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Biological properties of chimeric West Nile viruses

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Abstract

Recently, we have described a lineage 2 attenuated WN virus suitable for the development of a live WN vaccine. To design vaccine candidates with an improved immunogenicity, we assembled an infectious clone of the NY99 strain and created several chimeric constructs with reciprocal exchanges of structural protein genes between attenuated W956 and virulent NY99 and investigated their biological properties. Our data indicated that, while the growth rates of NY99 and chimeric viruses in tissue culture are determined primarily by properties of the structural proteins, determinants responsible for a highly cytopathic phenotype of NY99 or lack thereof for W956 are located within the nonstructural protein region of the WN genome. The high virulence of NY99 and the attenuated phenotype of W956 were found to be associated with determinants in the nonstructural region. Chimeric viruses carrying the NY99 structural proteins were attenuated in neuroinvasiveness and demonstrated an immunogenicity superior to W956.

Keywords: West Nile; NY99 strain; Infectious clone; Chimeric virus; Vaccine; Antibody; Neutralization; Pathogenicity; Mouse

Introduction

West Nile (WN) virus isolated over 60 years ago (the prototype B956 strain; Smithburn et al., 1940) has been known in Europe and the Middle East for decades as a causative agent of self-limiting epidemics and epizootics (Murgue et al., 2001; Savage et al., 1999). Since its sudden emergence in the U.S. (Lanciotti et al., 1999), hundreds of human mortality cases and thousands of confirmed illness cases have been reported to the CDC (2005). Although highly related to certain strains circulating in the Middle East, the NY99 strain is perhaps the most pathogenic and virulent WN strain known to date (Monath, 2001), especially for mice that succumb to encephalitis after peripheral inoculation of very small doses (Beasley et al., 2002).

In humans, WN infection is often inapparent or occurs as a mild febrile disease (Monath and Heinz, 1996). However, this virus was also associated with severe neurological symptoms (Flatau et al., 1981; Smithburn et al., 1940); recent outbreaks of WN infection were characterized by an increased CNS involvement (Roehrig et al., 2002; Solomon and Vaughn, 2002). In the mouse model, in which flaviviruses are inherently neurovirulent, both neurovirulence and neuroinvasiveness have been positively associated with determinants in the envelope proteins (Cecilia and Gould, 1991; Chambers et al., 1999; Gualano et al., 1998; Hasegawa et al., 1992; Holzmann et al., 1990, 1997; Jiang et al., 1993; McMinn, 1997; Pletnev et al., 1992; Pletnev et al., 1993). Other evidence indicates that mutations in other parts of the flavivirus genome can also contribute to loss or acquisition of neurovirulence (Butrapet et al., 2000; Duarte dos Santos et al., 2000; Dunster et al., 1999; Muylaert et al., 1996; Ni et al., 1995; Xie et al., 1998). Based on serological data and genetic characterization, West Nile viruses have been grouped into at least two distinct lineages (Berthet et al., 1997; Price and O'Leary, 1967). Representatives with moderate and high virulence have been found in both WN virus groups (Beasley et al., 2002). The NY99 strain belongs to lineage 1, which also includes other WN strains that have been associated with human and equine outbreaks (Roehrig et al., 2002). The envelope protein (E) of many flaviviruses is glycosylated, and WN virus is not an exception to this rule, although a few non-glycosylated strains have been identified (Beasley et al., 2001; Berthet et al., 1997; Wengler et al., 1985). The importance of E glycosylation for expression of the virulent phenotype of lineage 1 WN viruses has been demonstrated experimentally (Beasley et al., 2005; Shirato et al., 2004).

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However, evidence documenting negative effects of E glycosylation on the WN virulence in mice or on its infectivity in cell culture has been reported as well (Chambers et al., 1998; Hanna et al., 2005).

A number of subunit or recombinant WN vaccines for veterinary and human use are currently under development (Kahler, 2003; Lai and Monath, 2003; Ng et al., 2003; Nusbaum et al., 2003; Pletnev et al., 2002; Tesh et al., 2002). In contrast to subunit or inactivated vaccines, a live WN vaccine may be expected to elicit a long lasting balanced humoral- and cell-mediated immune response normally seen after YF17D vaccination (Yamshchikov et al., 2005). However, the high virulence and pathogenicity of the NY99 strain (Beasley et al., 2002; Roehrig et al., 2002) make it questionable for use in development of a live-attenuated WN vaccine. Recently, we have reported characterization of a lineage 2 WN isolate, W956 (Yamshchikov et al., 2004), which appears sufficiently attenuated, immunogenic and cross-protective to be considered as a candidate for further development of live WN vaccine. One may expect that increasing the antigenic match to the strain circulating in the U.S. could improve the immunogenicity of a candidate for vaccine development. As a first step in evaluating this approach, we have assembled an infectious clone of the NY99 strain and created several chimeric viruses carrying reciprocal exchanges of the W956 and NY99 structural protein genes. In this study, we compared biological properties of the parent and chimeric viruses in tissue culture and in the mouse model and evaluated their immunogenicity in mice.

Results

Design of NY99 and other infectious DNA constructs

Earlier we reported the assembly of an infectious clone of WN lineage 2 virus (pSP6**W956**; Yamshchikov et al., 2001b). To simplify handling, it was converted to the infectious DNA (iDNA) format by engineering the CMV promoter transcription start to the beginning of WN genome cDNA. The antisense strand hepatitis δ ribozyme followed by the bovine growth hormone transcription termination signal (BG) was engineered to the end of WN cDNA for an increased fidelity of 3′-end formation, giving rise to the final construct pCMV**W956**δBG (further referred to as pCMV**W956**; Fig. 1).

A NY99 infectious clone was assembled using genetic material of isolate 385–99 (Xiao et al., 2001) according to the strategy we developed earlier (Yamshchikov et al., 2001a) with artificial introns inserted into viral genome cDNA for stabilization of the plasmid in *E. coli*. Upon transfection of eukaryotic cells, introns are spliced out resulting in precise restoration of viral ORF and initiation of the viral infectious cycle. Two such introns were inserted at positions 2383 and 3472 of the NY99 genome. The infectivity of the final construct CMV**NY99**(i2383i3472)δBG (further referred to as CMV**NY99**) is demonstrated in Fig. 2. Except for the two introns and an introduced *Mfe*I site at the end of E gene, NY99 genome cDNA in CMV**NY99** is authentic to the 385–99 genome (GenBank #DQ211652). The presence of both i2384 and i3472 was found to be crucial for the stability of the plasmid.

The pCMV**W956** and pCMV**NY99** constructs were used to create plasmids carrying reciprocal exchanges of the structural protein genes of two viruses (Fig. 1). The pCMV**[CprME$_{NY99}$]W956** chimera carries genes of all NY99 structural proteins instead of those of W956. In the pCMV**[prME$_{NY99}$]W956** chimera, only the prM-E region of NY99 (not including its prM signal sequence) from position 466 to position 2405 was used to replace the corresponding region in the pCMV**W956** construct. The E protein of NY99 is glycosylated at the NYS site ($E_{154–156}$). A variant of pCMV**[prME$_{NY99}$]W956** was prepared that encodes NY99 E in which this glycosylation site was mutated (NYS → SYS). The last chimeric construct pCMV**[CprME$_{W956}$]NY99** was created by transferring the *Bgl*II–*Mfe*I fragment coding for CprME of W956 into pCMV**NY99**.

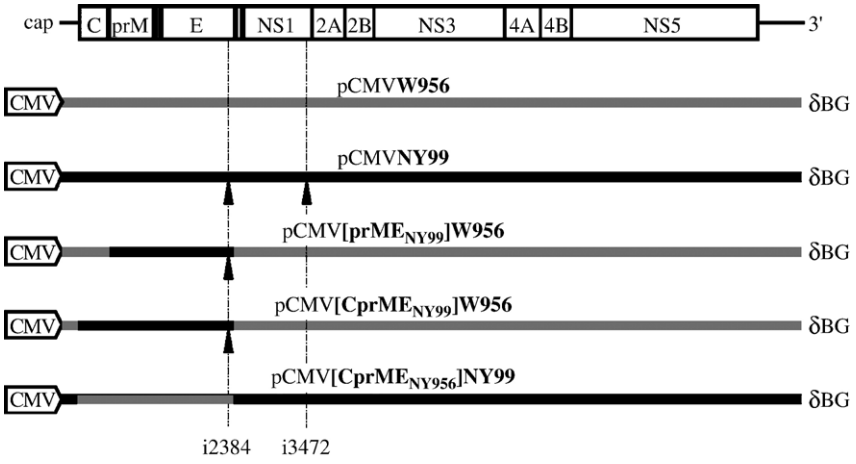

Fig. 1. Parent and chimeric infectious DNA constructs used in this study. A schematic representation of the WN virus genome with genes of recognized virus-specific proteins (Lindenbach and Rice, 2001) is shown on the top. Approximate locations of the intron present in particular constructs are marked by a filled arrowhead, genome positions of intron insertions are shown below. CMV—human cytomegalovirus promoter/enhancer, δ—antisense hepatitis δ ribozyme, BG—bovine growth hormone transcription termination and polyadenylation sequence. For clarity, the schematic is not drawn exactly to the scale.