High Interferon Producing Line of Transformed Murine Cells

(Accepted 4 June 1972)

During a comparative study of the interferon system in normal and virus-transformed murine cells, a clonal line was observed to produce unusually high yields of interferon. This report describes some variables which influence the interferon yield.

Procedures for the establishment of Moloney sarcoma virus (MSV)-transformed cell clones have been described previously (Bassin, Tuttle & Fischinger, 1970). The twice cloned line C-243-3 was received from Dr. Bassin, National Cancer Institute, Bethesda, Maryland. The line was derived by transforming 3T3FL cells, a subline of the original Swiss 3T3 cell line (Todaro & Green, 1963) under conditions permitting infection by MSV in the absence of associated ‘helper’ leukaemia virus. The cells contain the MSV genome but do not release infectious MSV or leukaemia virus. However, the cells do release small quantities of non-infectious, non-transforming, virus-like particles. Such cells have been termed S+L- cells (Bassinet al. 1970, 1971). The cloned line, C-243-3, was grown in McCoy’s 5a medium (modified) supplemented with 10% foetal bovine serum (FBS). The cells grew rapidly and were split four- to sixfold twice each week. Vigorous shaking was sufficient to disperse the cells for passage, thus making trypsinization unnecessary. For maintenance, Eagle’s reinforced medium (Bablanian, Eggers & Tamm, 1965) plus 2% FBS was used. A stock of Newcastle disease virus (NDV), HERTS strain, was propagated in embryonated chicken eggs (Henle & Hilleman, 1969); its titre was $10^{9.2}$ p.f.u./ml in chicken embryo cells. Chikungunya virus (CV) was produced in the brains of suckling mice (Hammon & Sather, 1969) and had an infectivity of $10^{9.5}$ p.f.u./ml in VERO cells.

Procedures for the preparation of polyinosinic-polycytidylic acid (poly I. poly C) (Field et al. 1967) and the induction of interferon production in cells using NDV (Dianzani et al. 1970), CV (Ho, 1966) or poly I. poly C plus diethylaminoethyl-dextran (DEAE-D) (Dianzani et al. 1971) have been reported. The input multiplicities of infection for NDV and CV were approximately 1. The concentrations of poly I. poly C and DEAE-D used were 30 and 100 µg/ml, respectively. Exposure time to poly I. poly C and DEAE-D was 2 h. Interferon was assayed by the GD-7 virus haemagglutinin yield reduction method in mouse L cells (Oie et al. 1972). By this assay the research reference mouse interferon had a titre of $10^{4.5}$ units/ml.

Production of interferon in C-243-3 cells was induced by NDV, CV, or poly I. poly C plus DEAE-D. The 24 h interferon yields from two experiments are shown in Table 1. All three stimulators induced interferon production in C-243-3 cells, but NDV was consistently better. In all the experiments with NDV the lowest titre obtained was $10^{4.2}$ units/ml, while the highest was $10^{5.6}$ units/ml. The interferon concentrations of most of the preparations ranged from $10^{5.2}$ to $10^{5.6}$ units/ml.

Measurement of interferon production by C-243-3 cells at different times after induction by NDV showed that at 6, 12 and 24 h after infection, $10^{5.4}$ units/ml, $10^{4.8}$ units/ml, and $10^{4.8}$ units/ml, respectively, of interferon were produced. Therefore, in all experiments interferon was harvested after 18 to 24 h incubation at 37°C.

The properties of the interferon produced by C-243-3 cells were determined. The interferon was stable at pH 2 for at least 5 days, sensitive to trypsin, not removed by frequent washings
Table 1. Interferon production by C-243-3 cells stimulated with various inducers

<table>
<thead>
<tr>
<th>Inducer</th>
<th>Interferon yield (log_{10} units/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly I, poly C (30 µg/ml) + DEAE-D (100 µg/ml)</td>
<td>Exp. 1: 3.9 Exp. 2: 4.3</td>
</tr>
<tr>
<td>Chikungunya virus (input multiplicity = 1 p.f.u./ml)</td>
<td>Exp. 1: 3.8 Exp. 2: 3.7</td>
</tr>
<tr>
<td>NDV (input multiplicity = 1 p.f.u./ml)</td>
<td>Exp. 1: 4.7 Exp. 2: 5.0</td>
</tr>
</tbody>
</table>

of interferon-treated cells, had antivirus activity against vesicular stomatitis virus, GD-7 virus and Sindbis virus, and was active in mouse L cells and secondary mouse embryo fibroblasts but not in chicken embryo cells. It induced antivirus activity in rat embryo cells but the activity was more than 100-fold less in rat cells than in mouse L cells. These properties are similar to those previously reported for mouse interferon (Lockart, 1966).

The effect of pre-treatment of cells with interferon on interferon production (Lockart, 1965; Stewart, Gosser & Lockart, 1972) was investigated. Pre-treatment of C-243-3 cells with 5 to 500 units/ml of interferon and then stimulation with either NDV or poly I.poly C plus DEAE-D did not alter interferon yields.

A pool of interferon having a titre of $10^{5.6}$ units/ml was successfully concentrated tenfold by dialysis against Aquacide II (California Biochemical Corp., Los Angeles) to a final titre of $10^{6.7}$ units/ml. The unconcentrated and concentrated interferon preparations were stable over a period of 4 months when stored at 4 °C, -20 °C, -70 °C and when frozen and thawed repeatedly. A possible contributing factor towards stability was the serum protein content of the preparations, i.e. 2 % FBS in the unconcentrated interferon and 20 % FBS in the concentrated material.

Production of interferon in both normal and transformed cells was highly variable (Freeman et al. 1970). Consistent with these observations was our finding that only 1 of 2 cloned S+L—cell lines from Moloney sarcoma virus-transformed 3T3FL cell culture was a high producer of interferon. The low producer cell line and the original 3T3FL culture produced $10^{2.5}$ interferon units/ml.

In this laboratory mouse L cells stimulated with NDV produced $10^{3.0}$ units/ml of interferon which was increased to $10^{3.9}$ units/ml when ‘priming’ was employed (Margolis, Oie & Levy, 1972). These primed interferon levels are four- to tenfold lower than those produced by C-243-3 cells.

The present system for production of mouse interferon offers several advantages and possibilities. The ease of handling and growing large volumes of C-243-3 cells, the high interferon yields, the simplicity of concentration and the high stability of the interferon preparation make this system suitable for production of large quantities of mouse interferon. The use of concentrated interferon preparations makes possible the administration of exogenous interferon at levels which approach those produced endogenously during many virus infections (Baron et al. 1966). Availability of large quantities of interferon may facilitate purification and analysis of the mouse interferon molecule.

H. K. OIE
A. F. GAZDAR
C. E. BUCKLER
S. BARON
REFERENCES


(Received 23 May 1972)