Veterinary Immunology and Immunopathology 126 (2008) 293–301

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm

Investigation of antigen specific lymphocyte responses in healthy horses vaccinated with an inactivated West Nile virus vaccine

Elizabeth G. Davis [a,*], Yuwen Zhang [a], John Tuttle [b], Kevin Hankins [b], Melinda Wilkerson [c]

[a] Department of Clinical Sciences, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506, USA
[b] Fort Dodge Animal Health, Inc., Fort Dodge Animal Health, Division of Wyeth, Overland Park, KS 66210, USA
[c] Department of Diagnostic Medicine Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506, USA

ARTICLE INFO

Article history:
Received 17 October 2007
Received in revised form 25 July 2008
Accepted 18 August 2008

Keywords:
Equine
Cell mediated immunity
WNV vaccination

ABSTRACT

West Nile virus (WNV) is a single-stranded, enveloped RNA virus capable of causing encephalitic disease in horses. Unvaccinated horses are at risk for developing WNV disease in endemic geographic regions. Effective vaccination reduces disease frequency and diminishes disease severity in vaccinated individuals that become infected with WNV. Recent data indicate CD4+ lymphocytes are required for effective protection against disease; in particular, cross talk between CD4+ and CD8+ lymphocytes must be functional. The objective of this project was to investigate immune responses in horses throughout a series of three vaccinations using a commercial inactivated vaccine under natural conditions.

Immune responses to vaccination were determined by neutralizing antibody titers with plaque reduction neutralization test (PRNT), IgM titer (capture ELISA), WNV specific antibody Ig subclass responses, WNV lymphocyte proliferative responses and intracellular cytokine expression. Horses were vaccinated with a series of three vaccines at 3-week intervals using an inactivated product. An initial measure of immune activation following vaccination was determined by evaluating changes in lymphocyte cytokine expression. Interferon (IFN) gamma and interleukin (IL)-4 expressing CD4+ lymphocytes significantly increased 14 days following initial vaccination compared to unvaccinated horses ($P < 0.05$). IFN-gamma expressing CD8+ lymphocytes also increased and remained elevated for 110 days. Antigen specific lymphocyte proliferative responses were significantly increased up to 90 days following the third vaccination ($P < 0.05$). As expected, vaccinated horses produced increased neutralizing antibody based on PRNT data and WNV antigen-specific Ig subclass responses compared with unvaccinated horses ($P < 0.05$). Our data indicate that WNV vaccination with an inactivated product effectively induced an antigen-specific antibody responses, as well as CD4+ and CD8+ lymphocyte activation.

1. Introduction

West Nile virus is a single-stranded, positive-sense, enveloped RNA virus classified within the *Flaviviridae* family (2005). West Nile virus was first detected in the Western Hemisphere in 1999 during a human outbreak of encephalitis in New York City (CDC, 1999). Subsequent to the 1999 outbreak the virus spread across the continental United States as well as north into Canada (Epp et al., 2007a,b) and south into the Caribbean Islands and Latin America (Blitvich, 2008; Dauphin et al., 2004). Although enzootics involving horses had previously been reported in Morocco in 1996 and 2003 (Schuffenecker et al., 2005), Italy in 1998, Israel in 2000, South Africa (Burt et al., 2002),

* Corresponding author. Tel.: +1 785 532 5700; fax: +1 785 532 4989.
E-mail address: edavis@vet.k-state.edu (E.G. Davis).

0165-2427/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetimm.2008.08.007

Southern France in 2000, 2003, and 2004 (Schuffenecker et al., 2005; Zeller and Schuffenecker, 2004) before 1999 WNV was not a pathogen of concern in the western hemisphere. Identification of effective prophylactic strategies for protection from serious disease has become a goal of health professionals and veterinarians throughout the continent. Subsequently to 1999 disease continued to traverse the continental US to the point that in 2007 CDC reports that included human, avian, mammalian or mosquito infections were reported throughout the continental United States.

The incidence of WNV disease is seasonal in the temperate zones of North America, Europe, and the Mediterranean basin with typical peak activity from July through October. A lengthened transmission season has been speculated, resulting in cases as early as April and as late as December.

West Nile virus is primarily transmitted by *Culex* mosquitoes, yet other genre are also capable of viral transmission (Hayes et al., 2005; Komar, 2003). Culex and other ornithophilic (avian feeding) species are important amplification vectors for West Nile virus. Mosquito species that have more general feeding habits are important for transmission of disease to mammalian hosts and humans. Completion of the enzootic transmission cycle for WNV requires mosquito bird interaction. Birds are the natural reservoir hosts for WNV (Hayes et al., 2005; Komar, 2003; McLean et al., 2002). Passeriformes (song birds), Charadriiformes (shorebirds), Stringiformes (owls), and Falconiforms (hawks) may have levels of viremia sufficient to infect most mosquito species (Hayes et al., 2005). Certain passerines that include common grackles (*Quiscalus quiscula*), various corvids (crows, jays and magpies), house finches (*Carpodacus mexicanus*) and house sparrows (*Passer domesticus*) have been reported to be highly infectious to mosquitoes resulting in high mortality rates (>40%). Although birds are known to play a roll in disease transmission other factors, such as local movement of residents, non-migratory birds, and long range travel of migratory birds all contribute to viral spread (Hayes et al., 2005).

Human infection with WNV typically manifests with pyrexia and flu-like symptoms. Elderly or immunocompromised human patients suffering from WNV-associated disease may have more serious disease, since neuroinvasiveness is more likely to occur. Similar to people, horses can become infected and demonstrate mild clinical signs. In more severe disease, such as when an unvaccinated individual has pronounced viral exposure or when immunocompromise has occurred, severe encephalitis may ensue. There is a strong affinity of this virus for neurotropism in the equine host. Evidence to support neuroinvasiveness of WNV is based on the frequency of clinical neurologic disease in horses and that lesions are rarely detected in non-neural tissues (Blitvich, 2008; Cantile et al., 2001; Castillo-Olivares et al., 2001; Castillo-Olivares and Wood, 2004). A primary goal of equine health maintenance is to prevent WNV-associated clinical signs and severe disease. Although the mortality rate for equine WNV is relatively low, when encephalitic disease leads to recumbency, the mortality rate rises (23–43%; Autorino et al., 2002; Durand et al., 2002; Salazar et al., 2004; Schuler et al., 2004; Ward et al., 2004; Ward, 2006).

Immune cell activation is a major factor for host protection from WNV-associated disease. Cytokine responses that are critical for protection from disease include alpha interferon (IFN-α), IFN-β, and IFN-γ (Keller et al., 2006; Liu et al., 2006; Samuel and Diamond, 2005; Sitati and Diamond, 2006). Evidence strongly supports the notion that control from dissemination to the central nervous system is dependent upon proper functioning of the immune system. In particular, CD4+ lymphocytes have been determined to play a major role in regulating manifestation of WNV-encephalitis as well as clearance of disease (Sitati and Diamond, 2006).

Based on the recognition that vaccination is effective at providing protection from severe WNV-associated disease (Davidson et al., 2005; Gardner et al., 2007; Salazar et al., 2004; Seino et al., 2007; Ward et al., 2006; Ward, 2006), we sought to determine what immune factors were responsible for the observed clinical efficacy of a proven vaccination protocol under natural conditions. Moreover, previous investigations have demonstrated the role of T-helper type 1 responses with regards to protection from disease; we chose to determine what lymphocyte subset responses and cytokine profiles were induced in healthy horses vaccinated with an inactivated WNV vaccine.

2. Materials and methods

2.1. Animals

Twelve mixed breed horses (6 males and 6 females; 7–14 months of age; weight range from 345 to 732 pounds) from a commercial herd were included in the study. One horse had detectable WNV antibody against WNV via the plaque reduction neutralization test (PRNT) at the commencement of the trial. This horse was not included in any of the statistical analysis, although he was maintained in the investigation for use as an internal control for assay development. Horses were housed in outdoor pens in groups of 2–4 that contained shelter, free choice grass/alfalfa hay mix, and covered but fully accessible water source. Horses were maintained in accordance with animal use and care guidelines of Institutional Animal Care and Use Committee at Kansas State University.

2.2. Vaccine

Immunization was performed with a commercial inactivated vaccine, West Nile Innovator™ (Fort Dodge Animal Health, Overland Park, KS), which contains inactivated WNV NVSL equine isolate licensed for the prevention of WNV in horses. A series of three vaccines were administered by deep intramuscular (mid-cervical region) injection at 3-week intervals.

2.3. Experimental design

Twelve horses were randomly assigned to one of two treatment groups. On days 0, 21, and 42 the skin surface on the left cervical area was cleansed with 70% isopropyl