Inhibitory Effect of Interferon Preparations on the Development of Foci of Altered Cells Induced in vitro by Mouse Sarcoma Virus

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Several investigators have shown that mouse sarcoma virus, MOLONEY strain (MSV-M), induces foci of altered cells in tissue culture of mouse or rat cells (Hartley & Rowe, 1966; Fischinger, Messore & O'Connor, 1967; Ting, 1966; Bernard, Boiron & Lasneret, 1967). The focus-forming capacity of MSV-M is now utilized for titrating the virus (Hartley & Rowe, 1966). Interferon has been proved to inhibit both the multiplication of oncogenic viruses and the cellular transformation induced by these viruses in vivo and in vitro. This inhibitory effect concerns DNA viruses such as polyoma or SV 40 (Atanasiu & Chany, 1960; Allison, 1961; Todaro & Baron, 1965) as well as RNA viruses such as Rous sarcoma virus (Strandström, Sandelin & Öker-Blom, 1962; Bader, 1962; Traub & Morgan, 1967) and murine leukaemia viruses (Gresser et al. 1966). We report here the inhibitory action of preparations of interferon on the development of foci of altered mouse cells induced in vitro by MSV-M.

The MSV-M used was kindly supplied by Dr J. B. Moloney and propagated by serial inoculations of cell-free extracts to BALB/c newborn mice. The virus was extracted from tumours by differential centrifugation (Chenaille et al. 1967) and stored in liquid nitrogen.

Preparations of interferon were obtained according to the technique of Finter from brains of BALB/c mice inoculated intracerebrally with West Nile virus (Finter, 1964). These preparations resisted pH 2, were not dialysable, could not be sedimented by a centrifugation at 80,000 g for 2 hr and inhibited the growth of different challenge viruses (vesicular stomatitis, encephalomyocarditis, vaccinia viruses) in cell cultures, provided that the assay was performed on mouse cells. They did not inhibit the same viruses when tested on rat and hamster cells. Interferon batches were titrated by a method already described (Peries, Boiron & Canivet, 1965). Titres were closely similar in the different stock preparations (1280 to 2560 units/ml.). A mock interferon was obtained by inoculating normal BALB/c mice intracerebrally with the medium used for the suspension of West Nile virus. Extracts of these brains were treated by the same technique as above and did not inhibit challenge viruses.

Secondary cultures of Swiss mouse embryo cells were prepared by standard techniques and allowed to grow in Falcon plastic flasks in a medium consisting of Eagle's minimal essential medium and 10% calf serum. As soon as the cells formed a monolayer the medium was changed and three series of three flasks each were prepared: (1) cells receiving 5 ml. of interferon preparation previously diluted 1/20 with Eagle's medium; (2) cells receiving the same volume of mock interferon similarly diluted; (3) cells receiving 5 ml. of Eagle's medium only. The bottles were placed at 37° and after an incubation of 24 hr the culture fluids were discarded and the cells were washed 3 times with Eagle's minimal essential medium. MSV-M was added to each flask at the dilution suitable to induce 50 to 200 foci per bottle and the volume was made up to
Table 1. Effect of interferon preparations on number of foci of altered cells induced by MSV on cultures of Swiss mouse embryo cells

<table>
<thead>
<tr>
<th>Expt*</th>
<th>MSV</th>
<th>MSV + Interferon</th>
<th>MSV + Mock interferon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of foci per flask</td>
<td>Σ†</td>
<td>m‡</td>
</tr>
<tr>
<td>1</td>
<td>81–96–72</td>
<td>249</td>
<td>83</td>
</tr>
<tr>
<td>4</td>
<td>33–53–71</td>
<td>157</td>
<td>52, 33</td>
</tr>
</tbody>
</table>

* Statistical evaluation using t test between MSV and MSV + interferon series. Expt 1, P = 0.003; Expt 2, P < 0.005; Expt 3, P < 0.005; Expt 4, P < 0.005.
† Total number of foci in three flasks.
‡ Mean on three flasks.
5 ml. with Eagle's medium + 5% calf serum. After 7 days' incubation at 37° the foci of altered cells were counted. Control uninoculated cells were observed at the same time (Table 1).

There was a highly significant reduction in the number of foci induced by MSV-M in the cells pretreated with interferon preparations. In vitro mixing of interferon preparations with MSV-M before the inoculation of cells did not inhibit the focus-forming capacity of the virus.

Experiments designed to rule out an eventual action of interferon preparations on viral adsorption to cells were carried out as follows: control and interferon-treated cells were inoculated with MSV-M at a multiplicity of 0.01. After a 3 hr incubation at room temperature, the inocula were harvested and titrated on Swiss mouse embryo cells. Similar adsorption rates were found in each series, that is, 100% of the focus-forming capacity had disappeared. This result was interpreted as indicating that interferon preparations did not impair adsorption of MSV-M to cells. Moreover, the virus yields in both series as evaluated by titration of focus-forming capacity in the culture fluids 5 days following inoculation were found to be $4 \times 10^4$ f.f.u./ml and $10^3$ f.f.u./ml respectively. Since the adsorption of the virus seemed normal, the decrease in virus yield could be due to an intracellular event, which is in keeping with the mechanism of action of interferon.

Laboratory of Experimental Haematology
Institut de Recherches sur les leucémies
Hôpital Saint-Louis
Paris Xe, France

J. PERIES
M. CANIVET
B. GUILLEMAIN
M. BOIRON

REFERENCES


Short communications


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