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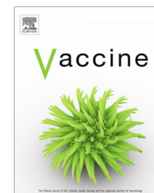

Japanese encephalitis virus/yellow fever virus chimera is safe and confers full protection against yellow fever virus in intracerebrally challenged mice

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ABSTRACT

Yellow fever (YF) is an acute viral haemorrhagic disease caused by the yellow fever virus (YFV), which remains a potential threat to public health. The live-attenuated YF vaccine (17D strain) is a safe and highly effective measure against YF. However, increasing adverse events have been associated with YF vaccinations in recent years; thus, safer, alternative vaccines are needed. In this study, using the Japanese encephalitis live vaccine strain SA14-14–2 as a backbone, a novel chimeric virus was constructed by replacing the pre-membrane (prM) and envelope (E) genes with their YFV 17D counterparts.The chimeric virus exhibited a reduced growth rate and a much smaller plaque morphology than did either parental virus. Furthermore, the chimera was much less neurovirulent than was YF17D and protected mice that were challenged with a lethal dose of the YF virus. These results suggest that this chimera has potential as a novel attenuated YF vaccine.

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1. Introduction

YFV is a mosquito-borne species that causes yellow fever YF over a wide area, especially Africa and South America. YFV, dengue virus (DENV), Japanese encephalitis virus (JEV), tick-born encephalitis virus (TBV) and Zika virus (ZIKV) are all members of the genus flavivirus [1]. YF is an acute viral haemorrhagic disease caused by YFV infection. The World Health Organization (WHO) estimates that between 84,000 and 170,000 cases of YF occur annually, and as many as 29,000–60,000 of these cases are fatal in Africa [2]. Even today, YF remains a potential threat to public health [3]. Up to 50% of severely affected persons will die without appropriate treatment after becoming infected. Since no effective antiviral drugs are available, vaccination is the most important preventive measure against YF.

The live-attenuated YF vaccine (17D strain) has been used for over 70 years [4,5]. The vaccine is safe, highly effective, and a single vaccination can confer 95% of recipients with life-long protection against YFV [6]. However, increasing adverse YF17D-associated vaccination events have been reported in recent years, despite a low average incidence [7–10]. The WHO developed and issued standard procedures for the surveillance, detection and investigation of serious adverse events following YF vaccinations to improve our understanding these events [11]. Alternative and safer vaccines, such as purified inactivated vaccines (PIV) [12,13], DNA-based vaccines encoding specific viral sequences [14] and adenovectors encoding YF17D antigens [15] have been developed but have not been licensed for market.

In this study, we constructed a chimeric JE/YF (Chimeri-JYF) virus by replacing the prM and E genes (prM/E) of the JEV SA14-14-2 strain with the corresponding genes from the YFV 17D strain. The Chimeri-JYF virus possesses the surface features of the YFV and the replication properties of the JEV SA14-14-2 strain. Because JEV SA14-14-2 and YFV 17D are both attenuated vaccine strains, the Chimeri-JYF virus is theoretically safer than either parental strain. To investigate whether this chimera can be used as a vaccine candidate strain to develop a novel YF vaccine, the biological characteristics of this chimera were preliminarily investigated. The resulting Chimeri-JYF virus was less neurovirulent than was YFV 17D, protected mice against the YFV challenge, and demonstrated great potential for further development.

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2. Materials and methods

2.1. Cells and viruses

Baby hamster kidney (BHK-21), primary hamster kidney (PHK) and African green monkey kidney (Vero) cells were cultured in minimal essential medium (MEM; Invitrogen, USA) supplemented with 10% foetal bovine serum (FBS) in a humidified 5% CO_2 atmosphere at 37 °C. JEV SA14-14-2 (GenBank accession No. D90195) was provided by the Chengdu Institute of Biological Products Co., Ltd. YFV 17D (GenBank accession No. FJ654700) was provided by Beijing Tiantan Biological Products Co., Ltd. The 50% lethal dose (LD50) was measured using groups of 3-week-old Kunming mice. Virus titer was determined by a standard plaque assay using BHK-21 cells, and viral stocks were stored in aliquots at −80 °C for further experiments.

2.2. Construction of a full-length cDNA clone of the JE/YF chimeric virus

A full-length cDNA clone of the JE/YF chimeric virus was constructed using methods similar to those used to generate the JEV/DENV4 and JEV/DENV1 chimeras described in our previous publications [16,17]. Briefly, a fragment containing the YF (17D strain) prM/E genes and a portion of the JEV SA14-14-2 NS1 gene were cloned into the vector pACNR to produce the plasmid pACNR-YF prM/E. The 5′-untranslated region (5′-UTR) and a fragment of the JEV C gene were then cloned into the pACNR-YF prM/E plasmid to generate a pACNR-JEV/YF 5′ half plasmid. Next, the 3′ half of the JEV cDNA (nt3446-10977) was isolated from the pACNR-JEV plasmid and inserted downstream of the pACNR-JEV/YF 5′ half plasmid to generate the recombinant full-length plasmid, pACNR-JEV/YF. The construction of pACNR-JEV was described previously [16].

2.3. Transcription and transfection

The recombinant plasmid pACNR-JEV/YF was linearized with the restriction enzyme *Xho*I (NEB, USA) and used as a template for *in vitro* transcription using the Ribomax Large Scale Production System (Promega, USA) in the presence of an m7GpppA cap analogue according to the manufacturer's protocols. The transcription products were purified using an RNeasy Mini Kit (QIAGEN, Germany) and used to transfect specific-pathogen-free(SPF) PHK cells by electroporation. The cells were incubated in MEM supplemented with 10% FBS. Once cytopathic effects (CPE) were observed, the supernatant was harvested, which contained the Chimeri-JYF virus. The plaque assay and Chimeri-JYF viral genome sequencing methods were the same as those previously used for JEV/DENV4, JEV/DENV1 and JEV/DENV2 [16–18].

2.4. Indirect immunofluorescence assays(IFA)

For indirect immunofluorescence staining, two groups of BHK-21 cells growing in 24-well plates were inoculated with either the Chimeri-JYF, JEV SA14-14-2 or YFV 17D virus. Twenty-four hours later, the cells were fixed with methanol for ten minutes and permeabilized with 1% Triton X-100 for 30 min. The cells were then sequentially incubated with an anti-YFV antibody (Ab) or a JEV E polyclonal antibody (Abcam, England) overnight at 4 °C and then with a fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG at room temperature for 1 h. After being washed with phosphate buffer saline (PBS), the cells were immediately observed for fluorescence, and images were captured using a fluorescence microscope (Carl Zeiss, Germany).

2.5. Growth characteristics of the Chimeri-JYF virus

To assess the growth characteristics of the Chimeri-JYF virus constructed from the parental viruses JEV and YFV, Vero and PHK cells were infected with the Chimeri-JYF virus, JEV or YFV at a multiplicity of infection (MOI) of 0.001. Virus samples were collected at different time points at 24-h intervals, and the titer were determined by a plaque assay using BHK-21 cells. Growth curves based on these cells were generated to assess the growth kinetics of the chimeric virus and to compare them with the parental viruses JEV and YFV.

2.6. Neuroinvasiveness and neurovirulence of the chimeric virus in mice

To study the neuroinvasiveness of the Chimeri-JYF virus, 3-week-old Kunming mice were inoculated subcutaneously (s.c.) with 0.1 ml of the chimeric virus (5.3lgPFU [$\log_{10}$ plaque forming units]) and intraperitoneally (i.p.) with 0.5 ml of the chimeric virus (6.0lgPFU). Each group contained 6 animals, and an additional group of mice was inoculated with MEM supplemented with 2% FBS as a control. The animals were observed for 21 days to record number of dead and surviving mice.

To study the neurovirulence of the Chimeri-JYF virus, 3-week-old Kunming mice were inoculated intracerebrally (i.c.) with 0.03 ml of the chimeric virus (3.5lgPFU) or MEM supplemented with 2% FBS. Each group contained 6 animals, and all the animals were observed for 21 days to record the number of dead and surviving mice.

2.7. Immunogenicity and protective efficacy

Chimeri-JYF virus immunogenicity was tested in 3-week-old Kunming mice. The animals were i.p. immunized with 0.5 ml of the chimeric virus (5.7lgPFU) and boosted with an equal dose of chimeric virus after 2 weeks. Blood samples were collected, and sera were isolated at 4, 8, 12, and 22 weeks after the initial immunization. Serum neutralizing antibody titer against YFV 17D were determined by plaque reduction neutralization tests (PRNT50) and were expressed as the reciprocal of the highest immune serum dilution that neutralized ≧50% of YFV.

To assess the protective efficacy of the Chimeri-JYF virus, 3-week-old Kunming mice were randomly distributed into 4 groups, with 12 mice in each group. For the test groups, each animal was i.p. immunized with 0.5 ml of the chimeric virus (5.7lgPFU). For the control groups, the animals were i.p. immunized once with 0.5 ml of MEM supplemented with 2% FBS, YFV 17D (5.7lgPFU) or JEVSA14-14-2 (5.7lgPFU/ml). Four weeks post-immunization, half the animals in each group were i.c. challenged with 500 LD50 of YFV 17D, while the remaining animals were identically challenged 8 weeks post-immunization. The animals were observed for 14 days to record number of dead and surviving mice. The protective efficacy was assessed based on the mortality reduction compared with the control groups.

2.8. Statistical analysis

Statistical analysis of the neutralizing antibody titer was performed using Student's *t*-test, and $P < 0.05$ was considered significant. All analyses were performed using SPSS version 17.0 (SPSS, USA).

3. Results

3.1. Recovery and characterization of the Chimeri-JYF virus

After the full-length JE/YF chimeric virus cDNA clone was constructed and verified, the linear full-length cDNA plasmids

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