Type 2 Bovine Adenovirus as an Adventitious Contaminant in Primary Bovine Embryonic Kidney Cell Cultures

SASHI B. MOHANTY AND MARY G. LILIE

Department of Veterinary Science, University of Maryland, College Park, Maryland 20740

Received for publication 31 October 1969

Three lots of primary bovine embryonic kidney cell cultures obtained from commercial sources were found to contain type 2 bovine adenovirus. These cell cultures, apparently derived from healthy fetuses, required long incubation periods before the virus could be detected.

A number of viruses have been found to be common adventitious contaminants in primary tissue cultures derived from human and simian sources. Primary bovine embryonic kidney (BEK) cell cultures are most frequently used for propagation of bovine viruses. Recently, a latent bovine adenovirus designated type 6 has been isolated from primary calf testicular cell cultures (3). This report describes the isolation of bovine adenovirus type 2 from three lots of commercially obtained primary BEK cultures.

The BEK cultures were obtained from Industrial Biological Laboratories, Inc., Rockville, Md. Kidneys were derived from 4- to 16-week-old bovine embryos and trypsinized in flasks in the conventional manner. The growth medium contained Eagle basal medium with Hanks salts, 10% fetal calf serum, and the usual concentration of antibiotics.

While attempting to isolate bovine rhinoviruses in BEK cell cultures, occasional controls were noted to degenerate spontaneously. The BEK cultures were incubated in roller drums at 33 C for isolation of bovine rhinoviruses. The maintenance medium contained Eagle basal medium with Earle salts, 2% normal rabbit serum, and the usual concentrations of antibiotics. On primary isolation, occasional uninoculated control cultures showed focal cytopathic effect (CPE) after 12 to 14 days of incubation. On subsequent passages, however, the isolates showed CPE within 7 days in roller drum cultures at 33 C and within 10 to 12 days in stationary cultures at 37 C. The maximum titer obtained was \(10^{6.5}\) TCID\(_{50}\)/ml even after repeated passages in BEK cultures. The isolates were inhibited in the presence of 5-fluorodeoxyuridine, were acid-stable and chloroform-resistant, and had characteristic adenovirus structure in negatively stained electron microscopic preparations. All isolates also shared group-specific soluble antigen with human adenoviruses (complement fixation titer 1:4 to 1:16). Hyperimmune sera were prepared in rabbits against

### TABLE 1. Identification of type 2 bovine adenovirus isolated from spontaneously degenerating BEK cultures using cross serum neutralization tests

<table>
<thead>
<tr>
<th>Virus</th>
<th>Reciprocal serum neutralization titer(^a)</th>
<th>Serum 1</th>
<th>Serum 2</th>
<th>Serum 3</th>
<th>Serum type 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolate 1</td>
<td></td>
<td>32</td>
<td>16</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Isolate 2</td>
<td></td>
<td>32</td>
<td>32</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Isolate 3</td>
<td></td>
<td>32</td>
<td>32</td>
<td>32</td>
<td>16</td>
</tr>
<tr>
<td>Bovine adenovirus type 2(^b)</td>
<td></td>
<td>32</td>
<td>32</td>
<td>16</td>
<td>32</td>
</tr>
</tbody>
</table>

\(^a\) Highest serum dilution that completely neutralized 100 TCID\(_{50}\) of virus.

\(^b\) Strain 19, originally isolated by Klein et al. (2), was obtained from American Type Culture Collection.

these isolates and cross serum neutralization tests were performed along with known bovine adenovirus type 2 immune serum. Type 2 bovine adenovirus obtained from the American Type Culture Collection was used to prepare immune serum in rabbits. The virus grew very poorly in BEK cultures and had a maximum titer of \(10^{8.5}\) TCID\(_{50}\)/ml even after repeated passages. Of the three rabbits immunized, only one had a serum neutralization titer of 1:32. The serum neutraliza-

---

1 Approved as Scientific Article no. A1559. Contribution no. 4268 of the Maryland Agricultural Experiment Station.
tion test results (Table 1) indicate that the isolates were type 2 bovine adenovirus. The isolates were not neutralized by types 1 and 3 bovine adenovirus antisera.

Type 2 bovine adenovirus was first isolated by Klein et al (2). The virus produces clinical respiratory disease syndrome in colostrum-deprived calves (1). Whether the virus produces in utero infection is not known. Antibody to type 2 bovine adenovirus is widespread in cattle population (1). The detection of type 2 bovine adenovirus in this study was probably due to natural endogenous contamination of BEK cultures. Since two types of bovine adenoviruses have now been shown to be endogenous contaminants in primary tissue culture of bovine origin, other types might well be present as contaminants. The adventitious contamination of BEK cultures with type 2 bovine adenovirus creates a particular problem for their use in virological studies.

LITERATURE CITED