Methods Enzymol* 1981, **79**, 149–161.
25. Vlodavsky I, Lui GM, Gospodarowicz D. Morphlogical appearance, growth behavior and migratory activity of human tumor cells maintained on extracellular matrix versus plastic. *Cell* 1980, **19**, 607–616.
26. Crickard K, Crickard U, Yoonessi M. Human ovarian carcinoma cells maintained on extracellular matrix versus plastic. *Cancer Res* 1983, **43**, 2762–2767.
27. Einhorn S, Strander H. Is interferon tissue specific. Effect of human leukocyte and fibroblast interferons on the growth of lymphoblastoid and osteosarcoma cell lines. *J Gen Virol* 1977, **35**, 573–577.
28. Ostlund L, Einhorn S, Robèrt K-H. Induction of 2′,5′-oligoadenylate synthetase and blast transformation in primary chronic lymphocytic leukemia cells following exposure to interferon *in vitro*. *Cancer Res* 1986, **46**, 2160–2163.
29. Hamburger AW, Salmon SE. Primary bioassay of human tumor stem cells. *Science* 1977, **197**, 461–463.
30. Fogh J, Giovanella B. *The Nude Mouse in Experimental and Clinical Research.* New York, Academic Press, 1977.