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An infectious West Nile Virus that expresses a GFP reporter gene

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Abstract

West Nile virus is a mosquito-borne, neurotropic flavivirus that causes encephalitis in humans and animals. Since being introduced into the Western hemisphere in 1999, WNV has spread rapidly across North America, identifying this virus as an important emerging pathogen. In this study, we developed a DNA-launched infectious molecular clone of WNV that encodes a GFP reporter gene. Transfection of cells with the plasmid encoding this recombinant virus (pWNII-GFP) resulted in the production of infectious WNV capable of expressing GFP at high levels shortly after infection of a variety of cell types, including primary neurons and dendritic cells. Infection of cells with WNII-GFP virus was productive, and could be inhibited with both monoclonal antibodies and interferon-β, highlighting the potential of this system in the development and characterization of novel inhibitors and therapeutics for WNV infection. As expected, insertion of the reporter gene into the viral genome was associated with a reduced rate of viral replication, providing the selective pressure for the development of variants that no longer encoded the full-length reporter gene cassette. We anticipate this DNA-based, infectious WNV reporter virus will allow novel approaches for the study of WNV infection and its inhibition both in vitro and in vivo.

Keywords: West Nile Virus; Flavivirus; Molecular clone; Reporter gene

Introduction

West Nile virus (WNV) is a neurotropic flavivirus within the Japanese encephalitis antigenic complex that is responsible for causing West Nile encephalitis in humans (reviewed in reference (Brinton, 2002)). Entry of WNV into cells is thought to occur via interaction of the viral envelope protein (E) with an unidentified cellular receptor (Chu and Ng, 2003). Once bound, virus is internalized and trafficked into an endosomal compartment where fusion occurs in a pH-dependent process (Gollins and Porterfield, 1986; Kimura et al., 1986). In the cytoplasm of infected cells, the 11 kb positive-stranded viral genomic RNA is translated into a single polyprotein that is subsequently cleaved by cellular and viral proteases into three structural (capsid, pre-membrane (prM), and E) and seven non-structural (NS) proteins (reviewed in reference (Rice, 1996)). As with other flaviviruses, the sequence encoding the WNV polyprotein is flanked by two untranslated regions (UTRs) that are required for efficient translation and genomic RNA replication (Cahour et al., 1995; Khromykh et al., 2003; Lai et al., 1991; Men et al., 1996). Viral RNA and protein synthesis occurs in association with intracellular membranes (Mackenzie and Westaway, 2001; Mackenzie et al., 1999), leading to the assembly and release of enveloped virions with icosahedral symmetry that are approximately 50 nm in diameter (Mukhopadhyay et al., 2003).

WNV is maintained in an enzootic cycle between birds and mosquitoes, although many other vertebrate species can be infected including humans and horses (Marfin and Gubler, 2001). While the majority of individuals exposed to WNV remain asymptomatic, a spectrum of disease has been reported that ranges from a mild febrile illness to

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severe meningoencephalitis (Petersen et al., 2003). Risk factors that influence the severity of WNV infection include age and the immune status of the affected individual (Chowers et al., 2001; Nash et al., 2001; Tsai et al., 1998). Currently, no specific therapy for WNV infection has been developed and treatment is supportive in nature.

While endemic in parts of Africa, Europe, Asia and the Middle East, introduction of WNV into the Western Hemisphere did not occur until the late summer of 1999, when 59 clinical cases were reported in the New York City area (Lanciotti et al., 1999; Marfin and Gubler, 2001). WNV has since spread rapidly across North America, infecting more than 9000 individuals in 2003 alone. To date, the 2004 epidemic of WNV in the US has involved approximately 2470 laboratory-confirmed human cases of WNV, resulting in at least 88 fatalities. The spread of WNV across much of the United States, Canada and Mexico in mosquito, avian and mammalian species makes this virus an important emerging pathogen in the Western Hemisphere.

At present, relatively little is known about WNV tropism and pathogenesis. While WNV is clearly neurotropic in vivo, it also infects several visceral organs including lymph node, spleen and kidney (Diamond et al., 2003a). WNV also exhibits a broad cellular tropism in vitro (Brinton, 2002). The cellular targets for WNV in non-neural tissues in vivo have not yet been determined, nor have the mechanisms by which WNV gains access to the central nervous system from these peripheral sites of viral replication. While the use of congenic strains of mice have proven to be a powerful tool for identifying host factors that contribute to WNV pathogenesis (Diamond et al., 2003a, 2003b; Shrestha et al., 2003; Wang et al., 2003), little progress has been made on the development of rapid and quantitative methods to study the viral determinants of WNV tropism and pathogenesis in vivo.

In this study, we have developed an infectious, replication competent WNV genome that encodes a reporter gene. The gene encoding green fluorescent protein (GFP) was cloned into the 3′ UTR of the viral genome under the translational control the of internal ribosome entry site (IRES) of encephalomyocarditis virus (EMCV). Transfection of cells with this infectious DNA plasmid resulted in the production of infectious WNV capable of mediating high-level expression of GFP in several different cell types, including primary rat neurons and human monocyte derived dendritic cells. This GFP-expressing WNV is a novel tool that provides a quantitative approach for investigating many aspects and inhibitors of the WNV lifecycle.

Results

Development of an infectious WNV that encodes GFP

To construct a DNA plasmid encoding an infectious WNV reporter virus, we modified a molecular clone of a lineage II strain of WNV (Yamshchikov et al., 2001a, 2001b). This vector, called SP6WN/Xba, encodes an infectious WNV cDNA under the control of the bacteriophage SP6 promoter (Fig. 1) (Yamshchikov et al., 2001a, 2001b). Using this vector system, the production of virus particles is accomplished after in vitro transcription of full-length, capped, viral RNA and subsequent transfection. However, recent studies indicate that the cDNA of a flavivirus can be engineered downstream of a constitutive eukaryotic promoter provided that the transcribed RNA begins and ends with the highly conserved terminal nucleotides of the viral genome (Hall et al., 2003; Liu et al., 2003; Mishin et al., 2001; Yamshchikov et al., 2001a, 2001b). Virus production using vectors with this configuration involves standard DNA transfection methods and eliminates the requirement for production and purification of genomic-length, 11 kb RNA molecules in vitro. To construct an infectious WNV plasmid, we modified SP6WN/Xba so that the WNV genome was placed under

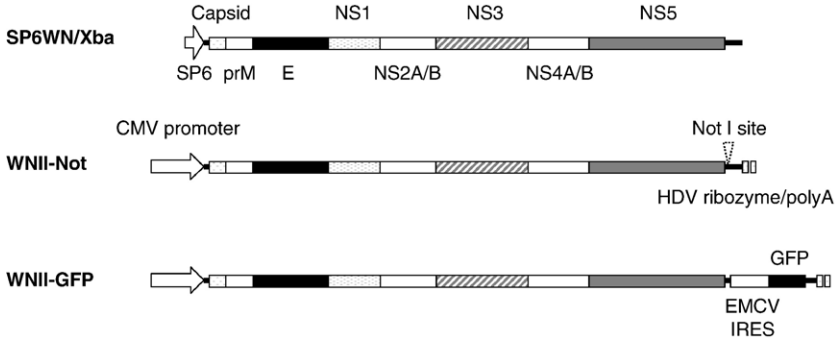

Fig. 1. Schematic of the WNII-GFP reporter virus. A plasmid encoding an infectious WNV genome was modified to allow the production of infectious WNV capable of expressing GFP using standard transfection methods. A previously described molecular clone of the lineage II WNV strain (956 D117 3B) was used as a starting point for the construction of two plasmids encoding WNV: WNII-Not and WNII-GFP. In both plasmids, the expression of WNV is regulated by the activity of the CMV promoter, allowing the production of virus particles following transfection of DNA into cells. In this configuration, the ribonucleotides at the terminus of the viral genomic RNA are determined by the transcriptional start site of CMV and by cleavage of the ribozyme of HDV. A variant of WNV was constructed that encodes a unique *Not*I restriction endonuclease recognition site. To generate a WNV capable of expressing a reporter gene, a cassette composed of GFP under the translational control of the EMCV IRES was cloned into this unique *Not*I site to produce WNII-GFP.