1995

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Studying Virus Cell Interactions: Finding New Ways to Prevent Infectious Bovine Rhinotracheitis in Cattle

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CATTLE 95-24

Summary

Infectious Bovine Rhinotracheitis (IBR; bovine herpesvirus 1 (BHV-1); Rednose) is one of several cattle respiratory viruses that plays a large role in the shipping fever complex. One of the major problems with IBR is that like other herpesviruses, once animals are infected they carry the virus for life. Investigations in our laboratory have been aimed at understanding how the IBR virus gets into cells. By understanding the mechanism that the virus uses we hope to develop strategies to prevent IBR infection. Our investigations indicate that IBR grows only in actively growing cells like those found in the upper respiratory tract of cattle. We also know that IBR does not grow well in slow growing cells like those found in the brain.

Key Words: IBR, Bovine Herpesvirus 1, Rednose

Introduction

Infection of cattle with BHV-1 (IBR virus) results in two major clinical syndromes: infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis (IPV). IBR is an infection of the epithelial cells of upper respiratory tract. IBR is seen throughout the world and is the most common form of BHV-1 infection in the U.S. It was first described in the U.S. in the 1950's. The disease is characterized by a 5- to 10-day incubation period with clinical signs that include fever (40.5 to 42°C), harsh cough, inappetence, depression, and a loss of milk production in lactating animals. A serous nasal discharge is present early in disease but progresses to a mucopurulent discharge. The animals may also develop a conjunctivitis. In animals that are under low stress, recovery is uneventful with animals returning to normal 5 to 10 days after onset of signs. However, in cattle that are stressed, the immunosuppressive effect of BHV-1 on the bacterial defense mechanisms of the lungs frequently results in lung complications due to secondary opportunistic bacterial infections. The most common and severe complication is the bovine respiratory disease complex commonly known as shipping fever. This complex results in a severe bronchopneumonia characterized by high morbidity and frequent mortality. Another frequent complication of IBR infection in pregnant cattle is abortion. Abortion occurs 3 to 6 weeks following infection mainly in cows between the 5th and 8th month of pregnancy and can result in an abortion rate of up to 25% of pregnant cattle.

IPV is the disease caused by BHV-1 in the reproductive tract of cows. Its counterpart in bulls is infectious balanoposthitis (IBP). IPV/IBP was first described in Europe over 150 years ago but was not identified as being caused by a herpesvirus until the late 1950's. This disease occurs 1 to 3 days following mating and results in a severe inflammatory reaction of the genital mucosa including edema, hyperemia, small pustules (1-2 mm in diameter) and a mucopurulent discharge. This disease frequently results in secondary bacterial infections. The acute phase of the disease lasts 2 to 4 days and is usually resolved by 10 to 14 days following onset. Another reproductive consequence of

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BHV-1 infection is infertility resulting from ovarian infection.

A third syndrome seen following BHV-1 infection is encephalitis. This is a rare disease of younger cattle that has been reported worldwide. This neurological disease results in signs that include incoordination, muscular tremors, recumbency, ataxia, and blindness that invariable leads to death. This neurogenic BHV-1 is genetically and antigenically related to the BHV-1 responsible for IBR and IPV. However, due to the serologic, genetic, and clinical differences, the name bovine herpesvirus 5 (BHV-5) has been suggested for this BHV-1-like agent.

BHV-1 establishes latent infections in cattle resulting in a lifelong carrier state. Reactivation and shedding of the virus can occur following stress (transport, parturition, etc.) or immunosuppression with glucocorticoids. The reactivation of the virus in the infected host results in inapparent clinical disease but serves as a source of infection for naive animals. We have been studying the interaction of BHV-1 and bovine cells to understand the mode the virus infects cells. An increased understanding of these interactions can be used to prevent infections and shedding of the virus.

Materials and Methods

The goal of this study was to investigate the cellular changes that occur when bovine herpesvirus 1 (BHV-1) interacts with target cells. This research project pursued three questions. First, do virus-host cell interactions trigger a cellular signal transduction cascade that involves changes in cytoplasmic [Ca^{2+}] or pH? Second, does virus infection alter host cell DNA synthesis? Third, is the efficiency of BHV-1 replication dependent on the cell cycle?

For the first question we used fluorescent probes to examine the possibility that interactions between BHV-1 and cellular receptors induce a signal transduction cascade that results in changes in intracellular Ca^{2+} and pH.

For the second question we measured the effect of BHV-1 on BT cell DNA utilizing the incorporation of (methyl-^{3}H) thymidine as an indicator of DNA synthesis. The use of UV inactivated BHV-1 (UV BHV-1) and quiescent cells makes these studies unique.

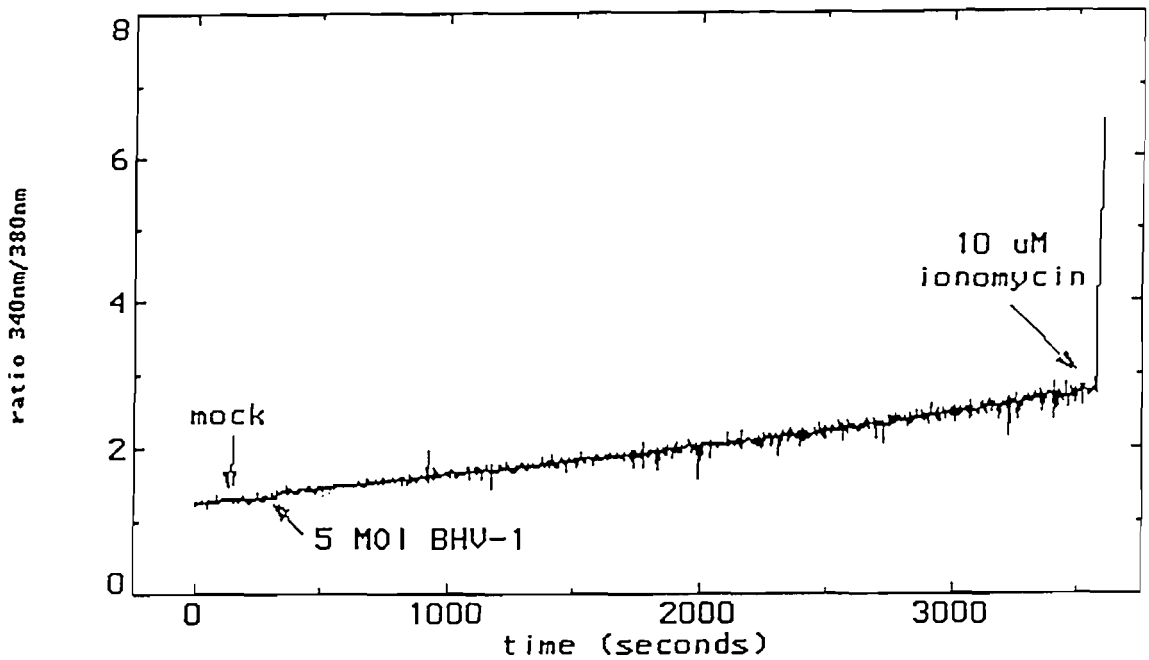
For the third question we compared BHV-1 replication in actively dividing and quiescent primary bovine turbinate (pBT) cells.

Results and Discussion

The results showed that BHV-1 does not stimulate changes in either cytoplasmic Ca^{2+} concentration (Figure 1) or pH (data not shown). A variety of cell types, probe loading procedures, and analysis techniques were used to measure the effect of BHV-1 on these two parameters of cell signaling. With these methods, a change in cytoplasmic free calcium concentration ($[Ca^{2+}]_i$) was detected following treatment of bovine turbinate cells (BT) and peripheral blood lymphocytes with growth factors. A change in cytoplasmic pH could not be detected in BT or Madin Darby bovine kidney cells treated with growth factors. These findings become important when interpreting the effect of BHV-1 on cell signaling. Growth factor stimulated changes in $[Ca^{2+}]_i$ provide a biological control for Ca^{2+} influx. The results with BHV-1 indicate that virus interactions with host cells do not trigger a Ca^{2+} dependent cell signaling pathway. This same interpretation cannot be made with cytoplasmic pH since the expected change in pH was not detected in cells treated with growth factors. This implies that either the probe, equipment, or some other factor was not functioning properly resulting in an undetected pH change. Assuming an undetected pH change did occur, the effect of BHV-1 on cytoplasmic pH can only be speculated.

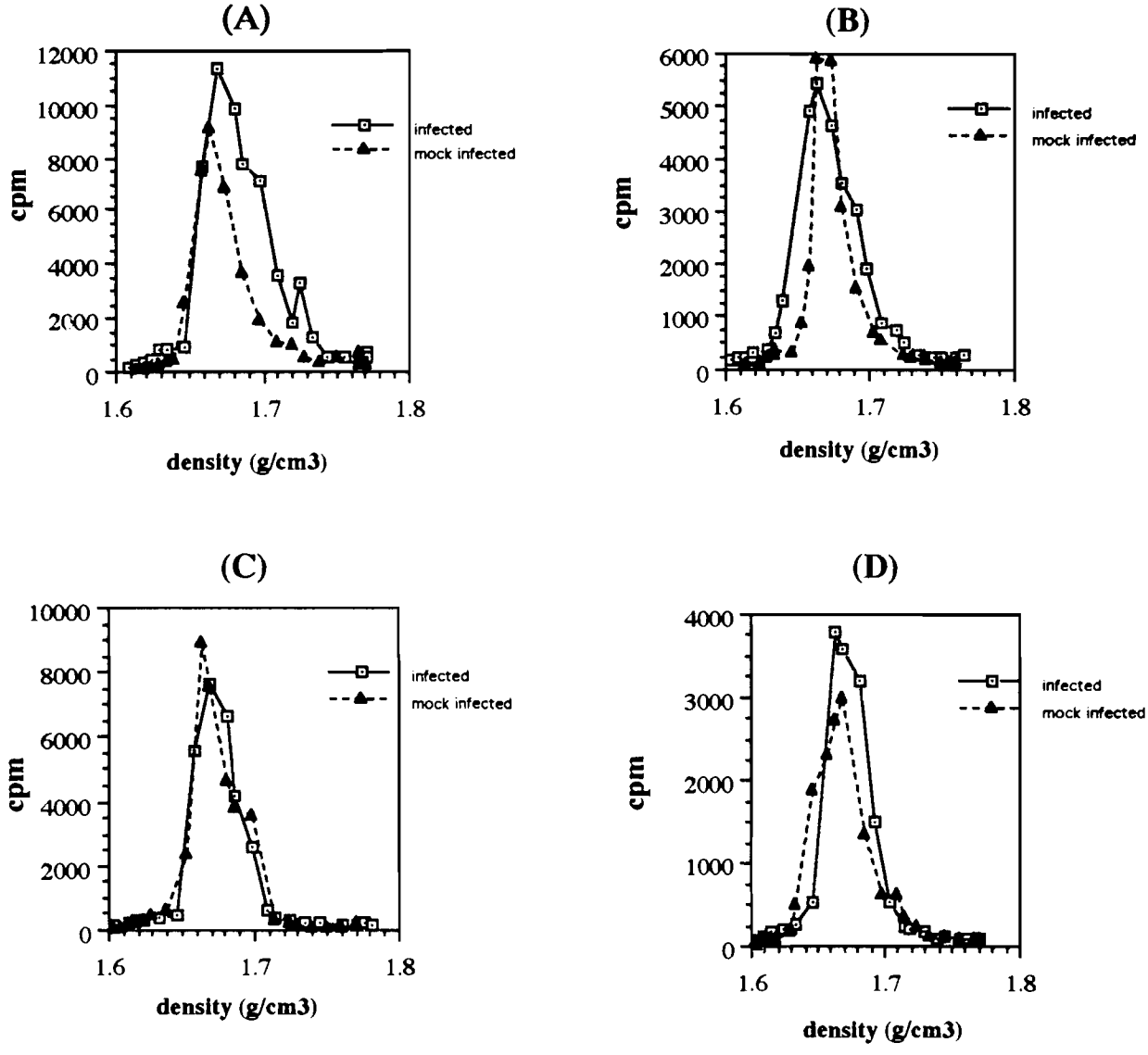
Neither BHV-1 nor UV BHV-1 stimulated cellular DNA synthesis in actively dividing or quiescent cells (Figure 2). Our studies demonstrated that BHV-1 infection of actively dividing cells did not completely inhibit host cell DNA synthesis. Interestingly, we could not detect viral DNA synthesis in BHV-1 infected quiescent cells. This was an important observation because it suggested that BHV-1 replication is cell cycle dependent.

Figure 1



PBL were loaded with 5 μM of fura-2 AM. 5 x 10^6 PBL were placed in a cuvette an mock infected with MEM followed by 5 MOI of BHV-1. Fluorescence measurements were made by alternating the excitation wavelength between 340 and 380 nm and collecting the emissions at 510 nm. 10 μM ionomycin was added at the end of the experiment to saturate the probe with Ca^{2+}. Additions are indicated on the graph with arrows and text.

Figure 2

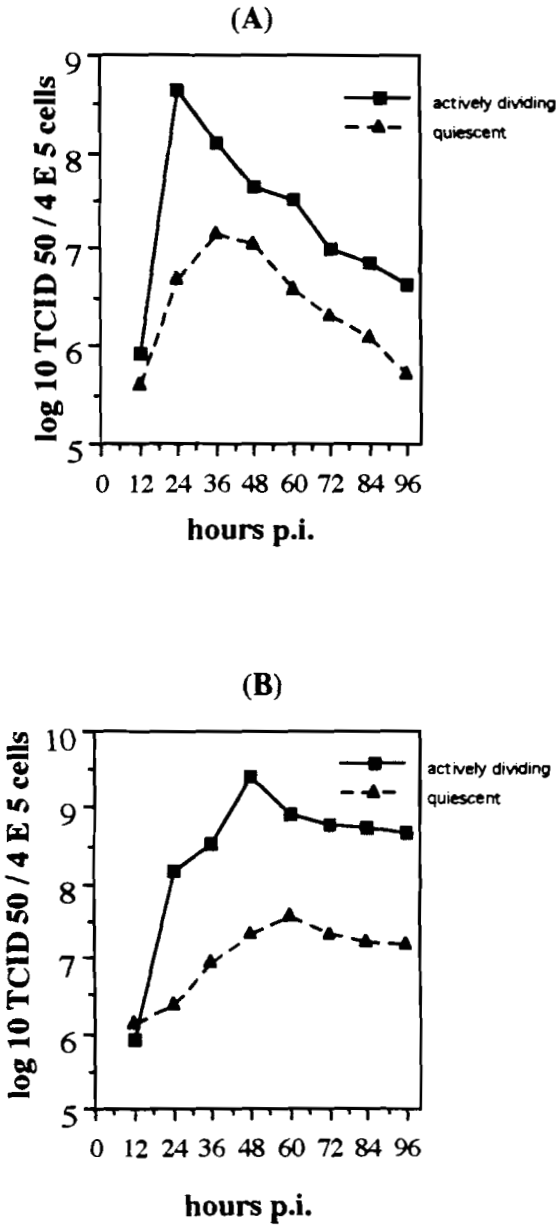


Actively dividing (A) and quiescent (B) BT cells were infected with 0.3 MOI of BHV-1 and treated with 1 mCi of (methyl-3H) thymidine for 24 hours. Actively dividing (C) and quiescent (D) BT cells were treated with UV inactivated BHV-1 and (methyl-3H) thymidine as described above. Total cellular DNA was isolated and isopycnic centrifugation in CsCl was used to separate virus and cellular DNA. The density of each fraction was determined using a refractometer and the radioactivity measured with a scintillation counter. Thymidine incorporation is expressed in counts per minute (cpm). The clear squares represent virus infected cell DNA and solid triangles represent mock infected cell DNA.

Higher virus yields (Figure 3) and more viral protein synthesis (data not shown) occurred in cells that were undergoing active DNA synthesis. These differences were not due to a reduced amount of BHV-1 virions attaching to or infecting the quiescent cells. This suggests that a factor associated with the quiescent cells is limiting BHV-1 gene expression and replication. Viral gene expression and replication in the quiescent cells may have been limited by a decreased supply of cellular machinery such as RNA polymerase II and transcription factors. Quiescent cells would have a decreased quantity of these proteins compared with cells that were actively dividing.

The co-evolution of a pathogen with its host leads to the development of some interesting relationships. Through selective pressure, viral phenotypes adapt and become better able to modify and use the host cell. This research represents a component of a model that envisions early virus-host cell interactions as a means for viruses to alter host cell physiology by triggering different signaling cascades. These cascades serve the virus by modifying the intracellular environment in a way that promotes viral synthetic events. Present data suggest that BHV-1 does not activate quiescent cells and that it replicates more efficiently in actively dividing cells.

Figure 3



Actively dividing and quiescent pBT cells were infected with 5 MOI of BHV-1 and the TCID50 of each cell associated (A) and cell free (B) virus sample was measured. Solid squares represent actively dividing cells infected with BHV-1 and solid triangles represent infected quiescent cells. Titers are expressed as the log10 TCID50 per 4 x 105 cells. Each data point is the average of triplicate samples.