ISSN 1466-2523

Animal Health Research Reviews **10**(2); 149–153
doi:10.1017/S146625230999020X

Update on viral pathogenesis in BRD

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Received 12 August 2009; Accepted 18 October 2009

Abstract

Many viruses, including bovine herpesvirus-1 (BHV-1), bovine respiratory syncytial virus (BRSV), parainfluenzavirus-3 (PI_3), bovine coronavirus, bovine viral diarrhea virus and bovine reovirus, have been etiologically associated with respiratory disease in cattle. This review focuses on the pathogenesis of BHV-1 and BRSV, two very different agents that primarily cause disease in the upper and lower respiratory tract, respectively.

Keywords: bovine herpesvirus-1, bovine respiratory syncytial virus, immune response, immunosuppression, bovine respiratory, bovine herpesvirus, latency-related (LR), bovine respiratory disease, open reading frame (ORF-E)

Bovine herpesvirus-1 (BHV-1): what are its characteristics?

First isolated in 1956 (Madin *et al.*, 1956), BHV-1 is a representative of the *Alphaherpesvirinae*, which as a group, cause similar upper respiratory tract diseases in a wide range of host species. These viruses are large (135 kB), double-stranded DNA viruses composed of an icosahedral nucleocapsid, and envelope that are connected by a 'tegment'. More than 70 proteins are encoded by temporally regulated sets of genes, immediate early (IE), early (E) and late (L), with some variability among members of the group. Alphaherpesviruses replicate in the nucleus of the host cell, and are thus subject to host cell proofreading mechanisms. This relatively conservative replication scheme results in minor genetic and antigenic changes in BHV-1 isolates over time, but these changes have not been associated with biologically significant antigenic alterations that are consistent with antibody escape mutation (Kaashoek *et al.*, 1996). Differences in virulence among various BHV-1 isolates have been described, but the mechanistic basis for this is not understood (Kaashoek *et al.*, 1996).

Latency, primarily in neurons in sensory ganglia of the head, notably the trigeminal ganglion, but also probably in other tissues, such as tonsils, is a characteristic feature of alphaherpesviruses (Muylkens *et al.*, 2007; Jones and Chowdhury, 2008). Mechanisms of maintenance of latency are not completely understood, but are associated with high levels of transcription of latency-related (LR) and open reading frame (ORF-E) genes. Conversely, recrudesence/reactivation of latent BHV-1 and resultant replication in epithelia of the upper respiratory tract are associated with reduced expression of LR and ORF-E genes together with increased expression of other viral genes (Muylkens *et al.*, 2007; Jones and Chowdhury, 2008). This reactivation of latent virus is thought to be a critical event in the transmission of the virus and maintenance of the virus in cattle populations. This phenomenon accounts for BHV-1 outbreaks in the absence of acute infection, i.e. in a 'closed herd' or in a pen of feedlot cattle derived from the same ranch.

BHV-1: what cells does it infect; what is the outcome of infection?

Following transmission, usually by direct nose to nose contact, but also by aerosol over short distances, BHV-1 enters epithelial cells (and eventually nerves) in the upper airways. Viral entry into cells is a three-step process involving an interaction between structural glycoproteins in the viral envelop and cellular receptors; first low affinity binding by gC and/or gB to heparin-like sugar moieties on the cell surface; then high affinity/stable binding between gD and the cellular receptor nectin-1, and finally fusion of the viral envelop with the cell

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membrane involving gB, gD, gH and gL (Muylkens *et al.*, 2007; Jones and Chowdhury, 2008). There is no apparent host cell tropism that is determined by the expression of specific receptors for viral glycoproteins, i.e. BHV-1 can infect a variety of cell types, in the respiratory tract and systemically. Once inside the cytosol, BHV-1 particles are transported to the nucleus by a poorly understood process, probably involving an interaction between viral tegument and capsid proteins with cellular cytoskeletal elements. Once in the nucleus, DNA replication, structural protein synthesis and capsid morphogenesis occur under tightly regulated processes (Muylkens *et al.*, 2007). Currently, mature capsids are thought to egress from the nucleus and mature through a three step process; first, naked, DNA-containing capsids acquire a primary envelop by budding through the inner nuclear membrane, then, they enter the cytoplasm by fusing with the outer nuclear membrane, and finally they acquire their mature tegument and secondary envelop by budding into a Golgi compartment (Muylkens *et al.*, 2007). Nascent viral particles can spread directly cell to cell, involving envelope glycoproteins gB, gD and gH/gL, thus avoiding neutralizing antibodies in the extracellular milleu.

Erosions and ulcers in the upper respiratory tract, notably the trachea, are the pathological hallmark of infectious bovine rhinotraceitis (IBR). How do these lesions occur? A characteristic of BHV-1 infection, *in vivo* and *in vitro*, is lysis of infected cells, an event that involves cell death by both necrosis and apoptosis, or programmed cell death (Muylkens *et al.*, 2007). Necrosis probably primarily results from the shut down of cellular protein synthesis, notably by the activity of a tegument protein, the 'virion host shutoff' (vhs) protein, encoded by the UL41 gene. Eventual loss of membrane integrity, Ca+ influx, and lysis results in the release of viral particles. Apoptosis, or programmed cell death, induced by BHV-1 infection, or simply binding of gD, has been well documented in CD4+ T cells (Winkler *et al.*, 1999), but not convincingly in epithelial cells (Geiser *et al.*, 2008), the primary target cell in infected airways. Nevertheless, from the virus's perspective it would be advantageous to inhibit apoptosis, thereby prolonging the life and virus-producing capacity of infected host cells or by playing a role in latency (Jones and Chowdhury, 2008). It has recently been suggested that bICP0 activated expression of viral encoded anti-apoptotic genes, does interfere with ongoing apoptosis (Geiser *et al.*, 2008) possibly by stimulating the cleavage of caspase-3, a critical enzyme in the apoptotic pathway (Muylkens *et al.*, 2007).

BHV-1: how do interactions with host defenses affect the pathogenesis of bovine respiratory disease (BRD)?

Cattle with uncomplicated BHV-1 infections have upper respiratory disease of variable severity that can resolve in 7–10 days (Kiorpes *et al.*, 1978). However, in many, if not most BHV-1-associated BRD cases there is a mixed infection with bacteria, notably *M. haemolytica* and/or *P. multocida*, that results in severe lower respiratory tract disease. Notwithstanding the critical role of environmental co-factors in disease progression, what accounts for this often fatal synergism? From a simple anatomical standpoint, widespread lysis of ciliated epithelium in the trachea disrupts the housekeeping functions of the mucocilliary escalator, and results in a failure to clear bacteria from the upper airways, thereby resulting in deposition of bacteria in alveoli (Allan and Msolla, 1980). Beyond that, BHV-1 affects several 'immunosuppressive' outcomes including down-regulation of type 1 interferon (IFN) by a BICP0 gene product (Henderson *et al.*, 2005), apoptosis of leukocytes, notably CD4+ T cells (Winkler *et al.*, 1999), and reduced expression of MHC I molecules by UL49.5 gene product, gN (Koppers-Lalic *et al.*, 2005), and MHC II molecules by vhs (Hinkley *et al.*, 2000), resulting in decreased removal of virus infected cells and reduced antigen presentation, respectively.

In addition, at least *in vitro*, proinflammatory cytokines, such as tumor necrosis factor α (TNFα), interleukin-1β (IL-1β), and IFNγ that are secreted in response to BHV-1 (and bacterial) infection increase the expression of the β2 integrin CD11a/CD18 (LFA-1) on bovine leukocytes, including alveolar macrophages and neutrophils (Czuprynski *et al.*, 2004). This molecule is the receptor for the leukotoxin of *M. haemolytica*. Enhanced binding of this toxin to leukocytes in the lung leads to death of these cells by apoptosis and a resulting vicious cycle of compromised immune function and inflammation. These phenomena correlate with the consistently observed increased susceptibility of BHV-1-infected to the development of bronchopneumonia; i.e. the classical manifestation of BRD in feedlot cattle.

Bovine respiratory syncytial virus (BRSV): what are its characteristics?

First isolated in 1970 (Paccaud and Jacquier, 1970), BRSV is a representative of the *Pneumoviriane* (*Paramyxoviridae*), which as a group, cause similar lower respiratory tract diseases in a wide range of host species. These viruses are relatively small (15 kB), single-stranded, negative sense RNA viruses composed of an icosahedral nucleocapsid, comprising N, P and L proteins, and envelop containing three viral proteins, G, F (both glycosylated) and SH, which are associated with a matrix or M protein. Genomic RNA is a template for replication and transcription, with transcription occurring sequentially 3′ to 5′. In the case of BRSV, 10 mRNA are transcribed and then translated into 11 proteins (Valarcher and Taylor, 2007). In contrast to BHV-1, BRSV replicates in the cytoplasm of the host cell. The error-prone viral

https://doi.org/10.1017/S146625230999020X Published online by Cambridge University Press

polymerase (RNA-dependent RNA polymerase; L gene product) together with the lack of exonuclease proof-reading leads to genetic and antigenic changes that constitute the quasi-specific nature of BRSV and other pneumoviruses. Variation of up to 11%, mostly in the G protein, has been reported in sequential isolates from outbreaks in the same herd (Larsen *et al.*, 2000). The biological significance of inter-isolate variation with regard to the propensity for reinfection, differential virulence and antibody escape mutation is debated (Larsen *et al.*, 2000; Deplanche *et al.*, 2007; Valarcher and Taylor, 2007).

In contrast to BHV-1, latency is not a feature of BRSV infections, nevertheless there is evidence of persistence, or long-term carriage of the virus in some animals (De Jong *et al.*, 1996). The mechanism(s) of this persistence are not completely understood, but cannot be explained by sequential reinfection of cattle with waning immunity, and may be due to long-term survival of the virus in the lymphoid or other tissues in some 'carrier' animals. Whatever the mechanism, this property could explain outbreaks in the absence of acute infection or introduction of new animals, for example, 'summer pneumonia' in a group of suckling beef calves on pasture.

BRSV: what cells does it infect; what is the outcome of infection?

Following transmission, by contact with nasal secretions or aerosol over short distances, BRSV can be found in a variety of ciliated and non-ciliated epithelial cells in the respiratory tract, including the airways and pulmonary parenchyma (Castleman *et al.*, 1985; Viuff *et al.*, 1996, 2002). Viral attachment and entry into cells is initiated by loose binding of the G protein with membrane glycosaminoglycans (GAG), notably heparin moieties followed by cleavage of the F protein into two subunits, F1 and F2. High affinity, species-specific binding of the F2 subunit to an unidentified receptor allows viral penetration into the cytoplasm (Schlender *et al.*, 2003). In contrast to BHV-1 there is scant evidence of significant infection of any cells beyond respiratory epithelium by BRSV in *vivo* (Viuff *et al.*, 2002). Recent studies *in vitro* (Goris *et al.*, 2009) failed to demonstrate BRSV infection in cultured differentiated ciliated bovine airway cells, in contrast to parainfluenza-3, and suggested that environmental or physiologic stimuli *in vivo*, maybe surfactant (Harris and Werling, 2003), render target cells susceptible to BRSV infection.

After the cytoplasmic RNA replication and transcription and translation of viral mRNA, nucleocapsids form in the cytoplasm and migrate with the M protein to the cellular membrane in which viral glyoproteins F and G are embedded. Viral particles then bud directly through the apical membrane. In polarized human cells infected with human RSV (HRSV) and probably BRSV-infected bovine cells (Valarcher and Taylor, 2007) this budding occurs without obvious cytopathology, so how do the characteristic and extensive lesions associated with BRSV infection occur? Studies *in vivo* (Viuff *et al.*, 2002) and *in vitro* (Michel *et al.*, 2008) indicate the primary role of death of infected cells by apoptotic mechanisms, with progressive loss of cells in the upper to lower airways and then in the pulmonary parenchyma (type I and type II pneumocytes). And, as is the case with BHV-1, there is a trade off between causing death of the host cell and prolonging its survival as a site for virus production. Similarly to BHV-1, studies with HRSV document virus-mediated triggering of anti-apoptotic pathways early in infection (Groskreutz *et al.*, 2007) that are also likely to occur in BRSV infected cattle. In addition to participating in viral entry, the F2 subunit of the fusion protein mediates syncytium formation, which is a characteristic feature of BRSV-mediated cytopathology, *in vivo* and *in vitro* (Valarcher and Taylor, 2007). The result of another post-translational modification of the fusion protein is the generation of virokinin (Zimmer *et al.*, 2003), which induces smooth muscle contraction and may contribute to bronchoconstriction and clinical respiratory disease in BRSV-infected cattle. A synergism between the cytopathological effects of BRSV infection and rumen-derived pneumotoxicants, as represented by 3-methylindole, has been demonstrated experimentally (Bingham *et al.*, 1999). The importance of this interaction in the context of nutritional changes and altered rumen metabolism in feedlot cattle and their enhanced susceptibility to BRD remain to be determined.

BRSV: how do interactions with host defenses affect the pathogenesis of BRD?

As with BHV-1 loss of ciliated cells in BRSV-infected airways could account for the disruption of the normal non-specific defense mechanism of mucocilliary escalator function, and result in deposition of bacteria in the lower respiratory tract. This, alone, could contribute significantly to secondary bacterial bronchopneumonia typical BRD.

One of the most controversial and unresolved issues in RSV pathobiology is the role of the host immune response in disease. Voluminous studies of mice infected with HRSV, and recently BRSV (Spilki *et al.*, 2006) that discuss immunopathologic mechanisms of disease uniformly disregard the obvious deficiencies of these models; rare if any clinical disease, the failure to document significant viral replication after inoculation of large amounts of cultured HRSV, and the absence of gross and histologic lesions that are similar to those found in either BRSV-infected cattle (Viuff *et al.*, 2002), or HRSV-infected humans (Johnson *et al.*, 2007). Although the findings in the mouse model have been extrapolated to cattle (Valarcher and Taylor, 2007; Gershwin, 2008), these studies will not be further addressed here.

https://doi.org/10.1017/S146625230999020X Published online by Cambridge University Press

Although BRSV is relatively resistant to IFN, as with BHV-1, BRSV-mediates inhibition of α/β (type 1) IFN. This activity of the non-structural proteins, NS1 and NS2 (Schlender *et al.*, 2000), could negatively affect antiviral responses and activity of phagocytes in the BRSV-infected lung, thereby contributing to the pathogenesis of BRD. Circulating leukocytes from calves experimentally infected with BRSV have enhanced secretion of proinflammatory cytokines including, IL-6, IFNγ, TNFα (Grell *et al.*, 2005a, b). This effect is age-dependent, with younger calves having more pronounced innate (cytokine) responses, which could account for more severe BRSV-associated disease in younger calves (Grell *et al.*, 2005a). There is evidence that BRSV-infection can predispose to allergic pulmonary disease in response to some antigens (Gershwin, 2007). The prevalence of this immunopathological phenomenon and its contribution to BRD in cattle populations is unclear. Although not formerly examined, it is likely that proinflammatory cytokines secreted in response to BRSV infection could contribute to increased susceptibility to the pathologic effects of the leukotoxin of *M. haemolytica*, as with BHV-1.

Summary/conclusions

BHV-1 and BRSV are very different viruses with very different lifestyles within infected cells. Yet, both viruses cause necrosis and/or programmed death in infected cells, stimulate the innate immune system to secrete proinflammatory cytokines, and mediate potentially immunosuppressive effects. This combination of factors results in variable respiratory disease in uncomplicated infections, and, if viral replication is not controlled, can predispose the lung to secondary bacterial infections typical of BRD.

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