Type I and Type II Interferons: Differential Antiviral Actions in Transformed Cells

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(Accepted 14 September 1979)

SUMMARY

In transformed mouse embryo cells, type II interferon had much less antiviral activity than type I interferon. In non-transformed cells, the two interferons had similar high activity. Reversal of the phenotype of Moloney sarcoma virus (MSV) transformed cells by sodium butyrate restored their sensitivity to the antiviral action of type II interferon. Additional evidence for a role of the cytoskeletal network in the action of type II interferon is that its antiviral effect is reduced by cytochalasin B, colchicine or vinblastine. MSV-transformed cells, selected for their resistance to the antiviral action of type I interferon, were sensitive to type II interferon. These differences in the effects of type I and II interferon on transformed cells are at present unexplained, but suggest that they have at least partially separate mechanisms of action.

INTRODUCTION

Recent experiments have demonstrated differences between type I and type II interferons in the relative potencies of their tumour-inhibitory, immuno-modulating and cell surface-modifying properties (Salvin et al. 1975; Sonnenfeld et al. 1978, 1979). It was of interest, therefore, to determine whether their antiviral actions would be similar in normal and transformed cells. For example, studies of type II interferon in a transformed cell line, selected for resistance to type I interferon, might show whether the two interferons have similar or different mechanisms of action (Chany & Vignal, 1970). In addition, the cytoskeletal system, which, as is well-documented, affects the cell membrane, could play a role in the action of type I interferon (Bourgeade & Chany, 1976). Thus, the question arises whether inhibitors of the synthesis of microfilaments and microtubules would affect the sensitivity of the cells to type II interferon. On the other hand, sodium butyrate, which stimulates the elaboration of these constituents in transformed cells, could increase their response to this type of interferon.

METHODS

Cells. Mouse embryonic fibroblasts (MEF) type 3T3 and SV-40-transformed 3T3 cells (obtained from D. Sergiescu, Hôpital St. Vincent de Paul, Paris) were grown in Eagle's minimum essential medium (MEM) supplemented with 10% calf serum. The MSV cell line was obtained by transforming Balb/c fibroblasts with Moloney sarcoma virus (MSV). MSV-IF cells were obtained by carrying the MSV cells used in the experiments in the
presence of interferon (IF) for over 200 passages. These cells lost the capacity to form colonies in agar, recovered contact inhibition and produced high quantities of type I interferon after induction, but became resistant to the antiviral action of the same interferon.

**Virus.** Encephalomyocarditis (EMC) virus, strain MM, was a generous gift from Dr S. Grossberg, Milwaukee, Wisconsin, and was routinely passaged on L cells.

**Interferons.** Type I interferon was produced in L cells using Newcastle disease virus and standard procedures (Chany & Vignal, 1970). Type II interferon was obtained from mice infected with *M. tuberculosis*, strain BCG. Six weeks after infection, they were given tuberculin intravenously and blood was collected 4 h later, according to the method described by Youngner & Salvin (1973). The type I interferon preparation was assayed on L cells and its titre, expressed in international reference units per ml, was 64000. Type II interferon was assayed either on L or MEF cells; the preparations used contained 1000 to 2000 units/ml.

**Chemicals.** Sodium butyrate was purchased from Merck, Darmstadt, Germany; and cytochalasin B, colchicine and vinblastine from Sigma, St. Louis, Mo., U.S.A.

**Experimental procedures.** In parallel sets of experiments, the cells were treated with decreasing concentrations of either type I or type II interferon and incubated for 24 h at 37 °C. The medium was then removed and cells challenged with EMC at a m.o.i. of 1. After an adsorption period of 1 h, the cells were washed to remove the unadsorbed virus and incubated for 18 h at 37 °C. The virus yield was measured as p.f.u. in L 929 cells.

## RESULTS

**Sensitivity of normal and transformed cells to type I and type II interferons**

As shown in Fig. 1(a), type I interferon induced a similar degree of virus yield inhibition in normal MEF and in transformed MSV cell lines. Type II interferon showed a dose-response effect comparable to type I in normal cells, but there was no significant antiviral protection in the transformed cells. Similarly, when the two interferons were assayed in parallel in normal or SV-40 transformed 3T3 cells (Fig. 1 b), the sensitivity of the transformed cells to type II interferon was significantly less.

**Effect of microtubule and microfilament modifiers on type II interferon action**

It is known that MSV-transformed cells are somewhat less sensitive to type I interferon than normal cells (Brailovsky et al. 1969). It has also been shown that transformed cells frequently have a defective cytoskeletal network (Brinkley et al. 1975; Pollack et al. 1975). Sodium butyrate induces changes in the morphology of MSV-transformed cells by increasing the polymerization of microfilaments and microtubules (Altenburg et al. 1976). In parallel, their interferon sensitivity to type I interferon increases significantly and becomes comparable to that of non-transformed mouse embryonic cells. This effect has been attributed at least in part to a butyrate-induced correction of the deficient cytoskeletal system observed in these transformed cells (Bourgeade, 1978). The same compound has no effect on the interferon sensitivity of normal cells. As shown in Fig. 2, the antiviral effect of type II interferon was increased, to an even greater extent than type I interferon, when butyrate was added at a concentration of 2 mM, 24 h before interferon treatment.

Because of the butyrate reversal of the diminished response of transformed cells to type II interferon, we decided to further examine the role of the cytoskeletal system in the action of this type of interferon. In Table 1, the effects of inhibitors of microtubule and microfilament formation on the action of both interferons are presented. A diminished interferon incubation period (6 h) was utilized in such a manner that no inhibitory action of these various drugs on cellular RNA or protein synthesis was observed (data not shown). However, as previously reported for L cells (Bourgeade & Chany, 1976), they substantially inhibited
Relative effect of type I and II interferons

Fig. 1. Dose–response curves of type I interferon (IF-I) and type II interferon (IF-II) in (a) normal (MEF) and MSV-transformed cells: ▲—▲, MEF+IF-I; △—△, MEF+IF-II; ■—■, MSV+IF-I; ●—●, MSV+IF-II; and (b) in normal 3T3 and SV-40 transformed 3T3 cells: ▲—▲, 3T3+IF-I; △—△, 3T3+IF-II; ■—■, SV-40 (3T3)+IF-I; ●—●, SV-40 (3T3)+IF-II.

Table 1. Effect of cytochalasin B, colchicine and vinblastine on the action of type I and II interferons in 3T3 cells (10^6/dish)*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>EMC virus yields (p.f.u. x 10^{-6}/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls (64 units)</td>
</tr>
<tr>
<td>Medium</td>
<td>Controls (64 units)</td>
</tr>
<tr>
<td>Cytochalasin B (5 μg)</td>
<td>440</td>
</tr>
<tr>
<td>Colchicine (5 μg)</td>
<td>240</td>
</tr>
<tr>
<td>Vinblastine (5 μg)</td>
<td>430</td>
</tr>
<tr>
<td>Cytochalasin B + colchicine</td>
<td>400</td>
</tr>
<tr>
<td>Cytochalasin B + vinblastine</td>
<td>520</td>
</tr>
<tr>
<td>Colchicine + vinblastine</td>
<td>150</td>
</tr>
</tbody>
</table>

* Cells were treated with type I or II interferon for 6 h together with cytochalasin B, colchicine, or vinblastine, then infected with EMC virus at a m.o.i. of 1 for 1 h. Medium was added, and virus yields determined 16 h later.

the action of type I interferon, especially when cytochalasin B and vinblastine were combined. The action of type II interferon was inhibited by these drugs in a similar fashion. Thus, the integrity of microfilaments and microtubules appears to be necessary for the action of both interferons.
Fig. 2. Effect of sodium butyrate (2 mM for 24 h) on the sensitivity of MSV-transformed cells to type I interferon (IF-I) and type II interferon (IF-II). ■—■, IF-I; •—•, IF-I + sodium butyrate; ○—○, IF-II; △—△, IF-II + sodium butyrate.

Fig. 3. Treatment in parallel of interferon-resistant MSV-IF cells with decreasing concentrations of type I interferon (IF-I) and type II interferon (IF-II). The IF-I preparation used in this experiment, assayed at the same time in control MEF cells at a concentration of 64 units/ml, inhibited the yield of EMC virus by 2.7 log. ■—■, IF-I; ○—○, IF-II.
Sensitivity of MSV-IF cells to type II interferon

To study the comparative actions of both interferons further, one more set of experiments was performed. As shown in Fig. 3, both type I and type II interferons were assayed in MSV-IF cells, selected for resistance to type I interferon (Chany & Vignal, 1970). In these cells, 5 units of type II interferon were active whereas 64 units of type I interferon were inactive. The relatively modest action of type II interferon in the MSV-IF cells is not surprising in view of their transformed nature.

DISCUSSION

In interpreting our results, it is important to remember that the non-transformed MEF cells used to calculate the units of activity of both interferon preparations were selected arbitrarily. Furthermore, it is possible that purification of type II interferon to homogeneity will reveal a molecule or molecules with specific activity very different from that of type I interferon. Therefore, in these experiments, a similar interferon effect might be due to markedly different amounts of active proteins. However, our own unpublished mixing experiments and those of others (Dianzani et al. 1978) indicate that there are no inhibitors of interferon action in type II interferon preparations which could explain its decreased action in transformed cells.

Interferon activity is mediated by cell surface events followed by intracellular modifications in metabolism (Baron & Dianzani, 1977). Other previous work (Epstein & Epstein, 1976; Wietzerbin et al. 1977) has suggested that some steps in the antiviral actions of type I and type II interferons are shared: our experiments do not allow us to identify a precise site for the differential action seen in transformed cells. Moreover, the sensitivity to type II interferon of MSV-IF cells (resistant to the type I variant) suggests differences in an as yet unidentified metabolic step in their actions. On the other hand, our experiments also show the existence of identical effects such as the sensitivity to inhibitors of the cytoskeletal system and, in contrast, the improvement of the antiviral response in the presence of butyrate in transformed cells.

A recent report (Dianzani et al. 1978) of differential rates of induction of intracellular resistance to virus infection by the two interferons also suggests that they may differ in their mechanism of actions. In the absence of labelled interferon, no differences in the rates of cell binding of the two interferons can be detected. Thus, the cell surface events involved may be more subtle than can be evaluated in such experiments which measured total ‘cell-associated’ interferon and, of necessity, were conducted with very high concentrations of interferon.

We are grateful for the skilful assistance of J. Lopez, I. Tardivel and the able secretarial support of L. Weiler and C. Girard. This work was supported by grants from the United States Public Health Service (AI-05629), the Délégation Générale à la Recherche Scientifique et Technique (n° 78-7-0360) and the Institut National de la Santé et de la Recherche Médicale (A T P n° 28 76 60).
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(Received 22 March 1979)