Suppression of Interferon Production in Mouse Spleen Cells by Cytochalasin D

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SUMMARY

Cytochalasin D is thought to impair microfilament function. The present study has investigated its effects on four different systems in which interferon is formed, namely (1) mouse fibroblasts induced with virus (2) mouse spleen cells induced with virus, or (3) with endotoxin or (4) by allogeneic stimulation.

Cytochalasin D did not suppress formation of interferon by fibroblasts (L cells) or spleen cells stimulated with either HVJ or NDV. However it did suppress production of interferon by spleen cells in response to endotoxin or an allogeneic stimulation; here its action was apparently not on the secretion of interferon, but on some earlier event. It also suppressed the production of interferon by mouse spleen cells induced with HVJ if this had been u.v. irradiated for more than 15 min: this suggests that cytochalasin D sensitive structures do play some role in interferon production by mouse spleen cells when stimulated with HVJ, as well as when they are stimulated with endotoxin or an allogeneic stimulus.

INTRODUCTION

We recently reported that both cytochalasin and colchicine inhibit the production of interferon by mouse spleen cells induced through contact with BHK cells persistently infected with HVJ (Ito et al. 1976a). These results suggest that microfilaments and microtubules may play an important role in this particular interferon production system. However, based on physicochemical and antigenic differences, four different types of mouse interferon and human interferon can now be distinguished, namely, fibroblast interferon, virus-induced leukocyte interferon, endotoxin-induced interferon, and immune (Type II) interferon.

The present study has examined the effects of cytochalasin D, which apparently impairs microfilament function (Puszkin et al. 1973), on four different systems in which mouse interferons are produced, in order to see whether microfilaments play any role in these systems.

METHODS

Viruses. The Nagoya strain of HVJ (haemagglutinating virus of Japan, Sendai virus) and the Miyadera strain of NDV (Newcastle disease virus) were used. Stock virus was prepared by allantoic inoculation of 10-day-old embryonated eggs with 0·1 ml of 10−2 or
10⁻³ dilution of infected allantoic fluid. After incubation for 3 days (HVJ) or 2 days (NDV) at 35 °C, allantoic fluid was harvested and stored at −80 °C.

Cell culture. Mouse L fibroblast cells were grown in Eagle's minimum essential medium (MEM) supplemented with 10% bovine serum, 10% tryptose phosphate broth, and antibiotics. The maintenance medium contained no bovine serum supplement.

Mice. Male C57BL/6 mice, weighing 25 to 30 g, were used. The titre of haemagglutination-inhibition antibodies to HVJ in their sera was less than 4.

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

Drugs. Cytochalasin D (Shionogi Pharmaceutical Company, Japan) was maintained in a stock solution containing 10 mg/ml in dimethylsulphoxide (DMSO). DMSO alone (0.1%) did not affect interferon production in L cells (Ito et al. 1976b) or mouse spleen cells (Ito et al. 1976a). Escherichia coli endotoxin was obtained from Difco Lab. Inc., Detroit, Michigan U.S.A.

Ultraviolet irradiation. Viruses were purified by two cycles of alternating low and high speed centrifugation and diluted with PBS to 200 times the original volume. One ml of the diluted virus was exposed in a 45 mm Petri dish to u.v. irradiation from a 15 W Toshiba germicidal lamp (80 erg/mm²/s).

Interferon titration. Interferon was assayed by a c.p.e. inhibition microassay method with mouse L cells and vesicular stomatitis virus as the challenge as previously described (Ito & Montagnier, 1977). The highest dilution of the titrated sample causing at least 50% protection was taken as the end point. One of our interferon units is equivalent to 2.0 reference research units of mouse interferon (in terms of the National Institutes of Health reference preparation, catalogue number Go02-904-511). All interferon samples were dialysed against MEM to remove cytochalasin D before assay of interferon.

RESULTS

Lack of effect of cytochalasin D on virus-induced interferon production in mouse fibroblasts and mouse spleen cells

Cultures of 1 x 10⁷ L cells which had been grown to confluence in a Petri dish, and similarly 5 x 10⁷ mouse spleen cells, were infected with 128 HA units of HVJ or 64 HA units of NDV in 2 ml of MEM containing various concentrations of cytochalasin D. After 20 h incubation at 35 °C, the culture fluid was collected and assayed for interferon. As shown in Fig. 1(a) and (b), interferon production was not influenced by cytochalasin D treatment in either system.

Effects of cytochalasin D on endotoxin-induced interferon production

Mouse spleen cells were incubated at 35 °C for 8 h with E. coli endotoxin (100 µg/ml) in MEM containing various concentrations of cytochalasin D. At the end of the incubation period, a sample of cell-free supernatant fluid was assayed for interferon. A 97% inhibition of interferon production was observed in the presence of 10 µg/ml cytochalasin D, and 94% inhibition in the presence of 1 µg/ml (Fig. 2a).

In another experiment, cytochalasin D (10 µg/ml) was added at 0, 1, 2 or 4 h after incubation of mouse spleen cells with endotoxin (100 µg/ml). As can be seen from Fig. 2(b), when cytochalasin D was already present at the start of the incubation, interferon
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production was reduced to less than 5% of that in control culture. If, however, the drug was added even after incubation for only 1 h, interferon was produced to an appreciable extent. Cytochalasin D, therefore, seems to inhibit an early stage of interferon production.

Effect of cytochalasin D on immune interferon production

Spleen cells obtained from mice previously injected with allogeneic cells produce immune interferon when they are co-cultivated in vitro with these cells (Borecky et al. 1971). The effect of cytochalasin D on interferon production in this system was studied. Spleen cells were derived from mice injected intraperitoneally with $1 \times 10^7$ L cells 7 days previously. When these were treated with various concentrations of cytochalasin D, there was a dose-dependent reduction in the amount of interferon in culture fluids collected 24 h after co-cultivation in vitro with $1 \times 10^7$ L cells (Fig. 3a). It appears that cytochalasin D inhibits an early stage of immune interferon production. When the drug ($10 \mu g/ml$) was added during the first 2 h of co-cultivation, it greatly inhibited interferon production, but when it was added after co-cultivation for 4 h, interferon was produced almost to the full extent (Fig. 2b).

Effect of cytochalasin D on interferon production in response to u.v.-irradiated HVJ

In a previous study (Ito et al. 1976a), we showed that cytochalasin D inhibits interferon production by mouse spleen cells co-cultivated with HVJ-carrier cells. Interferon production in this system is initiated by membrane-membrane interaction between the spleen cells and the virus-infected cells. We have recently obtained data suggesting that the mechanism of membrane-membrane interaction may also be involved when interferon is produced by mouse spleen cells in response to HVJ (Ito et al. 1978). However, our finding that interferon production by mouse spleen cells stimulated with HVJ is not influenced by cytochalasin D treatment is inconsistent with this speculation. Ultraviolet-irradiation does not affect the ability of HVJ to induce interferon formation in mouse spleen cells, and the actual inducer of interferon in mouse spleen cells is highly resistant to u.v.-irradiation (Ito et al. 1978). We found that interferon production by mouse spleen cells could be suppressed by cytochalasin D when the cells were stimulated with HVJ which had been

Fig. 1. Lack of effect of cytochalasin D on virus-induced interferon production in (a) L cells and (b) mouse spleen cells. Cytochalasin D was present throughout the 20 h duration of the experiment. 
\( \Delta \cdots \Delta \), cultures treated with HVJ; \( \bullet \cdots \bullet \), cultures treated with NDV.
Fig. 2. (a) Effect of cytochalasin D on endotoxin-induced interferon production in mouse spleen cells. Mouse spleen cells were incubated at 35 °C for 8 h with *E. coli* endotoxin (100 μg/ml) in MEM containing various concentrations of cytochalasin D. (b) The effect of adding cytochalasin D to ongoing interferon production. Cytochalasin D (final concentration 10 μg/ml) was added at 0, 1, 2 or 4 h after incubation of mouse spleen cells with endotoxin (100 μg/ml). Each point is the mean of two experiments. †, Time when inhibitor added; ●—●, control without cytochalasin D.

Fig. 3. (a) Effect of cytochalasin D on immune interferon production. Mouse spleen cells derived from C57BL/6 mice intraperitoneally injected with 1 × 10⁷ L cells 7 days previously were cocultivated at 35 °C for 24 h with 1 × 10⁷ cells in the presence of various concentrations of cytochalasin D. At the end of the incubation period, cell-free supernatant fluid was assayed for interferon. (b) The effect of adding cytochalasin D to ongoing interferon production. Cytochalasin D was added at 0, 1, 2 or 4 h after mixing L cell sensitized mouse spleen cells and L cells. The final concentration of cytochalasin D was 10 μg/ml. Each point is the mean of two experiments. †, Time when inhibitor added; ●—●, control without cytochalasin D.

Irradiated for 2 h, but not when they were stimulated with virus irradiated for only 15 min. However, in L cells treated with cytochalasin D, HVJ irradiated for 1 min induced as much interferon as in untreated control L cells (Fig. 4).

The infectivity of HVJ for eggs was completely lost with 5 min u.v.-irradiation. The neuraminidase and haemolytic activities remained almost unchanged after 15 min u.v.-irradiation, but were decreased to less than 1% of those of untreated HVJ after 2 h
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Fig. 4. Effect of cytochalasin D on interferon production induced by u.v.-irradiated HVJ. Cultures of \(1 \times 10^7\) L cells and \(5 \times 10^7\) mouse spleen cells were incubated for 20 h at 35 °C with u.v.-irradiated HVJ in MEM containing various concentrations of cytochalasin D, and cell-free supernatant fluids were assayed for interferon. •–•, 15 min u.v.-irradiated HVJ and mouse spleen cells; ▲–▲, 2 h u.v.-irradiated HVJ and mouse spleen cells; ■–■, 1 min u.v.-irradiated HVJ and L cells.

u.v.-irradiation (Ito et al. 1978). HA activity was the most resistant against u.v.-irradiation and a considerable amount of this activity remained even after 2 h u.v.-irradiation (Ito et al. 1978). These findings indicate that resistance to cytochalasin D treatment in the mouse spleen cells – HVJ interferon production system may result from virus activities other than haemagglutinating activity.

DISCUSSION

The present studies show that cytochalasin D suppresses interferon production by spleen cells in response to endotoxin or an allogeneic stimulation, but that interferon production in mouse fibroblasts (L cells) stimulated with HVJ or NDV is not influenced by cytochalasin D treatment.

Endotoxin-induced interferon production was suppressed by cytochalasin D treatment, provided this was added together with the inducer. Similar results were obtained in immune interferon production system when the drug was added during the first 2 h of co-cultivation between L cells and mouse spleen cells sensitized to L cells. These findings indicate that the action of cytochalasin D is not on secretion of interferon, but rather on an earlier event. In a previous study (Ito et al. 1976a) we showed that cytochalasin B inhibited interferon production by mouse spleen cells co-cultivated with virus carrier cells because some necessary interaction between these cells was prevented. It seems likely that also in the case of interferon induced by endotoxin or immune stimulation, cytochalasin D may affect the processing of the inducer by mouse spleen cells.

Although interferon production in mouse spleen cells stimulated with untreated HVJ was not influenced by cytochalasin D treatment, such treatment did suppress interferon production when HVJ was u.v.-irradiated for more than 15 min. Thus cytochalasin D sensitive structures seem to play some role in interferon production by mouse spleen cells stimulated with HVJ.

These results demonstrate that cytochalasin D sensitive structures play an essential role
in the initial stage of interferon production by mouse spleen cells stimulated with endotoxin, an allogeneic stimulus or HVJ. It is likely that these structures correspond to a cortical layer of microfilaments which interact directly with membrane elements (Wessels et al. 1971).

On the other hand, since interferon production by L cells stimulated with untreated or u.v.-irradiated HVJ was not influenced by cytochalasin D treatment, no essential role of microfilaments could be detected in this fibroblast-virus system.

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REFERENCES


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