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EFFECT OF MOUSE INTERFERON ON RETROVIRUS PRODUCTION BY CHRONICALLY INFECTED RAT CELLS

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Mouse interferon (IFN) inhibited retrovirus release by both mouse and rat cells with the same efficiency. However, the antiviral state developed more slowly in rat than in mouse cells, and after removal of IFN it also persisted for a longer time in rat than in mouse cells. Under conditions where IFN strongly inhibited virus production it had no effect on cell replication nor on cellular RNA or protein synthesis.

interferon retroviruses reverse transcriptase

INTRODUCTION

Interferon (IFN) has been shown in numerous studies to inhibit the replication of retroviruses [4,6]. Most of these studies have been limited to a single system, consisting of murine leukemia virus (MLV), mouse cells and mouse IFN. A few studies have used other systems. Thus, mouse IFN has been reported to inhibit murine mammary tumor virus production by mouse cells [15,16], and human fibroblasts and lymphoblastoid as well as monkey IFNs have been found to inhibit replication of the feline RD-114 retrovirus in the human cell line, RD-114 [12].

Antiviral activities of IFN [5–7], including the inhibitory effect on retroviruses [1,12], are usually species specific. However, several exceptions have been reported [5–7]. In the present study we examined the effect of mouse IFN on retrovirus replication in heterologous cells and found it as effective in rat cells as in mouse cells.

MATERIALS AND METHODS

Cells and viruses

The cells used were: a NIH/3T3 mouse cell line continuously releasing MLV/MSV complex (NIH/3T3(MLV/MSV)), a 'normal rat kidney' (NRK) cell line that was product-

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ively infected with MLV/MSV (NRK(MLV/MSV)) and the human cell line, RD-114, chronically releasing the feline RD-114 virus. The cells were grown in Dulbecco's modified Eagle's medium with 10% newborn calf serum. In experiments with IFN the serum concentration was reduced to 2.5% [14].

Interferons

Mouse IFN was prepared as previously described [2] and contained 10^6 international units per mg protein. Human fibroblast (10^7 units/mg protein) and lymphoblastoid (5×10^5 units/mg protein) were prepared as previously described [12] and were kindly provided by L. Chen and M. Revel of the Weizman Institute of Science, Rehovot, Israel.

Reverse transcriptase assay

Virus release was monitored by assaying viral reverse transcriptase activity in aliquots of the culture medium as described elsewhere [2].

Radioactive labeling

Cells were labeled with 20 μCi/ml of either [^{3}H]uridine (New England Nuclear, 50 Ci/mmol) or [^{35}S]methionine (New England Nuclear, 690 Ci/mmol). At various time intervals, samples of the cells were washed three times with cold phosphate-buffered saline (PBS) and lysed with 0.5 ml of 0.5% sodium dodecyl sulfate (SDS). After collecting the lysate each dish was further washed with 2 ml of distilled water which was combined with the lysate. The lysates were stirred vigorously until viscosity disappeared. Aliquots of 50 μl were spotted in duplicate on 3 MM Whatman filter discs, which were dried and suspended in toluene-based scintillation fluid for estimating total radioactivity in the lysate. The remainder of each lysate was precipitated by 10% trichloroacetic acid (TCA) and counted with the same filters and scintillation fluid. Cytoplasmic pools of the radioactive precursors were estimated by subtracting the TCA-precipitated radioactivity from the total radioactivity of the lysate.

RESULTS

Kinetics of development of the IFN-induced antiviral state

NRK(MLV/MSV) and NIH/3T3(MLV/MSV) cells were treated for 24 h with various concentrations of mouse IFN, washed and further incubated with fresh IFN-free medium. After 3 h incubation the viral reverse transcriptase activity was assayed in the culture medium as a measure for virus release by the cells. Fig. 1 shows almost identical dose–response curves of the IFN effect on virus release in both rat and mouse cells. As a control we examined the sensitivity of retrovirus release by rat cells to human fibroblast IFN. For comparison, the effect of this IFN was tested also on human RD-114 cells. As shown in Fig. 1, even doses which were strongly inhibitory for virus release from the human cells were completely inactive in rat cells. Similar results were obtained with human lymphoblastoid IFN (data not shown).

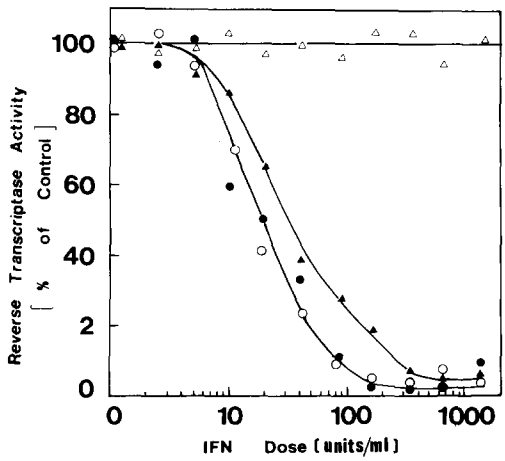

Fig. 1. Effect of increasing doses of IFN on retrovirus release. Cells were treated for 24 h with different doses of mouse or human fibroblast IFN. The cells were then washed, refed with IFN-free fresh medium and further incubated for 3 h. Viral reverse transcriptase activity appearing in the medium was assayed as a measure for virus release. Mouse IFN was tested on NRK(MLV/MSV) (○) and NIH/3T3(MLV/MSV) (●) cells. Human fibroblast IFN was tested on NRK(MLV/MSV) (△) and RD-114 cells (▲).

The antiviral activity of mouse IFN in rat cells was also tested using VSV as a challenge virus. Both NRK(MLV/MSV) and NIH/3T3(MLV/MSV) cells were treated for 24 h with various concentrations of mouse IFN and then infected with vesicular stomatitis virus (VSV) at a multiplicity of about 1 plaque-forming unit/cell. Unadsorbed virus was removed 2 h after inoculation and the cytopathic effect (CPE) was examined after additional incubation for 24 h. In mouse cells 50% inhibition of CPE was observed with 5–10 IFN units/ml, while in rat cells this inhibition was obtained with 10–20 IFN units/ml. In view of the limited accuracy of the CPE inhibition method, these results indicate that mouse and rat cells were about equally sensitive to the general antiviral effect of mouse IFN.

In order to study the kinetics of development of the antiviral state, NIH/3T3(MLV/MSV) and NRK(MLV/MSV) cells were incubated with 100 units/ml of mouse IFN and virus release was followed by assaying viral reverse transcriptase activity in aliquots taken from the culture medium at various time intervals. Fig. 2A demonstrates that the antiviral state was fully established in mouse cells after about 8 h of IFN treatment. On the other hand, as illustrated in Fig. 2B, the development of the antiviral state in rat cells was much slower, reaching its full establishment only after 20 h of IFN treatment.

Cellular RNA and protein synthesis

IFN-mediated induction of antiviral state is known to require cellular RNA and protein synthesis [6,7,14]. It was therefore of interest to clarify whether slower develop-

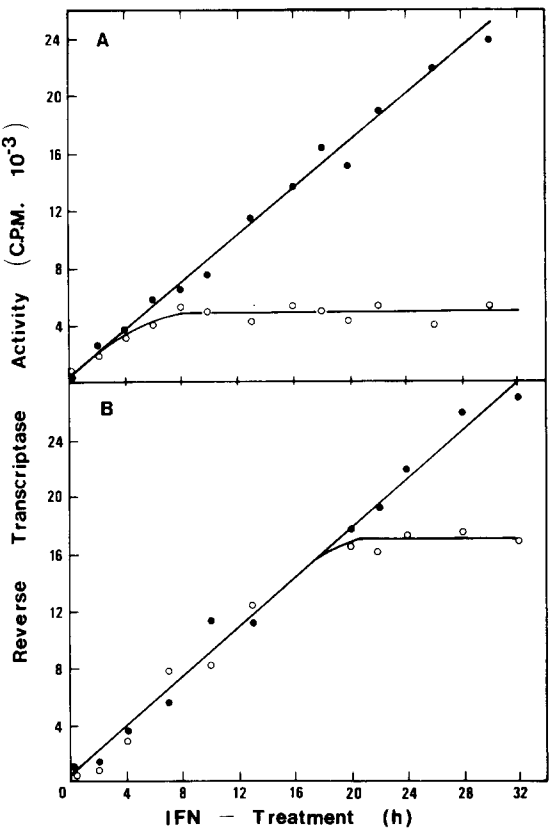

Fig. 2. Kinetics of development of IFN inhibitory effect on retrovirus release. IFN (100 units/ml) was added to A) NIH/3T3(MLV/MSV) and B) NRK(MLV/MSV) cells, and viral reverse transcriptase activity was assayed in aliquots taken from the culture medium at various time intervals. ○, IFN-treated cells; ●, untreated control cells.

ment of antiviral state in rat cells reflected a lower rate of these cellular metabolic processes. Thus cells were labeled with either [^{3}H]uridine or [^{35}S]methionine. At various times after label addition, samples of cells were lysed with 0.5% SDS and analysed for the cytoplasmic pool size of the labeled precursors and for incorporation of precursors into cellular macromolecules. The results in Fig. 3A reveal a higher amount of TCA-precipitable [^{35}S]methionine in lysates of NRK(MLV/MSV) cells than in those of NIH/3T3(MLV/MSV) cells. Since, as shown in the inset of Fig. 3A, the cytoplasmic pool of this labeled precursor was the same in both cells, the TCA precipitation results may be interpreted as demonstrating that the protein synthesis rate was higher in NRK(MLV/MSV) than in NIH/3T3(MLV/MSV) cells. The same was concluded about cellular RNA synthesis. Fig. 3B shows comparable TCA-precipitable [^{3}H]uridine in both cells, but since the pool size of this label was lower in rat cells (see inset in Fig. 3B), the TCA precipitation results of this experiment were interpreted as suggesting a higher RNA synthesis rate in NRK (MLV/MSV) cells.

IFN treatment for 24 h before labeling had no effect on either the cytoplasmic pool size or the incorporation into cellular macromolecules of any of these two radioactive precursors (Fig. 3).

Resumption of virus release after IFN removal

NIH/3T3(MLV/MSV) and NRK(MLV/MSV) cells were treated for 24 h with mouse IFN. After removal of IFN the cells were further incubated with fresh, IFN-free medium and virus release was followed by assaying reverse transcriptase activity in aliquots taken from the culture medium at various time intervals. Fig. 4 indicates that the antiviral state persisted in rat cells for a longer time than in mouse cells. Virus release from NIH/3T3 (MLV/MSV) cells resumed at about 10 h after IFN removal (Fig. 4A), whereas in NRK (MLV/MSV) cells this resumption was apparent only 29 h after IFN removal (Fig. 4B). Nevertheless, it is interesting to note that in both cases virus release resumed in a rather abrupt manner.

The IFN-induced antiviral state has been shown to depend on binding of IFN molecules to specific receptors on the cell membrane [5–7]. It is thus conceivable that after IFN

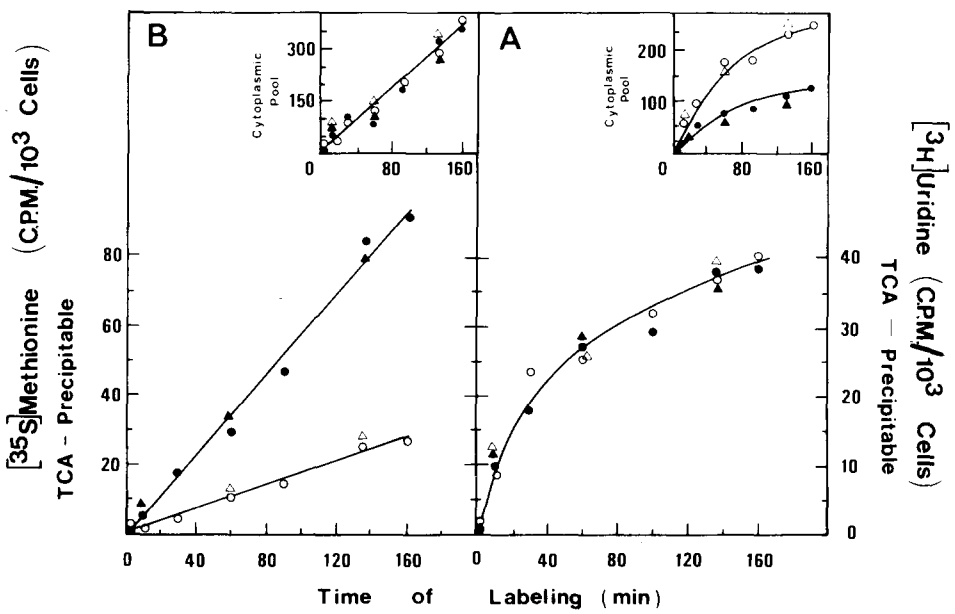

Fig. 3. Accumulation of radioactive methionine and uridine in the cytoplasm and their incorporation into cellular macromolecules. Cells were labeled with either [^{35}S]methionine (A) or [^{3}H]uridine (B). At various time intervals duplicate cultures were lysed and analysed for the cytoplasmic pools of radioactive precursors and for their incorporation into TCA-precipitable materials as detailed in Materials and Methods. The symbols represent: NRK(MLV/MSV) cells treated with IFN (▲); untreated with IFN (●); and NIH/3T3(MLV/MSV) cells treated with IFN (Δ); untreated with IFN (○).

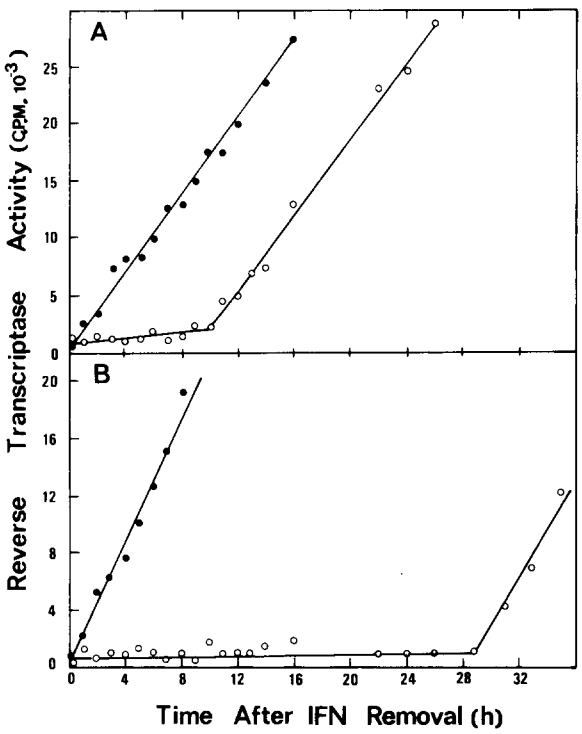

Fig. 4. Decay of the antiviral state after IFN removal. Cells were treated for 24 h with 100 units/ml of mouse IFN, then washed to remove unadsorbed IFN, and further incubated with fresh medium. Virus release was followed by assaying reverse transcriptase activity in aliquots taken from the culture medium at various time intervals. A) NIH/3T3(MLV/MSV) cells. B) NRK(MLV/MSV) cells. ○, IFN-treated cells; ●, mock IFN-treated cells.

removal from the culture medium, the antiviral state persists as long as a sufficient number of IFN molecules remain bound to the cell surface. Accordingly, recovery from the antiviral state could result from a reduction in the amount of IFN bound to each cell as a consequence of cell replication. Therefore, we examined whether the different recovery rates of mouse and rat cells were perhaps associated with different cell replication rates. For this purpose, cells were seeded and their replication rate was determined by counting the cells in duplicate cultures sampled at various times after plating. Fig. 5 shows that NIH/3T3(MLV/MSV) cells grew much faster, with a generation time of about 17 h, whereas the generation time of NRK(MLV/MSV) cells was, under these conditions, about 30 h. It is also shown that IFN had no effect on cell replication.

DISCUSSION

IFN action is usually species specific, though several exceptions are well known [5–7]. Such an exception is illustrated in this study, in which mouse IFN was found

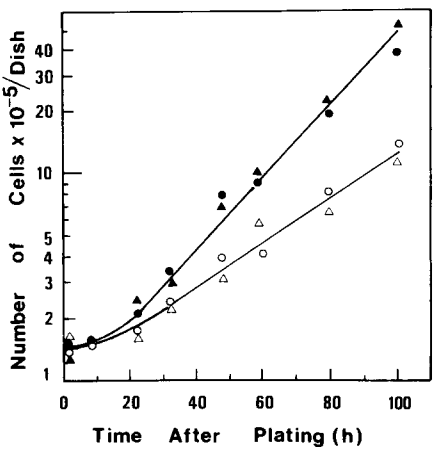

Fig. 5. Replication rate of IFN-treated and untreated cells. Cultures were seeded at 1.5×10^5 cells per 5 cm diameter Petri dish in the presence or absence of 100 units/ml of mouse IFN. At various time intervals after plating, cells were trypsinized and counted. Data are average values of duplicate cultures. △, IFN-treated NRK(MLV/MSV) cells; ○, mock IFN-treated NRK(MLV/MSV) cells; ▲, IFN-treated NIH/3T3(MLV/MSV) cells; ●, mock IFN-treated NIH/3T3(MLV/MSV) cells.

to be as effective in inhibiting MLV/MSV release in a rat kidney cell line as in mouse NIH/3T3 cell line. Similar effects of mouse IFN were observed with low-passage rat embryo cells productively infected with the same MLV/MSV complex (data not shown). On the other hand, human fibroblasts and lymphoblastoid IFNs were inactive in this respect. This latter finding is consistent with the inability of human fibroblast [13] and leukocyte [5,13] IFNs to protect rat cells against cytocydal viruses. Human IFNs have also been found incapable of inhibiting retrovirus production by chronically infected mouse cells [1,12], although they can bind to both rat [5] and mouse [1] cells. The antiviral activity of mouse IFN in rat cells could also be demonstrated with a cytocydal virus such as VSV. It is interesting to mention several studies illustrating that rat IFN also exhibits a cross-species antiviral effect, particularly in mouse cells, and to a lesser extent also in other species [3,8,9].

Despite the comparable response to mouse IFN dose, the antiviral state development was much slower in rat than in mouse cells. However, this should not necessarily be surprising. Similarity in dose–response relationships may reflect a similar affinity of the cell membrane receptors for the applied IFN. The subsequent development of the antiviral state is a distinct event, which may proceed at different rates in different cell types. IFN-mediated induction of the antiviral state is known to require cellular RNA and protein synthesis [5–7,14]. It could thus be suggested that the different rates of the antiviral state development reflected a difference in the rate of these cellular metabolic processes. However, our data seem to indicate just the reverse; both RNA and protein synthesis appeared to be faster in rat than in mouse cells. Early studies have indicated that, although both cellular RNA and protein synthesis are required for anti-

viral action of IFN, conditions lowering the rate of these processes are more favorable for the antiviral development [10,14], which may explain the faster establishment of IFN effect in mouse cells despite their slower macromolecules synthesis. Alternatively, the slower rate of the antiviral state development in rat cells may be related to their lower multiplication rate or to the fact that they are larger in size than the mouse cells.

Antiviral state expression depends on IFN molecules bound to the cell membrane [5–7]. It is therefore plausible that, after IFN removal from the culture medium, its antiviral effect will persist at least as long as sufficient IFN molecules remain bound to the cell membrane. Consequently, cell multiplication can be expected to reduce the amount of IFN bound to each cell and when this reduction reaches a certain critical level, the cells can be expected to recover rapidly from the antiviral state. We found that rat cells recovered from the antiviral state more slowly than mouse cells. This perhaps was also due to the slower replication of the rat cells. Nevertheless, both mouse and rat cells recovered from the effect of IFN in a rather abrupt manner, which is consistent with our previous observations [11]. The significance of this abrupt recovery needs a further elaboration.

ACKNOWLEDGEMENT

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