ANALYSIS OF VIRUS HOST INTERACTIONS FOLLOWING INFECTION WITH BOVINE HERPESVIRUS 1 (BHV-1)

by

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ANALYSIS OF VIRUS HOST INTERACTIONS FOLLOWING INFECTION WITH BOVINE HERPESVIRUS 1 (BHV-1)

Luciane Teresinha Lovato, Ph.D.

University of Nebraska, 2002

Advisor: Clinton Jones

Bovine herpesvirus 1 (BHV-1) is an important pathogen of cattle that causes respiratory and reproductive disease. BHV-1 induces latency in sensory ganglia that innervate the site of primary infection. The latency-related (LR) gene is the only BHV-1 gene abundantly expressed during latency, suggesting that LR gene products regulate the latency/reactivation cycle. A mutant virus with three stop codons at the beginning of the open reading frames within the LR gene has been constructed in our laboratory (Inman, M, Lovato, L, Doster, A, Jones, C. J. Virol. 75:8507-8515, 2001). To determine the effects of LR gene products on distinct aspects of BHV-1 pathogenesis, calves were infected with the LR mutant virus or wt BHV-1 and virological and molecular studies performed. *In situ* hybridization demonstrated that the LR mutant virus does not efficiently colonize the trigeminal ganglia (TG) or conjunctiva of infected calves when compared to the wt BHV-1. Apoptosis analysis by TUNEL assay and immunohistochemistry for cleaved capase 3 indicated that the LR mutant virus induced higher amounts of apoptosis in TG when compared to wt. The ability of the virus to induce latency in lymphoreticular tissues was also examined. Viral DNA was consistently

detected in peripheral mononuclear cells (PBMC) of calves infected with wt BHV-1 or LR mutant virus after acute infection, suggesting viral DNA persisted in these cell types. The levels of heat shock proteins (hsp) were examined in cultured cells following infection with wt BHV-1 or the LR mutant virus. Hsps 60, 70, 90, and 25, protein levels were not dramatically altered after infection. In summary, a mutation in the LR gene of BHV-1 impaired establishment and reactivation from latency, in part, because LR gene products inhibit apoptosis. In contrast, the LR gene mutation does not appear to affect viral persistence in PBMC.

DEDICATION

This work is dedicated to Dr Nereu A. Streck, my husband.

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Chapter 1

Detection of BHV-1 nucleic acid in trigeminal ganglia and conjunctiva following infection of cattle

Abstract

The RNA encoded by the latency related (LR) gene of BHV-1 is the only viral transcript abundantly expressed in trigeminal ganglia (TG) of infected calves during latency. The latency-associated transcript (LAT), the correlate gene in Herpes simplex virus, is essential for efficient establishment and reactivation of latency in mice and rabbits. It is hypothesized that expression of wild type (wt) LR gene products has an important role in establishment, maintenance, and reactivation from latency. A LR mutant virus containing three stop codons at the beginning of the LR open reading frames was constructed in our laboratory (Inman, M, Lovato, L, Doster, A, Jones, C, J. Virol, 2001). Calves were infected with the LR mutant or the wt BHV-1 virus, and conjunctiva and TG tissues were examined for the presence of viral nucleic acid by *in situ* hybridization at different time points after infection. During acute infection, wt BHV-1 nucleic acid was detected at days 2 and 4 post-infection in conjunctiva tissues, but not at 6, 10 and 14dpi. The LR mutant virus was not detected in conjunctiva at the same time points. The nucleic acid of wt BHV-1 was also detected in the nuclei of TG neurons during acute infection, latency and reactivation from latency. However, viral nucleic acid was not detected in TG neurons from calves infected with the LR mutant virus at any

time after infection as judged by *in situ* hybridization. Taken together, these findings suggest that LR gene products are essential for the latency-reactivation cycle in cattle.

Introduction

BHV-1 is an important pathogen of cattle and is classified in the *Herpesviridae* family, *Alphaherpesvirinae* subfamily. The virus contains an icosaedral nucleocapsid containing 162 capsomeres that is surrounded by tegument and a lipid bilayer envelope, forming virions 150-200 nm in diameter (Tikoo et al, 1995). It has a double-stranded DNA genome of approximately 135-140 kbp (Tikoo et al, 1995, Jones, 1998). Infection of permissive cells, MDBK (Madin Darby Bovine Kidney) for example, induces cythopathic effect (CPE).

BHV-1 infects the respiratory or genital tract of bovine, inducing a variety of clinical symptoms including conjunctivitis, rhinotracheitis, vulvovaginitis, balanopostitis, meningoencephalitis and abortion (Kahrs, 1977, Tikoo et al, 1995, Jones, 1998). This virus has worldwide distribution as demonstrated by prevalence of antibodies directed against BHV-1 in serum of bovine from different countries (Jones, 1998).

BHV-1 gene expression during lytic infection of bovine cells goes through a temporal cascade with immediate-early (IE), early (E) and late (L) genes being expressed (Wirth et al, 1989, Wirth et al, 1991, Jones, 1998). BTIF, a viral protein in the tegument, interacts with a cellular transcription factor (Oct-1) and stimulates IE gene expression. Subsequently, IE gene stimulates E gene expression, viral DNA replication occurs, L gene expression occurs leading to virion assembly and release (reviewed in Jones, 1998).

BHV-1 gene expression in trigeminal ganglia of acutely infected bovine was altered (Schang and Jones, 1997). Full-length and poly-adenylated transcripts of ribonucleotide reductase (RR) or bICP22 (early genes) were detected earlier during

infection than full-length and poly-adenylated transcripts of bICP0 or bICP4 (immediate-early genes). Changes in gene expression may favor the establishment of latency .

Viruses from the *Alphaherpesvirinae* subfamily induce latency in sensory neurons that innervate the site of acute infection (Roizman and Sears, 1996). Bovine herpesvirus 1 (BHV-1) induces latency in trigeminal ganglionic neurons of rabbits (Rock et al, 1986) and cattle (Ackermann et al, 1982).

Latency can be divided into three important steps: establishment, maintenance, and reactivation. Viral and cellular factors contribute to each of these steps to regulate latency. The latency related transcript (LRT) is the only abundant BHV-1 gene expressed during latency in rabbits or bovine, suggesting that this gene is important for one or more of the steps in latency (Jones, 1998).

The presence of a latency-related transcript during BHV-1 infection was first described by Rock et al (1986). LR-RNA, but not viral DNA, was readily detected in trigeminal ganglia of BHV-1 latently infected rabbits by *in situ* hybridization. Conversely, viral DNA and RNA were detected during acute infection. The results suggested that the BHV-1 genome is transcriptionally active during latent infection. Further studies showed that transcription in neurons of BHV-1 latently infected rabbits was restricted to a region of 1.9kb of the viral genome (Rock et al, 1987).

The analysis of the LR RNA sequence indicated the presence of a promoter at the 5' terminus and two open reading frames on the sense strand, and another open-reading frame on the complementary strand (Kutish et al, 1990). It is believed that species specific as well as tissue-specific, factors regulate the LR promoter. When compared to the SV40 promoter, the LR promoter was at least 10 times more efficient in neuronal

cells, but 6 times less efficient in other bovine, rodent, or monkey cells (Jones et al, 1990). In addition, the deletion of a 146-bp fragment had different effect on bovine cells as compared to rabbit neurons, monkey fibroblasts, or rodent cells, suggesting that the promoter is regulated by a bovine-specific factor(s).

Exonuclease III footprinting (ExoIII) experiments and electrophoretic mobility shift assay (EMSA) demonstrated that neuronal cell specific factors bind to defined regions of the LR promoter (Bratanich and Jones, 1992, Delhon and Jones, 1997). Sequences within the protected LR promoter region were able to cis-activate the herpes simplex type 1 (HSV-1) thymidine kinase promoter when transfected into neuronal cells, but cis-activation was absent in transfected non-neuronal cells (Bratanich and Jones, 1992). These results were later confirmed by EMSA showing that abundant factors in dorsal root ganglia of cattle and rat pheochromocytoma cells (PC12) bound to a smaller fragment of the LR promoter (Delhon and Jones, 1997).

Differential activation of viral promoters has also been observed in tissues from HSV-1-infected mice (Mitchell, 1995, Loiacono et al, 2002). Transgenic mice containing the ICP4, ICP0, and/or ICP27 (immediate-early genes) promoter fused to the bacterial beta-galactosidase gene were generated. The beta-galactosidase activity was detected in nervous tissues, but not in non-neural tissues when non HSV-1 infected mice tissues were analyzed. In addition, activation of ICP0 and ICP27 promoters was different for distinct subsets of neurons (Mitchell, 1995, Loiacono et al, 2002).

The beta-galactosidase assay was also used to examine the activity of an IE promoter or the promoter that directs expression of HSV-1 latency-associated transcript (LAT) in the mouse ear model (Lachmann et al, 1999). In this study, a lacZ reporter gene