Cytochalasin B-induced Activation in the Synthesis of L-cell Virus Particles

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SUMMARY

Some but not all of the mouse L cell strains carry virus particles which have been found to be morphologically, biochemically and antigenically indistinguishable from known RNA tumour viruses. Present electron microscopic study has revealed that cultures of two of the clones derived from NCTN 929 cells when grown in Eagle's medium enriched with foetal calf serum contained a few intracisternal A-type virus-like particles but were continuously negative for C-type virus particles. Exposure of the cultures to the drug cytochalasin B resulted in the appearance of abundant intracytoplasmic and extracellular C-type virus particles. Possible mechanisms of the activation of virus synthesis and release by cytochalasin B are discussed.

The L cells derived from an explant of methylcholanthrene-treated connective tissue of a C3H mouse were found to be highly malignant when injected into C3H mice (Earle, 1943). Clone NCTN 929 derived from a strain of L cells (Sanford, Earle & Likely, 1948) retained its malignant property (Earle, Shelton & Schilling, 1950). The clone NCTN 929, and several clones subsequently derived, were used in a wide variety of virological and biochemical studies. The presence of virus particles morphologically indistinguishable from known RNA tumour viruses has been reported in some but not all of the strains of L cells (Dales & Howatson, 1961; Kindig & Kirsten, 1967; Cromack, 1968). Moreover, the virus particles in L cells not only share certain biochemical characteristics with the known RNA tumour viruses (Kindig, Karp & Kirsten, 1968; Faras & Erikson, 1969; Nichols, Quade & Luftig, 1973) but are antigenically identical with murine leukaemia viruses in complement fixation tests (Hall et al. 1967). The fungal (Helminthosporium dematioides) metabolite, cytochalasin B, has been used in the enucleation of mammalian cells (Carter, 1967; Prescott, Myerson & Wallace, 1971). During our studies on the preparation of enucleated L cells by cytochalasin B treatment (Sethi et al. 1973), electron microscopic examination showed that two of the clones derived from clone NCTN 929, when exposed to cytochalasin B, developed a large number of mature and budding extracellular C-type virus particles in addition to intracytoplasmic C-type particles. In contrast, electron micrographs of the untreated control L cells revealed a few intracisternal A-type particles but no C-type particles. These observations are described below.

The two clones derived from clone NCTN 929 were maintained unless stated otherwise, as monolayers in Eagle's basal medium (BM) containing 100 units/ml penicillin, 100 μg/ml of streptomycin and supplemented with 10% foetal calf serum (BM). Confluent sheets of cells were treated with cytochalasin B at a concentration of 2 or 10 μg/ml. Cytochalasin B (Serva, Heidelberg, West Germany) was dissolved in 1 mg/ml dimethyl sulphoxide (DMSO) and then added to the BM at the required concentration. After incubation of L cell monolayers for 12 h with BM containing 2 μg/ml of cytochalasin B, the medium was removed and fresh BM without cytochalasin B was added. Incubation continued, as before, for 20 h. L cell sheets incubated with 10 μg/ml of cytochalasin B for 12 h were subjected to centrifuging at 37 °C for 1 h at 3000 g. Cells were then rinsed with fresh BM and reincubated in
Fig. 1. Micrograph of a normal L cell showing rare intracisternal A-type particles (arrows).
Fig. 2. Enlarged intracisternal A-type particle.
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Table I. Number of L cell virus particles observed in ultrathin sections of normal and cytochalasin B treated L cells

<table>
<thead>
<tr>
<th>Treatment of L cells</th>
<th>No. of sections of cells examined</th>
<th>A-particles</th>
<th>C-particles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive cells (%)</td>
<td>Particles/cell</td>
</tr>
<tr>
<td>Normal</td>
<td>32</td>
<td>79</td>
<td>7.4</td>
</tr>
<tr>
<td>Cytochalasin B (2 μg/ml)</td>
<td>54</td>
<td>80</td>
<td>6.3</td>
</tr>
<tr>
<td>Cytochalasin B (10 μg/ml)</td>
<td>25</td>
<td>74</td>
<td>5.6</td>
</tr>
</tbody>
</table>

The numbers of particles shown do not account for the particles in culture fluid or those washed away during processing of cells.

fresh BM for 20 h. This procedure produced 90 to 95 % enucleated L cells. Controls included L cells incubated in BM without cytochalasin B and L cells incubated with BM containing only 1 % DMSO.

For electron microscopy, cell sheets were removed with the aid of glass beads and the suspensions centrifuged at 250 g for 10 min. The resulting pellets were then fixed for 1 h at 4 °C in 6:25 % glutaraldehyde in 0.1 M-Sörensen's buffer solution (pH 7.2), post-fixed in 1 % osmium tetroxide in buffer solution, dehydrated in acetone and embedded in Vestopal. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in the Zeiss AM 9 or Siemens Elmiskop I electron microscope.

Electron microscopy revealed that most, if not all, of the untreated control L cells, or cells incubated with DMSO alone, contained few structures which could be classified (Anonymous, 1966) as A-type particles. These particles, measuring 70 to 80 nm in diam. with a 30 to 35 nm diam. electron-lucent zone, were detected in the cisternae of the endoplasmic reticulum (Figs. 1, 2). In sharp contrast to the control preparations, L cells exposed to cytochalasin B showed large numbers of intracytoplasmic and extracellular C-type particles. The criteria for classifying the particles as C-type were size (average size 100 nm), lack of surface spikes and eccentric nucleoids (Figs. 3, 4).

Very frequently, C-type particles budded from the plasma membrane (Fig. 5) and from intracytoplasmic cisternae (Figs. 6, 7). A-type particles were found only rarely in cytochalasin B treated L cells. The 28 C-type particles per L cell exposed to 2 μg/ml of cytochalasin B was similar to the number in cells treated with 10 μg/ml and largely enucleated (Table I). However, the number of virus particles decreased on subculturing the treated cells in BM without cytochalasin B.

The relationship between A-type virus-associated particles and C-type particles is uncertain (Hall, Hartley & Sandford, 1968; Kimura et al. 1972). The intracisternal A-type particles may represent the precursors of C-type particles, whereas in another hypothesis, there may be no relationships between these particles. Furthermore, whereas there are numerous reports on the association of the C-type particles with solid tumours and leukae-mias in animals, A-type particles have shown no biological activity. The appearance of many intracytoplasmic and extracellular C-type particles in cytochalasin B treated L cells, and the apparent rarity of intracisternal A-type particles, suggests that either cytochalasin B fails to stimulate the synthesis of A-type particles or that A-type particles undergo rapid differentiation into C-type particles. The cytochalasin B induced activation in the synthesis of L cell virus particles suggests that the information for virus synthesis is present in every
Fig. 3. L cell enucleated with cytochalasin B. Note (arrows) the abundance of intracytoplasmic and extracellular C-type particles. Large arrow heads locate intracisternal and extracellular budding virus particles.
Fig. 4. Free extracellular C-type particles with central nucleoid (arrows) within cytochalasin B-treated L cell.

Fig. 5. Budding C-type particles. The nucleocapsid (arrow) is a crescent-like structure below the site of budding. Another budding virus particle is detaching from the plasma membrane.

Fig. 6. Single budding intracytoplasmic C-type virus particles (arrow) and doublet intracisternal particle (long arrow head) in cytochalasin B-treated L cell.

Fig. 7. Intracisternal production of many virus particles (arrow).
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L cell. In one earlier study, lymphocyte-mediated degenerative changes in a clone of L cells increased the frequency of observation of associated virus particles (Weiss, 1968). On the other hand, another clone of L cells was converted to a virus producing state only when the cell cultures were grown in the presence of foetal calf serum (Kindig et al. 1968). In addition to enucleation of mammalian cells, cytochalasin B has been reported to prevent the cell cytokinesis which results in multinucleation (Carter, 1967), as also does the pulverization of chromosomes (O'Neill, 1972). Cytochalasin B also exerts on living cells a variety of other reversible effects which involve contraction of microfilaments (Wessels et al. 1971).

It is not known whether any of the above events initiated by cytochalasin B result in the activation of L cell virus particles. We are studying the role of cytochalasin B in activation or stimulation of the expression of virus genomes in normal and virus-transformed cells.

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