Interferon Induced Growth Depression in Diploid and Heteroploid Human Cells (36379)

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Paucker, Cantell, and Henle (1) first reported that the growth rate of suspended mouse L-cells was markedly depressed when grown in the presence of interferon prepared in L-cells. This growth depression effect (GDE) was found to be closely associated with the interferon activity in either crude or purified (2) preparations. Cantell (3) Gresser *et al.* (4) and O'Shaughnessy, Lee, and Rozee (5) recently also observed a similar inhibition of cell growth in cultures exposed to homologous interferon. At present, very little is known of the mechanism by which interferon preparations depress cell growth except that it may be related to the control of synthesis of cellular DNA (5). Experiments herein reported were designed to extend the original observation of Paucker, Cantell, and Henle (1) to diploid and heteroploid human cell lines.

Methods and Results. Cell cultures. HeLa cells were routinely grown as monolayer cultures in 1 liter Blake bottles in Eagle's minimal essential medium (MEM) supplemented with 10% heat-inactivated fetal bovine serum (growth medium).

Two strains of human embryo fibroblast of skin and muscle cells, designated as HESM-90 and HESM-92 were grown in Eagle's diploid medium supplemented with 10% inactivated fetal bovine serum. These cells were subcultivated every week; and continuing diploidy in these cultures was ensured by karyotype analysis in approximately 100 metaphase cells at every 10th subculture.

Preparation of interferon from normal human leukocytes. Human interferon was prepared by infection of normal peripheral leukocyte suspensions with ultraviolet-light-inactivated Newcastle disease virus (UV-NDV) or Sendai strain of parainfluenza virus type 1. As previously described in detail (6) washed suspensions of leukocytes in Eagle's diploid medium containing 10% fetal calf serum were exposed to either UV-NDV or Sendai virus for 18–24 hr. The cell free supernate was then collected and was usually found to contain interferon at 400–500 plaque reduction doses, 50% (PRD_{50}) units per milliliter. Control fluid (spent medium) was similarly prepared with uninfected leukocyte suspensions.

All preparations were subjected to acid (pH 2) treatment, back dialysis against growth medium, ultracentrifugation and filtration as described in our previous publication (5).

In the current study, interferon assays were carried out in monolayer cultures, in 60 mm petri dishes grown at 37° in a 5% CO_2 incubator, by the plaque reduction technique. Cell cultures of either HeLa or HESM were treated with dilutions of interferon or control spent medium preparations for 18 hr prior to challenge with about 100 PFU of vesicular stomatitis virus (VSV). Interferon titers were expressed as plaque reduction doses 50% (PRD_{50}) units. A PRD_{50} unit was defined as the reciprocal of the dilution of interferon at which the number of plaques was reduced to 50% of values found in control cultures.

Assay for growth depression effect. The effect of human leukocyte interferon preparations on the growth of human cell types was carried out in cultures of monodispersed cells in 60-mm petri dishes.

These monodispersed cell cultures were

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customarily prepared from a 2–3-day-old monolayer culture which was harvested into 6 ml of growth medium by 0.25% trypsin treatment. The detached cells were washed and then vigorously dispersed by pipetting and then were aspirated into a 5-ml pipette. The cell suspension was retained in the pipette with the help of a "ProPipette" device. The pipette was then placed in a vertical position; and the cells were allowed to settle for 10 min at room temperature. The top 0.5–1 ml of the cell suspension was then carefully collected and served as the source of monodispersed cells. When this suspension was stained and observed by microscopy only occasional paired cells were seen.

Success in demonstrating the growth depression effect was dependent upon careful attention to the number of seeded cells. Cultures were seeded with a number of monodispersed cells sufficient to ensure that a light, evenly dispersed, noncontiguous monolayer of cells adhered to the glass after 16–18 hr incubation at 37° in a 5% CO_2 incubator. Too heavy an inoculum resulted in an inability to reliably demonstrate a growth depression effect. The number of cells per culture was determined by hemacytometer count on at least 4 randomly chosen cultures so as to ascertain the uniformity of the cultures before use. The optimum seeding density was found to be about 10^4 cells in 4-ml seeding volume.

Treatment of the cultures with interferon was carried out by replacing the culture medium at 17–18 hr with fresh grown medium containing the desired dilution of interferon using triplicate cultures per dilution. As control, spent medium was used, similarly diluted in growth medium. The respective medium was replaced at 3-day intervals during the duration of the incubation.

After an appropriate period of time, usually 6 days, the cultures were rinsed 3 times with phosphate buffered saline free of calcium and magnesium (PBSA) and 2–3 ml of 0.25% trypsin in PBSA was added to each culture to detach the cells. The suspension was then dispersed thoroughly; and the cells from both interferon treated and control cultures were stained with neutral red and counted with a hemacytometer. The results of such an experiment are reported in Fig. 1. The HeLa cells appeared more sensitive to the growth depression activity of human leukocyte interferon preparations than did the HESM cells of a human diploid strain.

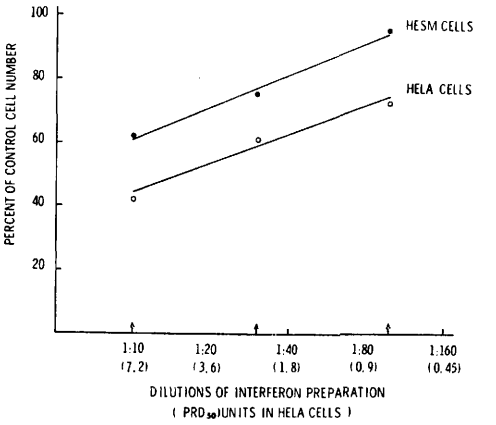

FIG. 1. The effect of human leukocyte interferon on the growth of monolayer cultures of HESM-92 and HeLa cells. Cells were counted 6 days posttreatment and control cultures averaged 5×10^5 and 10^6 cells/culture, respectively.

In order to measure more accurately this effect, experiments were done in which the growth depression activity of various leukocyte preparations was measured in growth depression effect, 50% (GDE_{50}) units. We defined a GDE_{50} unit as the reciprocal of the dilution of interferon preparation at which the number of cells in treated cultures was reduced to a value 50% of that found in control cultures. In Table I these values are compared for the heteroploid HeLa cell line and the diploid HESM-92 cell line. It can be seen that the preparations had a more pronounced growth depression effect on HeLa cells, being 40–50% more active than against HESM-92. However they were much less efficient in HeLa cells than in HESM-92 when their ability to initiate antiviral activity was measured. The antiviral activity of the leukocyte preparations in HESM-92 was 6 to 7 times more effective than in HeLa cultures.

In order to determine if the passage number of the human diploid cells influenced their sensitivity, several experiments were

TABLE I. Comparative Antiviral Activity of Human Leukocyte Interferon Preparations in Human Embryonic Skin–Muscle (HESM) and HeLa Cell Monolayer Cultures.

Leukocyte interferon preparation	(GDE_{50}/ml)		(PRD_{50}/ml)	
	HeLa	HESM-92	HeLa	HESM-92
4	10.0	7.2	71	497
5	18.0	5.5	72	495
6	10.5	6.6	70	553
7	8.0	5.0	70	455
Av	11.6	6.1	70.8	497.5

performed on HESM-92 cell line which had passage numbers from primary culture of 4 to 11. These were compared to the HESM-90 cell line whose passage number was 28-32. There was no indication in either growth depression or antiviral studies that these two cell lines differed significantly.

The factor responsible for growth depression activity in these leukocyte preparations had all the biological characteristics by which interferon is usually described. It was nonsedimentable at 105,000*g*, nondialyzable, stable at pH 2.0, destroyed by trypsin and stable at 56° for 1 hr. It was also species-specific in that it was totally without activity when used to treat mouse L-cell cultures.

Discussion. Moehring and Stinebring (7) have recently reported that interferon at a concentration of 12 PRD_{50} antiviral units, determined in human foreskin cells, had no effect on the life-span of these cells even after 48 passages. This is explicable on the grounds that no growth depression effect could be detected in our HESM diploid cell system until at least 20 PRD_{50} antiviral units, measured in HESM cells, was used to treat the cultures. Obviously too low a dose of interferon was employed in their case.

The foregoing results confirm the reports from our own (5) and other laboratories (1, 3, 4) that growth of cells in the presence of homologous interferon preparations is markedly reduced. The active factor responsible for this growth depression activity is dose dependenat and has biological characteristics that closely resemble that of interferon. These experiments, in addition support the view that the growth depression activity is similar to the antiviral activity in that not all homologous cell types are equally affected.

Of particular interest is the finding that the heteroploid HeLa cell is less responsive to the antiviral activity than to growth depression by interferon preparations when the reverse is true with the diploid HESM cell. The reason for this is obscure at the moment, but the phenomenon leads to interesting speculations. Whether or not the cellular property of heteroploidy with its attendant neoplastic characteristic is a determining factor for sensitivity to growth depression remains to be elucidated.

One facet of the activities of interferon preparations may be related to growth depression. Earlier (5) we have reported that DNA sysnthesis was delayed by interferon in synchronized L-cell cultures. This delay may signal a change in the control of DNA synthesis in cells and depressed cellular multiplication may be one result of such a change. Perhaps in nature interferon is selectively more active in the regulation of DNA synthesis in cells that differ from normal.

Summary. A simple method for reliably demonstrating the growth depression activity of interferon preparations in monolayer cultures is described. Using this method interferon preparations from normal human leukocytes was found to be relatively more active in inhibiting the growth of heteroploid HeLa cells than diploid HESM cells. The antiviral factor of the preparations was, however, 6-7 times more active in HESM cells.

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