Interferon-mediated Inhibition of Mouse Mammary Tumour Virus (Type B) and Mouse Leukaemia Virus (Type C) in the Same Culture

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SUMMARY

The sensitivity of mouse mammary tumour virus (MMTV) and murine leukaemia virus (MuLV) to interferon was tested by investigating the effect of interferon in cultures of mouse mammary tumour cells simultaneously synthesizing the two viruses. The dose-response curves showed that the extracellular production of both viruses by these cells was equally inhibited by interferon treatment. In contrast, the intracellular steady-state concentration of virus-specific RNA of both MMTV and MuLV was not significantly affected in these cells. This was the case regardless of whether total cellular RNA or polysomal poly(A)-containing RNA was examined. These results are consistent with the suggestion that interferon-caused inhibition is related to a host-mediated effect on a post-transcriptional step in virus synthesis.

Interferon inhibits the replication of a wide range of viruses. The characteristics of this inhibition are apparently related to specific cell–virus interaction. The replication of a given virus may be strongly inhibited in one cell type and slightly or not at all in another cell type. Similarly, two different viruses replicating in cells from the same species may respond differentially to interferon treatment (for review, see Stewart, 1979). Such differential interferon effects have been reported even for closely related viruses of the same family or genera. For example, Billiau et al. (1975) reported that the production of a murine leukaemia virus (MuLV) (a type C retrovirus) by chronically infected murine JLS-V5 cells was strongly inhibited by mouse interferon while that of mouse mammary tumour virus (MMTV) (a type B retrovirus) by a productively infected C57B1 mouse mammary tumour cell line was not affected. Both of these cell lines were otherwise responsive to interferon when challenged with vesicular stomatitis virus. While the production of extracellular MMTV by another mouse mammary tumour cell line (MJY-α) is reportedly refractile to interferon inhibition (Yagi et al., 1980), the release of MMTV by the C3H and GR mouse mammary tumour-derived cell lines is strongly inhibited by interferon (Strauchen et al., 1977; Arya et al., 1980; Sen & Sarkar, 1980). Furthermore, the host-dependent sensitivity of MuLV in mouse cell lines can vary significantly as a feature of the cell line rather than the virus or the cell species (Allen et al., 1976; Pitha et al., 1976). To further elaborate on the host dependence, we have investigated the effect of interferon on MMTV and MuLV which were being produced simultaneously by the same cell line. In our study, two types of cultures derived from mouse (C3H) mammary tumour Mm5mt/c₁ cells were used: one from a single cell clone producing both MMTV and MuLV (Schochetman et al., 1978; Schidlovsky et al., 1978) and the other obtained by continuous and regular passage of Mm5mt/c₁ cells to a passage level where they produce both MMTV and MuLV (Schochetman et al., 1978; Arya, 1980b). We report that the extracellular production of both MMTV and MuLV is equally inhibited by interferon in these two cultures while the synthesis of viral RNA is not affected.

To determine the effect of interferon on extracellular virus production, monolayer cultures were incubated with interferon for 24 h in Dulbecco–Vogt culture medium containing 2 μg/ml dexamethasone and supplemented with 10% foetal calf serum and antibiotics. The culture medium was harvested, clarified of any cells or cell debris by low speed centrifugation and
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Fig. 1. Dose-response curves for the effect of interferon on the release of MMTV and MuLV by cultured mouse mammary tumour cells. The cultures were treated with the indicated concentrations of interferon for 24 h and virus production was estimated by particle-associated RNA-directed DNA polymerase assays. ■, ■, Single cell clone-derived cultures; ○, ○, mid-passage cultures (see text). ■, ■, MMTV production; ○, ○, MuLV production.

subjected to centrifugation at 25000 rev/min for 90 min in a Beckman SW27 rotor at 4 °C. The pellet was suspended in a small volume of 0.01 M-tris–HCl pH 7.5, 0.1 M-NaCl, 1 mM-EDTA, layered on 20% glycerol in the above buffer and centrifuged at 30000 rev/min for 90 min in a Beckman SW50.1 rotor at 4 °C. The pelleted virus was suspended in 0.01 M-tris–HCl pH 7.9, 0.01 M-NaCl, and aliquots were used to estimate MMTV and MuLV by RNA-directed DNA polymerase assays using poly(C). oligo(dG) as a template primer in the presence of Mg$^{2+}$ and Mn$^{2+}$ as cofactors respectively (Dion et al., 1974; Arya et al., 1979). The polymerase reaction mixture contained 50 mM-tris–HCl pH 7.9, 50 mM-NaCl, 20 mM-dithiothreitol, 0.1% NP-40, 50 mM-$^3$H-dGTP (600 ct/min/pmol), 50 μM-poly(C). oligo(dG), 20 mM-MgCl$_2$ or 1 mM-MnCl$_2$ and aliquots of virus preparation. The mixture was incubated at 37 °C for 30 min. Under these assay conditions, the relative effect of Mg$^{2+}$ to Mn$^{2+}$ as cofactors in the synthesis of poly(G) catalysed by MMTV and MuLV (AKR) was about 6 to 10 and 0.055 to 0.084 respectively. Thus, DNA synthesis measured with Mg$^{2+}$ and Mn$^{2+}$ reflected predominantly MMTV and MuLV respectively. The concentration of intracellular viral RNA was determined by hybridizing cellular RNA with MMTV (C3H) and MuLV (AKR) cDNA. Total cellular RNA was extracted by a guanidine–HCl extraction procedure described by Chirgwin et al. (1979), followed by CsCl centrifugation (Gisin et al., 1974). Polysomal poly(A)-containing RNA was prepared essentially according to Sala-Trepat et al. (1978) as previously described (Arya et al., 1980; Arya, 1980b). The viral cDNAs were prepared by transcribing respective viral RNAs with avian myeloblastosis reverse transcriptase using calf thymus DNA oligomers as primers (Arya & Young, 1980; Strauchen et al., 1980). Hybridization was performed at 65 °C in 0.38 M-NaCl, 0.038 M-sodium citrate, 1 mM-EDTA pH 7, 0.02% sodium lauroyl sarcosinate and hybrids were scored by S1 nuclease digestion (Arya, 1980a; Strauchen et al., 1980). The mouse interferon (sp. act. 1 x 10$^8$ to 2 x 10$^8$ units/mg) was kindly provided by Dr Robert M. Friedman of the U.S. National Institutes of Health.

Fig. 1 shows the dose-response curves for the effect of interferon on extracellular virus production by cultures derived from a single cell clone of Mm5mt/c$_4$ cells (previously designated SC13D; Schochetman et al., 1978), and by mid-passage Mm5mt/c$_4$ cells (Arya, 1980b). The two cultures showed some apparent difference in their response to interferon but both MMTV and MuLV were equally inhibited as measured by DNA polymerase assays. The DNA polymerase activity, whether measured using Mg$^{2+}$ or Mn$^{2+}$ as cofactor, was
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Fig. 2. Effect of interferon on intracellular virus-specific RNA in mouse mammary tumour cells. (a) Total cellular RNA from single cell clone-derived cultures and (b) Polysomal poly(A)-containing RNA from mid-passage cultures (see text) were hybridized with MMTV cDNA (●, ○) or MuLV cDNA (■, □). The cultures were treated with 100 units/ml interferon for 24 h. ●, ■, RNA from control cultures; ○, □, RNA from treated cultures.

proportionately equally reduced, within experimental error, for particles from interferon-treated cultures relative to control cultures. Thus, regardless of the actual magnitude of Mg\(^{2+}\) versus Mn\(^{2+}\) differential response of the two virus-associated DNA polymerases, interferon treatment affected the production of the two viruses to about the same extent. The dose of interferon resulting in 50% inhibition of both viruses was about 20 units/ml for single cell clone-derived cultures and about 5 units/ml for mid-passage cultures. The effect of interferon on viral RNA transcription was investigated by hybridizing cellular RNAs from control and interferon-treated cultures with MMTV and MuLV cDNA. The two viral RNAs and cDNAs did not show any cross-hybridization; thus, the probes were specific for the respective viral RNAs (Arya, 1980a, b). The hybridization was carried out in large mass excess of cellular RNA. In the case of the single cell clone-derived culture, the R\(_{0.5t/2}\) of hybridization of MMTV cDNA with total cellular RNA from control and interferon-treated cultures was about 6 (Fig. 2a). Compared with the R\(_{0.5t/2}\) of pure viral RNA and its cDNA of about 0-01 (Arya 1980a, b) the intracellular concentration of MMTV-specific RNA in both control as well as treated cultures was about 0.16%. The hybridization of the same preparations of RNAs with MuLV cDNA gave an R\(_{0.5t/2}\) of 8 to 10 and no significant difference between control and interferon-treated RNA was observed (Fig. 2a). Both cultures contained about 0.10 to 0.12% of total RNA as MuLV-specific RNA. Thus, interferon failed to affect the steady-state levels of either MMTV-specific or MuLV-specific RNA in these cells.

Considering the possibility that interferon may specifically affect processing of virus-specific RNA, its effect on the concentration of viral RNA in polysomes from control and interferon-treated cultures was investigated. The mid-passage cultures were used for these experiments and polysomal poly(A)-containing RNA was hybridized with cDNA probes. As
shown in Fig. 2(b), interferon did not significantly affect the concentration or complexity of either MMTV-specific or MuLV-specific RNA associated with polysomes. The R_{0.5} of hybridization of polysomal RNA from control and interferon-treated cultures with MMTV cDNA was 1.2 and 1.0 respectively, and corresponding values for hybridization with MuLV cDNA were 0.4 and 0.6. If interferon affects the processing of viral messages in these cells, this effect must be of a magnitude and quality not detectable by the present approach. These results are consistent with the interpretation that interferon-mediated inhibition of retrovirus synthesis is a result of some post-transcriptional event (Fan & MacIsaac, 1978; Arya et al., 1980; Sen & Sarkar, 1980). It has been suggested that interferon affects the assembly or release of MuLV (Billiau et al., 1974; Friedman et al., 1975). Whatever the exact mechanism, it is clear that both MMTV and MuLV synthesis are equally sensitive to interferon inhibition in these cells.

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