Effect of Interferon on the Cell Cycle of BALB/c 3T3 Cells

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SUMMARY

The effect of interferon on the exponential growth phase of BALB/c 3T3 cells was studied. Although interferon reduced the growth rate and the proportion of cells in both the M and S phases (mitotic index and labelling index), there were no appreciable differences in the duration of these phases between control and interferon-treated cells. Moreover, the shape of the first peak of the fraction of labelled mitoses (FLM) curve was not altered by treatment with interferon, which indicates that the duration of the S and G2 phases was not affected. However, the height of the second peak of the curve in interferon-treated cells was extremely reduced as compared to control culture. These results are compatible with the idea that the suppressive effect of interferon is exerted mainly in the G1 phase (A-state) of the cell cycle of BALB/c 3T3 cells.

In addition to their inhibitory effect on virus multiplication, interferons have been shown to inhibit the growth of cells (Knight, 1976; Stewart et al. 1976; Collyn d'Hooghe et al. 1977; Sokawa et al. 1977). In a previous report (Sokawa et al. 1977), we showed that mouse L cell interferon preparations reduced the frequency of initiation of DNA synthesis by BALB/c 3T3 cells in the transition from the quiescent state to the serum-stimulated state. Applying the A-B transition theory proposed by Smith & Martin (1973) to the results, we suggested that interferon reduced the ‘transition probability’ from the A-state to the B-phase of the cell cycle. Interferon molecules might suppress certain cellular events in the G1 phase, since the ‘transition probability’ reflects the activities of cells in the A-state which is equivalent to the G1 phase. However, it remains to be clarified whether the same holds true also for cells in the exponential growth phase and whether stages other than the G1 phase are affected or not. Here we have studied the effect of interferon on the growth rate, mitotic index, labelling index and labelled mitosis of BALB/c 3T3 cells under conditions of exponential growth. The inhibitory effect of interferon was found to reside mainly in the G1 phase (A-state), and no appreciable alterations were observed in the durations of the S, G2 and M phases.

The interferon preparations used contained $2 \times 10^2$ units/mg protein. They were obtained from the supernatant media of monolayer cultures of mouse L cells inoculated with Newcastle disease virus, and were purified by column chromatography using DEAE-Sephadex and CM-Sephadex according to Yamamoto et al. (1974). The titres are expressed in mouse reference standard units (u) in terms of the National Institute of Health Research Reference Mouse Interferon Preparation, catalogue number G002-934-511.

BALB/c 3T3 cells (Flow Laboratories) were plated at 50000 cells in 35 mm Falcon plastic plates containing 2 ml of Dulbecco’s modified Eagle’s medium (Gibco) with 10% foetal bovine serum (Microbiological Associates) in the presence or absence of interferon (1000 or 10000 u/ml) and they were grown at 37 °C in a 10% (v/v) CO2 atmosphere. The increase in cell number was measured as follows: cell sheets were washed with phosphate buffered saline (PBS) and fixed for 15 min in 5% perchloric acid. After rinsing with ethanol and
air-drying, the cells were stained with Giemsa solution. Cells were counted under the microscope with a micrometer (a square of 660 × 660 μm in size) in the ocular. The relative number of cells on a plate was determined by counting over 10 fields moved across the plate along a diameter. The error of the estimation was lower than 3%. Regression lines for the growth rate were estimated by the method of least squares. The fraction of cells in mitosis (mitotic index, MI) was determined by examination of 1000 cells.

As shown in Fig. 1(a) BALB/c 3T3 cells grew exponentially whether or not interferon was present in the medium but there was an increase in doubling time and a decrease in MI when interferon was present in the medium.

Fig. 1(b) shows the proportion of cells with labelled nuclei (labelling index, LI). At each time indicated, cells were exposed to $^3$H-thymidine (2 μCi/ml; 5 Ci/mmol) for 30 min. The labelled cells were washed with PBS, extracted with 5% perchloric acid, rinsed with ethanol and air-dried. The procedures for autoradiography were as described previously (Sokawa et al. 1977). After exposure for 2 weeks at 4 °C, autoradiographs were developed and stained with Giemsa solution. Cell number and labelling indices were determined as previously. The LI of interferon-treated cells was significantly reduced as compared to that of control cells (Fig. 1(b)). The values of MI and LI thus obtained in both control and interferon-treated cultures were nearly constant during the period of growth, affirming that the cells grew exponentially with a constant rate.

The duration of the M phase ($T_M$) and that of the S phase ($T_S$) can be calculated from the growth rate constant ($K_p$), the mitotic index (MI) and the labelling index (LI) according to the following equations: $T_M = MI/K_p$, and $T_S = LI/K_p$. The $K_p$ which can be obtained from the slope of the regression line of the growth curve and the mean values of MI and LI were obtained from the data of Fig. 1(a) and (b). The values of $T_M$ and $T_S$ were calculated from the above equations and all these are summarized in Table I. The results show that although interferon reduced the proportion of cells in both the M and S phases of the cell cycle, there were no appreciable differences in the duration of these phases between control and interferon-treated cells.

The durations of the G2 and S phases ($T_g$ and $T_s$) can be estimated by measuring the fraction of labelled cells in mitosis (fraction of labelled mitoses, FLM) as a function of incubation time after pulse labelling of cells with $^3$H-thymidine (Quastler & Sherman, 1959). The duration of the G2 phase ($T_g$) is roughly equal to the time from zero to the 50% point on the ascending limb of the first peak of the FLM curve, and the width of the peak at the 50% point corresponds to $T_s$. We have obtained the FLM curves of BALB/c 3T3 cells growing exponentially with or without added interferon (10000 u/ml). Cells (20000) were plated on 14 mm plastic coverslips (Wako Pure Chemical Industries), submerged in a well of a Linbro multidish tray (Linbro 24-16-TC) containing 1 ml of medium, and cultured at 37 °C in a 10% (v/v) CO2 atmosphere. After cultivation for 40 h, the cells were labelled for 20 min with $^3$H-thymidine (10 μCi/ml; 5 Ci/mmol). The labelled cells were washed with PBS containing non-labelled thymidine (20 μM) to dilute the remaining radioactivities in the cells, and were placed in the same media as before labelling, except that they contained non-labelled thymidine (20 μM). At the times indicated, coverslips were removed, washed with PBS and fixed for 20 min in acetic acid-methanol (1:3). After air-drying, they were processed for autoradiography. Autoradiographs which were exposed for 3 weeks at 4 °C were developed and stained with Giemsa solution. The fraction of labelled mitoses was determined from examination of about 100 cells in metaphase. The values of the growth rate constant ($K_p$) of control and interferon-treated cells in this experiment were 0.035 h$^{-1}$ and 0.022 h$^{-1}$, respectively. The shapes of the first peaks of the curves of the two cultures were
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Fig. 1. Effect of interferon on the growth of BALB/c 3T3 cells. (a) Growth rate and mitotic index. Cell number: O--O, control; △--△, interferon (10000 u/ml) present. Mitotic index: ◦--◦, control; ▲--▲, interferon (10000 u/ml) present. (b) Growth rate and labelling index. Cell number: O--O, control; □--□, interferon (1000 u/ml) present; △--△, interferon (1000 u/ml) present. Labelling index: ◦--◦, control; ■--■, interferon (1000 u/ml) present; ▲--▲, interferon (10000 u/ml) present. (c) Fraction of labelled mitoses. ◦--◦, Control; ■--■, interferon (10000 u/ml).

closely similar to each other (Fig. 1c), and $T_{G_1}$ and $T_s$ were estimated to be 4 h and 8 h, respectively, for both cultures. This, together with the results in Table 1, indicates that the addition of interferon exerted no appreciable effect on the $T_{M_i}, T_s$ and $T_{G_1}$ of exponentially growing BALB/c 3T3 cells.

As shown in Fig. 1(c), the height of the second peak of the FLM curve in interferon-treated cells was extremely reduced, and indeed no peak was apparent. This result indicates that interferon induces a delay in entering into the S phase, that is, the duration of the $G_1$ phase is extended by the treatment of interferon.

According to the A-B transition theory (Smith & Martin, 1973), variations of the 'transition probability' ($P$) alter the height of the second peak of the FLM curve without
Table 1. Parameters of the cell cycle of BALB/c 3T3 cells growing in the presence and absence of interferon

<table>
<thead>
<tr>
<th>Expt.*</th>
<th>Interferon (u/ml)</th>
<th>Doubling time (h)</th>
<th>Growth rate constant [Kp] (h⁻¹)</th>
<th>Mitotic index [MI] (%)</th>
<th>Labeling index [LI] (%)</th>
<th>Duration of phase†</th>
<th>M phase (h)</th>
<th>S phase (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a)</td>
<td>0</td>
<td>19.3</td>
<td>0.036</td>
<td>2.4±0.2</td>
<td>—</td>
<td>0.7±0.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>10000</td>
<td>3.4</td>
<td>0.023</td>
<td>1.7±0.1</td>
<td>—</td>
<td>0.7±0.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(b)</td>
<td>0</td>
<td>26.1</td>
<td>0.027</td>
<td>—</td>
<td>26.2±0.7</td>
<td>9.7±0.3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>29.0</td>
<td>0.024</td>
<td>—</td>
<td>24.4±0.8</td>
<td>10.2±0.3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>10000</td>
<td>31.2</td>
<td>0.022</td>
<td>—</td>
<td>21.1±0.7</td>
<td>9.6±0.3</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* (a) and (b) refer to the data shown in Fig. 1 (a) and (b), respectively.
† Calculated from the equations, $T_m = MI/K_p$ and $T_s = LI/K_F$.

changing its position when the duration of the B-phase ($T_B$) is constant; a lower second peak results from a lower $P$. The results obtained previously for the serum-stimulated culture of quiescent cells (Sokawa et al. 1977) and those obtained in this report are compatible with each other, and indicate that interferon reduces the ‘transition probability’ from the A-state to the B-phase in BALB/c 3T3 cells; the effect of interferon resides mainly in the G₁ phase of the cell cycle.

As in the previous report (Sokawa et al. 1977), we confirmed that the growth inhibitory effect of the interferon preparations observed here was lost in heating (but preserved if heated in the presence of SDS) and by trypsin digestion, in parallel to the antiviral activity (data not shown).

Although the multiplication of mouse leukaemia L1210 cells was inhibited by interferon, no effect on the generation time was detected (Macieira-Coelho et al. 1971) and, moreover, this inhibition of cell division was not due to a block in any one phase of the cell cycle (Killander et al. 1976). However, in mouse EMT6 tumour cells, interferon induced a marked and progressive increase in the intermitotic times (Collyn d’Hooghe et al. 1977). Recently, Matarese & Rossi (1977) reported that in cultures of Friend leukaemia cells, interferon extends the duration of the G₂ phase as well as that of the G₃ phase.

The discrepancies between these results may be due to the particular cell lines used; their sensitivities to interferon in respect to the reduction of cell growth may be different from each other. BALB/c 3T3 cells were rather resistant to interferon as compared to other cell lines, since a concentration of 10000 u/ml was required to exhibit the effect distinctly. However, it seems to be common in all cell lines that the initiation frequency of DNA synthesis is reduced by treatment with interferon, i.e. the duration of the G₁ phase is extended. There are some uncertainties in the case of L1210 cells, but interferon inhibited selectively the incorporation of ³H-thymidine into acid-precipitable material under steady-state growth conditions in a chemostat (Tovey et al. 1975).

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Institute for Virus Research
Kyoto University
Sakyo-ku, Kyoto, Japan

YUJI WATANABE
YOSHIHIRO SOKAWA
REFERENCES


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