

*Full Length Research Paper*

# Immunological enhancement of a retrovirus causing non-suppurative chronic arthritis in adult goats

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Accepted 19 April, 2015

Antibody-mediated enhancement of caprine arthritis encephalitis (CAE) virus in goat synovial membrane (GSM) cells was investigated with regards to time of onset and severity of lesions. Homologous antibody was serially diluted to enhance the growth of the virus as this provided both optimal and subnormal concentrations of the antibody. The cells were incubated for five days and later stained with Giemsa stain. Marked cytopathic effects (CPE) were observed early at sub-optimal concentrations of the antibody indicating that the virus was enhanced at these concentrations. Possible epidemiological implications of these are discussed with relevance to the pathogenesis of retrovirus infections in man and animals.

**Key words:** CAEV, GSM cells, antibody-mediated enhancement, cytopathic effects.

## INTRODUCTION

Immune enhancement is a biological phenomenon that requires interaction between virus and Fc receptor bearing cells in the presence of sub-neutralizing concentrations of antibody (Halstead and O'Rourke, 1977). This phenomenon has been postulated to account for increased severity or early death in certain diseases (Halstead, 1982; Porterfield, 1981) and has been established as a major mechanism in the pathogenesis of Dengue Haemorrhagic Fever/Shock Syndrome (DHF/SS) (Fagbami and Ojeh, 1981). There are many other important viruses of man and animals that have not been studied with regards to immune enhancement phenomenon. This paper therefore focused on the study of immunological enhancement of a retrovirus causing non-suppurative chronic arthritis in adult goats and a demyelinating leuko-encephalitis syndrome in goat kids using goat synovial membrane (GSM) cell line derived from the synovium of day old goat-kid's carpal joints.

## MATERIALS AND METHODS

### Culture of goat synovial membrane (GSM) cells

Day-old goat kids from CAEV-free dams were sacrificed for the preparation of these cells. The goat kids were euthanized by intravenous injection of 1ml Beuthanasia®. The kids were decapitated,

amputated around the elbow and flayed. Following this, the fore limbs were thoroughly rinsed with 70% ethanol and quickly transferred to the Laminar Flow hood (NuAire, US Autoflow, NuAire Corp, Plymouth, MN, 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<sup>3</sup> cell culture flask into which 7 ml of Dulbecco's Minimum Essential Medium (DMEM) containing 20% Foetal Bovine Serum (FBS) had been dispensed for growth and incubated at 37°C in a humidified chamber with an atmosphere of 5% CO<sub>2</sub> and 95% air (NuAire US Autoflow, NuAire Corp, Plymouth, MN, USA) and allowed to stay for 4 - 5days before changing the medium.

The cells that grew from this preparation were either used immediately or trypsinized, centrifuged, then re-suspended in the growth medium and stored in liquid nitrogen (-196°C) using a freezing medium for future use.

### Test sera

Serum samples were obtained from West African Dwarf goats brought for "big knee" treatment at FUTA Vet Clinic but later confirmed of natural infections at the Federal University of Technology, Akure Diagnostic Laboratory (Adebayo, 2005). The sera were stored at -20°C until used. Serum inactivation was carried out to remove heterophilic substances from the serum samples as described below. After thawing, 5 ml of the stored serum was transferred into a test tube. This was immersed in a water-bath already preheated to 56°C. This was allowed to remain in the water

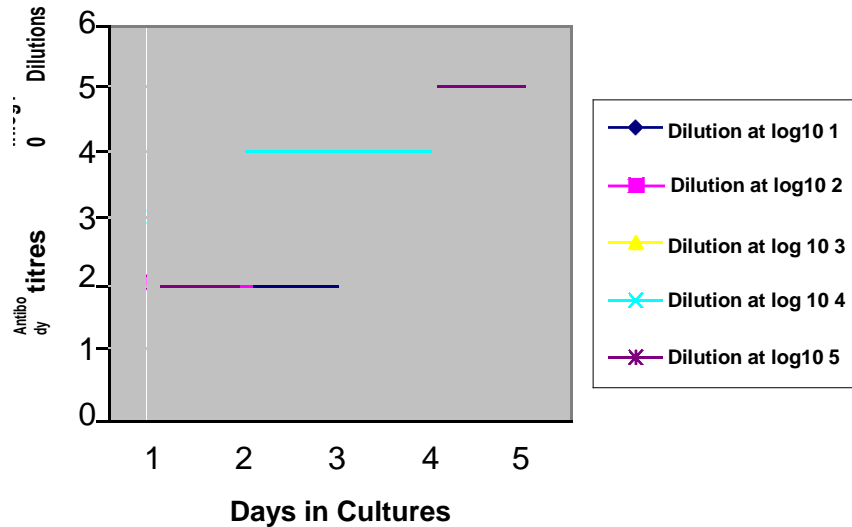


Figure 1. CPE curves of CAE Virus in GSM cell cultures.

water - bath till the temperature rose up to 56°C and then timed for 30 min before the serum was removed, cooled and serially diluted as in  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$  and  $10^{-5}$  for running the assay.

#### Virus inoculum

The CAE virus strain-g63 - 75 used in this experiment was obtained from American type cell culture (ATCC), Rockville, MD. USA. The virus was the prototype strain, which had previously undergone two passages in GSM cells. The virus stock with Tissue Culture Infective Dose (TCID<sub>50</sub>) of  $6.5 \times 10^{-6}$  was aliquoted in 0.5 Tris Ethylenediamine tetra acetate disodium (TEN) buffer (pH 7.4) cryovials and stored in liquid nitrogen until used.

10 l of virus stock was added to 20 ml of specially formulated medium containing the test goat serum already serially diluted for the assay and incubated in a humidified chamber for 30 min at 37°C in an atmosphere of 5% carbon dioxide and 95% air before it was added to the 75 cm<sup>3</sup> tissue culture flasks containing GSM cells at 80% confluence at a low multiplicity of infection (MOI). This was incubated for 48 h after which the medium was changed and replaced with fresh 5 ml of the medium and incubated for 5 days after which the plates were stained and scored for cytopathic effects (CPE). Cultures were assayed in triplicates. Daily observations were made on the cultures so as to determine the time of onset and severity of CPE of the virus on the cells at the sub-optimal concentrations. A CAE virus infected cell may appear round, jagged, multinucleated, ballooned and fuse with others to form giant cells; it is not unusual to see some of them transforming to ghost cells. The number of these dead and dyeing cells in the cultures was counted in ten fields of microscope-view per tissue culture flask and the mean value from three flasks prepared from the same serum dilution was calculated for each dilution. Analysis of variance (ANOVA) was done to determine the dilution at which the CPEs were very significant ( $p < 0.05$ ). An average CPE count of 25 was taken as the assay threshold value while culture flasks with undiluted serum samples served as the control.

#### Cell staining

Following incubation of the experimentally infected cells for the specific periods allowed for the infection to take place, the growth

medium was removed and the cells fixed in absolute methanol for 10 min at room temperature. Giemsa stain, already diluted 1: 20 and filtered (using Whatman 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 viral cytopathic effects (CPE).

#### Neutralizing antibody determination

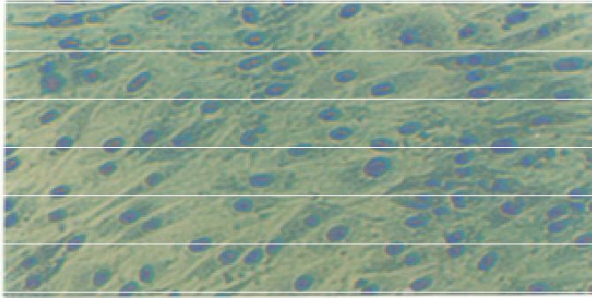
GSM cells at the third stage passage were seeded at  $5 \times 10^4$  per ml and grown for three days in 75 cm<sup>3</sup> cell culture flasks (Fisher Scientific, Swanne, GA, USA). A mixture of 500 µl inactivated homologous goat serum in 3.5 ml of growth medium already containing 50 µl of the CAE virus was incubated for 30 min in a humidified chamber of 5%CO<sub>2</sub> and 95% air. Following this, the supernatants from the cell culture flasks were removed and the mixture of the serum and virus added to the cells and incubated for determination of the neutralizing antibody titre.

## RESULTS

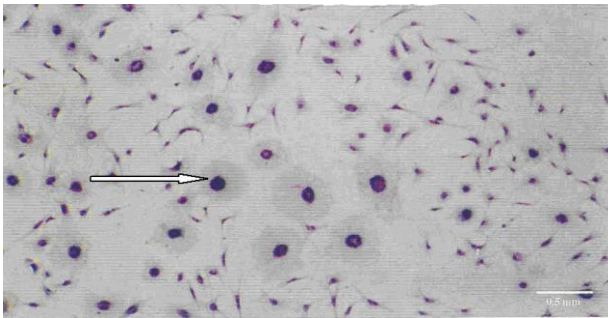
There were rapid reductions in cell population following infection of the cells with CAE virus at the sub-optimal concentrations of the serum due to the formation of CPEs (Figure 1). A normal GSM cell is spindle shaped with a single nucleus (Figure 2) while a CAE virus infected cell may appear roundish, jagged, multinucleated, ballooned and fuse with others to form giant cells.

CPE was observed on day 3 under the  $10^{-4}$  dilution while only slight cellular changes were observed at  $10^{-2}$  dilution on day 3. On day 5, one could hardly make out the cellular outlines thus forming typical ghost cells.

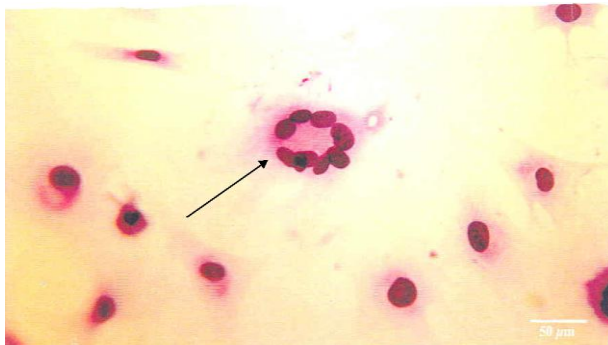
Virus growth occurred at both antibody supplemented and non-antibody supplemented cultures. High virus titres were consistently detected in cultures containing antibody at sub-optimal concentrations than in those without. At the highest MOI ( $10^{-1}$  dilution) high virus titres and CPEs



**Figure 2.** Normal (spindle shape) GSM cells. Magnification X 1,250.



**Figure 3.** Infected GSM cell at serum dilution of  $10^{-2}$  on day 5. Arrow shows a pyknotic goat synovial membrane cell on day 5. Magnification: X1250.



**Figure 4.** Multinucleated (infected) GSM cell with  $10^{-4}$  serum dilution (arrowed) on day 5. Arrow shows a multinucleated giant cell. Magnification: X1250.

were detected on day 1 post infection (pi) in the antibody supplemented cultures (Figure 3) and the controls. In contrast, cultures infected at low MOI of  $\log 10^{-5}$  had late virus growth and higher peak fold enhancement. CPE was detected in test cultures on days 2 and 3 pi under  $\log 10^{-3}$  to  $\log 10^{-5}$ ; peak-fold enhancement was recorded on days 3 and 4 with  $\log 10^{-4}$  and  $\log 10^{-5}$  on days 3 and 4 pi. On day 5, there was preponderance of ghost cells with faint cellular architecture and formation of multinucleated giant cells (Figure 4).

## DISCUSSION

CAE virus is a lentivirus capable of inducing chronic inducive mastitis, non-suppurative carpal arthritis and interstitial pneumonia in adult goats. In goat kids under six months of age, the virus causes leucencephalomalacia and ataxia resulting in wobbled gait, paraplegia an eventual death. The present assay in GSM cells showed that the virus can be enhanced in cultures by sub-neutralizing homologous antibody concentration. The enhancement capacity of homologous antibody can be attributed to the presence of enhancing antibody directed against both type and group specific determinants in the homologous antisera. This phenomenon has been shown to be a major pathogenic mechanism in some viral infections such as severe dengue infections (Halstead, 1982) and this may be a contributory factor in early deaths from rabies.

In natural infections, goats infected with CAE virus are known to produce large amounts of anti-envelope antibodies with negligible levels of neutralizing antibodies which unfortunately are not recognized by the virus neutralizing epitopes. This notwithstanding, the antibodies still bind to the CAE virions and the opsonized virions are endocytosed by macrophages. Unfortunately, this virus replicates in macrophages so that the antibody merely speeds up the virus replication cycle (Tizard, 2000). The implication of this is that vaccination may not give the desired result as this would only lead to more severe disease. This is of veterinary and public health significance.

The role of this phenomenon is far from being fully unraveled and exploited in disease onset and prevention. This then calls for more investigations in determining the role of antibody mediated enhancement in disease severity, acceleration of onset or course of disease, increased viraemia and pathological lesions.

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