Efficient Transfer of Interferon-Induced Virus Resistance between Human Cells

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SUMMARY

The rate of development of interferon-induced virus resistance in a mixture of two human cell types (U and WISH) is determined by the cell type (WISH) in the mixture which responds first. This phenomenon has been shown with two types of interferon assay procedure, and with both vesicular stomatitis virus and Sindbis virus. The transfer of virus resistance from one human cell (WISH) to another (U) (homospecific transfer) is much more efficient than the transfer from mouse L cells to WISH cells (heterospecific transfer), as shown by a much lower ratio of donor to recipient cells required for maximum transfer as well as a more rapid transfer. Thus, virus protection afforded by the interferon system is amplified more efficiently in mixtures of different human cells than in mixtures of mouse and human cells. These results suggest that, in a mixed population of cells such as occurs in vivo, more slowly responding cells might be influenced by cells which respond more rapidly to interferon. A defensive role is suggested for this mechanism which amplifies protection due to interferon.

INTRODUCTION

We have previously shown that interferon-treated mouse L cells can transfer their virus resistance to co-cultivated cells from another species (Blalock & Baron, 1977). More recently, the donor cells and corresponding interferons with which such transfer has been observed have expanded to three species, namely mouse, human, and rabbit, and the recipient cells to four species, namely human, hamster, chicken and monkey (Hughes et al. 1978).

Since cells from a relatively large number of species demonstrate the transfer of resistance, this process may be a general occurrence that represents a fundamental feature of the interferon system. Furthermore, this could be a general phenomenon among adjacent cells within the body and play an important role in the pathogenesis of virus infections. Evidence relevant to this hypothesis would come from the demonstration not only of transfer but also of efficient transfer of protection between cells of the same species.

Our experimental approach has been to use two human cell lines which differ in the rate at which they develop resistance in response to interferon. If there was a transfer of resistance between the rapidly responding and slower responding cell types, then in a mixture of cells treated with interferon the rate of development of resistance would be accelerated in the more slowly responding cells. In this report, we confirm this possibility by showing that the rate of development of interferon-induced resistance in a mixture of two human cell types is determined by the cell type responding first in the mixture.
METHODS

Human fibroblast interferon was obtained from Dr Jan Vilcek. Titres are expressed in terms of the NIH research reference standard of human leukocyte interferon (catalogue number G-023-901-527).

Human WISH and U cells were cultured in Eagle's medium (supplemented with 10% foetal calf serum) in wells of Micro Test II tissue culture plates (Falcon Plastics, Oxnard, Calif.) either alone or mixed in various ratios. The total number of cells in each well (about 28 mm²) was 1 x 10^8 to 2.25 x 10^9. After overnight incubation at 37 °C in an atmosphere of 4% CO₂, the supernatant medium was removed, and replaced with interferon or an equivalent volume of medium. When the cultures had been further incubated for various times, the supernatant fluids were decanted, the cell cultures washed, and then challenged with vesicular stomatitis virus (VSV) at 3 p.f.u./cell, or with Sindbis virus at 50 TCID₅₀/cell. After 1.5 h at 37 °C, the inoculum was decanted and the cell sheets were washed and replenished with fresh medium. With VSV challenge, the virus yields were determined approx. 24 h later on pooled medium from triplicate cultures, by a slightly modified microplaque assay in which methylcellulose was substituted for carboxymethylcellulose (Campbell et al. 1975). In studies with Sindbis virus, triplicate cultures were pre-treated with each of a series of 0.5 log dilutions of interferon. After overnight incubation, the cultures were stained with 1% crystal violet in 20% methanol, and from the amount of cytopathic effect (c.p.e.) as thus determined, the 50% c.p.e. inhibition titre of the interferon preparation was calculated.

The plating efficiency of VSV and of Sindbis virus was the same on both WISH and U cells. With each cell type, or mixtures of the two, the virus yield at 24 h was about 7.6 log p.f.u./ml of VSV.

RESULTS

Transfer of interferon-induced virus resistance between human cells

Preliminary experiments had shown that human amnion (U) cells developed interferon-induced virus resistance 2 to 3 h later than human amnion (WISH) cells. An experiment was performed to determine whether mixed U and WISH cell cultures developed antiviral activity in response to interferon at times and rates similar to pure cultures of either cell type alone, or at an intermediate time and rate. WISH cells, U cells or a 1:1 mixture of the two were treated for various times with human interferon (30 units/ml) and challenged with VSV at an input multiplicity of infection (m.o.i.) that would infect 95% of the cells. The results of a typical experiment are shown in Fig. 1. It can be seen that WISH cells alone developed significant resistance (50% inhibition of virus yield) to virus infection after 30 min treatment with human interferon, while U cells alone required 2.5 h to develop an equivalent (58% inhibition) degree of resistance. The mixed population of cells developed significant resistance (63% inhibition) after 30 min and thus the kinetics of development of virus resistance was that of the more rapidly responding (WISH) of the two cell types rather than an average. This finding suggests that a highly efficient transfer of interferon-induced virus resistance can occur between cells of the same species.
Transfer of interferon-induced virus resistance between human cells. U cells and WISH cells were cultured individually or mixed at a 1:1 ratio in wells of tissue culture plates at a total number of $2.25 \times 10^5$ cells/well. After overnight incubation at $37$ °C, the supernatant fluids were removed and replaced with 30 units/ml of human fibroblast interferon. At the times shown, the interferon was removed, and the cells were washed and challenged with 3 p.f.u./cell of VSV. Virus yields in the media from pooled triplicate cultures approx. 24 h later were measured. •••, U and WISH cells; ○—○, WISH cells alone; □—□, U cells alone.

**Effect of the ratio of WISH to U cells on the homologous transfer of interferon-induced virus resistance**

It was shown previously that the degree of inhibition of virus yield in mixed cultures of cells from heterospecific species in the presence of mouse interferon was directly related to the ratio of mouse L cells to WISH or BHK cells (Blalock & Baron, 1977): the higher the proportion of L cells, the greater was the transfer of resistance to the heterospecific cells. To test this in the present system, the effect of varying the ratio of donor (WISH) to recipient (U) cells at a constant cell density was studied. Specifically, a constant number of cells ($1 \times 10^6$/well) was seeded in the proportions indicated in Fig. 2, and the cells formed monolayers overnight with essentially no divisions. The number of harvested cells approximately equalled the number added. Thus, since the plating efficiency approached 100%, the proportion of cells in a culture did not change appreciably from that added. Dilutions of interferon were then added to monolayers and removed at the times indicated. Immediately following interferon removal and washing, the cultures were infected with Sindbis virus at an m.o.i. of 50 TCID$_{50}$/cell, and after overnight incubation, the interferon titres were calculated.
Fig. 2. Effect of the ratio of WISH to U cells on the transfer of interferon-induced virus resistance. Triplicate cultures of WISH cells, U cells and mixtures of the two in defined proportions at a total of $1 \times 10^5$ cells/culture plate well were treated with dilutions of interferon for the times indicated. The cultures were washed and challenged with 50 TCID$_{50}$ of Sindbis virus, and from the c.p.e. observed after overnight incubation, the interferon titres obtained at each time of challenge were calculated. • • •, WISH cells; © © ©, 3 WISH:1 U cell; • • •, 1 WISH:3 U cell; ± ± ±, 1 WISH:1 U cell; [ ] [ ], U cells.

As shown in Fig. 2, the U cell cultures alone were not protected following 2 to 3 h treatment with interferon, whereas WISH cell cultures alone, even when treated for as short a time as possible, developed antiviral activity (Dianzani & Baron, 1975). With all the ratios of mixed cell cultures tested, protection developed very quickly and at the same rate as with the WISH cells. Hence, the WISH cells appeared to control the time and rate of development of protection over a range of cell mixtures, and this was so even with a proportion of WISH cells as low as 25%.

These results and those in the previous section also show that a highly efficient transfer process could be demonstrated with two different assay procedures for interferon (virus yield reduction and inhibition of virus c.p.e.) which employed two different challenge viruses (VSV and Sindbis virus).

Comparison of the efficiency of homospecific and heterospecific transfer of interferon-induced virus resistance

Fig. 3 shows a comparison of the effect of donor to recipient cell ratio on the transfer of virus resistance in a heterospecific and homospecific cell mixture. The degree of transfer of resistance from mouse L cells to heterospecific human WISH cells was directly related to
Transfer of interferon-induced virus resistance

Fig. 3. Effect of varying the proportion of donor human (WISH) or mouse (L) cells to recipient human (U or WISH) cells on the homospecific and heterospecific transfer of interferon-induced virus resistance. Transfer of virus resistance in the WISH and U cell mixtures was determined as in the experiment shown in Fig. 2 (100% transfer in this system is defined as the rate at which WISH cell alone developed resistance). Transfer of resistance in the L and WISH cell mixtures was determined as described by Blalock & Baron (1977): Maximum transfer in this heterospecific system was defined as the 92% inhibition of expected VSV yield obtained at a 2:1 ratio of L cells to WISH cells. •—•, WISH:U cells + human interferon; ——•, L:WISH cells + mouse interferon.

Table 1. Rate of transfer of interferon-induced virus resistance between different human cells (WISH and U) and between mouse (L) and human (WISH) cells

<table>
<thead>
<tr>
<th>Cells (donor:recipient)</th>
<th>Interferon</th>
<th>Hours of treatment required for 50% inhibition of VSV yield*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 WISH:1 U</td>
<td>Human</td>
<td>0.4</td>
</tr>
<tr>
<td>WISH only</td>
<td>Human</td>
<td>0.5</td>
</tr>
<tr>
<td>U only</td>
<td>Human</td>
<td>2.5</td>
</tr>
<tr>
<td>1 L:1 WISH</td>
<td>Mouse</td>
<td>1.0</td>
</tr>
<tr>
<td>L only</td>
<td>Mouse</td>
<td>0.4</td>
</tr>
</tbody>
</table>

* The time required for 50% inhibition of VSV yield was determined from a kinetic curve of interferon action, as described in Fig. 1.
the ratio of L cells to WISH cells, and maximum transfer occurred at a ratio of 2:1. In the WISH and U cell mixtures, maximum transfer of resistance was observed with ratios as low as 1 WISH cell to 3 U cells. These data indicate that, in terms of maximum transfer of resistance, the homospecific process is at least six times more efficient than the heterospecific.

The transfer process also seems to occur more rapidly between homologous than heterospecific cells. Table 1 shows that in a mouse L and human WISH cell mixture, WISH cells required 1 h for the development of significant resistance in response to mouse interferon, while L cells alone required only 0.4 h. This is in contrast to the human WISH and human U cell mixture, where U cells in the mix developed resistance in response to human interferon, as rapidly as WISH cells (0.4 to 0.5 h). Since WISH cells with human interferon and L cells with mouse interferon developed resistance at very similar rates, these data show that the slower rate of development of resistance in WISH cells in the presence of L cells does not result from an inherent slower response by these cells. These findings indicate that transfer between the two types of human cell occurs almost immediately whereas the heterospecific transfer requires 1 h. This again suggests a greater efficiency of homospecific transfer of virus resistance.

This suggestion of greater efficiency in the homospecific system is also supported by the observation that in a WISH and L cell mixture in the presence of mouse interferon, WISH cells only reach 1% to 10% of the level of resistance of L cells alone while in a WISH and U cell mixture with human interferon both cell types reach the same level of resistance.

**DISCUSSION**

The findings in this study indicate that transfer of interferon-induced virus resistance occurs between cells of the same species. The transfer process also appears then to be much more efficient than when there is transfer between cells from different species. Transfer of virus resistance is demonstrable with more than one type of interferon assay, and protection occurs against at least two viruses.

An explanation for this difference in efficiency might be found in the mechanism of transfer between cells. For example, if transfer of virus resistance occurs through gap junctions (Blalock & Baron, 1977) which allow cells to communicate between themselves (Pitts, 1971; Sheridan et al. 1975; Pitts & Finbow, 1976), then the efficiency of the transfer should be a reflection of the relative ability of the cells to communicate. Recently, specificity of junctional communication was shown and appeared to occur more frequently between homospecific than heterospecific cells (Fentiman et al. 1976; Pitts & Burk, 1976). Hence, the demonstration that a lower percentage of donor cells is required in a homospecific cell mixture for maximum transfer of virus resistance may be explained in terms of the relative ability of these cells to communicate.

Probably the most intriguing implication of these findings is that in a mixed population of cells in vivo, the more slowly responding cells may be influenced by cells which respond more rapidly to interferon. Thus, the efficiency of the virus protection afforded by the interferon system could be amplified. Since transfer of virus resistance between different human cells occurs almost immediately and with low percentages of donor cells, this process probably represents an important component of the interferon system in its defence against virus infections.

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REFERENCES


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