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Dissociation of Interferon Effects on Murine Leukemia Virus and Encephalomyocarditis Virus Replication in Mouse Cells

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Two subclones of Swiss mouse cells infected with Moloney murine leukemia virus (M-MuLV) were tested for their response to interferon (IFN). Whereas M-MuLV production in the two subclones was inhibited to the same extent, one of the subclones was significantly more sensitive to IFN when the antiviral effect was measured by replication of encephalomyocarditis (EMC) virus. The same subclone was also more sensitive to the anticellular activities of IFN. Additionally, NIH 3T3 cells infected with M-MuLV were completely resistant to IFN actions when EMC virus replication or the anticellular activities were tested. However, under the same conditions, M-MuLV production was completely inhibited by IFN. These results indicate that IFN may affect cell growth functions and EMC replication through mechanisms different from those by which MuLV production is inhibited.

Interferon (IFN) treatment of cells induces an antiviral state which limits replication of a variety of viruses. The major basis of this antiviral activity for a lytic virus like encephalomyocarditis virus (EMC) or vesicular stomatitis virus appears to be the inhibition of viral protein synthesis (5). In contrast, the IFN-induced suppression of retroviruses such as murine leukemia virus (MuLV) in chronically infected cells appears to occur at a late stage of virus growth since viral protein synthesis is unaltered, but proper maturation and virus release are inhibited (5). Apart from its antiviral activity, IFN has been shown to alter cellular parameters, in particular those involved in cell growth (6). There may be a correlation between this property of IFN, termed the anticellular activity, and the antiviral (EMC, vesicular stomatitis virus) activity since cells which respond to the anticellular activity of IFN also appear to be sensitive to the antilytic virus effect (8). It is not clear, however, whether similar correlations exist between the IFN-induced inhibition of MuLV production and the anticellular or antilytic virus activities of IFN. One could argue, for instance, that the inhibition of MuLV is mediated through the anticellular effect since production of MuLV has been shown to be affected by the cell cycle (10) and IFN extends both the G_1 and $S+G_2$ phases (2, 4, 13).

In the present study we compared the anti-MuLV, anti-EMC virus, and anticellular activities of IFN. Three parameters were used for measuring the anticellular effects: inhibition of (i) cell division, (ii) DNA synthesis, and (iii) ornithine decarboxylase induction. Inhibition of ornithine decarboxylase induction has been shown to be an independent parameter for the anticellular activity of IFN (11a, 12). The results presented here indicated that the anti-MuLV effect of IFN can be dissociated from both its anticellular and antiviral activities.

The approach used consisted of the following. Swiss 3T3 cells (14), susceptible to the anticellular and antiviral effects of IFN (2), were infected with Moloney MuLV (M-MuLV) at a multiplicity of 1. The chronically infected cells were cloned by plating in a microtiter dish. Two of the clones were tested for susceptibility to IFN by assaying three parameters: (i) anti-MuLV effect; (ii) anti-EMC virus activity, and (iii) anticellular effects. Figure 1a shows a dose-response effect of IFN on the production of M-MuLV in two cell clones, D-8 and H-2. The virus released into the medium was assayed for reverse transcriptase activity, and activity was corrected for cell number in the cultures. The sensitivity of the clones was similar since 50% inhibition of M-MuLV production was achieved with 6 U of IFN per ml. Virus production was also monitored by focus formation on S^+L^- cells (3), and parallel reduction curves were obtained (results not shown), indicating that the residual virus released from IFN-treated cells was fully infectious. In contrast to the anti-MuLV effect, EMC virus was inhibited by IFN differentially in D-8 and H-2 cells. IFN, at 50 U/ml, reduced EMC virus yield by over 4 logs in H-2 cells but

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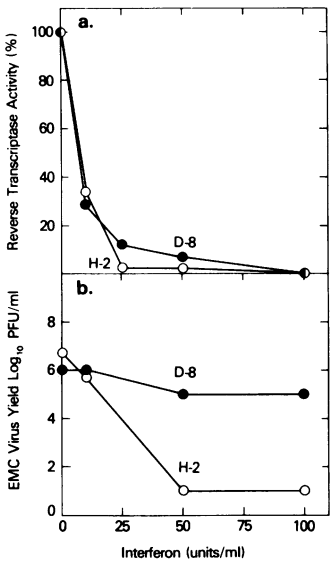

FIG. 1. *Effects of IFN on virus production in two Swiss 3T3 subclones. (a) Subconfluent cultures of M-MuLV-infected cells, D-8 (●) and H-2 (○) (10^5 cells per 100-mm petri dish in 10 ml of Dulbecco-modified Eagle medium containing 10% fetal calf serum), were incubated for 24 h with L-cell IFN (specific activity, 2×10^7 U/mg of protein). Culture fluids were replaced with fresh media containing IFN, and after 24 h, M-MuLV released into culture fluids was measured by assay of reverse transcriptase activity. Fluids were clarified by low-speed centrifugation, and virus was pelleted by sedimentation for 1 h at 105,000 × g. Pellets were suspended in 100 μl of TNE (20 mM Tris-hydrochloride, pH 7.5; 100 mM NaCl; 1 mM EDTA). Reverse transcriptase activity in the virus pellet was determined by incorporation of [^{3}H]TMP in the presence of polyadenylic acid-oligodeoxythymidylic acid template primer (9). Since cell number changed with IFN concentration, results were corrected for original fluid volume and cell number at time of harvest. M-MuLV from control cultures of D-8 and H-2 without IFN (100% activity) catalyzed the incorporation of 0.27 and 0.53 pmol of [^{3}H]TMP per min, respectively. (b) Parallel cultures of D-8 (●) and H-2 (○) were infected with EMC virus (multiplicity of infection of 0.1) after 24 h of treatment with IFN. After an additional 24 h, fluids were harvested and clarified by low-speed centrifugation, and EMC virus was titrated by infecting L-cells in 96-well microtiter plates with serial virus dilutions. Cytopathic effect was recorded at 24 and 48 h postinfection.*

only by 1 log in D-8 cells.

Next, the anticellular effects of IFN on these two clones were examined. Figure 2 shows a dose-response curve of IFN effects on (i) cell division; (ii) DNA synthesis, as measured by thymidine incorporation; and (iii) induction of ornithine decarboxylase enzyme activity. All of these activities were inhibited. Comparison of D-8 and H-2 sensitivities indicated that for D-8 cultures, the amount of IFN required for 50% reduction was 5-fold more for cell number, 45-fold more for DNA synthesis, and 3-fold more for ornithine decarboxylase activity, as compared to H-2 cultures. Thus, the H-2 subclone appeared to be more sensitive than the D-8 subclone in terms of both the anti-EMC virus and anticellular activities of IFN. However, M-MuLV inhibition in the two subclones was similar. These results suggested that IFN may affect cell growth functions and EMC replication through pathways different from those by which M-MuLV production is inhibited.

To test this hypothesis further, several additional cell lines were infected with M-MuLV and analyzed for their ability to develop antiviral states against EMC virus and M-MuLV after IFN treatment. Of the cells tested, the results obtained with NIH 3T3 cells (7) chronically infected with M-MuLV were intriguing. Figure 3 demonstrates that IFN, at 1,500 U/ml, inhibited M-MuLV production by over 95%, whereas this high dose had no effect on EMC virus replication. Next, we tested the anticellular activity of IFN on these cells (NIH 3T3-MuLV). IFN treatment had no inhibitory effects on cell division, DNA synthesis, or ornithine decarboxylase enzyme induction, as shown in Table 1. Similar resistance to IFN in terms of these parameters was also observed in NIH 3T3 cells not infected with M-MuLV obtained from a different source (results not shown). This cell line (NIH 3T3) thus showed a complete dissociation of the anti-MuLV activity from the anti-EMC or anticellular effects induced by IFN.

The finding that two subclones isolated from a single culture of Swiss 3T3 cells chronically infected with M-MuLV exhibited different ranges of sensitivity to IFN suggested variability among cells in a population. Whereas variability was not observed with the anti-MuLV activity, the clones differed in their sensitivities to both the anti-EMC virus and anticellular activities. Such a variability could have developed at one of two stages: (i) during cell passage or (ii) subsequent to infection by M-MuLV. Since M-MuLV infection of fibroblasts in a culture appears to have no effect on the cellular phenotype, the latter possibility is less likely.

Three different parameters were assayed for the anticellular effect of IFN. Of the three, cell division appeared to be the most sensitive to IFN. This can be explained by the fact that IFN affects not only the G_1 and S phases (analyzed by ornithine decarboxylase induction and DNA synthesis), but also the G_2 phase of the cell cycle (2). In the two subclones D-8 and H-2, DNA synthesis was more sensitive to the inhibitory effect of IFN than was induction of ornithine

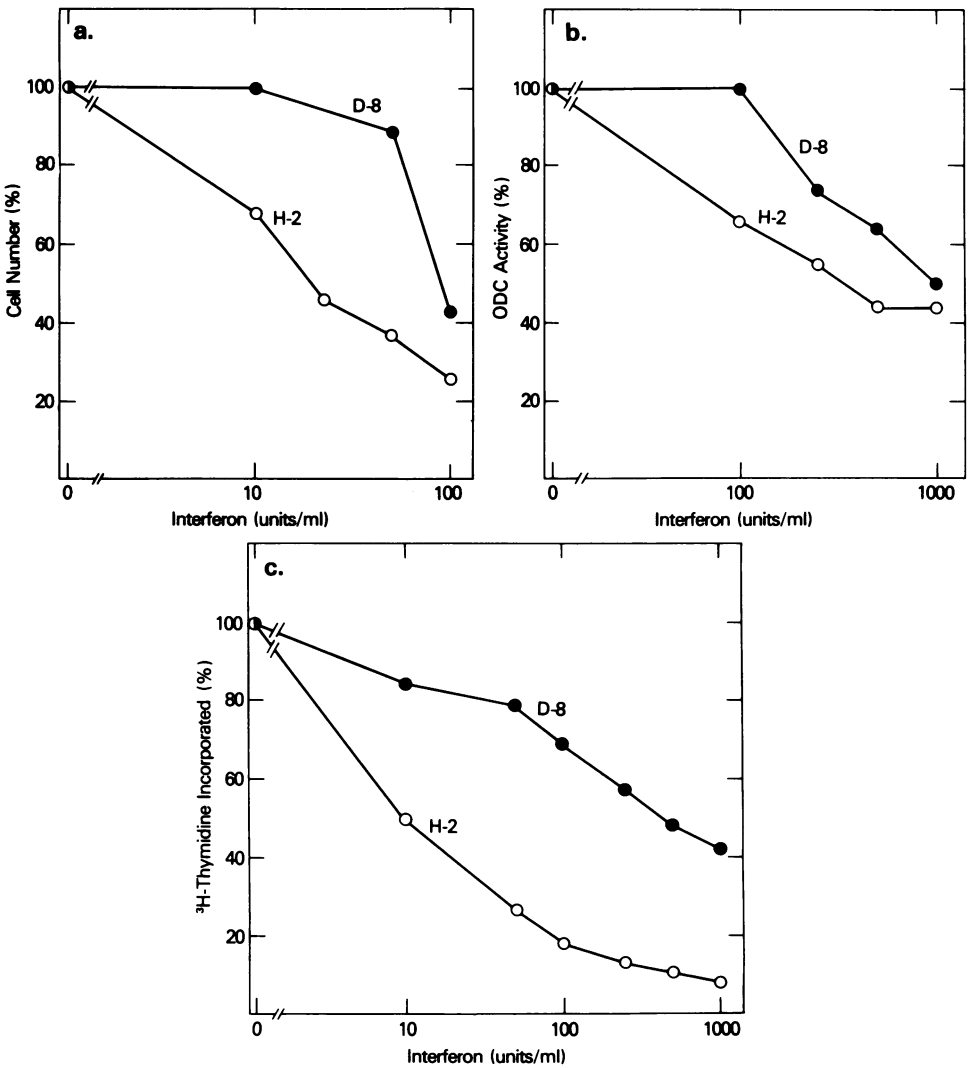

FIG. 2. *Anticellular effects of IFN in D-8 and H-2 subclones. (a) Inhibition of cell multiplication. Cultures were treated with IFN for 48 h as described in Fig. 1. After fluids were harvested for reverse transcriptase assay, the monolayers were trypsinized for determination of cell number. Control cultures of D-8 and H-2 without IFN (100%) gave cell counts of 3.2 × 10^5 and 6.5 × 10^5, respectively. (b) Inhibition of ornithine decarboxylase (ODC) induction. Quiescent cultures were prepared by serum depletion (12). The cultures were stimulated with 10% serum in the presence of increasing concentrations of IFN, and ornithine decarboxylase activity in the cells was measured at the end of 6 h after serum addition (12). Values are expressed as percent of control cultures stimulated in the absence of IFN. The level of enzyme activity in unstimulated cultures was less than 0.1 nmol of $^{14}CO_2$ released per mg of cell protein per h. Enzyme activities in serum-stimulated D-8 and H-2 subclones were 2.58 and 2.26 nmol of $^{14}CO_2$ per mg per h, respectively. (c) Inhibition of DNA synthesis. The incorporation of [^{3}H]thymidine in quiescent cultures stimulated with serum was measured (12). After 30 h of incubation, incorporation into acid-precipitable DNA was determined and is expressed as percentages of control cultures which were stimulated in the absence of IFN. Quiescent and serum-stimulated D-8 cultures incorporated 1.6 × 10^4 and 5 × 10^5 cpm per culture, respectively, whereas similar values for H-2 were 2 × 10^3 and 2.7 × 10^5 cpm per culture, respectively. D-8 (●), H-2 (○).*

decarboxylase (Fig. 2b and c). This is consistent with the observation that the inhibition of DNA synthesis in IFN-treated Swiss 3T3 cells is not a direct consequence of the inhibition of ornithine decarboxylase induction (11a).

Resistance of cells to IFN might be attributed to either a lack of receptors for IFN or a deficiency in an intracellular factor required for

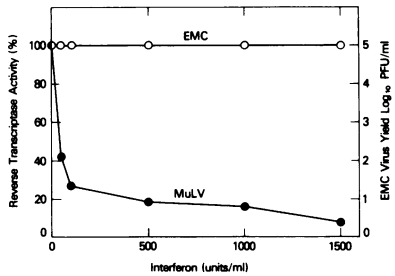

FIG. 3. *Effect of IFN on virus production in NIH 3T3 cells. Subconfluent NIH 3T3 cells chronically infected with M-MuLV were incubated with IFN as described in Fig. 1. M-MuLV production (●) and EMC replication (○) in these cultures were assayed as described in Fig. 1. M-MuLV production in control cultures without IFN (100% activity) catalyzed the incorporation of 1.16 pmol of [^{3}H]TMP per min. Replication of EMC is illustrated as percentage of virus from cells without IFN which yielded an EMC titer of 10^5 PFU/ml (100%).*

TABLE 1. *IFN has no apparent anticellular effects on NIH 3T3 cells*

IFN (U/ml)	Cell no.[a] ($\times 10^5$)	[^{3}H]thymidine incorporated[b] (cpm $\times 10^5$)	Ornithine decarboxylase activity[b] (nmol of $^{14}CO_2$ per h per mg of protein)
None	3.6	1.80	2.53
500	4.0	1.83	2.50
1,000	3.3	1.75	2.33
5,000	ND[c]	1.73	2.63

[a] Cell counts of subconfluent cultures 48 h after addition of IFN as described in Fig. 2a.

[b] M-MuLV-infected NIH 3T3 cultures were arrested by serum depletion and released by addition of 10% serum, with or without IFN. [^{3}H]thymidine incorporation into DNA and ornithine decarboxylase activity were assayed as described in Fig. 2a and b.

[c] ND, Not done.

establishment or maintenance, or both, of the antiviral and anticellular activities. NIH 3T3 cells appeared to be completely resistant to IFN in terms of the anti-EMC and anticellular activities, but retained sensitivity to the anti-MuLV activity. There are at least two possible explanations for the differential inhibitions of M-MuLV and EMC virus in NIH 3T3 cells: (i) two different receptors for IFN are responsible for the establishment of antiviral states against EMC and M-MuLV, and NIH 3T3 cells are devoid of the receptors necessary to initiate the anti-EMC viral state; or (ii) there is only one type of receptor for IFN, but NIH 3T3 cells are deficient in an intracellular factor needed for the expression of the anti-EMC virus activity. Several enzymatic systems have been implicated in the anti-EMC viral state induced by IFN (11). One pathway involves induction of a synthetase activity which catalyzes the polymerization of 2′,5′-oligoadenylic acid, and this oligonucleotide induces a latent endonuclease activity in the IFN-treated cells. Preliminary observations of Epstein et al. (personal communication) have indicated that 2′,5′-oligoadenylic acid synthetase is induced in the same NIH 3T3 cell line in which EMC virus replication is not inhibited after IFN treatment. This observation suggested the existence of receptors for IFN for the initiation of what is thought to be an anti-EMC virus activity. Work is in progress to determine whether or not this pathway in NIH 3T3 cells is aborted at a later step, such as at the level of the 2′,5′-oligoadenylic acid-dependent endonuclease.

Finally, it has been shown that retroviruses as well as lytic viruses can be used for assaying IFN activity (1). In view of the present results, use of the standard assays (e.g., EMC virus inhibition) may not be sufficient for the determination of cell sensitivity to IFN, since cells resistant to the antilytic virus activity of IFN may still respond to the anti-MuLV effect.

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