A review of the biology of bovine herpesvirus type 1 (BHV-1), its role as a cofactor in the bovine respiratory disease complex and development of improved vaccines

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Received 14 August 2007; Accepted 12 October 2007

Abstract
Infection of cattle by bovine herpesvirus type 1 (BHV-1) can lead to upper respiratory tract disorders, conjunctivitis, genital disorders and immune suppression. BHV-1-induced immune suppression initiates bovine respiratory disease complex (BRDC), which costs the US cattle industry approximately 3 billion dollars annually. BHV-1 encodes at least three proteins that can inhibit specific arms of the immune system: (i) bICP0 inhibits interferon-dependent transcription, (ii) the UL41.5 protein inhibits CD8⁺ T-cell recognition of infected cells by preventing trafficking of viral peptides to the surface of the cells and (iii) glycoprotein G is a chemokine-binding protein that prevents homing of lymphocytes to sites of infection. Following acute infection of calves, BHV-1 can also infect and induce high levels of apoptosis of CD4⁺ T-cells. Consequently, the ability of BHV-1 to impair the immune response can lead to BRDC. Following acute infection, BHV-1 establishes latency in sensory neurons of trigeminal ganglia (TG) and germinal centers of pharyngeal tonsil. Periodically BHV-1 reactivates from latency, virus is shed, and consequently virus transmission occurs. Two viral genes, the latency related gene and ORF-E are abundantly expressed during latency, suggesting that they regulate the latency-reactivation cycle. The ability of BHV-1 to enter permissive cells, infect sensory neurons and promote virus spread from sensory neurons to mucosal surfaces following reactivation from latency is also regulated by several viral glycoproteins. The focus of this review is to summarize the biology of BHV-1 and how this relates to BRDC.

Keywords: bovine herpesvirus type 1 (BHV-1), bovine respiratory disease complex (BRDC), pneumonia, immune suppression, latency, pathogenesis

Disease and clinical symptoms induced by bovine herpesvirus type 1 (BHV-1)

BHV-1 is an α-herpesvirinae subfamily member that causes significant economical losses to the cattle industry (Turin et al., 1999). Three BHV-1 subtypes, BHV-1.1 (1), BHV-1.2a (2a) and BHV-1.2b (2b), have been identified based on antigenic and genomic analysis (Metzler et al., 1985). Subtype 1 virus isolates are the causative agents of infectious bovine rhinotracheitis (IBR) and are frequently found in the respiratory tract as well as aborted fetuses. Subtype 1 strains are prevalent in Europe, North America and South America. Subtype 2a is frequently associated with a broad range of clinical manifestations in the respiratory and genital tracts such as IBR, infectious pustular vulvovaginitis (IPV), balanopostitis (IPB) and abortions (Oirschot, 1995). Subtype 2a is prevalent in
Brazil and was present in Europe prior to the 1970s (Oirschot, 1995). Subtype 2b strains are associated with respiratory disease and IPV/IPB, but not abortion (Oirschot, 1995; D’Arce et al., 2002). Subtype 2b strains are less pathogenic than subtype 1 and are frequently isolated in Australia or Europe, but not Brazil (Edwards et al., 1990).

In feedlot cattle, the respiratory form of BHV-1 is the most common (subtype 1 strains). In breeding cattle, abortions or genital infections tend to be more common. Genital infections can occur in bulls (IPB) and cows (IPV) within 1–3 days of mating or close contact with an infected animal. Transmission can also occur in the absence of visible lesions and through artificial insemination with semen from sub-clinically infected bulls.

The incubation period for the respiratory and genital forms of BHV-1 is 2–6 days (Yates, 1982). With respect to respiratory disease, clinical symptoms include high fever, anorexia, coughing, excessive salivation, nasal discharge, conjunctivitis with lacrimal discharge, inflamed nares, and dyspnea if the larynx becomes occluded with purulent material. Nasal lesions consist of numerous clusters of grayish necrotic foci on the mucous membrane of septal mucosa. In the absence of bacterial pneumonia, recovery typically occurs 4–5 days after the onset of clinical symptoms. Abortions can occur at the same time as respiratory disease, but may also be seen up to 100 days after infection, which is likely due to reactivation from latency.

With respect to genital infections, the first clinical signs are frequent urination and a mild vaginal infection (Yates, 1982). It is also common to observe swollen vulva or small papules followed by erosions and ulcers on the mucosal surface. In bulls, similar lesions occur on the penis and prepuce. If secondary bacterial infections occur, there may be inflammation of the uterus and transient infertility with purulent vaginal discharge for several weeks. In the absence of bacterial infections, animals usually recover within 2 weeks after infection. Regardless of the involvement of secondary bacterial infection, BHV-1 establishes lifelong latency following acute infection.

The seroprevalence of BHV-1 ranges from 14 to 60% in Africa and from 36 to 48% in Central and South America (Straub, 1990). In The Netherlands, 49% of cows that give birth to their first calf are seropositive, whereas 91% of older cows are seropositive (Wuyckhuise et al., 1994). Serological testing and removal of infected animals has been successfully used to eliminate BHV-1 from Denmark, Switzerland and Austria (Ackermann and Engels, 2005). In these countries, cattle populations are relatively small and movement of cattle can be controlled. With respect to the United States and other countries, eradication would be difficult, perhaps impossible, and very expensive.

The seroprevalence of BHV-1 in bison raised on a ranch is 43.8% (Sausker and Dyer, 2002), indicating that infection can readily occur in bison. One would assume that BHV-1 is readily transmitted from buffalo to cattle and vice versa. Since more buffalo meat is being consumed each year in the United States, BHV-1 infections will have an impact on the emerging bison industry. It is not clear what the percentage of sero-positive animals is due to vaccination. Nevertheless, it is clear that BHV-1 is widely disseminated in the field.

**Relationship between BHV-1-induced immune suppression and bovine respiratory disease**

Bovine respiratory disease complex (BRDC), also referred to as 'shipping fever', costs the US cattle industry at least $3 billion year⁻¹ (Tikoo et al., 1995a, b; National Agricultural Statistics Service (NASS), 1996; Bowland and Shewen, 2000). In addition to the clinical symptoms described above for BHV-1, BHV-1 can initiate BRDC by transiently suppressing the immune system of infected cattle (Yates, 1982). BHV-1-induced immune suppression leads to secondary bacterial infections (Pasteurella haemolytica, Pasteurella multocida and Haemophilus somnus for example) that can cause pneumonia (Yates, 1982). Increased susceptibility to secondary infection correlates with depressed cell-mediated immunity after BHV-1 infection (Griebel et al., 1987a, b, 1990; Carter et al., 1989). CD8⁺ T-cell recognition of infected cells is impaired by repressing expression of major histocompatibility complex (MHC) class I and the transporter associated with antigen presentation (Hariharan et al., 1993; Nataraj et al., 1997; Hinkley et al., 1998). CD4⁺ T-cell function is impaired during acute infection of calves because BHV-1 infects CD4⁺ T-cells and induces apoptosis (Winkler et al., 1999). Two viral genes, bICP0 and UL41.5, can inhibit specific immune responses in the absence of other viral genes. A discussion of these genes and how they inhibit immune responses is provided below.

**The bICP0 protein activates viral gene expression and inhibits interferon (IFN) signaling pathway**

The bICP0 protein is the major transcriptional regulatory protein because it activates expression of all viral promoters (Everett, 2000), and the bICP0 transcript is constitutively expressed during productive infection (Fraefel et al., 1994). The ICP0 homologues encoded by BHV-1 and herpes simplex virus type 1 (HSV-1) contain a well-conserved $C_4H_4$ zinc RING finger near their respective N-termini (Fig. 1A). Mutational analysis has demonstrated the importance of the $C_4H_4$ zinc RING finger domain of bICP0 and ICP0 (Everett, 1987, 1988; Everett et al., 1993; Inman et al., 2001c). ICP0 (Maul et al., 1993; Maul and Everett, 1994; Everett et al., 1997, 1999a, b) and bICP0 (Parkinson and Everett, 2000; Inman et al., 2002).
et al., 2001c) colocalize with and disrupt the proto-oncogene promyelocytic leukemia protein-containing nuclear domains. Disruption of the bICP0 zinc RING finger prevents trans-activation of a simple viral promoter (Zhang and Jones, 2005) and impairs the ability of bICP0 to stimulate plaque formation following transfection of BHV-1 DNA into cultured bovine cells (Inman et al., 2001c; Geiser and Jones, 2003). The bICP0 protein contains two transcriptional activation domains (TAD) and a nuclear localization signal (NLS) that is necessary for efficient transcriptional activation (Zhang and Jones, 2005) (Fig. 1A). Unlike most transcription factors, bICP0 or ICP0 does not apparently bind to specific DNA sequences, suggesting that bICP0 activates transcription by interacting with cellular transcriptional machinery. Support for this prediction comes from the finding that bICP0 associates with chromatin remodeling enzymes, histone deacetylase 1 (HDAC1) (Zhang, 2001) and p300, a histone acetyl transferase (Zhang and Jones, 2001). The ability of bICP0 to associate with HDAC1 and p300 correlates with activating viral transcription.

In the absence of other viral genes, bICP0 inhibits IFN signaling (Henderson et al., 2005). bICP0 (directly or indirectly) reduces IRF3 (IFN regulatory factor 3) protein levels in human or bovine cells, which consequently leads to reduced IFN-β promoter activity (Saira et al., 2007). In addition, bICP0 inhibits the ability of IRF7 to activate IFN-β promoter activity, but bICP0 does not dramatically reduce IRF7 protein levels (Saira et al., 2007). The RING finger of ICP0 (Van Sant et al., 2001; Boutell et al., 2002; Boutell and Everett, 2003) and bICP0 (Dia et al., 2005) is an E3 ubiquitin ligase, suggesting that the bICP0 RING finger mediates IRF3 degradation. The finding that a functional proteasome is necessary for bICP0-induced IRF3 degradation (Saira et al., 2007) supports the notion that the bICP0 RING finger mediates IRF3 degradation. The RING finger is not the sole component necessary for bICP0-induced IRF3 degradation because specific mutations near the C-terminus of bICP0 are also important (Saira et al., 2007). Additional studies are necessary to understand how bICP0 inhibits IRF7 induction of IFN-β promoter activity as well as the mechanism by which bICP0 induces IRF3 degradation.

What role does IRF3 play during IFN production, and why is it important for bICP0 to target IRF3 functions? Following virus infection, two cellular protein kinases, IKKε or TBK-1, phosphorylate serine residues at the C-terminus of IRF3, which induces IRF3 homodimerization and nuclear translocation (Fitzgerald et al., 2003; Sharma et al., 2003). Nuclear IRF3 associates with other transcriptional activators resulting in direct binding and stimulation of IFN-β promoter activity (Wathelet et al., 1998). IRF3 also directly binds several consensus DNA-binding sites, including ISRE (IFN response elements), and can consequently stimulate transcription of IFN-stimulated genes in the absence of IFN (Guo et al., 2000; Mossman et al., 2001). IRF3 activation is an immediate early regulator of the IFN response, whereas IRF7 is believed to be a component of the early response (Yoneyama et al., 1998; Yuan et al., 1998; Sato et al., 2001). Surprisingly, IRF7 is more important with respect to

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Fig. 1. Schematic of bICP0, LR gene and ORF-E genes within the BHV-1 genome. (A) Schematic of BHV-1 genome, location of bICP0 and ORF-E. Domains within bICP0 include an NLS (nuclear localization sequence), TAD (transcriptional activation domain), acidic domain and the C3HC4 zinc RING finger. (B) The start sites for LR-RNA transcription during latency and productive infection were previously described (Devidrey and Jones, 1998; Hossain et al., 1995). The C-termini of bICP0 and ORF-E overlap the LR gene and are antisense with respect to bICP0 and ORF-E. Reading frames B and C (RF-B and RF-C) each contain an open reading frame (ORF) that lacks an initiating Met. The (*) denotes the position of stop codons that are in frame with the respective ORF.
inhibiting viral infection when IRF3 versus IRF7 knockout mice are compared (Honda et al., 2005). Since BHV-1 does not grow in mice unless they lack IFN receptors (Abril et al., 2004), it is clear that the ability of BHV-1 to inhibit innate immune responses, IFN for example, is crucial for pathogenesis in cattle and host range.

**UL49.5**

The BHV-1 UL49.5 open reading frame (ORF), also known as glycoprotein N (gN), encodes a 96-amino-acid protein with an apparent molecular mass of 9 kDa (Liang et al., 1993). The UL49.5 ORF contains a signal peptide (N-terminal 22 amino acids), an extracellular domain of 32 amino acids, a transmembrane region of 25 amino acids and a 17 amino acids long cytoplasmic tail (Liang et al., 1993) (Fig. 2). The UL49.5 protein is expressed as a non-glycosylated type 1 membrane protein (Liang et al., 1993, 1996).

All gN herpesvirus orthologues studied to date form complexes with glycoprotein M (gM), which is encoded by the UL10 ORF (Jonsson et al., 1998; Wu et al., 1998; Rudolph et al., 2002). gM is important for secondary envelopment because in PRV secondary envelopment in the Golgi and subsequent egress require gM, in the absence of gE (Brack et al., 1999). The gN homologues encoded by PRV and BHV-1 inhibit transporter-associated antigen processing (TAP)-mediated transport of cytosolic peptides into ER, which consequentially blocks the assembly of peptide-containing ternary MHC-I complexes in *vivo* in virus-infected cells (Koppers-Lalic et al., 2005; Lipiriska et al., 2006). Furthermore, the BHV-1 gN targets the TAP complex for proteosomal degradation (Koppers-Lalic et al., 2005). The TAP complex consists of a TAP1/TAP2 heterodimer, both of which are members of the ATP-binding cassette transporter superfamily (Androlewicz et al., 1993; van Endert et al., 2002; Koch et al., 2004).

Peptide transport by TAP is a critical step in MHC class I antigen presentation (Ahn et al., 1996; Hughes et al., 1997; Ambagala et al., 2004; Koppers-Lalic et al., 2005). In the absence of a functional TAP transporter, most MHC class I molecules are not loaded with peptides (Hughes et al., 1997). However, they are retained within the ER and ultimately directed for degradation by the proteasome (Hughes et al., 1997). Structurally, both gM and TAPs are similar because they both contain multiple membrane spanning segments (Wu et al., 1998; Koch et al., 2004). A gN mutant that lacks the cytoplasmic tail can still bind to the TAP complex and block peptide transport, but this mutant gN protein does not degrade TAP (Koppers-Lalic et al., 2005). Therefore, sequences within the gN transmembrane domain are likely to interact with TAP. The phenotype of a viral strain expressing the various gN mutant proteins has not been tested in the context of a virus and/or virally infected cells. In summary, the ability of gN to inhibit TAP is hypothesized to prevent virus-infected cells from being killed by CD8+ T-cells.

**Viral encoded structural proteins mediate cell entry and pathogenesis**

To fully understand the role that BHV-1 plays in BRDC, one must understand how BHV-1 initiates productive infection. Consequently, this section summarizes what we know about how the virus enters cultured cells and disseminates *in vivo*.

α-Herpesvirus glycoproteins are required for viral particles to bind and penetrate permissive cells. In addition, a subset of viral glycoproteins is necessary for virus release, cell fusion and cell-to-cell spread. Ten of the 12 BHV-1 envelope proteins (gB, gC, gD, gE, gG, gH, gI, gM and gK) are glycosylated and two (gN and Us9) are non-glycosylated (Van Drunen Littel-van den Hurk and Babuik, 1986; Liang et al., 1993; Baranowski et al., 1995, 1996; Khattar et al., 1996; Schwyzer and Ackermann, 1996; Brideau et al., 1998; Meyer et al., 1998; Mettenleiter, 2000, 2002; König et al., 2002; Nakamichi et al., 2002; Chowdhury et al., 2006). Five envelope proteins (gB, gD, gH, gL and gK) are essential for growth in cultured cells, whereas the remaining seven (gC, gE, gG, gM, gN and Us9) are not essential for growth in cultured cells (Schwyzer and Ackermann, 1996; Brideau et al., 1998; Butchi et al., 2007).

All α-herpesvirus gC homologues (including BHV-1) facilitate attachment of virus particles to the cell surface by binding to heparan sulfate proteoglycan (Okazaki et al., 1991). In the absence of gC, gB complements the attachment function of gC by binding to heparan sulfate
following initial attachment mediated by gB and/or gC, the next step in viral entry requires the interaction of gD with the cellular receptor nectin 1, also known as HveC (Spear, 1993, 2004; Campadelli-Fiume et al., 2000, 2007; Spears et al., 2000; Mettenleiter, 2002; Spear and Longnecker, 2003; Campadelli-Fiume and Gianni, 2006), or HVEM, a member of the tumor necrosis factor family of proteins (Montgomery et al., 1996). Nectin 1 belongs to a family of intercellular adhesion molecules whose ectodomain contains three immunoglobulin-like domains (Ogita and Takai, 2006). Certain members of the tumor necrosis factor family can induce apoptosis (Schmitz et al., 2000), suggesting that an interaction between gD and HVEM induces apoptosis in certain cell types (Hanon et al., 1998). Following gD-receptor binding, gB and/or a gH–gL complex interacts with cellular membrane protein(s) leading to fusion of the viral envelope to the cell membrane (Pertel et al., 2001; Mettenleiter, 2002; Spear et al., 2006; Campadelli-Fiume et al., 2007; Reske et al., 2007). The mechanism of viral and cellular membrane fusion is not well understood other than the fact that gD-receptor binding must occur prior to the fusion process (Mettenleiter, 2002; Spear et al., 2006). Viral entry into an adjacent cell (cell-to-cell spread) also requires gD because gD-deleted viruses are incapable of cell-to-cell spread (Fehler et al., 1992). In stably transformed cells expressing gD, cell-to-cell spread of wild-type (wt) virus is restricted (Dasika and Letchworth, 1999), suggesting that gD binds to putative cellular receptors, which are present on the apical cell membrane at the cell surface and lateral cell membrane located at intercellular junctions (Dasika and Letchworth, 1999; Campadelli-Fiume et al., 2000, 2007; Sakisaka et al., 2001; Campadelli-Fiume and Gianni, 2006). Although gD is an essential glycoprotein for virus entry into the cell, a compensatory mutation in gH at amino acid residue 450 (mutation of glycine to tryptophan) mediates gD-independent entry and cell-to-cell spread of a gD-deleted virus (Schröder et al., 1997). Interactions between gE and gI also promote cell-to-cell virus spread, and gE and/or gI deletion mutants are defective in cell-to-cell spread (Dingwell and Johnson, 1998; Chowdhury et al., 1999; Mettenleiter, 2000, 2002). Based on results from HSV-1 studies, it appears that gE binds to a putative cellular receptor located at cellular junctions (Dingwell and Johnson, 1998). Of the non-essential envelope proteins, gE has received a lot of attention because it is required for anterograde neuronal spread in vivo and neurovirulence (Enquist et al., 1999). gE-deleted viruses are transported retrogradely from the nose and eye via the maxillary and ophthalmic branch of trigeminal ganglia (TG) where they establish latency in sensory neurons (Enquist et al., 1999; Chowdhury et al., 2000; Liu et al., 2007). gE-deleted viruses do not reactivate from latency because they cannot spread in an anterograde direction from sensory neurons in TG to non-neuronal cells in the nose or ocular cavity (Enquist et al., 1999; Liu et al., 2007).

The envelope protein (Us9) is also important for anterograde neuronal spread because calves latently infected with Us9-deleted viruses do not shed virus in the nose and eye following dexamethasone-induced reactivation (Butchi et al., 2007). Interestingly, Us9-deleted BHV-1 and pseudorabies virus (PRV) do not have a phenotype following infection of epithelial cells in culture (S. I. Chowdhury, unpublished data). With respect to PRV, the Us9-deleted virus has a defective anterograde neuronal spread phenotype because in the absence of Us9, axonal transport of viral glycoproteins or vesicles containing viral glycoproteins does not occur (Tomishima and Enquist, 2001). Therefore, the Us9 function is neuron-specific and is required only in the context of anterograde neuronal transport.

BHV-1 gG is secreted after proteolytic processing. In addition, gG is present on the virus envelope and is associated with infected cell membranes (Bryant et al., 2003). Like other α-herpesvirus gG homologues, the BHV-1 gG has chemokine binding activity because it blocks the interaction of chemokines with cellular receptors and glycol-aminoglycans (GAGs). The chemokine binding activity of gG also occurs on cell membranes because membrane-anchored forms of gG bind to various chemokines (Bryant et al., 2003). Deletion of the BHV-1 gene encoding gG leads to viral attenuation in calves because the mutant virus is more immunogenic (Kaashoek et al., 1996b). The chemokine binding activity encoded by BHV-1 gG is responsible for the attenuated phenotype following infection of calves (Bryant et al., 2003). In summary, these studies indicate that numerous viral genes are necessary for initiating productive infection and cell-to-cell spread.

The BHV-1 latency–reactivation cycle of BHV-1

Acute infection leads to high levels of virus production

In the field, it is recognized that BRDC is not always associated with acute BHV-1 infection. It is generally believed that the ability of BHV-1 to reactivate from a latent infection can initiate BRDC. The goal of this section is to summarize our current knowledge of the BHV-1 latency–reactivation cycle.

Acute BHV-1 infection is initiated on mucosal surfaces and results in high levels of programmed cell death (Winkler et al., 1999). Infection of permissive cells (Devireddy and Jones, 1999) with BHV-1 also leads to rapid cell death, in part, due to apoptosis. Viral gene expression is temporally regulated in three distinct phases: immediate early (IE), early (E) or late (L). IE gene expression is stimulated by a virion component, α-TIF (Misra et al., 1995). Two IE transcription units exist:
IE transcription unit 1 (IEtu1) and IEtu2. IEtu1 encodes functional homologues of two HSV-1 IE proteins, ICP0 and ICP4. IEtu2 encodes a protein that is similar to the HSV IE protein, ICP22 (Wirth et al., 1991). In general, IE proteins activate E gene expression, and then viral DNA replication occurs. L gene expression is also activated by bICP0, culminating in virion assembly and release. As discussed above, bICP0 is important for productive infection because it transcriptionally activates all viral promoters, and is expressed at high levels throughout infection (Wirth et al., 1991, 1992; Fraefel et al., 1994). Acute infection leads to high levels of virus production and secretion in ocular, oral or nasal cavities. If acute infection is initiated in the genital tract, virus shedding can be readily detected in genital tissues. Regardless of the site of infection, virus shedding can last for 7–10 days after infection (Jones, 1998, 2003).

Summary of the latency–reactivation cycle in cattle

Viral particles, or perhaps subviral particles, enter the peripheral nervous system via cell–cell spread. If infection is initiated via the oral cavity, nasal cavity or ocular orifice, the primary site for latency is sensory neurons within TG. Relatively high levels of viral gene expression (Schang and Jones, 1997) or infectious virus (Inman et al., 2002) can be detected in TG from 1–6 days after infection (see Fig. 3, for a summary of the latency–reactivation cycle of BHV-1). Viral gene expression and detection of infectious virus are subsequently extinguished, but viral genomes can be detected in TG (establishment of latency). In contrast to productive infection in cultured cells, a significant number of infected neurons survive, and these neurons harbor latent genomes. A hallmark of latency is the abundant level of transcription that occurs from the latency related (LR) gene (Jones, 1998, 2003; Jones et al., 2006) and ORF-E (Inman et al., 2004). It appears that the LR transcript is the first viral transcript expressed in infected neurons because we have detected a spliced LR transcript in TG at 24 h after calves were infected (Devireddy and Jones, 1998). In contrast, we were not able to detect IE or E gene expression at the same time using sensitive RT-PCR assays, suggesting that LR gene products play a pivotal role in programming the outcome of virus infection in sensory neurons. Support for this prediction comes from the finding that LR gene products promote establishment of latency by inhibiting apoptosis (Ciacci-Zanella et al., 1999; Henderson et al., 2004) and viral gene expression (Bratanich et al., 1992; Geiser et al., 2002). ORF-E may promote efficient establishment of latency because it can induce neurite-like outgrowth in mouse neuroblastoma cells (Perez et al., 2007). Consequently, ORF-E may enhance restoration of mature neuronal functions following infection. During maintenance of latency, infectious virus is not detected using standard virological methods. Since the LR gene and ORF-E are abundantly expressed during the maintenance of latency, they are predicted to play an active role in maintaining latency.

Elevated corticosteroid levels (stress) and/or immune suppression can initiate reactivation from latency. The stress associated with moving cattle from one location to another is one obvious stimulus that can trigger reactivation from latency and BRDC. During reactivation from latency, three significant events occur: (i) productive viral
gene expression is readily detected in sensory neurons, (ii) ORF-E and LR gene expression decrease dramatically and (iii) infectious virus is secreted from nasal or ocular swabs (Jones, 1998, 2003; Jones et al., 2006). Administration of dexamethasone to calves or rabbits latently infected with BHV-1 reproducibly leads to activation of viral gene expression and reactivation from latency (Rock et al., 1992; Jones, 1998, 2003; Jones et al., 2000, 2006; Inman et al., 2002). Although many latently infected neurons do not apparently produce infectious virus, a much higher number of neurons are detected in which viral gene expression occurs (Rock et al., 1992), suggesting that virus-producing neurons are not common.

Non-neural sites of latency–persistence in cattle

Although establishment of latency in ganglionic neurons is the main site of latency for BHV-1 and other α-herpesvirinae subfamily members, latent or persistent infections also occur in non-neural sites, tonsils and lymph nodes for example. BHV-1 DNA is consistently detected in tonsils (Winkler et al., 2000), peripheral blood cells (Fuchs et al., 1999), lymph nodes and spleen of latently infected calves, even when infectious virus is not detected (Mweene et al., 1996). Pseudorabies virus (Sabo and Rajanci, 1976; Cheung, 1995), equine herpesvirus type 4 (Borchers et al., 1999) and canine herpesvirus type 1 (Miyoshi et al., 1999) DNA is also detected in lymphoid tissue during latency. It remains to be seen what non-neural cell types are latently infected with BHV-1, and whether viral genes are expressed in these latently infected cells. In contrast to latency in sensory neurons, LR-RNA is not abundantly expressed in latently infected lymphoid tissue (Winkler et al., 2000). Infectious virus can be detected when germinal centers from tonsil of latently infected calves are explanted (Perez et al., 2005) adding support to the hypothesis that BHV-1 has established a latent or persistent infection in cells of lymphoid origin.

The LR gene is abundantly expressed during latency and is necessary for the latency–reactivation cycle

As discussed above, LR-RNA is abundantly transcribed in latently infected neurons (Rock et al., 1987, 1992; Kutish et al., 1990), and is antisense relative to the bICP0 gene (Fig. 1A). The lytic start site for LR-RNA is downstream of the start site used in TG (Bratanich et al., 1992; Hossain et al., 1995) (Fig. 1B). The LR gene has two ORFs (ORF-1 and ORF-2), and two reading frames that lack an initiating ATG (RF-B and RF-C) (Fig. 1B). A peptide antibody directed against ORF-2 recognizes a protein encoded by the LR gene (Hossain et al., 1995; Jiang et al., 1998, 2004). A fraction of LR-RNA is polyadenylated and alternatively spliced in TG, suggesting that more than one LR protein is expressed (Hossain et al., 1995; Devireddy and Jones, 1999). LR gene products inhibit cell proliferation, bICP0 RNA expression (Bratanich et al., 1992, Schang et al., 1996; Geiser et al., 2002), and apoptosis (Ciacci-Zanella et al., 1999). LR protein expression is necessary for inhibiting apoptosis, but not cell growth (Geiser and Jones, 2005) or bICP0 expression (Bratanich et al., 1992; Schang et al., 1996; Geiser et al., 2002).

A mutant BHV-1 strain with three stop codons at the N-terminus of ORF-2 (Inman et al., 2001b) does not express ORF-2 or RF-C following infection of bovine cells (Jiang et al., 2004). As expected, wt or the LR rescued virus express ORF-2 and RF-C. Calves infected with the LR mutant virus exhibit diminished clinical symptoms, and reduced shedding of infectious virus from the eye, TG or tonsil (Inman et al., 2001b, 2002; Perez et al., 2005). The LR mutant virus does not reanimate from latency following treatment with dexamethasone, whereas all calves latently infected with wt virus or the LR rescued virus shed infectious virus following dexamethasone treatment (Inman et al., 2002). Thus, the LR gene is necessary for the latency–reactivation cycle in calves. It is not clear whether LR proteins are necessary for stimulating reactivation from latency or whether these products establish and maintain latency in a pool of neurons that are capable of supporting reactivation from latency.

Previous studies determined that the LR transcript is alternatively spliced in TG during acute versus latent infections (Devireddy and Jones, 1998; Devireddy et al., 2003). A novel alternatively spliced cDNA is expressed in TG at 7 days after infection (7d cDNA), which is the transition from acute infection to establishment from latency. The 7d cDNA has the potential to encode the N-terminal domain of ORF-2 fused to ORF-1, suggesting that this novel fusion protein promotes establishment of latency. The 7d cDNA was used in a two-hybrid screen to identify cellular proteins that interact with the novel LR fusion protein encoded by the 7d cDNA (Meyer et al., 2007). The LR fusion protein interacts with two proteins that can induce apoptosis (Bid and Cdc42), and with the CCAAT enhancer-binding protein α (C/EBP-α). Bid links the extrinsic pathway of apoptosis to the intrinsic pathway because following cleavage by caspase 8, Bid interacts with mitochondria (Li et al., 1998), which leads to cytochrome c and Smac/Diablo release (Wang, 2001). Bid is also specifically cleaved by granzyme B (Barry et al., 2000; Alimonti et al., 2001; Pinkoski et al., 2001), suggesting that the ability of LR proteins to interact with Bid impairs cytotoxic T-lymphocyte (CTL)-induced death of infected neurons. Neuronal stress, including removal of nerve growth factor from neuronal cultures (Kanamoto et al., 2000; Xu et al., 2001), induces Cdc42 activity, a Rho GTPase family member (Harwood and Braga, 2003). When PC12 cells (rat neuronal-like cell line) are treated with nerve growth factor, they differentiate and behave like mature sympathetic neurons (Greene and Tischler, 1976; Greene et al., 1987). If PC12 cells or primary sympathetic neurons are deprived of nerve growth factor,
C/EBP-α is activated resulting in apoptosis (Kanamoto et al., 2000; Xu et al., 2001). Since dexamethasone is a potent stressor and reproducibly induces BHV-1 reactivation from latency (Sheffy and Davies, 1972; Ackermann et al., 1982; Homan and Easterday, 1983; Ackermann and Wyler, 1984; Brown and Field, 1990; Rock et al., 1992; Jones et al., 2000; Winkler et al., 2002), Cdc42 may be activated in TG.

The interaction between the LR fusion protein and C/EBP-α may also influence BHV-1 gene expression because C/EBP-α stimulates lytic gene transcription of other herpesviruses, EBV, HHV-8 and HVS for example (Wang et al., 2005b; Wu et al., 2004; Wakenshaw et al., 2005; Huang et al., 2006). Three lines of evidence support the finding that C/EBP-α enhances BHV-1 productive infection: (i) C/EBP-α protein expression is stimulated during productive infection, (ii) there is a positive correlation between viral gene expression in TG and C/EBP-α protein expression and (iii) wt C/EBP-α, but not a DNA binding mutant, enhances BHV-1 plaque formation (Meyer et al., 2007). It will be of interest to determine whether C/EBP-α directly stimulates viral promoters or if it indirectly activates productive infection.

The BHV-1 LR gene and HSV-1 LAT (latency-associated transcript) share structural and functional properties. For example, LR-RNA and LAT are transcribed in an antisense direction of bICP0 and ICP0 respectively, and both are abundantly expressed during latency (Rock et al., 1987; Kutish et al., 1990; Hossain et al., 1995; Schang and Jones, 1997; Devireddy and Jones, 1998; Winkler et al., 2000). Furthermore, the LR gene (Ciacci-Zanella et al., 1999) and LAT (Perng et al., 2000; Inman et al., 2001a; Ahmed et al., 2002) inhibit apoptosis. To test whether the LR gene restores spontaneous reactivation to a HSV-1 LAT deletion mutant (dLAT2903), a 2 kb fragment containing the LR promoter and LR coding sequences was inserted into the LAT locus of dLAT2903, and the recombinant virus designated CJLAT (Perng et al., 2002). Insertion of the LR gene into the HSV-1 LAT locus restores high levels of spontaneous reactivation in the rabbit eye model and in explant-induced reactivation. Rabbits infected with CJLAT have higher levels of recurrent eye disease (stromal scarring and detached retinas). Further evidence for the expanded pathogenic properties of CJLAT came from the finding that CJLAT is more lethal in mice relative to LAT+ or LAT− strains of HSV-1. In contrast, insertion of the LR gene with stop codons into dLAT2903 generated a virus that behaves like the parental LAT-null mutant adding further support that LR protein expression regulates the latency–reactivation cycle (Mott et al., 2003).

**ORF-E encodes a protein that is expressed in TG of latently infected calves**

A small ORF that is present within the LR promoter has been designated ORF-E (Fig. 1A). ORF-E is antisense to the LR transcript and downstream of the bICP0 ORF. A transcript that encompasses ORF-E is expressed in productively infected bovine cells and TG of latently infected calves (Inman et al., 2004). When ORF-E protein coding sequences are fused in frame with green fluorescent protein (GFP) sequences, GFP protein expression is detected in the nucleus of mouse or human neuroblastoma cells. In contrast, the ORF-E-GFP fusion protein is expressed throughout rabbit skin cells.

Polyclonal antiserum directed against an ORF-E peptide or the entire ORF-E protein specifically recognizes the nucleus of sensory neurons in TG of latently infected calves and mouse neuroblastoma cells transfected with an ORF-E-expressing plasmid (Perez et al., 2007). ORF-E also induces neurite-like projections in these same mouse neuroblastoma cells. In contrast, neurite-like projections are not observed following transfection of mouse neuroblastoma cells with an empty vector, suggesting that ORF-E induces changes in the physiology of neuronal cells. It will be necessary to construct a BHV-1 mutant that does not express the ORF-E protein to test whether ORF-E plays a role in the latency–reactivation cycle.

### Immune response to BHV-1 following acute infection

Although BHV-1 has the potential to immune-suppress cattle, a potent immune response ultimately occurs, which prevents systemic infection. With respect to BRD, this implies that immunosuppression initiated by BHV-1 is short-lived.

The host immune response to BHV-1 infection includes innate and adaptive immune responses (Rouse and Babiuk, 1978). Innate immune responses include the antiviral action of IFN, alternative complement pathway and local infiltration of lymphoid cells, macrophages, neutrophils or natural killer (NK) cells for example. Following BHV-1 infection, IFN-α and IFN-β are detectable in nasal secretions as early as 5 h post-infection, reach a maximum level at 72–96 h post-infection and can persist for up to 8 days after infection (Straub and Ahl, 1976). During early times after infection, IFN-α and IFN-β promote leukocyte migration, activate macrophages and increase NK cell activity (Babiuk et al., 1985; Lawman et al., 1987; Jensen and Schultz, 1990). Activation of macrophages and increasing NK cell activity stimulate cytolytic activities against virus-infected cells (Rouse and Babiuk, 1978). NK cells are a diverse population of non-adherent effector cells that lack T- and B-cell markers (Cook and Splitter, 1989). These cells require a long incubation period with the target cell for optimum lysis (Campos and Rossi, 1986). In addition, NK-like cytotoxicity is also associated with a population of CD3+ CD45+, Fc receptor-positive lymphocytes, which may represent a subset of γδ T-cells (Amadori et al., 1992).

Adaptive or humoral immune responses lead to production of neutralizing antibodies that bind virus particles and
inhibit productive infection. Envelope glycoproteins gB, gC, gD and gH are the most potent inducers of virus neutralizing antibodies (Van Drunen Littel-van den Hurk, 1986; Marshall et al., 1988). In addition, non-neutralizing antibody may mediate the destruction of enveloped virus or cells expressing viral proteins on the cell membranes, and this process is referred to as antibody-mediated cell cytotoxicity (Van Drunen Littel-van den Hurk et al., 1993). Neutralizing and non-neutralizing antibodies produced against envelope proteins can also inhibit virus infection by several distinct mechanisms: (i) membrane attack complex (MAC) lysis of virus envelope and virus-infected cells mediated by antibody and complement and (ii) antibody-mediated cell cytotoxicity in which either IgG interacts with Fc receptor-positive cells (macrophages) or (iii) binding of C3b to IgM mediates binding to C3b receptor-positive cells (lymphocytes, macrophages). In all cases, virus-infected cells are lysed (Rouse et al., 1976, 1977a, b; Rouse and Babiker, 1977, 1978). Production of virus neutralizing and non-neutralizing antibodies (IgG) can be detected 8–12 days post-infection (Rouse and Babiker, 1978). Local/mucosal immunity depends on secreted neutralizing antibodies (IgA molecules) and systemic humoral immunity depends on IgG.

Cell-mediated immune (CMI) responses play an important role in killing virus-infected cells that express viral antigens on the cell surface (Van Drunen Littel-van den Hurk, 2007). For example, a CD8+ CTL response is an important defense against BHV-1 because cell-to-cell spread in upper respiratory epithelium occurs before hematogenous spread (Van Drunen Littel-van den Hurk, 2007). Cytotoxic and proliferative T lymphocyte responses occur in the blood approximately 8 days after infection. CTL responses have been induced by gB and gD DNA vaccines in mice (Deshpande et al., 2002; Huang et al., 2005). Further, gC and gD have been identified as targets for CTL responses in cattle (Denis et al., 1993) and gB DNA vaccines elicit a CTL response in cattle (Huang et al., 2005). Other structural and non-structural viral proteins may also play a role in CMI response because only a limited number of BHV-1 proteins have been evaluated for CTL activity (Van Drunen Littel-van den Hurk, 2007). In addition to destruction of infected cells, T-lymphocytes release a number of lymphokines that modulate specific and non-specific immune response. For example, IFN-γ and other factors that further activate macrophages (Campos et al., 1989) are produced. BHV-1 proteins (gB-, gC-, gD- and VP8) are recognized by CD4+ T helper cells from immune cattle (Hutchings et al., 1990). Cells expressing gB, gC or gD on their membranes have also been identified as targets for CD4+ T-cells (Leary and Splitter, 1990; Tikoo et al., 1995a, b). Finally, CD4+ T-cells are important for the development of antibody response and for developing effective CD8+ T-cell memory (Janssen et al., 2003; Shedlock and Shen, 2003). Therefore, both CD4+ and CD8+ T cells as well as antibodies are required for long-term protection.

A prolonged CMI response occurs in the peripheral nervous system during latency

Several independent studies have demonstrated that T-cells, CD8+ T-lymphocytes in particular, control HSV-1 infection in sensory ganglia during latency (Nash et al., 1987; Simmons and Tscharke, 1992; Simmons et al., 1992; Cantin et al., 1995; Shimeld et al., 1995; Halford et al., 1996; Liu et al., 1996; Shimeld et al., 1996, 1997). Viral antigen-positive neurons surrounded by non-neural cells expressing TNF-α, IL-2, IL-6, IL-10 or IFN-γ can also be detected in mice latently infected with HSV-1 (Halford et al., 1996; Liu et al., 1996; Shimeld et al., 1997). No cells with lymphoid cell morphology are detected in TG of uninfected mice. A persistent infiltration of lymphoid cells in sensory ganglia and spinal cord is also detected in mice or guinea pigs latently infected with HSV-2 (Milligan et al., 2005). Following infection of calves with BHV-1, foci of infiltrating lymphocytes can be readily detected during acute infection (Winkler et al., 2002). Finally, in human TG latently infected with HSV-1, a chronic immune response has also been observed (Theil et al., 2003; Verjans et al., 2007). Collectively, these studies indicate that long-term persistence of T-cells in sensory ganglia during latency has the potential to regulate the latency-reactivation cycle.

Persistence of immune cells in TG may be due to viral protein expression in a rare neuron or satellite cell. Careful examination of TG neurons for viral gene expression in HSV-1 latently infected mice (37–47 days after infection) demonstrated that abundant viral transcripts, viral protein and viral DNA replication occur in approximately 1 neuron per 10 TG (Feldman et al., 2002). Infectious virus is not detected in these mice, confirming that they are latently infected. Neurons expressing high levels of HSV-1 transcripts are invariably surrounded by foci of infiltrating white blood cells. The term ‘spontaneous molecular reactivation’ has been coined to describe these rare neurons (Feldman et al., 2002). Rare cells in TG of calves latently infected with BHV-1 can be detected that express viral transcripts and proteins (Winkler et al., 2002), suggesting that molecular spontaneous reactivation occurs during BHV-1 latency.

CD8+ T-cells that produce IFN-γ are believed to play an important role in preventing reactivation from latency in sensory neurons in mice latently infected with HSV-1 (Liu et al., 2000, 2001). Two independent studies have also concluded that IFN-α and IFN-γ control recurrent herpetic lesions (Cunningham and Mikloska, 2001; Mikloska and Cunningham, 2001). In addition to IFN, lymphocyte-mediated cytotoxicity could inhibit virus spread in TG. Lymphocyte-mediated cytotoxicity induces two potent apoptotic pathways: the granule exocytosis and the Fas–Fas ligand pathway (Kagi and Hengartner, 1996; Shresta et al., 1998). The granule exocytosis pathway is employed predominantly by CD8+, NK and lymphokine-activated killer cells. It appears that the BHV-1 LR gene
plays a role in regulating lymphocyte infiltration because enhanced infiltration of lymphocytes occurs in TG of calves acutely infected with the LR mutant virus (Perez et al., 2006). The ability of dexamethasone to induce apoptosis of inflammatory cells in TG of calves latently infected with BHV-1 correlates with reactivation from latency (Winkler et al., 2002).

**BHV-1 vaccines**

Many commercially available vaccines directed against BHV-1 can cause disease in small calves, in part because these vaccines are immunosuppressive. Consequently, certain vaccines have the potential to induce BRDC in small calves. A discussion of the current status of BHV-1 vaccines and how they can be improved is included in the following paragraphs.

**Commercially available vaccines**

Commercially available vaccines directed against BHV-1 consist of modified live attenuated virus (MLV) or killed whole virus (KV) (Van Drunen Littel-van den Hurk, 2005). In many cases, MLVs are derived by serial passage in tissue culture. KV vaccines are usually produced by chemical treatments, such as formaldehyde, β-propiolactone or binary ethyleneimine. MLVs generally induce both humoral and cellular immune response because virus replication in infected cells leads to presentation of viral antigen on MHC class I+ and II+ molecules. Safety is a concern for MLVs because these strains can establish latency and upon reactivation can be transmitted to pregnant cows, which can lead to abortion. MLVs can also be pathogenic in small calves because their immune system is not fully developed, and most MLVs can be immunosuppressive. KV vaccines are usually safe but they are not as efficacious because they usually produce only humoral immunity but no cellular immune responses. Additionally, KV vaccines always require more than one injection to achieve acceptable neutralizing antibody levels. In the case of formaldehyde-inactivated KV, antigens may also be denatured, which may affect the immunogenicity of vaccine preparations. KV also requires suitable adjuvant formulations and some adjuvants may induce injection site reactions.

Commercially available BHV-1 vaccines are primarily evaluated on induction of neutralizing antibodies and the duration as well as level of virus shedding following challenge of vaccinated animals. As discussed earlier, the CMI response against the virus is important to prevent cell-to-cell spread of the virus. Therefore, a direct measure of CMI directed against a vaccine virus is important. Traditionally, IFN-γ levels in vaccinated calves have been an indicator of cellular immune responses. However, a recent study demonstrated that there is an increase in CD25 by CD4+, CD8+ and γδT lymphocytes from a BHV-1 MLV-vaccinated group (Endsley et al., 2002). Therefore, increased expression of CD25, in addition to IFN-γ production by T-cells after vaccination, appears to be a useful cellular immunity marker (Van Drunen Littel-van den Hurk, 2007).

Recently, there has been an apparent increase in IBR outbreaks in vaccinated feedlot cattle (commonly referred to as vaccine outbreaks) (Van Drunen Littel-van den Hurk et al., 2001; Ellis et al., 2005). Many of these vaccine outbreaks occur in feedlots that have used several different BHV-1 MLVs without serological markers, suggesting that they are not vaccine-specific. Since the vaccines used in the feedlots did not have any serological marker, it is difficult to determine by serology whether an animal is infected with an MLV or field strain. Thus, determining the source of origin from a particular vaccine break is not possible nor is it possible to test whether these outbreaks were due to changes in the vaccine strain. It is also possible that there is the emergence of a new IBR strain that is not covered by existing MLVs. A study investigating several isolates from such outbreaks determined that at least one such isolate had mutations within sequences comprising the gB-specific neutralizing epitope (Van Drunen Littel-van den Hurk et al., 2001).

**Development of genetically engineered gene-deleted BHV-1 vaccines**

During the last 10–15 years, the usefulness of genetically engineered gene-deleted vaccines has become increasingly apparent because they can be attenuated and serologically distinguished from wt field strains (Van Drunen Littel-van den Hurk, 2005). Numerous viral mutants (gC-, gE-, gG-, Us9-deleted) (Kaashoek et al., 1998; Chowdhury et al., 1999; Butchi et al., 2007), thymidine kinase (TK)-deleted (Chowdhury, 1996; Kaashoek et al., 1996a, b) and LR gene mutant virus (Inman et al., 2002) have been constructed and their in vivo pathogenic properties, reactivation properties and immunogenicity analyzed. Based on recent studies, both gE- and Us9-deleted viruses were safe in calves because they do not reactivate from latency and they are highly attenuated (Kaashoek et al., 1996a, b; 1998; Chowdhury et al., 1999; Butchi et al., 2007; Liu et al., 2007). Studies with gC-, gG- or TK-deleted viruses showed that they either reactivate from latency and/or they retain some degree of virulence (Kaashoek et al., 1996a, b, 1998). Studies with a LR mutant virus showed that the virus does not reactivate from latency and has reduced pathogenicity in calves (Inman et al., 2002). Therefore, considering the virulence and reactivation properties of gene-deleted vaccine strains, gE-, Us9-deleted or the LR mutant virus may have the potential to be safer vaccine candidates. Comparative vaccine efficacy studies showed that relative to gC- and gG-deleted viruses, gE-deleted virus is less...
efferacous (Kaashoek et al., 1998). However, the 
G-E-deleted vaccine has been used successfully to er-
adicate IBR from a number of European countries.
Although the G-E-deleted marker vaccine is not as
efficacious as others noted above, it will be used until a
better genetically engineered vaccine is developed.
Recent efforts to improve the IBR marker vaccine are
directed to delete viral gene sequences that are immuno-
suppressive. As noted above, GN inhibits TAP and
down-regulates MHC-I antigen presentation. Therefore,
a vaccine virus lacking the gN TAP binding domain may
stimulate better cellular immune responses. Our recent
studies show that a G-E cytoplasmic tail truncated virus is
similarly attenuated in calves infected with the virus as the
entire G-E ORF-deleted virus. Notably, like the G-E-deleted
virus, the G-E cytoplasmic tail truncated virus does not
reactivate from latency (Liu et al., 2007). Since the
cytoplasmic tail-specific amino acid sequences generate
antibodies that immunoprecipitate G-E, antibodies specific
to the cytoplasmic tail sequence may serve as a sero-
logical marker to distinguish vaccinated versus infected
calves (Whitbeck et al., 1996; Chowdhury et al., 2000).
Furthermore, the Us9 gene is located immediately down-
stream of the G-E cytoplasmic tail gene; therefore, a
recombinant BHV-1 can be constructed in which the G-E
cytoplasmic tail and the Us9 coding regions are deleted.
A vaccine virus that lacks Us9, the cytoplasmic tail of G-E, and
gN TAP binding domain may be a superior vaccine
candidate because it: (i) should stimulate better cellular
immune responses against BHV-1, (ii) will incorporate a
serological marker and (iii) should not be pathogenic.
Concerns have been raised about recombination occur-
ing between two vaccine strains, which could lead to the
presence of virulent viral strains in vaccinated herds (Thry
et al., 2005). Although it is clear that recombination can
occur, the process requires that both viruses must replicate
at the same time in the same cell of an infected calf.

**Vectored vaccine strains**

Intranasal vaccination of young calves with a replication
competent (E3-deleted) bovine adenovirus (BAV)-3
that expresses full-length BHV-1 gD induced protective
gD-specific neutralizing antibodies and cellular immune
responses (Zakhartchouk et al., 1999). Several studies also
concluded that gD-specific bacterial plasmid-based DNA
vaccines can serve as an effective subunit vaccine.
Specifically, an expression plasmid that encodes the
secreted form of gD (tgD) induces significant protection
against BHV-1 challenge following intradermal injection
(Van Drunen Littel-van den Hurk et al., 1998; Braun et al.,
1999). Relative to killed BHV-1 vaccine, the tgD DNA
vaccine induced significant IFN-γ production in PBMCs.
Regardless of maternal antibody levels, the plasmid
encoding tgD also triggered neutralizing antibodies,
lymphoproliferative responses and IFN-γ-secreting cells
in newborn lambs, suggesting that this approach may
be efficacious for neonate immunization (Van Drunen
Littel-van den Hurk et al., 1999).

Mucosal delivery of gB-based plasmid DNA vaccines
has also shown promising results. Delivery of plasmid-
based BHV-1 gB DNA vaccine in the female vulvomu-
cosal epithelium induced gB-specific antibodies and
cellular immune responses. Furthermore, vaccinated
animals have significantly reduced genital lesions upon
genital challenge (Huang et al., 2005). Recent studies
using a combined gB–gD DNA vaccine in mice indicated
that mice immunized with the combined vaccine contain-
ing the secreted forms of the respective glycoproteins
developed higher titers of anti-BHV-1 neutralizing anti-
odies and increased cellular immune response com-
pared to single plasmid injected groups (Caselli et al.,
2005).

**Use of BHV-1 as a vaccine vector**

BHV-1-based vectors have significant advantages relative
to other potential virus vectors for immunization of cattle
against economically important viral and bacterial
diseases, including those that initiate BRDC. Cattle are
routinely immunized against BHV-1 and, therefore, the
use of BHV-1 as a vector would not pose a new risk to
cattle or humans. Gene-deleted BHV-1 strains could be
used to express other bacterial and viral gene coding
sequences. BHV-1 expressing the BVD (bovine diarrhea
virus) gP53 (E2) protein has been constructed (Kweon
et al., 1999; Wang et al., 2003a). While the protective
efficacy of the BHV-1 vectored vaccine against BVD
challenge has not yet been reported, BHV-1 expressing
the E2 protein induced BVD gP53-specific neutralizing
antibody in vaccinated calves (Kweon et al., 1999). BVD is
well known to be immunosuppressive and, like many
RNA viruses, can readily mutate (Peterhans et al., 2006).
Such complications from modified live BVD vaccines
could be avoided by using BHV-1 as live attenuated
vector to deliver BVD-specific protein(s). Similarly, BHV-
1-expressing bacterial proteins could be used to immu-
nize against bacterial diseases that affect dairy and beef
cattle. These may include Mannheimia hemolytica protec-
tive antigens, which are a significant cause of pneumonia
that occurs as a result of shipping fever. Similarly,
*Mycobacterium avium* subspecies *paratuberculosis*
(MAP) is a slow growing bacterium with zoonotic
potential (Grant, 2005). Development of an effective
vaccine is hindered due to its extremely slow growth and
difficulties in protective bacterial antigen purification.
Several mycobacterial proteins trigger cellular immune
responses (Rossels et al., 2006), which are important for
protective immunity against MAP. BHV-1-expressing MAP
proteins would certainly have a potential as a vaccine
against MAP and BHV-1. Although BHV-1 can be used as
a potential vector for delivering foreign antigens to calves,
a thorough understanding of the immune-suppressive nature of BHV-1 and the latency–reactivation cycle is essential.

Acknowledgments

The laboratory of CJ is supported by two USDA grants (2005-01554 and 2006-01627), a grant from NIAID (R21AI069176) and, in part, a Public Health Service grant 1P20RR15635 to the Nebraska Center for Virology. The laboratory of SC is supported by two USDA grants (00-02103 and 2004-35204-14657).

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