

Full Length Research Paper

A study of caprine brain cells with caprine arthritis encephalitis virus by direct virus application

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One of the constraints in unraveling the mysteries blurring the advancement of research in the quest to totally put HIV problems under control is getting the appropriate animal model that would truly simulate human cases. This problem is more apparent in studies involving the central nervous system. Consequently, a viable animal model to generate information for the production of drugs and vaccines for the prevention and or control of lentiviral induced dementia in affected host animals is pertinent and vital. In this study, explant cultures prepared from the brain of new-born goat-kid were infected with Caprine Arthritis Encephalitis (CAE) virus- a retrovirus affecting goats. The specific brain cell types infected by the (CAE) virus were determined using reverse-transcription polymerase chain reaction (RT-PCR) and transmission electron microscopy (TEM techniques). TEM showed that in 85 – 90% cases, microglia were the cells specifically infected by the virus. Amplification of the genomic sequence of the *envelope* and the *gag genes* by RT- PCR confirmed the presence of CAEV proviral DNA in the brain cells of affected animals. No productive infection of the astrocytes was observed. The results of this study showed a lot of similarities in the tropism of CAE virus infection of goat brain cells to that of HIV infection in humans thus suggesting the potential usefulness of the caprine model for the study of HIV neuropathology. The goat model system as a non-primate model therefore could be more adaptable as a simple animal model than primate models with their complexity of anthropological, environmental and safety problems.

Key words: CAEV, HIV, affinity, brain cells, RT-PCR, TEM.

INTRODUCTION

Caprine arthritis encephalitis virus (CAEV) is a type lentivirus of goats causing neuro- degenerative changes and non-suppurative arthritis ('big knee') in adult goats while wobbling gait with ataxia are the main features in goat kids. The virus was first identified in the USA (Cork et al., 1974). To-date, eight lentivirus species have been isolated (Narayan and Clements, 1990; Coffin, 1996). The discovery of a lentivirus, human immunodeficiency virus (HIV), as one of the causes of Human Acquired Immunodeficiency Syndrome (AIDS), has greatly stimulated interest in the animal lentiviruses. Lentivirus infections are characterized by a very long period of latency lasting

from several months to several years. At times, incubation period may exceed the natural lifespan of the host.

CAEV shares similar morphological, biochemical and genetic features as well as replicative mechanisms and neuro-pathological features with those observed in AIDS due to HIV (Chiu et al., 1985; Gendelman and Gendelman, 1992). As part of efforts to find alternative animal models for studying lentiviral induced neural effects, most especially, AIDS Dementia Complex (ADC), attempts were made to identify those brain cells that are supportive to the CAE virus. Consequently, this study was designed essentially to culture, identify and infect caprine brain cells with CAE virus by direct virus application, and reverse transcription-polymerase chain reaction (RT-PCR) and transmission electron microscopy (TEM) techniques were employed in establishing the spe-

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cific brain cell types that normally support the growth and productive infection of the virus in goats.

MATERIALS AND METHODS

Culture of brain cells

Explants of the frontal lobe of the cerebrum of day old goat kids were made and cultured in DMEM containing 20% FBS and 0.1% Fungizone. They were incubated at 37°C in a humidified chamber containing 5% CO₂ and 95% air. The cells were seeded at the rate of 3 X 10⁶ per 75 cm tissue culture flasks and left untouched for 72 h after which the old medium was removed and a fresh 20% FBS/DMEM added. The culturing continued until the cells became 80% confluent.

Culture of microglia

Following the confluence of the brain cells, the flasks containing the growing cells were rocked on a table rocker for one hour at room temperature. The medium containing some floating cells (floaters) was transferred into 15 ml tubes and centrifuged at 1000 rpm for 10 min, thus separating the brain cells by simple centrifugation method. The supernatant was removed and the cells were re-suspended in 7 ml of 10% FBS/DMEM and grown in 75 cm tissue culture flasks at 37°C in a humidified chamber for 5% CO₂ and 95% air. Pure cultures of these cells were later infected with CAE virus by direct virus application.

Culture of astrocytes

Following the differential separation of the microglia from the astrocytes by simple centrifugation method, a specially formulated (selective) medium containing 45 mg/L glucose without L-glutamine and sodium pyruvate (Hyclone, Logan, UT, USA) was added to the tissue culture flasks for the growth of the astrocytes. Since the medium contained no glutamine and pyruvate, the survival of other types of cells were thus jeopardized. The cells were grown until they reached 80% confluence after which they were used for subsequent experiments.

Infection of the brain cells by direct virus application

Cultures of microglia and astrocytes in the sixth passage in 75 cm³ culture flasks were grown to 70% confluence and infected with 10 µl of the stored CAE virus stock (TCID₅₀ of 2.5 X 10⁻⁶) diluted in 10 ml of 5% FBS/DMEM at a low multiplicity of infection (MOI) and incubated for one hour before the volume was made up to 10 ml using 10% FBS/DMEM.

Reverse-transcriptase polymerase chain reaction (RT-PCR)

RNA was extracted from the infected goat brain cells using the chloroform/ether method as described by Frederick et al. (1999). Following this, a cocktail of reagents were assembled for the synthesis of the cDNA for the reverse transcriptase reaction in a thermocycler. Polymerase chain reaction was done using the primer sequence constructed from the nucleotide sequence of Saltarelli et al. (1990). The primers sequence were ...5' AGA AFT ATT GGC CAT GAT GCC T-3' (sense from nucleotide 982); and 5' -CCA CAT CTC TAC ATG CTT GAC TT- 3' (antisense from nucleotide 1472). 16 µl of nuclease free water was added while a drop of mineral oil was also added to make a total reaction volume

of 49 µl. The tube containing these reagents was heated to 94°C before adopting the following cycle parameters for optimizing the PCR products: 95°C for 5 min, 80°C for 8 min, 55°C for 30 s and 72°C for 40 s; this cycle was repeated 30 times before moving to 72°C for 5 min and ending up in soak file (4°C) following the products which were electro-phoresed in 0.8% low melting agarose gel to clarify the PCR product.

Transmission electron microscopy

Prepared macrophages were grown on MillicellTM-HA membranes (Millipore Corporation, Bedford, MA) inserts using sterile forceps in a 24-well microtitre plates at 37°C in the humidified chamber of 95% air and 5% CO₂. Three days later, the cells were infected with CAEV at low multiplicity of infection (MOI). Thin sections of about 60 – 90 nm thick were stained with uranyl acetate and lead citrate, after which they were examined and photographed with Transmission Electron Microscope (Philips EM 201). Identified viral particles were also photo-documented.

RESULTS AND DISCUSSIONS

In the RT-PCR reaction, the genomic RNA obtained from the infected brain cell cultures gave a product that has a size of 414 base-pair (bp) which corresponds to the expected product size thus confirming the presence of CAE proviral DNA in the macrophages (Figure 1). This aptly demonstrated the permissiveness of these cells for the virus and further proved that microglia are most frequently infected in these animal species (Cunningham et al., 1997). The budding of viruses from the brain cells as observed during the transmission electron microscopy (Figure 2) goes a long way to suggest productive infection of the microglia and that the brain can be a rich reservoir of the virus *in vivo*.

Mononuclear phagocytes were observed as the cell types with high affinity (tropism) for CAE virus in this work. The typical morphological changes of the cultured microglia infected with CAE virus including swelling, fusion and formation of syncytium observed in this work are similar to those of microglia infected with HIV-1 in culture (Watkins et al., 1990).

Infection of astrocytes with subsequent production of the virus was not observed in this study (Figures 3A and 3B). Astrocytes are not known to be natural targets of retroviruses; though infection of these cells (astrocytes) by HIV-1 may occur only in children (Saito et al., 1994) and human astroglial cells may be infected *in vitro* producing infective but not productive infections (Cheng-Mayer et al., 1987; Funke et al., 1987). The enlargement of astrocytes during infection with CAE virus noticed in this study (Figure 3B) may be their cellular response to products released by virus-infected microglia component of brain response to injuries (Eddleston and Mucke, 1993). Macrophages and microglia have been shown to produce neurotoxic substances which mediate CNS injury through soluble factors like nitric oxide, reactive oxygen species, quinolinic acid, glutamate, arachidonic acid metabolites and cytokines (TNF-, IL-1, IL-6, IL-8

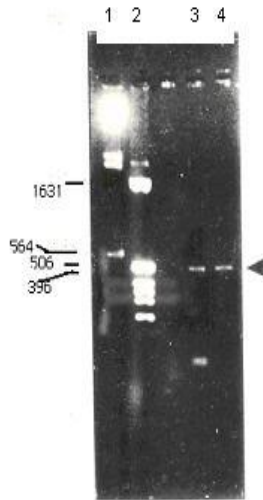


Figure 1. Amplification of the *gag* gene of CAE virus by RT-PCR. Lanes 1 and 2: Lambda (molecular marker); lanes 3 and 4: the amplified 414 bp fragment is indicated by the arrow.

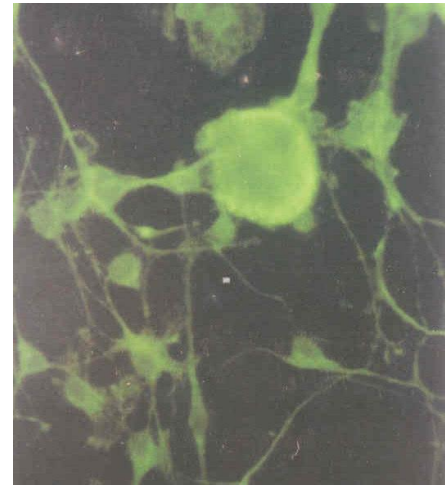


Figure 3B. Astrocytes co-cultured with supernates from infected microglia cells. The cells were stained with fluorescein isothiocyanate (FITC). Magnification = X1200.

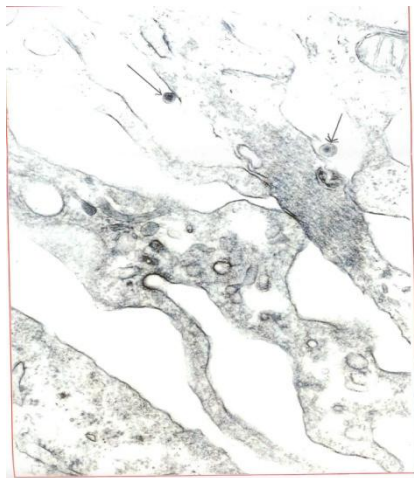


Figure 2. Virus budding from goat brain cells infected with CAE virus (*in vitro*). Magnification = X71,000.

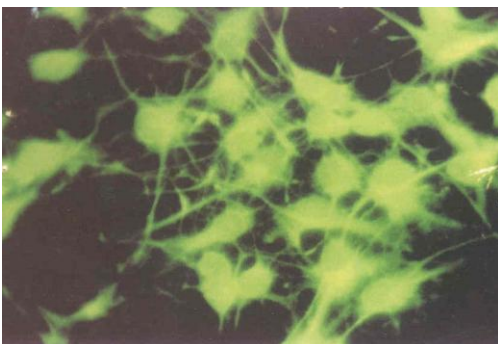


Figure 3A. Normal astrocytes stained with fluorescein isothiocyanate (FITC). Magnification = X1200.

and IL-10) among others (Gulian et al., 1990; Merrill and Chen, 1991; Adebayo and Adeyemo, 2003). These neurotoxic sub-stances released by CAEV-infected macrophages and microglia which were the cells were observed to be infected. This study was implicated in the increased necrotic rate of neurons co-cultured in the supernatant of CAE virus infected mixed brain cells compared to neurons treated with supernatants of uninfected cultures (Adeyemo et al., 1996). These cells are capable of contributing significantly to the brain lesions *in vivo*.

In conclusion, this study has revealed that the cells of the monocyte-macrophage lineage are the principal target cells of the brain affected by CAE virus thus establishing the many similarities that exist in the neurotropic nature of the two viruses. Consequently, the goat system (as a non-primate model) could be more acceptable as a simple and viable alternative animal model than primate models with their complexity of anthropological, environmental and safety problems for the study of lentiviral induced neuropathology.

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