A SYSTEMATIC STUDY OF INTERFERON PRODUCTION BY MOUSE L-929 CELLS INDUCED WITH POLY(I)·POLY(C) AND DEAE-DEXTRAN

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1. Introduction

Interferons are proteins with antiviral activity [1]. For purification, and application in animal and man, large amounts of potent interferon preparations are needed. Therefore, searches for new methods of large-scale interferon production, and improvements of current techniques are of great importance.

It is well known that viruses are excellent interferon-inducing agents (reviewed [2]). At present, Sendai virus is routinely used for large-scale production of human leukocyte [3] and lymphoblast [4] interferon, and Newcastle disease virus for preparation of large amounts of mouse interferon [5–8].

In addition to viruses, double-stranded polyribonucleotides, e.g., polyinosinic·polycytidylic acid (poly(I)·poly(C)), are the most potent interferon inducers available (reviewed [9,10]). Nevertheless, poly(I)·poly(C) has only been applied for large-scale production of human fibroblast type interferon [11–14]. Although it has been shown, that mouse cell lines do produce interferon upon incubation with poly(I)·poly(C) [15–18], our knowledge concerning the optimal conditions of interferon synthesis is rather limited. Data presented in [15–19] indicate that poly(I)·poly(C) is only a moderate interferon inducer in these cell species. In contrast to these earlier findings, the results presented in this study show that, under well-chosen conditions, L-929 cells are excellent producers of potent mouse interferon.

Parts of this study have been presented in [20].

2. Experimental

2.1. *Materials*

Poly(I)·poly(C) was purchased from Microbiological Associates (Bethesda), diethylaminoethyl (DEAE)-dextran (mol. wt 5×10^5) from Pharmacia (Uppsala), cycloheximide from Sigma (St Louis), actinomycin D from Boehringer (Mannheim) and amphotericin B from Squibb and Sons (Princeton). Culture media were obtained from Flow Labs (Irvine, Scotland).

2.2. *Cell culture*

L-929 cells (Flow Labs.) were grown in monolayer in Eagle's minimum essential medium (MEM) supplemented with 10% foetal calf serum, non-essential amino acids and gentamicin (160 μg/ml) at 37°C in a 5% CO_2 atmosphere.

2.3. *Interferon induction*

To induce interferon synthesis, L-929 cells, grown to confluency in 25 cm^2 culture flasks (Corning, NY), were incubated in 1 ml MEM containing appropriate amounts of poly(I)·poly(C) and DEAE-dextran (as indicated in the figure legends). After an appropriate period, cell layers were washed twice with 5 ml MEM and incubated overnight in 5 ml MEM. Subsequently, the culture fluid was collected, centrifuged for 10 min at $800 \times g$ and assayed for its interferon content.

2.4. *Interferon assay*

Interferon was assayed by measuring the reduction of the cytopathic effect of vesicular stomatitis virus on L-929 cells. The microtitre method used was essentially as in [21]. In this assay 1 unit is equivalent to 2.5 reference units (reference standard G-002-904-511, NIH, Bethesda). All interferon titres in this report are expressed in terms of reference units/ml.

2.5. *Protein determination*

Protein concentrations were determined by the Lowry method [22] using bovine serum albumin as a standard.

3. Results and discussion

3.1. *Kinetics of interferon production*

It is well known, that L cells produce interferon after triggering with poly(I)·poly(C). Addition of substances which promote the adsorption of poly(I)·poly(C) to the cell membrane, or which stimulate its penetration into the cell, is, however, of crucial importance [15–18]. In this study we investigated in detail interferon production induced by poly(I)·poly(C) and DEAE-dextran, by modifying the method in [15].

The time period after which interferon activity can be detected in the culture fluid, upon incubation with poly(I)·poly(C), appears to be dependent on the system used [12,16]. For L-929 cells we found (fig.1, see also [16]) that production of interferon starts about 7 h after addition of poly(I)·poly(C) and DEAE-dextran, almost independent of the induction period and concentrations of poly(I)·poly(C) and DEAE-dextran. Possibly, interferon production starts somewhat earlier at high DEAE-dextran concentration (fig.1c).

After its start, interferon production continues for ~9 h and then stops, irrespective of the incubation conditions, and even if poly(I)·poly(C) and DEAE-dextran are present during the whole incubation period (fig.1b). The reason for this shut-off remains to be established. It has been suggested that for human fibroblasts a specific repressor is responsible for this shut-off [23,24]. However, our data can also be explained by a non-specific cytotoxic effect, since

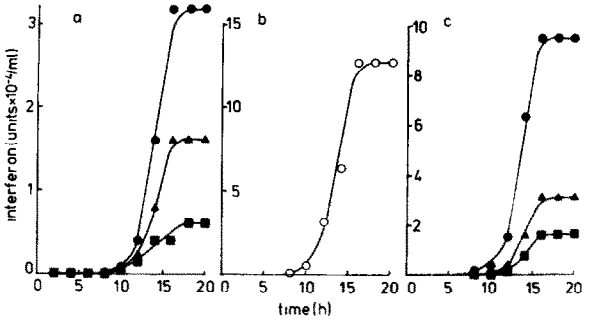

Fig.1. Kinetics of interferon production. Interferon induction was performed as in section 2.3, except for fig.1b, 20 h induction. In this case interferon production was measured during the induction period (in 1 ml induction medium in contrast to 5 ml culture fluid used in the other experiments). (a,b) Effect of induction period on interferon production. Poly(I)·poly(C) 50 μg/ml, DEAE-dextran 200 μg/ml; induction period, respectively, 2 h (■–■); 4 h (▲–▲); 8 h (●–●); 20 h (○–○). (c) Effect of poly(I)·poly(C), DEAE-dextran concentrations on interferon production. Induction period 8 h; poly(I)·poly(C), DEAE-dextran, respectively, 5 μg/ml, 200 μg/ml (■–■); 50 μg/ml, 200 μg/ml (▲–▲); 50 μg/ml, 400 μg/ml (●–●).

protein synthesis tends to decline during the last part of the incubation period (data not shown).

Figure 1 shows that the final yield of interferon is strongly dependent on the experimental conditions used. If high concentrations of poly(I)·poly(C) and DEAE-dextran (50 μg/ml and 400 μg/ml, respectively) are used, a considerable increase in the interferon production is observed. The optimal induction period is about 8 h. Prolonged induction periods do not give higher interferon yields, and, obviously, lead to production of interferon during the induction phase, whereas shorter induction periods lead to incomplete induction.

3.2. *Effect of incubation conditions on interferon production*

Figure 2 shows in more detail the effect of different incubation conditions on interferon production.

In summary, maximum amounts of interferon are produced after an induction period of 6–8 h using 20–50 μg/ml of poly(I)·poly(C) and 400–800 μg/ml of DEAE-dextran. Under these conditions 64–128 × 10^3 units/ml of interferon are formed. This is equivalent to 12–25 × 10^3 units/cm^2 of cell

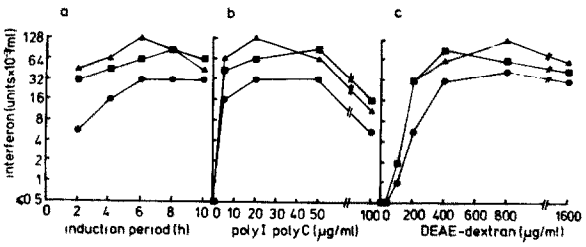

Fig.2. Effect of induction period and poly(I)·poly(C), DEAE-dextran concentrations on interferon production. Interferon induction was carried out as in section 2.3. Total incubation period was 20 h. (a) Poly(I)·poly(C) 20 μg/ml; DEAE-dextran, respectively 200 μg/ml (●–●); 400 μg/ml (■–■); 800 μg/ml (▲–▲). (b) Induction period 6 h; DEAE-dextran, respectively 200 μg/ml (●–●), 400 μg/ml (■–■) and 800 μg/ml (▲–▲). (c) Poly(I)·poly(C) and induction period, respectively 20 μg/ml, 2 h (●–●); 20 μg/ml, 6 h (▲–▲); 50 μg/ml, 6 h (■–■).

culture surface or 50–100 units/10^3 cells. These data further show that previous experiments concerning interferon synthesis by mouse L cells have been carried out under sub-optimal conditions [15–17,19].

All experiments were done in serum-free medium. Interferon produced in this way, under optimal conditions as above, has spec. act. 1.5–3 × 10^6 units/mg protein. Addition of serum during the induction or interferon production phase did not lead to a higher interferon yield. Also, the addition of cycloheximide and actinomycin D during part of the induction period (super induction) and 'priming' with interferon, both known to enhance interferon synthesis in human fibroblasts, had no effect in our system [12,23,25].

We were able to confirm earlier observations which showed that amphotericin B [17] and Ca^{2+} [18], in the presence of small amounts of DEAE-dextran, or in its absence, stimulate interferon production by L-929 cells induced with poly(I)·poly(C). However, using optimal conditions (20 μg poly(I)·poly(C)/ml, 800 μg DEAE-dextran/ml, 6 h induction) no beneficial effect of these substances was noticed.

3.3. *Large-scale interferon production*

In a preliminary study on large-scale interferon preparation 10^8 units interferon were produced on 6000 cm^2 culture surface (fourty 150 cm^2 culture flasks). These results are comparable to or even better than those obtained by others who use virus as inducing agent to produce mouse interferon [5–8,26].

It is concluded that induction of interferon on L-929 cells by poly(I)·poly(C) and DEAE-dextran under the experimental conditions described in this report is an excellent system for production of large amounts of potent mouse interferon.

Experiments to purify and characterize this type of interferon are in progress.

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