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Summary

Bovine herpesviruses cause acute disease in cattle. Bovine herpesvirus 1 (BHV-1 or IBR) is a respiratory virus, while bovine herpesvirus 5 (BHV-5) affects the brain and causes a viral encephalitis. Studies in the laboratory showed no difference in the growth rate of BHV-1 or BHV-5 in blood vessel, brain, or kidney cells. The ability of BHV-1 to cause cells to die is not caused by apoptosis (programmed cell death). Further studies on the pathogenesis of bovine herpesviruses need to be conducted to improve control and prevention measures.

Key Words: Bovine, Herpesvirus, Pathogenesis

Introduction

Bovine herpesviruses (BHV) cause a myriad of clinical diseases in cattle. Infectious bovine rhinotracheitis (IBR) caused by bovine herpesvirus type 1 (BHV-1) is a contagious viral disease of cattle and the most common form seen in North America. Symptoms include upper respiratory tract disease that predisposes animals to shipping fever and abortion. Coughing, anorexia, depression, decreased milk production (in milking cows), weight loss, and increased salivation may also accompany these respiratory tract problems. A nasal discharge along with nasal congestion may develop and is referred to as red nose. Animals in a stressful environment such as a feedlot will often develop more complicated conditions of IBR and death will result. A second BHV-1 syndrome, infectious pustular vulvovaginitis (IPV) in the cow or infectious balanoposthitis (IBP), causes pustular lesions of the genital tract in females or males but rarely causes abortions and is seen in Europe. Disease of the reproductive system, IPV, is observed 1 to 3 days after mating and often leads to painful inflammation. The first signs of IPV are frequent micturition (urination) and a tail out of normal position followed later by small pustules on the vulva. Outbreaks of both the respiratory form, IBR, and genital disease, IPV, together are rare. A third syndrome, viral encephalitis, is caused by a virus closely related to BHV-1, bovine herpesvirus 5 (BHV-5). Clinical signs include incoordination, muscular tremor, aimless circling, blindness, confusion, recumbency, and death. BHV-5 (encephalitis) has been observed in scattered cases worldwide, with the highest prevalence in Australia and Argentina.

BHV-1 is transported by monocytes and white blood cells to target organs and then causes a life-long infection of the trigeminal nerve of cattle. This occurs with either natural BHV-1 infections or vaccination with modified live BHV-1. Recent research has suggested that BHV-5 is passed to the brain from the upper respiratory tract by branches of the trigeminal nerve. Some studies indicate the virus invades the blood stream by infecting the endothelial cells that line blood vessels and then directly infects the brain. By understanding the route of infection, improved methods of treatment and prevention can be targeted to the appropriate route.

BHV-1 causes massive cell destruction in any tissue infected with the virus. The cause of this cell death is unknown. Many viruses have been shown to kill cells by activating the programmed cell death cycle (apoptosis). Apoptosis results in a characteristic breakdown of the cell's genome, resulting in a DNA ladder.

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Other viruses kill cells through necrosis, the process where the virus totally disrupts the cell. No DNA ladder is formed in this process. Understanding which process is involved will allow the use of prevention techniques that can block apoptosis. Two experiments were conducted to measure 1) any difference in vitro in the replication of BHV-5 and BHV-1 that might explain why BHV-5 infects the central nervous system and 2) apoptosis in BHV-1 infected cells.

Materials and Methods

Cells. Primary endothelial cells were harvested from third trimester fetuses obtained from a slaughter house. Mardin Darby kidney (MDBK) and bovine turbinate (BT) cells were obtained from American Tissue Culture Collection (ATCC, Rockville, MD). Bovine neuronal (BN) cells were a gift from Dr. C. Jones, University of Nebraska, Lincoln, NE.

Viruses. BHV-1 (Cooper strain) was obtained from ATCC. BHV-5 was a gift from Dr. C. Whetstone, National Animal Disease Center, Ames, IA.

Virus Growth Curves. Flasks containing either MDBK, BT, or BN cells were inoculated with either BHV-5 or BHV-1 at a multiplicity of infection (MOI) of 10. Two flasks per virus were used each time period. The time periods used were 0, 12, 24, 36, and 48 hours. At time period 0 the virus was allowed to be in the flask for 1 hour and then removed. At each time point, the liquid media was removed, placed in a 3-ml snap cap tube, and frozen. The flask was then filled with 3 ml of minimum essential media (MEM) and placed in the -70°F (0°C) freezer. This was repeated for all time periods. The flasks were freeze-thawed three times and then the fluid centrifuged and removed from the cell pellet and placed in snap cap tubes and stored in the freezer. Tissue culture infectious dose 50 (TCID 50: the amount of virus required to kill 50% of the cells) was determined on the liquid media (supernatant) and the cell portion. MDBK cells were used in 96 well plates to measure the amount of each virus at the different time periods.

Apoptosis Measurements. Confluent monolayers of MDBK or BT cells were infected with BHV-1 at a MOI of 1. The cells were harvested at 0, 6, 12, 18, or 24 hours post infection or 0, 12, 24, 36, or 48 hours post infection. The cellular DNA was extracted and quantified and 800 to 1000 μg were loaded on a 1.0% agarose gel and electrophoresed in a horizontal gel apparatus. The DNA present in the gel was stained with ethidium bromide, visualized with UV light, and photographed.

Results and Discussion

Virus Growth. Virus production was measured in three different bovine cell lines: endothelial (Figure 1), central nervous system (CNS; data not shown), and kidney (data not shown). The virus yield from the three different cell types was similar for both viruses as shown in Figure 1. The other two cell types had similar growth curves (data not shown).

Apoptosis. The extracted cellular DNA ran at a high molecular weight greater than 23 Kb. The results indicated no evidence of the multiple bands (DNA ladder) characteristic of apoptosis (data not shown).

Pathogenesis studies of bovine herpesviruses have been limited. The first experiment led us to conclude that the difference in disease syndromes produced by BHV-1 or BHV-5 did not correlate with in vitro growth in endothelial cells, the cell type that plays a role in the blood-brain barrier or in bovine CNS cells, the target of BHV-5 in vivo. The spread of BHV-5 into the brain to cause a viral encephalitis can not be explained by faster growth in either nerve or blood vessel cells. The second experiment indicated that BHV-1 infection of bovine cells in vitro does not trigger apoptosis. Other mechanisms need to be investigated to determine the factors responsible for the rapid cell death seen in BHV-1 infections. Further study needs to occur to define the pathogenesis of the infection to improve bovine herpesvirus treatment and prevention measures.

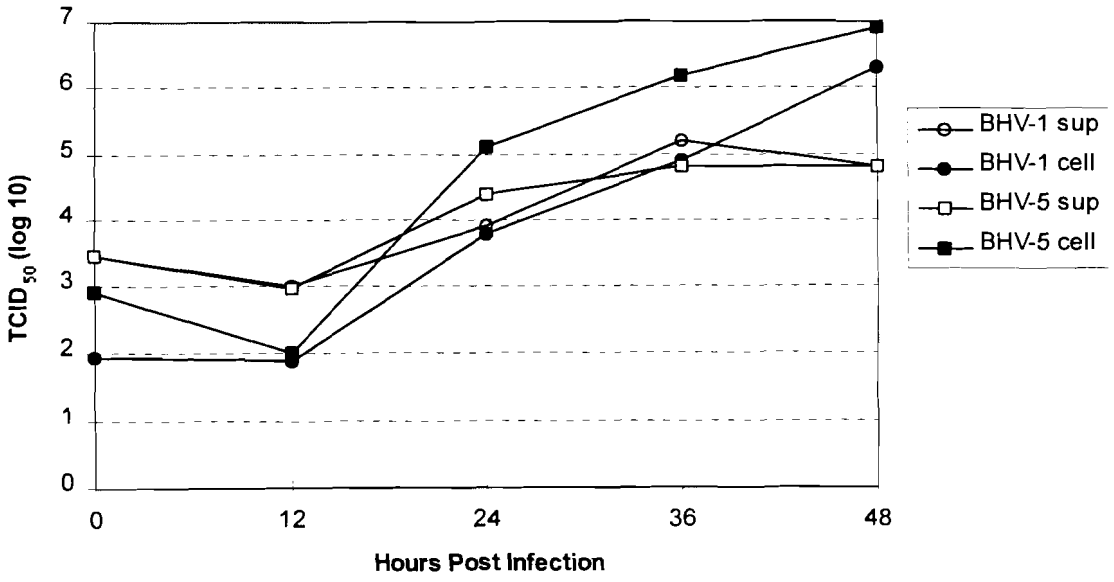

Figure 1. Growth of BHV Subtypes in Endothelial Cells