Interferon Inhibits Mouse Leukaemia Virus Release: 
an Electron Microscope Study

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SUMMARY

Scanning electron microscopy of AKR cells chronically infected with AKR mouse leukaemia virus revealed that the number of budding virions was greatly increased in interferon-treated cells. These results, together with previous biochemical findings, suggest that in this system, interferon inhibits a late stage of virus assembly or release.

When mouse cells of the AKR, C+ line, chronically infected by and producing a mouse leukaemia virus, were treated with interferon for 16 h or 9 days, virus yields were reduced by 90 to 95% but levels of mouse leukaemia virus group-specific antigen (internal non-glycosylated protein, p 30) and reverse transcriptase activity were not decreased (Friedman & Ramseur, 1974; Friedman et al. 1975). Thus, interferon appeared to inhibit a late step in virus assembly, and/or a step or steps involved in virus release. To complement these previous biochemical studies, we have now used (a) transmission electron microscopy (TEM) to study ultracellular structures and the mode of virus assembly and extrusion, and (b), scanning electron microscopy (SEM) to examine the cell surfaces, a technique already used to study oncornavirus extrusion (de Harven, Lampen & Sato, 1973; Panem & Kirsten 1975; Wong & MacLeod, 1975).

The mouse embryo cell line AKR, C- (AKR-2B) was grown in monolayers in McCoy's modified 5a medium supplemented with 10% heat-inactivated foetal bovine serum. Mouse leukaemia virus group-specific (p 30) antigen and reverse transcriptase activity are not expressed in these cells or in their culture fluid (Rowe et al. 1971; Friedman et al. 1975). The cells of the mouse embryo line, AKR, C+, originally did not produce a leukaemia virus, but later became activated spontaneously, and then produced a leukaemia virus infectious for NIH Swiss mice but not BALB/c mice (i.e. an N-tropic virus – Hartley, Rowe & Huebner, 1970). Both cell lines were kindly provided by Dr W. P. Rowe, National Institute of Allergy and Infectious Diseases, Bethesda.

Mouse interferon, prepared by the method of Ogburn, Berg & Paucker (1973) was supplied by Dr K. Paucker, Medical College of Pennsylvania, and had a specific activity of at least 5 x 10^7 mouse interferon research standard units per mg protein. For TEM studies, cells were washed five time with cacodylate buffer, pH 7.3, containing 0.05 M-sucrose, and then scraped off and pre-fixed in 1.25% glutaraldehyde in cacodylate buffer, pH 7.3, containing 0.05 M-sucrose, at room temperature for 1 h. The cell pellets were resuspended in a small volume of 3% agarose, cut into blocks, and post-fixed with Dalton’s chrome osmium tetroxide, pH 7.3, for 1 h at 4 °C. The grid was stained with 5% uranyl acetate for 1 h at room temperature, then counterstained with Reynolds’ lead citrate (1963). Specimens were examined in a Phillips EM 200 electron microscope.

For SEM, AKR cells were seeded in Petri dishes containing 6 mm glass coverslips (Corning) 48 h before sample collection. Interferon was added during the final 18 h at a
concentration of 30 units/ml. The 80% confluent monolayers were washed five times in phosphate buffered saline (PBS), pH 7.4, and fixed in 2.5% glutaraldehyde in PBS, pH 7.4, for 1 h at room temperature. After washing three times with PBS, the monolayers were post-fixed for 30 min in OsO₄, rinsed and dehydrated through gradients of ethanol and amyl acetate. Immediately after dehydration, the cells were dried at the critical point in CO₂ in a Denton Vacuum (Cherry Hill, N.J.) apparatus, DCP-1 (Anderson, 1951) and then rotary coated with a 15 to 20 nm layer of gold palladium in a High Vacuum Equipment Corporation (Hingham, Mass.) vacuum evaporator. Samples were viewed in an Etel scanning electron microscope at a nominal resolution of approx. 15 to 20 nm.

By TEM, AKR, C- cells were predominantly fibroblasts with a single unlobed nucleus. There was no difference in the appearance of interferon-treated and untreated cells, and no intracellular or extracellular virus particles were detected in either case.

The morphology of cellular organelles in interferon-treated and in control AKR, C+ cells was similar to that of AKR, C- cells. Both intracellular and extracellular C-type virus particles were seen in AKR, C+ cells, whether treated with interferon or not. However, interferon treatment appeared to reduce the number of extracellular virus particles, in agreement with the previous finding of reduced extracellular reverse transcriptase activity and infectivity (Friedman & Ramsaur, 1974; Friedman et al. 1975). Thus, of the total virus particles seen in 200 AKR, C+ cells in three independent experiments examined by TEM, approx. 80% were classed as budding virions in the interferon-treated cells, as compared to only 30% in untreated cells, the remaining virions in both cases being extracellular. The striking finding was the large number of budding virions seen in the interferon-treated cells in spite of their poor production of extracellular virus. The reliability of the quantitative data obtained from thin sections was, however, limited by the extremely small portion of each cell that was observed, even though interferon-treated and control samples from an experiment were carefully processed together, so that the results could be directly compared.

In SEM studies, several characteristic features were revealed on the surfaces of both C- and C+ cells. These included microvilli about 100 nm in diam. (all measurements are corrected for the thickness of the metal coating) and of varying length, occasional blebs of different sizes intracellular filaments or cell processes and web-like structures with a fine embossed appearance. AKR, C- cells appeared as elongated fibroblasts in monolayer. No difference was seen in their outer surface architecture whether or not they were treated with interferon, and in either instance, no virus particles were seen on the surface. AKR, C+ cells, while similar in surface morphology, characteristically had fewer microvilli, except for cells in mitosis. Numerous budding virions of 100 to 110 nm diam. (de Harven et al. 1973; Panem & Kirsten, 1975; Wong & MacLeod, 1975) were observed on their surface. The virus particles were more numerous on the surface of interferon-treated cells than on untreated controls. Thus, in those illustrated in Fig. 1 (a) and (b), there were 2.17 virus particles/μm² in controls and 6.06 in interferon-treated cells, a difference which is highly significant (P < 0.001).

Interferon treatment can reduce virus production if administered before or during the initial exposure of cells to a virus (Sonnabend & Friedman, 1973). The marked inhibition by interferon of mouse leukaemia virus production in AKR, C+ and other chronically infected mouse cells has been reported (Billiau, Sobis & De Somer, 1973; Friedman & Ramsaur, 1974; Friedman et al. 1975; Pitha, Rowe, & Oxman, 1976). However, although amounts of extracellular virus were reduced as detected by reverse transcriptase assay, amounts of intracellular virus in terms of reverse transcriptase and virus group-specific (p 30) antigen were equal or higher in interferon-treated C+ cells than in control untreated cells. These find-
Fig. 1. Scanning electron micrograph of (a) an interferon-treated AKR, C⁺ cell. The density of budding virions on the surface of these cells is considerably greater than in untreated cells, (b).
ings suggest that the virus genome is at least partially expressed in the interferon-treated cells. In our present studies, intended to complement the previous biochemical investigations, a significant accumulation of budding virus was observed on the surface of interferon-treated cells. Together, these findings suggest that in this system interferon may act by inhibiting a late stage in virus assembly or virus release.

Recently, a system of mouse thymus and bone marrow cells (TB) infected with a temperature-sensitive mutant \( (ts\ 3) \) of Moloney leukaemia virus has been described (Wong & MacCarter, 1974; Wong & MacLeod, 1975). There are marked similarities in scanning and transmission electron micrographs between interferon-treated AKR, C\(^+\) cells and TB cells infected with this \( ts\ 3 \) mutant virus and grown at a non-permissive temperature (39 °C). In both, there is significant accumulation of budding virions at the cell surface, suggesting that similar stages in virus maturation may be inhibited by interferon action or the \( ts\ 3 \) mutation.

Since the action of interferon on mouse leukaemia virus in AKR cells appears to be associated with membrane phenomena involved in a late stage of virus assembly or extrusion, the mechanism of interferon action in this virus-host system appears to differ from that reported for other systems (Joklik & Merigan, 1966; Friedman, 1968; Oxman & Levin, 1971; Friedman et al. 1972; Sonnabend & Friedman, 1973). It is possible, however, that in our system interferon inhibits the translation of a single virus protein necessary for virus assembly and release, presumably a protein similar or identical to that involved in the \( ts\ 3 \) mutation (Wong & MacCarter, 1974; Wong and MacLeod, 1975). The production of other virus proteins in this system is not inhibited possibly because interferon may not affect transcription and translation of virus genetic information which is integrated into the host genome (Oxman & Levin, 1971).

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REFERENCES


Short communications


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