Interferon Induction: Rate of Cellular Attachment of Poly IC

(Accepted 3 January 1972)

Cells exposed to the complex of polyinosinic and polycytidylic acid (poly IC) develop resistance to direct virus challenge and simultaneously release interferon (Field et al. 1967; Younger & Hallum, 1968). The mechanisms by which poly IC triggers the production of interferon are unknown. The effect of the time of exposure of cells to poly IC on interferon induction was examined in order to clarify the nature of 'triggering'. Since all the antivirus activity of poly IC is not necessarily due to the induction of interferon (Kjeldsberg & Flikki, 1971), we determined simultaneously the yield of interferon and the intracellular resistance to virus as functions of the poly IC concentration and the time of exposure. Our results indicate that the critical adsorption of molecules of poly IC by human cells is much more rapid than previously suspected.

The polynucleotides poly I and poly C (Miles Laboratories) were dialysed before use for 24 hr at 4° against 0.001 M-sodium phosphate, pH 7.4. The concentration is given in molarity of phosphate residues in polynucleotides. The equimolar complex between poly I and poly C was prepared in 0.01 M-sodium phosphate, pH 7.4, 0.001 M-MgCl₂, 0.15 M-NaCl and equilibrated at 5° for 24 hr. Human neonatal cells (Carter et al. 1971) were grown in monolayers (60 x 15 mm. Petri dishes) under standard conditions in minimal Eagle's medium (MEM) (Eagle, 1959) containing 15% foetal calf serum (FCS). Bovine vesicular stomatitis virus (VSV), New Jersey serotype, was harvested from mouse embryo cells at an infectivity of 10⁷ p.f.u./ml.

Human fibroblast cells (MA 184, Microbiological Associates) (6 x 10⁵), grown in monolayers, were exposed for various times to different concentrations of poly IC in MEM (with 6% FCS), washed twice in 2 ml. MEM and replaced in fresh medium at 37°. Resistance to virus and interferon were measured colorimetrically 18 hr later (Finter, 1969). Collected medium (3 ml.) with interferon was first pre-incubated with pancreatic RNase (40 µg./ml.) for 1 hr at 37°. Resistance to virus (%) is expressed as the ratio of the number of viable cells after infection with VSV to the number of viable cells after mock infection (Pitha & Carter, 1971a) with medium instead of virus under identical conditions. Interferon activity is expressed in units/ml., which is the reciprocal of the dilution giving 50% of the absorbance of control cells (Finter, 1969). For the virus yield reduction assay, cells (6 x 10⁵) were treated with VSV (10⁵ p.f.u.) at 37° for 45 min. and the unadsorbed virus removed. The cells were incubated in fresh MEM (with 6% FCS) for an additional 24 hr at 37°. The medium was collected and the virus infectivity determined by plaque assay in mouse L cells. To measure the attachment of poly IC, cells (4 x 10⁶) were exposed to poly IC* (poly IC containing tritiated polycytidylic acid, 10⁻⁴ M, 2.3 x 10⁵ counts/min./ml.) for 60 min. at 4°, then washed with MEM as indicated and the acid insoluble fractions (10% TCA, 1 hr at 65°) determined (Colby & Chamberlin, 1969).

The percentage of cells resistant to virus infection after treatment with 10⁻⁴, 10⁻⁵ or 10⁻⁶ M poly IC is shown in Fig. 1. At 37° (Fig. 1A), exposure of cells for 1 min. to a given concentration of poly IC was sufficient to initiate the maximum protection against virus. The magnitude of this effect depended primarily on the initial extracellular concentration and was not influenced significantly by the presence of free poly IC in the medium.
Fig. 1. Rate of initiation of resistance to virus at 4° and 37°. Human fibroblast cells were exposed to
different concentrations of poly IC at 37° (A) or 4° (B). The experiment was made twice in duplicate
and mean values are given. Standard errors computed on each point ranged from 4 to 6% of the
mean. Poly IC concentrations (molarity of phosphate residues in polynucleotides) 10⁻⁴ M (○—○),
10⁻⁵ M (×—×), and 10⁻⁶ M (●—●).

At each concentration of inducer a plateau of level resistance was reached within
several minutes. To test whether this saturation effect was due to a depletion or inacti-
vation of extracellular poly IC, we replaced the medium at a constant concentration of
poly IC (10⁻⁵ and 10⁻⁶ M) every 10 min. for 1 hr. The resistance to virus was not increased
by these medium replacements. Any late events, such as the attachment to cells of additional
poly IC molecules, were therefore unimportant in the development and maintenance of
resistance to virus.

We made similar kinetic studies at 4° (Fig. 1 B). At this temperature, energetic processes
such as those associated with the penetration of cells by macromolecules are usually inhibited
while physical events, such as the attachment of macromolecules to cell membranes, are
unimpeded (Garen & Puck, 1951). The observation that treatment of cells with RNase, to
which poly IC has been attached at 4°, prevents interferon production (Bausek & Merigan,
1969; Pitha & Carter, 1971 b) indicates that the inducer is retained on cell surfaces at low
temperatures. Cells were exposed to poly IC at 4° for various times, washed, and incubated
in fresh medium at 37°. The percentage of cells resistant to virus (Fig. 1 B) after poly IC
treatment at 4° was similar to that obtained at 37°, although the rate of the reaction was
decreased, and maximum effects were not reached until 10 to 15 min. The delay is probably
due to the temperature dependent diffusion of poly IC molecules to cell surfaces (Tanford,
1963).

In order to test the irreversibility of the adsorption of poly IC to cells, we monitored the
radioactivity removed with successive washes after binding poly IC* to cells. The amount
of poly IC* bound did not change significantly after the second of 10 washes and less than
0·5 % of the input concentration (10⁻⁴ M) was firmly bound, even with extended exposure
times (Table 1). We conclude that the attachment of poly IC to cells is sufficiently strong
to be uninfluenced by its total removal from the medium within several minutes.

The question arises as to whether the production of interferon, like the resistance to virus,
can be initiated by brief exposures to double-stranded polynucleotides. There may be a
Table 1. The persistence of poly IC* attachment to human fibroblasts

<table>
<thead>
<tr>
<th>No. of washes</th>
<th>Cell radioactivity (counts/min./4 x 10^6 cells)</th>
<th>% poly IC* bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22,152</td>
<td>9.8</td>
</tr>
<tr>
<td>2</td>
<td>9,503</td>
<td>4.2</td>
</tr>
<tr>
<td>3</td>
<td>1,175</td>
<td>0.57</td>
</tr>
<tr>
<td>4</td>
<td>1,151</td>
<td>0.51</td>
</tr>
<tr>
<td>5</td>
<td>1,147</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Cells (4 x 10^6) were exposed to poly IC* (poly IC with tritiated polycytidylic acid, 10^-4 M, 2.3 x 10^5 counts/min./ml.). The experiment was made twice in duplicate and mean values are presented.

Table 2. Course of induction of interferon and resistance to virus by poly IC

<table>
<thead>
<tr>
<th>Poly IC (M)</th>
<th>Exposure time (min.)</th>
<th>% of cells resistant to virus infection</th>
<th>Virus yield (p.f.u./ml.)</th>
<th>Interferon activity (units/ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10^-3</td>
<td>1</td>
<td>100</td>
<td>&lt;10</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>100</td>
<td>&lt;10</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>10^-5</td>
<td>100</td>
<td>&lt;10</td>
<td>100</td>
</tr>
<tr>
<td>10^-4</td>
<td>1</td>
<td>100</td>
<td>9.0 x 10^3</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>100</td>
<td>9.0 x 10^3</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>10^-5</td>
<td>100</td>
<td>9.0 x 10^3</td>
<td>28</td>
</tr>
<tr>
<td>10^-5</td>
<td>1</td>
<td>60</td>
<td>5.4 x 10^5</td>
<td>&lt;5</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>75</td>
<td>6.0 x 10^5</td>
<td>&lt;5</td>
</tr>
<tr>
<td></td>
<td>10^-5</td>
<td>70</td>
<td>1.8 x 10^5</td>
<td>&lt;5</td>
</tr>
<tr>
<td>10^-6</td>
<td>1</td>
<td>15</td>
<td>3.0 x 10^5</td>
<td>&lt;5</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>25</td>
<td>1.5 x 10^5</td>
<td>&lt;5</td>
</tr>
<tr>
<td></td>
<td>10^-5</td>
<td>23</td>
<td>9.0 x 10^5</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Absent</td>
<td>–</td>
<td>6</td>
<td>1.0 x 10^8</td>
<td>–</td>
</tr>
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</table>

The values are the means for three separate experiments.

delay in reaching the maximum level of interferon production, but yields were certainly not augmented by exposure beyond 60 min. (Table 2). In agreement with our previous studies (Pitha & Carter, 1971a), we observed that the cells treated with poly IC for 60 min. released no interferon below a critical concentration of 10^-3 M and established a maximum yield at a polymer concentration of 10^-3 M. The resistance of the cells to direct virus challenge showed a similar dependence on poly IC concentration, although protection was observed at concentrations at which the release of interferon was undetectable. Interferon was detected only when cells were completely resistant to virus infection. This suggests that interferon may be released only when the intracellular concentration is higher than a 'limit' value.

Our work indicates that the long exposure times widely used in experiments in vitro (Colby & Chamberlin, 1969; DeClercq et al. 1971; Field et al. 1968) may not be necessary for maximum interferon production. Moreover, the continued exposure of cells to these polyanions may make interpretation more difficult since undesirable secondary effects, such as an increase in toxicity (Absher & Stinebring, 1969; Lindsay et al. 1969), may not be accompanied by a further blockade of virus replication. Thus, biological stability may be an unnecessary and undesirable property of interferon inducers. It should be possible to develop polynucleotides which are of sufficient structural specificity to trigger rapid resistance to virus yet are easily hydrolysed (Pitha & Carter, 1971b) so that secondary effects are absent.
Short communications

This work was supported by grants from The Council for Tobacco Research, no. 694, U.S.P.H.S. CA06973 and AI10289, The Jane Coffin Childs Memorial Fund for Medical Research, no. 286, and The American Cancer Society, Maryland Division, nos. 69-01 and 71-16. W. A. C. is recipient of a Public Health Service Career Development Award.

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REFERENCES


(Received 18 October 1971)

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