Initial Interaction of Human Fibroblast and Leukocyte Interferons with FS-4 Fibroblasts

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SUMMARY

Human FS-4 cells were exposed to human fibroblast interferon for various times and further incubated in the absence of interferon until challenged with vesicular stomatitis virus. Addition of antibody to fibroblast interferon at the time of removal of interferon did not alter the development of the antiviral state. If cells were exposed to interferon for 45 min at either 0 or 37 °C, they developed resistance upon subsequent incubation at 37 °C. However, less resistance developed if the cells were initially incubated at 0 °C. Our results indicate that a single interaction of fibroblast interferon with susceptible cells, either at 0 or 37 °C, is sufficient for the subsequent development of an antiviral state, at least in the short term experiment.

The kinetics of development of the antiviral state were compared with fibroblast and leukocyte interferon. The rise in the degree of antiviral resistance was steeper and maximal levels of resistance were reached sooner when FS-4 cells were incubated with increasing concentrations of fibroblast interferon than with leukocyte interferon. This suggests a greater affinity of fibroblast interferon for these cells.

INTRODUCTION

Although many details of the pathways leading to the induction of antiviral resistance by interferon are still unknown, an interaction of the interferon molecule with the surface of target cells must be the first event occurring in this process. It is possible that interaction of interferon with a specific receptor site on the cell surface is the crucial event that triggers the expression of cellular functions leading to the antiviral state and other alterations associated with interferon action.

Different interferons exert variable degrees of antiviral activity in different types of cells in culture (Edy et al. 1976; Vilček et al. 1977a). These differences could be partly due to various affinities of interferons and cells. The binding of interferons to cells can be studied directly. The usual experimental approach involves incubation of cells with interferon for a short time; the cells are then washed to remove all unattached interferon and the amount of bound interferon is measured in the cell homogenate. Such studies showed that human fibroblast or mouse interferon attaches to homologous cells in culture quite rapidly at 37 °C, the amount of cell-bound interferon reaching a maximum in about 30 min. When interferon is removed from the culture fluid, the cell-bound interferon elutes (Stewart et al. 1972; Berman & Vilček, 1974; Kohno et al. 1975). The drawback of this experimental approach is that it may be difficult to distinguish between specific and non-specific binding.

In a recent study Dianzani & Baron (1977) concluded that a short exposure of cells to human leukocyte interferon at 37 °C could make them resistant to virus infection. However, cells exposed to interferon briefly at 0 °C did not develop the antiviral state upon subsequent
incubation at 37 °C unless interferon was allowed to elute from the cells into the culture medium and re-attach at 37 °C.

One aim of this study was to determine whether the development of the antiviral state during exposure of human fibroblasts to fibroblast interferon entails (a) a single interaction of the interferon molecule with a target cell, or (b) several cycles of elution and re-attachment of the same interferon molecule. Our results do not support this second contention. Also, from the kinetics of the development of the antiviral state and other results, we conclude that fibroblast interferon has a higher affinity for cultured human fibroblasts than leukocyte interferon.

METHODS

Cell cultures. Cells of the FS-4 strain of human diploid fibroblasts (Vilček & Havell, 1973) were grown in Eagle's minimal essential medium (MEM) containing 5% heat-inactivated foetal bovine serum (FBS), 2.5 μg/ml amphotericin B (fungizone) and 10 mM-HEPES [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid]. Experiments were performed with confluent cell cultures, usually 12 days old, grown in 35 mm plastic Petri plates (Falcon Plastics, Los Angeles, Calif., U.S.A.) in a 37 °C humidified incubator provided with 5% CO₂. During the experiments, cultures were kept in 1 ml maintenance medium consisting of MEM supplemented with 2% FBS and 10 mM-HEPES. When incubation at 0 °C was required, cultures were placed on crushed ice. Mouse L cells grown in 60 mm plastic Petri dishes were employed in the plaque assay for vesicular stomatitis virus (VSV). All tissue culture media and sera were purchased from Grand Island Biological Co., Grand Island, N.Y.

Interferons. Human fibroblast interferon was induced in FS-4 cells grown in roller bottle cultures, using poly(rI), poly(rC) and superinduction with metabolic inhibitors as described by Havell & Vilček (1972). The interferon used in this study was concentrated by ultrafiltration in an Amicon model 2000 stirred cell with PM 10 membrane and had a final titre of $1 \times 10^5$ units/ml and a specific activity of about $1 \times 10^4$ units/mg protein. Human leukocyte interferon with a titre of $6 \times 10^4$ units/ml and a specific activity of $2.4 \times 10^4$ units/mg protein was kindly provided by Dr Kari Cantell. Leukocyte interferon preparations contain a small fraction (<1% of total activity) of fibroblast-type interferon (Berg et al. 1975; Havell et al. 1975) and to abolish its activity, 0.1% of antiserum to fibroblast interferon was added to the preparation of leukocyte interferon before use. This did not affect the activity of the major component of the leukocyte interferon preparation.

Interferon titrations. Both types of human interferons were titrated in a semi-microassay based on inhibition of VSV cytopathic activity in the FS-7 strain of human foreskin fibroblasts (Armstrong, 1971; Havell & Vilček, 1972). A human leukocyte interferon standard G-023-901-527, obtained from the National Institute of Allergy and Infectious Diseases, was used as the reference preparation for both leukocyte and fibroblast interferon and all results are expressed in units in terms of this standard.

Anti-interferon sera. An antiserum to interferon obtained from a rabbit immunized with highly purified fibroblast interferon (Vilček et al. 1977b) was kindly supplied by Dr Edward A. Havell. The neutralization titre of this serum was 16000, i.e. this was the reciprocal of the highest dilution of antiserum that inhibited the antiviral activity of 10 units/ml of fibroblast interferon, after incubation for 1 h at 37 °C. When used at a dilution of 1/100, it completely inhibited the activity of 30 units/ml of interferon within 15 min. Antiserum to leukocyte interferon prepared in sheep (Anfinsen et al. 1974), kindly supplied by Dr C. B. Anfinsen, had a neutralizing titre of 20000 against homologous interferon. Neither antiserum neutralized the activity of the heterologous human interferon. In all experiments, controls were run to ensure that interferon at the concentration used was indeed neutralized by the antibody concentration employed.
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**RESULTS**

Effect of antibody to interferon on the development of the antiviral state

Groups of FS-4 cell cultures were exposed to fibroblast interferon (30 units/ml) at 37 °C for varying times. When the interferon was removed, excess antibody to fibroblast interferon was added to one half of the cultures. All cultures were inoculated with VSV at 6 h 45 min after the original time of exposure to interferon. The results of two independent experiments (Fig. 1) showed that there was a very rapid rise in resistance to VSV in the first 5 min of exposure to interferon, followed by a gradually diminishing increase upon further incubation. The addition of interferon antibody at the time of removal of interferon did not alter the rate of development or the final level of the antiviral state. Similar results were obtained using a dose of 300 units/ml of fibroblast interferon (data not shown).

Effect of the incubation temperature on the interaction of interferon with cells

Cultures were exposed to fibroblast interferon (30 units/ml) at 0 or 37 °C for 45 min. Thereafter, the cells were washed and further incubated at 37 °C with or without antibody to fibroblast interferon. To determine the degree of antiviral resistance established, groups of cultures were inoculated with VSV immediately after removal of the interferon (zero time) and at different times thereafter, and the virus yields were determined. The results indicated that cells exposed to interferon at either 0 or 37 °C developed resistance when
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Fig. 2. The rate of development of the antiviral state in cells exposed to fibroblast interferon (30 units/ml) at 0 °C (○, ●) or 37 °C (▲). After 45 min incubation with interferon (zero time on the abscissa) the cells were washed and replenished with maintenance medium (○, ▲) or medium containing 1% antibody to fibroblast interferon (○), and further incubated at 37 °C. At the times indicated the cells were washed and challenged with VSV.

subsequently incubated at 37 °C (Fig. 2). However, there were clear differences in the rate of development and magnitude of the antiviral state produced after initial exposure of cells to interferon at 0 or 37 °C. In cells exposed at 37 °C, there was a near maximal antiviral state after 45 min incubation with interferon (zero time in Fig. 2). In contrast, cultures incubated with the same dose of interferon at 0 °C were fully sensitive to virus at the end of the initial 45 min incubation and further incubation at 37 °C was required for the antiviral state to be established. Again, no differences were observed in the antiviral state developed in the presence or absence of antibody to interferon. However, the maximum level of antiviral resistance was significantly lower in the cultures exposed to interferon at 0 °C than in those exposed at 37 °C.

Effect of temperature on the interaction of cells with varying doses of interferon

To determine if this lower degree of resistance in cells exposed to fibroblast interferon at 0 °C could be the function of the amount of interferon bound, cultures were incubated with various concentrations of interferon at either 0 or 37 °C for 45 min, washed free of extracellular interferon and further incubated with or without antibody to fibroblast interferon at 37 °C. After enough time had been allowed for the development of the antiviral state all cultures were inoculated with VSV (Fig. 3). With interferon doses between 3 and 100 units/ml, the antiviral state was greater in cells exposed to interferon at 37 °C. Cells incubated with interferon at 37 °C attained a maximum demonstrable resistance to VSV with interferon doses between 30 and 100 units/ml. Cells treated with interferon at 0 °C reached the same level of resistance only after contact with 300 units/ml. Development of the antiviral state cannot have required elution and re-attachment of interferon because the presence of antibody to interferon did not significantly alter the degree of the antiviral state developed.

The effect of antibody to interferon on the maintenance of the antiviral state

Cultures were incubated with 10 units/ml of fibroblast interferon at 37 °C. At 5 h, enough antibody to fibroblast interferon was added to one group to neutralize all interferon present, whereas other cultures were further incubated in the presence of the original interferon-
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Fig. 3. Resistance to VSV challenge in cells exposed to various concentrations of fibroblast interferon at 0 °C (Δ, ▲) or at 37 °C (○, ●). After 45 min the cells were washed, replenished with 1 ml of maintenance medium (○, Δ) or medium containing 1 % antibody to interferon (●, ▲) and further incubated at 37 °C. At 6 h all cultures were washed and challenged with VSV.

Fig. 4. The effect of antibody to interferon on the maintenance of the antiviral state. Cultures were incubated with 10 units of fibroblast interferon in 1 ml of medium (○). In other cultures antibody to fibroblast interferon was added at 5 h (■). On the day indicated, cultures were drained, washed and challenged with VSV. Control cultures that had received no interferon treatment were inoculated with VSV on each day.

Fig. 3- Resistance to VSV challenge in cells exposed to various concentrations of fibroblast interferon at 0 °C (Δ, ▲) or at 37 °C (○, ●). After 45 min the cells were washed, replenished with 1 ml of maintenance medium (○, Δ) or medium containing 1 % antibody to interferon (●, ▲) and further incubated at 37 °C. At 6 h all cultures were washed and challenged with VSV.

Fig. 4. The effect of antibody to interferon on the maintenance of the antiviral state. Cultures were incubated with 10 units of fibroblast interferon in 1 ml of medium (○). In other cultures antibody to fibroblast interferon was added at 5 h (●). On the day indicated, cultures were drained, washed and challenged with VSV. Control cultures that had received no interferon treatment were inoculated with VSV on each day.

containing culture fluid without medium change and without the addition of antibody. The antiviral state in the two groups was compared at daily intervals in terms of the inhibition of VSV yield (Fig. 4). The antiviral state decayed faster in the presence of the antibody, suggesting that extracellular interferon plays an important role in the long-term maintenance of the antiviral state. The antiviral state in cells which did not receive antibody also decreased gradually, albeit at a slower rate. Although the levels of interferon in the culture fluids were not measured at the time of the VSV inoculation, this decrease in the antiviral state occurring in the continuous presence of interferon may in part be due to the gradual inactivation of interferon in the culture fluids.

Effect of incubation temperature on the interaction of leukocyte interferon with cells

The above experiments employed fibroblast interferon. In light of previous studies with leukocyte interferon (Dianzani & Baron, 1977) we compared some aspects of the action of leukocyte and fibroblast interferons in FS-4 cells. Groups of cultures were exposed to either leukocyte or fibroblast interferon (100 units/ml) at 0 or 37 °C for 45 min. The cultures were then washed and further incubated at 37 °C. The cells were inoculated with VSV 4 h after exposure to interferon (Table 1). There was only a small difference in the antiviral state developed in cells exposed to fibroblast interferon at 0 or 37 °C. In contrast, cells exposed to a comparable dose of leukocyte interferon developed a lesser degree of antiviral resistance at 37 °C and virtually no antiviral resistance after 45 min contact at 0 °C. However, cells incubated with leukocyte interferon for 6 h at 37 °C developed the same degree of antiviral state as cells exposed to comparable doses of fibroblast interferon (see Fig. 5).
Table 1. The antiviral state developed after 45 min contact with 100 units/ml of fibroblast or leukocyte interferon

<table>
<thead>
<tr>
<th>Interferon</th>
<th>Incubation temperature during contact (°C)</th>
<th>VSV yield (log p.f.u./ml)</th>
<th>Log inhibition of VSV yield</th>
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</thead>
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<tr>
<td>None</td>
<td>--</td>
<td>5.8</td>
<td>--</td>
</tr>
<tr>
<td>Fibroblast</td>
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<td>3.7</td>
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</tr>
<tr>
<td></td>
<td>0</td>
<td>4.0</td>
<td>1.8</td>
</tr>
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<td>Leukocyte</td>
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<td></td>
<td>0</td>
<td>5.6</td>
<td>0.2</td>
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Fig. 5. Kinetics of development of the antiviral state in FS-4 cells after incubation with various concentrations of (a) fibroblast interferon or (b) leukocyte interferon. Cultures were exposed to 10 units/ml (∆), 30 units/ml (○) or 100 units/ml (●) of interferon for the indicated times, then washed, replenished with maintenance medium and challenged at 360 min with VSV.

Kinetics of development of the antiviral state with leukocyte or fibroblast interferon

Cell cultures were exposed to three different concentrations of leukocyte or fibroblast interferon for varying times at 37 °C. At the time of removal of the interferon the cells were washed and replenished with medium containing 2% FBS. All cultures were inoculated with VSV 6 h after the initial exposure to interferon and the virus yields were determined (Fig. 5). The kinetics of development of the antiviral state with various concentrations of fibroblast interferon resembled the results shown in Fig. 1. There was a sharp rise in resistance in the first 5 min of exposure to interferon followed by a more gradual increase until about 135 min and a subsequent levelling off in the degree of resistance attained. In contrast, with leukocyte interferon the development of the antiviral state was much more gradual throughout the 6 h exposure of cells to interferon. These differences with the two interferons probably reflect a lower affinity of leukocyte interferon for FS-4 cells.

Discussion

Induction of the antiviral state by interferon requires (a) contact of interferon with target cells and (b) an incubation period that leads to the state of resistance to virus infection. Under some conditions these two events can be delineated from each other. For instance, contact of cells with interferon at 0 to 4 °C usually will result in cellular resistance to viruses, but only if, after contact with interferon at the low temperature, cells are further incubated...
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at 37 °C (Vilček & Rada, 1962; Friedman, 1967). It is quite generally assumed that during the initial contact interferon binds to cells (possibly via specific receptors on the cell surface) and that binding is a prerequisite for subsequent development of antiviral resistance. By measuring the amount of interferon recovered from extracts of cells exposed to interferon several groups of investigators concluded that interferon binds to cells more rapidly at 37 than at 4 °C (Stewart et al. 1972; Berman & Vilček, 1974; Kohn et al. 1975).

Recently Dianzani & Baron (1977) reported that the same amount of human leukocyte interferon bound to fibroblasts in culture at 0 °C and at 37 °C. They also found that if antibody to interferon was added at the time when interferon was removed from cells after contact at 0 °C, the development of the antiviral state was prevented and they suggested that resistance established in cells exposed to leukocyte interferon at 0 °C is caused by interferon which eluted from the cell surface upon subsequent incubation at 37 °C. Our results using fibroblast interferon indicate that initial interaction of interferon with fibroblasts at 0 °C is sufficient to establish antiviral activity upon further incubation at 37 °C. This conclusion is based on the finding that addition of antibody to fibroblast interferon after initial contact of cells with interferon at 0 °C did not significantly alter the development of the antiviral state. Antibody to interferon present in the culture medium would rapidly neutralize interferon eluted from cells and prevent its contact with other cells. Thus under these conditions fibroblast interferon apparently did not need to elute and re-attach to cells in order to produce antiviral activity.

On the basis of the degree of antiviral state which developed on subsequent incubation at 37 °C, it can be concluded that the affinity of interferon for cells is probably lower at 0 °C than at 37 °C, since less resistance developed after contact of cells with fibroblast interferon at 0 °C than after initial contact with the same interferon dose at 37 °C (Fig. 3). Since non-specific attachment could not be ruled out in previous studies which measured the amount of interferon recovered from homogenates of cells exposed to interferon, our present system probably measures more accurately the specific binding of interferon to cellular receptors.

Results obtained in our experiments with leukocyte and fibroblast interferon suggest that fibroblast interferon may have a higher affinity for FS-4 cells than leukocyte interferon. This conclusion is based on the more rapid development of antiviral state with fibroblast interferon. Our experiments corroborate previous reports that human fibroblasts differ in the response to fibroblast and leukocyte interferons (Edy et al. 1976; Vilček et al. 1977a). Einhorn & Strander (1977) observed that fibroblast interferon has greater cell growth inhibitory activity on osteosarcoma cell lines than leukocyte interferon; conversely, leukocyte interferon inhibited the growth of lymphoblastoid cells to a greater extent than fibroblast interferon. They suggested that fibroblast and leukocyte interferon may be to some degree tissue-specific, with leukocyte interferon having a greater activity in cells of lymphoid origin and fibroblast interferon being more active in some other types of cells. These differences may also be due to different binding affinities of the two interferons for various cells. Thus, although our results show that fibroblast interferon is more efficient in producing an antiviral state in cultures of human fibroblasts than leukocyte interferon, the order of efficiency with which these two interferons exert biological activity in cells of lymphoid origin may be reversed.

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