Cytochemical and Immunofluorescence Study of an Oncogenic Avian Adenovirus (CELO) in Mammalian Cell Cultures

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Received for publication 31 March 1971

Chicken embryo lethal orphan (CELO) virus replication studies in adult hamster kidney and green monkey kidney cell cultures by means of infectivity, cytochemistry, and fluorescent-antibody techniques indicated that replication in hamster kidney cells was limited to the production of neoantigen. Production of virus in green monkey kidney cells was limited but appeared to exceed the production of virus in hamster kidney cells. The data indicated that the replication cycle of CELO virus is abortive in hamster kidney and green monkey kidney cells.

The chicken embryo lethal orphan (CELO) virus, a type I avian adenovirus (12), replicates to a high titer in host epithelial cell cultures, to a lesser degree in whole embryo cultures, and very poorly or not at all in embryo fibroblastic cell cultures (Miller et al., manuscript in preparation). The virus transforms a variety of mammalian cells in culture (1, 10).

The preparation and purification of CELO stock virus (Phelps) has been described elsewhere (12). The method of preparing chicken embryo kidney cell (CEK) cultures and the techniques for performing cytochemical tests as well as fluorescent-antibody (FA) studies have been described (9; Miller et al., manuscript in preparation). Cell suspensions of African green monkey kidney (GMK) cells were prepared as described by Melnick (8). When confluent, the cell sheets were inoculated with cell-associated CELO virus obtained from CEK cell cultures or with virus obtained from infectious allantoamniotic fluid (AAF). Virus was inoculated at a multiplicity of 50 to 100 plaque-forming units (PFU) per cell and allowed to adsorb at 37 C for 2 hr. The cell sheets were then washed with phosphate-buffered saline and overlaid with nutrient medium containing 1.5% agar (7). Replication in these cells was determined by the plaques produced. Titration of CELO virus were performed by inoculating decimal dilutions of these samples into 10- or 11-day-old developing chicken embryos or by plaquing the dilutions in CEK cell cultures.

Cover-slip cultures were stained by hematoxylin and eosin (H&E) for intranuclear inclusions and by direct and indirect FA for viral and neoantigens, respectively. In control infectivity trials, replication of CELO virus was examined in GMK cell cultures and in CEK cell cultures. Viral replication was also examined in mixtures of these two cell types made by combining equal volumes of the two cell suspensions containing $2 \times 10^5$ to $3 \times 10^6$ cells/ml.

Cell suspensions of adult hamster kidney cells were prepared as described by Fong et al. (4, 5). When confluent, Leighton tube cultures were inoculated with CELO virus at a multiplicity of 100 PFU/cell. Cover slips were harvested daily for 8 days postinoculation (PI). These were stained and examined for intranuclear inclusions, viral antigen, and neoantigen.

Limited replication of CELO virus was observed in both types of mammalian cells used. Table 1 shows that, when CELO virus obtained from CEK cells was inoculated into pure cultures of GMK cells, virus was not detected up to 30 hr after infection. At that time the virus had reached a titer greater than $10^6$ PFU/ml in CEK cells. When the virus was inoculated into cultures consisting of mixtures of GMK cells and CEK cells, the titer at 30 hr after infection approached that attained in CEK cells.

GMK cells were inoculated with AAF having a titer of $10^6$ ELDD$_{50}$ (median embryo lethal dose) per ml. Extracellular virus harvested from these cells had a titer of $3.7 \times 10^6$ ELDD$_{50}$/ml at 6 days PI. Embryos inoculated with CELO virus from GMK cells contained CELO virus at a titer of $10^6$ ELDD$_{50}$/ml in the AAF at 48 hr PI. The virus in one portion of this AAF was neutralized by anti-CELO serum. CELO virus in another por-
tion of the infectious AAF inoculated undiluted into four embryos (0.1 ml/embryo) produced 100% mortality within 3 days.

A third portion of the infective AAF was used to inoculate cultures of GMK cells. Seven 3-oz bottles were inoculated with 0.5 ml/bottle, giving a total of $10^4 \cdot 14$ virus particles. Thirty-five plaques resulted from this inoculation.

This observation indicated either that 1 out of every $10^2 \cdot 16$ particles was infectious for GMK cells or that a small fraction of the cells was susceptible to CELO virus infection.

The FA studies indicated that CELO virus was capable of infecting GMK cells. However, the infection seemed to have been primarily abortive, since viral protein was not detected in the cells by direct FA, whereas neoantigen was evident in about 50% of the cells at 48 hr PI (Fig. 1).

Whereas limited replication was seen in GMK cells, CELO virus apparently did not replicate in hamster kidney cells. During the 8-day observation period, no intranuclear inclusions were observed and viral antigen was not produced. Neoantigen production occurred only after 8 days (Fig. 2), and infectivity was not demonstrable by embryo inoculation. The absence of inclusions and infectivity along with the retarded appearance of neoantigen and the inability to demonstrate viral antigen by FA indicated that it may be even more difficult for CELO to replicate in hamster kidney cells than in GMK cells. It would appear, therefore, that CELO has an abortive replicative cycle in these cells also. This type of abortive cycle in non-host cells appears to be one of the characteristics of adenoviruses, since observations of this type have been reported previously (2-6, 10).

**Table 1. Titer of CELO virus propagated in green monkey kidney cells, chick embryo kidney cells, and mixed cultures of these cells**

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Log ELD50</th>
</tr>
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<tbody>
<tr>
<td>Green monkey kidney</td>
<td>0</td>
</tr>
<tr>
<td>Chicken embryo kidney</td>
<td>5.5</td>
</tr>
<tr>
<td>Green monkey kidney plus chick embryo kidney (1:1)</td>
<td>4.6</td>
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</tbody>
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* Equal portions of avian and simian cells were mixed and seeded. All cell sheets were inoculated when confluent with CELO virus at 10 PFU/cell, incubated at 37°C for 30 hr, and assayed for virus in embryos.

* Median embryo lethal dose.

* Green monkey kidney cells seeded at $10^5$ cells/ml.

* Chick embryo kidney cells seeded at $10^5$ cells/ml.

Experiments are continuing to determine whether the plaques observed in GMK cells were due to susceptibility of a portion of the cells to CELO virus infection or to the infectivity of a small fraction of virus particles.

This is contribution no. 1389 of the Rhode Island Agricultural Experiment Station, Kingston, R. I. 02881.

**LITERATURE CITED**

