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Virulence determinants between New York 99 and Kunjin strains of West Nile virus

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ABSTRACT

An attenuated Australian strain of West Nile virus (WNV), Kunjin (KUN), shares ~98% amino acid homology with the pathogenic New York 99 NY99 strain (NY99). To investigate the viral factors involved in NY99 virulence we generated an infectious cDNA clone of the WNV NY99 4132 isolate from which virus was recovered and was shown to be indistinguishable from the parental isolate. We then introduced the regions of the NY99 non-structural (NS) proteins and/or untranslated regions (UTRs) into the KUN backbone. Chimeric KUN viruses containing NY99 5′UTR and the parts of NS coding region were more virulent in mice than parental KUN virus. Chimeric NY99 viruses, containing KUN NS2A protein with alanine 30 to proline substitution were significantly less cytopathic in cells and less virulent in mice. Our results identify the 5′UTR and NS proteins as WNV virulence determinants and confirm a role for the NS2A in WNV cytopathicity and virulence.

Introduction

West Nile virus (WNV) is a member of the Japanese encephalitis serogroup of flaviviruses which includes other medically important, neuro-invasive viruses, such as Japanese encephalitis virus (JEV), Murray Valley encephalitis (MVE), and St Louis encephalitis virus (SLEV) (Calisher et al., 1989). The virus is endemic throughout Africa, the Middle East, parts of Asia, and Europe, however since a 1999 outbreak in the USA, WNV has emerged as the most common cause of arboviral encephalitis in North and Middle America (Petersen, 2009). An unusually high percentage of neurological infections associated with WNV circulated in the Americas compared to the relatively low neuro-invasiveness of WNV strains previously circulated in the Old World initiated studies on identifying viral determinants of the high neuro-invasiveness of the American strain. Based on serological and genetic data, WNV strains have been grouped into two distinct lineages, lineage 1 and lineage 2 (Lanciotti et al., 2002). The highly neuro-invasive New York 99 strain (NY99) belongs to lineage 1 which also contains a relatively benign Australian strain Kunjin (KUN) (Lanciotti et al., 2002). KUN virus was first isolated in 1960 in North Queensland (Doherty et al., 1963) and since then has been found to be endemic in Australia (Hall et al., 2002). KUN causes mainly asymptomatic infection and was associated with only a handful number of cases of mild encephalitis and no death (Hall et al., 2002). Immunization of mice with KUN virus or plasmid DNAs encoding a full-length cDNA copy of the KUN genome provides highly effective protection against NY99 (Hall et al., 2003) and therefore KUN has been considered as potential vaccine candidate against NY99. Although both NY99 and KUN are lethal after peripheral injection in weanling (less than 21 days old) mice, the 50% lethal dose (LD_{50}) for NY99 is substantially lower than for KUN. In contrast, only NY99 and not KUN is lethal after peripheral injection in adult (more than 21 days old) mice, thus providing a convenient model for identifying virulence determinants.

Studies comparing the neuro-invasiveness of NY99 with other WNV strains as well as with genetically engineered viral mutants in mice identified a number of virulence determinants residing in both structural and non-structural genes as well as in the 3′ untranslated region (UTR) (Beasley et al., 2004; Beasley et al., 2001, 2002; Beasley et al., 2005; Davis et al., 2007; Wicker et al., 2006; Zhang et al., 2006). Analysis of chimeric WNV viruses between NY99 strain and lineage 2 attenuated W956 strain showed that high cytopathicity and high virulence of NY99 strain was associated with determinants in the non-structural coding region (Borisevich et al., 2006). Previous studies with KUN demonstrated a role for NS2A in virus-induced cytopathicity in cells and virulence in mice and showed that a single point mutation from alanine to proline at position 30 (A30P) in NS2A significantly reduced virus-induced cytopathicity in cells and

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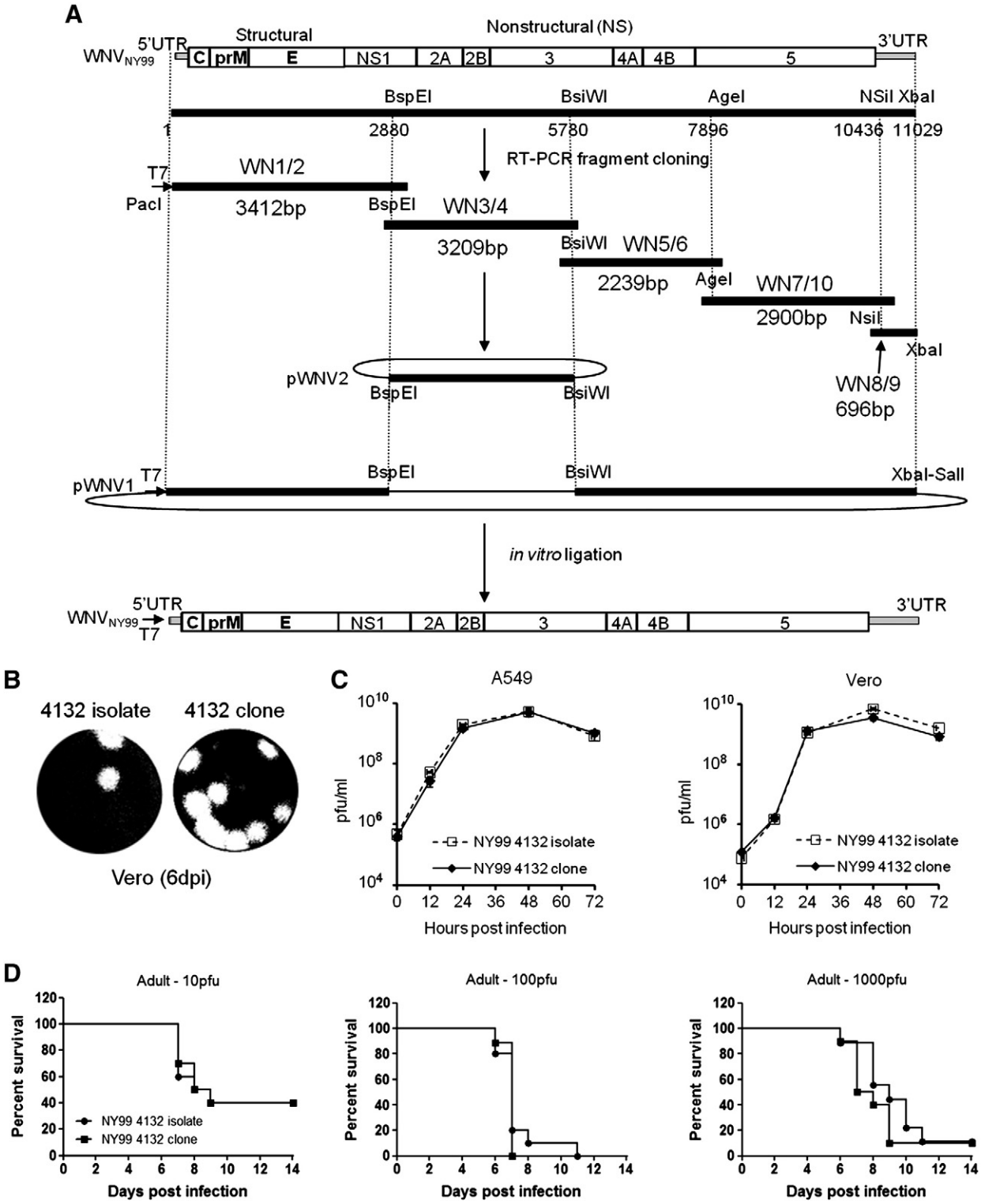

Fig. 1. Construction of a WNV NY99 clone and recovery of infectious NY99 virus. (A) Schematic representation of the cloning strategy. NY99 viral RNA was reverse transcribed and the cDNA fragments were ligated to form pWN NY99 1 (fragments 1–2880 and 5781–11029 nt) and pWN NY99 2 (fragments 2881–5780). To create infectious virus pWN NY99 1 and 2 were digested with BspEI and BsiWI, *in vitro* ligated, digested with SalI and *in vitro* transcribed to form full-length viral RNA. The RNA was then electroporated into BHK-21 cells, and virus was recovered. (B) Comparison of plaque morphology of BHK, vero, and A549 cells infected at an MOI of ~1 with wt NY99 and virus recovered from *in vitro* ligated pWN NY99. The cells were then overlaid with 0.75% LMT agarose in DMEM containing 2% FCS. At the indicated time, the cells were fixed with 4% formaldehyde and stained with 0.2% crystal violet. (C) Growth kinetics of wt NY99 and cloned NY99 in A549 cells infected at an MOI of ~1. At the indicated time post-infection culture supernatants were collected and viral titres were determined by plaque assay on Vero cells. (E) Survival of adult (4–5 weeks old) Swiss outbred mice infected with NY99-4132 isolate and virus recovered from NY99-4132 infectious clone. Groups of 10 mice were i.p. injected with 10, 100 or 1000 pfu of each virus. The mice were monitored for 14 days post-infection for signs of encephalitis at which point the mice were sacrificed. All mouse experiments in this were conducted with approval from the University of Queensland Animal Experimentation Ethics Committee in accordance with the guidelines for animal experimentation as set out by the National Health and Medical Research Council, Australia.