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Research article

Equine herpesvirus-1 infected peripheral blood mononuclear cell subpopulations during viremia

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ABSTRACT

Infection with equine herpesvirus-1 (EHV-1) causes respiratory disease, late term abortions and equine herpesvirus myeloencephalitis (EHM) and remains an important problem in horses worldwide. Despite increasing outbreaks of EHM in recent years, our understanding of EHM pathogenesis is still limited except for the knowledge that a cell-associated viremia in peripheral blood mononuclear cells (PBMCs) is a critical link between primary respiratory EHV-1 infection and secondary complications such as late-term abortion or EHM. To address this question our objective was to identify which PBMC subpopulation(s) are infected during viremia and may therefore play a role in transmitting the virus to the vascular endothelium of the spinal cord or pregnant uterus.

PBMCs from 3 groups of animals were collected between days 4 and 9 following experimental infection with EHV-1 strain Findlay/OH03 or strain Ab4. PBMCs were labeled with primary antibodies selective for CD4+ or CD8+ T lymphocytes, B-lymphocytes, or monocytes and positively selected using magnetic bead separation. Cell numbers and EHV-1 genome numbers in each subpopulation were then determined using quantitative PCR for β-actin and the EHV-1 glycoprotein B, respectively.

Viral genomic DNA was found in all PBMC subpopulations; the CD8+ lymphocytes were most frequently positive for viral DNA, followed by B-lymphocytes. These differences were statistically significant in horses infected with the EHV-1 strain Findlay/OH03, and ponies with Ab4. These results differ from what has been reported in *in vitro* studies, and indicate that different PBMC subpopulations may play different roles in EHV-1 viremia.

1. Introduction

Equine herpesvirus-1 (EHV-1) is an ubiquitous virus in horse populations world-wide. It is associated with mild, respiratory disease and fevers in the adult horse, while it causes more moderate to severe respiratory disease in the juvenile or immunological naïve animal. In addition, EHV-1 causes late-term abortions in the pregnant mare, and equine herpesvirus myeloencephalopathy (EHM), which can affect 10% of adult infected horses in outbreaks. The spinal cord is the part of the central nervous system (CNS) that becomes most severely affected during EHM, which results in ataxia and weakness, that can progress to recumbency requiring euthanasia (Goehring and Lunn, 2008). This clinical disease is the result of an inflammatory cascade associated with EHV-1 infection of the endothelial cells of the CNS, resulting in damage to the microvasculature secondary to microthrombosis and local hemorrhage, and extravasation of mononuclear cells causing perivascular cuffing (Edington et al., 1986; Slater, 2007)

Despite the increasing occurrence of EHM, we currently have a very limited understanding of its pathogenesis,

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beyond the essential role of viremia, in transmitting the virus from the primary site of infection or recrudescence to the vasculature of the CNS, where endothelial infection occurs. Cell-associated viremia is usually detected between days 4 and 10 following primary infection of the respiratory tract and its associated lymphoid tissue. Previous studies have found that the onset of viremia commonly follows or is accompanied by a secondary fever (Hussey et al., 2006; Soboll et al., 2006, 2010) and EHM occurs following this viremic phase. Most importantly, Allen et al. compared viremia data of EHV-1 strains that are known to have a low neuropathogenic potential with those of a high neuropathogenic potential using real time PCR and found that viruses with a high neuropathogenic potential (D_{752}) are characterized by a longer duration and greater magnitude of viremia when compared to EHV-1 strains with a low neuropathogenic potential (N752) (Allen and Breathnach, 2006; Nugent et al., 2006). This observation has subsequently been confirmed by others (Goodman et al., 2007). This evidence supports the argument that prolonged exposure of the CNS vascular endothelium to high viral loads increases the risk of EHM. Therefore, the magnitude and duration of EHV-1 viremia as well as occurrence of EHM likely depends on a number of different host and viral factors including virus strain, pre-existing antigen-specific cytotoxic T lymphocyte precursor frequencies, as well as other host factors (Allen, 2008; Goehring et al., 2009).

While the importance of cell-associated viremia within peripheral blood mononuclear cells (PBMCs) in the pathogenesis of EHM is well recognized, only incomplete information is available on which PBMC subpopulations are infected, and the viral load in each cell subpopulation. This crucial piece of information is the first step to understanding how the virus is transferred from the blood to the spinal cord endothelium. An early *in vivo* study identified the T lymphocyte population as the primary PBMC subpopulation infected with EHV-1 during viremia (Scott et al., 1983). Subsequent *in vitro* work by van Der Meulen et al. (2000) identified EHV-1 in the resting monocyte subpopulation following *in vitro* infection of PBMCs, as well as in T lymphocytes following mitogenic stimulation. A recent *in vivo* study suggested that the majority of virus-positive cells in the mandibular lymph node and PBMCs were of the monocyte lineage (CD172a+) rather than of T lymphocyte (CD5+) lineage and that EHV-1 is concentrated in only a few circulating PBMC (Gryspeerdt et al., 2010). Caution may be warranted to designate CD172a+ cells as the main virus carrier during viremia, as a limited number of animals may have generated this data. In a recent *in vitro* study, Goodman et al. (2007) used GFP expressing EHV-1 strains of either the D_{752} or N_{752} genotypes. Both strains infected all subsets of PBMCs, with the subpopulation infection frequency decreasing in the following order for both strains; monocytes > B-lymphocytes > CD4+ lymphocytes > CD8+ lymphocytes. While the frequency of infected PBMC is substantial *in vitro* (as high as 10–30% using GFP-EHV-1 (Goodman et al., 2007)), this situation is very different *in vivo* where the frequency of infection is reported to be 1–10 positive cells/10^7 PBMC using virus isolation techniques (van der Meulen et al., 2006). Because of this low frequency of infection, there have been no comprehensive studies to date of PBMC subpopulation infection *in vivo*. With the development of quantitative real time PCR (qPCR) to measure EHV-1 viremia (Hussey et al., 2006) it has now become more feasible to address this question because qPCR is more sensitive, makes daily testing more feasible and allows for detection of viral load in cell subpopulations.

Because our long term goal is to identify how EHV-1 is transmitted from viremic PBMC subpopulations into the endothelial cells of the CNS, the purpose of this study was to identify the EHV-1-infected subpopulation(s) of PBMC collected from horses and ponies infected with neuropathogenic EHV-1 strains during viremia. For this, PBMCs were separated into 4 separate subpopulations (CD4+, CD8+, B-lymphocytes and monocytes) by using equine-specific PBMC markers and magnetic bead separation. Viral DNA quantification in each collected cell subpopulation was then performed using qPCR. By identifying the subpopulation(s) of viremic PBMC we may be able to better understand the early pathogenesis of EHM.

2. Materials and methods

2.1. Animals

Three groups of animals were selected for this study. These groups included horses or ponies that were part of independent EHV-1 infection experiments, and were composed of placebo-treated, experimentally infected, control animals. Three studies were performed several months apart and at different times of the year. Animals for each experiment were selected based on their low virus neutralizing antibody (VN) titer (<1:16) against EHV-1 in serum. All animals had free choice access to water and a grass/alfalfa hay mixture, and all received a commercial grain mixture and daily mineral and vitamin supplementation. Group 1 consisted of eight yearling horses, 2 males and 6 females. Group 2 consisted of seven yearling ponies, 2 females and 5 males, and group 3 contained 4 yearling horses, 2 males and 2 females. All animals were randomly assigned to their respective groups. All experiments were performed in accordance with the United States Welfare Act and under the supervision of the Colorado State Animal Care and Use Committee.

2.2. EHV-1 challenge infections

Group 1 was inoculated with 1×10^7 PFU of EHV-1 strain Findlay OH03, and Group 2 and group 3 were inoculated with EHV-1 strain Ab4 at 1×10^7 PFU and 5×10^7 PFU respectively. Both strains are neuropathogenic expressing the D_{752} genotype, and were kindly provided by Dr. Klaus Osterrieder, Cornell University, NY, USA. All viral inocula were prepared as a 6th passage in equine dermal cells for Findlay OH03, and for Ab4 as a 6th passage on rabbit kidney cells (RK13). On the day of infection (D0) each animal was inoculated by intranasal instillation of the virus in 4 ml of saline using a 20 ml syringe attached to a short length (12 cm) of tubing with an atomizer on the end.