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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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Abstract: 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: Caprine arthritis encephalitis, amplification, neurological dysfunctions

INTRODUCTION

One important biological property common to all lenti-viruses that cause neurologic disease (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: 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 scapel blade (Fisher Scientific, Swanne, GA). The membranous surfaces of the carpal joints were excised and dropped into 75 cmn 3 tissue culture flasks 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 either used immediately or stored at 196°C for future use.

Brain cells: Brain explants from the frontal lobe of the goat kid cerebrum was enzymatically digested and seeded in 75 cm 3 tissue culture flasks containing 20% FBS, 0.25 μg mL^{-1} fungizone. 100U mg penicillin, 100 μg mL^{-1} streptomycin, DMEM in an atmosphere of 5%CO_2 and 95% air at 37. 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 100 rpm for 45b min 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 2 mM glutamine with 10% FBS and antibiotics with or without 5 (v/v) Glial Cell Tumor (GCT) supernatant. Cells not detaching from the flasks after the orbital shaking procedure were grown as strocytes enriched cultures in astrocytes-culture medium without L-glutamine containing 5% FBS and antibiotics. The media on cultures were changed every two 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.

The stock virus was titrated according to the method of Reed and Muench (1938) and had an infectivity titre of 3.2×10^{-6} Tissue Culture Infective Dose ($TCID_{50}$) per mL.

Infection of cultures and rna extraction: The prepared brain cells were infected by direct application of the CAE virus. The production of the virus was monitored by screening for cytopathic effects of the virus two days post inoculation (pi) and by application of the culture supernatant to the cultured GSM cells.

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. The cDNA obtained was 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 by Saltarelli *et al.* (1990): 5'-AGA AGT 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). 16 µL of the nuclease free water was added while a drop of mineral oil was layered on it to make a total reaction volume of 49 µL . This was heated to 94°C before the addition of 1 unit of Taq-polymerase to make a total reaction volume of 50 µL. The cycle parameters adopted for optimising the PCR products were: 95°C for min, 80°C for 8 min, 55°C for 30 sec and 72°C for 40°C sec. This cycle was repeated 30 min for chain elongation before moving to 72°C for 5 min and ended in soak file (4°C).

10 µL of the reaction mixture was analysed on 2% agarose and stained with ethidium bromide prior to photo-documentation.

Using the cDNA as a template, 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 copiously infected by the virus was aptly demonstrated to be microglia as CAE proviral DNA was detected in them following the direct experimental infection. The astrocytes were not infected.

The PCR product obtained has a size of 414bp which corresponds to the expected product size (Fig. 1). Nested PCR product of 219 bp was obtained as a further confirmation of the integration of the CAE virus in the infected goat brain cells (Fig. 2).

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.

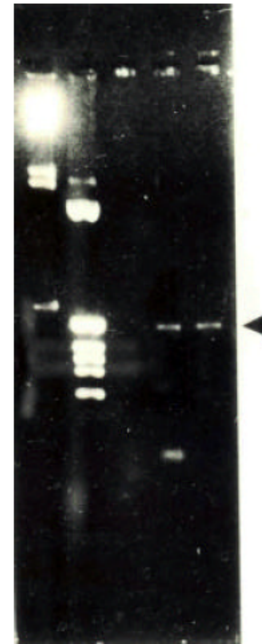

Fig. 1: Amplification of the gag gene of CAE virus by RT-PCRLanes 1 and 2: lambda (molecular marker) Lanes 4 and 5 The amplified 414 bp is indicated by the arrow

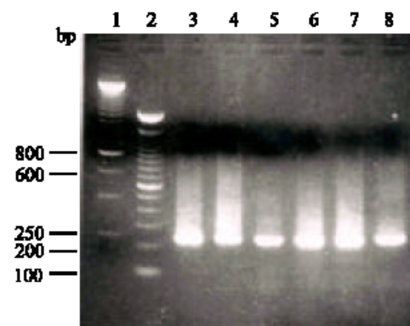

Fig. 2: Amplified product from the gag gene of CAE virus Lanes 1 and 2: Lambda bacteriophage DNA cut with Hind III Lanes 3-8: Nested PCR product was used to amplify a 219 bp gag gene fragment of CAE virus from the genomic DNA of experimentally infected goat kid

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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