Actions of Interferon in Tissue Cultures Harboring Mouse Leukemia Virus

Early observations[1] and experiments[2] show that murine leukemia viruses are sensitive to interferon. However, primary tissue cultures deriving from mice with viral leukemia produce only small amounts of interferon[1,3]; and established cultures harboring mouse leukemia viruses are devoid of interferon activity[4–6]. Thus, mouse leukemia viruses appear as poor inducers, or even antagonists, of interferon. This report describes a system in which mouse leukemia virus is present and the effect of exogenous interferon on another virus is altered.

A tissue culture line was developed from spleen and thymus cells of normal suckling Swiss mice and has been maintained by serial passages of the cells since August 1964, both as uninfected (protocol No. 491) and infected with the Rauscher mouse leukemia virus (protocol No. 479) lines, respectively[5]. Early passages of these lines were exposed to vesicular stomatitis virus (VSV); this virus was received from Dr. W. HENLE and has been propagated in our laboratory in cultures of normal mouse embryo cells. The cytopathic effect and replication of VSV were not significantly altered in the cell line harboring the leukemia virus[5], as compared with the behavior of the VSV in the uninfected line and in normal mouse embryo cultures. The cytopathic effect of VSV at multiplicities of approximately 1 : 1 (or less than 1 ID_{50} VSV/cell) in these cultures consisted of the early formation of a few cells with 4 nuclei followed by rapid disintegration of the culture, i.e. within 24–48 h after infection. Interferon prevented and significantly reduced the extent of the cytopathic effect and replication of the VSV. Interferon was obtained from the serum of mice killed 8 h after the i.v. injection of a neurotropic strain of the Newcastle disease virus[5,7]. This serum was acidified and re-alkalinized and stored deep frozen in dry ice.

The culture line harboring the Rauscher virus (protocol No. 479) has gradually undergone 'malignant transformation' 2 years after its initiation[8]. Prior to its transformation, this cell line consisted of a monolayer of elongated cells which replicated mouse leukemia virus particles, as

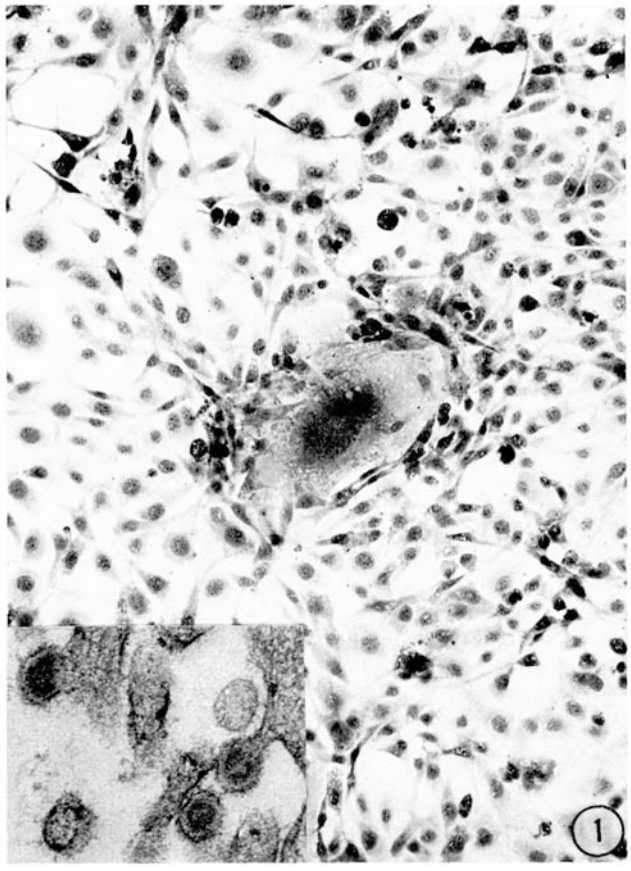

Fig. 1. Giant cell with 1 large nucleus in transformed line No. 479. Papanicolaou stain, × 25 objective, × 10 ocular. Insert: mouse leukemia virus particles in this culture. Electron microscopy. × 66,000.

Cytopathic effects and sensitivity to interferon of vesicular stomatitis virus (VSV) with extensive replication

Protocol No. of cultures tested	CP	VSV Release	Sensitivity to IF
ME	rapid, complete	rapid	strong
491	rapid, complete	rapid	strong
479 early passages	rapid, complete	rapid	mediocre
479 transformed (late passages)	altered, delayed	delayed	weak or none
1078 transformed	altered, delayed	delayed	weak or none

Replication; extensive, titer of VSV in fluid exceeds dilution 10^{-4}. ME, normal mouse embryo cultures, secondary. No. 491, suckling mouse thymus and spleen cell line not infected intentionally with leukemia virus (this line harbors virus particles resembling mouse leukemia virus)[8]. No. 479, suckling mouse thymus and spleen culture infected with Rauscher virus[5,8]. No. 1078, culture of myxofibrosarcoma[8] of mouse inoculated with transformed cells of culture No. 479. CP, cytopathic effect; rapid, complete, disintegration of culture within 48 h; altered, delayed, formation of multinucleated giant cells and syncytia followed by disintegration from 48–96 h. Release; rapid, titers exceeding dilution 10^{-4} of VSV appear in fluid within 24–48 h; delayed, similar titers appear after 48 h. Sensitivity to interferon (IF), strong, dilution 1:2 of mouse serum (IF) inhibits approx. 50–75% of focus formation or CP of approx. $10^{4.7}$ ID_{50} of VSV in a sheet of approx. $10^{4.7}$ cells; mediocre, approx. 25–50% of VSV inhibited; weak, less than 25% of VSV inhibited.

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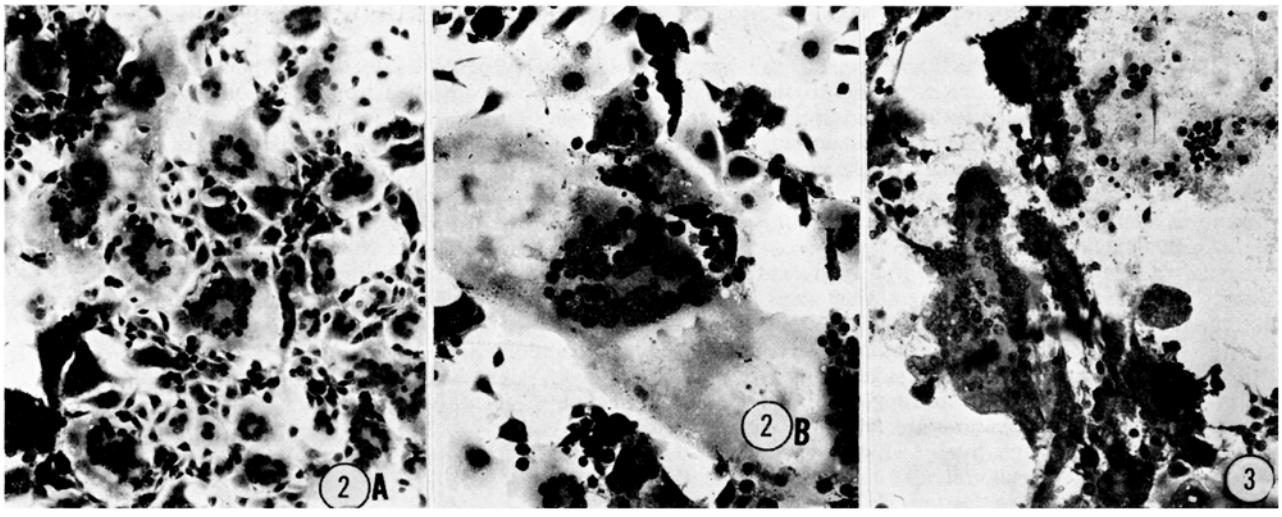

Fig. 2. Formation of multinucleated giant cells by cell-fusion in cultures No. 479 (A) and No. 1078 (B) infected with VSV. Wright stain, × 20 objective, × 8 ocular.

Fig. 3. Disintegration of syncytia in culture No. 1078 pretreated with interferon and infected with VSV. Wright stain, × 20 objective, × 8 ocular.

shown by electron microscopy[9], fluorescent antibody staining[9] and bioassays[5]. After the transformation, giant cells with large multilobulated nuclei and several large nucleoli were formed (Figure 1) and the growth pattern has become irregular and multilayered[8]. The transformed culture continues to manufacture mouse leukemia virus particles but the cells grow out as myxofibrosarcoma in new-born mice[8]. A tissue culture line from such a myxofibrosarcoma has also been initiated (protocol No. 1078); this culture also manufactures mouse leukemia virus particles[8]. The reactions of the transformed cultures (protocol Nos. 479 and 1078) to infection with VSV and to interferon have changed. Addition of VSV to the transformed cultures at a multiplicity of 1:1, or less, i.e. less than 1 ID_{50} VSV/cell, results in the formation of numerous multinucleated giant cells containing 20–50 nuclei and of syncytia containing over 100 nuclei. These cells (Figure 2) persist for 48–72 h and release only small amounts of VSV into the tissue culture fluid. Between 48 and 96 h, a slow disintegration of the multinucleated giant cells and syncytia occurs with release of VSV into the medium. Interferon added to the transformed cultures prior to infection with VSV failed to prevent the formation and later disintegration of multinucleated giant cells and syncytia (Figure 3) and the release of VSV. The interactions of interferon and VSV with these cultures are summarized in the Table.

These observations suggest that not the presence of the leukemia virus in the transformed cultures carries the main responsibility of the altered course of infection with VSV and lost sensitivity to interferon, inasmuch as mouse leukemia virus was present in the same cell line before transformation occurred. Before the transformation, however, the sensitivity of the leukemia virus carrier cultures to exogenous interferon was already decreased. It appears more likely that the transformed cells adopted a new attitude toward an exogenous virus and interferon. It is not as yet known whether this change in cellular behavior occurred 'spontaneously' or under the effect of a defective sarcoma-inducing variant of the mouse leukemia virus. The formation of multinucleated giant cells and syncytia appears to be the result of cell-fusion; thus, a major structural change of the cell surface and that of the lysosomal systems of the transformed cells must have taken place. Under these conditions, it appears, a highly cytocidal virus first elicits cell-fusion and the cell-disintegration becomes delayed; and interferon is not able to inhibit virus synthesis, i.e. the incorporation of viral messenger RNA into the polyribosomes was not prevented. Release of the new virus generations was retarded in the transformed cultures and probably occurred after the exogenous interferon has already been eliminated. Thus, transformed cells are excellent media for virus replication. Tumors have since long been known to promote replication of passenger and oncolytic viruses[10,11]. It may then be expected that stimulons[12] or other anti-interferon agents[13] occur in tumor cells and that 'depression of interferon formation' in mice infected with leukemia virus[14,15] might be due to anti-interferon agents or to loss of sensitivity to interferon[16].

Zusammenfassung. Das Virus der vesikulären Stomatitis verursacht Zellverschmelzung und Syncytienbildung in mit Mäuse-Leukämie-Virus infizierten Gewebskulturen. Interferon konnte diese Zellzerstörung in der Kultur nicht verhindern.

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