Lack of Syncytium Formation by a Type C Virus-producing XC Cell Line in the Mixed Culture Cytopathogenicity Test

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SUMMARY

XC cells, derived from a Rous sarcoma virus-induced Wistar strain rat tumor, form syncytia when cultured in the presence of murine leukemia virus-producing mouse cells. However, one XC cell culture (designated as XC-v cells), found to produce type C virus particles, fails to form syncytia in the presence of murine leukemia virus-producing mouse cells. Coculture of XC-v cells and XC cells negative for type C virus particles leads to a moderate degree of syncytium formation. Infection of XC cells with either the Moloney (M) strain of mouse leukemia virus or type C virus particles released by XC-v cells results in the loss of ability of XC cells to form syncytia in the mixed culture cytopathogenicity test. The syncytium-forming ability of XC cells, therefore, is altered by the presence of a type C virus in these cells.

INTRODUCTION

The XC cell line established from a Wistar rat sarcoma induced by Rous sarcoma virus has many interesting properties that have previously been reviewed (9). Exposure of XC cells to mouse cells infected with MuLV\(^3\) (10) leads to cytopathic effect of cell vacuolization and syncytium formation (10). This so-called MCC test (10) was the basis for the subsequent development of an assay for MuLV (15). Later, it was reported that syncytium formation could also be induced by exposure of XC cells to purified MuLV (8), to cat or dog cells infected with feline leukemia virus (13, 14), or to human cells producing MSV-M (3).

In this communication, 3 interesting observations are reported on an XC cell line obtained from another laboratory. (a) A type C virus (of yet unidentified origin) has been found in the XC cell subline designated XC-v. (b) This virus-producing XC-v cell subline no longer forms syncytia in the presence of purified, concentrated MuLV or MuLV-producing mouse (3T3) cells. (c) The XC-v cells are capable of inducing syncytium formation when cocultivated with cells of an XC line that does not contain a type C virus.

MATERIALS AND METHODS

Tissue Cultures

**XC-v Cells.** The type C virus-producing XC-v cells were obtained from Dr. N. Biswal of the Baylor College of Medicine at Passage 39. These XC cells came originally from Dr. S. A. Aaronson (17) and have not been cloned. At the time of their receipt, a portion of the cells was immediately frozen at \(-70^\circ\) and a portion was subcultured. The XC-v cells were subcultured twice weekly (with trypsin-EDTA as dispersing agent) in Auto Pow Eagle's minimum essential medium (APMEM, purchased from Flow Laboratories, Inc., Rockville, Md.) supplemented with 10% inactivated FCS and neomycin, 100 µg/ml.

**XC Cells.** Another XC cell line was provided by Contract E-73-2001-N01 within the Virus Cancer Program, NIH, USPHS, through the courtesy of L. E. Hoosier, Cell Culture Laboratory, University of California, Berkeley, Calif. Cells of this line were subcultured twice weekly in TMEM containing 10% heat-inactivated PCS; penicillin, 500 units/ml; and streptomycin, 100 µg/ml, pH 7.4.

**MuLV-M-producing Mouse 3T3 Cells.** The MuLV-M-producing Swiss mouse 3T3 cell line (IC-3T3-19) was kindly provided by Dr. P. J. Fischinger of the National Cancer Institute. Cells of this line were subcultured weekly in McCoy's 5A medium supplemented with 10% heat-inactivated FCS and neomycin, 100 µg/ml.

Syncytium Formation Test

MuLV-M-producing IC-3T3-19 cells were seeded in 60-mm plastic Falcon dishes at \(1 \times 10^5\) to \(10^6\) cells/dish in 5 ml McCoy's 5A medium. Twenty-four hr later, media were aspirated and the mouse cells were overlaid with 5 ml medium containing \(5 \times 10^4\) XC or XC-v cells. Forty-eight to 72 hr after coculture, dishes were washed with phosphate-buffered saline, fixed with absolute methanol, and stained with Giemsa stain. The number of multinucleated giant cells with 5 or more nuclei were enumerated with an AO Zoom stereoscopic microscope. The test for syncytium formation by XC and XC-v cells was carried out essentially the same way, except that \(5 \times 10^4\) XC-v cells were used in place of the virus-producing mouse cells.
RESULTS

Failure of XC-v Cells to Produce Plaques. Initially, XC-v cells were used in the MCC test of MuLV as described by Rowe et al. (15). Mouse cells (NIH/3T3, 3T3, or BALB/3T3) were infected with serially diluted MuLV produced by IC-3T3-19 cells or with Soehnlein-Dmochowski murine sarcoma virus (MSV-SD) produced by rat bone tumor cells (4, 5) and, 6 days after infection, were overlaid with 1 x 10⁶ XC-v cells. However, repeated attempts failed to yield plaque formation. This failure to observe plaque formation led to investigation of the possible source of the problem. After a series of experiments, the problem was traced to the XC-v cell line.

Presence of a Type C Virus in XC-v Cells. XC-v cells originally frozen at the time of receipt (Passage 39) were retrieved from the freezer and propagated. Reexamination of the retrieved XC-v cell culture showed the presence of giant cells containing 10 or more nuclei (Fig. 1) in some areas of the monolayer. However, these giant cells disappeared after 2 to 3 passages, and the morphology and growth rate of XC-v cells appeared similar to that of other XC cells.

The presence of multinucleated giant cells within the population of XC-v cells at Passage 41 led to an examination of these cells by electron microscopy for the possible presence of virus. Electron microscope examination demonstrated that at the time of receipt the XC-v cells (Passage 40) were already releasing some type C virus particles (Fig. 2a). The same XC-v cells appeared to produce more virus particles (by electron microscope examination) after some 40 passages (Fig. 2b). In comparison, the XC cell line did not contain type C virus particles by electron microscope examination.

The presence of type C virus particles in XC-v cells was confirmed by rate zonal centrifugation of concentrated fluids from uridine-³H-labeled cultures. High-molecular-weight RNA found in the culture fluids of XC-v cells (Passage 88) had a sedimentation value of 58 S (Chart 1A). In contrast, in the same labeling experiment, no heavy RNA could be found in fluids of XC cells (Chart 1B). However, when an equal number of XC and XC-v cells were cocultivated, a peak of high-molecular-weight (58 S) RNA was observed that was twice the size of that from XC-v cells alone (Chart 1C). This latter observation may indicate that XC cells are susceptible to infection by the virus released from XC-v cells.

Failure of XC-v Cells to Form Syncytia. The 2 XC cell lines (XC-v and XC) were tested for their ability to form syncytia in the presence of MuLV-producing mouse cells. Results summarized in Table 1 show that neither the XC-v cells at Passage 40 nor those at Passage 96 formed syncytia with MuLV-M-producing mouse cells, whereas XC cells did. The number of giant cells formed was dependent upon the number of IC-3T3-19 cells seeded.

XC cells that did not contain any type C virus particles (as determined by electron microscopy and by uridine-³H-labeling studies) responded by vigorous syncytium formation with MuLV-producing IC-3T3-19 cells (Fig. 3a). In contrast, XC-v cells failed to form any multinucleated giant cells (Fig. 3b). Giant cells, originally present at an early passage (Passage 41, Fig. 1) in the XC-v control, could not be found at Passage 88 or later (Fig. 3c). XC cells alone did not contain multinucleated cells (Fig. 3d).

Syncytium Formation between XC and XC-v Cells. Cocultivation of an equal number of XC and XC-v cells resulted in syncytium formation. As shown in Table 2, within 24 hr of cocultivation about 400 giant cells per culture, with 5 or more nuclei per cell, were observed (Fig. 4). The number of giant cells decreased by 48 hr, indicating perhaps more fusion among giant cells. Either XC or XC-v alone did not produce comparable syncytia. The results of further experiments showed that exposure of XC cells to a high concentration of purified MuLV-M (1 to 10 x 10¹¹ virus particles per ml, obtained from Electro-Nucleonics, Inc., Fairfield, N. J., through the courtesy of Dr. J. Gruber, Virus Cancer Program, National Cancer Institute) produced many giant cells. Exposure of XC-v cells to the same MuLV-M did not induce formation of giant cells. The failure of XC-v to respond to concentrated MuLV or to MuLV-producing mouse cells indicates that the presence of a type C virus may have altered the syncytium-forming ability of the XC-v cells.

Loss of Syncytium-forming Ability after Virus Infection of XC Cells. Experiments have been carried out in which XC cells were deliberately infected with either the virus released from XC-v cells (at Passage 83) or with the MuLV-M...
TABLE 1
Lack of syncytium formation by XC-v cells cocultivated with MuLV-M-producing IC-3T3-19 cells

<table>
<thead>
<tr>
<th>No. of IC-3T3-19 cells seeded</th>
<th>XC-v Passage 40</th>
<th>XC-v Passage 96</th>
<th>XC Passage 126</th>
</tr>
</thead>
<tbody>
<tr>
<td>$1 \times 10^4$</td>
<td>1</td>
<td>0</td>
<td>*</td>
</tr>
<tr>
<td>$1 \times 10^5$</td>
<td>0</td>
<td>0</td>
<td>115</td>
</tr>
<tr>
<td>$1 \times 10^6$</td>
<td>0</td>
<td>0</td>
<td>12</td>
</tr>
</tbody>
</table>

* Average of 2 dishes per group; giant cells with 5 or more nuclei per cell.

Too many to count; some giant cells have fused together.

TABLE 2
Giant cell formation following coculture of XC-v and XC cells

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of cells added ($\times 10^5$)</th>
<th>No. of giant cells/culture$^a$ after cocultivation$^b$ at 6 hr</th>
<th>24 hr</th>
<th>48 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>$0.5 \times 10^6$</td>
<td>0</td>
<td>377</td>
<td>303</td>
</tr>
</tbody>
</table>

$^a$ Giant cells with 5 or more nuclei per cell.

$^b$ XC-v (Passage 83) or XC cells, or a mixture of both, were plated in 60-mm Falcon plastic dishes. At different times, 2 cultures from each group were fixed with absolute methanol and stained with Giemsa.

for the following reasons. Both XC and XC-v cell lines share great similarity in their characteristic morphology and rapid growth rate. Furthermore, in subsequent experiments in which virus-negative XC cells were deliberately infected with the undiluted cell-free culture fluid of XC-v cells or with MuLV-M virus preparations, similar changes were again observed. The infected XC cells initially showed a few syncytia which disappeared later and, after 3 passages, the infected XC cells also failed to form syncytia with MuLV-M-producing IC-3T3-19 cells (Table 3). The available data lend strong support to the conclusion that the failure of XC-v cells to form syncytia is connected in some way with the presence of an intracellular virus. The mechanism by which a type C virus alters the property of the XC-v cells remains to be investigated.

Recently, Ebbesen et al. (6) reported that a double-cloned hypotetraploid XC cell line forms plaques with MuLV in the XC test with a decreased efficiency. The hypotetraploid cells also contained budding type C particles of yet unidentified origin. This observation is somewhat similar to the results reported here.

DISCUSSION

In the MCC test, XC cells form syncytia when placed in contact with MuLV-infected mouse cells. The process of syncytium formation is thought to be by fusion rather than by endomitosis (10).

In an attempt to explain some of the reported observations, several possibilities have been considered. Among these, the possibility of contamination of XC-v cells by mouse cells was ruled out by a modified mixed hemadsorption test (11) with rat antismouse sera. The results summarized in Table 4 clearly indicate that both XC-v and XC cells are rat cells and do not contain any mouse cells. The possibility that XC-v is not an XC cell line but rather a type C virus-infected rat cell culture is considered highly unlikely.
The origin and identity of the type C virus particles found in XC-v cells are not known. It may be a laboratory type C virus inadvertently introduced into the XC-v cells. Alternatively, it could be a spontaneously activated virus (2, 12, 16). Experiments are now underway to identify the type C virus in the XC-v cells by molecular hybridization and immunological assays and to analyze further the possible mechanism by which an intracellular type C virus alters the syncytium-forming ability of XC cells in the MCC test.

ACKNOWLEDGMENTS

We thank Catherine Green for her excellent technical assistance.

ADDENDUM

During revision of the manuscript, Hampar et al. (7) reported that human lymphoblastoid cells chronically infected with either of 2 feline type C viruses (FeLV and RD-114) become refractory to induction of syncytia by the virus they carry.

REFERENCES


Fig. 1. Formation of multinucleated giant cells by XC-v cells alone at Passage 41. XC-v cells (1 x 10⁸) were seeded in a T-75 Falcon plastic flask for 48 hr. The cells were fixed with absolute methanol and stained with Giemsa. Photomicrograph, x 400.

Fig. 2. Electron micrographs of thin sections of XC-v cells showing the presence of type C virus particles: a, at Passage 40, x 37,500; b, at Passage 88, x 45,000.

Fig. 3. Test of syncytium formation by XC or XC-v cells in the presence of MuLV-M-producing IC-3T3-19 cells. IC-3T3-19 cells (1 x 10⁸) were seeded in 60-mm Petri dishes and, 24 hr later, were overlaid with: a, 0.5 x 10⁴ XC cells; b, 0.5 x 10⁴ XC-v cells, Passage 96, alone; and d, 0.5 x 10⁴ XC cells. Plates were incubated for 72 hr at 37°C. Giemsa stain. Photomicrographs, x 400.

Fig. 4. Giant cell formation by coculture of XC and XC-v cells. XC (5 x 10⁴) and XC-v (5 x 10⁴) cells were plated in 60-mm Falcon plastic dishes for 48 hr at 37°C, fixed, and stained with Giemsa, as in Fig. 1. Many multinucleated giant cells can be seen throughout the coculture. Photomicrograph, x 170.
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