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Research paper

Infection of peripheral blood mononuclear cells with neuropathogenic equine herpesvirus type-1 strain Ab4 reveals intact interferon-α induction and induces suppression of anti-inflammatory interleukin-10 responses in comparison to other viral strains

Bettina Wagner[a,*], Christine Wimer[a], Heather Freer[a], Nikolaus Osterrieder[b], Hollis N. Erb[a]

[a] Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA
[b] Institut für Virologie, Freie Universität Berlin, Philippstrasse 13, 10115 Berlin, Germany

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ABSTRACT

The recent increase in incidence, morbidity, and mortality of neurological disease induced by equine herpesvirus type 1 (EHV-1) has suggested a change of virulence of the virus. The exact mechanisms by which EHV-1 induces neurologic disease are not known. Environmental, viral, and host risk factors might contribute to neurological manifestation. Here, we investigated innate interferon-α (IFN-α), interleukin-10 (IL-10) and IL-4 responses after infection of equine peripheral blood mononuclear cells (PBMC) with EHV-1 using an available cytokine multiplex assay. Three viral strains representing an older isolate (RacL11), a recent abortigenic (NY03) and a neuropathogenic isolate (Ab4) were compared to identify differences in cytokine induction that might explain the increased pathogenicity of Ab4. Cytokine concentrations were also compared between foals, mares after birth, pregnant and non-pregnant mares to investigate whether immune responses to EHV-1infection are influenced by age or pregnancy status. PBMC from all groups secreted high concentrations of anti-viral IFN-α in response to EHV-1. A reduced response was observed in foals compared to non-pregnant mares. EHV-1 infection induced moderate IL-10 and overall low IL-4 secretion. Ab4 infection resulted in a significant reduction of IL-10 responses in adult horses. IL-10 and IL-4 responses were lower in foals than in most mare groups. These data suggested that EHV-1 induces robust IFN-α secretion without major differences between viral strains. However, anti-inflammatory IL-10 production was significantly reduced after infection with neuropathogenic Ab4. The ability of this EHV-1 isolate to down-regulate IL-10 production might contribute to increased local inflammation and a higher risk for neurological manifestation of the disease after infection with Ab4.

1. Introduction

Herpesviruses are widely distributed in nature, causing disease in organisms as diverse as bivalves and primates. Each virus appears to have established a long-standing relationship with its host. The viruses manipulate and control the metabolism of host cells, as well as the innate and adaptive anti-viral immune responses. Herpesviruses ultimately achieve escape from the host's

Abbreviations: CMV, cytomegalovirus; CNS, central nervous system; EHV-1, equine herpesvirus type 1; HSV, herpes simplex virus; MOI, multiplicities of infection; pDC, plasmacytoid dendritic cell; RM ANOVA, repeated-measures analysis of variance; TLR, toll-like receptor.

* Corresponding author. Tel.: +1 607 253 3813; fax: +1 607 253 3440.
E-mail address: bw73@cornell.edu (B. Wagner).

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immune responses by maintaining themselves in a latent state resulting in virus persistence for years – usually for the life span of the hosts (Roizman, 1996; Roizman and Sears, 1996). Despite differences in disease manifestation, all herpesviruses establish latency in either neurons or leukocytes, and some herpesviruses – invariably those with a distinct lymphotropism – are associated with severe disease in immunocompetent and/or immunosuppressed hosts (Roizman, 1996).

Equine herpesvirus type-1 (EHV-1) is member of the *Varicellovirus* genus in the *Alphaherpesvirinae* subfamily that is highly prevalent in most equine populations (Gilkerson et al., 1999; Patel and Heldens, 2005; Lunn et al., 2009). EHV-1 has an enormous medical impact on equine industries worldwide through respiratory disease, abortion, and encephalomyelopathy and in some cases death of affected animals (Lunn et al., 2009; Perkins et al., 2009). The disease manifestations also have substantial economic impact from the resulting treatments, quarantine measures and the lost training and competition times (Goehring et al., 2006; Lunn et al., 2009). Most horses are first infected very early in life, usually before weaning, and remain latently infected for life. Latently infected, lactating mares are the source of infection for their foals, which in turn infect other foals and weanlings (Gilkerson et al., 1999; Foote et al., 2004; Patel and Heldens, 2005). The virus spreads via respiratory secretions during direct contact or via contact with fomites. EHV-1 first infects the respiratory epithelium and quickly enters lymphocytes in the retropharyngeal lymphoid tissues. From here viruses spread systemically via a cell-associated viremia, and establish latency in $CD8^+$ T-cells and neurons of the trigeminal ganglion (Kydd et al., 1994; Slater et al., 1994). EHV-1 reactivates and is shed again during times of stress, and all clinical manifestations may be seen during recrudescence (Rebenko-Molla et al., 2006; Lunn et al., 2009). The recent increase in incidence, morbidity, and mortality of neurological EHV-1 suggests a change in virulence of the virus (Kohn et al., 2006; Henninger et al., 2007; Perkins et al., 2009) and prompted the USDA to classify Equine Herpesvirus Myeloencephalopathy (EHV-1 – EHM) as an emerging disease (APHIS, 2007).

Currently, both inactivated and modified-live virus vaccines for prevention of EHV-1 disease are available in the US and Europe. The use of these vaccines and improved management practices has decreased the occurrence of abortion storms. However, outbreaks of EHV-1 continue to occur and neurological manifestation (EHM) is increasing in the face of widespread vaccination. Improvement of vaccines requires further elucidation of the protective immune response to EHV-1, and vaccination strategies must be constantly adjusted to the challenge that this virus presents to the host immune system (Kydd et al., 2006; Van de Walle et al., 2008, 2010).

Studies investigating the adaptive immune response to EHV-1 suggest that increased pre-infection levels of EHV-1-specific cytotoxic T lymphocytes (CTL) protect from progression to disease, whereas serum neutralization antibody levels do not. CTL activity is routinely measured by increased IFN-γ production of $CD8^+$ T-cells induced by EHV-1 challenge in natural or experimental infection models (Allen et al., 1995; Kydd et al., 2003; Breathnach et al., 2005; Paillot et al., 2006, 2007; Allen, 2008). In adult horses, pre-existing EHV-1-specific IFN-γ producing cells protected from developing clinical signs and viral shedding after experimental infection (Coombs et al., 2006). In foals, IFN-γ production in response to EHV-1 infection increased with age (Paillot et al., 2005). IFN-γ producing cells in foals and young horses were mainly composed of $CD8^+$ CTL (Paillot et al., 2007). In older horses, the EHV-1 specific T-cell response were shifted towards a $CD8^-$ phenotype resulting in a clear decrease of EHV-1-specific, IFN-γ producing CTL in aged horses (Paillot et al., 2007). These phenotypic variations in EHV-1-specific effector T-cells offered an explanation for the observed age-dependent increase in severity of clinical disease in older horses (Goehring et al., 2006; Henninger et al., 2007; Allen, 2008; Lunn et al., 2009).

Host innate immune responses stimulate and modulate adaptive immunity to pathogens. Appropriate innate immunity results in protective adaptive immune responses to EHV-1, which are essential for preventing severe clinical signs upon viremia caused by primary infection, re-infection or reactivation of latent infection (Allen, 2008; Wuest and Carr, 2008). Nevertheless, innate immune responses to EHV-1 have not yet been thoroughly investigated. Very recent data in horses have shown that chemokine expression differed between neuropathogenic (Ab4) and abortigenic (RacL11, NY03) virus strains (Wimer et al., 2011). Here, we focused on the induction of three cytokines, IFN-α, IL-10 and IL-4, after *in vitro* infection of PBMC with EHV-1. IFN-α is an anti-viral cytokine of the innate immune response (Ito et al., 2005), while IL-10 (Moore et al., 2001; Trinchieri, 2001) and IL-4 (Mosmann and Coffman, 1989; Min et al., 2004; Mitre et al., 2004) can be produced by both innate and adaptive immune cells. We compared the influence of neuropathogenic and abortigenic EHV-1 strains on cytokine secretion. We also compared cytokine responses in foals, pregnant and non-pregnant mares to identify age-dependent differences in cytokine secretion or variations of the response resulting from pregnancy and that might influence susceptibility to initial infection, re-infection or re-activation of latent EHV-1.

2. Materials and methods

2.1. Horses

Blood samples were obtained from 17 clinically healthy adult mares to determine an optimal viral concentration for cytokine induction. The group consisted of 14 Thoroughbreds, two Standardbreds, and one pony. Horses were between ages 4 and 28 years (median 15 years). For the comparison of EHV-1 strains and infection in foals and mares, we used blood samples from 11 foals at day 5 of life (foals), 11 dams of the foals at day 5 after birth (dams), nine pregnant mares during the last month of pregnancy (samples were taken 5–22 days before birth), and seven adult, non-pregnant mares (adults). The dams were 10–19 years old (median 13 years) and the group was composed of 10 Warmbloods and one Thoroughbred. The pregnant