Effect of Interferon on Murine Leukaemia Virus Infection. IV. Formation of Non-infectious Virus in Chronically Infected Cells

By P. M. PITHA, N. A. WIVEL,* B. F. FERNIE AND H. P. HARPER

Oncology Center, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205 and *Bldg. 8, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014, U.S.A.

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SUMMARY

Interferon (150 units/ml) was used to treat SC-1 and AKR-2B cells which were chronically infected with murine leukaemia virus (MuLV). This led to a 100-fold decrease in the amount of infectious virus released into the medium and a 10-fold decrease in the number of virus particles measured by the virion-associated reverse transcriptase assay. However, there was little change in the amount of cell-associated infectious virus, though nearly twice as many cell-associated virions were counted in electron micrographs. With both types of cells, interferon blocked MuLV replication at the post-budding stage, but it did not change the morphology of the particles produced or their content of virion 70S RNA.

Infectious virus assembled on the cell membranes of interferon-treated cells was less stable at 37 °C than that grown in the absence of interferon. Release of infectious virus from interferon-treated cells was not inhibited by actinomycin D or cycloheximide, though both agents inhibited virus production in controls.

These results show that interferon inhibits MuLV replication through effects on virion assembly; these lead both to the formation of non-infectious particles and of fewer virions. Kinetic analysis further shows that interferon affects MuLV assembly rapidly and induction of an antiviral protein may not be required.

INTRODUCTION

Treatment of mouse cells (2B cloned cell line) infected with AKR-L1 virus (MuLV) with purified mouse interferon, either prior to or after infection, has been shown to inhibit the production of infectious virus (Pitha et al. 1976, 1977). While in acute infection the production of virus particles paralleled the appearance of infectious virus, in chronically infected cells interferon treatment led to the formation of non-infectious particles.

Other workers (van Griensven et al. 1971; Billiau et al. 1973, 1974, 1975, 1976a, b; Friedman & Ramseur, 1974; Swetly & Ostertag, 1974; Friedman et al. 1975; Chang et al. 1977; Luftig et al. 1977; Wong et al. 1977), using predominantly cells chronically infected with MuLV, show results in general agreement with these data. It has been suggested that the interferon block is due to the inhibition of release (Billiau et al. 1973; Chang et al. 1977) or to ‘trapping’ of the virus (Billiau et al. 1976a) by the cell membranes of the infected
cells. In these reports, however, the particles which accumulated on the cell surface of interferon-treated cells were examined only morphologically and no attempts were made to correlate the number of virions with their infectivity. In contrast, our previous results pointed more to a defect in virus assembly and maturation. We have attempted in this report to compare the cell-associated virions produced in the presence and absence of interferon and similarly the released virions. Given the fact that the effects of interferon on MuLV are reversible and that the inhibition occurs after virus RNA and protein synthesis has occurred, there is a strong possibility that the differences are both transient and subtle. Accordingly, the virions were compared on the basis of infectivity, content of virus genome RNA and their morphology.

METHODS

Cells and viruses. The virus-negative mouse embryo clonal cell line AKR-2B (Rowe et al. 1971), the III6A clone of the SC-1 cell line (Hartley & Rowe, 1975) and XC cells (Svoboda et al. 1963) were grown as described recently (Pitha et al. 1976). The AKR-L1 virus was isolated from a spontaneous leukaemic AKR mouse and grown as described previously (Pitha et al. 1976). The titre was $10^7$ p.f.u./ml in SC1-cells. Cell-free virus was titrated on SC-1 mouse cells by the u.v. XC plaque procedure (Rowe et al. 1970). For the assay of cell-associated virus, cells were extensively washed with phosphate buffered saline (PBS), scraped, and disrupted by pipetting three times through a 0.3 mm diam. needle; infectious virus was assayed by the XC test either before or after freezing.

To remove the virus associated with the external parts of the plasma membranes, cells were treated with 0.075 % trypsin (for 5 min).

Vesicular stomatitis virus (VSV, New Jersey serotype) was propagated in mouse L cells. The titre of clarified supernatant was $10^9$ p.f.u./ml in mouse L cells.

Radioisotopic labelling of virus particles. Chronically infected cells in confluency were incubated in leucine-free medium for 15 min at 37 °C then in leucine-free medium containing $10 \mu$Ci/ml of $^3$H-leucine (15 Ci/mmol, New England Nuclear) and for 3 or 12 h at 37 °C. Virus radioactively labelled with $^3$H-uridine (20 $\mu$Ci/mmoll, Moravek Biochemicals) was prepared by incubating the cells with 100 $\mu$Ci/ml of $^3$H-uridine for 2 h; the medium was then replaced with medium containing 5 % foetal calf serum and incubated for another hour, after which the medium was harvested and virus particles were purified as described below.

Virus purification. Virus was purified from the medium by the method of Hunter et al. (1976). The harvested medium was clarified at 10000 $g$ for 10 min and then the supernatant was layered on top of a discontinuous gradient consisting of 20 % (w/v) sucrose in standard buffer (Duesberg et al. 1968) and 40 % (w/v) potassium tartrate in standard buffer (sucrose: tartrate ratio 5:1, v/v). The gradient was centrifuged at 286000 $g$ in a Beckman SW 41 rotor for 1 h or in an SW 27 rotor at 131000 $g$ for 3 h. The virus banded at the tartrate-sucrose interface was collected, diluted twofold with standard buffer and layered on top of a continuous 24 % (w/v) to 48 % (w/v) sucrose gradient which was centrifuged to equilibrium in an SW 40 rotor at 286000 $g$ for 3 h. Gradients were fractionated and fractions containing the virus were pooled, diluted with standard buffer and pelleted by centrifugation at 337000 $g$ for 1 h in a SW 60 rotor.

Isolation of the virion RNA. Virus RNA was isolated from gradient-purified virions by a modification of the Palmiter method (1974). The pooled fractions (about 1 to 2 ml) were made up to 4 ml with standard buffer (pH 9). Washed phenol (4 ml) was added together with 1 drop of stock mercaptoethanol (Eastman), 0.1 ml of 0.5 M-EDTA and 15 $\mu$l rat liver
Effect of interferon on MuLV infection

RNA and finally (1 mg/ml) SDS to give 1% (v/v). The mixture was vigorously vortexed for a few seconds at room temperature. Buffer-saturated phenol (4 ml) was added, shaken for 3 min, allowed to settle for 5 min and then shaken for 3 min longer. The phenol-RNA mixture was then centrifuged for 10 min at 8000 rev/min in an SS 34 (Sorvall) rotor. The lower phase was removed, leaving the interphase. After the aqueous phase had been warmed to room temperature, 8 ml of chloroform–isoamyl alcohol (24:1) was added. The procedure of shaking and centrifugation was repeated with chloroform–isoamyl alcohol until the interphase disappeared or no longer changed (about three times). After the final centrifugation, the aqueous phase only was removed and precipitated with 2 vol. of cold (−20 °C) 0.2 M-NaCl, 80% ethanol.

Polyacrylamide gel electrophoresis. Electrophoresis of the RNA species isolated from the exogenous virions was performed according to the method of Levin et al. (1974) in 18% polyacrylamide, 0.5% agarose gel containing 0.5% SDS. The gels were run at 4 mA/gel for 90 min. After electrophoresis the gels were sliced into 2 mm sections, placed in scintillation vials and dissolved in 0.5 ml 30% hydrogen peroxide at 85 °C for several hours. Ten ml of Aquasol (New England Nuclear) were added to each vial, and the samples were counted.

Electron microscopy. The preparation of the monolayers for transmission electron microscopy (TEM) was carried out as described previously (Wivel & Smith, 1971). Thin sections were examined in a Hitachi HU-11E (Tokyo, Japan) microscope with an accelerating voltage of 75 kV.

For scanning electron microscopy (SEM), cells were grown on 6 mm² glass cover slips. The cells were fixed in 2% glutaraldehyde in 0.05 M Na cacodylate buffer, pH 7.4, post-fixed in chrome osmium for 1 h and dehydrated through a series of graded ethanols in 0.5% uranyl acetate. After two rinses with 100% ethanol, the samples were immersed in 50% ethanol, 50% Freon 113, followed by two rinses in 100% Freon 113. Without being allowed to dry, the cover slips were transferred to the chamber of a Bomar SPC 50/ex (Decoma, Washington, U.S.A.) critical point drying apparatus and treated under conditions previously described (Malech & Wivel, 1976). The cover slips were mounted on specimen stubs and shadowcasted with a 25 to 20 nm layer of carbon and gold on a rotary stage at an angle of approx. 15°. The specimens were examined in a Hitachi HHS-2R scanning electron microscope. Virus particles were counted by means of a computer-directed image analysis system (LeMont Scientific Inc., State College, Pennsylvania, U.S.A.) interfaced to the scanning electron microscope. This analysis gives a diameter for a particle which is an average of diameters through eight separate planes. Only those particles which had an aspect ratio near one were recorded; the aspect ratio is the ratio of the maximum diameter to the minimum diameter and when this is approx. 1, the particle is a near-perfect sphere.

It has been previously established that SEM can be successfully used to study the surfaces of cells infected with oncornaviruses (de Harven et al. 1973; Panem & Kirsten, 1975; Wong & MacLeod, 1975). The degree of resolution is sufficient for the identification of the 100 to 110 nm particles which are budding from or contiguous to the plasma membrane of the cell. The use of image analysis lends itself to the counting of virus particles of this size. All measurements were corrected for the heavy metal coating, and particles in the size range of 100 to 110 nm were identified. During the calibration of the system, the computer-generated particle counts and measurements were compared with the results from pictures of the selected fields. There was essentially a complete correlation among the sets of data.

Reverse transcriptase. The virus was pelleted from the supernatant fluid at 150,000 g in a SW 60 rotor for 60 min and the activity of the virion-associated reverse transcriptase was
assayed as described previously (Pitha et al. 1976), with poly A. oligo dT (Collaborative Research, Waltham, Mass., U.S.A.) as an exogenous template.

Interferon. The mouse interferon was a generous gift from Dr E. Knight. It was produced in L cells by induction with MM virus and purified as described previously (Knight, 1975). Its specific activity was $10^6$ units/mg protein. The amount of interferon used was standardized against the reference mouse interferon (National Institutes of Health, catalogue no. Go02-906-511) that has an assigned activity of $1.2 \times 10^4$ units/ml and gave an end point titre of $1.6 \times 10^4$ when assayed by a VSV yield reduction assay.

RESULTS

Kinetics of interferon-induced inhibition of virus production

In our previous report (Pitha et al. 1976), mouse 2B cells infected with MuLV virus were treated with interferon for 24 h. This procedure decreased the production of infectious virus 100- to 500-fold, and virion production as assayed by the activity of virion-associated reverse transcriptase was decreased 5- to 10-fold. In the present work we examined the kinetics of this inhibition at earlier time points. The cells were treated with interferon (150 units/ml) and at various times the amount of virus in the medium was determined by titration in SC-I cells or by assaying reverse transcriptase activity. Fig. 1 shows that as early as 6 h after addition of interferon the amount of infectious virus present in the medium was reduced 90% compared to the virus from the untreated cells, while at this same time the reverse transcriptase activity in the medium was reduced only 20%. This indicates that interferon primarily affects the infectivity of the released virions, while reduction in yield of physical particles released is a slower process. This observation was confirmed by quantification of the radioactive virus released.

Cells were pulse labelled either with $^3$H-leucine (10 $\mu$Ci for 30 min) or $^3$H-uridine (100 $\mu$Ci for 1 h; data not shown) and chased in the presence or absence of interferon. The amounts of radioactive virus released in the medium were determined at 3-h intervals. Fig. 1 indicates that the first inhibition in the virus release was observed 6 h after the addition of interferon. By 12 h the amount of virus released in the presence of interferon was reduced 1000-fold when assayed by infectivity, eightfold when assayed by reverse transcriptase activity, but only twofold when compared to the amount of labelled virus released in the untreated cells.

We have shown previously that infection with MuLV does not interfere with the development of the antiviral state in AKR-2B cells, as detected by reduction in VSV yield (Pitha et al. 1976). In order to examine the kinetics of induction of the antiviral state in infected SC-I cells, the cells were treated with interferon (150 units/ml) for various lengths of time. The interferon was removed, the cells were washed and infected with VSV at high multiplicity and the virus yield in the medium was determined after a single replicative cycle (Fig. 1 b). It was found that the cells had to be incubated with interferon for at least 8 h for there to be a 50% inhibition of VSV replication. Thus there is a decrease in both the infectivity and the number of MuLV virions released into the medium before an antiviral state is established in these cells.

In all these experiments we assayed only the virus released into the medium. In order to examine whether the observed inhibition in virus particle production in interferon-treated cells could be solely explained by inhibition of virus release, we examined the effect of interferon on the amount of cell-associated virus.
Effect of interferon on MuLV infection

Fig. 1. Effect of interferon (150 units/ml) on the replication of MuLV and VSV in SC-1 cells. (a) The amount of MuLV in the medium of infected cells was assayed at various times after adding interferon: ○ ○, p.f.u./ml; □ □, reverse transcriptase activity; △ △, virus labelled with $^3$H-leucine for 12 h before addition of interferon. (b) Infected cells were treated with interferon for various times. The interferon was then removed and the cells were washed and superinfected with VSV (m.o.i. 10): ● ●, yield of VSV in the fluids of a single replication cycle, estimated by titration in L cells.

Effect of interferon on the virus assembly and release

The number of cell-associated virus particles produced in interferon-treated MuLV infected mouse cells was estimated both by a semi-quantitative electron microscope survey and by assay of infectious virus. Two types of infections, acute and chronic, in two types of host cells were compared (Table 1). Interferon treatment of AKR-2B cells prior to acute infection with MuLV leads to a decrease in the total number of newly synthesized virus particles in the cells (Pitha et al. 1976). The block seems to occur before bud formation. When measured by thin section electron microscopy in 100 cells, there were ten times fewer buds and four times fewer virions associated with the plasma membrane in interferon-treated cells than in infected controls. At the same time the amount of cell-associated infectious virus was fifty times less. The inhibition of the accumulation of the virus in the medium was more pronounced. Both infectious virus and the total number of released virions were inhibited by 99%.

In chronically infected AKR-2B cells treated with interferon for 24 h, only the accumula-
Table 1. Effect of interferon (150 units/ml on MuLV release

<table>
<thead>
<tr>
<th>Cells</th>
<th>Infection</th>
<th>Interferon treatment (h)</th>
<th>Infectious virus* (p.f.u./ml)</th>
<th>Reverse transcriptase activity (%)†</th>
<th>Plasma membrane associated virus</th>
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<td>$3 \times 10^5$</td>
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<td>47</td>
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<td>$3 \times 10^5$</td>
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<td>0 → 24</td>
<td>$5 \times 10^4$</td>
<td>1069</td>
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</table>

* The assay was done on cells subjected to one cycle of freezing and thawing.
† 100% = 140 pmol $^3$H-dTMP incorporated in response to poly rA-dT (13-14) per 50 μl of pelleted virus suspended from 3 ml of culture fluid.
‡ Transmission electron microscopy: 100 cells counted.
§ Scanning electron microscopy: number of particles per 100 unit area (each approx. 121 μm²).

Fig. 2. Transmission electron microscopy: representative sections of SC-I cells (a) infected with MuLV (AKR-L1), (b) infected with MuLV and treated for 72 h with interferon.
Effect of interferon on MuLV infection

The effect of interferon on virus production was further examined in mouse SC-1 cells, which are known to be highly susceptible to MuLV infection. In this cell line the interferon block occurred at the post-budding stage both in acute and chronic infections (Table I). The number of plasma membrane-associated virus particles, as detected by electron microscopy, was increased twofold in interferon-treated cells in the acute infection but somewhat less in the chronic infection (Fig. 2a and b). Also, the amount of infectious virus detected in the cells was not significantly different in interferon-treated cells and controls, while the amount of virus released into the medium was again markedly inhibited by interferon. As with AKR-2B cells, the increase in cell-associated virus in the interferon-treated cells was not reflected by increase of infectious virus detected in the cells, indicating that some of the virus which accumulates during interferon treatment is non-infectious. However, the virus particles formed in the presence of interferon were not morphologically different from those in the control cells. In both cell lines, the accumulation of virus particles in the interferon-treated cells was not sufficient to explain the reduction in the amount of virus in the medium, so that it is rather unlikely that inhibition of virion release into the medium (Billiau et al. 1973; Chang et al. 1977) is the entire interferon effect.

While the TEM data allowed budding particles and extracellular forms to be distinguished, quantification was subject to a significant sampling error because of the small number of cells examined in relation to the total number of cells in a given experiment. In order to assess the reliability of these observations, acutely infected SC-1 cells from the experiment indicated in Table I were processed for SEM.

Uninfected cells had no surface virus particles and interferon did not appear to alter the architecture of infected cells. Most of the cells had moderately irregular surfaces and there were scattered areas containing filamentous structures, but very few microvilli.

Particle counting was done at a magnification of 6000 and each field covered a unit area of about 121 μm². Some 100 such fields were scanned and the results are expressed in terms of the number of particles per unit area, since one area does not cover the entire surface of a cell. The acutely infected cells contained 2.67 virus particles per unit area and the infected interferon-treated cells 10.67 virus particles, a difference that is statistically significant (P < 0.001; Fig. 3a and b). Thus these SEM results confirm and augment the TEM results.

Effect of cycloheximide and actinomycin D treatment on the production of MuLV in interferon-treated cells

To test whether the release of MuLV accumulated on the cell surface of the interferon-treated cells requires protein synthesis, we tested the effect of cycloheximide on the production of infectious virus in cultures treated with interferon for 24 h prior to cycloheximide treatment and compared it to the virus production in the cycloheximide-treated controls.

Infected SC-1 cells were treated with 10 μg/ml of cycloheximide, a concentration which decreased cellular protein synthesis by 95% within half an hour, in agreement with previous studies (Levin & Rosenak, 1976). At this concentration, cycloheximide led to a sharp decrease in virus production (Fig. 4). After 4 h the relative amount of infectious virus and
Fig. 3. Scanning electron microscopy: (a) view of a portion of the surface of an infected SC-1 cell from the experiment illustrated in Fig. 2(a); (b) view of an infected, interferon-treated cell from the experiment illustrated in Fig. 2(b). Arrows indicate some of the virus particles on the cell surface.
Effect of interferon on MuLV infection

Fig. 4. Effect of cycloheximide and actinomycin D on the synthesis of infectious virus in SC-1 infected cells (a) treated either with cycloheximide (10 \(\mu\)g/ml) for the time indicated or with actinomycin D (1 \(\mu\)g/ml) for 60 min; (b) treated with interferon (150 units/ml) for 24 h, washed to remove interferon and then treated with cycloheximide or actinomycin D (as in a). The amount of infectious MuLV in the culture fluids at the time shown was estimated by the u.v. XC test: ○, no cycloheximide treatment; ●, cycloheximide-treated cultures; ■, actinomycin D treatment.

reverse transcriptase activity (data not shown) in the medium was reduced by 85 to 95\%, which confirms that virus production in these chronically infected cells is strongly dependent on protein synthesis. However, if cells were first treated with interferon, cycloheximide had practically no effect on the production of infectious virus in the first 8 h. Thus after 7 h of cycloheximide treatment, the production of infectious virus in interferon-treated cells decreased by twofold, while that in the controls was inhibited by more than 1000-fold. Similarly, actinomycin D (1 \(\mu\)g/ml) treatment significantly inhibited virus production, but not in cells which had been exposed to interferon for 24 h before actinomycin D treatment.

These results indicate that the release of the infectious virus assembled on the plasma membranes of interferon-treated cells does not require further protein synthesis or gene expression, while in control cells the production of infectious virus is dependent on both protein and RNA synthesis.

Heat lability of the infectious virus

Our data indicate that in interferon-treated cells, more of the non-infectious virus was present in the medium than in the cells. This suggests that the virus released from interferon treated cells was inactivated faster than that associated with the cells. To test this hypothesis we treated infected SC-1 cells with interferon and assayed the stability of both the virus associated with the cells and that in the medium by incubation at 37 °C before and after freezing. It can be seen (Fig. 5a) that the stabilities of the virus present in the medium of interferon-treated cultures and of control cultures did not differ.

The inactivation kinetics of the virus associated with the cells (Fig. 5b) or of the virus removed from the cells by mild (0.075 %) trypsin treatment (cell wash: Fig. 5c) was much faster in interferon-treated cultures than in the controls. Thus, cell-associated virus from the controls had a half-life of 72 min, while that from interferon-treated cultures was only
Fig. 5. The stability at 37 °C of MuLV made in SC-1 infected cells in the presence and absence of interferon (150 units/ml for 24 h). (a) Virus in cell fluid; (b) cell-associated virus; (c) virus in cell wash (cells were washed with medium and 0.075% trypsin was added for 5 min. Its action was stopped by addition of 10% FCS and the cells and fluid were separated by centrifugation). The values represent the average of duplicate cultures from two independent experiments. Solid lines represent samples before freezing, and dotted lines represent samples after freezing; ○, virus from control cultures; ●, virus from interferon treated cultures.

48 min at 37 °C (Fig. 5b). In the wash, the difference was smaller—54 min for the controls and 36 min for the interferon-treated cultures (Fig. 5c). However, no difference in virus stability between the control and interferon-treated cultures was found when the samples were frozen before the assay. Nearly identical results were obtained with interferon-treated AKR-2B cells chronically infected with MuLV, or when the chronically infected SC-1 cells were grown in interferon for 2 to 4 weeks (data not shown).

**RNA of virions synthesized in interferon-treated cells**

The specific infectivity of the virus synthesized in the presence of interferon was found to be much lower than that of the virus from the control cells. The lack of infectivity could be due to the lack of virus 70S RNA (as suggested by van Griensven et al. 1971) or virus structural polypeptides in the virions.

We have shown that treatment with interferon for 6 h caused a marked decrease in the infectivity of the released virus, while the number of virions produced was not greatly changed. It was therefore of interest to examine whether the change in infectivity was due to degradation of the virion RNA. Cells were pulse labelled with ³H-uridine (100 μCi/ml) for 1 h and chased with unlabelled medium in the presence or absence of interferon. The radioactive virus which accumulated between the third and sixth hours of chase was collected and the virion RNA was extracted and analysed by gel electrophoresis (Fig. 6).
Effect of interferon on MuLV infection

Fig. 6. Radioactivity profile of virion RNA. SC-1 infected cells were labelled with \(^{3}H\)-uridine (100 \(\mu\)Ci/ml) for 1 h and chased with cold medium in the absence or presence of interferon (150 units/ml) for 6 h. The virus RNA was isolated from the purified virus collected between 3 to 6 h during chase, and analysed by gel electrophoresis as described in Methods. • – •, Control virus; ■ – ■, virus from interferon-treated cultures.

profiles of the two RNA preparations were identical, and in both cases the 70S RNA could be dissociated into the 35S subunit (data not shown). Thus it is unlikely that the rapid degradation of virus RNA is the cause of the loss of virus infectivity in interferon-treated cultures.

DISCUSSION

Interferon treatment of mouse cells chronically infected with MuLV leads to the inhibition of virion synthesis (Billiau et al. 1973, 1974; Friedman & Ramseur, 1974; Swetly & Ostertag, 1974; Pitha et al. 1976; Luftig et al. 1977; Chang et al. 1977). It has been shown that the interferon-induced inhibition of MuLV replication occurs in two steps. The first, which has been detected only in acute MuLV infection and MSV-induced transformation, is relatively inefficient (Pitha et al. 1977; Pitha & Staal, 1978; Aboud et al. 1978; Morris & Clegg, 1978) and affects an early step in virus replication; there is a twofold reduction in the relative amounts of virus RNA, major virus core protein p30 and glycoprotein gp71. The second and major interferon-mediated inhibition of virus production observed in both acute and chronic MuLV infection occurs after the steps of virus transcription and translation and seems to be due to interference with the late stages of virus maturation.

The results presented in this paper indicate that the exact location of this late interferon block is determined both by the host cell and by the type of infection. When measured in the first cycle of virus replication in AKR-2B cells, the inhibition of MuLV maturation occurred before virus assembly. Thus in interferon-treated cells the amount of infectious virus and the number of virus buds and assembled virus particles were reduced proportionately both in the cells and in the culture fluids. However, after infection had been
established, interferon treatment did not prevent virus assembly but led to an accumulation of virions on the cell surface. A high proportion of the virus particles released in the presence of interferon was non-infectious. In both acute and chronic infection in mouse SC-1 cells, interferon led to accumulation of assembled virions on the plasma membranes and to the presence of non-infectious particles in the culture fluids.

Morphologically there was no noticeable difference between the appearance of virus particles from interferon-treated cells and the controls. It was reported recently that interferon treatment of Friend cells yields cell-associated particles which are morphologically different from the virions produced in the control cells (Luftig et al. 1977). However, in this system and unlike the AKR virus system, there are frequent particles which have an electron-lucent core and which are designated as ‘immature C particles’. It is quite probable that these ‘immature C particles’ are the precursors of the aberrant forms seen after interferon treatment of Friend erythroleukaemia cells. It should be emphasized, nonetheless, that in both cell systems, the decrease in infectious virus in interferon-treated cells could not be explained entirely by the inhibition of virus release.

The dependence on the host cell of the interferon inhibition of MuLV replication may explain the wide variation in the results which have been reported. Thus an accumulation of assembled virions on the cell surface of interferon-treated cells was found in several cell lines chronically infected with MuLV (Billiau et al. 1974, 1976a; Chang et al. 1977), and led to a suggestion of interferon block (trapping) of virus release. Unfortunately, these studies did not correlate the number of the cell-associated virions with their infectivity. It has been shown recently that interferon treatment of TB cells infected with MuLV leads to the presence of non-infectious particles in the medium (Wong et al. 1977), which is in accordance with our findings.

We have shown previously that in the chronically infected cells interferon did not affect the transcription of virus RNA (within the limits of detection of the assay) and the relative synthesis of virus p30 protein (Pitha et al. 1976, 1977). The present data show that the release of infectious virus from interferon-treated cells is not inhibited by cycloheximide or actinomycin D, while virus production in the control cells is greatly affected by both inhibitors. It is thus rather unlikely that the interferon-induced inhibition of virus assembly and release is due to the lack of synthesis of a virus protein essential for this process. It was shown recently by Wong et al. (1977), who used a temperature-sensitive mutant (ts3) (Wong & Carter, 1974) which at non-permissive temperatures produces particles accumulating on the cell surface, that interferon (like cycloheximide) was not able to prevent the dissociation of formed particles from the cells at permissive temperatures. However, though this ts mutant has been shown to be a late mutant with respect to the stage at which virus replication is impaired, it has not been characterized biochemically and the defect in virus gene expression has not been established. Thus, this system does not throw light on the site of action of interferon.

Kinetic analysis of the interferon effect revealed that the observed inhibition of the MuLV replication may be not mediated through the antiviral effect of interferon (Taylor, 1965). The decrease in the infectivity of the MuLV preceded the reduction in yield of virus particle production and was observed as soon as 6 h after the addition of interferon; the antiviral state (measured by the inhibition of VSV replication) was detected only after 8 h of interferon treatment. This difference in the time required for the inhibition of MuLV and VSV is not caused by a higher sensitivity of MuLV to interferon, since we have previously shown that MuLV is threefold less sensitive to interferon than VSV (Pitha et al. 1976) when measured in the first replicative cycle.
Effect of interferon on MuLV infection

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