The Effects of Cytochalasin and Colchicine on Interferon Production

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SUMMARY

The present study was undertaken to investigate the mechanism of interferon production by mouse spleen cells co-cultivated with BHK-HVJ cells, i.e. baby hamster kidney cells persistently infected with the HVJ (or Sendai) strain of para-influenza I virus. Cytochalasin B appears to inhibit an early stage in the process and colchicine a relatively late stage. It is suggested that microfilaments and microtubules may play an important role at an initial stage of interferon production in this system.

INTRODUCTION

We have recently reported that an interferon with the species specificity of mouse interferon is released into the supernatant fluid of mixed cultures of mouse spleen cells and BHK-HVJ cells. Its production is initiated by membrane-membrane interaction between lymphoid cells and HVJ-infected cells (Ito et al. 1974), and Henney & Bubbers (1973) reported that cytolysis mediated by T cells was inhibited by agents acting on microfilaments or microtubules. The present study was undertaken to investigate further the mechanism of interferon production in our system, and in particular to investigate what roles microfilaments and microtubules might play in the process.

METHODS

Mice. The mice used in the present study were male C57BL/6 mice weighing 25 to 30 g and with serum haemagglutination inhibition titres against HVJ of less than 4.

Cell cultures. The cells used in the present study were mouse L cells and BHK-HVJ cells (Nagata et al. 1972). They were grown in Eagle’s minimum essential medium (MEM) supplemented with 10% calf serum and penicillin, streptomycin and amphotericin B at 100 units/ml, 100 µg/ml and 0.2 µg/ml respectively.

Mouse spleen cells. Whole spleens were removed aseptically and teased on steel mesh immersed in chilled medium 199 in a plastic dish. The cells which passed through were washed twice with medium and resuspended in growth medium.

Drugs. Cytochalasin B was obtained from Imperial Chemical Industries, Ltd., England and cytochalasin D from Shionogi Pharmaceutical Company, Japan. They were maintained as stock solutions containing 10 mg/ml in dimethylsulphoxide (DMSO). Colchicine was purchased from E. Merck AG, Darmstadt, Germany.

Interferon titration. Interferon was assayed by the plaque reduction method with mouse L cells and vesicular stomatitis virus (VSV) as challenge virus (Ito et al. 1974). Titres of
RESULTS

Effect of cytochalasin B and cytochalasin D on interferon production

Mouse spleen cells were co-cultivated for 8 h with BHK-HVJ cells in MEM containing various concentrations of cytochalasin B or cytochalasin D. At the end of the co-cultivation period, the cell suspension was centrifuged and a sample of cell-free supernatant fluid was dialysed against MEM to remove the drug, and then assayed for interferon. The effects of cytochalasins on interferon production are shown in Fig. 1, a, b. Complete inhibition of interferon production was observed in the presence of 10 μg/ml cytochalasin B and 0.37 μg/ml cytochalasin D.

To test the reversibility of inhibition by cytochalasin B, spleen cells were pre-incubated for 2 h with cytochalasin B (10 μg/ml), washed three times, and then co-cultivated with BHK-HVJ cells for a further 8 h. Spleen cells thus tested produced as much interferon as those unincubated with cytochalasin B (compare lines 1 and 4, Table 1). Thus, the effect of cytochalasin B is reversible and cannot be attributed to generalized cell damage. When DMSO (the solvent for the stock 10 mg/ml solution of cytochalasin B) was diluted in MEM to the same final concentration (0.1%) as when the cytochalasin was added, it did not affect interferon production (see lines 1 and 2, Table 1).

In another experiment, spleen cells were pre-incubated for 2 h with BHK-HVJ cells in the presence or absence of cytochalasin B (10 μg/ml), after which the spleen cells alone were isolated, washed three times and re-incubated for 8 h without BHK-HVJ cells. Interferon production was completely abolished by 2 h pre-incubation with cytochalasin B (see lines 5 and 6, Table 1).

Additional evidence that cytochalasin B inhibits an early stage of interferon production in this system was provided by the following experiment in which the effect of adding the drug at different times was studied. As can be seen from Fig. 2, when cytochalasin B (10 μg/ml)
Initial step in interferon production

Table 1. Interferon production by mouse spleen cells: induction and inhibition by cytochalasin B

<table>
<thead>
<tr>
<th>First incubation</th>
<th>Incubated for (h)</th>
<th>IF titre*</th>
<th>Second incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHK-HVJ cells present</td>
<td>Inhibitor</td>
<td></td>
<td>BHK-HVJ cells present</td>
</tr>
<tr>
<td>+</td>
<td>–</td>
<td>8</td>
<td>97</td>
</tr>
<tr>
<td>+</td>
<td>DMSO†</td>
<td>8</td>
<td>116</td>
</tr>
<tr>
<td>+</td>
<td>CB‡</td>
<td>8</td>
<td>&lt;3</td>
</tr>
<tr>
<td>–</td>
<td>CB</td>
<td>2</td>
<td>&lt;3</td>
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<tr>
<td>+</td>
<td>–</td>
<td>2</td>
<td>&lt;3</td>
</tr>
<tr>
<td>+</td>
<td>CB</td>
<td>2</td>
<td>&lt;3</td>
</tr>
</tbody>
</table>

* Interferon titre.
† Dimethylsulphoxide (DMSO) was diluted to a final 0.1% in medium to match the concentration present in the culture with cytochalasin B added.
‡ Cytochalasin B (10 µg/ml).

Fig. 2. The effect of adding cytochalasin B to an ongoing interferon production. Cytochalasin B was added at 0 (△), 1 (□), 2 (○) or 4 (◇) hours after mixing mouse spleen cells and BHK-HVJ cells. The final concentration of cytochalasin B was 10 µg/ml. Each point is the mean of two experiments. (↓) Addition of inhibitor; •—•, controls without cytochalasin B.

was already present at the start of the co-cultivation, there was no interferon production. If, however, the drug was added after co-cultivation for 1 h, interferon was produced almost to the full extent (Fig. 2). Cytochalasin B, therefore, seemed to inhibit an initial stage of interferon production.

Effect of colchicine on interferon production

Mouse spleen cells were co-cultivated for 8 h with BHK-HVJ cells in MEM containing various concentrations of colchicine. At the end of the co-cultivation period, the cell suspen-
Fig. 3. Effect of colchicine on interferon production. Mouse spleen cells were co-cultivated for 8 h with BHK-HVJ cells in MEM containing various concentrations of colchicine. At the end of the co-cultivation period, a sample of cell-free supernatant fluid was dialysed against MEM to remove the inhibitor, and then assayed for interferon. Each point is the mean of two experiments.

Fig. 4. Effect of adding colchicine to an ongoing interferon production. Colchicine was added at 0 (△), 1 (○), 2 (○) or 4 (○) hours after mixing mouse spleen cells and BHK-HVJ cells. The final concentration of colchicine was 10⁻² M. Each point is the mean of two experiments. (↓), Addition of inhibitor; •—•, controls without colchicine.
sion was centrifuged. A sample of cell-free supernatant fluid was dialysed against MEM to remove the drug, and assayed for interferon. As shown in Fig. 3, colchicine (10^-3 to 10^-2 M) also inhibits interferon production. When this inhibitor (10^-3 M) was added during the first hour of co-cultivation, it inhibited interferon production in clear contrast to the finding with cytochalasin B (Fig. 4). If, however, the drug was added after co-cultivation for 2 h, interferon was produced almost to the full extent (Fig. 4). Thus, colchicine seemed to inhibit a relatively late stage of interferon production. Moreover, unlike cytochalasin B, the inhibitory effect of colchicine was partially reversible.

DISCUSSION

The present studies clearly show that both cytochalasin B and colchicine inhibit interferon production in the system studied. Cytochalasin B inhibits an early stage of interferon production whereas colchicine inhibits a relatively late stage. It is also noteworthy that neither cytochalasin B nor colchicine inhibits the release of interferon in this system (Fig. 2 and 4).

When spleen cells were pre-incubated for 2 h with BHK-HVJ cells in the presence of cytochalasin B, interferon production was completely abolished, although the BHK-HVJ cells adsorbed spleen cells to their surfaces. This observation shows that cytochalasin B does not inhibit the cell-to-cell contact between spleen cells and BHK-HVJ cells and therefore indicates that contacts alone are not sufficient to trigger interferon production in this system. Since even co-cultivation for 1 h resulted in production of an appreciable amount of interferon (Ito et al. 1974), it seems likely that cytochalasin B may prevent the necessary interactions between the mouse spleen cells and BHK-HVJ cells. On the other hand, colchicine, when added during the first hour of co-cultivation, inhibited interferon production, but when added after co-cultivation for 2 h, allowed interferon production to almost the full extent. This shows that colchicine can inhibit the interferon production at stages following cell-to-cell interaction.

Cytochalasin B inhibited interferon production at concentrations (5 to 10 µg/ml) comparable to those which affect microfilament function in other systems, and this inhibition was reversible (Wessells et al. 1971). On the other hand studies on the mode of action of colchicine have indicated that it binds to subunits of microtubules, thus preventing their aggregation (Borisy & Taylor, 1967). Therefore, it seems reasonable to conclude that cytochalasin B and colchicine may inhibit interferon production in our system by suppressing the function of microfilaments or microtubules, which may therefore be important at early stages of interferon production.

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REFERENCES


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