CHARACTERISTICS OF THE PORCINE KIDNEY CELL LINE IB-RS-2 CLONE D10 (IB-RS-2 D10) WHICH IS FREE OF HOG CHOLERA VIRUS

J. A. HOUSE, C. HOUSE, AND M. E. LLEWELLYN

United States Department of Agriculture, Animal and Plant Health Inspection Services, National Veterinary Service Laboratories, Foreign Animal Disease Diagnostic Laboratory, P. O. Box 848, Greenport, New York 11944

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SUMMARY

A Brazilian stock of clone C17 of the IB-RS-2 porcine kidney cell line which was contaminated with hog cholera virus (HCV) was cloned. One clone designated IB-RS-2 D10 was determined to be free of HCV, 20 other viruses, and Mycoplasma. IB-RS-2 D10 cells possessed the same viral susceptibility pattern as the contaminated parent cells to the viruses of foot-and-mouth disease, swine vesicular disease, vesicular exanthema of swine, transmissible gastroenteritis, and several other viruses. The IB-RS-2 D10 cells had a median chromosome count of 34, were morphologically epithelioid cells, and were resistant to HCV infection. Freedom from HCV affords advantages for vaccine production and avoids laboratory contamination.

Key words: cell culture; hog cholera; IB-RS-2; porcine kidney; foot-and-mouth disease.

INTRODUCTION

The IB-RS-2 cell line was developed from the kidney cells of a normal, 3-m.-old female pig by Dr. Maria de Castro at the Instituto Biologico in Rio de Janeiro, Brazil (5). The name, IB-RS-2, is derived from “Instituto Biologico” (IB), “rim suino” (RS), and pig kidney number 2.

The cell line was cloned at the 154th and 188th passages (6). Clone C17 and a few others maintained a high susceptibility to foot-and-mouth disease virus (FMDV) (6). Clone C17 is used for FMDV isolation at the Pan American Foot-and-Mouth Disease Center in Rio de Janeiro, Brazil. The IB-RS-2 cell line is used for FMDV isolation and serology at the Laboratorio de Investigaciones Medicas Veterinarias (LIMV) in Bogota, Colombia (personal communication), for vaccine production (3,13) and to assay bovine interferon (1). At the Foreign Animal Disease Diagnostic Laboratory (FADDL) in Plum Island, NY, IB-RS-2 cells have been used for virus isolation and for serologic studies of FMD and swine vesicular disease (SVD) viruses.

Extensive chromosome analysis of IB-RS-2 cell clones indicated a relationship between susceptibility to FMDV and certain chromosome patterns (6). Spontaneous degeneration of certain clones of IB-RS-2 was related to the presence of a togavirus contaminant [hog cholera virus (HCV)] by de Castro (7). Ribeiro (14) reported on the successful immunization of swine to HCV with IB-RS-2 cells. Laude isolated a cytopathogenic strain of HCV from IB-RS-2 cells (10) and Laude and Golfi (11) used it for HCV serologic studies. Persistent infection of IB-RS-2 cells with HCV prevented the licensing of a pseudorabies vaccine in Denmark (9). In addition, the persistent shedding of HCV represents a source of laboratory contamination.

This paper describes cloning of the IB-RS-2 cell line to obtain a clone free of HCV but which maintains the useful characteristics of the parent cells.

MATERIALS AND METHODS

IB-RS-2 cell line. Clone 17 of the IB-RS-2 cell line was received in the 201st total cell passage (including cloning) from the Pan American Foot-and-Mouth Disease Center in Rio de Janeiro, Brazil, in September 1981. The cell line was propagated in Eagle's minimum essential medium (EMEM) supplemented with 5 to 10% fetal bovine serum (FBS). It was passaged at weekly intervals by detaching cells with a trypsin-versene solution followed by standard culture procedures. Selected passages were cryopreserved in EMEM supplemented with 15% FBS and 7.5% dimethyl sulfoxide (DMSO).

Detection of HCV and Bovine Viral Diarrhea Virus (BVDV)

Direct examination. Cell cultures of IB-RS-2, and of normal and HCV-infected PK-15 cells (National Veterinary Services Laboratories, Ames, IA) were propagated on chamber slides (Lab Tek slides, Miles Scientific, Naperville, IN), fixed with cold (~20°C) acetone, and stained with fluorescein isothiocyanate (FITC) conjugat-
ed HC antiseraum (National Veterinary Services Laboratories). The slides were examined under X160 and X400 magnification with a mercury lamp ultraviolet light microscope (Leitz Co., Inc., Rockaway, NJ).

Examination after passing in PK-15 cell cultures. Confluent cell cultures of IB-RS-2 were frozen and thawed and their fluids clarified by low speed centrifugation (900 g). Supernatant fluid was used to inoculate chamber slides of PK-15 cells which were 80-90% confluent. After 24 h of incubation, the cells were fixed with cold acetone and stained with anti-HCV serum or anti-BVDV serum conjugated with FITC (National Veterinary Services Laboratories). The slides were examined under X160 and X400 magnification as above.

Animal inoculation. Nine- to fourteen wk-old pigs were held in high containment (P3 or greater) animal rooms at the FADDL. A cell suspension of the IB-RS-2 parent cells for inoculation was prepared by freezing and thawing confluent cell cultures growing in a 75-cm² flask (Costar Division of Data Packaging Corp. Cambridge, MA), then clarifying the fluid by centrifugation at 900 g for 10 min. Two pigs were inoculated with 2 ml of the supernatant i.m. and were observed daily for a febrile response. Two control pigs were housed with the inoculated pigs. Twenty-eight days postinoculation (DPID) all four animals were challenged i.m. and were necropsied when moribund or at 10 DPC. Sera collected from 0 and 28 DPI from all four animals, and from the cell culture inoculated swine 8 d postchallenge (DPC). Animals were necropsied when moribund or at 10 DPC. Sera collected from the pigs were tested by the virus neutralization test (2) to detect antibodies to HCV.

Cloning. The parent IB-RS-2 cell line was propagated using EMEM supplemented with 2% bovine serum containing antibodies to BVDV (neutralization index = 4.5) and with 10% procine serum containing antibodies to HCV. After 6 d, the cells were dispersed and 10-fold dilutions made to give an estimated 200, 20, and 2 cells/well in 96-well microtiter plates (Costar). Eleven wells from the plate seeded with an estimated two cells/well showed growth of a single clone. Four of these 11 clones, designated B6, C1, D10, and F12, showed acceptable morphology and growth characteristics and were cryopreserved for further studies.

Karyology and morphology. When the cell monolayers reached 70% confluence, 0.1 ml Vinblastine (Eli Lilly, Indianapolis, IN) was added for each 75 cm² of growth surface. After 3.5 h at 37°C, the cells were harvested, exposed briefly to hypotonic saline to enlarge the cells, and then fixed in glacial acetic acid and methanol (1:3 vol/vol). Slides were prepared, stained with Giemsa stain, and examined for mitotic figures. Fifty mitotic figures were counted per preparation, and the median number of chromosomes determined.

Morphology of the IB-RS-2 D10 cells was documented by phase microscopy of living cells using a Diavert inverted microscope (Leitz Co., Inc., Rockaway, NJ). All photographs were taken with an Ortho-Variomat camera (Leitz Co.).

Growth of HCV and BVDV in parent and IB-RS-2 D10 cells. Stocks of HC and BVD viruses propagated in the PK-15 cell line were diluted to contain approximately 2000, 400, and 80 TCID₅₀ and inoculated into chamber slides of PK-15, parent IB-RS-2, or IB-RS-2 D10 cells. After 18-24 h of incubation at 37°C in 5% CO₂, and 80-90% humidity, cultures were fixed with cold acetone and stained with anti-BVDV and anti-HCV FITC conjugates. Slides were examined by ultraviolet light microscopy. In addition, 25-cm² tissue culture flask of IB-RS-2 D10 cells were inoculated with 10⁵ TCID₅₀ of HCV. The inoculated cells were passaged at 48-72-h intervals twice, in flasks and then chamber slides that were fixed with cold acetone 18 to 24 h after seeding then stained with anti-HCV FITC conjugate.

Detection of Mycoplasma with bissbenzimidazole stain. IB-RS-2 D10 cells were passed 3 times in antibiotic-free EMEM with 5% FBS, and the supernatant was used to inoculate antibiotic-free Vero cells. Cultures on chamber slides were stained with bissbenzimidazole (Hoechst 33258, Hoechst Pharmaceuticals, Somerville, NJ) according to McGrartty et al. (12). Vero cells infected with Acholeplasma laidlawii served as the positive control. Slides were examined by ultraviolet microscopy.

Safety test on the IB-RS-2 D10 cell line. The IB-RS-2 D10 cell line and its parent have been passed in laboratories where numerous animal disease agents, many exotic to the United States, were propagated. A safety test used at the FADDL involves inoculation of susceptible animal hosts by various routes followed by clinical observation and serologic tests on serum samples taken at 0 and 21 DPI. Two cattle were inoculated intradermally (IDL) to test for FMD and vesicular stomatitis (VS) and i.v. to test for rinderpest (RP), lumpy skin disease (LSD), and Akabane (AKA) viruses. Two swine were inoculated intradermally (ID) to test for FMD, VS, vesicular exanthema of swine (VES), San Miguel sea lion viruses (SMSV), and swine vesicular disease (SVD) virus, and i.v. or i.m. to test for HC and African swine fever (ASF). Two sheep were inoculated i.v. to test for sheep pox (SP), goat pox (GP), peste des petits ruminants (PPR), bluetongue (BT), and epizootic hemorrhagic disease (EHD). The protocol requires the inoculation of at least 10⁴ cells/test system (e.g., one test system is the i.v. inoculation of cattle). Zero and 21 DPI sera were tested for antibody to AKA, HC, LSD, SP, GP, PPR, RP, and SVD by the virus neutralization test; for antibody to ASF, VES, and SMSV by the indirect fluorescent antibody test (IFA) and for antibody to BT, EHD, and FMD by the agar gel immunodiffusion (AGID) test. For detection of avian disease agents, one dozen embryonating chicken eggs, 8-11 d old, were inoculated via the allantoic route with 0.3 ml/egg. The eggs were candled daily, any deaths noted, and materials collected for further diagnostic procedures for Newcastle disease (ND) and avian influenza (AI) viruses if necessary.

Detection of selected extraneous agents. Direct fluorescent antibody conjugates were used to stain fixed monolayers of IB-RS-2 D10 and positive control cells infected with HC, BVD, swine adenovirus (SAV), porcine parvoviruses (PPV), rabies virus (RAB) (positive control