Establishment of Carrier-State Infection of a Feline Renal Cell Line with Feline Syncytial Virus

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ABSTRACT. Although feline syncytial virus (FSV) is normally highly cytopathogenic in Crandell feline kidney cells, a non-cytopathic persistent infection was established in the cells after cocultivation of the initially infected cells with uninfected cells 4 times. More than 90% of the persistently infected cells were positive for FSV antigen, and electron microscopy showed that the culture produced morphologically normal FSV. Virus from the carrier culture was infectious, however, the titer of the virus from the culture was lower than that from the cytoidally infected CRFK cells. — Key words: carrier culture, CRFK cell, feline syncytial virus, spumavirus.

Feline syncytial virus (FSV) is a member of the spumaviruses, a genus of the Retroviridae [2]. It has been isolated from normal and neoplastic tissues of cats from Europe [10–12], the U.S.A. [7, 9, 20], Japan [15], Australia [4–6], and other countries. Diseases tentatively associated with FSV infection include arthritis in old male cats [18] and urolithiasis [22]. However, it is difficult to make specific association with disease because of the widespread asymptomatic infection in the cat population. Besides FSV, spumaviruses have been isolated from many mammalian species including hamsters, cows, sea lions, a variety of nonhuman primates, and man [2, 14].

The infection of cats with FSV is thought to persist for life, and the virus can be isolated from all cats which are positive for anti-FSV antibody [7, 22], suggesting that cells infected with FSV persist in the infected cat and continue to stimulate the immune system to produce antibodies by expressing FSV antigen.

Spumaviruses have a broad host cell range and are highly cytopathogenic in vitro [9, 14, 17]. However, some strains of simian foamy virus (SFV) have been shown to establish a carrier state in certain cell lines infected in vitro [1, 8, 16, 19, 21]. In this study, we established a carrier culture in FSV-infected Crandell feline kidney (CRFK) cells, after cocultivation of initially infected CRFK cells with uninfected CRFK cells several times.

MATERIALS AND METHODS

Virus and cells: CRFK cells [3] were maintained in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal calf serum in a humidified atmosphere of 5% CO₂ in air. The Coleman strain [7] of FSV used in this study was kindly supplied by Dr. J. M. Gaskin (The University of Florida, Gainesville, Fl., U.S.A.).

Indirect immunofluorescence assay: Cells were grown on cover slips and fixed in cold acetone for 30 min, then incubated at 37°C for 30 min with serum from an FSV-infected cat and an uninfected specific pathogen-free cat. After incubation, the cover slips were washed twice with phosphate-buffered saline (PBS), and rabbit anti-cat immunoglobulin G conjugated with fluorescein isothiocyanate was applied. After incubation for 30 min at 37°C, the cover slips were rinsed twice with PBS, mounted in buffered glycerol and examined by fluorescence microscopy.

Electron microscopy: The cells were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4), post-fixed in 1% osmium tetroxide in the same buffer, dehydrated, and embedded in epoxy resin. Sections were stained with uranyl acetate and lead citrate, and observed with a JEOL model 1200 EX electron microscope.

Virus titration: To obtain intracellular virus, the cells were frozen and thawed twice, sonicated for 10 seconds, and centrifuged at 1,600 g for 10 min to remove the cell debris. Ten-fold serial dilutions of the lysate were distributed into 4 wells of a 24-well plate at 100 μl/well and suspension of 1x10⁵ CRFK cells was added to each well. After incubation for 3 days, the cells were passaged and cultured for 4 additional days. Infection by FSV was judged by CPE observed by microscopy. The virus titers were expressed as 50% tissue culture infective dose (TCID₅₀).

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RESULTS

Establishment of carrier culture of FSV in CRFK cells: Non-confluent monolayers of CRFK cells were inoculated with the Coleman strain of FSV at an approximate multiplicity of infection (MOI) of 0.1 TCID₅₀. After the second passage (4 fold dilution) of the infected CRFK cells, almost all the cells became detached from the culture bottle following severe CPE. Surviving cells started to grow very slowly to form colonies following cocultivation with sufficient numbers of uninfected CRFK cells 4 times at intervals of 7 to 10 days. After the cells grew to confluence, the infected CRFK cells were able to be passaged at 2 to 4 fold dilution weekly. As the passage-level proceeded, the cells grew faster, and at the tenth passage, the characteristics of the culture were defined.

Morphology of the carrier culture: The carrier culture, cytocidally infected CRFK cells, and uninfected CRFK cells were stained by May-Grünwald-Giemsa stain. The

Fig. 1. Morphologies of cytocidal infection of CRFK cells (A), the carrier culture (B), and uninfected CRFK cells (C). The cells were stained by May-Grünwald-Giemsa. (× 200).

Fig. 2. Cells infected with FSV were examined by indirect immunofluorescence assay. Those shown are cytocidally infected CRFK cells (A, B), the carrier culture (C, D), and uninfected CRFK cells (E, F). The cells were treated with serum from a FSV-infected cat (A, C, E) and from an uninfected SPF cat (B, D, F). (× 200).
results showed that cytoidal infection is characterized by formation of syncytia in the CRFK cells (Fig. 1A). The cell morphology of the carrier culture (Fig. 1B) was similar to the uninfected CRFK cells (Fig. 1C), however, the carrier culture sometimes contained small syncytia.

**FSV antigens in the cultures:** The carrier culture, cytoidally infected CRFK cells, and uninfected CRFK cells were examined for the presence of FSV antigens by indirect immunofluorescence assay. As shown in Fig. 2, more than 90% of the cells were positive for FSV antigen in both chronically and cytoidally infected cultures. Cells of both cultures showed strong cytoplasmic fluorescence and undetectable nuclear fluorescence. The fluorescence intensity of the carrier culture was slightly weaker than that of the cytoidally infected CRFK cells.

**Electron microscopic analysis:** The morphology of both
FSV and the cells in the cultures of chronic and cytoidal infection were examined by electron microscope. In cytoidically infected CRFK cells, most mitochondria were swollen with a markedly less dense matrix and fewer cristae when compared with the controls (Fig. 3A). In the carrier culture, some cells contained numerous vacuoles (Fig. 3C) and many cells produced FSV. The morphology of the virus particles in the culture was identical to that of the viruses in cytoidically infected CRFK cells (Fig. 3B, D). The virus concentration of the carrier culture was lower than that of the cytoidically infected cells. In both chronic and cytoidal infections, virus was seen as particles budding at the plasma membrane or at the endoplasmic reticulum.

Virus production from the cultures: Extra- and intracellular virus from the carrier culture and cytoidically infected CRFK cells was titrated on CRFK cells. The titers of the intracellular virus were higher than those of the extracellular virus in both chronically and cytoidically infected cultures. The total virus titer of the culture was lower than that of the cytoidically infected CRFK cells. In the carrier culture, the titer of the intracellular virus was $10^{15.25}$ TCID$_{50}$/ml and that of the extracellular virus was $10^{1.25}$ TCID$_{50}$/ml. In the cytoidically infected CRFK cells in which CPE was observed in 60 to 80% of cells, the maximum titer of the intracellular virus was $10^{8.25}$ TCID$_{50}$/ml and that of the extracellular virus was $10^{4.75}$ TCID$_{50}$/ml. When the viruses from the chronically and cytoidically infected cultures were inoculated to CRFK cells at the same MOI of 0.01, we could not find any differences in the onset day and the severity of CPE.

DISCUSSION

In the present study, we succeeded in establishing a carrier culture of FSV in CRFK cells, although FSV is highly cytopathogenic in dividing susceptible cells in vitro [6, 8, 15, 22]. To our knowledge, this is the first report of a carrier culture of FSV which can be stably passaged for a long time.

By electron microscopic analysis, we could not find any morphological difference in FSV particles from either the carrier culture or cytoidically infected CRFK cells. Moreover, the FSV from the carrier culture can grow and cause CPE in CRFK cells as efficiently as normal virus from cytoidically infected CRFK cells. From these data, it is suggested that the viruses from the carrier culture contain non-defective ones.

Establishment of carrier cultures of SFV type 1 (SFV-1) in both HEP-2 and BHK-21 cells [1, 8] and that of SFV type 3 (SFV-3) in Vero cells [21] was reported. The carrier cultures of SFVs in both HEP-2 and Vero cells produced little or no infectious virus [1, 21]. On the other hand, the carrier culture of SFV-1 in BHK-21 cells [1] and that of FSV in this study produced relatively high amount of the viruses, although the total virus titer of the carrier culture was much lower than that of the cytoidically infected cells. In addition, it was reported that there are differences in the distribution of virus antigens between cytoidically and chronically infected HEp-2 cells [1, 8], i.e., nuclear fluorescence was commonly seen with cytoplasmic fluorescence in cytoidically infected cells, while only cytoplasmic fluorescence was seen in chronically infected cells. Gould and Hartley [8] suggested that envelope components of SFV were missing in the chronically SFV-1-infected cells. On the other hand, nuclear fluorescence was weak or undetectable in both cytoidically and chronically SFV-1-infected BHK-21 cells [1]. In this study, we could not find any differences in antigen-distribution between cytoidically and chronically infected CRFK cells and nuclear fluorescence was undetectable in either culture. From the standpoint of both virus production and antigen distribution, the situation of the carrier culture of FSV in CRFK cells is similar to that of SFV-1 in BHK-21 cells. The morphology of the cells of the carrier culture of SFV-1 in BHK-21 cells was reported to be quite different from uninfected BHK-21 cells, i.e., parallel orientation of uninfected cells was not evident in the chronically infected cells [1]. However, we could not find such a difference between the carrier culture of FSV and uninfected CRFK cells.

Immunofluorescence analysis revealed that more than 90% of the cells of both chronically and cytoidically infected cultures were positive for FSV antigen. These observations, together with the data from virus titrations and electron microscopy suggest that most of the cells of the carrier culture were infected with FSV, however cells of the carrier culture produced lower amounts of infectious virus than those of the cytoidically infected CRFK cells.

The precise mechanism of the establishment of the carrier culture is unknown at present. However, it is possible that viruses from the carrier culture contain many defective and/or slowly growing viruses which might interfere the infection or efficient growth of the wild-type viruses. Another possibility is that cells which are relatively resistant to FSV, producing little infectious virus are present in the original population of CRFK cells, and during cocultivation several times and subsequent passages, the resistant cells were selected. Further studies will be necessary to understand the mechanism of this persistent infection in vitro.

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