An Effect of Interferon on the Uncoating of Murine Leukaemia Virus Not Related to the Antiviral State

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SUMMARY

Adsorption of murine leukaemia virus (MLV) to NIH/3T3 cells, as determined by analysing its reverse transcriptase activity in the cell membrane, was found to be unaffected by interferon (IFN). Virus penetration and uncoating were followed by quantifying intracellular virions in terms of sedimentable reverse transcriptase activity in the cytoplasmic fraction. The penetrating virions were found to accumulate to a higher level in IFN-treated cells than in untreated controls. Intracellular virions were uncoated in untreated cells shortly after their penetration, whereas their uncoating was delayed in the IFN-treated cells for 2 to 3 h. Neither virus uncoating nor the effect of IFN on this process appeared to require new protein synthesis, since both were unaffected by cycloheximide (CH).

In the past few years particular attention has been focused on the effect of interferon (IFN) on RNA tumour viruses (for reviews, see Friedman, 1977; Billiau, 1977). Experiments with chronically infected cells have suggested that IFN blocks some late step in the replication of murine leukaemia virus (MLV), such as post-translational processing of virus proteins (Chang & Friedman, 1977), virion assembly (Van Griensven et al. 1970; Pitha et al. 1976; Chang et al. 1977a) or its final release from the cell surface (Billiau et al. 1974, 1976a, b; Chang et al. 1977b). Pitha et al. (1976, 1977) and Wong et al. (1977) claim that in any exogenous infection with MLV, IFN also interferes only with a late event and not with any of the early steps occurring before the integration of virus DNA into the cellular genome. In contrast, work from our laboratory (Aboud et al. 1978) has strongly suggested that, in addition to its late effects, IFN interferes with one or more of the early events of exogenous infection. Support for this view has been more recently provided by Morris & Clegg (1978) who showed that IFN inhibits cell transformation by murine sarcoma virus (MSV) only if added sufficiently long before virus infection, but not when added to cells already transformed. This finding suggests that IFN inhibits some step before the integration of virus DNA into the cell genome. As a consequence of these observations, we investigated the effect of IFN on MLV adsorption, penetration and uncoating, i.e. on the earliest events of virus infection. For this purpose we used our procedure for detecting membrane-bound and intracellular virus particles in terms of the virus-associated reverse transcriptase as described elsewhere (Aboud et al. 1979).

Our cells were maintained in Dulbecco's modified Eagle's minimal medium supplemented with 10 % calf serum. In experiments with IFN, the serum concentration was reduced to 2.5 %. MLV stocks for infection were prepared from NIH/3T3 mouse fibroblasts chronically infected with the Moloney strain of MLV [NIH/3T3(MLV)]. These cells were plated at a density of 2 x 10^6 cells per 9 cm Petri dish (Nunc, Denmark) in 10 ml medium containing 10 % serum. After 24 h the culture medium was replaced with 6 ml fresh medium. After 16 h incubation this medium was collected, clarified and supplemented with 8 µg/ml polybrene before it was used for infection of other cells. Uninfected NIH/3T3 cells were used as host cells for the exogenous infections. These cells were plated at a density of 2 x 10^6 cells per 9 cm Petri dish.
Fig. 1. Effect of IFN (80 IU/ml) on accumulation and uncoating of intracellular virions. Cells were plated with (●—●) or without (○—○) IFN. After 16 h the cells were infected with MLV. (a) At various times after virus addition the cells were washed, dispersed by trypsinization and disrupted. (b) After 30 min of infection the cells were washed and further incubated with fresh IFN-free medium. At various times afterwards cells were dispersed and disrupted as before. Intracellular virions were sedimented by a high-speed centrifugation from the cytoplasmic fractions in both experiments and quantified by assaying their reverse transcriptase activity. Broken lines indicate the activity of comparable preparations from mock-infected control cells.

2.5 × 10⁶ cells per 9 cm dish 16 h before being infected. Mouse IFN was prepared from L929 cells infected with Newcastle disease virus (NDV) and partially purified as described elsewhere (Aboud et al. 1976). The IFN preparation used in most experiments in this study contained 10⁵ international units (IU)/mg protein. Other batches of IFN at different stages of purity were used in various control experiments. IFN was used at a concentration of 80 IU/ml.

The effect of mouse IFN on MLV adsorption was examined first. For this purpose cells were plated with or without IFN. After 16 h the culture medium was removed and the cells were infected for 20 min. Unadsorbed virus was then removed by three washes with cold phosphate-buffered saline (PBS) and the cells were scraped off with a rubber policeman, suspended in a hypotonic buffer and disrupted by homogenization and sonication as described previously (Aboud et al. 1979). The cell debris was sedimented, resuspended in a small volume of 0.01 M-tris-HCl pH 8.2 and assayed for reverse transcriptase activity in the presence of 50 μg/ml actinomycin D. Cell debris prepared from mock-infected cells served as control. The enzyme activity of debris from infected cells was four- to fivefold higher than that of the uninfected control, this higher activity representing membrane-bound virions as proved in our previous study (Aboud et al. 1979). However, no difference could be detected in this activity between IFN-treated and untreated cells, indicating that IFN treatment had no effect on virus adsorption.

Next, an attempt was made to elucidate the effect of IFN on virus penetration. IFN treated and untreated cells were infected with MLV and at various times after virus addition, the cells were washed and dispersed by 0.4% trypsin to destroy external virions. The cells were disrupted and the cytoplasmic fraction was centrifuged at 105,000 g for 45 min to sediment intracellular virions. This high speed pellet was suspended in a small volume of 0.01 M-tris-HCl pH 8.2 and assayed for reverse transcriptase activity, with a comparable pellet from mock-infected cells serving as a control. As shown in Fig. 1(a), penetrating virions accumulated in the untreated cells during the first 30 min up to a certain maximum level and then gradually disappeared. This disappearance of intracellular virus particles most probably reflected their uncoating, with conversion of their reverse transcriptase into a soluble form. In IFN-treated cells the penetrating virions accumulated to a higher level
Fig. 2. Effect of IFN (80 iu/ml) and CH (5 μg/ml) on uncoating of intracellular virus. Cells were
plated and 16 h later were infected with MLV for 30 min after receiving various treatments. ••••
IFN added at the time of plating; ▲—▲, IFN added at the time of plating and CH at 30 min
before infection; □——□, IFN added 30 min before infection; ■——■, IFN and CH added 30
min before infection; ◆——◆, CH added 30 min before infection; ○——○, no additions. After 30
min of infection, the cells were washed and analysed for intracellular virus particles by their
reverse transcriptase activity either immediately or after an additional 2 h incubation with fresh
IFN-free medium. CH was added to this medium in all CH-treated cultures.

with no subsequent decline. This finding may suggest that IFN treatment resulted in inhibi-
tion of virus uncoating. To substantiate this possibility more directly, IFN-treated and un-
treated cells were infected with MLV. After 30 min, unadsorbed virus was removed and the
cells were further incubated with fresh IFN-free medium. At various times afterwards the
cells were analysed for intracellular virus particles. Fig. 1 (b) shows that in control cells virus
penetration was followed by immediate uncoating, whereas in IFN-treated cells, virus un-
coating was delayed for 2 to 3 h. This effect was not due to impurities in the particular IFN
preparation used, since IFN preparations of different purity (from 2 × 10⁴ to 5 × 10⁶ iu/mg
protein) had the same effect, whereas mock IFN was ineffective. It is thus evident that IFN
treatment results in inhibition of virus uncoating, but from these experiments, its effect on
virus penetration is still unclear. The higher accumulation of virions observed during the
first 30 min of infection does not necessarily reflect a higher penetration. It seems probable
that uncoating of virus begins immediately after its penetration and thus the kinetics of
virus accumulation is merely the balance between virus penetration and its uncoating. Since
virus uncoating is inhibited in IFN-treated cells, even a lower rate of penetration could lead
to a higher accumulation.

One possible explanation for the effect of IFN on virus uncoating can be obtained from
data suggesting that IFN blocks virus protein synthesis (Friedman, 1977). It could be
suggested that uncoating is exerted by virus-induced enzymes and that IFN inhibits their
synthesis: if so, inhibitors of protein synthesis, such as cycloheximide (CH), should also
interfere with virus uncoating. To explore this possibility, 5 μg/ml CH was added from
30 min before infection up to the end of the experiment. This CH concentration was found
in preliminary experiments to inhibit protein synthesis almost completely (about 97 %)
within 15 min. However, from the results presented in Fig. 2 it is evident that CH has no
effect on virus uncoating: this excludes the possibility that IFN exerts its effect on uncoating
by inhibiting virus-induced protein synthesis, since this process does not depend on newly
synthesized proteins. The IFN-mediated antiviral state is known to be an inducible process
which requires several hours for its full establishment and is strictly dependent on protein synthesis (Friedman, 1977). It was of interest to ascertain whether the effect of IFN on uncoating is related to this antiviral state. Cells were therefore treated with IFN for only 30 min, a time insufficient for IFN to induce an antiviral state in our system (Salzberg et al. 1978). This short IFN treatment was found, in the experiment presented in Fig. 2, to have the same effect on virus uncoating as the 16 h treatment. Moreover, this effect was exerted with the same efficiency even when IFN was added for 30 min together with 5 μg/ml CH (Fig. 2). The possibility cannot be excluded that IFN acts faster than CH and consequently some IFN-induced proteins might have been synthesized even in the presence of CH. However, as noted before, CH at this concentration blocks protein synthesis almost completely within 15 min. It is unlikely that under such conditions IFN will be capable of inducing the synthesis of cellular proteins that block uncoating to the same extent as in cells without CH treatment. Therefore, from our findings it seems more likely that the effect of IFN on MLV uncoating is relatively independent of cellular protein synthesis. From both points of view this effect of IFN is quite unlike that involved in the conventional IFN-mediated antiviral state.

In other experiments (Aboud et al. 1980) we found that human fibroblast IFN has the same effect on MLV uncoating in NIH/3T3 cells. In their induction of the antiviral state, interferons are generally considered to be species specific. However, IFN binding to the cell surface is not species specific. Furthermore, this binding is rapid and requires no protein synthesis (Friedman, 1977). It therefore appears that the mere binding of IFN to the cell surface is sufficient to inhibit virus uncoating. Since virus uncoating might be a membrane-related process, and since IFN binding leads to various changes in the cell membrane (Lindahl et al. 1974; Knight & Korant, 1977; Chang et al. 1978) it is quite possible that IFN binding to the cell surface is sufficient for interference with virus uncoating without the need to induce the processes leading to the establishment of the antiviral state.

Studies with other viruses (Ho, 1961; DeSommer et al. 1962; Grossberg & Holland, 1962; Wiebe & Joklik, 1975) have indicated that IFN does not interfere with virus uncoating. However, unlike these other viruses, the outer envelopes of RNA tumour viruses contain some cell membrane components resulting from the budding process by which they are released from the cell surface (Gillespie & Gallo, 1977). It is therefore quite possible that uncoating of these tumour viruses involves particular membrane interactions of a type which alone are affected by IFN.

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