Differential Sensitivity of Rauscher Murine Leukaemia Virus (MuLV-R) to Interferons in Two Interferon-Responsive Cell Lines

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SUMMARY

The effect of interferon on the replication of vesicular stomatitis virus (VSV) and type-C oncornavirus in two Balb/c mouse cell lines, JLS-V5 and JLS-V9R, infected with MuLV-R was examined. VSV replication was inhibited threefold (0.5 log₁₀) in both cell lines by 10 to 20 units of interferon/ml. In JLS-V5 cells C-type virus yields, as measured by ³H-uridine incorporation and reverse transcriptase activity, were also reduced threefold by 10 to 20 units of interferon/ml. However, in JLS-V9R cells, C-type virus replication was refractory to interferon at concentrations up to 1×10⁴ units/ml. Infectious C-type virus transmitted from JLS-V9R cells to Balb/3T3 cells was as sensitive to interferon as virus transmitted from JLS-V5 cells, indicating that resistance of C-type virus in JLS-V9R cells is a feature of the cells rather than of the virus strain.

It has been demonstrated in several laboratories that type-C oncornaviruses may be inhibited by interferon under conditions where cytopathogenic viruses, such as VSV, are also inhibited (Van Griensven et al. 1971; Billiau, Sobis & De Somer, 1973a; Billiau et al. 1974, 1975; Friedman & Ramseur, 1974). Although there are numerous differences in the modes of replication of VSV and type-C viruses, we have nevertheless observed that approximately the same amount of interferon is needed to bring about a threefold (0.5 log₁₀) reduction in the yield of the two types of viruses in several cell lines continuously infected with type-C viruses (Billiau et al. 1975). It was therefore considered unusual when initial data obtained by one of us (H. Schellekens) indicated that type-C virus production in JLS-V9R cells (i.e. JLS-V9 cells infected by MuLV-R) was not suppressed by interferon at concentrations which severely inhibited VSV replication in the same cells. The results presented here document this phenomenon with JLS-V9R cells and contrast the results in cells of another line (JLS-V5).

The preparation of mouse L cell interferon has been described in a previous paper (Billiau et al. 1973). West Nile virus-induced interferon was prepared according to Finter (1964). Interferon titrations were done with a microtitre procedure (Schellekens, Huffmeyer & Van Griensven, 1975) or with a dye-uptake method (Finter, 1969). All interferon concentrations were expressed in terms of the NIH-reference preparation.

Cells were grown in CO₂-incubators using Eagle's minimum essential medium with Earle's salts, 2.2 g/l bicarbonate, added non-essential amino-acids and 10 % foetal bovine serum. JLS-V9 cells were derived by Wright et al. (1967) from the bone marrow of weanling Balb/c mice. Derived cell lines infected with MuLV-R were obtained by Chopra & Shibley (1967) by inoculation with virus from two sources: (a) virus harvested from JLS-V5 cells and (b) virus prepared from plasma of mice infected with an unspecified strain of MuLV-R. The cell line JLS-V9R used in the present work is derived from one of these. JLS-V5 cells
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were obtained by Wright & Lasfargues (1965) by infecting JLS-V6 cells (which they derived from the spleen and thymus of normal Balb/c mice) in their 6th passage with MuLV-R obtained from the plasma of mice infected with a strain termed P-314, obtained from Dr F. Rauchser. Both JLS-V9R and JLS-V5 cells had had many passages before they reached our laboratories, and so the presence of C-type viruses, other than the original MuLV-R cannot be excluded. Balb/3T3 cells were originally described by Aaronson & Todaro (1968).

The amount of MuLV-R in the culture fluid was determined by two methods: (i) by radioactive uridine incorporation into sedimentable particles as first described by Robinson (1967); and (ii) by the amount of RNA-dependent DNA polymerase, an enzyme specific for oncogenic RNA-viruses (Temin & Mizutani, 1970; Baltimore, 1970). Culture supernatant fluids were processed for analysis of MuLV-R as follows. After clarification for 10 min at 10000 rev/min, the virus in each sample was pelleted by centrifugation at 100000 g for 60 min, resuspended in NT buffer (100 mM NaCl, 10 mM tris-HCl, pH 7.4), sedimented again as above, and finally resuspended in 0.5 ml of NT buffer.

To determine the quantity of 5-3H-uridine incorporated into acid-insoluble RNA in virions, 10 nl of boiled bovine pancreatic ribonuclease A at 100 μg/ml was added to duplicate 100 nl portions of each sample. After incubating for 1 h at 37 °C, 100 μg of yeast RNA was added as carrier, and 10 vol. of ice-cold trichloroacetic acid (5%, w/w) were added. Acid-insoluble material was collected on membrane filters of 0.45 μm pore size, and washed extensively with the same acid solution. The filters were then washed with ethanol to remove the acid, heat-dried, and counted in toluene-based scintillant.

The method used for determination of reverse transcriptase is described in a separate paper (Billiau et al. 1976).

Isopycnic sucrose gradient analysis has been described in a previous report (Billiau et al. 1973a). Polyacrylamide gel electrophoresis of virus RNA was done as follows. Cells were washed and incubated for 16 h with medium containing 15 μCi/ml of 3H-uridine. Virus pellets (131000 g, 45 min) were purified by centrifugation (131000 g, 150 min) in discontinuous sucrose gradients (20 ml, 20/50%, w/w). The interphase material was dissolved in 100 μl of phosphate buffered saline. Virus was disrupted by incubation for 30 min at room temperature with sodium dodecyl sulphate (100 mg/ml) and polyvinyl sulphate (30 μg/ml). Polyacrylamide gel electrophoresis was carried out on 180 minigels, using 50 μl samples of virus RNA as described by Tiollais et al. (1972), for 3-25 h at 18 °C, 6.5 V/cm and 4 mA/gel; 2 mm gel slice fractions were eluted with 1 ml of water (15 h, 60 °C). Radioactivity was determined using Bray’s scintillant.

Fig. 1 (a and b) compares the effect of interferon on the growth of exogenous VSV and endogenous C-type viruses in JLS-V9R cells and in JLS-V5 cells. Cell monolayers in 75 cm² T-flasks were exposed for 24 h to dilutions of interferon. The medium was removed and VSV was added at a multiplicity of infection of 1. Virus yields were determined in the supernatant fluid harvested after 24 h. It can be seen (Fig. 1a) that JLS-V9R cells exhibited approximately the same response to mouse interferon as JLS-V5 cells; yields of VSV were reduced threefold (0.5 log10) at 18 and 14 reference units/ml of interferon. In contrast, when the effect of interferon on the production of C-type oncornaviruses by these cells was examined, the results were strikingly different. Again cell monolayers in T-flasks were exposed for 24 h to different amounts of interferon. The cultures were then refed with medium containing the appropriate concentration of interferon and 20 μCi/ml of 3H-uridine. After 24 h incubation, medium was harvested for determination of particle-associated acid-insoluble label and of reverse transcriptase. The same cultures were also challenged with VSV to verify adequate development of resistance in both cell lines. The data (Fig. 1b) indicate
that in JLS-V5 cells, the release of particles exhibiting reverse transcriptase activity and containing RNase-resistant acid-insoluble $^3$H-uridine, was suppressed by interferon at a concentration similar to that required to inhibit VSV replication. In this and other experiments with JLS-V5 cells, $^3$H-uridine incorporation into virus particles was somewhat more sensitive to interferon than was the release of particles containing reverse transcriptase. However, regardless of the virus assay method, MuLV-R production was inhibited threefold...
by treatment of the cells with 10 to 20 reference units/ml of mouse L cell interferon. In the same cells, VSV was inhibited threefold by 6 reference units/ml of interferon. In contrast, MuLV-R release in JLS-V9R cells was quite refractory to interferon. A threefold reduction in MuLV-R yield was not achieved even with as much as $10^4$ units of L cell interferon. In the same cultures, VSV replication was inhibited much more than threefold by $10^2$ reference units/ml of interferon, the lowest concentration used on JLS-V9R cells in this experiment.

In control experiments it was found that treatment of JLS-V5 cells or MOP cells (a C3H-mouse cell line infected with Kirsten murine sarcoma virus – Billiau et al. 1973b) with mouse brain interferon at $10^2$ reference units/ml suppressed virus production. MuLV-R production in JLS-V9R however, was not significantly inhibited by treatment with this interferon preparation either. Experiments were also done to examine the inhibition of MuLV-R in JLS-V5 cells and JLS-V9R cells by treatment of the cells with poly(rI), poly(rC) rather than interferon (data not shown). A concentration of $10 \mu g/ml$ led to 65% inhibition of MuLV-R in JLS-V5 cells but did not inhibit MuLV-R production in JLS-V9R cells as measured by $^3$H-uridine incorporation into particles of 1.16 g/ml in sucrose gradients. Finally two additional strains of JLS-V9R cells obtained from different laboratories were examined; each one was found to behave in a similar way to that described above.

Resistance of MuLV-R to interferon in otherwise interferon-sensitive JLS-V9R cells may be due to the predominance of a poorly sensitive MuLV-R variant in these cells as opposed to a sensitive variant in most other cell lines examined. In order to test this possibility Balb/3T3 cells were inoculated with concentrated virus preparations obtained from either JLS-V5 or JLS-V9R cells. After three passages the cell lines, now termed 3T3-V5 and 3T3-V9, were actively releasing C-type viruses as indicated by reverse transcriptase levels in the supernatant medium. The non-infected 3T3-cells remained negative. At this passage level both 3T3-V5 and 3T3-V9 cultures in 60 mm Petri dishes were exposed to interferon concentrations, ranging from 0 to 300 reference units/ml. After 24 h the cultures were washed, and fresh medium with the appropriate concentration of interferon was added. After a second incubation period of 24 h, the supernatant media were harvested and processed for determination of particle-associated reverse transcriptase. The cell monolayers, on the other hand, were challenged with VSV for determination of interferon-mediated resistance to exogenous infection. Fig. 1 (c and d) shows that both cell lines were equally responsive to interferon, when tested for resistance to VSV. Also, the C-type virus populations which were transmitted from JLS-V5 and JLS-V9R cells to the 3T3 lines were both very sensitive to interferon. These results prove that JLS-V9R cells as well as JLS-V5 cells contain virus populations which are inherently sensitive to interferon and therefore lend support to the theory that failure of C-type virus to respond to interferon in JLS-V9R cells is a feature of cells rather than of the virus. This view is further confirmed by preliminary results showing that virus populations transmitted from JLS-V5 or JLS-V9R cells to uninfected JLS-V9 cells both resisted the action of interferon. However, in these experiments it became evident that such cultures produce both eco- and xenotropic virus populations. Further study is needed to establish whether both populations respond in a similar manner to interferon.

The results shown in Fig. 1 were based on the observation of particulate elements which contained ribonuclease-resistant, acid-insoluble $^3$H-uridine, and reverse transcriptase activity. To eliminate the possibility that the particles released from JLS-V9 cells in the presence of interferon were physically different from those released by untreated cells, virus pellets from $^3$H-uridine-labelled cultures were examined by (i) isopycnic sucrose gradient analysis in which the labelled particles banded sharply at density 1.16 g/ml, and (ii) by polyacrylamide gel electrophoresis of the virus RNA (Fig. 2), which showed a major, sharp, slow
moving peak among a minor, composite, fast moving peak. The mobility profiles of the viral RNA from interferon-treated JLS-V9R cultures were indistinguishable from those of virus RNA from control cultures, indicating that the particles from treated cultures were not demonstrably different from those in control cultures.

We cannot as yet rule out the possibility that JLS-V9R cells produce a mixed population of C-type viruses. One population (e.g. ecotropic MuLV-R) might be sensitive to interferon and easily transmissible to Balb/3T3 cells. The second one (e.g. an endogenous xenotropic virus) may be insensitive to interferon and not transmissible to other mouse cells. Further characterizations of the virus population in JLS-V5 and JLS-V9R cells, using infectivity assays for both ecotropic and xenotropic viruses, as well as serotyping, are needed.

In spite of the uncertainties mentioned above, this report represents the only experimental evidence known to the authors demonstrating interferon resistance of a type-C oncornavirus replicating in cultured cells which are demonstrably interferon-responsive when tested by challenge with a cytopathogenic virus such as VSV. It should be pointed out that Friedman and his colleagues (1975) have also observed the failure of interferon to suppress oncornavirus production in MuLV-R infected JLS-V9 cells. However, their cells also were not responsive to interferon as measured by VSV challenge.

The availability of two interferon-responsive Balb/c mouse cell lines which produce an oncornavirus (presumably MuLV-R), but which differ in their response to interferon with respect to oncornavirus production may provide a useful system for studying the process of oncornavirus replication. The system may also be useful in elucidating the process by which interferon acts in inhibiting the production of these viruses.
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