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DETECTION OF CAPRINE ARTHRITIS ENCEPHALITIS VIRUS GAG-GENE BY RT-PCR FROM EXPERIMENTALLY INFECTED GOAT BRAIN CELLS

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SUMMARY

Different brain cell types obtained from goat cerebrum explant cultures were prepared from newborn goat kids and infected by direct Caprine Arthritis Encephalitis (CAE) virus application. Predominantly, cells of the monocyte-macrophage lineage were specifically infected by the virus as proviral DNA was detected in infected cultures by amplification of a 414 base-pair (bp) fragment of the viral gag-gene by Reverse Transcription- Polymerase Chain Reaction (RT-PCR) technique. The present study revealed that microglia are the target brain cells infected by the CAE virus. This finding will undoubtedly help in devising some immuno-prophylactic measures to be adopted in the management of neurological dysfunctions resulting from retroviral infections of man (HIV) and animals.

KEY WORDS: CAEV, Gag-gene, Brain cells, RT-PCR

INTRODUCTION

One important biological property common to all lenti-viruses that cause neurologic disease (Human Immunodeficiency Virus-1 (HIV-1), Maedi-Visna Virus, CAE virus) is tropism for cells of the monocyte- macrophage lineage during natural infection (Embreaston, *et al.*, 1993). Though the involvement of monocytes in the process of viral infection of the brain cells *in-vivo* has been suggested for HIV-1 (Hickey, 1990), this has not been practically documented. To investigate the involvement of monocytes in CAE virus infection of goats, experiments were designed to determine the specific cells for which the virus has tropism in the brain and the fate of these cells when infected.

MATERIALS AND METHODS

Cell culture

a. Culture of the Goat Synovial Membrane (GSM) cells-the indicator cells

Day-old goat kids from CAE-free dams were euthanized, amputated around the elbow, flayed, thoroughly rinsed in 70% ethanol and quickly transferred to the Laminar Flow Hood (NuAire®, USA) for further dissection. The synovial membrane was exposed using a sterile size 40 scalpel blade (Fisher Scientific, Swanne, GA). The membranous surfaces of the carpal joints were excised and dropped into 75cm^3 tissue culture flasks (with fenestrated caps) into which 7ml of DMEM containing 20% FBS had been dispensed and incubated at 37°C in a humified chamber containing 5%CO_2 and 95% air (NuAire Autoflow, NuAire Corp, Plymouth, MN) and allowed to stay for 4-5 days before changing the medium. The cells that grew from this preparation were trypnized, washed in 10%DMEM and seeded in four 75cm^3 tissue culture flasks at a rate of 4.5 X 10^4/ml medium. Cells from two of these flasks were infected by direct virus application on the third day after reaching about 70-80% confluence while the rest were processed and stored at 196°C for future use.

b. Brain Cells

Brain explants from the frontal lobe of the goat kid cerebrum was digested in 10ml of HDB-collagenase (Worthington Inc, USA) for enzymatic dissociation of the cells and incubated at 37°C for 20 minutes before thorough trituration and re-incubation as before and seeded in 75cm^3 tissue culture flasks with DMEM containing 20% FBS and 0.25μg/ml fungizone. 100U/mg penicillin, 100μg/ml streptomycin, in an atmosphere of 5% CO_2 at 37°C. To enrich for different glial cells, the method of Da-Cunha and Vitcovic (1991) was adopted. The flasks containing the 3-day old primary cell culture were shaken at 100rpm for 45 minutes at room temperature on an orbital shaker. The cells floating in the supernatant were separated and grown as microglia enriched culture in Ham's F12 medium/DMEM containing 2mM glutamine with 10% FBS and antibiotics with or without 5% (v/v) giant cell tumor (GCT) supernatant. Cells not detached from the flasks after the orbital shaking procedure were grown as astrocytes enriched cultures in astrocytes-culture medium without L-glutamine containing 5% FBS and antibiotics (Hyclone®). This special medium was changed every 2 days.

Virus

CAE virus strain 75-g63 obtained from American Type Culture Collection (ATCC) Rockville, MD USA was propagated in the indicator cells goat synovial membrane (GSM) cells grown in Dulbecco's Minimum Essential Medium (DMEM) containing 10% foetal bovine serum. After the propagation of the stock virus, the method of Reed and Meunch (1938) was employed in titrating and calculating the tissue culture infective dose ($TCID_{50}$). An infectivity titre of 3.2 X10^{-6}/ml was obtained.

Infection of cultures and RNA extraction

The prepared monolayers of brain cells were infected at a low multiplicity of infection (MOI) by direct application of 10μl of the CAE virus ($TCID_{50}$ of 3.2 X 10^{-6}) in 5ml of DMEM containing 5%FBS. The production of the virus was monitored by screening for cytopathic effects (CPE) of the virus two days post inoculation (pi) and by application of the culture supernatant to the cultured GSM cells. The cytopathic effects (CPE) of the virus on GSM cells and microglia were observed by using Giemsa stain. Following the period allowed for the infection to take place in the experimentally

infected cells, the growth medium was removed and the cells fixed in absolute methanol for 10 minutes at room temperature. The Giemsa stain, already diluted 1: 20 and filtered (using Whattman Filter paper size I) was added to the cells after decanting the absolute methanol and incubated at room temperature for five hours or left overnight. Following this, the cells were viewed under the light microscope for morphological changes. The Cells-to-cDNA Kit® was used according to the manufacturer's instructions (Ambion Inc., Texas, USA) in extracting RNA from the goat-brain infected cells).

Reverse transcription-polymerase chain reaction RetroScript® (Ambion Co., Austin, Texas, USA) kit containing a cocktail of reagents for the synthesis of complimentary DNA (cDNA) was used for the Reverse transcription reaction. 10µl of template (extracted) RNA was dispensed into a PCR tube while 4µl of dNTP was added. 2µl of 1st strand primers was added. All were gently mixed together and briefly centrifuged. The mixture was heated to 80°C and flash-cooled on ice. 2µl of 10X RT-PCR buffer, 1µl of Placental RNAse Inhibition and 1µl M-MLV Reverse Transcriptase were added; all making 20µl total reaction volume. Mixing and spinning was done briefly. The reactants were incubated at 42°C for one hour. Following this, the mixture was incubated at 92°C for 10 minutes and thereafter stored at 20°C till use.

PCR was carried out using the method of Saiki *et al.*, (1980.The primers used were constructed from the published nucleotide sequence of CAE virus (Saltarelli *et al.*, 1990): 5'-AGAAGT ATT GGC CAT GAT GCC T-3' (sense from nucleotide 982); and 5' - CCA CAT CTC TAC ATG CTT GAC TT-3' (anti-sense from nucleotide 1472). 36.5µl of nuclease free water, 2.5µl of dNTP, 2.5µl each of the two primers (oligos) and 5µl of 10X PCR buffer was prepared and a drop of mineral oil gently layered on it to make a total reaction volume of 49µl. This was heated to 94°C before the addition of 1 unit of Taq-polymerase to make a total reaction volume of 50µl. The cycle parameters adopted for optimising the PCR products were: 95°C for 5mins, 80°C for 8 min, 55°C for 30 sec and 72°C for 40secs. This cycle was repeated 30 times for chain elongation before moving to 72°C for 5mins and ended in soak file (4°C). 10µl of the reaction mixture was analysed on 2% agarose and stained with ethidium bromide prior to photo-documentation.

A 414 bp fragment of the virus gag-gene was amplified by the RT-PCR technique. Plasmid pBR 322 cut with restriction endonuclease, Hif-III was used as a size marker for the base-pair. A nested PCR was conducted to further amplify a 219 bp gag fragment of CAE virus from the genomic DNA of the experimentally infected goat brain cells.

RESULTS AND DISCUSSION

In this study, the specific brain cell type mostly infected by the virus was aptly demonstrated to be microglia as CAE proviral DNA was detected in them following the direct experimental infection. CPE was observed as rounding of cells, formation of giant cells with their nuclei arranged circumferentially (syncitia formation) with lots of mis-shapened and jagged cell membranes. Astrocytes were not infected.

The PCR product obtained has a size of 414bp (lane 2) which corresponds to the expected product size (Figure 1). Nested PCR product of 219 bp (Figure 1 lane 3 & Figure 2 lanes 3-8) was obtained as a further confirmation of the integration of the CAE virus in the infected goat brain cells. The technique employed here (RT-PCR) was an advancement over the previous work of Reddy *et al.*, (1993) in which direct PCR was employed in the diagnosis of CAE virus infection in goats.

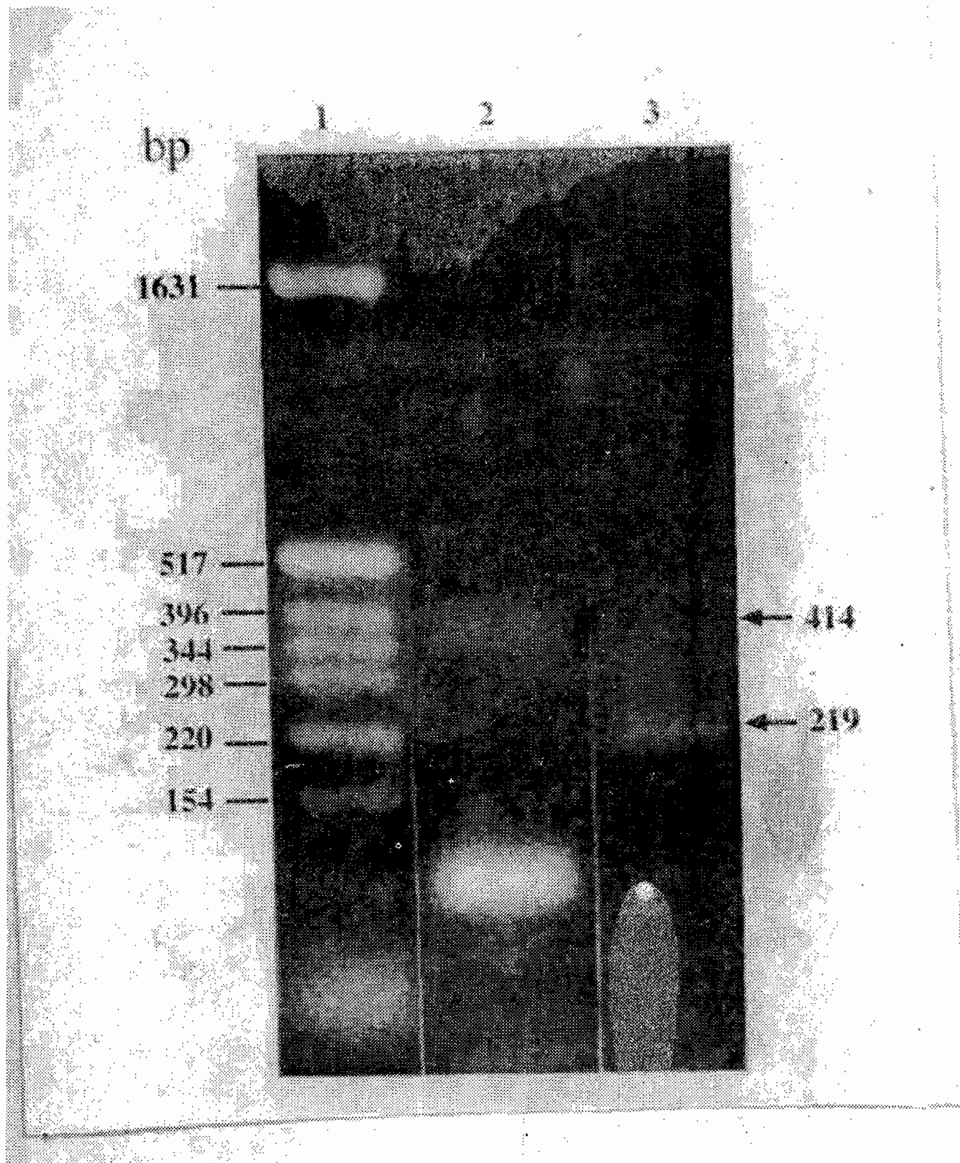

Figure 1: RT-PCR product of the amplified gag gene.

Lane 1: Molecular marker

Lane 2: Expected product of 414bp

Lane 3: Nested PCR product of 219bp fragment of the gag-gene of CAE virus.

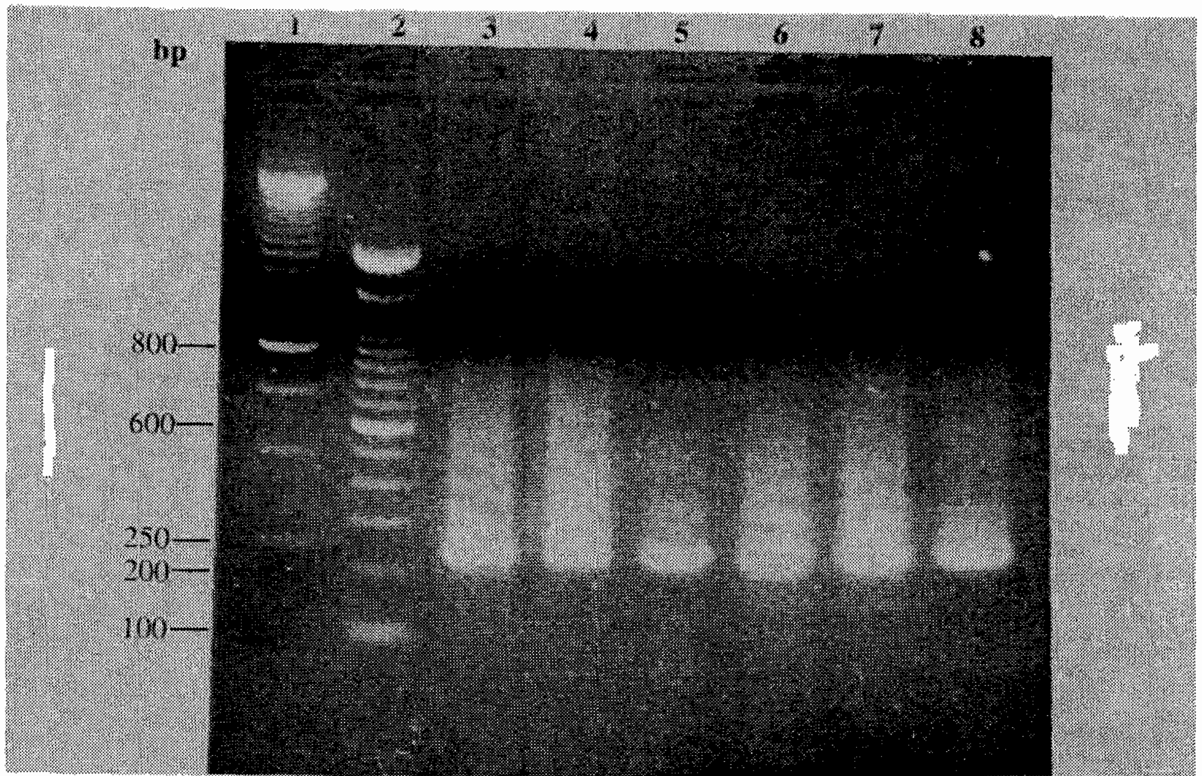

Figure 2: Amplified product from the gag gene of CAE virus
Lanes 1 & 2: Lambda bacteriophage DNA cut with Hind III
Lanes 3-8: Nested PCR product was used to amplify a 219 bp gag gene fragment of CAE virus from the genomic DNA of experimentally infected goat kid.

In conclusion, this study has demonstrated the tropism as a well as productive nature of microglia of the brain for CAE virus. The investigation also showed that RT-PCR technique could be readily employed as a modern diagnostic tool in the early detection of retroviral infections. Consequently, prophylactic measures aimed at combating the virus in the brain should take the tropism of the virus into due consideration along with the capacity of such preparations to cross the blood-brain barrier. Because of the many similarities of the neuropathology of CAE infection of goat brain to HIV-1 infection of man (Cunningham *et al.*, 1997), the result from this investigation also suggests a potential usefulness of the goat CAE model for the study of neuropathogenesis of AIDS dementia complex (ADC) in man.

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