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Interferon Production in Glia and Glioma Cell Lines

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Interferon (IF) was produced in glia and glioma cell lines in titers comparable to those produced by human fibroblasts. It was inducible by both Sendai virus and polyriboinosinic:polyribocytidylic acid. "Superinduction" resulted in up to 500-fold-higher titers of IF. The IF appeared to be of the fibroblast type, as revealed by experiments using heat treatment, assay of antiviral activity in heterologous cell lines, and neutralization with specific antisera. Since large amounts of IF may easily be produced with glioma cell lines, such cells may be suitable for mass production of IF.

It is well established that interferon (IF) preparations are potent antitumor agents in mouse models (9, 14), and in current clinical studies on human neoplastic disease similar results are accumulating (H. Strander, Production of Human Interferon and Investigations of Its Clinical Use, a symposium workshop, Lake Placid, N.Y., 1977). In most studies leukocyte IF has been used. The IF was induced by stimulation of short-term buffy coat leukocyte cultures with Sendai virus (3). Recently fibroblast IF has been produced in sufficient amounts to be used for clinical trials as well (V. G. Edy, A. Billiau, and P. de Somer, Production of Human Interferon and Investigations of Its Clinical Use, a symposium workshop, Lake Placid, N.Y., 1977).

Several authors have shown that resistant clones develop in vitro after prolonged exposure to IF (7, 13). Such resistance seems to be confined to the particular kind of IF to which the cells have been exposed (H. Strander, personal communication). In vitro studies have demonstrated that malignant cells of different tissue origin display different sensitivity to the two kinds of human IF available, i.e., leukocyte and fibroblast IF (6).

Several laboratories have screened cell lines for the ability to produce large amounts of IF for different clinical uses (2, 17; Edy et al., a symposium workshop). In this laboratory some glia and glioma cell lines have been available (15) which have turned out to be producers of IF and are easy to cultivate in standard media. In this paper we show that the IF produced by these cells is of the fibroblast type and may easily be produced in large quantities. It is concluded that glioma IF may be potentially useful for the treatment of malignant diseases.

MATERIALS AND METHODS

Cell lines and cell culture. The fibroblast cell lines used were derived from skin of human fetuses obtained after induced abortions during weeks 16 to 24 of pregnancy. The cells were used between passages 6 and 20. The human embryonal glia (HEG) cells were derived from the same fetuses by mincing brain tissue and suspending it in a small amount of culture medium until the pieces had attached to the bottom of the dishes. The glioma cell lines (MG and T) were kindly provided by B. Westermark, Uppsala, Sweden. The lymphoblastoid cell lines 43915 and 43909, which carried Epstein-Barr virus markers, were kindly provided by K. Nilsson at the same laboratory.

The cell lines growing as monolayers were cultivated in 90-mm Nunclon plastic dishes (NUNC, Roskilde, Denmark), using Eagle minimum essential medium supplemented with antibiotics and 10% newborn calf serum. The lymphoblastoid cell lines were grown in glass bottles in Ham F10 medium also supplemented with fetal calf serum. The cultures were incubated in humidified air with 5% CO_2 at 37°C.

Reference IF preparations. Purified human leukocyte IF with a specific activity of 2.5×10^5 U/mg of protein was kindly supplied by the Blood Service of Finland's Red Cross. To produce lymphoblastoid IF, a culture volume of about 100 ml was used, and the cell number was adjusted to 5×10^6 cells per ml. The cells were inoculated with 600 hemagglutination units of Sendai virus per ml, and medium was harvested after 24 h. Fibroblast IF was induced by Sendai virus as described below for glia cell IF. All IF preparations produced in our laboratory were treated at pH 2.0 after adjustment with 1.0 M HCl for 24 h, and thereafter the pH was raised to 7.3 with 1.0 M NaOH. Fibroblast IF preparations were concentrated by precipitation with 30% polyethylene glycol 4000 to achieve a final specific activity of 2.5×10^4 U/mg of protein. Fibroblast IF produced in normal diploid skin fibroblasts was also kindly supplied by V. G. Edy, Leuven, Belgium (specific activity, 7×10^6 U/mg of protein). Y. H. Tan, Calgary, Alberta, Canada, kindly provided fibroblast IF produced by a simian virus 40-transformed, mutagenized and selected cell line (18) with a specific activity of 10^5 U/mg of protein.

Production of IF in glia and glioma cells. For screening purposes Sendai virus or polyriboino-

sinic:polyribocytidylic acid [poly(IC)] was used as the inducer. The cells were inoculated with 600 hemagglutination units of Sendai virus per ml. Virus was inactivated at pH 2 after 24 h as described above. Poly(IC) was used in a concentration of 100 μg/ml for a period of 6 h. For superinduction, 2.5 μg of cycloheximide per ml was added simultaneously with poly(IC), and actinomycin D was present during the last 30 min in a final concentration of 2 μg/ml. The inducers were removed by three washes in 37°C phosphate-buffered saline, and IF was collected during the following 24 h in minimum essential medium with 2% fetal calf serum. Priming was achieved by adding 100 U of leukocyte IF per ml of culture medium 12 h before poly(IC) induction started.

IF assay. The microplate method described by Havell and Vilček (11), using cells cultivated in minimum essential medium with 5% fetal calf serum and vesicular stomatitis virus for challenge, was used for titration of IF. The international reference preparation 69/19 of human IF (National Institute of Medical Research, London, England) or a laboratory standard which was titrated against 69/19 was included in each titration.

IF neutralization assay. Sheep antileukocyte IF serum (anti-L) with a neutralization titer of 45,000 U/ml was a gift from K. Cantell, Helsinki, Finland. Rabbit antifibroblast IF serum (anti-F) with a neutralization titer of 400 U/ml was kindly provided by K. Paucker, Boston, Mass. The neutralization tests were performed in microtiter plates. Anti-L was used in a dilution of 1/5,000, and anti-F was used in a dilution of 1/50. These dilutions were found to reduce IF activity by 50% when tested against 100 U of the homologous IF per ml. Fifty microliters of serial twofold dilutions of IF were preincubated in the plates for 1 h at 37°C with 50 μl of antiserum before addition of the cell suspension. The antisera did not effect the replication of Sendai virus in amnion U cells.

TABLE 1. *IF titers obtained by poly(IC) treatment or Sendai virus infection in glia and glioma cell lines*

Cell line	Inducer	
	Poly(IC)	Sendai virus
F13HEG	NT[a]	8,000
F16HEG	NT	1,000
F21HEG	NT	2,000
373MG	62	<31
138MG	<31	NT
87MG	1,000	4,000
105MG	1,000	500
489MG	<1	NT
178MG	125	NT
563MG	250	250
539MG	250	NT
399MG	1,000	NT
251MG	<4	2,000
706T	125	500
410MG	250	NT
1231MG	9	4,000

[a] NT, Not tested.

TABLE 2. *Superinduction of IF in glioma cell lines*[a]

Cell line	IF activity (U) of cells treated with:		
	Poly(IC)	Poly(IC) + cycloheximide	Poly(IC) + cycloheximide + Act D
1231MG	8	500	4,000
251MG	2	64	500
87MG	1,000	6,000	32,000
105MG	1,000	4,000	61,000

[a] Cells were treated with poly(IC), cycloheximide, and actinomycin D (Act D) according to the text. IF activity was assayed 24 h after addition of poly(IC).

RESULTS

IF titers obtained from glioma and glia cell lines. Almost all glia and glioma cell lines tested produced detectable quantities of IF after induction with poly(IC) or Sendai virus (Table 1). The titers were reproducible by one or two titer steps when induction of cells from various passages was performed. No extremely high producers were found, and only a few cell lines consistently produced more than 2,000 U/ml in this screening.

In some of the cell lines superinduction with cycloheximide and actinomycin D was tried. This was readily obtained and resulted in up to 500-fold increases in IF titers (Table 2). Priming of the cells with low doses of IF (100 U/ml) resulted in a further two- to threefold increase in the IF yield up to 80,000 U/ml, corresponding to 220 U/1,000 cells.

Attempts were made to obtain large-scale production of IF by using the glioma cell line 105MG, which was the most rapidly growing glioma line and also was most convenient to handle. This cell line reached a final density of 5.6×10^4 cells per cm^2 of growth surface. In five roller bottles (1,300-cm^2 growth area, 150-ml harvest volume) a titer of 33,600 U/ml (standard error, 6,400) was achieved, corresponding to 70 U/1,000 cells or 14 cells per reference unit of IF. No difference in titer was observed between passages 50 and 100.

Characterization of glia-glioma IF. All of the IF preparations used in the present study had been subjected to dialysis against pH 2, which means that activity was not due to occurrence of so-called immune IF in the preparations (10).

All the tested IF preparations were stable during ribonuclease treatment, whereas they were destroyed by trypsin.

IF from glia-glioma cells differed from IF of leukocyte or lymphoblastoid origin with respect to heat lability. Thus, after incubation at 56°C for 30 min, leukocyte and lymphoblastoid IF activity was essentially unaffected, whereas fi-

broblast and glia-glioma IF activity was reduced by more than 90%.

The neutralizing activities of the two antisera used, anti-L and anti-F, are shown in Table 3. It is obvious that anti-L neutralized leukocyte and lymphoblastoid IF as well as, to a smaller degree, IF produced by glia-glioma cell lines and fibroblast cells. Anti-F did not neutralize leukocyte or lymphoblastoid IF, whereas it inhibited glia-glioma and fibroblast IF. Thus, glia-glioma IF seemed to be of the fibroblast type.

It has been established that some cross specificity of human IF exists. Thus, high activity of leukocyte and lymphoblastoid IF can be achieved in bovine porcine and feline cell lines (5, 8). Leukocyte (purified) and lymphoblastoid (43915) IF gave higher titers in bovine kidney cells (MDBK) than in human amnion U cells (Table 4). Fibroblast IF, on the other hand, never displayed higher activity in heterologous cells than in the human reference line; again, glia and glioma IF behaved as fibroblast IF.

DISCUSSION

The glia-glioma cell lines tested in this study seemed to produce the fibroblast type of IF as the main component. This was concluded from studies on heat stability, ability of IF to establish an antiviral state in heterologous cell systems, and the effect of neutralizing antisera raised against leukocyte or fibroblast IF. The finding that anti-leukocyte IF partially neutralized the activity of glia-glioma IF agrees with the notion that leukocyte IF preparations contained two antigenic determinants, one of which is shared with fibroblast IF (1).

The maximum amounts of IF produced per cell by glia or glioma cell lines were higher than those obtained after stimulation of commonly used fibroblast cells (12) and comparable to titers obtained with the osteosarcoma cell line described by Billiau et et al. (2) that produced 120 U/1,000 cells. The reproducibility of the results and the ease with which the glioma cells could be cultured may make them suitable for large-scale production of IF. In contrast to fibroblast lines, no effect of aging was observed because the glioma line 105MG produced the same amount of IF at passage 50 as at passage 100. Glia lines, on the other hand, seem less suitable, because they only grow for 15 to 25 passages during routine culture conditions.

At present the production cost of fibroblast IF is rather high, which has made it prohibitive for use in tumor diseases and other conditions where there is need for treatment with high doses over long periods of time. So far, diploid cell lines derived from human embryos or normal non-neoplastic adult tissue have been rather good producers, but much higher titers per cell have been obtained in cell lines of neoplastic origin (2) or in cell lines from simian virus 40-transformed cells (18). The use of human neoplastic cell lines such as the glioma lines used in the present study as producers of IF, however, has a distinct disadvantage in that it is difficult to ensure that they do not contain oncogenic virus

TABLE 3. *Effect of anti-F and anti-L sera on human IF from different sources*[a]

Type of IF	IF titer after absorption with:		
	Medium (control)	Anti-L	Anti-F
Lymphoblastoid (43909)	32	<2	32
Glioma (105MG)	64	16	4
Glia (F13HEG)	64	16	4
Fibroblast (F11HED)	64	8	2
Fibroblast (Edy)	64	<2	4
Leukocyte (purified)	125	<2	125

[a] IF titers are given as the reciprocal of the dilution at which 50% of the cells were protected from vesicular stomatitis virus.

TABLE 4. *Antiviral activity of glioma IF on cells from different species*[a]

Test cell	Species	IF activity on:				
		PIF[b]	43915[c]	Edy[d]	Tan[e]	Glioma
Amnion U	Human	512	128	1,024	2,048	4,096
GMK	Monkey	8	2	32	64	128
RK-13	Rabbit	2	2	2	8	4
SIRC	Rabbit	8	NT	128	2	32
MDBK	Cow	12,288	1,024	128	24	32
Feline	Cat	1,024	192	4	16	48
PK15	Pig	256	NT	3	12	16

[a] IF titers are expressed as the reciprocal of the dilution at which 50% of the cells were protected from lytic infection by vesicular stomatitis virus. NT, Not tested.

[b] Leukocyte IF.

[c] Lymphoblastoid IF.

[d] Fibroblast IF.

[e] Fibroblast IF from a simian virus 40-transformed, mutagenized cell line.

or transforming units of viral nucleic acids. Therefore, for the treatment of virus diseases which are not life-threatening such preparations should be avoided.

For the treatment of tumor disease the situation is different. First, most tumors represent a threat to life. Moreover, many of the currently used therapeutic regimens are mutagenic and may be tumorigenic. This is especially true for radiation and treatment with immunosuppressive and cytostatic drugs, and it seems possible that the use of IF of neoplastic cell origin would be less hazardous than using such forms of treatment.

Concerning IF from glioma cell lines, it is not known if any oncogenic virus is present in the IF preparation. Oncogenic RNA virus has been found in human brain tumors (4), but to our knowledge it has not been proved to cause transformation of human cells in in vitro systems.

As long as this hypothetical risk of tumor virus contamination remains, the glioma-derived fibroblast IF preparations should be used with caution. Large-scale production of glioma cell IF under optimal conditions has, however, been initiated in our laboratory with the aim to test it in malignant disease.

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LITERATURE CITED

1. **Bert, K., C. Ogburn, K. Paucker, K. E. Mogensen, and K. Cantell.** 1975. Affinity chromatography of human leukocyte and diploid cell interactions sepharase-bound antibodies. J. Immunol. **114**:640–644.
2. **Billiau, A., V. G. Edy, H. Heremans, J. van Damme, J. Desmyter, and P. de Somer.** 1977. Human interferon: mass production in a newly established cell line, MG-63. Antimicrob. Agents Chemother. **1**:11–15.
3. **Cantell, K., S. Hiervonen, K. E. Mogensen, and L. Pyhälä.** 1974. Human leukocyte interferon: production, purification, stability and animal experiments. In Vitro **3**:35–38.
4. **Cuatico, W., and S. Spiegelman.** 1973. Particles with RNA of high molecular weight and RNA-directed DNA polymerase in human brain tumors. Proc. Natl. Acad. Sci. U.S.A. **70**:2789–2793.
5. **Desmyter, J., and W. E. Stewart II.** 1976. Molecular modification of interferon: attainment of human interferon in a conformation active on cat cells but inactive on human cells. Virology **70**:451–458.
6. **Einhorn, S., and H. Strander.** 1977. Is interferon tissue specific? Effect of human leukocyte and fibroblast interferons on the growth of lymphoblastoid and osteosarcoma cell lines. J. Gen. Virol. **35**:573–577.
7. **Gresser, I., M.-T. Bandu, and D. Brouty-Boyé.** 1974. Interferon and cell division. IX. Interferon-resistant L1210 cells: characteristics and origin. J. Natl. Cancer Inst. **52**:533–559.
8. **Gresser, I., M.-T. Bandu, and D. Brouty-Boyé.** 1974. Pronounced anti-viral activity of human interferon on bovine and porcine cells. Nature (London) **251**:543–545.
9. **Gresser, I., and C. Bourali.** 1970. Antitumor effects of interferon preparations in mice. J. Natl. Cancer Inst. **45**:365–376.
10. **Haahr, S., L. Rasmussen, and T. C. Merigan.** 1977. Lymphocyte transformation and interferon production in human mononuclear cell microcultures for assay of cellular immunity to herpes simplex virus. Infect. Immun. **14**:45–54.
11. **Havell, E. A., and J. Vilček.** 1972. Production of high-titered interferon in cultures of human diploid cells. Antimicrob. Agents Chemother. **2**:476–484.
12. **Horoszewicz, J. S., S. S. Leong, M. Ito, L. di-Berardino, and W. A. Carter.** 1978. Ageing in vitro and large-scale interferon production by 15 new strains of human diploid fibroblasts. Infect. Immun. **19**: 720–726.
13. **Kuwata, T., A. Fuse, and N. Morinaga.** 1976. Effects of interferon upon cell and virus growth in transformed human cell lines. J. Gen. Virol. **33**:7–15.
14. **Levy, H. B.** 1970. Interferon and interferon inducers in the treatment of mouse malignancies. Arch. Intern. Med. **126**:78–83.
15. **Nilsson, K., and J. Pontén.** 1975. Classification and biological nature of established human hematopoetic cell lines. Int. J. Cancer **15**:321–341.
16. **Pontén, J., and E. H. MacIntyre.** 1968. Long term culture of normal and neoplastic human glia. Acta Pathol. Microbiol. Scand. **74**:465–586.
17. **Strander, H., K. E. Mogensen, and K. Cantell.** 1975. Production of human lymphoblastoid interferon. J. Clin. Microbiol. **1**:116–117.
18. **Tan, Y. H., and W. Berthold.** 1977. A mechanism for the induction and regulation of human fibroblast interferon genetic expression. J. Gen. Virol. **34**:401–411.