Persistent Infection of Mouse Fibroblasts with Coxsackievirus

By

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Summary

Infection of fibroblast cell lines initiated from BALB/c or NFR mice with coxsackievirus B3 (CBV-3) or B4 (CBV-4) resulted in infections which persisted for a limited number of subpassages of the infected cells in most cases, but for over a year in one case. In all instances primary acute infections were characterized by cytopathology and release of infectious virus progeny. Viral antigen could be detected during the acute phase of infection, but not in subcultured infected cells. Infectious center assays showed that every cell was infected during the acute phase of infection, but that from the first subcultivation on, the numbers of cells which were able to initiate infection were greatly reduced. The long term persistent CBV-3 infection was characterized by wide fluctuations in titers of virus released into the supernatant fluids. Interferon did not appear to play a role in maintenance of the persistent infection. Information derived from studies on mechanisms of CBV persistence in the in vitro model may help to elucidate the role of CBV in chronic human diseases such as myocarditis.

Introduction

Persistent infection of cells in culture has usually been observed with viruses which are not highly cytoltyc, although the cell type is also an important factor. In general, enteroviruses are cytopathic in cell cultures which support their replication (15). When persistent cell culture infections
have been established with enteroviruses they have usually required the
presence of specific antibody (1—3), addition of 10 percent human serum
(19), or daily washing of cells and addition of fresh medium (16). However,
a persistent infection of a human fetal diploid cell line was established
following infection with a strain of coxsackievirus type B2 (CBV-2) in the
absence of specific antibody or human serum and without frequent changes
of medium (13).

Persistent infection of L929 cells by Theiler’s murine encephalitis virus,
a picornavirus, did not require specific antibody (18). In this case interferon
was detected in the supernatant fluids of the cultures, and this may have
had a role in maintaining the noncytoidal nature of the infection.

These persistent infections could best be described as carrier cultures.
That is, a cytoidal virus was kept in check by inhibitory factors in the
medium or by the presence in the culture of a minority of susceptible cells
and a majority of genetically resistant cells (4).

Persistent infection of cell cultures by coxsackievirus is of interest as
a possible in vitro model for persistence of coxsackieviruses in chronic
diseases such as cardiomyopathies. The possibility that mouse cells might
be subject to persistent infection was suggested to us by the observation
that primary skin fibroblasts set up for assay of cell mediated immunity
were not completely lysed by CBV-3 in 7 days. This report describes certain
features of a persistent coxsackievirus infection of mouse fibroblasts which
was established without specific antibody or human serum, and without
frequent washing or medium changes.

**Materials and Methods**

**Viruses**

The myocarditic “M” strain of CBV-3, adapted to the murine heart by Dr. Jack
Woodruff, was obtained through the courtesy of Dr. Lilian P. Job, and was passed
twice in a continuous line of fetal rhesus monkey kidney cells developed in this
laboratory, and once in Buffalo green monkey kidney (BGMK) cells. A CBV-4 field
strain was isolated in this laboratory in rhesus monkey kidney (RhMk) cells from the
heart of a 10-day-old female with myocarditis, and was passed twice in RhMk cells
and twice in BGMK cells. Identity of the virus strains was confirmed by neutralization
tests with antisera produced in this laboratory. Vesicular stomatitis virus (Indiana
strain) was obtained from the Naval Bioscience Laboratory, Oakland, CA, and was
passed once in baby hamster kidney cells.

**Infected Cell Cultures**

Skin fibroblast cultures were initiated from trypsinized skin of neonatal NFR or
BALB/c mice. Cells were subcultured when they reached confluency, usually 2—4
days with a range of 1—14 days. Confluent cultures were infected with CBV-3(M)
or CBV-4 at various multiplicities of infection (MOI). Persistently infected cells were
established by dispersing cells with a mixture of 0.25 percent trypsin and 0.02 percent