Use of Chicken Cell Line LSCC-H32 for Titration of Animal Viruses and Exogenous Chicken Interferon

SUSANNE ROTH and OSKAR R. KAADEN*
Institute for Virology, Hannover Veterinary School, D-3000 Hannover, Federal Republic of Germany

Received 6 August 1984/Accepted 12 November 1984

The chicken embryo cell line LSCC-H32 was tested for the propagation and titration of several animal viruses of the families Toga-, Reo-, Rhabdo-, Herpeto-, Orthomyxo-, Paramyxo-, and Poxviridae and compared with secondary chicken embryo cells. The LSCC-H32 cells were demonstrated to be as susceptible for most of the tested viruses as were secondary chicken embryo cells. Both produced comparably sized virus plaques. The titers of Sindbis and Semliki Forest viruses in LSCC-H32 cells were 5- to 40-fold higher than in secondary chicken embryo cells or BHK-21 cells, respectively. Furthermore, exogenous chicken standard interferon was titrated in the LSCC-H32 cells, and a 50% plaque titer reduction of the challenging vesicular stomatitis virus was achieved by 0.12 IU of a standard chicken interferon preparation. Endogenous chicken interferon could not be induced by treatment of the cells with polyinosinic acid-polycytidylic acid. Due to its high plating efficiency and metabolic activities, the LSCC-H32 cell line provides a useful cell system for the titration and large-scale production of the tested animal viruses and for the titration of exogenous chicken interferon.

Established cell lines provide a useful tool for the diagnosis of viral diseases and in vitro studies of viral replication. Furthermore, cell lines are potentially immortalized and therefore especially useful for the large-scale production of viruses. The growth and test conditions for cultured cells can thus be standardized to ensure reproducible results.

Most avian cell lines are of lymphoblastoid origin, obtained from tumors of lymphoid leukemia (6, 9) or Marek’s disease (1, 3, 8, 9). Attempts to establish lines of chicken embryo cells (CEC), which are the most frequently used avian cells, have generally been unsuccessful (5) or the cell lines have been difficult to cultivate (10).

This report describes the usefulness of the recently established CEC line LSCC-H32 (7) for the propagation and titration of different viruses and exogenous interferon by plaque tests. The cell line was demonstrated to be comparably susceptible to secondary CEC for titration of most animal viruses and for detection of exogenous chicken fibroblast interferon.

MATERIALS AND METHODS

Cells. The cell line LSCC-H32 was established from CEC, spontaneously transformed at 32°C (7). Briefly, the line consists of fibroblastoid and polygonal cells and has a subtel-plastid karyotype of 2N = 130 to 140. The cells show an increased plating efficiency of 53 versus 7% for secondary CEC. The cells of line LSCC-H32 also have increased metabolic activities and release avian leukemia viruses of subgroups A and B.

Secondary CEC used as control cells were prepared from 10-day-old embryos according to the published method (2). The fertilized eggs were obtained from Valo-SPF breedings (Lohmann, Cuxhaven, Federal Republic of Germany [FRG]) and were certified to be free of avian leukemia viruses and mycoplasmas. The cells were grown in growth medium consisting of equal parts Hanks balanced salt solution and tissue culture medium 199 (Serva, Heidelberg, FRG) supplemented with 0.5% lactalbumin hydrolysate (Difco Laboratories, Detroit, Mich.), 2% heat-inactivated calf serum (56°C, 30 min), 0.04 IU of insulin (Hoechst A.G., Frankfurt, FRG) per ml, and antibiotics. In some experiments BHK-21 clone 13 cells were grown in Eagle minimal essential medium supplement with 5% neonatal calf serum.

Viruses. Gumboro disease virus (Vemie A.G., Kempen, FRG), Newcastle disease virus strain Rostock, and fowl plague virus strain Montana were kindly provided by Erich Kaleta, Hannover, FRG; Semliki Forest virus (SFV) was from K. Simmons, Heidelberg, FRG; and Sindbis virus (SINV) was from M. Horzinek, Utrecht, The Netherlands. Pseudorabies virus strain phylaxia was from H.-R. Frey, Hannover, FRG; vesicular stomatitis virus strain Indiana was from B. Dietzschold, Tubingen, FRG; and vaccinia virus strain Elstree was from J. Marquardt, Hannover, FRG. Marek’s disease virus (MDV), strains GA and HPRS-16, attenuated, was provided by V. von Buelow, Tuebingen, FRG, as CEC-adapted virus. Gumboro disease virus, Newcastle disease virus, fowl plague virus, MDV, SFV, and SINV were used as seed virus pools grown in secondary CEC. Pseudorabies and vesicular stomatitis viruses were previously propagated in BHK-21 cells. The source of vaccinia virus was virus-infected chorioallantoic membranes.

Plaque test. The different viruses used were diluted in SPA solution (0.022 M sucrose, 0.0038 M K2HPO4, 0.0072 M KH2PO4, 10 g of bovine serum albumin per liter) from 10−3 to 10−11. LSCC-H32 cells and secondary CEC were seeded in 35-mm petri dishes. After reaching confluence the supernatant was discarded and 50 μl of virus inoculum was applied. Series of three petri dishes each of both cell types were infected with different virus dilutions. Three mock-infected dishes served as controls. The inoculated dishes were incubated for 30 min at room temperature followed by 30 min at 37°C in a 3.5% CO2 atmosphere. They were then covered with 2 ml of an agarose overlay consisting of 0.75% agarose in tissue culture medium 199, 2% calf serum, 2% tryptose phosphate broth, 2% beef embryo extract (GIBCO, Karlsruhe, FRG), and antibiotics. The cell cultures were refed every 2 days with a 1-ml agarose overlay of the same

* Corresponding author.
composition. After 3 to 7 days they were fixed with 2 ml of 5\% formaldehyde for 2 h at room temperature. The agarose layer was then removed, and the fixed cells were rinsed with distilled water and stained with Giemsa solution for 20 min at room temperature. The plaques were counted and the number of PFU was calculated.

Titration of interferon. A reference standard interferon (67/18) with 80 IU per container was provided from the National Institute for Biological Standards and Control, London, U.K. Titration of exogenous chicken interferon was performed by a plaque reduction test, using 100 PFU of vesicular stomatitis virus, strain Indiana, as the challenging virus. The interferon samples, which had previously been heated at 56\^\circ C for 30 min, were serially diluted in Eagle minimal essential medium containing 5\% fetal calf serum. The plaque reduction of the interferon-treated cells compared with untreated control cells was determined, and the interferon titers were expressed in international units per milliliter.

Induction of chicken fibroblast interferon. The CEC were exposed for 1 h at 37\^\circ C to polyinosinic acid-polycytidylic acid (Sigma Chemie, Munich, FRG) dissolved at a concentration of 50 \mu g/ml in minimal essential medium containing 2\% fetal calf serum at 37\^\circ C (4). The cultures were then washed three times with Eagle minimal essential medium to remove the inducer. They were then replenished with 2 ml of growth medium. Culture fluids were harvested after 24 h of incubation at 37\^\circ C. The samples were stored at -20\^\circ C after low-speed centrifugation and tested for interferon activity as described above.

RESULTS

Plaque test. All virus strains were first propagated in roller bottles of LSCC-H32 cells or secondary CEC, and the infectious supernatants were then titrated by using the homologous cell system. The results of the titration of different viruses by plaque tests are given in Table 1. The plaque titers are the mean values of three assays. The data determined for the diameter and titer of plaques demonstrated no significant differences in susceptibility between LSCC-H32 and secondary CEC for most of the tested viruses, with the exceptions of SFV and SINV. The titers of both alphaviruses in the LSCC-H32 cell system were between 5- and 40-fold higher than in secondary CEC or BHK-21 cells (SINV, 10^6.1; SFV, 10^0.1 PFU/ml). The LSCC-H32 cells, however, were not permissive for either the oncogenic GA or the attenuated HPRS-16 strain of MDV.

Titration and induction of interferon. The titer of the standard chicken fibroblast interferon batch 16/80 was comparably high in LSCC-H32 cells and secondary CEC. A 50\% plaque titer reduction was obtained with 0.012 IU of chicken interferon in the LSCC-H32 cells. The corresponding value for secondary CEC was 0.010 IU. Endogenous chicken interferon could not be induced by treatment of the LSCC-H32 cells with polyinosinic acid-polycytidylic acid.

DISCUSSION

In comparative plaque assays with nine virus strains of seven different virus families, LSCC-H32 cells and secondary CEC were demonstrated to be comparably susceptible for most virus strains (Table 1). The LSCC-H32 cells can be grown on roller bottles for large-scale production of viruses and have a population doubling time during the logarithmic growth phase of 17.5 h if seeded at a density of 10^6 cells per ml. The permanent chicken fibroblasts were extremely useful for the propagation of SFV and SINV, and the virus yields were 5- to 40-fold higher than in secondary CEC. Use of the LSCC-H32 line ensures reproducible results and is cheaper and less time-consuming than using secondary CEC. It can be anticipated that the cell line will be able to replace secondary CEC for most laboratory purposes.

There is no explanation as to why LSCC-H32 cells did not support the growth of attenuated or oncogenic strains of MDV. A direct viral interference can be excluded as the LSCC-H32 cells were proven to be free of contaminating MDV (7). LSCC-H32 cells were shown to be constitutively infected with exogenous avian lymphoid leukosis viruses of subgroups A and B (7). The avian lymphoid leukosis virus released by the LSCC-H32 was shown to be oncogenic and induced lymphoid tumors in chickens 11 to 15 weeks post-infection. The expression of avian lymphoid leukemia virus by the chicken cell line thus limits its use for the production of vaccine virus for inactivated vaccines only.

In other experiments, the LSCC-H32 cells were used to study the metabolism and polymorphism of mitochondrial DNA (Morais, personal communication). Their sensitivity to the antiviral effect of chicken but not mouse fibroblast interferon was confirmed (Marcus, personal communication). In summary, chicken fibroblasts of line LSCC-H32 were shown to support the growth of several ortho-, para-, rhabdo-, herpes-, and togaviruses and can be used for large-scale production in roller bottles. They were also suitable for the titration of exogenous chicken fibroblast interferon. The LSCC-H32 line thus provides an excellent cell system for the titration and general laboratory use, as well as large-scale production, of the tested viruses.

ACKNOWLEDGMENTS

We thank Saimé Tan and Regine Neth for skillful technical assistance.

LITERATURE CITED

4. Colby, C., and M. J. Chamberlin. 1969. The specificity of

TABLE 1. Titers and diameters of plaques of different viruses generated in cell line LSCC-H32 and in secondary CEC

<table>
<thead>
<tr>
<th>Virus*</th>
<th>LSCC-H32 cells</th>
<th>Secondary CEC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Titer (PFU/ml)</td>
<td>Diam (mm)</td>
</tr>
<tr>
<td>FPV</td>
<td>8.2</td>
<td>5</td>
</tr>
<tr>
<td>Vaccinia virus</td>
<td>8.13</td>
<td>ND^d</td>
</tr>
<tr>
<td>NDV</td>
<td>8.5</td>
<td>1</td>
</tr>
<tr>
<td>Gumboro disease virus</td>
<td>5.66</td>
<td>2-4</td>
</tr>
<tr>
<td>VSV</td>
<td>7.24</td>
<td>1-2</td>
</tr>
<tr>
<td>PRV</td>
<td>6.96</td>
<td>1-3</td>
</tr>
<tr>
<td>SINV</td>
<td>9.8</td>
<td>1-3</td>
</tr>
<tr>
<td>SFV</td>
<td>10.5</td>
<td>2-5</td>
</tr>
<tr>
<td>MDV</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* FPV, Fowl plague virus; NDV, Newcastle disease virus; VSV, vesicular stomatitis virus; PRV, pseudorabies virus.
* Titters are expressed as logarithmic values.
* Mean plaque diameter.
* ND, Not determined.