Biochemical Analysis and Electron Microscopic Study on Intracellular Virions in NIH/3T3 Mouse Cells Chronically Infected with Moloney Murine Leukaemia Virus: Effect of Interferon

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SUMMARY

Radioactively labelled virus particles of intracellular origin were isolated from the cytoplasmic fraction of disrupted NIH/3T3 cells chronically infected with Moloney murine leukaemia virus [NIH/3T3 (MLV)]. Interferon (IFN) treatment for 48 h, which arrested more than 90% of virus release, resulted in a remarkable accumulation of these intracellular virions. However, no major effect of such treatment was apparent on their structural properties. Transmission electron microscopic examination revealed that these intracellular virions were located within cytoplasmic vacuoles. IFN treatment resulted in a considerable increase in the number of virus-containing vacuoles, as well as the total number of vacuolar virions. It seems that IFN inhibits the final release of vacuolar virions from the cells, thus leading to their intracellular accumulation.

INTRODUCTION

The replication cycle of retroviruses is generally considered to end up with virus assembly at the inner side of the plasma membrane and its final release from the cell surface by a budding process (Bishop, 1978; Fan, 1978; Montelaro & Bolognesi, 1978). However, in a recent study (Salzberg \textit{et al.}, 1980), we were able to isolate reverse transcriptase-containing particles from the cytoplasmic fraction of disrupted NIH/3T3 cells chronically infected with Moloney murine leukaemia virus [NIH/3T3 (MLV)]. These particles, sedimenting at a density of 1.14 g/ml, were isolated by a procedure that minimized contamination of the cytoplasmic fraction with extracellular or membrane-associated virus particles, and therefore seemed to be of intracellular origin. It is of interest to mention in this context several early electron microscopic studies that have shown the presence of intracellular retrovirus particles in cytoplasmic vacuoles of various chronically infected cells (Dalton, 1966; Dalton \textit{et al.}, 1964; De Harven & Friend, 1966; Ikawa \textit{et al.}, 1973; Sato \textit{et al.}, 1971, 1972; Sugano \textit{et al.}, 1973; Yumato \textit{et al.}, 1966).

In the present study we provide additional biochemical characterization of the cytoplasmic virions isolated from interferon (IFN)-treated and untreated NIH/3T3 (MLV) cells and illustrate their location in cytoplasmic vacuoles by transmission electron microscopy.
Cells and viruses. NIH/3T3 (MLV) cells were used throughout this study. They were grown in Dulbecco’s modified Eagle’s medium containing 10% newborn calf serum. In experiments with IFN serum concentration was reduced to 2.5%.

Interferon. Mouse fibroblast IFN was prepared as described previously (Aboud et al., 1976). The preparation used in this study contained 10^6 international units (IU) per mg protein. Cells were treated for 48 h with 80 IU/ml. This treatment arrested 90 to 95% of the virus release, as determined by measuring reverse transcriptase activity in the culture medium (Aboud et al., 1976).

Radioactive labelling and isolation of intracellular virions. Cells were incubated with 50 μCi/ml of either [3H]uridine (50 Ci/mmol), [35S]methionine (690 Ci/mmol) or [3H]glucosamine (59 Ci/mmol) (all from New England Nuclear) for 48 h, in the presence or absence of IFN. Intracellular virions were isolated from the cytoplasmic fraction of these cells by the procedure of Salzberg et al. (1980) with a slight modification introduced by Aboud et al. (1981) in order to dissociate polyribosomes and hydrolyse labelled RNA outside of virus particles.

Immunoprecipitation and gel electrophoretic analysis of purified virus. Proteins and glycoproteins of purified extracellular or intracellular labelled virions were immunoprecipitated with goat MLV-specific antiserum and analysed by SDS-10% polyacrylamide gel electrophoresis (SDS-PAGE) as described by Aboud et al. (1981). For virus RNA analysis, [3H]uridine-labelled, cytoplasmic virions were lysed with 0.5% SDS, boiled for 2 min, and subjected to 0.75% agarose-1.8% polyacrylamide slab gel electrophoresis. 3H-labelled 28S, 18S and 4S cellular RNA served as markers.

Transmission electron microscopy. Cells were plated at a density of 3 × 10^6 cells per 9 cm tissue culture dish (Nunc), with or without IFN. After 36 h the cells were washed four times with phosphate-buffered saline to remove extracellular virions, and gently scraped off the dishes by a rubber policeman to avoid damage to membrane-associated virus buds. The collected cells were fixed in 1% glutaraldehyde in 0.1 M-phosphate buffer pH 7.4, post-fixed in osmium tetroxide and embedded in Epon 812. Thin sections were cut by an LKB ultratome III and examined by a JEOL 100C electron microscope.

Results

Radioactive labelling of the intracellular virions

In order to characterize the biochemical properties of the intracellular virions their RNA, proteins and glycoproteins were labelled for 48 h with [3H]uridine, [35S]methionine and [3H]glucosamine respectively. The intracellular virions were isolated from the cytoplasmic fractions of the cells and banded in 15 to 60% sucrose gradients. Fig. 1 shows that in all cases the labelled intracellular virions banded at a density of 1.12 to 1.14 g/ml. Similarly to our previous observations with the reverse transcriptase-containing particles (Salzberg et al., 1980), we found that IFN treatment of the cells during their labelling resulted in accumulation of the intracellular virions, regardless of which of these labels was used.

Gel electrophoresis of virus components

Various structural changes have been noticed by several investigators in virions released from IFN-treated cells (Van-Griensvan et al., 1971; Chang & Friedman, 1977; Pitha et al., 1979a, 1980). It was therefore of interest to examine whether IFN had any effect on the structural components of the intracellular virions in our system. Thus, [3H]uridine-labelled
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Fig. 1. Accumulation of intracellular labelled virions in IFN-treated cells. NIH/3T3 cells were plated in the presence of 50 μCi/ml of (a) [3H]uridine, (b) [35S]methionine and (c) [3H]glucosamine with (●) or without (○) 80 IU/ml IFN. After 48 h intracellular labelled virions were isolated from the cytoplasmic fractions of the disrupted cells and analysed on a 15 to 60% sucrose gradient.

Fig. 2. Gel electrophoresis of virus RNA, protein and glycoprotein. The peak fractions of virions banded in the sucrose gradients presented in Fig. 1 (a) were pooled and the virus particles were pelleted and resuspended in small volumes. Aliquots containing 3000 to 5000 ct/min [3H]uridine-labelled virions (●) were lysed by boiling for 2 min in the presence of 0.5% SDS and subjected to 0.75% agarose–1.8% polyacrylamide gel electrophoresis. [3H]uridine-labelled 28S, 18S and 4S cellular RNAs served as markers. The [35S]methionine-labelled (b) and the [3H]glucosamine-labelled (c) virions were subjected to SDS–10% polyacrylamide gel electrophoresis as described elsewhere (Aboud et al., 1981). The gels were cut into 2 mm slices which were extracted with soluene (Packard) and counted in a toluene-based scintillation liquid. ●, Virions from IFN-treated cells; ○, virions from untreated cells.

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Fig. 3. Transmission electron micrographs of uninfected NIH/3T3 cells and of vacuolar virions in untreated and IFN-treated NIH/3T3 (MLV) cells. Cells were fixed and sectioned for transmission electron microscopy. (a) Uninfected NIH/3T3 cells containing virus lacking cytoplasmic vacuoles; bar marker represents 2 μm. (b) Chronically infected NIH/3T3 (MLV) cells with many virus-containing cytoplasmic vacuoles; bar marker represents 2 μm. (c) Higher magnification of a typical virus-containing vacuole from NIH/3T3 (MLV) cells illustrating type C particles; bar marker represents 0.2 μm. (d) Cytoplasmic vacuole crowded with numerous C-type particles observed in IFN-treated NIH/3T3 (MLV) cells; bar marker represents 0.5 μm.

Electron microscopy of the intracellular virions

In order to visualize the existence of the intracellular virions suggested by our biochemical observations, we examined a large number of thin sections of NIH/3T3 (MLV) cells by transmission electron microscopy as described in Methods. For comparison, thin sections of uninfected NIH/3T3 cells were prepared and examined in the same manner. While no virus
Table 1. Electron microscopic quantification of vacuolar virions in IFN-treated and untreated cells*

<table>
<thead>
<tr>
<th>Cells</th>
<th>Virus-containing vacuoles</th>
<th>Total vacuolar virions</th>
<th>Virions per vacuole</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-treated</td>
<td>12,252</td>
<td>42,100</td>
<td>3.4</td>
</tr>
<tr>
<td>Untreated</td>
<td>6477</td>
<td>18,517</td>
<td>2.8</td>
</tr>
</tbody>
</table>

*Quantification was determined by counting virions and virus-containing vacuoles in 100 cellular thin sections of IFN treated or untreated cells.

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...particles were observed in uninfected cells (Fig. 3 a), many cell-associated virions were seen in each section of the infected cells; most of them were located in cytoplasmic vacuoles whereas only a few were attached to the outer cell membrane. Fig. 3 (b) presents a typical example. Fig. 3 (c) illustrates a higher magnification of a representative virus-containing vacuole, showing complete type C virions in various stages of maturation. No virus particles could be detected in the cytoplasm outside the vacuoles.

Accumulation of vacuolar virions in IFN-treated cells

As indicated by our biochemical data, IFN treatment leads to a considerable accumulation of the intracellular virions. It was of interest to confirm these observations using transmission electron microscopy. Thus, vacuolar virions were counted in 100 thin sections of IFN-treated and untreated cells. The results summarized in Table 1 indicate a three- to four-fold increase in the total number of intracellular virions in IFN-treated cells. This increase was mainly due to a higher number of virus-containing vacuoles, whereas the average number of virions per virus-containing vacuole was only slightly higher. However, vacuoles crowded with a very high number of virions, like the one presented in Fig. 3 (d), were occasionally observed in IFN-treated cells but never in untreated cells.

DISCUSSION

We have previously demonstrated the isolation of reverse transcriptase-containing particles from the cytoplasmic fraction of disrupted NIH/3T3 (MLV) cells (Salzberg et al., 1980). These particles, sedimenting at a density typical of MLV, have been isolated by a procedure that minimizes contamination of the cytoplasmic fraction with extracellular or membrane-bound virions, strongly suggesting their intracellular origin. In the present study, we confirmed this finding by isolating labelled cytoplasmic virus particles by a similar procedure, slightly modified in order to dissociate polyribosomes and degrade RNA which was outside the assembled virions (Aboud et al., 1981). Moreover, using transmission electron microscopy, we could illustrate that such intracellular type C particles are located in cytoplasmic vacuoles of these cells. No similar virus particles were found in the cytoplasm outside such vacuoles. Sato et al. (1971, 1972), who have occasionally observed such vacuolar virions in their infected cells, claim that these are extracellular virus particles that have been phagocytosed by the cells. This is rather unlikely to be the case in our system, in which vacuolar virions were not just found occasionally. They appeared in large numbers in every cell. Furthermore, their number was even higher after IFN treatment, which extremely reduced the number of extracellular virions. Moreover, by following the kinetics of radioactive labelling, we have demonstrated elsewhere (Aboud et al., 1981) that labelled intracellular virions appear long before the labelled extracellular virions, further eliminating the possibility that the vacuolar virions are extracellular particles that have been phagocytosed. An alternative possibility is that the vacuolar virions are a result of self phagocytosis by the cells of virus particles which are still bound to their own surface. The
higher number of vacuolar virions found in IFN-treated cells does not contradict this possibility since several studies (Billiau et al., 1976; Chang & Friedman, 1977; Pitha et al., 1979b) have illustrated an accumulation of membrane-bound virions in IFN-treated cells. However, although this possible mechanism cannot be totally excluded, it seems rather unlikely, because careful analysis of many sections of IFN-treated and untreated cells revealed that virions bound to the cell surface are quite rare in our system. It is therefore more likely that, at least in these particular cells, MLV particles are formed intracellularly, apparently by budding into cytoplasmic vacuoles, as appeared in several sections (M. Aboud et al., unpublished results).

The accumulation of the intracellular virions in IFN-treated cells could be demonstrated with various parameters used in this and the previous study (Salzberg et al., 1980). However, no major structural difference between intracellular virions of IFN-treated and untreated cells could be detected. Both contained 35S RNA which has been previously shown to be polyadenylated and to efficiently anneal to MLV-specific cDNA (Salzberg et al., 1980) and both contained typical virus proteins and glycoproteins. Nevertheless, some minor IFN-induced structural defects like those reported by Chang & Friedman (1977), Pitha et al. (1979a, 1980) and Van-Griensvan et al. (1971) cannot be excluded by the procedures we used. We also cannot evaluate, as yet, the infectious capacity of the intracellular virions of IFN-treated or untreated cells, since our procedure of isolating these virions was found to destroy mechanically the infectivity of infectious extracellular virions added to the cells at the beginning of the procedure. A further elaboration of this question is therefore still required.

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REFERENCES


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