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Disparate response of encephalomyocarditis virus and MM virus to interferon in JLS-V9R cells

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Summary

JLS-V9R cells, a Balb/c mouse bone marrow cell line chronically infected with Rauscher leukemia virus, were treated with mouse interferon and inoculated with several different lytic viruses. Relatively low interferon concentrations protected the cells against Sindbis virus, vesicular stomatitis virus and MM virus. In contrast, encephalomyocarditis virus replication was inhibited by less than 1 log even with an interferon concentration of 1000 U/ml. These findings provide further evidence that interferon-induced antiviral effects are mediated through multiple mechanisms and demonstrate that even viruses which are classified within the same family (MM and encephalomyocarditis virus) can exhibit differential interferon sensitivities.

encephalomyocarditis virus; MM virus; interferon; response

Experimental

Treatment of cells with interferon (IFN) establishes an antiviral state which confers protection against infection with a wide range of RNA and DNA viruses. Cell lines in which viruses classified in different families vary in their sensitivities to the antiviral effects of IFNs have been described and these have been reviewed by Stewart II [13]. Thus, it is clear that the antiviral state can be established through multiple mechanisms. The dissociation of IFN-induced inhibition of retroviruses from that of various lytic viruses has been described in both mouse [3–5,10] and human cell systems [7,11]. Additionally, several cell systems have been described which demonstrate partial antiviral states with respect to various lytic viruses [6,9,12,14]. Biochemical character-

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izations of these cell systems have been done to measure the two well-studied pathways activated by IFN treatment, i.e. the 2–5A-dependent RNase and dsRNA-dependent protein kinase pathways. Both correlations and lack of correlations between these pathways and the IFN-induced antiviral state have been reported. However, no cell system has been described in which the inhibition of encephalomyocarditis virus (EMCV) replication does not correlate with activation of the 2–5A-dependent RNase pathway.

In contrast to many other cell systems, JLS-V9R cells possess extremely low constitutive levels of 2–5A-dependent RNase activity and this enzyme activity is induced by IFN treatment [8]. The replication of VSV is efficiently inhibited in these cells by IFN treatment [3]. We were therefore interested in comparing the response of selected lytic viruses to IFN treatment in JLS-V9R cells. Our results indicate that JLS-V9R cells represent a unique cell system in which the anti-VSV and cell growth inhibitory effects of IFN are expressed in the absence of IFN-induced inhibition of EMCV replication.

To examine the inhibitory effects of IFN on the replication of viruses that differ in their modes of replication, viruses were chosen from three taxonomic groups. Two viruses, EMCV and MMV, are non-enveloped positive-stranded picornaviruses. Of the enveloped viruses chosen, Sindbis virus (SBV), a group A togavirus, possesses a positive-stranded genome while vesicular stomatitis virus (VSV), a rhabdovirus, is negative-stranded. A homogeneous population of JLS-V9R cells [3] was established by cloning the cells in 96-well microtiter dishes and a single clone, clone 4, was chosen for the studies reported here.

As shown in Fig. 1a, treatment of JLS-V9R cells with semipurified mouse L-cell IFN (specific activity 6.7×10^7 IU/mg) provided a differential range of protection depending on the challenge virus. SBV replication was reduced by several orders of magnitude with IFN doses as low as 10 U/ml. VSV and MMV demonstrated intermediate sensitivities since 1 log reduction in virus yield occurred with 12 and 50 U/ml IFN, respectively. Although EMCV and MMV are classified within the same family, these two viruses differed in their response to IFN treatment. EMCV replication was relatively resistant to the antiviral effects with IFN doses as high as 1000 U/ml, resulting in less than 1 log reduction in virus yield. These viruses also exhibit a disparity in their abilities to induce IFN. Whereas EMCV induces little or no IFN in L929 cells (P.T.A., unpublished results), MMV is a potent inducer, resulting in yields of $\geqslant 2 \times 10^4$ U/ml [2]. However, neither virus induced detectable IFN in JLS-V9R cells when virus input ranged from 50 to 0.0005 PFU/cell (data not shown). It is interesting to note that treating JLS-V9R cells with a recombinant human hybrid IFN, IFN-αAD (BglII), which has been purified to homogeneity and which has been shown to have equal antiviral activities on both human and mouse cells [15], resulted in the same pattern of differential sensitivities as treatment with mouse L-cell IFN.

We also examined the ability of IFN treatment to protect JLS-V9R cells against virus-induced cytopathic effect (CPE), by staining surviving cells with naphthol blue black (1 mg/ml naphthol blue black dye, 1 M acetic acid, 0.2 M sodium acetate). Cell-associated dye was quantitated by elution in 50 mM NaOH and determination of the A_{600} via an EIA reader (Bio-Tek Instruments, Inc., Burlington, VT). The dose

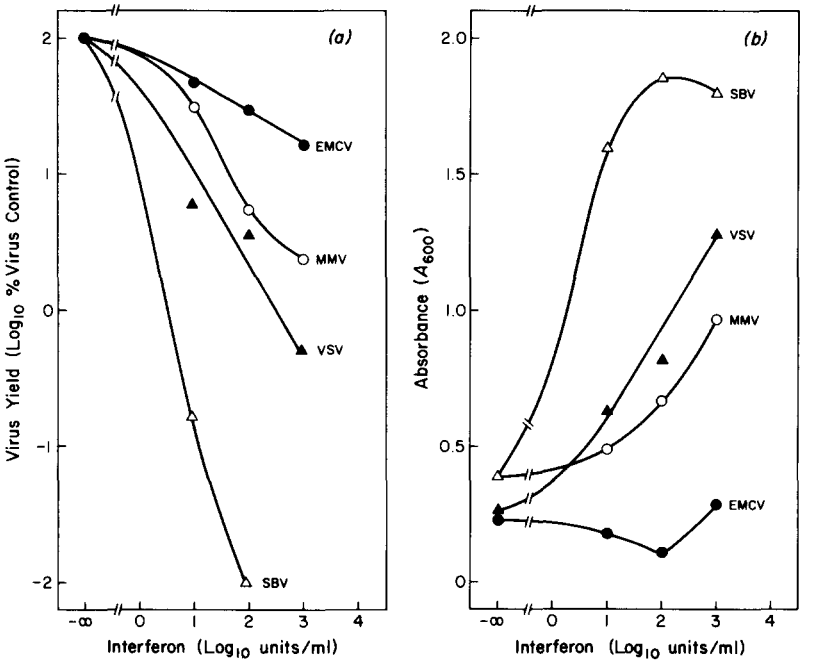

Fig. 1. (a) Inhibition of virus replication. Cell cultures (5×10^5 cells per 22.6 cm^2 well) were incubated with IFN for 16 h and infected with EMCV (●——●), MMV (○——○), VSV (▲——▲), or SBV (△——△) at equivalent multiplicity of infection. After virus adsorption, cell cultures were washed with PBS and 1 ml of media was added. Culture fluids were harvested 24 h post-infection and stored at -20°C for later plaque assay on BHK cells. Virus yields are expressed as the $\log_{10}$ % of the yield of virus produced in control cultures. Virus titers (PFU/ml) from control cultures were 1.2×10^8 (EMCV), 2.0×10^8 (MMV), 7.5×10^8 (VSV) and 4.3×10^8 (SBV). (b) Inhibition of virus-induced CPE. Virus-infected, IFN-treated cell cultures were stained with naphthol blue black as a measure of intact cells. Cell-associated dye was quantitated by elution in 50 mM NaOH and determination of the A_{600} (see text for details).

responses, reflecting inhibition of virus replication (Fig. 1a), correlated well with the inhibition of virus-induced CPE (Fig. 1b). IFN treatment was most effective in protecting the cells against CPE caused by SBV infection and virtually ineffective in establishing protection against CPE caused by EMCV infection.

These cells were chronically infected with Rauscher leukemia virus (MuLV-R). Consistent with earlier findings [3], JLS-V9R cells were more sensitive to the anti-VSV effects than the anti-MuLV-R effects of IFN. An IFN concentration of 1000 U/ml inhibited MuLV-R replication by only 66% (data not shown). The anticellular effects of IFN were also expressed in JLS-V9R cells. Maximal inhibition of cell growth was observed 48 h after addition of IFN and 50% inhibition occurred with IFN concentrations of 100 U/ml.

A differential response of different lytic viruses to inhibition by IFN treatment was reported by Nilsen et al. [9]. In that report, IFN protected undifferentiated mouse embryonal carcinoma (EC) cells against EMCV but not against VSV or SBV infec-

tion, while the corresponding differentiated cells were protected by IFN against VSV infection. This differential response was not observed by Aguet et al. [1], who found the undifferentiated embryonal carcinoma cells to be completely resistant to the inhibitory effects of IFN on the multiplication of VSV as well as EMCV in contrast to the sensitivity of the differentiated embryonal carcinoma cells. The 2–5A synthetase activity was induced by IFN treatment in both types of cells; however, protein kinase activity was demonstrated only in the differentiated EC cells treated with IFN [17]. Vandenbussche et al. [14] described two cell lines derived from human choriocarcinomas which were sensitive to the IFN-induced anti-EMCV effects but refractory to IFN treatment with respect to the antiviral effect toward VSV and the anticellular effect. The 2–5A-dependent RNase and dsRNA-dependent protein kinase pathways were both operative in these cells.

Analyses of IFN-induced enzymatic activities in IFN-treated JLS-V9R cells indicated levels of dsRNA-dependent protein kinase and 2–5A synthetase activities which were comparable to those found in similarly treated L-cells (H. Jacobsen, pers. commun.). Additionally, Jacobsen et al. [8] showed that these cells are unusual in the sense that 2–5A-dependent RNase activity is also induced by IFN-treatment and is present in low levels (approximately 5% of that found in L-cells) in control, untreated cells. Maximal levels of this protein, which approached levels found constitutively in Ehrlich ascites tumor cells, were induced by IFN concentrations in excess of 2000 U/ml. In this study we have shown that the concentration of IFN required to inhibit EMCV replication by 90% correlated well with that concentration needed for maximal induction of 2–5A-dependent RNase activity (Fig. 1a). These results are consistent with the theory that an operative 2–5A-dependent RNase pathway is necessary for inhibition of EMCV.

In summary, we have extended the characterization of JLS-V9R cells by examining the effects of IFN on the replication of different viruses in these cells. Our results demonstrate that not only can the IFN-induced inhibition of MuLV-R be dissociated from that of VSV, but replication of SBV and VSV can be inhibited in the absence of effects on EMCV replication. Also, our data clearly indicate that two viruses (EMCV and MMV) classified as picornaviruses responded differently to the antiviral effects of IFN. JLS-V9R cells therefore represent an excellent cell system for the study of differential IFN-induced inhibition of virus replication.

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