Sequential Suppression by Actinomycin D of Interferon Production and Cellular Resistance Induced by Poly I: C

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Cellular RNA and protein synthesis are required both for interferon synthesis (Heller, 1963; Wagner, 1963) and the expression of the antiviral activity of interferon (Taylor, 1964). The suppression of the antiviral activity of interferon by metabolic inhibitors has been interpreted to mean that interferon acts as a de-repressor for the synthesis of a cellular antiviral protein. While direct proof for the interferon-induced antiviral protein is still lacking, recent circumstantial evidence supports this interpretation (Dianzani, Buckler & Baron, 1968; Stewart & Lockart, 1970).

In a recent publication, Kjeldsberg & Flikke (1971) reported that polyinosinic-polycytidylic acid (poly I:C) inhibited poliovirus RNA synthesis in cells whose DNA-dependent RNA synthesis had been suppressed by actinomycin D. They suggested that, rather than acting through interferon induction, low concentrations of poly I:C may directly interfere with virus replication by binding to the virus-specific RNA polymerase.

Our experiments were done in rabbit kidney cell cultures prepared as previously described (Vilček et al. 1968). Poly I:C (double-stranded sodium salt, lyophilized) was purchased from P-L Biochemicals, Milwaukee, Wisconsin. The methods of titration of vesicular stomatitis virus (VSV) in cultures of chick embryo cells (Vilček & Freer, 1966) and of interferon titration by a plaque-reduction test with VSV in secondary rabbit kidney cell cultures (Vilček & Ng, 1971) have been described previously.

Cultures in 60 mm Petri dishes were incubated with 0.5 μg./ml. of actinomycin D or control medium for 45 min. They were then treated with various concentrations of poly I:C or with rabbit kidney cell interferon. Six hr later all cultures were thoroughly washed and inoculated with VSV at a multiplicity of 3 p.f.u./cell. The virus was adsorbed for 1 hr. The cultures were then washed to remove extracellular virus and replenished with medium. The virus yield was titrated from culture fluids collected 16 hr after inoculation. The inhibitory effect of three different concentrations of poly I:C on growth of VSV was abolished by previous exposure of cells to actinomycin D. The antiviral action of interferon was also suppressed by actinomycin D (Fig. 1).

This experiment indicated the necessity of a cellular function for the expression of the antiviral action of poly I:C. However, it did not demonstrate that interferon was a necessary intermediate in this reaction. It could not be ruled out that poly I:C served as a direct stimulus for the synthesis of the cellular antiviral protein and that, strictly speaking, interferon synthesis was not required. This idea was tested experimentally.

Cultures in 60 mm Petri dishes were incubated with 10 or 50 μg./ml. of actinomycin D for 30 min. and were then thoroughly washed. At various intervals two cultures each received 0.5 μg./ml. of actinomycin D for 30 min. They were then washed and replenished with medium. Media were collected 6 hr after exposure to poly I:C for interferon determinations. Thereafter, the same cultures were inoculated with VSV as in the preceding experiment. Samples of culture fluids for virus titrations were collected 17 hr after inoculation. Interferon production was greatly reduced by actinomycin D added ½ hr after the exposure of cells to poly I:C. However, the addition of actinomycin D at 1 hr reduced the interferon yield by
only about 50% and at 1½ hr treatment with actinomycin D no longer caused a significant decrease in the amount of interferon produced. On the other hand, the addition of actinomycin D at 1 hr completely suppressed the inhibitory effect of poly I:C on VSV multiplication and the development of resistance to VSV was greatly reduced in cells treated with actinomycin D 1½ hr after the addition of poly I:C (Table 1).

Table 1. Effect of actinomycin D interferon production and the development of cellular resistance

<table>
<thead>
<tr>
<th>Dose of poly I:C</th>
<th>50 µg./ml.</th>
<th>10 µg./ml.</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Interferon yield*</td>
<td>VSV yield†</td>
<td>Interferon yield*</td>
</tr>
<tr>
<td>½ hr with actinomycin D</td>
<td>34</td>
<td>ND</td>
<td>20</td>
</tr>
<tr>
<td>1 hr with actinomycin D</td>
<td>140</td>
<td>1,600</td>
<td>70</td>
</tr>
<tr>
<td>1½ hr with actinomycin D</td>
<td>195</td>
<td>450</td>
<td>125</td>
</tr>
<tr>
<td>Control (no actinomycin D)</td>
<td>220</td>
<td>2½</td>
<td>130</td>
</tr>
</tbody>
</table>

* Units/2 ml. † Vesicular stomatitis virus. ‡ p.f.u./ml. × 10⁴. ND = Not done.

The fact that interferon production was suppressed by actinomycin D treatment at ½ hr, but not at 1½ hr, suggested that interferon messenger RNA synthesis in rabbit kidney cells was virtually completed by 1½ hr after the addition of poly I:C. This result is in agreement with our earlier findings (Vilček, 1970).

The finding that the development of resistance to virus was prevented or greatly suppressed by treatment with actinomycin D at a time when interferon production in the same cultures was no longer inhibited suggests that poly I:C does not induce cellular resistance to virus directly. Rather, resistance to virus infection appears to be the result of two separate steps. The first step is the synthesis of interferon, stimulated by poly I:C. The second step is likely to be the induction of the hypothetical antivirus protein by interferon. An identical conclusion was reached by Dianzani et al. (1968), who studied the nature of cellular resistance in cultures exposed to a virus interferon inducer.

The conclusion that interferon is the mediator of the antivirus activity of poly I:C in cell
cultures is in agreement with the findings of other investigators (Stewart, Scott & Sulkin, 1969; Schafer & Lockart, 1970). The actinomycin D-resistant and, therefore, apparently indirect effect of poly I:C on poliovirus RNA synthesis reported by Kjeldsberg & Flikke (1971) was observed in cultures treated with poly I:C at the time of virus inoculation. Under these conditions, there was probably insufficient time for interferon-mediated resistance to develop. On the other hand, if poly I:C treatment precedes virus inoculation by several hr, a direct effect on virus replication is much less likely to be observed, the main reason being that poly I:C is rapidly degraded in cultured cells (Bausek & Merigan, 1969). The results of this study show that in rabbit kidney cells treated with both low or high concentrations of poly I:C 6 hr before inoculation with VSV the antivirus activity is indeed only indirect.

Double-stranded polynucleotides have often been shown to induce cellular resistance in the absence of detectable interferon production. For instance, as little as 0.001 μg./ml. of poly I:C is sufficient to render rabbit kidney cells resistant to VSV while a considerably higher concentration is required to produce demonstrable interferon in the culture fluids (Field et al. 1968; Vilček et al. 1968). In view of the results presented in this paper, it has to be assumed that cellular resistance produced by such low poly I:C concentrations is mediated by endogenous cellular interferon that becomes undetectable once released into the culture medium.

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REFERENCES


Short communications


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