Susceptibility of Various Cells Treated with Interferon to the Toxic Effect of Poly(rI).poly(rC) Treatment

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SUMMARY

Various cells were treated with interferon and then exposed to polyriboinosinic–polyribocytidylic acid complex [poly(rI).poly(rC)]. With mouse L cells, there was a marked cytotoxic effect from low doses of interferon and poly(rI).poly(rC), whereas chick embryo cells showed an effect only after high doses. When primate cells (LLC.Mk2, BSC.B, Vero and human embryo cells) were treated with human or monkey interferon, poly(rI).poly(rC) was not cytotoxic.

Stewart et al. (1972) found that interferon treatment of L929, mouse embryo or human embryo cells, followed by exposure to poly(rI).poly(rC), resulted in a marked cytotoxic effect. Subsequent work has shown that cytotoxicity after interferon treatment only occurs if double-stranded ribonucleic acids are added (Stewart et al. 1973). The degree of cytotoxicity after interferon and poly(rI).poly(rC) treatment is related to the size of the poly(rI) strand of the synthetic inducer (Stewart & De Clercq, 1974), as is interferon production (Tytell et al. 1970). The mechanism of the effect and its relationship to interferon production and action is obscure. However, if it is related to the interferon response, it should be demonstrable in all cells that possess a complete interferon system. We have therefore investigated the phenomenon in a number of cell lines and primary cell cultures.

An initial experiment was performed with interferon-treated chick embryo cells. The interferon used was a partially purified preparation (11000 units in terms of the British Medical Council research reference preparation A62/4 per mg protein) generously provided by G. Bodo (Arzneimittelforschung, GmbH, Vienna). Confluent monolayers of chick embryo cells in glass scintillation vials (approx. 5 × 10⁶ cells) were treated with dilutions of chick interferon in growth medium [Dulbecco’s modified Eagle’s medium supplemented with serum (Morgan et al. 1973)]. After 24 h at 37 °C this was removed, the cells were washed with Hank’s basal salts solution, and poly(rI).poly(rC) diluted in PBS was added (0.5 ml per vial). After incubation for 1 h growth medium (1.5 ml) was added to the vials (without removal of the inducer) and cytotoxicity was measured by neutral red dye uptake (Finter, 1969) after a further 24 h incubation at 37 °C. There was a reduction in dye uptake of 20% of control values in chick cells treated with 125 units per vial of this interferon followed by 50 µg/ml of poly(rI).poly(rC) (Table 1a). It was concluded that chick embryo cells are relatively insensitive to the cytotoxic effects of treatment with interferon and poly(rI).poly(rC).

To test the validity of the methods used for determining cytotoxicity, L929 cells were obtained from W. E. Stewart II (Rega Institute for Medical Research, Leuven, Belgium). Procedures were as previously described for chick cells except that when the poly(rI).poly(rC) was removed after 1 h, the cells were washed before the addition of the growth medium (2 ml). Neutral red uptake was measured after 24 h. A cytotoxic effect was observed with as little as 2 µg/ml poly(rI).poly(rC) after treatment with 13 or 63 units of Sindbis virus-induced mouse L cell interferon (Table 1b). After treatment with 63 units of interferon
Table 1. Toxicity of poly(rI).poly(rC) for (a) chick embryo cells
and (b) mouse cells pre-treated with interferon

<table>
<thead>
<tr>
<th>Interferon (units/ml)</th>
<th>Amounts of poly(rI).poly(rC) (µg/ml)</th>
<th>Cytotoxicity (%)*</th>
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<tr>
<td></td>
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<td>10</td>
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<tr>
<td>(a) Chick interferon</td>
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<td>(b) Mouse interferon</td>
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<td>63</td>
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<td>59</td>
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* Measured in terms of uptake of neutral red by treated cells.

and 10 µg/ml of poly(rI).poly(rC), the cytotoxic effect was greater than 50% which is comparable with the results of Stewart et al. (1972).

We next studied primate cells able to synthesize interferon. The origins of these cells and their cultural conditions are described in the accompanying paper (Emeny & Morgan, 1979). LLC.Mk2 Rhesus monkey cells were treated (as for mouse L929 cells) with 30 or 60 reference units of human interferon and up to 50 µg/ml of poly(rI).poly(rC). No cytotoxicity was observed after 48 h. A similar experiment conducted with human embryo cells and up to 75 units of interferon also yielded negative results. Further experiments were carried out with LLC.Mk2, Vero and BSC. B cells using small amounts of interferon derived from Sindbis-virus infected LLC.Mk2 cells (Emeny & Morgan, 1979) with essentially the same results. Various modifications of the method, including the use of serum-free medium and incubation for up to 72 h were tried, but no enhanced cytotoxicity was observed. The amounts of interferon and poly(rI).poly(rC) added were comparable with those used by Stewart et al. (1972) which caused cytotoxicity in human embryo cells. The difference in the response which we observed in human cells may be due to differences in the cell types used. Stewart et al. (1972) used skin cells: we used cells derived from the trunk and limbs of whole embryos. Continuous lines of primate cells may be insensitive or may require larger doses of both interferon and poly(rI).poly(rC) before cytotoxicity is seen.

It appears likely that L929 cells are unusual in their sensitivity to treatment with interferon and poly(rI).poly(rC). These cells are also sensitive to lysis by vaccinia virus if pre-treated with interferon. Lysis is prevented by treatment of the cells with actinomycin-D or cycloheximide or by heat treatment of the virus (Stewart et al. 1973) and is probably caused by the production of double-stranded RNA during virus infection.

The cytotoxic effect of interferon and poly(rI).poly(rC) treatment of cells is not sufficiently reproducible or universal to give information on the interaction of synthetic inducers with all cells and may therefore be of only limited interest. This cytotoxicity may
however, be useful for the isolation of resistant L929 cells. Depending on the mechanism of action of the cytotoxic effect, it might be possible to isolate variants with a phenotype similar to Vero cells, i.e. insensitive to the antiviral activity of poly(rI):poly(rC). Such variant cells would be useful tools in the analysis of interferon induction and action.

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REFERENCES


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