Suppressive Effects of Interferon on Syncytium Formation by RD-114 Virus in Human Transformed Cells

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SUMMARY

The RD-114 virus rapidly induced syncytia in the human transformed cell lines, RSa, RSb and IFr. Treatment of the virus with heat or ultrasonic vibration completely eliminated the syncytium-forming activity. Irradiation with u.v.-light or treatment with β-propiolactone (BPL) reduced but did not completely destroy the activity. Pre-treatment of the cells for 16 h with 25 to 500 units/ml of human leucocyte interferon (Le-IF) or fibroblast interferon (F-IF) significantly reduced formation of syncytia by active virus or inactivated (u.v. or BPL) virus. This activity of interferon was inhibited by treatment of the cells with cycloheximide. Interferon did not increase the binding of 3H-uridine-labelled RD-114 virus to the cells. It is postulated that interferon treatment altered the plasma membrane of the cells and thus reduced their capacity to fuse.

INTRODUCTION

RD-114 virus was first isolated as a typical type C oncornavirus from a line of human rhabdomyosarcoma cells (McAllister et al. 1972), and was subsequently identified as an endogenous virus of the domestic cat (Fischinger et al. 1973; Okabe et al. 1973), closely resembling an endogenous baboon type C virus (Benveniste & Todaro, 1974). This virus (Rand & Long, 1972; 1973) and other type C viruses (Ahmed et al. 1974; Rand et al. 1974) have been shown to induce syncytium formation in Rous sarcoma virus transformed human cells (KC cells), when virus-producing cells were co-cultivated with KC cells or when concentrated virus was added to KC cell cultures. Rand & Long (1973) found that the ability of RD-114 virus to induce syncytia in KC cells is sensitive to trypsin, heat or ultrasonic vibration, but not to BPL. Furthermore, under conditions where macromolecular synthesis was inhibited, KC cells still fused in response to RD-114 virus. It has been proposed that the mode of fusion is similar to that of KC cells by murine leukaemia virus (Rand & Long, 1973).

Interferons cause various effects on cells such as development of an antiviral state (Friedman, 1977), inhibition of cell and tumour growth (Paucker et al. 1962; Kuwata et al. 1976b; Gresser, 1977a; De Maeyer-Guignard et al. 1978) and regulation of the immune system (Gresser, 1977b). Recently, it has been shown that interferon treatment of cells can alter their plasma membrane, and these changes have been discussed in relation to the development of the antiviral state (Lindahl et al. 1973; Kohn et al. 1976; Pitha et al. 1976; Knight & Korant, 1977). Since cell fusion is a membrane function, we decided to investigate the possibility that it is also influenced by interferon. A good opportunity to carry out such a study was offered to us since we had established lines of virus-transformed human cells.
(RSa, RSB and IFr) which are nonproducer and also highly sensitive to the anti-cellular action of interferon and to fusion by RD-114 cells. In the present paper, we report that fusion is induced in our transformed human cells by RD-114 virus but that this is significantly suppressed by pre-treatment with human interferon. An alteration in the plasma membrane of the cells is discussed in relation to this activity of interferon.

METHODS

Cells. The cells used have been described in detail by Kuwata et al. (1976a, b). RSa and RSB cells were derived from human embryonic fibroblasts doubly transformed by Rous sarcoma virus and simian virus 40; IFr cells were isolated from RSa cells and are relatively insensitive to the anticellular effects of Le-IF. RD-114 cells were a gift from Dr T. Takano, Keio University, Tokyo, Japan. These cells were cultivated in plastic dishes (60 or 100 mm diam.) with Eagle's minimum essential medium (MEM) supplemented with 10% calf serum in a humidified atmosphere containing 5% CO2.

Preparation of RD-114 Virus. Culture fluids from RD-114 cells were harvested at 24 h intervals and clarified by low speed centrifugation at 8000 g for 10 min. Then, the virus was pelleted by centrifugation at 27000 rev/min for 90 min in a Spinco SW-27 rotor and resuspended in culture medium by shaking for 30 min at room temperature. The 20-fold concentrated suspension was homogenized using a Potter's homogenizer in an ice bath and stored at −80 °C.

Direct syncytium assay. Cultures of RSa and IFr cells were grown to about 90% confluence in 60 mm dishes and 1.5 ml of concentrated RD-114 virus was added. After incubation at 37 °C for 20 h, the cells were fixed with methanol and stained with 10% Giemsa stain. The number of syncytia which contained four or more nuclei were counted using a microscope at 100× magnification. The total number of syncytia per dish was calculated from the average number in twenty randomly chosen square fields (about 38 mm²) in two dishes. The number in control cultures was subtracted from the number in test cultures. Unconcentrated virus preparations were incubated with cells for 2 to 3 days and the number of syncytia was counted.

Heat inactivation. Concentrated RD-114 virus was treated in a water bath at 56 °C for 30 min or at 100 °C for 5 min, then chilled on ice. Heated virus in 1.5 ml amounts was added to about 90% confluent monolayers of RSa and IFr cells to test for syncytium formation.

Inactivation by ultrasonic vibration. About 9 to 15 ml of virus preparation were sonicated on ice at 20 kHz, 100 W for 2 to 15 min. At various time intervals, 3 ml of the sample were withdrawn and used for syncytium assay.

β-propiolactone treatment. The procedure of Rand & Long (1973) was followed. BPL (Nihon Kayaku) was mixed with the virus preparation in a serum-free medium at a 1:2000 dilution and incubated at 5 °C for 16 h. Then the virus was pelleted by centrifugation and resuspended in 1/20 of original volume.

U.v.-irradiation. Two ml of virus in 60 mm plastic dishes or 6 ml in 100 mm dishes were exposed to u.v. light at a distance of 150 mm using a germicidal lamp (Hitachi GL-15; about 60 erg/mm²/s) for 2 min; 1.5 ml aliquots of the sample were added to 60 mm dishes containing confluent monolayers of IFr or RSa-cells.

Interferons. Human Le-IF preparation (sp. act. 6 × 10⁶ units/mg protein), mock-IF, and sheep antiserum against Le-IF (anti-Le-IF) were kindly supplied by Dr K. Cantell, Central Public Health Laboratory, Helsinki, Finland. The F-IF (sp. act. 3.9 × 10⁶ units/mg protein)
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was prepared at the Rentschler Laboratory, Laupheim, Germany and provided through the courtesy of Dr R. Skoda and the National Institutes of Health, Bethesda, Maryland.

Neutralization of interferon. Equal volumes of Le-IF (1000 units/ml) and anti-Le-IF (1000 units/ml) were mixed and incubated at 37 °C for 1 h and then diluted 10-fold with MEM containing 10% calf serum; samples were used to treat cells. One unit/ml of the antiserum neutralized 10 units/ml of Le-IF.

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Binding of 3H-uridine labelled RD-114 virus. RD-114 cells were cultured in the presence of 3H-uridine (5 μCi/ml) for 16 h. The culture fluid was harvested, clarified and homogenized according to the procedures described above. One ml of the virus suspension (27 000 ct/min) was added to IF cells which had been treated for 16 h with different concentrations of interferon. The cell cultures were disrupted by mild sonic vibration and precipitated by adding 1 ml of cold 10% trichloroacetic acid (TCA). The acid-precipitable materials were collected on a glass fibre filter (Whatman GF/C), and the radioactivities were counted as described previously (Tomita & Kuwata, 1976).

Reverse transcriptase assay. RD-114 virus was assayed by measuring the reverse transcriptase activity. The 100 μl reaction mixture contained 25 mM-tris-HCl, pH 7.8, 20 mM-dithiothreitol, 0.02% NP40, 0.01 A260 units of poly(A).d(T)12-18, 0.8 μCi of 3H-TTP (47 Ci/mmol) and 50 μl of 30-fold concentrated tissue culture fluid. The mixture was incubated at 35 °C for 60 min, and then acid-precipitable radioactivity was estimated as described by Tomita & Kuwata (1978).

RESULTS

Syncytium formation with concentrated or non-concentrated RD-114 virus

To determine whether RD-114 virus induced fusion of RSa and IF cells, a concentrated virus preparation was added to cell cultures, and after 20 h these were fixed and stained as described in the Methods. RD-114 virus rapidly induced syncytia in IF cells. The fused cells contained between two and thirty nuclei, and most frequently between five to ten nuclei each. Since a small percentage of IF cells fused spontaneously, giant cells containing one to five nuclei were found in control cultures. Syncytium formation occurred also but less efficiently in RSa cell cultures treated with RD-114 virus. Detectable numbers of syncytia were also found when the cells were incubated with an unconcentrated virus preparation for 3 days. Syncytia with similar morphology were induced also in RSb cells.

Stability of syncytium forming activity

When a RD-114 virus preparation was treated at 56 °C for 30 min or at 100 °C for 5 min, the syncytium-forming activity of the virus was completely eliminated. Exposure of the virus to sonic vibration decreased its activity depending on the duration of treatment; exposure for 15 min completely destroyed its activity.

Effect of u.v.-irradiation or BPL-treatment on the cell fusion activity of RD-114 virus

To determine whether infection and replication of virus are necessary for syncytium formation, virus preparations were inactivated by u.v.-irradiation or BPL-treatment and tested for syncytium-forming activity. BPL-treatment reduced but did not eliminate the fusion activity of the virus. Likewise, u.v.-irradiation decreased the activity depending on time of the irradiation period; after 2 min irradiation (about 7200 erg/mm²) the number of syncytia induced per dish of IF cells was still 46% of the control value; with RSa cells, the number was 19% of the control value. However, when RD-114 virus, which had been irradiated for 2 min, was passaged twice in IF cells, no infectivity was detected when it was
Table 1. Effect of interferon on syncytium formation in IFr-cells by u.v.-irradiated or BPL-treated RD-114 virus

<table>
<thead>
<tr>
<th>Interferon treatment* (units/ml)</th>
<th>u.v. irradiated</th>
<th>BPL-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Le-IF</td>
<td>F-IF</td>
</tr>
<tr>
<td>0</td>
<td>2400</td>
<td>2400</td>
</tr>
<tr>
<td>25</td>
<td>1200</td>
<td>410</td>
</tr>
<tr>
<td>50</td>
<td>870</td>
<td>260</td>
</tr>
<tr>
<td>250</td>
<td>260</td>
<td>310</td>
</tr>
<tr>
<td>500</td>
<td>51</td>
<td>100</td>
</tr>
<tr>
<td>Mock-IF (equivalent to 500 units/ml)</td>
<td>2000</td>
<td>---</td>
</tr>
<tr>
<td>IF neutralized by antiserum†</td>
<td>2100</td>
<td>---</td>
</tr>
</tbody>
</table>

* Cells treated for 20 h as indicated and challenged with 1·5 ml of concentrated RD-114 virus.
† Le-IF (100 units/ml) was neutralized with 100 units/ml of antiserum as described in Methods.

tested in IFr cells in terms of syncytium formation or the presence of reverse transcriptase activity in the culture fluid (with RD-114 cells, the reverse transcriptase activity in the culture medium ranged from 6000 to 20000 ct/min/80 μl reaction mixture).

Suppression of cell fusion by interferon

To study the effect of interferon on fusion, RSa and IFr cells were pre-treated with various concentrations of Le-IF or F-IF for 16 h, and then the cells were washed and assayed for syncytium formation in response to concentrated infectious RD-114 virus. Treatment of RSa or IFr cells with 25 or 50 units/ml of Le-IF, or 32 or 74 units/ml of F-IF reduced the formation of syncytia to 11 to 27% of the control levels, while no effect was observed when these cells were pre-treated with mock interferon. Furthermore, when Le-IF was pre-incubated with anti-Le-IF serum, its activity was neutralized. The amount of virus (estimated by reverse transcriptase activity) produced from these cells during syncytium formation (16 h) was very small compared with the total virus added to the cultures (data not shown). However, to eliminate further the possibility that the decreased syncytium formation might be caused mainly as the results of the inhibition of virus replication by the antiviral activity of interferon, we carried out experiments using u.v.-irradiated or BPL-treated virus. Table 1 shows that fusion in IFr cells was still significantly suppressed by pre-treatment with Le-IF or F-IF interferon even when the syncytia were induced by u.v.-irradiated or BPL-treated virus. Thus the interferon treatment must have directly affected the ability of the cells to fuse.

Effect of cycloheximide on the establishment of interferon activity

It is known (Kuwata et al. 1977) that cycloheximide blocks the establishment of an antiviral state in cells treated with interferon. It was of interest therefore to examine whether cycloheximide would also block the effect of interferon on cell fusion. Table 2 shows that treatment of IFr cells with 250 units/ml of Le-IF reduced the induction of syncytia by u.v.-irradiated virus to 10% of the control level. However, if the cells were simultaneously treated with 50 μg/ml of cycloheximide, the amount of syncytium formation recovered to 77% of the control level. This effect was dependent on the dose of cycloheximide and treatment of IFr-cells with cycloheximide alone did not inhibit syncytium formation. These results suggest that for the development of the suppressive activity of interferon on syncytium formation, protein synthesis of cells is required.
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Table 2. Effect of treating cells for 16 h with interferon, or cycloheximide, or both, on formation of syncytia in IF? cells in response to u.v.-irradiated RD-114 virus

<table>
<thead>
<tr>
<th>Le-IF (units/ml)</th>
<th>Cycloheximide (µg/ml)</th>
<th>Number of syncytia/dish</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>2500</td>
</tr>
<tr>
<td>250</td>
<td>0</td>
<td>250 (10.0)*</td>
</tr>
<tr>
<td>0</td>
<td>5.0</td>
<td>2710 (108.4)</td>
</tr>
<tr>
<td>250</td>
<td>5.0</td>
<td>1920 (76.8)</td>
</tr>
<tr>
<td>250</td>
<td>1.0</td>
<td>1380 (55.2)</td>
</tr>
<tr>
<td>250</td>
<td>0.5</td>
<td>970</td>
</tr>
</tbody>
</table>

* % of control culture.

Binding of 3H-uridine labelled virus to the cells

To examine whether the treatment of the cells with interferon decreases the binding of RD-114 virus on the cell surface, IF? cells were treated with 100, 250 and 500 units/ml of Le-IF for 16 h. Then 3H-uridine labelled virus was added, and the cultures were incubated for 1 h at 37 °C. Virus binding was estimated as described in Methods. About 0.14 % of the input virus was bound to the cells in the control cultures. In cultures treated with 100, 250 and 500 units/ml of Le-IF, binding amounted to 0.08, 0.09 and 0.11 % respectively. Thus, interferon treatment did not affect binding of virus.

DISCUSSION

Several RNA tumour viruses including Mason-Pfizer monkey virus (Ogura et al. 1978) induce cell fusion in XC or KC cells. Such cell fusion has been considered to be 'fusion from without' (Bratt & Gallaher, 1969), since it is insensitive to inhibition of cellular macromolecular synthesis and since inactivated virus retains fusion ability (Johnson et al. 1971; Rand & Long, 1973). We studied cell fusion induced by RD-114 virus in RSa, RSb and IF? cells. When concentrated virus was added to monolayers, syncytia were rapidly induced in all these cells, with cells of the IF? line responding the most quickly to a particular concentration of virus (Table 1). The fusion activity of RD-114 virus was sensitive to heat and ultrasonic vibration, but relatively insensitive to u.v.-irradiation or treatment with BPL. From these results it seems that the replication of RD-114 virus is not essential for cell fusion and thus that the phenomenon may be considered as 'fusion from without' as described by Hampar et al. (1973) and Rand & Long (1973).

Since interferons have been shown to exert pleiotropic actions on cells, we examined their effect on cell fusion induced by infectious or by inactivated RD-114 virus. Pre-treatment of IF? and RSa cells with interferon suppressed fusion in these cells in response to infectious or u.v.-irradiated or BPL-treated RD-114 virus preparations. The phenomenon was observed with both Le-IF and F-IF preparations, but not with a mock-IF preparation. Furthermore, pre-incubation of Le-IF with anti-Le-IF serum completely eliminated the activity.

Syncytium formation was reduced when the cells were treated with as little as 25 units/ml of Le-IF or F-IF. Although the growth of RSa cells has been shown to be very sensitive to interferon (Kuwata et al. 1976b), it would not be affected by such a small concentration of interferon. IF? cells were first isolated as a line partially resistant to the growth inhibitory action of Le-IF, but still sensitive to its antiviral action (Kuwata et al. 1976b). This cell line was found to be very sensitive to inhibition by interferon of syncytium formation. Furthermore the effect of interferon on cell fusion in IF? cells was blocked by cycloheximide as is
the antiviral activity (Kuwata et al. 1977). These results suggest that the effect of interferon on cell fusion may be associated in some way with antiviral action of interferon and not with the growth inhibitory effect.

Recently, there have been several reports concerning change in the cytoplasmic membrane induced by interferon and such changes have been discussed in relation to the development of the antiviral state. Knight & Korant (1977) showed that interferon treatment of mouse L cells changes their electrophoretic mobility. Kohn et al. (1976) showed that interferon treatment alters the binding of cholera toxin or thyrotropin to plasma membranes. Moehring et al. (1971) reported that interferon decreases the sensitivity of cells to diphtheria toxin and Stewart et al. (1972) showed that it increases the susceptibility of cells to the toxicity of poly(rI).poly(rC). Furthermore, Chang et al. (1978) pointed out recently that interferon treatment changes the concentration of some of the membrane glycoproteins of AKR mouse cells and increases the total density of the plasma membrane. Thus, a decreased fusion capacity of cells to fuse after interferon treatment may be one reflection of alterations in the cytoplasmic membrane elicited by interferon. The prevention of assembly and release of RNA tumour viruses in interferon-treated cells (Billiau et al. 1976; Friedman, 1977) may also be caused by these alterations.

It could be argued that the suppression was specific for cell fusion in response to RD-114 virus. This is unlikely, since the suppression was also observed when interferon-treated HeLa or IFc cells were fused by u.v.-irradiated Sendai virus (Y. Tomita & T. Kuwata, unpublished data). Rand et al. (1975) isolated from RD-114 virus a fusion-inhibition factor which is a glycoprotein analogous to the gp70 of RD-114 virus. This factor was irreversibly bound to the surface of KC cells and inhibited fusion of the cells by intact RD-114 virus, but not by BPL-inactivated Sendai virus. Therefore, RD-114 virus and Sendai virus are probably bound to different attachment sites on the cell surface when they induce cell fusion. Considering these facts, we suggest that the suppressive effect of homologous interferon on cell fusion is not specific for fusion by RD-114 virus, but is a general phenomenon.

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