Survey of Interferon Production and Sensitivity in Human Cell Lines

J. M. MOEHRING, W. R. STINEBRING, AND D. J. MERCHANT

Department of Medical Microbiology, University of Vermont, College of Medicine, Burlington, Vermont 05401

Received for publication 26 March 1971

Seven presumed diploid and 11 established cell lines were studied for their ability to produce free interferon in response to a standardized Newcastle disease virus challenge. Interferon production was evaluated in both serum-containing and serum-free medium. The ability of these cell lines to respond to the application of a standard interferon preparation by becoming resistant to virus was also examined. The diploid lines were distinctly more efficient producers of interferon than were the established lines. They also evidenced a greater requirement for serum to produce their maximum titers, but some were able to produce good titers in serum-free medium. The diploid lines were uniformly more sensitive to the application of exogenous interferon than were the established cell lines and attained greater degrees of virus resistance, but all lines tested displayed measurable sensitivity to interferon.

Interferon may be the body's first line of defense against invading viruses. Interest in the therapeutic use of this specific native protein has been growing, and it is at least theoretically feasible that human interferon can be produced in cell culture and processed for clinical use. It was, therefore, of interest to us to determine what cell lines were the most prolific producers of interferon. We have studied 18 cell lines (7 presumed diploid and 11 established) for their ability to produce interferon in response to a virus challenge. In addition, the different abilities of these cell lines to become resistant to virus in response to the application of a standard preparation of human interferon has been evaluated.

MATERIALS AND METHODS

Cell cultures. All cell cultures were maintained routinely in Eagle basal medium (BME) supplemented with the seven nonessential amino acids, 10% fetal calf serum, 100 units of penicillin per ml, and 100 μg of streptomycin per ml. Cultures were incubated at 36 °C in an atmosphere of 5% CO₂ in air and were passaged every 3 or 4 days. Primary cell cultures were prepared by trypsinization of tissue samples, as previously reported (J. M. Moehring and W. R. Stinebring, Proc. Soc. Exp. Biol. Med., in press). These presumed diploid cell lines were always transferred by two for one splitting, so that their passage number was approximately equal to their generation in culture (1).

Production of interferon in cell cultures. For each cell line, equal numbers of cells were seeded in 10 ml of growth medium in 8-oz (ca. 240 ml) prescription bottles. Inocula varied from 10⁶ to 2.5 × 10⁸ cells/ml, governed by the rate of growth of the cell line. When the cell sheets were almost confluent, from 48 to 72 hr, a count was made of the cells in one or two replicate cultures. Medium was removed from the other cultures by aspiration, and Newcastle disease virus (NDV; vaccine strain obtained from Ben R. Forsyth, Univ. of Vermont) was added at a concentration of 4 × 10⁸ hemagglutination units/cell in 2 ml of serum-free BME. The virus was allowed to adsorb to cells for 1 hr at 36 °C, with shaking of the bottles at 15-min intervals. The supernatant medium was then removed, and cell sheets were washed with 10 ml of Hanks balanced salts solution (HBSS). A 10-ml amount of test medium was then added, and cultures were incubated for 24 hr at 36 °C. Three test media were employed in these studies: (i) BME containing 5% fetal calf serum; (ii) Neuman and Tytell (NT) medium (5), a complex serum-free medium; and (iii) "2XE" medium, unsupplemented BME in which 12 amino acids, 8 vitamins, and glutamine are incorporated at twice their usual concentration (4).

Cytopathic effect of the virus on the cells was scored, and culture fluid was harvested at 24 hr. Samples were titrated to pH 2 with 1 N HCl and were stored at 4 °C for 4 to 8 days. Samples were then neutralized with 2 N NaOH before assay. A random selection of eight samples for sterility testing was made on two occasions. A 0.1-ml amount of each sample was inoculated into two 9-day-old embryonated hen's eggs, and the eggs were incubated and candled over a 5-day period. All embryos were alive after this period, and complete inactivation of live virus was assumed. Some samples were also centrifuged at 100,000 × g after the pH 2 treatment to remove any residual virus.

Interferon assay. Interferon content of all samples

1 Present address: W. Alton Jones Cell Science Center, Lake Placid, N. Y. 12946.
was assayed by the plaque reduction method on monolayers of human foreskin fibroblasts in 60-mm plastic petri dishes (Falcon Plastic). Samples were serially diluted in BME containing 2% fetal calf serum and were left in contact with full 48-hr monolayers of HF cells for 18 to 22 hr. HF-5 cells of passage number 20 to 38 were used in all interferon production assays. After draining and one 4-ml wash with HBSS, plates were inoculated with 40 to 80 plaque-forming units of vesicular stomatitis virus in 0.5 ml of serum-free medium. Virus was adsorbed for 1 hr at 36°C in the CO2 incubator, with shaking at 15-min intervals. A 4-ml amount of an overlay medium consisting of Eagle minimum essential medium (MEM) containing 5% inactivated fetal calf serum and 0.85% Noble agar (Difco) was then added, and plates were incubated for 48 hr. A second agar overlay containing 0.1% 2-(p-iophenyl)-3- (p-nitrophenyl)-5-phenyltetrazolium chloride (reference 2; Aldrich Chemical Co.) was added, and the plates were incubated for 8 hr or longer, until the living cells reduced the tetrazolium salt to a purple-red formazan and plaques could be counted. One unit of interferon was that dilution which reduced the plaques by one-half, and the reciprocal of that dilution was the titer of a given preparation.

An interferon standard was included in all assays, as a check on cell condition and reproducibility. A standard interferon preparation, HIF-3, was produced in human foreskin cells by the same procedure utilized in these studies. It was exposed to pH 2 for 5 days before being neutralized, centrifuged at 100,000 X g for 1 hr, and divided into small samples for routine use. Its titer on HF-5 cells averaged 500 in 11 assays.

RESULTS AND DISCUSSION

Table 1 shows the yields of interferon from 18 cell lines when challenged with NDV. Yields are given as units of interferon produced per cell to eliminate the differences in titer which were due to different numbers of cells in the challenged cultures. Although cultures were used when grown to almost confluent layers, the varying sizes and growth habit of the cell lines caused considerable differences in cell number per 8-oz bottle; e.g., a "full" bottle of HF-5 had 3.2 X 10⁶ cells, a number characteristic of the diploid lines used. HeLa showed 5.6 X 10⁴ cells and typified most of the established epithelioid lines, and RPMI 2650 had 10.5 X 10⁴ cells (it was a notably smaller cell than most).

Although it has been observed that some continuous cell lines, especially those of human epidermoid origin, are poor producers of interferon, it has been difficult to draw any firm generalizations from the available data regarding comparative production in established and diploid lines (3, 7). When interferon is produced by cell-virus interaction, the specific interactions must be evaluated separately for each individual case. Certain cell lines which produce little or no interferon in response to challenge with one virus may produce considerable interferon in the presence of another virus. By using in our study only one virus which is known to be capable of simulating high levels of interferon in some human cell lines and by carefully regulating the numbers of virus added per cell and the length of exposure, we have attempted to achieve a valid comparison of the production capabilities of the various lines. Our study, which included cells of varied histories and origins, clearly indicated that, on a unit per cell basis, established human cell lines produce much less interferon than diploid human lines. The aneuploid lines produced low titers of interferon; only one of them (HCAAT) even approached the production of any of the presumed diploid lines. The diploid lines varied considerably among themselves with respect to interferon production, two normal synovial membrane lines (NS-1 and NS-3), and a human embryonic lung line (HEL) being exceptionally good producers. The average units per cell figured for all the established aneuploid lines was 3.7 X 10⁻⁴. The average for the diploid lines was 28 X 10⁻⁴.

The established quasidiploid tumor cell RPMI 2650 was unique in that it was the only cell in this study which produced no detectable interferon in any of the media used when challenged with the vaccine strain of NDV. Some further experiments were done with this line to determine whether it could produce interferon in response to a different challenge. No detectable interferon was produced when cultures were exposed to Sindbis virus or to polynosinic: polycytidylic acid by the method used by Youngner and Hallum (9). When cultures were exposed to the Roakin strain of NDV, a small amount of antiviral activity could be detected in supernatant medium, with a titer of less than 6.

For some experimental procedures and for ease of purification and concentration, it would be desirable to produce interferon in a medium free of any serum. We tested the interferon production of several aneuploid and diploid human cell lines in two serum-free media as well as medium with 5% fetal calf serum. Our findings are shown in Table 2. All of the cell lines which produced interferon in 5% serum medium also produced in serum-free medium. The diploid lines in most cases had considerably reduced titers with no serum, but there were two notable exceptions: the HEL line gave an excellent yield in 2XE medium and the VB line had increased titers in both NT and 2XE medium. The aneuploid cells were affected less by the omission of
### Table 1. Interferon yields from various human cell lines

| Type                               | Designation | Tissue of origin      | Units of interferon produced per cell |
|------------------------------------|-------------|-----------------------|---------------------------------------|
| Established aneuploid cell lines   | Chang liver<sup>b</sup> | Normal liver         | 12<sup>c</sup>                        |
|                                    | Chang (HT line)<sup>b</sup> | Derivative of Chang liver | 11                                    |
|                                    | Detroit 562<sup>b</sup> | Foreskin (Down's syndrome) | 4                                     |
|                                    | HeLa<sup>d</sup> | Carcinoma, cervix     | 5                                     |
|                                    | HCAAT<sup>b</sup> | Derivative of HeLa    | 135                                   |
|                                    | HEP-2<sup>b</sup> | Carcinoma, larynx     | 51                                    |
|                                    | Intestine 407<sup>b</sup> | Embryonic intestine   | 1                                     |
|                                    | J-111<sup>b</sup> | Monocytic leukemia    | 29                                    |
|                                    | KB<sup>r</sup> | Amnion                | 57                                    |
|                                    | WISH<sup>b</sup> | Carcinoma, oral       | 65                                    |
| Established quasidiploid cell line | RPMI 2650<sup>b</sup> | Tumor                 | 0                                     |
| Presumed diploid cell lines        | Detroit 532, passage 12<sup>c</sup> | Foreskin (Down's syndrome) | 217                                   |
|                                    | HEL, passage 28<sup>b</sup> | Embryonic lung        | 330                                   |
|                                    | HF-5, passage 8<sup>e</sup> | Foreskin (normal)     | 176                                   |
|                                    | HF-6, passage 28<sup>e</sup> | Foreskin (normal)     | 273                                   |
|                                    | NS-1, passage 4<sup>e</sup> | Synovial membrane (normal) | 425                                   |
|                                    | NS-3, passage 4<sup>e</sup> | Synovial membrane (normal) | 417                                   |
|                                    | VB, passage 22<sup>e</sup> | Synovial membrane (rheumatoid arthritis) | 119                                   |

<sup>a</sup> Passage numbers given only for finite, presumed diploid cell lines.
<sup>b</sup> Laboratory of D. J. Merchant.
<sup>c</sup> Values expressed X10<sup>-5</sup>.
<sup>d</sup> Laboratory of W. I. Schaeffer, University of Vermont.
<sup>e</sup> Laboratory of T. J. Moehring, University of Vermont.
<sup>f</sup> Laboratory of C. S. Stulberg, The Child Research Center, Detroit, Mich.
<sup>g</sup> Produced from tissue.

### Table 2. Interferon yields in serum-containing and serum-free media

| Cell line     | Units of interferon produced per cell |
|---------------|---------------------------------------|
|               | MEM<sub>c</sub> | FCS<sup>b</sup> | NT<sup>c</sup> | 2XE<sup>d</sup> |
| Diploid lines |Detroit 532, passage 12| 217 | 90 | 56 |
|               | HEL, passage 28| 330 | 174 | 399 |
|               | HF-5, passage 8| 176 | 38 | 35 |
|               | NS-1, passage 4| 425 | 127 | 202 |
|               | VB, passage 22| 119 | 259 | 259 |
| Aneuploid lines | KB| 65 | 71 | 76 |
|               | HEP-2<sup>e</sup>| 51 | 70 | 51 |
|               | HCAAT<sup>b</sup>| 135 | 102 | 145 |
|               | J-111<sup>b</sup>| 29 | 15 | 11 |
|               | WISH<sup>r</sup>| 57 | 57 | 57 |

<sup>a</sup> Values expressed X10<sup>5</sup>.
<sup>b</sup> Eagle minimum essential medium supplemented with 5% fetal calf serum.
<sup>c</sup> Neuman and Tytell serum-free medium (5).
<sup>d</sup> Eagle basal medium containing twice the original concentration of amino acids, vitamins and glutamine and no serum added (4).

In general, they made nearly the same amounts of interferon per cell in NT and 2XE medium as they did in BME with 5% fetal calf serum.

It has been noted that there seems to be a relationship between the ability of cells to produce interferon and their sensitivity to the action of externally applied interferon (8). It has also been noted that some continuous cell lines have a low sensitivity or are even totally insensitive to interferon action (3). We exposed 8 diploid lines, 10 aneuploid lines, and the quasidiploid tumor cell RPMI 2650 to serial dilutions of our standard human interferon preparation HIF-3. The results of these experiments are shown in Table 3. Although none of the cell lines tested was totally insensitive to the action of the interferon, it was evident that the established cell lines were much less sensitive than were the diploids. In general, it requires the application of approximately 10 times as many units of interferon to an established cell culture as are needed on a diploid culture to confer equal antiviral resistance. The quasidiploid tumor cell RPMI 2650 clearly falls in with the aneuploid lines with respect to its sensitivity to interferon. It is, how-
TABLE 3. Sensitivity of various cell lines to standard interferon HIF-3

| Cell line          | Titer of HIF-3 on this cell line |
|--------------------|----------------------------------|
| Diploid lines      |                                  |
| Detroit 532, passage 14 | 820                             |
| HEL, passage 24    | 100                              |
| HF-5, passage 8    | 368                              |
| HF-5, passage 26   | 500                              |
| HF-6, passage 26   | 640                              |
| NS-1, passage 6    | 310                              |
| NS-3, passage 6    | 520                              |
| RS-1*, passage 5   | 540                              |
| Quasidiploid line  |                                  |
| RPMI 2650          | 56                               |
| Aneuploid lines    |                                  |
| Chang liver        | 48                               |
| Chang (HT)         | 28                               |
| Detroit 562        | 48                               |
| HeLa               | 48                               |
| HCAAT              | 32                               |
| HEp-2              | 48                               |
| Intestine 407      | 22                               |
| J-111              | 17                               |
| KB                 | 43                               |
| WISH               | 82                               |

a Synovial membrane (rheumatoid arthritis).

ever, very distinctly sensitive to the action of interferon despite the fact that it is a virtual non-producer.

It has been observed (6) that human cell lines of embryonic origin are less sensitive to the action of interferon than are cell lines of neonatal origin. We included only one cell line of embryonic origin in our studies, HEL, a human embryonic lung line, and it is indeed considerably less sensitive to the action of HIF-3 than were the other presumed diploid lines of neonatal and adult origin. Siewers et al. (6) also observed that, when considering human diploid cell lines only, high sensitivity to interferon action did not correlate with ability to produce high titers of interferon in culture. This is also true of the diploid lines in our study: the most sensitive lines were not necessarily the best producers of interferon.

ACKNOWLEDGMENT

This investigation was supported by Public Health Service grant 7 ROI CA 11802-01 from the National Cancer Institute.

LITERATURE CITED

1. Hayflick, L., and P. S. Moorhead. 1961. Serial cultivation of human diploid cell strains. Exp. Cell. Res. 25:585–621.
2. Herrmann, E. C., J. Gablik, C. Engle, and P. L. Perlin. 1960. Agar diffusion method for detection and bioassay of antiviral antibiotics. Proc. Soc. Exp. Biol. Med. 103:625.
3. Ho, M. 1966. The production of interferon, p. 36–38. In N. B. Finter (ed.), Interferons. North Holland Publishing Co., Amsterdam.
4. Merchant, D. J., and K. B. Hellman. 1962. Growth of L-M strain mouse cells in a chemically defined medium. Proc. Soc. Exp. Biol. Med. 110:194–198.
5. Neuman, R. E., and A. A. Tytell. 1960. Serumless medium for cultivation of cells of normal and malignant origin. Proc. Soc. Exp. Biol. Med. 104:252–256.
6. Siewers, C. M. F., C. E. John, and D. N. Medearis, Jr. 1970. Sensitivity of human cell strains to interferon. Proc. Soc. Exp. Biol. Med. 133:1178–1183.
7. Vilcek, J. 1969. Synthesis of interferon, p. 31–32. In Interferon. Springer-Verlag, New York.
8. Vilcek, J. 1969. The action of interferon, p. 74–75. In Interferon. Springer-Verlag, New York.
9. Youngner, J. S., and J. V. Hallam. 1969. Inhibition of induction of interferon synthesis in L-cells pretreated with interferon. Virology 37:473–475.