Equine herpesviruses type 1 (EHV-1) and 4 (EHV-4)—Masters of co-evolution and a constant threat to equids and beyond

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A B S T R A C T

The equine herpesviruses type 1 (EHV-1) and 4 (EHV-4) are ubiquitous pathogens that affect horse populations on all continents. Despite widespread vaccination, EHV-1 and EHV-4 infections remain a permanent risk. While the two viruses share a high degree of genetic and antigenic similarity, they differ significantly in host range and pathogenicity. Compared to EHV-4, which mainly infects horses and causes respiratory disease, EHV-1 has a broader host range and can result in respiratory disease, abortions, neonatal death, and equine herpesvirusmyeloencephalopathy (EHM). Recent studies have elucidated a number of mechanisms that may, at least partly, explain the differential pathogenic potential of the two viruses. While both EHV-1 and EHV-4 can escape host immune responses and establish latent infection, there are differences with respect to virus entry and their ability to interfere with the innate immune response. Understanding the virus’ repertoire of immunomodulatory mechanisms may lead the way to develop more efficient vaccines.

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1. Introduction

The latest taxonomy summarizes herpesviruses within a new order, Herpesvirales, which is divided into three families: Herpesviridae, Alloherpesviridae and Malacoherpesviridae (Davison et al., 2009). The family Herpesviridae, representing the mammal, bird and reptile viruses, consists of three subfamilies namely the Alpha-, Beta- and Gammaherpesvirinae (Davison et al., 2009). In equids, nine herpesviruses have been identified so far, of which EHV-1 (equine abortion virus), EHV-3 (equine coital exanthema virus), EHV-4 (equine rhinopneumonitis virus), EHV-6 (asinine herpesvirus 1), EHV-8 (asinine herpesvirus 3), and EHV-9 (gazelle herpesvirus 1) belong to the genus Varicellovirus of the subfamily Alphaherpesvirinae. The other three viruses, EHV-2, EHV-5, and EHV-7 (asinine herpesvirus 2), belong to the Gammaherpesvirinae subfamily (Davison et al., 2009). The horse is the natural host to EHV-1, -2, -3, -4 and -5, while the donkey is the host to EHV-6, -7 and -8 (Patel and Heldens, 2005). EHV-9 is a neurotropic virus and was originally isolated from gazelle (Fukushi et al., 1997). More recently, a new member of the Gammaherpesvirinae subfamily, zebra herpesvirus 1, was identified by PCR from blood samples collected from a mountain zebra in Namibia (Ehlers et al., 2008).

EHV-1 and EHV-4 are arguably the most relevant herpesviruses affecting equids and were considered subtypes of one and the same virus species until 1981 (Sabine et al., 1981; Studdert et al., 1981). Both EHV-1 and EHV-4 harbor linear double-stranded type “D” DNA genomes, which are separated into a unique long (UL) region and a unique short (US) segment, the latter being bracketed by two inverted repeat regions called the internal repeat (IR) and terminal repeat (TR) (Telford et al., 1998, 1992). The EHV-1 genome is approximately
150-kbp in size and contains at least 76 distinct genes, four of which (gene 64, 65, 66 and 67) are duplicated resulting in a total of 80 open reading frames (ORFs). EHV-4, with a slightly smaller genome size of 146-kbp, also contains 76 genes, but only three of those (gene 64, 65 and 66) are duplicated. The nucleotide identity between EHV-1 and EHV-4 genes ranges from 55% (gene 76) to 84% (gene 42) (Telford et al., 1998, 1992). Although both viruses show a high degree of genetic and, hence, antigenic similarity, they are strikingly different with respect to their pathogenesis. While EHV-4 infection usually is limited mainly to the upper respiratory tract (URT), EHV-1 has a systemic distribution affecting multiple organ systems and causing different diseases that range from mild rhinopneumonitis to abortion and lethal myelencephalopathy (Patel and Heldens, 2005).

Both EHV-1 and EHV-4 have considerable economic impact on the horse industry. Despite widespread vaccination, the two viruses are still causing substantial problems. In recent years, efforts have been made to more comprehensively understand the mechanisms that might explain the persistence and the different pathogenicity of both viruses, and to develop more rational vaccines. In this review, we will summarize the current knowledge, focusing particularly on the developments during the past five years, and include new findings on virus epidemiology, entry, molecular pathogenesis, immune evasion and vaccines.

2. Epidemiology and diagnosis

EHV-1 and EHV-4 are known as ubiquitous pathogens in horse populations throughout the world. It is noteworthy, however, that serological surveys of these two closely related but distinct viruses have always been complicated by the extensive antigenic cross-reactivity and the virtual absence of type-specific antibodies (Patel and Heldens, 2005). A big milestone was reached in the early 1990s when one of the viral glycoproteins, gC, was identified to elicit type-specific antibodies. Epitopes present in the carboxy-terminal, variable region of the gC's of both viruses were shown to elicit type-specific humoral responses in the natural host and allowed distinction between EHV-1 and EHV-4 infection by a type-specific ELISA (Crabb et al., 1992; Crabb and Studdert, 1993).

Sero-epidemiological investigation on a large Thoroughbred stud farm in the Hunter Valley of New South Wales showed that more than 99% of the tested mares and foals were EHV-4-antibody positive, while the prevalence of EHV-1 antibody positive mares and foals were 26.2% and 11.4%, respectively (Gilkerson et al., 1999b). Mare and foal populations were found to be a reservoir of EHV-1, from which the virus can be transmitted before and after weaning and as young as 30 days of age (Gilkerson et al., 1999a). Even after vaccination with inactivated whole virus EHV-1 and EHV-4 vaccines, both viruses continued to circulate in vaccinated mares and their unweaned foals (Foote et al., 2006). Serological surveys from different countries have consistently shown that EHV-4 appears to have a significantly higher prevalence than EHV-1, which could be partially explained by the finding that EHV-4 infection can occur throughout the year, but EHV-1 infection mainly occurs in the winter (pregnancy) season (Patel and Heldens, 2005).

In recent years, a number of PCR-based assays have been developed for diagnosis and discrimination of EHV-1 and EHV-4 infection from different clinical samples. It is well known that EHV-1 and EHV-4 are transmitted by direct contact and aerosols after shedding from the nasal cavity or by large virus loads that are present in aborted fetuses and the placenta (reviewed in Lunn et al., 2009). Hebia-Fellah et al. (2009) studied the possible risk of horizontal transmission of EHV-1 and EHV-4 via semen and their effect on stallion fertility using conventional PCR. In their study, EHV-1 DNA was detected in 51 out of 390 total semen samples (13%). In contrast, no EHV-4 DNA was detected in these samples. The presence of EHV-1, however, did not appear to affect the fertility of infected stallions (Hebia-Fellah et al., 2009). In another study, using real-time PCR, EHV-1 shedding could be detected in semen on day 20 after onset of fever in naturally infected stallions, which seems not to be directly associated with spermatozoa (Walter et al., 2012). It must be emphasized that none of these studies have demonstrated infectious virus in the semen, therefore, it is still unclear whether the virus can actually spread by the venereal route. Ataseven et al. (2009) examined the prevalence of EHV-1 and EHV-4 infections in equid species in Turkey using both ELISA and multiplex PCR. The results showed that 14.5%, 37.2%, and 24.2% of the tested horses, mules, and donkeys, respectively, were seropositive for EHV-1, while EHV-4-specific antibodies were detected in 81.7% of the tested horses. Both EHV-1 and EHV-4 DNA was detected from nasal swabs taken from symptomatic horses (Ataseven et al., 2009).

More recently, series of studies have focused on the so-called neurovirulent and non-neurovirulent EHV-1 strains. The distinction is based on a single nucleotide polymorphism (SNP) A/G2254 in ORF30 encoding viral DNA polymerase (Goodman et al., 2007; Nugent et al., 2006). Viruses amplified from equine herpesvirus myelonecephalopathy (EHM) cases were more strongly associated with a DNA polymerase sequence containing a G2254 that encodes an aspartic acid residue at position 752 (D752), compared to an A2254 that encodes asparagine (N752). In several follow-up studies, the occurrence of G2254 and A2254 genotypes was assessed. While the Thoroughbred broodmare population of central Kentucky seems to have a large proportion of horses harboring latent G2254, which was also corroborated by findings of EHV-1 in aborted fetuses (Allen et al., 2008; Smith et al., 2010), the G2254/A2254 ratio seems smaller in other areas (Perkins et al., 2009; Pusterla et al., 2010).

There is increasing evidence that the host range of EHV-1, in contrast to EHV-4, goes far beyond equine species. It has been reported that non-equids, including llamas (Lama glama), alpacas (Vicugna pacos), blackbuck (Antelopa cervicapra), and Thomson’s gazelle (Eudorcas thomsoni), can be occasionally infected by EHV-1 or EHV-1-related viruses (Chowdhury et al., 1988; Ibrahim et al., 2007; Rebhun et al., 1988). Recently, neurotropic EHV-1 strains...
were isolated from black bears (*Ursus americanus*), Thomson’s gazelles, and guinea pigs (*Cavia porcellus* f. dom.) that suffered from severe neurological disorders (Wohlsein et al., 2011). In addition, a recombinant variant between EHV-1 and the newest equine herpesvirus and close relative of EHV-1, EHV-9, was found to be the culprit in the death of zoo polar bears in Germany. The virus seems to have originated in zebras, but it is unclear at present whether the recombination occurred in captive zebras in zoos or wild zebras in natural habitats before importation. The recombination resulted in a pathogen fatal for polar bears, while zebras remain asymptomatic and likely carry the virus as the definitive host (Greenwood et al., 2012). It is still unclear whether EHV-1/EHV-9 recombination is a single event or happens more frequently. However, it is clear that zebra-derived EHV-1 is widespread in zoos and its potential effect on non-equid and endangered animals must be considered.

In horses, both EHV-1 and EHV-4 can establish life-long latency after primary infection and become invisible to immune surveillance and, hence, elimination. Some studies demonstrated that latency was mainly established and maintained in lymphoid tissues and circulating lymphocytes (Carvalho et al., 2000; Chesters et al., 1997; Smith et al., 1998; Welch et al., 1992), while some other studies claim latency is established predominantly in the trigeminal ganglia (Borchers et al., 1997, 1999; Slater et al., 1994). Regardless of the biologically relevant site of latency, recrudescing viruses may be transmitted to other horses and cause clinical disease and viral shedding (Pusterla et al., 2009b). The establishment, maintenance, and reactivation from latency are of great importance not only for the viral life cycle but also are central to the epidemiology of EHV-1 and EHV-4, yet it is difficult to study and much of our understanding stems from comparisons with related viruses (Lunn et al., 2009; Patel and Heldens, 2005).

### 3. Virus entry

As mentioned above, EHV-1 has a much broader host spectrum than EHV-4. The difference in host spectrum is also reflected by differences in cellular tropism. In vitro, EHV-1 is able to enter and replicate in cultured cells of various origins including equine, human, swine, bovine, canine, feline, and rabbit, while EHV-4 infection is mainly restricted to equine cells (Trapp et al., 2005; Whalley et al., 2007). In horses, EHV-1 can infect at least three distinct cell types in three different organ systems that include epithelial cells, mononuclear cells in lymphoid tissue and peripheral blood (PBMCs), and endothelial cells of inner organs (Osterrieder and Van de Walle, 2010). On the other hand, lytic infection with EHV-4 is thought to be limited mainly to the epithelial cells of the URT, although recent in vitro studies showed that EHV-4 was also able to infect endothelial cells (Osterrieder and Van de Walle, 2010).

The difference in cellular tropism between EHV-1 and EHV-4 can be associated with their different abilities to enter cells. For the prototypic herpes simplex virus 1 (HSV-1) and most other alphaherpesviruses including EHV-1 and EHV-4, productive infection is initiated by a relatively unstable attachment to heparan sulfate moieties on cell surface proteoglycans that is mediated by gC and gB, followed by binding of gD to one of the specific receptors expressed on the cell surface (Azab et al., 2010; Osterrieder, 1999; Spear, 2004). Receptor engagement leads to conformational changes enabling complex interactions between gB and gH/gL (Spear, 2004). It has been shown that EHV-1 can infect cell lines without any of the gD receptors identified for HSV-1 and other alphaherpesviruses, for example, CHO-K1 and J1.1-2. The results indicated that EHV-1 utilizes alternative receptor(s) for entry (Frampton et al., 2005). Recently, equine major histocompatibility complex class I (MHC-I) was shown to serve as a receptor for EHV-1 entry, which is targeted by gD and critical for access into equine dermal (ED) cells as well as equine brain microvascular endothelial cells (Kurtz et al., 2010; Sasaki et al., 2011a). Since MHC-I is expressed on virtually all cell types (David-Watine et al., 1990), the distribution of MHC-I may not have a direct correlation with EHV-1 tissue or cell tropism (Sasaki et al., 2011a). Further studies showed that not all of the many different equine MHC-I alleles, but predominantly those with an alanine at position 173 in α2 domain, served as functional EHV-1 entry receptors (Sasaki et al., 2011b).

In contrast to EHV-1, the entry pathways of EHV-4 have been studied to a much lesser extent. With the successful construction of EHV-4 genome as an infectious bacterial artificial chromosome (BAC) clone, the mutagenesis of EHV-4 genes and study of the roles of EHV-4 proteins in host–virus interaction have been greatly facilitated (Azab et al., 2009). By swapping gD between EHV-1 and EHV-4, the differential cellular tropism of the two viruses was compared. Most of the cell lines permissive for EHV-1 became resistant to EHV-1 when gD (gD1) was replaced by EHV-4 gD (gD4). Vice versa, endowing EHV-4 with gD1 allowed entry into previously resistant cell lines, providing direct evidence that the cell tropism of EHV-1 and EHV-4 in vitro was mainly dependent on gD (Fig. 1) (Azab and Osterrieder, 2012). This recent study also showed that EHV-1 entered CHO-K1 cells and PBMCs via an integrin-independent mechanism, which is different from previous findings showing that αV integrins are important for EHV-1 entry into these two cell types (Van de Walle et al., 2008). More surprisingly, EHV-4 apparently is able to infect PBMCs as efficiently as EHV-1 in vitro and entry of neither EHV-1 nor EHV-4 into PBMCs was inhibited by blocking cell surface integrins or MHC-I (Azab and Osterrieder, 2012). The results clearly indicated that yet unidentified cellular receptors are involved in the entry of EHV-1 and EHV-4 into PBMCs or that the number of available receptors (i.e. MHC-I) was too high for complete blockade with antibodies or soluble receptor-binding gD. Besides gD, the role of gH, another protein involved in viral entry, was examined by swapping gH between EHV-1 and EHV-4. These experiments showed that EHV-1 or EHV-4 gH do not have a major effect on the host range of either virus in vitro (Azab et al., 2012).

Alphaherpesviruses have been shown to use different pathways to enter cells, including direct fusion at the plasma membrane or from within endosomes, either at neutral or acidic pH, depending on the cell type and
available receptor(s) (Geraghty et al., 1998; Hutchinson et al., 1992; Mercer et al., 2010; Shukla et al., 1999). Recently, our laboratory investigated the entry pathway of EHV-1 and EHV-4, for which the cellular and viral determinants routing virus entry are unknown. We showed that EHV-1 enters equine epithelial cells via direct fusion at the plasma membrane, while EHV-4 does so via an endocytic pathway, which is dependent on dynamin-II, cholesterol, caveolin-1, and tyrosine kinase activity (Azab et al., 2013). Exchanging gH between both viruses resulted in re-routing of EHV-1 to the endocytic pathway, as did blocking the interaction with α4β1 integrins on the cell surface. Furthermore, a point mutation in the SDI integrin-binding motif of EHV-1gH also directed EHV-1 to the endocytic pathway (Azab et al., 2013). Cumulatively, we could confirm that viral gH and cellular α4β1 integrins are important determinants in the choice of alphaherpesvirus cellular entry pathways.

In conclusion, the available data on EHV-1 and EHV-4 entry into target cells clearly suggest that gD plays a critical role in determining the cellular tropisms of EHV-1 and EHV-4. EHV-1 is able to bind various cellular receptors through gD and enter host cells by different pathways, resulting in a maximized host range. On the other hand, EHV-4 gD has a limited range of receptors that it can bind to, which, consequently, reduces the host range of the virus. Glycoprotein gH has been shown for the first time to act as a router, which decides, together with α4β1 integrins as the cellular counterpart, the entry pathway of the virus.

4. Disease and molecular pathogenesis

EHV-1 is highly contagious and usually transmitted by direct contact, mainly through infected nasal discharge. While rare, EHV-1 can also be transmitted by aerosol or contaminated feed, water, and equipment (Allen and Bryans, 1986). After inhalation, the virus replicates in epithelial cells of the URT, resulting in virus shedding and distinct herpetic lesions of mucosal membranes. In natural and experimental infections, both EHV-1 and EHV-4 can cause respiratory disease characterized by fever, anorexia, as well as nasal and ocular discharge (Patel and Heldens, 2005). After respiratory infection, EHV-1 invades the lamina propria using migrating mononuclear cells as vehicles for further distribution. As a result, EHV-1 can easily spread throughout the body via a cell-associated viremia, reaching the secondary sites of virus replication, mainly the vasculature of the pregnant uterus and/or the central nervous system (CNS) (Osterrieder and Van de Walle, 2010). The widespread infection of endothelial cells, along with the host’s repair program in response to such insult, ultimately induce abortion or neurological disease as a result of vasculitis, thrombosis, and ischemic damage (Smith et al., 1992; Wilson, 1997). It seems likely that the leukocyte-associated viremia and the infection of endothelial cells are key prerequisites for EHV-1 pathogenicity. In contrast to EHV-1, EHV-4 infection usually remains limited to the URT and is rarely accompanied by cell-associated viremia (Fig. 2).

The differences in the pathogenic potential between the two closely related viruses have not been fully understood. It was long thought that the difference was, at least in part, caused by the restricted cellular tropism, mainly determined by EHV-4 gD. EHV-4 is, however, able to replicate in endothelial cells both in vitro and in vivo (Blunden et al., 1995; Osterrieder and Van de Walle, 2010). Even in PBMCs in vitro, EHV-4 has similar infection efficiency as EHV-1 (Azab and Osterrieder, 2012). This finding was consistent with previous studies on EHV-4 replication kinetics in PBMCs during a field outbreak, which showed that EHV-4 genomic DNA was detected initially in PBMCs of every infected foal. Viral DNA loads were low and lasted only for a short period of time (Pusterla et al., 2005). In contrast, high levels of EHV-1 viremia were detected for prolonged periods of time in PBMCs, although the onset and duration of viremia differ between EHV-1 strains (Allen and Breathnach, 2006; Nugent et al., 2006). These data suggested that the difference in cellular entry does not play the decisive role in determining pathogenicity of EHV-1 and EHV-4.

Using nasal mucosal explants as an infection model, it was shown that EHV-1 could spread from epithelium to the connective tissue below the basement membrane, where mononuclear leukocytes were infected, whereas EHV-4-infected mononuclear leukocytes were extremely rare (Vandekerckhove et al., 2011). Interestingly, a viral
Chemokine binding protein (vCKBP), gG, expressed by EHV-1 and EHV-4 has different chemokine binding properties, with the former being able to modulate chemokine activity, while the latter is not (Van de Walle et al., 2009b, 2007). The inability of EHV-4 gG to modulate chemokines that are crucial for modulating leukocyte migration during both innate and adaptive immune responses was also thought to be one of the reasons why EHV-4 infection is restricted to the URT (Osterrieder and Van de Walle, 2010).

Considering the infection of endothelial cells, it was hypothesized that endothelial cell infection is initiated upon close contact following adhesion of PBMCs and direct cell-to-cell transfer of virus, but not by viral egress from PBMCs (Smith et al., 2002). A recent study supported the hypothesis of direct cell-to-cell spread of EHV-1 infection as the primary route of virus transfer from PBMCs to endothelial cells demonstrating successful infection in the presence of neutralizing antibodies (Goehring et al., 2010a). PBMCs circulate in the blood as non-adherent cells, but can become adherent upon stimulation, as is presumably the case after EHV-1 infection. This “on-off” adhesion behavior is controlled by the activity of adhesion molecules expressed on the cell surface. The question of how EHV-1-infected PBMCs adhere to endothelial cells and whether EHV-4-infected PBMCs have the same propensity has not been addressed. One previous study (Smith et al., 2002) has shown that the process of endothelial cell infection may be regulated by local mediators that are theoretically capable of directing tissue tropism. It has long been known that both PBMCs and endothelial cells possess a repertoire of adhesion molecules that can mediate the interactions between the two cell types (Dustin et al., 1986; Oppenheimer-Marks et al., 1991). Our recent data show that EHV-4, but not EHV-1, is able to downregulate VLA-4, the receptor for the adhesion molecule VCAM-1 expressed predominantly on the surface of infected PBMCs, which may reduce the ability of EHV-4-infected PBMCs to adhere to endothelial cells (Said et al., 2012). Taken together, we hypothesize that systemic spread and higher pathogenic potential of EHV-1 when compared to EHV-4 is caused by several factors including differential ability of both viruses to establish viremia and reprogram PBMCs, modulate chemokines, and regulate cellular molecules that are important for adhesion to endothelial cells.

Over the last decade, the incidence of EHM induced by EHV-1 has increased significantly in North America and Europe (Allen et al., 2008; Perkins et al., 2009; Pusterla et al., 2009a). As outlined above, a single nucleotide exchange at position 2254 (A/G2254) in the catalytic subunit of EHV-1 DNA polymerase encoded by ORF30 is strongly associated with the occurrence of EHM (Goodman et al., 2002).
et al., 2007; Nugent et al., 2006; Van de Walle et al., 2009a). Compared to the non-neurovirulent genotype of EHV-1 (A2254), the neurovirulent genotype (G2254) was found to replicate more efficiently in horses, resulting in higher levels and longer duration of cell-associated viremia (Goodman et al., 2007; Van de Walle et al., 2009a). Furthermore, the production of IL-10 was significantly reduced after infection with neurovirulent Ab4, which might contribute to increased local inflammation and a higher risk of neurological manifestation (Wagner et al., 2011). At the port of entry in epithelia of the URT, a neurovirulent EHV-1 strain was able to infect larger numbers of leukocytes under the basement membrane of nasal mucosa when compared to non-neurovirulent EHV-1 strains (Grysepeert et al., 2010). It should be noted that a minority of EHM cases were shown to be associated with the A2254 genotype; on the other hand, the G2254 genotype did not necessarily lead to EHM (Perkins et al., 2009; Pronost et al., 2010; Smith et al., 2010; Vissani et al., 2009) indicating that other viral factors might also contribute to the occurrence of EHM. Lately, the UL24 (ORF37) gene product was shown to play a role in neuropathogenesis in a mouse encephalitis model (Kasem et al., 2010). Besides viral genetics, host and environmental factors, such as age, physical condition, immune status of the host and infection route, also have a significant impact on the clinical outcome following EHV-1 infection (Nugent et al., 2006).

5. Immune evasion

EHV-1 and EHV-4, like other herpesviruses, establish lifelong presence in the infected host after primary infection. It is generally accepted that the long-term latency in the presence of an active host immune system is facilitated by immunomodulatory strategies that have been developed by the viruses during their long history of co-evolution with their hosts. In the last two decades, a number of mechanisms that herpesviruses utilize to counteract the host’s innate and acquired immune response have been elucidated. For EHV-1, specifically, the immune evasion strategies include interference with antibody-dependent cell lysis (ADCC) and cytotoxic T lymphocytes (CTL)-mediated lysis of infected cells as well as subversion of host cytokine action (van der Meulen et al., 2006b).

5.1. Evasion from humoral immunity

As one of the most important immune defense mechanisms, antibody-dependent humoral immunity plays a crucial role in the neutralization of cell-free viruses as well as the clearance of virus-infected cells by ADCC. To clear virus-infected cells, virus-specific antibody needs to recognize viral antigens that are expressed on the cell surface in order for ADCC and CTL activity to occur. It is clear that strong antibody responses can be elicited after vaccination or EHV-1 infection in horses, but that recognition of EHV-1-infected cells by antibody seems to be problematic if not impossible. Both in vitro and in vivo studies have shown that the majority of EHV-1-infected PBMCs do not express viral envelope proteins on the cell surface. As a result, it was speculated that infected cells are insensitive to ADCC-mediated elimination (van der Meulen et al., 2006a, 2003). It was also reported that viral envelope proteins were undetectable intracellularly and only immediate early (IE) and some early proteins were expressed, indicating that the replication of EHV-1 in circulating PBMCs is restricted to the early phase of infection or that PBMCs expressing viral envelope proteins might be selectively removed from the circulation (van der Meulen et al., 2006a).

Besides the ability of passively hiding its envelope proteins, EHV-1 is also able to interfere with the activation of the complement cascade by binding of gC to complement component C3 (Huemer et al., 1995). Similar findings were reported for gC of EHV-4 (Azab et al., 2010; Huemer et al., 1995) and related herpesviruses including suid herpesvirus 1 (PRV) (Huemer et al., 1992), bovine herpesvirus 1 (BHV-1) (Huemer et al., 1993), and HSV-1 (Fries et al., 1986) (Fig. 3A). HSV-1 gC has been studied in great detail and was demonstrated to bind complement C3 and its activation products, C3b, iC3b, C3c resulting in an acceleration of the decay of C3 convertase (Fries et al., 1986; Kostavasili et al., 1997).

To ensure efficient evasion from antibody-dependent immunity, herpesviruses are equipped with an envelope-resident heterodimer, gE–gI, which can function as a receptor for the Fc domain of immunoglobulin G (IgG) (FcyR) and allows the viruses to interfere with C1q binding and antibody-dependent attack (Dubin et al., 1991). The gE–gI complex of PRV, but not BHV-1, also binds IgG via the Fc domain and aids to protect PRV-infected cells from complement-mediated lysis (Favoreel et al., 1997; Van de Walle et al., 2003; Whitbeck et al., 1996). Whether the gE–gI complex of EHV-1 or EHV-4 also interferes with Fc-mediated activities is yet to be elucidated.

5.2. Evasion from CTL-mediated immunity

Cellular immunity mediated by CD8+ CTLs is an essential defense mechanism against many virus infections. The frequency of precursor CTLs specific for EHV-1 antigens is possibly correlated with protection against disease (Kydd et al., 2003). Compared to the short-lived complement-fixing antibody, CTL activity is of long duration after EHV-1 infection (Kydd et al., 2003). Unfortunately, it was revealed that the lymphocytes isolated from animals exposed to virulent strains of EHV-1 showed reduced activity and did not respond optimally to incubation with either inactivated or live viruses (Charan et al., 1997; Hannant et al., 1999).

CTL-based immunity is dependent on the efficient recognition of viral peptides presented by MHC-I on the cell surface. EHV-1 and EHV-4, like other herpesviruses, have developed different strategies to interfere with MHC-I antigen processing and presentation, which results in the downregulation of cell surface MHC-I expression in infected cells (Griffin et al., 2010). Recently, the products of the UL49.5 orthologues (pUL49.5) of members of the Varicellovirus genus including BHV-1, PRV, EHV-1 and EHV-4 were identified as a novel class of inhibitors that target the transporter associated with antigen processing (TAP).
In contrast, pUL49.5 is expressed rather late and is only detectable from 4hpi. On the other hand, deletion of ORF1, but not UL49.5, from Ab4 restored MHC-I surface expression. The results indicated that downregulation of cell surface MHC-I, at least in the early stage of the infection, is mainly induced by pUL56 (Ma et al., 2012). Similar results were also reported for EHV-4 (Said et al., 2012). It is likely that the long-term effect of pUL49.5-TAP interference is masked by the massive and early effect of pUL56 and that the removal of surface MHC-I is a consequence of coordinated and functional cooperation of both pUL56 and pUL49.5 (Fig. 3B). It should be noted that pUL56 by itself is unable to downregulate MHC-I, and its function dependent on virus infection, indicating that pUL56 needs to interact with at least another, yet unidentified, viral protein(s) to exert its function (Ma et al., 2012). Rappocciolo et al. (2003) reported that an early viral protein(s) was responsible for the observed surface MHC-I downregulation, probably by enhancing endocytosis of MHC-I molecules (Rappocciolo et al., 2003). In combination with the findings of our study, it can be assumed that pUL56 is this early viral protein. The detailed mechanism of pUL56 in downregulating MHC-I remains to be elucidated.

Interestingly, downregulation of cell surface MHC-I by EHV-1 seems to be locus- or allele-specific (Rappocciolo et al., 2003). This is considered an important evasion
strategy as virus-infected cells can avoid the action of natural killer (NK) cells by selectively downregulating only some of the multiple MHC-I molecules on the cell surface. The downregulation of cell surface MHC-I was also observed in EHV-1-infected PBMCs in vitro, but not in vivo. The controversial results might be due to the restricted replication of EHV-1 in PBMCs in vivo relative to in vitro, and to the fact that only the absolute percentage of MHC-I positive PBMCs, instead of the number of MHC-I molecules per infected PBMC, was evaluated in these studies (van der Meulen et al., 2006a,b).

5.3. Interference with chemokine responses

Alpha-, beta- and gamma-herpesviruses are known to encode vCKBP or chemokine receptors (Bryant et al., 2003; Lalani et al., 2000; Murphy, 2001). For alphaherpesviruses, including EHV-1 but not EHV-4, envelope protein gG, which is present both in cellular and viral membranes and secreted into the supernatant of infected cells, can bind a broad range of chemokines with high affinity and block their activity (Bryant et al., 2003). In vitro studies showed that EHV-1 gG inhibited the migration of equine neutrophils in response to IL-8 and keratinocyte-derived chemokine (KC, the murine orthologue of IL8) (Fig. 3C). Using a murine infection model, it was shown that neutrophil migration in lungs was significantly inhibited by EHV-1 gG (Van de Walle et al., 2007). EHV-1 gG interferes with migration of murine macrophages in vitro and in vivo induced by the proinflammatory chemokine macrophage inflammatory protein 1α (MIP1α) (Van de Walle et al., 2008). The epitope responsible for chemokine binding was mapped to a region spanning 40 amino acids (aa) in the hypervariable region (aa 301–340) of the protein by engineering loss- and gain-of-function gG’s (Van de Walle et al., 2009b). However, the hypervariable region is not solely responsible for the immunomodulatory potential in a viral background, as replacement of this region of EHV-1 (strain OH-03) with that of EHV-4 (strain KT-4) did not completely abolish chemokine-binding function. Also, re-insertion of the hypervariable region of EHV-1 gG into the EHV-1 mutant with EHV-4 gG (vOH-gG4hyp1) did not completely restore chemokine binding, indicating that other regions of gG might be also involved in the chemokine binding function (Thormann et al., 2012).

In summary, EHV-1 and EHV-4, similar to other herpesviruses, have developed multiple strategies to evade both innate and acquired immunity. As a result, EHV-1-infected cells cannot be completely cleared and re-infection can occur shortly after primary infection. The importance of evasion of CTL-mediated immunity has been highlighted by the discovery of an impressing number of herpesvirus proteins that are involved in the interference of MHC-I antigen presentation pathway.

6. Vaccines
6.1. Modified live virus and inactivated vaccines

Both modified live virus (MLV) and inactivated vaccines are currently available for protection against EHV-1 and EHV-4-induced diseases. The MLV vaccines based on EHV-1 Raci strain are licensed as Rhinomune in the United States (now by Boehringer IngelheimVetmedica, Inc., St. Joseph, MO) and as Prevaccinol in Europe (Merck MSD-Intervet, Munich, Germany). At present, they represent the only EHV-1 MLV vaccines in North America and Europe, respectively. The MLV vaccines have an excellent safety record and can protect horses against clinical disease; however, their efficacy in preventing viremia, abortion, and neurological disease is unclear (Kydd et al., 2006b). Recently, protection against neurological disease under experimental conditions and abortion under field conditions using the Raci-based vaccines was reported (Bresgen et al., 2012; Goodman et al., 2006).

 Concerning the safety of MLV vaccines, the currently available EHV-1 vaccines are made almost exclusively from inactivated preparations, the majority of which are combination vaccines with EHV-4 and/or other equine pathogens. Only three products [Pneumabort K and Duvaxyn-1,4(Zoetis) and Prodigy (MSD-Intervet)] claim to protect against EHV-1-induced abortion. Vaccines that are labeled for prevention of EHV-1-induced neurological diseases are not available (Rosas et al., 2006). In general, inactivated vaccines are able to induce high levels of virus neutralizing (VN) antibodies, which, theoretically, play an important role in neutralizing lytically replicating EHV-1. The action of VN antibody should reduce virus shedding in the nasal cavity and dampens clinical symptoms, especially with respect to the respiratory symptoms. Apart from that, humoral immunity is not considered to be the most effective mechanism in protection against abortion or EHM. It has in fact been shown that abortions occur irrespective of high levels of VN antibodies in pregnant mares (Mumford et al., 1994).

In recent studies, the efficacy of inactivated and MLV vaccines were compared. In the study conducted by Goodman et al. (2006), the MLV vaccine (Rhinomune) induced significantly lower VN antibodies than a 6-way combination inactivated vaccine also containing inactivated EHV-1 (Fluvac Innovator 6 combination vaccine, Fort Dodge). The MLV vaccine reduced virus shedding after challenge to almost undetectable levels. It also prevented neurological signs but only slightly reduced viremia (Goodman et al., 2006). In another study, the efficacy of the MLV vaccine was compared to an inactivated vaccine with high antigen content for abortion control (Pneumabort K, Pfizer) in an optimized 3-dose vaccination regimen. Clinical signs and nasal shedding after challenge were significantly reduced in both vaccine groups, with a reduction rate greater in case of the MLV group. However, the duration of viremia was only reduced in the inactivated vaccine group (Goehring et al., 2010b). Bresgen et al. (2012) performed another study comparing inactivated and MLV vaccines in pregnant mares and foals on a large study. Here, a 2-dose application regimen of the MLV vaccine and a 3-dose of EHV-1/EHV-4 inactivated combination vaccine induced a comparable neutralizing antibody response. No significant differences were observed with respect to abortion rates (Bresgen et al., 2012).
Table 1
Types of vaccines that are commercially available or under development.

<table>
<thead>
<tr>
<th>Type of vaccine</th>
<th>Availability on the market</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactivated vaccines</td>
<td>Yes</td>
<td>Safe induction of humoral immune responses</td>
<td>Poor inducer of cell-mediated immunity</td>
<td>The dominant vaccines in the market</td>
</tr>
<tr>
<td>Conventional MLV</td>
<td>Yes</td>
<td>Induce both humoral and cell-mediated immunity</td>
<td>Safety is a concern</td>
<td>RacH-based MLV has good safety records, but it is genetically remote to the epidemic strains</td>
</tr>
<tr>
<td>New generation MLV</td>
<td>No</td>
<td>based on current strains</td>
<td>Some deletion mutants are not fully attenuated or over-attenuated</td>
<td>Targeted genes are not optimized Immune evasion protein genes are alternative candidates</td>
</tr>
<tr>
<td>Subunit/vector vaccines</td>
<td>No</td>
<td>Safe</td>
<td>Only trigger limited immune response</td>
<td>Different viral proteins including glycoproteins and IE have been expressed Give only partial protection</td>
</tr>
<tr>
<td>DNA vaccines</td>
<td>No</td>
<td>Low cost</td>
<td>Poor antigenicity</td>
<td>Often used in prime-boost regimens with vector-based subunit constructs</td>
</tr>
</tbody>
</table>

6.2. Subunit and recombinant vaccines

In contrast to VN antibody, the frequency of MHC-I-restricted cytotoxic T-lymphocyte precursors (CTLp) has been discussed as a correlate of clinical protection after EHV-1 infection (Allen, 2008; Kydd et al., 2003). It was found that the IE protein (ICP4) encoded by ORF64 is one of the EHV-1 proteins that contain CTL epitopes presented by the equine MHC-I B2 allele of the A3 haplotype (Kydd et al., 2006a; Soboll et al., 2003). The expression of ICP4 alone by using a vaccinia virus-based vector, however, was unable to protect against challenge infection (Paillot et al., 2006). The efficacy of DNA vaccines has also been evaluated. A combination of plasmids expressing gB, gC, and gD or plasmids expressing the IE protein (ICP4) and the early UL5 gene product were used to vaccinate ponies and were shown to induce only limited immune responses and protection against EHV-1 challenge (Soboll et al., 2006). The failure to more completely protect may be due to the fact that the genetic background of animals was not taken into account in these studies. In a later study, ponies of the MHC-I A3/B2 (A3.1 haplotype) were vaccinated with a recombinant modified vaccinia virus Ankara (rMVA) expressing ICP4. Upon challenge, both clinical signs and viremia in vaccinated animals were significantly reduced, even though the antibody titers were low before challenge (Soboll et al., 2010).

By deleting virulence-associated genes, a number of EHV-1 mutants have been constructed and tested for vaccine efficacy. An EHV-1 mutant with a deletion in gE and gl was found to be safe for horses, but provided only partial protection against EHV-1 challenge (Matsumura et al., 1998). Similar findings were observed in another study, where a gE-negative EHV-1 mutant did not cause any signs of respiratory disease in foals vaccinated intranasally or intramuscularly and reduced virus loads in nasal secretions and viremia after challenge (Tsujimura et al., 2009). EHV-1 mutants devoid of gB or gM were apathogenic for mice and provided protection against challenge infection; however, their safety and protective potential are unknown in horses (Neubauer et al., 1997). The types of vaccines available on the market or under development are summarized in Table 1.

All in all, while a number of vaccine candidates have been developed, their protective efficacy is still unknown or unsatisfactory. Ideal vaccines should be able to induce both high levels of neutralizing antibody at mucosal surfaces and a strong and broad CTL immune response in horses with different haplotypes. From this point of view, MLV vaccines, if they are safe, should be the prime targets. Since EHV-1 is known to modulate immune responses, the deletion of genes involved in immune evasion, such as UL56 (ORF1), UL49.5, and gC, in addition to the deletion of virulence-associated genes, might influence immunogenicity and potentially result in more robust and long-lived immunity.

7. Conclusions

The availability of molecular tools to manipulate both EHV-1 and EHV-4 has allowed considerable progress over the past decade in terms of an understanding of virus replication and immune evasion. However, the main roadblocks the community is facing, namely truly translational research with respect to pathogenesis and immune control of the disease syndromes induced by the two viruses, will continue to be hard to overcome. This is mainly due to the difficulties associated with designing and performing experiments in the target species. It has been recognized that the currently available small animal models are questionable at best. For some of the open questions associated with lytic replication and virus transfer from one compartment to the next, the use of nasal explants and systems involving cultured endothelial cells and infected PBMCs may yield more meaningful information. The main challenge for the community in the near and mid-term future will, therefore, be further refinement and use of such surrogate models. It is unclear,
however, if and how the models can contribute to the development and testing of novel, more efficacious vaccines. This realization underscores the need for the design and execution of meaningful animal experiments that should ideally follow agreed-upon rules.

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