Production of immunogenic West Nile virus-like particles using a herpes simplex virus 1 recombinant vector

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ABSTRACT

West Nile virus (WNV) is a flavivirus that swept rapidly across North America in 1999, declined in prevalence, and then resurged in 2012. To date, no vaccine is available to prevent infection in the human population. Herpes simplex virus (HSV) replication-defective vaccine vectors induce a durable immunity characterized by strong antibody and CD8$^+$ T cell responses even in HSV-immune animals. In this study, a WNV protein expression cassette was optimized for virus-like particle (VLP) production in transfection studies, and the cassette was recombined into an HSV-1 d106-WNV virus vector, which produced extracellular VLPs, as confirmed by immunoelectron microscopy. Immunization of mice with the d106-WNV recombinant vector elicited a specific anti-WNV IgG response. This study highlights the flavivirus coding sequences needed for efficient assembly of virus-like particles. This information will facilitate generation of additional vaccine vectors against other flaviviruses including the recently emerged Zika virus.

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1. Introduction

The North American distribution of the flavivirus West Nile virus (WNV) expanded dramatically after its introduction into New York in 1999. It is now found throughout the United States, southern Canada, Central and South America, and the Caribbean (Cruz et al., 2005; Dauphin et al., 2004; Estrada-Franco et al., 2003; Komar, 2003; Reisen et al., 2004). WNV, which is transmitted primarily through *Culex* mosquito bites (Kulasekera et al., 2001), is spread to new areas *via* infected birds. WNV has caused significant human disease in the United States with estimates of 780,000 illnesses from 1999 to 2010 (Petersen et al., 2013), and elderly individuals are at greatest risk of neuroinvasive disease (Carson et al., 2012; Hayes et al., 2005). Neuroinvasive disease cases peaked in 2002–2003 and then declined until 2012 when WNV re-emerged with over 2800 neuroinvasive cases in the United States

(Centers for Disease Control and Prevention, 2013). With the wide distribution and significant human morbidity associated with WNV, it continues to be an important target for vaccine development.

Despite extensive efforts, no effective WNV vaccine is approved to protect the susceptible human population (Heinz and Stiasny, 2012; Volz et al., 2016); however, several vaccine approaches for animals or humans have been pursued, including inactivated virus (Samina et al., 2005); chimeric attenuated flavivirus viruses expressing WNV premembrane (prM) and envelope (E) proteins (Dayan et al., 2012; Monath et al., 2006); WNV virus-like particles (VLPs) (Qiao et al., 2004); pox vectors (Heinz and Stiasny, 2012; Siger et al., 2006); a lentivirus vector (Iglesias et al., 2006); a subunit vaccine (Chu et al., 2007; Watts et al., 2007); and DNA vaccines (Yang et al., 2001). The WNV proteins prM and E are necessary and sufficient for VLP production. When prM and E are expressed together within a cell, they self-assemble into VLPs that are released into the extracellular environment (Allison et al., 1995). VLPs derived from the co-expression of prM and E in cell culture systems independent of other viral factors are structurally and antigenically similar to genuine West Nile virions and have been shown to elicit neutralizing antibody titers in immunized mice. The protease required for cleaving E from prM is cellular, so E is released as a separate protein when expressed along with prM.

We have previously generated replication-defective HSV vaccine vectors expressing simian immunodeficiency virus (SIV) proteins that successfully protected non-human primates against mucosal challenge with virulent SIV (Kaur et al., 2007; Watanabe et al., 2007). Replication-defective HSV vectors are attractive because of their safety, as demonstrated in animal models (Hoshino et al., 2008), and ability to induce durable immune responses that include both B and T cell responses (Brehm et al., 1999, 1997; Brockman and Knipe, 2002; Brubaker et al., 1996; Da Costa et al., 2000, 1999; Dudek and Knipe, 2006; Jones et al., 2000; Kaur et al., 2007; Morrison et al., 1998; Murphy et al., 2000; Watanabe et al., 2007), and they are immunogenic even in the face of pre-existing HSV immunity (Brockman and Knipe, 2002). The replication-defective HSV-1 *d*106 virus contains multiple deletions that remove the coding sequences of the immediate-early proteins ICP4 and ICP27 and the promoter regions of the *ICP22* and *ICP47* genes (Samaniego et al., 1998). The loss of ICP4, ICP27, and ICP22 results in a dramatic decrease in the number of HSV proteins expressed during infection on non-complementing cells; however, *d*106 expresses the immediate-early ICP0 protein, which stimulates heterologous protein expression from transgenes encoded within the viral genome through its effects on the chromatin associated with the HSV genome (Cliffe and Knipe, 2008; Lee et al., 2016). We have therefore applied the HSV vector technology to the design of a WNV vaccine, and in this study we describe a recombinant replication-defective herpes simplex virus (HSV) vector, *d*106-WNV, which expresses the WNV structural proteins prM and E and part of the capsid (C) protein.

2. Results

With the continued prevalence of WNV, there is renewed interest in developing a safe and effective human WNV vaccine. To determine if a replication-defective HSV vaccine vector could serve as a candidate for flavivirus vaccine development, we constructed a recombinant HSV vaccine vector that expresses the WNV structural proteins premembrane (prM) and envelope (E).

2.1. Design of WNV expression constructs

The WNV structural proteins prM and E require a signal sequence for proper orientation in the host cell membrane (Lindenbach et al., 2013). During natural infection, this signal sequence is derived from the C-terminal transmembrane region of the capsid (C) protein (Fig. 1A). We first generated plasmid expression constructs expressing WNV E protein that lacked or contained a leader sequence for comparison with the WT C-prM-Env plasmid containing the coding sequences for the structural region starting with the methionine of the C protein (Fig. 1B): (1) The EnvΔss construct lacked a signal sequence. (2) The E-3XFlag construct expressed a modified WNV E protein containing the preprotrypsin
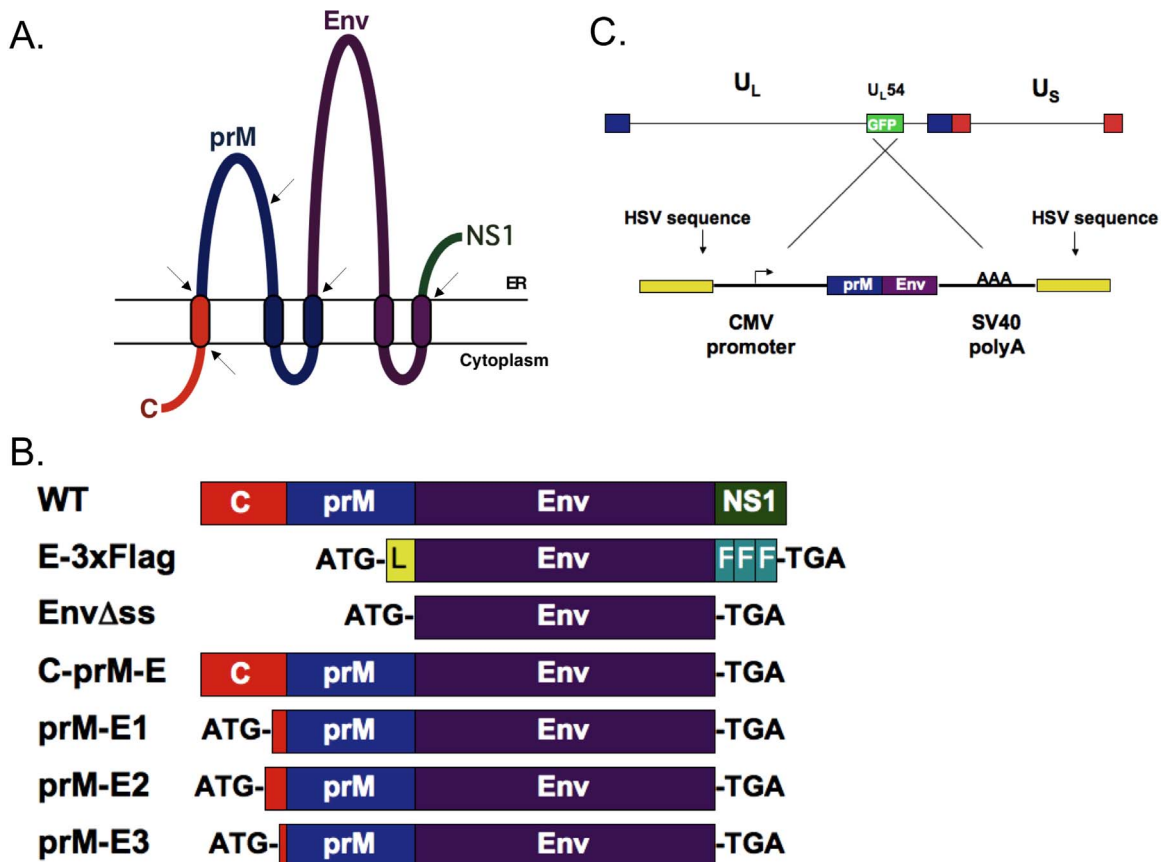

Fig. 1. Diagrams of the constructs used in this study. A. Graphical representation of the membrane orientation of the structural proteins in WNV including the initial portion of the nonstructural NS1 protein. Transmembrane regions are denoted by ovals passing through the lipid bilayer. Arrows indicate where cellular or viral proteases cleave the polyprotein to free the individual proteins: capsid (C), premembrane (prM), and envelope (Env). B. Comparison of the various plasmid constructs to the wildtype (WT) structural protein coding sequences. All plasmids used the CMV promoter/enhancer to drive transcription. The addition of start (ATG) and stop (TGA) codons are indicated. E-3xFlag contains the preprotrypsin leader sequence (L) and three sequential FLAG epitope tags (F). EnvΔss expresses the E protein lacking C and prM sequences. C-prM-E contains the WNV coding sequences for C-prM-Env-NS1. prM-E1–3 contain varying amino acid residues of C as described in Table 2. (C) Top: the *d*106 genome with the location of the *U*L54 (ICP27) region containing the GFP transgene indicated. Bottom: the pd27-WNV plasmid used to generate the recombinant *d*106-WNV vaccine vector. The crossed lines show the homologous recombination event between the reversed plasmid sequences with the *d*106 genome that generated the *d*106-WNV recombinant virus.