Large Scale Production of Mouse Interferons from Monolayers of Ehrlich Ascites Tumour Cells

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SUMMARY

Conditions are described for the large scale production of mouse interferons from Ehrlich ascites tumour cells cultured as monolayers in roller bottles. With the procedure reported here, we have used 50 to 65 600 cm$^2$ roller bottles to produce routinely $2 \times 10^8$ to $3 \times 10^8$ International units of crude mouse interferon/week with a specific activity of $1 \times 10^8$ to $1.5 \times 10^8$ units/mg protein.

Two decades elapsed between the discovery of interferons (Isaacs & Lindenmann, 1957) and the purification of some of them to apparent homogeneity (Knight, 1975, 1976; Berthold et al. 1978; DeMaeyer-Guignard et al. 1978; Fujisawa et al. 1978; Iwakura et al. 1978a, b; Kawakita et al. 1978; Cabrer et al. 1979; Rubinstein et al. 1979). The main cause of this delay was the difficulty in obtaining sufficient amounts of crude interferons for use as a starting material for purification.

The first interferons to be produced on a relatively large scale were from mouse cells and human cells. The mass production of crude mouse interferon is performed usually with one of two cell lines. L929 cells are grown in monolayers and usually induced by infection with Newcastle disease virus (NDV; Henle et al. 1959; Cantell & Paucker, 1963), although a more potent inducer, reovirus inactivated by u.v. irradiation, was reported recently (Henderson & Joklik, 1978). C243, a better producing line, was obtained by transforming mouse 3T3 cells with mouse sarcoma virus (Oie et al. 1972). This line can be used in suspension culture for interferon production (Tovey et al. 1974). To boost the yield of interferon, the cultures have usually been primed by exposure to low concentrations of interferon prior to induction (Isaacs & Burke, 1958; Stewart et al. 1971); after induction with live NDV, they have been treated with metabolic inhibitors (superinduction; Vilcek et al. 1969; Tan et al. 1970, 1971; Sehgal et al. 1975; Wiranowska-Stewart et al. 1977).

We describe here a procedure in which fractionated foetal calf serum was used in the production of mouse interferons from monolayer cultures of Ehrlich ascites tumour (EAT) cells in roller bottles. This procedure was used to prepare the starting material for the purification of mouse EAT cell interferons to apparent homogeneity (Kawakita et al. 1978; Cabrer et al. 1979; Taira et al. 1980).

The procedure developed for producing interferon in stationary flasks (Slattery et al. 1980) was adapted for large scale production from cell monolayers in glass (or plastic) roller bottles on a bottle rotator. For this purpose EAT cells were grown in monolayers in 150 ml monolayer growth medium in glass roller bottles ($118 \times 260$ mm; Wheaton) at 0.5 rev/min and 37 °C. When the cells reached confluency (usually 3 to 4 days after seeding; $1 \times 10^8$ to $2 \times 10^8$ cells/roller bottle) they were passaged at a split ratio of 1:10.

To induce interferon production, the medium was removed from bottles containing monolayers of cells which had just reached confluency and replaced with 10 ml of interferon induction medium (Slattery et al. 1980) containing 5% (v/v) carboxymethyl (CM)-Sephadex-treated foetal calf serum and supplemented with 20 p.f.u./cell of NDV. After 2 h of incubation, the induction medium was replaced by 20 ml of production medium containing
Table 1. **CM-Sephadex-treated foetal calf serum can substitute for foetal calf serum in supporting interferon production***

<table>
<thead>
<tr>
<th>Addition to the production medium</th>
<th>Yield of interferon (units/cell)</th>
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<tr>
<td>0·5% (v/v) foetal calf serum</td>
<td>0·082</td>
</tr>
<tr>
<td>5% (v/v) CM-Sephadex-treated foetal calf serum†</td>
<td>0·075</td>
</tr>
<tr>
<td>0·5% (v/v) foetal calf serum plus 6 mM-theophylline</td>
<td>0·30</td>
</tr>
<tr>
<td>5% (v/v) CM-Sephadex-treated foetal calf serum† plus 6 mM-theophylline</td>
<td>0·35</td>
</tr>
<tr>
<td>6 mM-theophylline (no serum)</td>
<td>0·0059</td>
</tr>
</tbody>
</table>

* EAT cells were infected with NDV at a m.o.i. of 15. Two h later, the medium was replaced by fresh medium containing no serum, or 0·5% (v/v) foetal calf serum, or 5% (v/v) CM-Sephadex-treated foetal calf serum and/or 6 mM-theophylline. At 25 h p.i., the medium was harvested from each culture and its interferon content was determined.† Because it is diluted 10-fold during preparation, 5% (v/v) CM-Sephadex-treated foetal calf serum corresponds to 0·5% (v/v) unfractionated serum.

5% (v/v) CM-Sephadex-treated foetal calf serum and supplemented with 6 mM-theophylline. After 3 additional hours of incubation, the production medium was replaced with 20 ml of fresh production medium containing 5% (v/v) CM-Sephadex-treated foetal calf serum and 6 mM-theophylline. Between 20 and 30 h p.i. (when the level of interferon is maximal; by 40 h it decreases) the medium was decanted. It was supplemented with 10 tablets of ashless cellulose carrier (Whatman) per litre medium and clarified by centrifugation at 7000 g and 4 °C for 30 min. The supernatant (crude interferon) was supplemented with 10 μM-phenylmethylsulphonyl fluoride and 3·1 mM-Na3 (to decrease proteolysis and microbial growth) and stored at 4 °C. This crude interferon was stable for up to 1 month.

The procedure resulted in the production of up to 0·8 International units of interferon/EAT cell with an average of 0·35 units. The titre of the crude interferon was between 1 × 10⁶ and 2 × 10⁶ units/ml with a specific activity between 1 × 10⁶ and 1·5 × 10⁶ units/mg protein.

The foetal calf serum used in large scale interferon production was fractionated by chromatography on CM-Sephadex as follows: 50 ml foetal calf serum were diluted to a final volume of 500 ml with PBS and applied to a 50 ml column of CM-Sephadex (Pharmacia) which had been equilibrated with PBS. The flow-through fraction was used as the serum in interferon induction and production media in the amounts indicated. This was done to facilitate purification of interferon: the proteins from the CM-Sephadex-treated serum fraction added to the production medium were quantitatively removed in the second step of the purification, i.e. chromatography on CM-Sephadex. As shown in Table 1, the serum fraction obtained after treatment with CM-Sephadex allowed the production of as much interferon as complete serum. The yield of interferon in a serum-free medium was less than 2% of that in the medium with serum.

The procedure has been in use in our laboratory for about 3 years. Using 50 to 65 (600 cm²) roller bottles with cells in confluent monolayers, we have routinely produced 2 × 10⁶ to 3 × 10⁹ units of interferon/week. In a series of 12 consecutive weeks of production, the average interferon yield/cell was 0·36 units. To obtain a high yield the cells should be induced as soon as they become confluent. The yield of interferon/cell is the same in glass and plastic roller bottles; it is, however, about 20% higher in stationary plastic flasks and 75 to 80% lower in suspension culture (data not shown).

The crude interferon produced is stable for at least 5 weeks if stored at 4 °C. Following the first two steps of the EAT cell interferon purification procedure of Cabrer et al. (1979), i.e. fractionation on controlled pore glass and CM-Sephadex, interferon with a specific activity of 2 × 10⁸ units/mg protein is obtained with no loss of activity. Further purification, involving chromatography on phosphocellulose and gel filtration, gives rise to an interferon
of specific activity of $1.4 \times 10^8$ units/mg with an 80% yield. The isolation of interferon in an apparently homogeneous state (sp. act. $2 \times 10^8$ to $3 \times 10^8$ units/mg protein) requires further purification by isoelectric focusing and hydrophobic chromatography. The overall yield after these steps is between 11 and 20% (Cabrér et al. 1979).

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Short communications


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