

Rat Tracheal Organ Culture Supports Replication of Parainfluenza 1 (6/94) Virus and Promotes 6/94 Virus Rescue From Latently Infected Human Brain Cells

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Rat tracheal organ culture (TOC) supported replication of parainfluenza 1 (6/94) virus. Cell-associated and cell-free viruses were found after primary infection of TOC. In contrast to other mammalian systems, rat TOC was capable of maintaining 6/94 virus infectivity after primary infection. Rat TOC may be considered a potential indicator system that could be used to detect virus latent in human tissue.

Key words: latent infection, organ culture, virus rescue

INTRODUCTION

In the past ten years, much evidence has been accumulated to indicate that chronic neurologic disease may be caused by persistence of viruses that normally produce acute disease [ter Meulen and Katz, 1977]. Exactly how virus persists despite a normal and often enhanced host immune response is still unclear. Even less well understood is the mechanism by which viruses establish latency in the human nervous system [Fenner and White, 1976] and periodically become activated.

The finding that measles virus is the causative agent of subacute sclerosing panencephalitis (SSPE), and that herpes simplex virus is latent in the human nervous system stimulated a search for a viral etiology in relatively common, chronic neurologic diseases such as multiple sclerosis (MS). Although a parainfluenza 1(6/94) virus has been isolated from the brains of two patients with MS [ter Meulen et al, 1972], subsequent attempts at isolation have failed. Furthermore, failure to isolate cell-associated measles virus from SSPE brain heavily laden with viral antigen is still a common occurrence [Katz and Koprowski, 1973].

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Expansion of the indicator systems and techniques used increases the chances of virus rescue. For example, Cytochalasin treatment of indicator cells has been shown to enhance the rescue of 6/94 virus from latently infected human brain cells [Wroblewska et al, 1978] after cocultivation. Since 6/94 is a parainfluenza virus that infects man most frequently through the respiratory tract, it became of interest to see if this favored entry site would be useful for an in vitro study of virus replication. It has already been shown that for some fastidious coronaviruses, tracheal organ culture (TOC) is the only host system useful for virus propagation in vitro [Tyrell and Hoorn, 1965; Tyrell and Bynoe, 1965; McIntosh et al, 1970; Thomas et al, 1976; Tyrrell et al, 1968]. For these reasons, we decided to study the replication of 6/94 virus in rat TOC and to determine its applicability for rescue of 6/94 virus from latently infected human brain cell cultures.

METHODS AND MATERIALS

Animals

Weanling Lewis rats were obtained from Microbiological Associates, Bethesda, Maryland.

Virus

6/94 virus (kindly provided by Dr. David Waters of The Wistar Institute) was originally isolated from multiple sclerosis brain tissue [ter Meulen et al, 1972] and characterized as a temperature-sensitive variant of parainfluenza type 1 virus, replicating more efficiently at 33°C than at 37°C [Lief et al, 1975]. 6/94 virus was propagated in embryonated hens eggs, and the titer of stock 6/94 virus was 10^8 egg infectious doses (EID)₅₀/ml.

Tracheal Organ Culture

TOC was obtained with slight modifications of previously described techniques [Collier et al, 1971]. Lewis rats were exsanguinated by cardiac puncture. Under aseptic conditions, the chest and anterior neck were opened and the trachea was exposed. The peritracheal tissue was trimmed, and the trachea was washed with Eagle's minimum essential medium (MEM). The proximal trachea was cut, the free end was grasped with a fine forceps, and the tracheal rings were cut separately to the level of the thyroid cartilage with a sterile scalpel. Each tracheal ring was placed on the scratched surface of a 30-mm plastic petri dish. Two to three rings were placed in each dish and incubated at 37°C in MEM supplemented with glutamine and 10% fetal bovine serum (E + 10). The beating motion of cilia was readily seen in healthy cultures for at least three weeks.

Virus Infection of TOC

In each experiment, 10 petri dishes each containing two or three tracheal rings were infected with virus 24 hours after explantation. The medium was removed, and the rings were washed in MEM and incubated for one hour at 33°C with 1.0 ml of MEM containing 10^8 EID₅₀ of 6/94 virus. Control TOC were treated with MEM only. The inoculum was then removed, and the TOC were rinsed twice with MEM, refed with 3 ml of E + 10, and incubated at 33°C.

Sampling of Infected Material

At various intervals after inoculation, samples of tissue culture medium from three infected and three control tracheal organ cultures were removed and stored at -80°C.

Tracheal rings were rinsed in sterile phosphate-buffered saline (PBS). One ring was cut frozen on a cryostat 4 microns thin, fixed for five minutes in cold acetone, and stored at -20°C for immunofluorescence (IF) studies. Another ring was fixed in 10% neutral buffered formalin for histopathologic examination, and the third ring was transferred to a 30-mm petri dish containing CV₁ cells (see Virus Detection).

6/94 Virus Passage Through TOC and CV₁ Cells

To determine whether 6/94 virus maintains its infectivity after passage through TOC, five days after infection with 6/94 virus tracheal rings were cocultivated with uninfected CV₁ cells. Cocultivated cultures were incubated for five days. The tracheal rings were removed, the CV₁ cells were washed three times in MEM, and then cocultivated with a fresh, uninfected TOC. Three to five days after the second cocultivation, these tracheal rings were transferred to uninfected CV₁ cells (see Virus Detection).

Recovery of 6/94 Virus From Human Brain Cells With TOC

Human brain cells latently infected with 6/94 virus have been described previously [Wroblewska et al, 1976]. Briefly, virus is cell-associated and no antigen is detectable by hemadsorption, immunofluorescence, or immunoprecipitation. Infectious virus can be recovered only after prolonged cocultivation or fusion of latently infected human brain cells with indicator cells.

Cocultivation of Latently Infected and Normal Human Brain Cells With TOC

Six rings of uninfected TOC were placed on to each of two T75 plastic Falcon flasks of latently infected human brain cell cultures, and two T75 flasks of normal brain cell cultures. Two "control" flasks of latently infected cells and normal brain cells were not cocultivated with TOC. All cultures were fed with E + 10 and incubated at 33°C in an atmosphere of 5% CO₂. At various intervals after cocultivation, samples of medium from all experimental and control cultures were removed and stored at -80°C . In addition, two to three rings from all cocultivated cultures were transferred to monolayers of uninfected CV₁ cells and incubated for five days. The tracheal rings were then removed, and the washed monolayer of CV₁ cells was checked for virus presence (see Virus Detection).

Virus Detection Procedure

Serial 10-fold dilutions of medium harvested from experimental and control cultures were inoculated intra-allantoically into ten-day-old embryonated hens eggs. Inoculated eggs were incubated for five days at 33° – 35°C , and the harvested allantoic fluid was tested for its ability to hemagglutinate chicken red blood cells as previously described [Lennette and Schmidt, 1969]. The titer of infectious virus was calculated according to the method of Reed and Muench. In addition, CV₁ cell cultures that had been cocultivated with 6/94 virus-infected TOC and uninfected CV₁ cells were tested by hemadsorption with 0.5% guinea pig red blood cells at 4°C [Lennette and Schmidt, 1969]. The results of the tests were expressed as an average of the percent of cells that were hemadsorption-positive in the culture. In addition, 6/94 virus in TOC was detected by indirect immunofluorescence [Gilden et al, 1976] using a 1:10 dilution of immune serum prepared in rabbits against 6/94 virus and a 1:10 dilution of fluorescein-conjugated goat anti-rabbit IgG [Cappel Laboratories, Downingtown, Pennsylvania]. Controls were provided by the substitution of normal rabbit serum for the immune rabbit serum. Observations were made with a Leitz Orthoplan fluorescent microscope illuminated with a mercury HBO 200 bulb, a Schott BG12 excitor filter, and a K510 barrier filter.

Identification of Virus Recovered From Latently Infected Human Brain Cells

Virus recovered from latently infected human brain cells by cocultivation with TOC was identified by indirect immunofluorescence [Gilden et al, 1976] and also by hemagglutination inhibition (HAI) using rabbit immune serum against 6/94 virus [Lennette and Schmidt, 1969]. HAI serum was treated with receptor-destroying enzymes before HAI testing [Lennette and Schmidt, 1969].

Histopathologic Examination

At various intervals after infection, sections of paraffin-embedded, formalin-fixed TOC were cut at 6 μ and stained with hematoxylin and eosin. Uninfected tracheal organ cultures were examined at identical intervals.

Parainfluenza 1 (6/94) Virus Infection of Rat TOC

Rat TOC infected with 6/94 virus appeared normal until six days post-infection (p.i.), when ciliary activity slowed. By day 10 ciliary beating was markedly decreased, and by day 12 it ceased entirely. Pathological changes were characterized by flattening of epithelial cells and loss of cilia (Figs. 1A and 1B). The kinetics of the release of infectious virus from cultures is illustrated in Figure 2. Cell-free virus peaked on day 5, and then decreased rapidly, although a small amount of infectious virus was released until day 24 (the total observation time). Cell-associated 6/94 virus, as determined by the ability of 6/94-infected TOC to produce infection in CV₁ cells (measured by hemadsorption), was detected by day 3, gradually increased until day 13, and was maintained at that level until day 24 p.i. Infectious 6/94 virus could always be obtained from these CV₁ cells by cocultivation with a fresh uninfected TOC and subsequent transfer of the TOC to uninfected CV₁ cells. Uninfected TOC failed to produce hemadsorption in CV₁ cells. In addition, 6/94 viral antigen was seen by indirect immunofluorescence in the cytoplasm of epithelial cells and in fibroblasts in the external layer of the tracheal rings from day 8 to day 24 p.i. (Figs. 3A and 3B). The intensity of the immunofluorescence was greatest at day 14; by day 24 only traces of viral antigen were detectable.

Enhancement of 6/94 Virus Detection by Rat TOC

The ability of rat TOC to promote 6/94 virus rescue from latently infected human brain cells is shown in Table I. When the brain cells were cocultivated with rat TOC, and

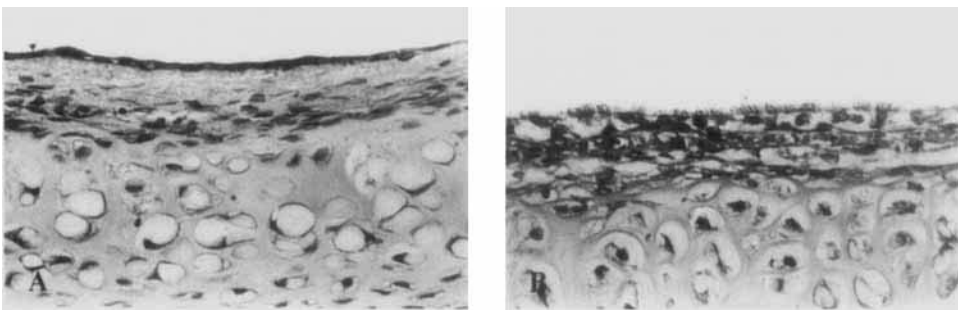


Fig 1. A) Portion of tracheal epithelium from tracheal organ culture infected with 6/94 virus. Note loss of cilia and flattening of epithelial cells (hematoxylin and eosin, original magnification, $\times 160$). B) Uninfected trachea (hematoxylin and eosin, original magnification, $\times 160$).

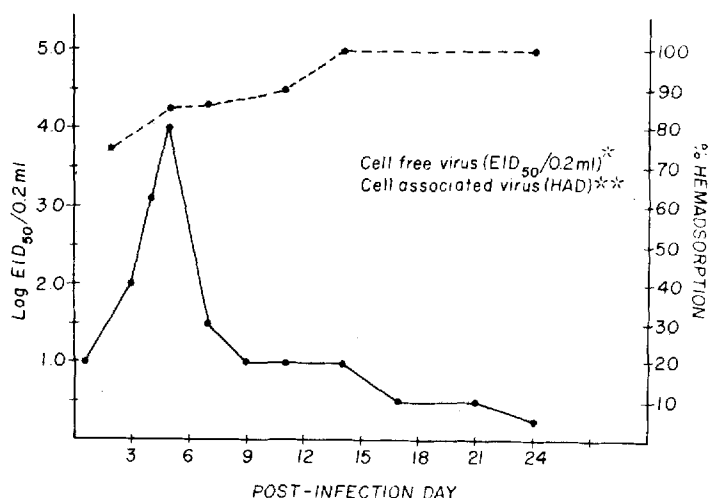


Fig. 2. Experimental infection of rat tracheal organ culture with 6/94 virus. Detection of cell-free (solid line) and cell-associated (dotted line) virus at various intervals after primary infection. (*Ability of medium from 6/94-infected TOC to infect embryonated hen eggs. **Ability of 6/94-infected TOC to infect CV₁ cultures.)

the rings were then transferred to uninfected CV₁ cells, 6/94 virus was detected as soon as day 1 p.i. and also on day 8 after the latter cocultivation. Transfer of rat TOC that had been cocultivated with normal human brain cells to CV₁ cells did not result in 6/94 virus detection. In addition, virus was not detected in any of the following controls: untreated latently infected or normal human brain cells, or untreated CV₁ cells. Furthermore, direct cocultivation of latently infected human brain cells with CV₁ cells did not result in virus rescue (Table I).

Virus that was recovered after cocultivation of latently infected brain cells with TOC was identified as 6/94 virus by both HAI and immunofluoresence.

DISCUSSION

These studies demonstrate that Lewis rat TOC is a favorable host system for replication of 6/94 virus. Infection of rat TOC with 6/94 virus led to the cessation of ciliary activity after 12 days and to the production of infectious virus. This finding is consistent with the observation of Tyrrell and Hoorn that parainfluenza types 1, 2, 3, and 4 are capable of growing in human and ferret TOC [Tyrrell and Hoorn, 1965]. Of interest is the fact that the only parainfluenza virus capable of damaging ciliary epithelium was the Sendai strain of type 1 parainfluenza virus. The close relationship between the Sendai and 6/94 strains of parainfluenza 1 virus has already been demonstrated [Lief et al, 1975]. Other RNA viruses, including coronaviruses and influenza virus, will also grow in TOC [McIntosh et al, 1970; Tyrrell and Hoorn, 1965]. Characteristic findings after primary infection include impairment of ciliary function followed by degeneration of cells 4–12 days after infection. Thus, TOC appears to be not only a susceptible host system for replication of a wide variety of viruses but also a convenient indicator system for monitoring virus presence.

Of considerable importance is the ability of rat TOC to support replication of fully infectious 6/94 virus. As has been reported previously, the infectivity of both Sendai and

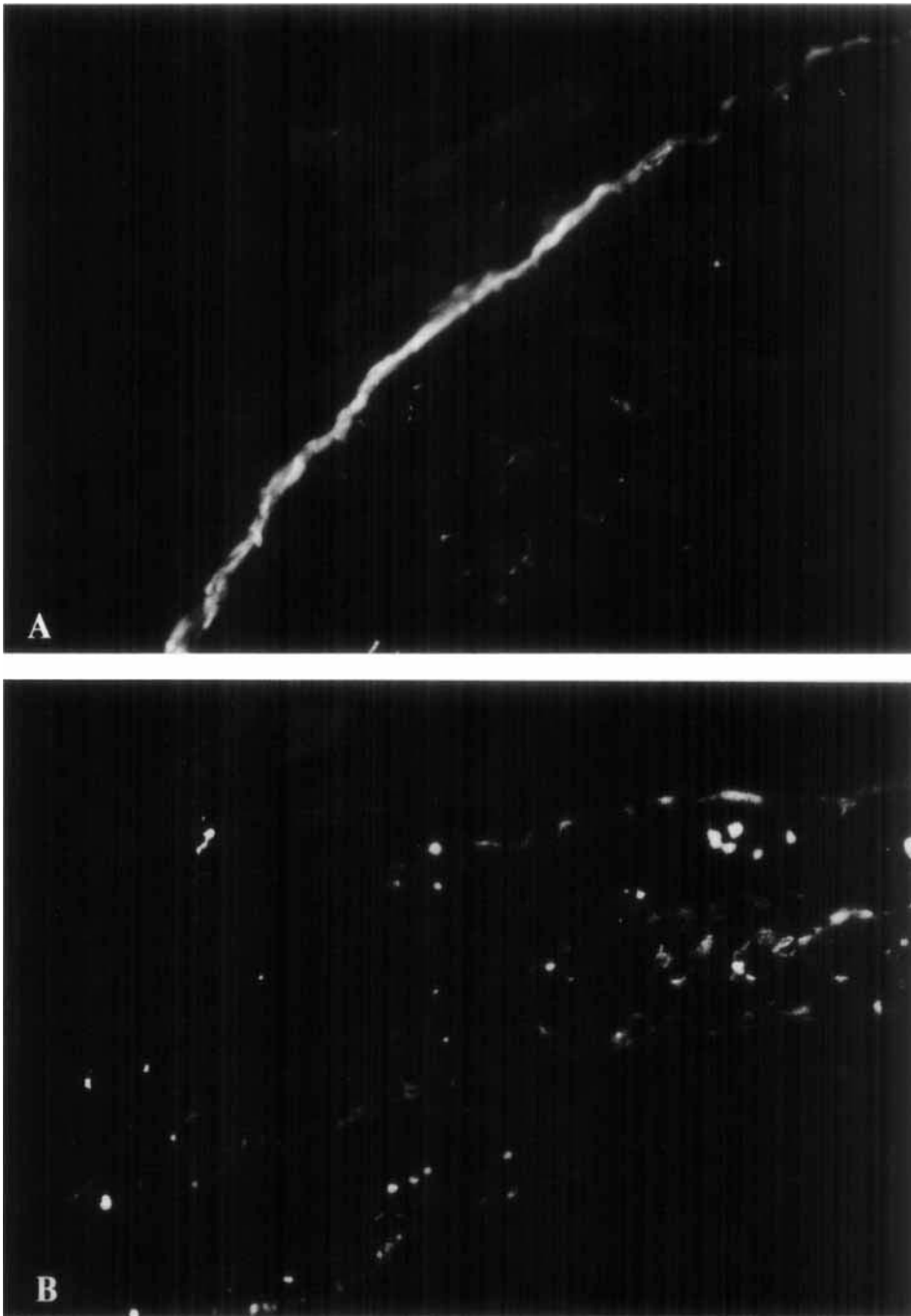


Fig. 3. A) Rat tracheal organ culture (TOC) 10 days after primary infection with 6/94 virus. Note infection of external layer of TOC. Indirect immunofluorescence using rabbit anti-6/94 hyperimmune serum (original magnification, $\times 225$). B) Absence of fluorescence in similarly infected TOC using rabbit serum free of 6/94 antibody (original magnification, $\times 85$).

TABLE I. Recovery of Parainfluenza 1 (6/94) Virus From Latently Infected Human Brain (LIHB) Cell Cultures by Cocultivation With Rat Tracheal Organ Cultures (TOC)

Experimental conditions	Virus presence (EID ₅₀ /0.2 ml)	
	Day 1 (p.i.)	Day 8 (p.i.)
LIHB + TOC	10 ^{1.45}	10 ^{2.45}
NHB ^a + TOC	< 1	< 1
LIHB + CV ₁	< 1	< 1
LIHB ONLY	< 1	< 1
NHB ONLY	< 1	< 1
TOC ONLY	< 1	< 1
CV ₁	< 1	< 1

After cocultivation with LIHB or normal (uninfected) human brain cells, TOCs were transferred to uninfected CV₁ cells, and 6/94 virus was assayed for by the ability of medium removed from the CV₁ cells to infect embryonated hen eggs. 6/94 virus was further identified by hemagglutination-inhibition and indirect immunofluorescence using rabbit anti-6/94 virus immune serum.

^aNormal human brain.

p.i. = post-infection.

6/94 virus was reduced when passaged in vitro in mammalian cells [Ishida and Homma, 1961; Lief et al, 1975]. This was associated with the development of a virus-induced specific glycoprotein in infected mammalian cell cultures [Choppin and Compans, 1975]. Virus infectivity could be restored by treatment with trypsin, which produced cleavage of this glycoprotein [Homma, 1971]. It is possible that organotypic cultures of trachea, as opposed to dissociated cell cultures, may produce a trypsin-like substance or other proteolytic enzymes that could result in maintenance of virus infectivity.

The usefulness of rat TOC as an intermediary for the rescue of virus from latently infected cells cannot be overstated. TOC provides a favorable growth environment for a large number of viruses as well as a system that is capable of rescuing virus from latently infected human brain cells. TOC may be a useful indicator system that could be applied to human tissue suspected of harboring latent virus.

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