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Review

Regulation of apoptosis by bovine herpesvirus-1

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ABSTRACT

Bovine herpesvirus-1 (BoHV-1), a ubiquitous bovine pathogen, belongs to the alphaherpesvirus family. BoHV-1 mostly causes respiratory and genital infections in cattle. In multicellular organisms, apoptosis or programmed cell death plays a crucial role in the cellular host defense against infections. Apoptosis is triggered by various stimuli through activation of intrinsic or extrinsic pathways. The execution of apoptosis is mediated by caspases, a set of cysteine proteases. Viruses are major modulators of apoptotic responses. According to several studies BoHV-1, like other herpesviruses, encodes multiple pro-apoptotic and anti-apoptotic proteins during productive infection. This review comprises the current information on the molecular mechanisms of the effects of multiple BoHV-1 proteins on apoptosis during infection, with reference to similar or divergent strategies of related alphaherpesviruses.

KEYWORDS: apoptosis, alphaherpesvirus, bovine herpesvirus-1, proteins.

ABBREVIATIONS

Apaf-1 - Apoptotic protease activating factor-1
ATM - Ataxia telangiectasia mutated protein
Bak - Bcl-2 homologous antagonist killer
Bad - Bcl2-associated death promoter
Bax - Bcl-2-associated X protein
Bid - BH3-interacting domain
BoHV-1 - Bovine herpesvirus-1
CK2 - Cellular kinase-2
c-JNK - c-Jun-N-terminal kinase
CHX - Cycloheximide
DNA - Deoxyribonucleic acid
EHV-1 - Equine herpesvirus-1
EP0 - Early protein-0
FADD - Fas-associated death domain
cFLIP - FLICE-inhibitory protein
gD - Glycoprotein D
HHV - Human herpesvirus -1
HSV-1 - Herpes Simplex Virus-1
HSP70 - Heat shock protein-70
ICP - Infected cell polypetide
IE - Immediate-early
IFN - Interferon
KSHV - Kaposi's sarcoma-associated herpesvirus
LR - Latency-related
MAPK - Mitogen-activated protein kinase
MDBK - Madin Darby Bovine kidney
MMP - Mitochondrial membrane potential
MPT - Mitochondrial permeability transition
NBS1 - Nijmegen breakage syndrome protein 1
NES - Nuclear export signal
NF-κβ - Nuclear factor-κβ
ORF - Open reading frame
PAMPs - Pathogen Associated Molecular Patterns
PDCD4 - Programmed cell death protein 4
PKR - Protein kinase R
PML - Promyelocytic Leukemia
PRV - Pseudorabies virus
RIG I - Retinoic acid-inducible gene-I
RING - Really interesting new gene

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RIP1 - Receptor-interacting protein kinase-1
ROS - Reactive oxygen species
SHV1 - Suid herpesvirus-1
TBK1 - TANK-binding kinase-1
TLRs - Toll-like receptors
TNF - Tumor necrosis factor
TNFR1 - TNF receptor-1
TRAF2 - TNF receptor accociated factor-2
TRADD - TNFR1-associated death domain
Tyk2 - Tyrosine kinase-2
UL - Unique long region
US - Unique short region
UV - Ultraviolet light
VZV - Varicella-zoster virus

1. Introduction

Alphaherpesviruses have a broad host range and are responsible for severe morbidity and mortality [1]. Human alphaherpesviruses include herpes simplex virus-(HSV)-1 and -2 (human herpesvirus (HHV)-1 and -2), and varicella-zoster virus (VZV) (HHV-3). Veterinary alphaherpesviruses include bovine herpesvirus-1 (BoHV-1), pseudorabies virus (PRV) [suid herpesvirus (SuHV)-1] and equine herpesvirus (EHV)-1 [2].

BoHV-1 is an important bovine pathogen that belongs to the subfamily *alphaherpesvirinae*. BoHV-1 strains are classified into subtypes 1.1, 1.2a, 1.2b and 1.3 [3]. This virus is responsible for multiple clinical symptoms in cattle, ranging from respiratory tract infections, genital disorders and encephalitis, to abortions [4]. The BoHV-1 subtype 1.1 is mostly associated with respiratory disease, whereas subtype 1.2a mostly causes respiratory and genital symptoms, and subtype 1.2b is predominantly associated with genital infections [5]. Subtype 1.1 strains are more pathogenic than subtype 1.2b strains [6]. Infection with BoHV-1, especially when followed by bacterial infections, lead to serious respiratory disease and cause a significant reduction in animal productivity including weight loss and a decrease in milk production [4, 7]. Thus, the severity of BoHV-1 infection and secondary infections results in significant losses to the cattle industry [8, 9].

BoHV-1 contains a large double-stranded DNA genome of ~140 kilobase pairs. The BoHV-1 virion is composed of four different compartments. The double-stranded DNA genome is enclosed within a capsid, which is surrounded by a layer containing inner and outer tegument proteins, and a lipid envelope with glycoproteins [10]. The BoHV-1 genome contains 73 open reading frames (ORFs), 33 of which encode structural proteins. Thirteen proteins are associated with the virion envelope, and 11 of them are glycosylated [7, 11].

Apoptosis, a prevalent form of cell death, is critical for development and tissue homeostasis in multicellular organisms. Apoptotis leads to the formation of apoptotic bodies, nucleosomal DNA fragmentation, membrane blebbing and chromatin condensation in the cells [12]. Caspases, a specific family of cysteine proteases, are responsible for the execution of apoptosis. Viruses are intracellular parasites that have adopted various strategies to utilize host cells to replicate and spread. Cell death is a common host defense mechanism against virus infection that is orchestrated to eliminate virus-infected cells prior to vast production of progeny viruses. As would be expected, viruses also developed strategies to escape cell death-based defenses. These evasion strategies promote virus infection and pathogenesis [13]. Therefore, it is not surprising that viruses have adopted multiple mechanisms to regulate cell death or apoptosis during productive infection. BoHV-1, like many other herpesviruses including HSV-1 [14], encodes both apoptotic and anti-apoptotic viral proteins to efficiently promote productive infection.

In this review, we summarize the current knowledge of the role of different BoHV-1 proteins, with reference to homologs in HSV-1, VZV and PRV, in modulating the apoptosis signaling pathways.

2. Mechanisms of apoptosis

Apoptosis is an energy-dependent process triggered by a variety of stimuli, and a critical component of the response to cellular injury [15]. Apoptotic cells exhibit morphological and biochemical changes including DNA fragmentation, apoptotic body formation, plasma membrane

blebbing, chromatin condensation and shrinkage of cells [12]. These apoptotic morphological features are the results of the action of proteolytic enzymes triggered by apoptotic stimuli. Induction of apoptotic pathways may occur by exposure of cells to endogenously synthesized [tumor necrosis factor (TNF-α) and reactive oxygen species (ROS)] or external (retinoic acid and ricin) components, alkylating agents, and chemotherapeutic agents [16]. Thus, a part of the cellular pathogenesis involves induction of apoptosis [17]. Viruses have adopted multiple mechanisms to modulate apoptotic responses. These evolutionary mechanisms confer survival advantages to either the host cell or to the virus.

Apoptosis involves highly complex and energy-dependent events consisting of three sequential parts, initiation, execution and termination [18]. Two distinct pathways may be engaged: i) extrinsic and ii) intrinsic or mitochondria-dependent pathways [18, 19].

The extrinsic pathway-mediated apoptotic signaling involves transmembrane receptor-associated interactions. These are classified as death receptors of the TNF superfamily [20], and contain "death domains" that are responsible for transmitting the death signal from the cell surface to intracellular networks. TNF-α/TNFR1 and the first apoptosis signal ligand/receptor (FasL/FasR) are considered death receptors and their corresponding ligands. The cascades of events involved in the extrinsic pathways are best characterized in TNF-α/TNFR1 and FasL/FasR systems [reviewed in [19]]. Binding to their corresponding homologous ligands triggers activation of death receptors. This results in the recruitment of cellular adaptor proteins [19]. For example, anchoring of TNF-α to the TNF receptor triggers recruitment of the TNFR1-associated death domain (TRADD) [21, 22]. Similarly, binding of FasL to the FasR causes engagement of the cytoplasmic adaptor protein Fas-associated death domain (FADD). Following FADD recruitment, complex-I triggers the formation of complex-II containing FADD, RIP1, and procaspase-8. Eventually, FADD interacts with and triggers activation of procaspase-8 [2, 14]. With the activation of procaspase-8, apoptosis is induced [23]. The active procaspase-8 then facilitates the cleavage of its downstream executioner procaspase-3 to generate active caspase-3. Finally, the active caspase-3 proceeds to the induction of apoptosis by initiating fragmentation of DNA [24]. TRADD also forms complex-I with TNFR1-associated factor-2 and receptor-interacting protein kinase-1 (RIP1) [15]. While TRADD can also activate caspase 8, the complex-I may also trigger nuclear translocation of nuclear factor-κβ (NF-κβ) to activate NF-κβ signaling, and thereby triggers expression of anti-apoptotic genes [15]. The termination phase of apoptosis involves the inhibitory function of the cellular FLICE-inhibitory protein (cFLIP) that binds to FADD or procaspase-8 to negatively regulate their activation [25].

In contrast, the intrinsic apoptotic signaling pathway is initiated in response to intracellular signals. This pathway is triggered by a set of stress stimuli within the cells such as DNA damage stress and ROS production [18]. It involves an array of non-receptor-associated stimuli that directly act on intracellular targets and also include mitochondria-dependent events [19]. All of these intracellular stimuli cause alterations in the inner mitochondrial membrane resulting in an opening of mitochondrial permeability transition (MPT) pores. Consequently, the mitochondrial transmembrane potential is lost, resulting in the induction of mitochondrial outer membrane permeabilization. This triggers the sequestration of pro-apoptotic proteins such as cytochrome-C from the intermembrane space into the cytoplasm [26]. Subsequently, the released cytochrome-C forms a complex with apoptotic protease activating factor-1 (Apaf-1) and caspase-9 denoted as "apoptosome" [27, 28]. The formation of apoptosome then leads to the activation of caspase-9 [29], and the activated caspase-9 triggers activation of executioner caspases including caspase-3 [30]. Finally, caspase-3 induces apoptosis by fragmentation of nucleosomal DNA. Apoptotic signaling through the intrinsic pathway is tightly regulated by the anti-apoptotic Bcl-2 family member proteins such as Bcl-1 and Bcl-xL and by the pro-apoptotic Bcl-2 family proteins, for example Bid (BH3-interacting domain death agonist), Bax (Bcl-2-associated X protein), and Bak (Bcl-2

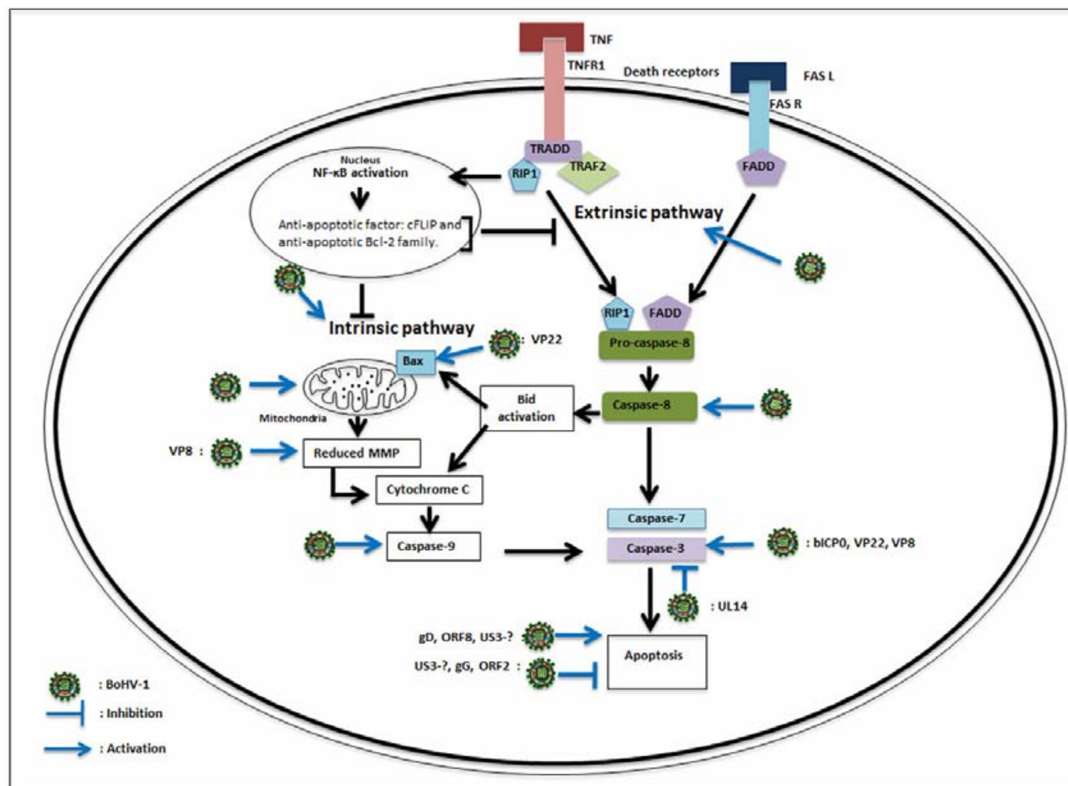

Figure 1. Modulation of apoptosis by BoHV-1. Apoptosis is triggered through activation of the extrinsic or intrinsic pathway. Binding of the death receptor, TNFR1 or Fas R, to its corresponding ligand, TNF or FasL, leads to the formation of a complex containing TRADD and FADD. The TRADD-associated complex triggers NF-κB activation followed by expression of anti-apoptotic genes, for example, anti-apoptotic Bcl2 family proteins and cFLIP. These anti-apoptotic factors block both the intrinsic and extrinsic pathways. In the extrinsic pathway, a secondary complex, consisting of FADD, pro-caspase-8 and RIP1, is formed, which triggers caspase-8 activation. Activated caspase-8 promotes caspase-3 and caspase-7 activation leading to apoptosis. In the intrinsic pathway, activation of Bax promotes the reduction of the mitochondrial membrane potential (MMP) leading to the release of cytochrome C. In parallel, caspase-8 also triggers Bid and Bax activation leading to cytochrome C release. Cytochrome C activates caspase-9 leading to caspase-3 and caspase-7 activation. These executioner caspases induce apoptosis. BoHV-1 is known to activate both the intrinsic and extrinsic pathways. In the extrinsic pathway, BoHV-1 activates pro-caspase-8. Several BoHV-1 proteins, such as bICP0, VP22 and VP8, trigger caspase-3 activation. gD, ORF8, and US3 also induce apoptosis. However, US3 has also been found to block apoptosis. BoHV-1 activates the intrinsic pathway through reduced MMP, Bax and Bid activation, cytochrome C release, and caspase-9 activation. Conversely, several anti-apoptotic proteins, such as gG and ORF2, block BoHV-1-induced apoptosis. Black arrows indicate the regular apoptotic pathways, whereas blue arrows show BoHV-1-modulated pathways.

homologous antagonist killer) [31]. A schematic diagram representing the overall apoptosis mechanism and modulation of apoptosis by BoHV-1 is shown in Figure 1.

3. Regulation of apoptosis by BoHV-1

A growing body of evidence suggests that like many other viruses BoHV-1 is involved in both induction and inhibition of apoptosis. BoHV-1 infection causes rapid cell death in permissive bovine cell lines [32, 33]. BoHV-1 was also found to induce apoptosis in peripheral blood mononuclear cells (PBMC) [34]. In addition, this virus causes apoptotic cell death in a large number of immune cells *in vitro* including T-lymphocytes, B-lymphocytes, monocytes/macrophages [35] and lymphoid tissue [33]. Induction of apoptosis by BoHV-1 is mediated by multiple mechanisms

[36-38]. Both live and inactivated BoHV-1 induces apoptosis in mitogen-stimulated PBMCs. Since triggering of apoptosis was observed with inactivated BoHV-1, it was postulated that the mechanism of apoptosis induction might include virus attachment or penetration, or the effect of viral structural proteins, such as glycoproteins or tegument proteins [34, 39]. A number of BoHV-1 proteins are involved in orchestrating the apoptotic response throughout the infection, all of which are discussed below.

3.1. Bovine ICP0 (bICP0)

One of the immediate early proteins of BoHV-1, bICP0, is encoded by an immediate early (IE) transcription unit-1 (IEtu1) [40]. bICP0 is constitutively expressed during productive infection and regulates IE and early (E) promoters, to transactivate multiple viral genes [41-43]. bICP0 expression causes toxic effects in transfected cells [44]. Moreover, bICP0 causes activation of caspase-3, a major hallmark of apoptosis, leading to apoptotic cell death [45]. Like its homolog, HSV-1 ICP0, bICP0 contains a zinc RING (really interesting new gene) finger domain at its N-terminus [41] [46], which is crucial to induce aggregated chromatin structures and toxicity [44]. It has been demonstrated that bICP0 plays a role in regulating cell death following BoHV-1 infection [47]. A bICP0 null mutant has 100-fold reduced titers compared to wild-type virus and induces a lower level of apoptosis compared to the bICP0-rescued virus [47]. This further indicates that bICP0 indeed functions as an apoptosis inducer during BoHV-1 infection of permissive cells.

Similarly, HSV-1 ICP0, a major homolog of bICP0, has been found to induce apoptosis. In the presence of a translational inhibitor, cycloheximide (CHX), wild-type HSV-1 triggers apoptosis, whereas a mutant virus with all five IE genes deleted was unable to induce apoptosis [48, 49]. Moreover, ICP0-deleted HSV-1 has a reduced ability to trigger apoptosis in the presence of CHX [50]. A recombinant HSV-1 expressing ICP0 but no other IE genes is capable of caspase-3 activation and induction of apoptosis during infection [50]. However, HSV-1 ICP0 has also been found to block ultraviolet (UV)-induced apoptosis, because expression of ICP0 provides protection against UV-induced apoptosis in HSV-1 infected U2OS cells [51]. It has been suggested that HSV-1 infection induces apoptosis and then prevents lethal apoptotic effects by producing anti-apoptotic proteins [14].

There is also some information about the role of the (b)ICP0 homolog VZV ORF61 (IE61) in apoptosis. Although VZV infection induces apoptosis [52-54] like BoHV-1 and HSV-1, unlike ICP0 and bICP0, VZV IE61 has been reported to inhibit apoptosis by regulating c-Jun-N-terminal kinase (JNK) and mitogen-activated protein kinase (MAPK) phosphorylation [55]. Like BoHV-1 and HSV-1, PRV induces apoptosis [56]. However, while a homolog of (b)ICP0 exists in PRV [early protein-0 (EP0)] the role of EP0 in regulating apoptosis is not known [57].

3.2. VP22

VP22, the U_L49 gene product of BoHV-1, is a tegument phosphoprotein and dispensable for virus growth in cell culture. However, the presence of VP22 during BoHV-1 infection contributes to increased virulence in cattle [58]. This is based on the fact that a UL49-deleted BoHV-1 was less virulent in cattle [58]. Recently, a cytotoxic effect was demonstrated in VP22-transfected Hela (human cervical carcinoma), D17 (canine osteosarcoma) and NSX2 (murine neuroblastoma) cells [38]. Moreover, caspase-3 activity was enhanced with increased time post-transfection. VP22 expression increased Bax expression and decreased Bcl-2 expression, which suggests that VP22 induces apoptosis in human tumor cells by upregulating the ratio of Bax to Bcl-2 [38].

Whether the VP22 homologs, HSV-1 VP22, VZV ORF9 and PRV VP22, play a role in apoptosis, is still unknown.

3.3. ORF2

In latently infected calves, BoHV-1-encoded latency-related (LR) RNA is abundantly expressed in trigeminal ganglia. The function of a LR-encoded protein is required for BoHV-1 to reactivate from latency and to protect neuronal cells from apoptosis. The LR gene product of BoHV-1 possesses anti-apoptotic activity and blocks programmed cell death [59], thereby promoting cell survival during acute BoHV-1

infection [59]. The LR gene has two open reading frames (ORF), ORF1 and ORF2. In transiently transfected cells the LR gene-encoded ORF2 inhibits apoptosis [60]. In mouse neuroblastoma cells (neuro-2A) the expression of ORF2 in the absence of other viral proteins blocks cold shock- or Fas ligand-induced apoptosis, while a frameshift mutant of ORF2 did not exhibit anti-apoptotic activity indicating that the protein expression is required to inhibit apoptosis [60]. In a separate study from the same group low-level expression of ORF2 was shown to retain its anti-apoptotic activity [61]. Thus, the LR gene product, ORF2, functions as anti-apoptotic protein to promote survival of infected neurons [60].

The homolog of ORF2 is absent in HSV-1 [62]. However, VZV ORF2 encodes a membrane-associated protein which is dispensable for latency establishment in dorsal root ganglia of cotton rats and also for growth in cell culture [63]. The role of VZV and PRV ORF2 in the regulation of apoptosis is still unknown.

3.4. ORF8

ORF8, located in the unique short (Us) segment of the BoHV-1 genome is a homolog of HSV-1 Us9 [64]. High-level expression of ORF8 induced a cytopathic effect and reduced cell viability in rabbit kidney cells (RK13). Analysis of genomic DNA fragmentation and nuclear condensation of ORF8-transfected RK13 cells revealed induction of apoptotic cell death in the presence of ORF8 [65]. To further demonstrate the role of ORF8 in inducing apoptosis an ORF8-deleted BoHV-1 mutant virus was used [66]. In RK13 cells infection with wild-type or ORF8-revertant BoHV-1 induced apoptosis, whereas infection with the ORF8-deleted virus resulted in reduced induction of apoptosis [66]. As a result, the ORF8-deleted BoHV-1 showed reduced release of progeny viruses into the extracellular supernatant when compared to BoHV-1. Thus, induction of apoptosis by ORF8 might facilitate the release of virus particles [66].

While the BoHV-1 ORF8 induces apoptosis, the role of its homologs, HSV-1, VZV and PRV US9 [67], in regulating apoptosis is not known.

3.5. US3

US3, a serine/threonine kinase, is conserved among all alphaherpesviruses including BoHV-1. US3 is a multifunctional protein involved in similar functions in different alphaherpesviruses. BoHV-1 US3 phosphorylates other viral proteins such as VP22 and VP8 [68, 69]. BoHV-1 US3 induces cytoskeletal changes during virus infection [70]. Takashima *et al.* demonstrated that both wild-type and US3-deleted BoHV-1 block sorbitol-induced apoptosis indicating that US3 does not affect apoptosis [71]. However, recently the role of US3 in regulation of apoptosis was re-evaluated [72], which demonstrated that the presence of US3 suppresses induction of apoptosis. BoHV-1 blocked apoptosis induced by infection, as well as protected cells from apoptosis triggered by other exogenous factors such as sorbitol or staurosporine [72]. US3-deleted BoHV-1 induced more apoptosis than wild-type and US3-revertant BoHV-1. Caspase-3 activity was also significantly higher in US3-deleted BoHV-1 compared to wild-type BoHV-1. In addition, US3-mediated inhibition of apoptosis is associated with phosphorylation of the Bcl2-associated death promoter (Bad), a pro-apoptotic member of the Bcl-2 family. These data suggest that US3 prevents apoptosis during BoHV-1 infection [72]. These contradictory results were attributed to the use of different cell lines, cell culture conditions, apoptosis-inducing agents, exposure time and intensity of the stimulus. Moreover, the concentration of sorbitol and the apoptosis detection method were different.

HSV-1 US3 plays a role in nuclear egress of infectious virions, evasion of antiviral responses and cytoskeletal rearrangements. HSV-1 US3 has also been shown to inhibit apoptosis [14] by blocking the release of cytochrome C to inhibit caspase-3 activation [73]. Additionally, HSV-1 US3 phosphorylates pre-apoptotic proteins Bad and Bid, to block their role in triggering apoptosis [74, 75]. HSV-1 US3 also phosphorylates procaspase-3 to enhance apoptosis resistance [76]. This shows that US3 prevents apoptosis by regulating the downstream effectors of the mitochondria-mediated pathway [74, 77]. Furthermore, US3 interacts with programmed cell

death protein 4 (PDCD4); knockdown of PDCD4 blocks apoptosis induced by an ICP4-deficient strain [78]. Thus, US3 modulates different pro-apoptotic proteins to block apoptosis.

PRV US3 functions as efficiently as HSV-1 US3 in preventing apoptosis [79]. Introduction of a point mutation in the PRV US3 ATP binding site prevents its ability to phosphorylate the pre-apoptotic protein Bad [80]. Moreover, transient transfection of PRV US3 triggers Bad phosphorylation, downregulates pro-apoptotic Bax, and upregulates anti-apoptotic Bcl2 to inhibit apoptosis [81]. Both HSV-1 and PRV US3 can block apoptosis induction triggered by Bcl2 family proteins indicating conserved mechanisms of inhibition of apoptosis by US3 [77].

ORF66, a protein kinase, is the VZV homolog of US3 [82]. Like US3, ORF66 is dispensable in cell culture, although kinase-deficient viruses show impaired replication in cell culture [83]. ORF66 is also known to inhibit apoptosis [84]. Wild-type VZV infection of T cells renders cells resistant to apoptosis by preventing caspase-3 activation, in contrast to an ORF66-deficient mutant virus. Thus, blocking or eliminating ORF66 protein increases susceptibility of T cells to apoptosis [84].

3.6. VP8

VP8, the U_L47 gene product, is the most abundant tegument protein of BoHV-1 [85]. VP8 is phosphorylated by US3 and cellular kinase-2 (CK2), and phosphorylated VP8 redistributes promyelocytic leukemia (PML) [86]. Recently, we demonstrated that VP8 also interacts with DNA damage response proteins, ataxia telangiectasia mutated protein (ATM) and Nijmegen breakage syndrome protein (NBS1) [87]. This interaction prevents phosphorylation of NBS1, blocks the downstream events of the DNA damage response and triggers DNA damage-induced apoptosis. Induction of apoptosis was stronger in cells infected with wild-type BoHV-1 and VP8-revertant virus compared to VP8-deleted virus [87]. Caspase-3 activation in the presence of VP8 supported the role of VP8 in inducing apoptosis during BoHV-1 infection [87]. Increased DNA damage in the presence of VP8 might trigger ROS production that leads to the induction of apoptosis. Two independent studies revealed that BoHV-1 infection triggers ROS overproduction and causes mitochondrial dysfunction to induce cell death [37, 88]. A recent report also showed that BoHV-1 infection induces oxidative DNA damage leading to cell damage [36].

The effects of VP8 homologs, such as HSV-1 VP13/14 [85], VZV ORF11 [89] and PRV UL47 [90], on apoptosis are unknown.

3.7. Glycoprotein D (gD)

Glycoprotein D (gD) of BoHV-1 is a major component of the viral envelope. The expression of gD in transfected cells causes cytotoxicity suggesting that gD plays a role in triggering cell death [91]. Glycoprotein H (gH) of BoHV-1 is crucial for virus penetration but not for attachment. A gH-deleted mutant was able to induce apoptosis indicating that attachment but not penetration is required for apoptosis induction by BoHV-1 [39]. Analysis of other glycoprotein-deleted viruses, such as gC-, gE-, gI- and gG-deleted mutants, indicated that these mutants are still able to induce apoptosis. Since gD expression has been demonstrated to be toxic and gD was present in the gH-deleted mutant, the authors concluded that gD is involved in apoptotic cell death [92]. It was also shown that a gD-deleted mutant did not induce apoptosis confirming the role of gD in triggering apoptotic cell death [92].

In contrast to BoHV-1 gD, HSV-1 gD is known to inhibit apoptosis [2, 14]. HSV-1 gD binds to the mannose-6 phosphate receptor and suppresses premature apoptosis [93, 94]. Additionally, gD inhibits Fas-mediated apoptosis, which involves NFκB activation to promote the expression of anti-apoptotic genes [95]. A gD-deleted mutant exhibits reduced ability to block apoptosis further supporting the role of gD in suppression of apoptosis [96]. However, the molecular mechanism of inhibition of apoptosis by HSV-1 gD is poorly understood. A gD homolog is present in PRV. However, the function of PRV gD in regulation of apoptosis is still unknown. While HSV-1, PRV and BoHV-1 encode gD, this protein is not encoded by VZV [97].

3.8. Glycoprotein G (gG)

Glycoprotein G (gG) of BoHV-1 is encoded by ORF4 within the unique short region of the viral genome [98]. BoHV-1 gG is required for maintaining cell-to-cell junctional adherence among infected cells [67]. BoHV-1 gG possesses anti-apoptotic properties [99]. DNA fragmentation and condensation analysis of RK13 cells infected with wild-type or a gG-negative virus revealed increased DNA fragmentation in the gG-negative virus. Moreover, gG-mediated inhibition of apoptosis increases virus replication [99]. Hence, BoHV-1 gG postpones apoptosis, stabilizes the cell structure and promotes efficient replication during BoHV-1 infection [99].

Homologs of gG are present in most alphaherpesviruses including HSV-1 and PRV [100, 101]. However, the gG homolog is absent in VZV [98]. HSV-1 gG is not essential for virus replication in cell culture [102], but the role of HSV-1 and PRV gG in apoptosis is not known.

3.9. UL14

UL14 is a tegument protein [103] and located in a gene cluster containing UL11, UL12, and UL13. The expression of BoHV-1 UL14 during transient transfection is sufficient to block sorbitol-induced apoptosis in human chronic myelogenous leukemia (K562) and Madin Darby kidney (MDBK) cells [104]. Furthermore, sorbitol-induced caspase-9 activation was suppressed in the presence of UL14 [104]. Another report supported the anti-apoptotic properties of UL14 [105]. This study indicated that UL14 expression in the absence of any other viral proteins is sufficient to inhibit stress-induced apoptosis. Stress agents, such as sorbitol, cause caspase-9 and -3 activation to trigger apoptosis [105]. However, the presence of UL14 prevented caspase-9 and-3 activation in sorbitol-treated cells further demonstrating the involvement of UL14 in blocking apoptosis [105]. These studies indicate that UL14 functions in promoting cell survival. However, the anti-apoptotic role of UL14 during BoHV-1infection is yet to be confirmed by examining the effects of a UL14-negative mutant virus.

The HSV-1 U_L14 gene product is a tegument protein synthesized later during infection [103]. HSV-1 UL14 can act as heat shock protein-70 (HSP70) [106]. Like BoHV-1 UL14, HSV-1 UL14 is known to inhibit apoptosis [107]. A UL14-deleted HSV-1 demonstrated decreased suppression of apoptosis compared to wild-type virus. Since HSP70 blocks caspase activation and apoptosis [108], the heat shock protein-like function of UL14 might facilitate inhibition of apoptosis [14]. The roles of the UL14 homologs in VZV and PRV in apoptosis are still unknown [109].

4. Conclusion

In this review the involvement of BoHV-1 proteins, and alphaherpesvirus homologs, in the modulation of apoptosis was summarized. Apoptosis is a prevalent mechanism of the host defense that contributes to eliminating virus-infected cells. However, BoHV-1 encodes multiple anti-apoptotic and pro-apoptotic proteins to regulate the balance of the apoptotic processes. For example, bICP0, gD, ORF8, VP22, and VP8 trigger apoptosis. However, the lethal effects of apoptosis are also prevented by the expression of some anti-apoptotic proteins, such as ORF2, gG, and UL14. In some cases, it has been observed that induction of apoptosis by some late proteins, such as VP8 and ORF8, facilitates the release of progeny viruses in the extracellular environment [66, 110]. This was attributed to the fact that infection with VP8- or ORF8-deleted virus produced significantly reduced extracellular virus compared to wild-type virus infection. Thus, it is conceivable that the induction of apoptosis later during infection also facilitates progression of infection through virus spread to the neighboring cells. Although BoHV-1 is released into the extracellular environment by exocytosis [111], this process can be expedited by apoptosis.

In contrast, the benefit of triggering apoptosis by caspase activation at the beginning of infection by the IE protein bICP0 is not completely understood. In a recent article the beneficial role of caspases in promoting replication of human gammaherpesviruses was discussed [112]. For instance, Epstein-Barr virus (EBV) utilizes caspases to inactivate cellular proteins with anti-EBV functions and also to process and activate viral factors to promote virus replication [113-115]. Thus, EBV has evolved strategies to thrive

in an environment with active caspases. In Kaposi's-sarcoma associated herpesvirus (KSHV)-infected cells induction of apoptosis results in lytic reactivation through activation of caspase-3 and -9 [116]. Moreover, recent studies revealed that caspase activation plays an important role in regulating antiviral type I interferon (IFN) signaling [112]. For example, during lytic infection, KSHV activates a caspase-dependent mechanism to block type I IFN, especially IFN-β, responses [117]. Viruses have developed multiple strategies to utilize caspase activity to facilitate replication [118]. Hence, induction of apoptosis by IE proteins might favor virus replication. Nevertheless, late anti-apoptotic proteins block this early apoptotic response during infection supporting the fine-tuned control of apoptosis to promote BoHV-1 infection.

Overall, an understanding of the BoHV-1 proteins and its homologs in other alphaherpesviruses that are involved in promotion or inhibition of apoptosis may provide valuable insight into the relevant mechanisms. A further understanding of specific molecular approaches in modulating apoptotic responses will advance the development of novel therapeutic strategies against BoHV-1 infection.

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CONFLICT OF INTEREST STATEMENT

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest or in financial conflict with the subject matter or materials discussed in the manuscript.

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