Effects of Interferon on Cell and Virus Growth in Transformed Human Cell Lines

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(Accepted 30 April 1976)

SUMMARY

The anticellular and antiviral effects of human leukocyte interferons were studied in vitro in the transformed human embryonic cell lines, RSA and RSb. The growth of these cells was inhibited and they began to deteriorate about 48 h after treatment with 500 units/ml of interferon. When interferon was washed out within 48 h, their growth recovered gradually. The effects of interferon on cell growth depended on the amount of interferon added per cell. A subline, named IFr, was isolated which grows in the presence of 2000 units/ml of interferon, whereas growth of vesicular stomatitis virus in these cells is suppressed by 10 units/ml of interferon, just as in the parent cells. The anticellular and antiviral effects of interferon on IFr cells are discussed in relation to cell surface receptors.

INTRODUCTION

It has been well established that interferon preparations exert antiviral and anticellular effects on homologous cells (Gresser, 1972). Several workers have tried to separate these activities by fractionating interferon preparations through various purification procedures (Borecky et al. 1973; Gresser et al. 1973; Ohwaki & Kawade, 1972; Borecky, Fuchsberger & Hajnicka, 1974; Matsuzawa & Kawade, 1974). Others have studied antiviral and anticellular expression in cells treated with interferon. Thus Fuchsberger, Borecky & Hajnicka (1974) described variant cell lines which were not inhibited in growth, but retained sensitivity to the antiviral effect of interferon. Gresser, Bandu & Brouty-Boyé (1974) isolated sublines of murine leukaemia L1210 cells which were resistant to the inhibitory effect of interferon on cell division and also to its antiviral effects.

We have established two clonal cell lines, RSA and RSb, from human embryonic cells transformed by Rous sarcoma virus and simian virus 40 (Kuwata et al. 1976). We report here the anticellular effects of human interferon on these cells under different conditions, and we attempt to explain the relation between these effects and its antiviral effects.

METHODS

Cells. Details of the biological characteristics of the two clonal cell lines, RSA and RSb, have been described (Kuwata et al. 1976). Cells were cultivated in 6 cm or 10 cm diam. plastic plates (Falcon) with Eagle’s Minimal Essential Medium (MEM) supplemented with 10% calf serum. For comparison, human fibroblast cultures of different origins were used in their early passages.
Fig. 1. Effect of human leukocyte interferon on the growth of RSB and normal human embryo cells. (a) RSB cells grown in the presence of various concentrations of interferon: O—O, control cells; △—△, 200 units/ml; □—□, 500 units/ml; ●—●, 1000 units/ml; ▲—▲, 2000 units/ml. (b) RSB cells treated with mock interferon and embryo cells treated with interferon: O—O, control RSB cells; ●—●, treated with mock interferon at the concentration equivalent to 1000 units/ml of interferon; O—O, control embryo cells; ●—●, treated with 1500 units/ml of interferon. —, Period of interferon treatment.

Interferon preparations. Human leukocyte interferon preparations were kindly supplied by Dr Kari Cantell of the Central Public Health Laboratory, Helsinki, Finland. The partially purified preparations (p-IF; Cantell et al. 1974) contained 13 to 15 x 10^6 units/ml, and had a sp. act. of 6.0 to 7.2 x 10^5 units/mg protein.

Mock interferon. Mock interferon, also provided by Dr K. Cantell, was prepared by treating leukocyte suspensions as in the production of interferon, except that Sendai virus was added only at the end of the incubation period. It was concentrated and purified in exactly the same way as the p-IF preparations.

Assay of anticellular activity of interferon. 10^5 to 10^6 cells were seeded into 6 cm Falcon plastic plates, which were divided on the following day into two groups. In one group, the culture medium was exchanged for fresh medium containing various concentrations of interferon. In the other group, which served as a control, the culture medium was exchanged for medium without interferon. Thereafter, cell multiplication was followed by counting, in a haemocytometer, the total number of cells detached from two plates with trypsin.

Virus. The Indiana strain of vesicular stomatitis virus (VSV) was obtained from Dr Shudo Yamazaki of the National Institute of Health of Japan. It was grown in monolayers of primary chick embryo cells and stored at -75 °C. For virus titrations, Linbro multi-well plastic trays (Fb-16-24-TC) were seeded with approx. 10^5 cells in each well, and VSV dilutions were added 2 days later. Virus titres are expressed as TCD_50/0.2 ml.

Recovery of cell-bound interferon. About 10^6 cells were seeded into 10 cm diam. plastic plates. When the cell sheets became confluent, they were treated with interferon for 2 h and then washed three times with MEM. The cells were scraped off into 10 ml of MEM with a
rubber policeman. The suspension was centrifuged at 800 rev/min for 10 min, and the cell pellet was resuspended in 2 ml of MEM, sonicated for 20 s (20 kHz, 15 W), and stored at −75 °C until assayed for antiviral activity.

RESULTS

Susceptibility of RSb cells to the anticellular action of human leukocyte interferon

The effects of various dilutions of a human leukocyte interferon preparation were compared on RSb cells and on normal human embryonic cells (Fig. 1). The growth of the RSb cells was reduced in proportion to the concentration of interferon applied, and when these cells were treated with more than 500 units/ml of interferon, many died. The mechanism underlying these phenomena is analysed in the following paper (Fuse & Kuwata, 1976). In contrast, normal embryonic cells (as well as fibroblasts from adult endometrium – data not shown) were less susceptible to the interferon preparation, as found by other investigators (e.g. Lee, O'Shaughnessy & Rozee, 1972), and were not killed by the treatment. Mock interferon preparation had no noticeable effect on the growth of RSb cells.

Cell density and interferon effects

RSb cells in cultures of two different densities were treated with the same dose (500 units/ml) of interferon and their growth was followed. As shown in Fig. 2, interferon suppressed growth in the cultures with $10^6$ cells, in agreement with findings in other interferon-cell systems (Gresser, 1972). However, with $2 \times 10^6$ cells, the interferon preparation also led to
Fig. 3. (a) Control and (b) interferon-treated RSb cells at the 9th day in the experiment shown with broken line in Fig. 2. Magnification x 370.

the death of the cells. Gresser et al. (1970) and Strander & Cantell (1974) also noticed that the effect of interferon on cell growth was more pronounced at low cell concentrations. There was some variability in the susceptibility of cells to 500 units/ml of interferon on different occasions as seen in Fig. 1 (a) and Fig. 2. One of the factors responsible may possibly be the particular cultural stage of the cells when seeded.

When 10^6 cells were seeded per plate and treated with 2500 units/ml of interferon, their
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Fig. 4. Effect of duration of interferon treatment on the growth of RSb cells. ○——○, Control cells. Cells treated with interferon: (a) with 500 units/ml for two (□——□) or three (□——□) days; (b) with 1000 units/ml for two (○——○) or three (○——○) days. •—•, Period of interferon treatment.

growth was suppressed during the first 2 to 4 days after addition of the interferon (Fig. 2) and some changes in their morphology began to appear. After 5 to 6 days the number of cells in the plates began to decrease and the cells became obviously degenerate. (Fig. 3).

Effect of the duration of interferon treatment

In the experiments described above, the cells were treated continuously with the interferon preparation. In the following experiments, cells were treated for only 2 or 3 days with interferon and were then washed twice with culture medium. When RSb cells were treated with 500 units/ml of interferon for 3 days, the cells first decreased in numbers and then recovered (Fig. 4a). Cells treated with 1000 units/ml of interferon for 3 days did not recover (Fig. 4b). Almost the same results were obtained with RSA cells.

A few RSb cells survived after treatment with 2000 units/ml of interferon for 3 days. These cells were then grown on interferon-free medium. Their susceptibility to interferon was tested and they were found to be as interferon-sensitive as the parent RSb cells.

Isolation of interferon-resistant cells

An interferon-resistant subline, IF^r, was derived by treating RSA cells with 200 units/ml of interferon and then gradually increasing the concentration of interferon applied. As shown in Fig. 5, IF^r cells were partially resistant to 2000 units/ml of interferon. IF^r cells have a morphology and growth pattern similar to that of the parental RSA cells. When 2 to 3 x 10^5 cells were seeded on to 6 cm Petri dishes and fed with interferon-free culture medium, they grew continuously for about 14 days and reached the same final number (2 to 5 x 10^7 cells/plate) as RSA cells. To maintain their interferon-resistant property, IF^r cells have usually been passaged in the presence of 1000 units/ml of interferon, though interferon was not always added. Passages of IF^r cells in the absence of interferon during a period of a month did not change their biological characteristics.
Fig. 5. Comparison of RSa and IF* cells in susceptibility to interferon. (a) RSa cells: ○—○, control cells; Δ—Δ, treated with 200 units/ml of interferon; □—□, treated with 500 units/ml interferon. (b) IF* cells: ○—○, control cells; ●●●●, treated with 1000 units/ml of interferon; △—△, treated with 2000 units/ml of interferon; —, period of interferon treatment.

Table 1. Comparison of VSV multiplication in interferon-sensitive RSa and interferon-resistant IF* cells

<table>
<thead>
<tr>
<th>Cells*</th>
<th>Interferon treatment (Units/ml)</th>
<th>Virus titre (log TCD₅₀/0·2 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>RSa</td>
<td>None</td>
<td>2·7</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>—</td>
</tr>
<tr>
<td>IF*</td>
<td>None</td>
<td>3·2</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>—</td>
</tr>
</tbody>
</table>

* Duplicate cultures with 2·0× 10⁶ cells/plate were treated with various concentrations of interferon for 24 h at 37 °C, washed once with MEM, and then infected with VSV at a multiplicity of 1·0. After 1 h of virus adsorption at 37 °C, cells were washed once with MEM and then MEM with 5 % calf serum was added to each culture.
† Cells were completely damaged and virus yields were not measured.

The susceptibility of IF* cells to the antiviral activity of interferon

When cells were not treated with interferon, VSV could multiply as well in IF* cells as in RSa cells (Table 1). When these cells were treated with 10 to 1000 units/ml of interferon before challenge with VSV, there appeared to be some differences in their virus susceptibility (Table 1), but this was not confirmed in other tests. K. Cantell (personal communication,
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Table 2. Recovery of interferon after 2 h from interferon-sensitive and resistant cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>Number of cells/plate</th>
<th>Interferon dose applied</th>
<th>Interferon recovered from sonicated cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSA</td>
<td>2.0 \times 10^7</td>
<td>1000</td>
<td>128</td>
</tr>
<tr>
<td>RSb</td>
<td>1.6 \times 10^7</td>
<td>1000</td>
<td>64</td>
</tr>
<tr>
<td>IFr</td>
<td>8.0 \times 10^5</td>
<td>1000</td>
<td>6</td>
</tr>
</tbody>
</table>

1975) assayed, by the plaque reduction method, a sample of his leukocyte interferon with our RSA, RSb, IFr cells and with his U line of human amnion cells; he observed little, if any, significant difference between their susceptibility to interferon.

Recovery of interferon from treated cells

IFr cells were thus apparently resistant to the anticellular but not to the antiviral activity of human interferon. To clarify these contradictory phenomena, the binding of interferon to these cells was investigated. As shown in Table 2, about 1.28 to 2.50 % of the total number of units of interferon applied were recovered from the interferon-sensitive RSA and RSb cells, whereas only 0.1 % or less was recovered from IFr cells.

DISCUSSION

Interferon preparations have a variety of activities in cells (De Clercq & Stewart, 1973). As reported in a previous paper (Kuwata et al., 1976), human interferon preparations had anticellular effects on human cell lines, but not on Rous sarcoma virus transformed mouse cells. The cell-growth depressing effects were proportional to the antiviral activity against VSV infection and were sensitive to trypsin treatment or heating at 60 °C for 30 min. Mock interferon had no anticellular activity (Fig. 1). When these results are taken in conjunction with others already reported (e.g. Adams, Strander & Cantell, 1975), it seems likely that the suppression and subsequent decrease of RSb cells are actually due to the interferon present in the preparations used.

Several factors influenced the expression of the anticellular action of interferon on RS cells. The density of the cell sheet is one of them, and another is the amount of interferon added per cell (see Fig. 1 and 2). To suppress cell growth a certain amount of interferon must presumably be bound to the cell surface, as verified for its antiviral action (Friedman, 1967; Stewart, De Clercq & De Somer, 1972; Chany et al., 1973; Berman & Vilcek, 1974). When there are more cells in a culture, more interferon is needed to affect them. When treatment of cells with interferon was limited to 2 days, the effect of interferon on the cells was rather slight, but with treatment for 3 days, cells were affected more markedly. These results suggest that interferon may affect cells in a certain stage of the cell cycle, and we are now analysing this by using synchronized cultures. The effect of interferon on the synthesis of macromolecules in RSA cells is described in the following paper (Fuse & Kuwata, 1976). In early passages, RSb cells were more susceptible to interferon preparations than in later passages and even 100 units/ml of interferon had marked anticellular effects. The stability of the susceptibility of these cells to interferon will be investigated further.

The IFr subline of RSA cells is resistant to the anticellular action of interferon, though still susceptible to its antiviral effect. This may be due to the fact that IFr cells apparently
bind less interferon than RSa or RSB cells (Table 2). The existence of interferon-binding sites at the cell surface has already been shown by those who have recovered interferon from treated cells (Stewart et al. 1972; Berman & Vilcek, 1974). Perhaps for interferon to express its antacellular effect, there must be a certain number of interferon-binding sites on the cell surface, while for the antiviral activity, fewer binding sites may be required. Gresser et al. (1974) isolated from L1210 cells several sublines which are insensitive to both the antacellular and antiviral effects of interferon. This may be due to the complete loss of interferon-binding sites in these resistant cells, because they could not recover interferon from them after interferon treatment. The relative effects of interferon on different cells may be associated with the number of interferon-binding sites. Adams et al. (1975) examined the susceptibility of 14 lymphoblastoid cell lines to human leukocyte interferon. Six of these were extremely sensitive while the remaining 8 were resistant to 10000 or more units per ml of interferon. However, it is not clear whether or not the susceptibility of cells to interferon from the homologous species depends exclusively on the number of interferon-binding sites. Our RSa and RSB cells are sensitive to human interferon, but from our data on the recovery of applied interferon, these cells do not seem to contain exceptionally large numbers of interferon-binding sites in comparison with results obtained with a human amnion cell line (Berman & Vilcek, 1974). This suggests that there may be some regulatory system in cells which control their sensitivity to interferon in addition to the role of the number of binding sites on the cells.

Alternatively, there still remains another possibility. As Stewart (1974) and Knight (1975) suggested, interferon preparations may not be composed of homogeneous molecules: there may be some interferon polypeptides which have only antiviral activity, while others may have both antiviral and antacellular activities. IfF' cells might thus be resistant to the antacellular fraction of interferon polypeptides, but remain susceptible to the antiviral fraction. Fuchsberger, Hajnicka & Borecky (1975) also discussed such a possibility. Further purification of human interferon may clarify this problem.

We wish to thank Dr K. Cantell for his interest and constant support in this work. We are grateful to Dr N. B. Finter for his advice and help in preparing manuscript. The work was supported by the Research Grant from the Ministry of Education, Japanese Government.

REFERENCES

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(Received 29 January 1976)