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Identification of a candidate standard strain of severe fever with thrombocytopenia syndrome virus for vaccine quality control in China using a cross-neutralization assay

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ABSTRACT

Severe fever with thrombocytopenia syndrome (SFTS) is caused by a phlebovirus of the *Bunyaviridae* family, which is designated as SFTS virus (SFTSV). To our knowledge, no efficient SFTSV vaccine exists. Here, we report the identification of a standard virus strain for the eight major SFTSV strains circulating in China for use in evaluating the SFTSV vaccine. Rabbits were immunized with the SFTSV strains and the cross-neutralization capacities of SFTSV anti-sera were determined in microculture cytopathic effect (CPE)-inhibition assays. The mean cross-neutralization capacity of the eight SFTSV anti-sera ranged from 62.4 to 142.6%, compared to autologous strains. The HB29 strain demonstrated strong cross-reactivity with heterologous antibodies, and 33 serum samples from SFTS patients efficiently neutralized HB29, suggesting its broad cross-reactivity. In addition, HB29 demonstrated good replication in Vero and MRC-5 cells (8.0 and 6.0 lg 50% cell culture-infectious dose/mL, respectively) and significant CPE, which satisfied the requirements for a standard virus strain. The HB29 isolate was proven identical to the reported HB29 strain by DNA sequencing, and showed high homology in the S segments with other SFTSV strains (94.8–99.7%). Our results suggest that HB29 may be the best candidate standard strain for use in SFTS vaccine development in China.

1. Introduction

Severe fever with thrombocytopenia syndrome (SFTS) is a newly emerged infectious disease first reported in 2010 in China. SFTS is caused by a novel SFTS virus (SFTSV) belonging to the *Phlebovirus* genus of the *Bunyaviridae* family [1]. SFTSV has also been found in other countries, including Japan and Korea [2,3]. Other SFTSV-like viruses that threaten human health have also been discovered. Heartland virus (HRTV), isolated from two patients in Missouri, USA, is also a phlebovirus and clusters phylogenetically with SFTSV [4]; furthermore, it was found to be cross-reactive with SFTSV [5]. A novel phlebovirus named Malsoor virus was reported in India in 2014; it was also phylogenetically related to SFTSV and HRTV [6]. SFTSV-like viruses may have even broader geographical distribution.

SFTSV can be transmitted by tick bites and direct contact with blood and other body fluids of infected individuals [1,7–13]. SFTSV infection is manifested by high fever, severe malaise, nausea, vomiting, and diarrhea often accompanied by bleeding [1,14], and

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cases of SFTSV infection-associated death have been reported in China. A deeper understanding of SFTSV biology and advances in virus diagnostics enabled recent progress in SFTS symptomatic treatment, accounting for an improved survival rate and a decrease in mortality of 10–15% according to 2011 surveillance data in China [15]. However, many individuals become ill due to SFTSV infection every year, particularly during peak season [16]. Therefore, effective vaccines and drugs are needed to prevent and treat SFTS.

Several companies are currently in the process of developing SFTSV vaccines in China [17]. A successful vaccine should be effective and pass quality control testing using appropriate methods. One key component in the quality control of the SFTSV vaccine is the assessment of its protective efficacy determined based on the levels of serum neutralizing antibodies (NTAbs) generated in response to vaccination, because serum NTAbs are major indicators of SFTSV infection and protective immunity. However, the potential for cross-reactivity of NTAbs with different SFTSV genotypes and subgenotypes is unclear. Given that the detection of NTAbs relies on the viral strains used, the identification of a standard strain that can potently cross-react with NTAbs generated by other SFSTV variants should warrant evaluation of the accuracy and efficiency of vaccines. Because SFSTV strains demonstrate considerable differences in the generation of cross-reactive NTAbs, the establishment of a standard strain exhibiting strong cross-reactivity with heterologous NTAbs would significantly facilitate SFTSV vaccine evaluation and promote its development. At present, no standard SFTSV strain has been identified in China. In this study, we tested eight major SFTSV strains circulating in China for cross-reactivity with anti-sera from immunized rabbits and serum samples obtained from 33 SFTS patients, with the aim of selecting a candidate standard strain for testing SFTSV vaccines in China.

2. Methods

2.1. Preparation of viruses and animal sera

All SFTSV strains used in the study were isolated from SFTS patients living in major epidemic regions in China, including the Anhui, Hubei, Shandong, Jiangsu, and Liaoning provinces. Overall, eight SFTSV strains most frequently circulating in China were investigated (Table 1). Viruses were plaque-purified and amplified in Vero cells for over nine passages. Vero cells were infected with SFTSV at a multiplicity of infection (MOI) of 0.01 and cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 2% fetal bovine serum at 37 °C in a 5% CO_2 incubator. Seven days after infection, Vero cells showed evident cytopathic effect (CPE). When the titer of virus particles in the medium approached 1×10^7–10^8/mL, culture supernatants were harvested, aliquoted, and stored at −70 °C until use. The same culture procedure was used with MRC-5 cells.

Table 1
Eight major SFTSV strains isolated in China.

SFTSV strain	Origin (province in China)	Year of isolation	Accession number
HB29	Hubei	2010	NC_018137.1
AH12	Anhui	2010	HQ141591.1
LN3	Liaoning	2010	HQ141612.1
SD4	Shandong	2010	HM802204.1
JS2007-01	Jiangsu	2010	JF837595.1
JS2011-013	Jiangsu	2011	KC505128.1
JS2010-018	Jiangsu	2010	JQ317177.1
JS3	Jiangsu	2010	HQ141603.1

To determine the 50% cell culture infectious dose ($CCID_{50}$) of virus stocks, the microculture CPE assay was used. Virus-containing supernatants were 10-fold serially diluted and used to infect Vero cells seeded in 96-well plates. Each dilution occupied eight wells of a 96-well plate, with each well containing 100 μL of the diluted virus and 100 μL of Vero cells at a concentration of 10^5 cells/mL. Seven days after infection at conditions of 37 °C and 5% CO_2, when the cytopathic effect (CPE) was evident in Vero cells, virus titers were calculated by the Spearman–Karber method [18,19].

To obtain immune sera, virus particles were inactivated with β-propiolactone and purified by ultrafiltration, precipitation, centrifugation, and chromatography [20]. The purified inactivated SFTSV was emulsified with aluminum adjuvants and used to immunize rabbits once every 2 weeks (three boosts in total) to generate sera containing high levels of SFSTV NTAbs for the following immunological analysis [20]. All serum samples were stored at −20 °C before use. All animal care and experimental procedures were consistent with the Guide for the Care and Use of Laboratory Animals, and the study protocol was approved by the Institutional Animal Care and Use Committee of National Institutes for Food and Drug Control.

2.2. Neutralization assay

Serum levels of NTAbs were determined by the microculture CPE inhibition assay using corresponding SFTSV strains [1,21,22]. Serum samples were 2-fold serially diluted and incubated with equal SFTSV volumes (50 μL) containing 100 $CCID_{50}$ for 90 min and then with Vero cells in 96-well plates for 7 days. Serum antibody titers were determined by comparing CPEs of immune serum-treated viruses with those of non-immune serum-treated viruses, used as controls. The cross-neutralization assay was performed by testing SFTSV strains (2000 $CCID_{50}$/mL) against each of the eight anti-sera, and the results were used to calculate neutralization titers. To assess the serum cross-neutralization potential against each SFTSV variant, relative neutralization titers against heterologous viruses were calculated as the ratio to the titer of the autologous virus set as 100%. The cross-reactivity of each virus was determined as a geometric mean titer (GMT) calculated based on the average of NTAb titers in eight SFTSV anti-sera.

2.3. Sequencing and phylogenetic analysis

To validate our candidate SFTSV strains and assess their genetic characteristics, we sequenced the relatively conserved small (S) genome segments of each virus isolate. Viral RNA was extracted from 140 μL of virus supernatants (7–8 lg $CCID_{50}$/mL) using the QIAamp Viral RNA Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The S segments were amplified using the following primers: F1 (5′-ACACAAAGACCCCCTTCATTTG-3′) and R1 (5′-ACACAAAGACCCCCAAAAAAGG3-′). Subsequently, traditional PCR was performed using the Platinum Taq DNA Polymerase Kit (Invitrogen, Carlsbad, CA, USA) with the following thermocycling conditions: 94 °C for 2 min; 35 cycles at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 3.5 min; and a final step at 72 °C for 5 min. Sequence alignment of the amplified fragments to the SFTSV reference sequences were retrieved from GenBank (Table 1) was performed, and phylogenetic analysis was conducted using the Mega 6.0 software.

2.4. Human serum collection

Thirty-three hospitalized patients diagnosed with SFTSV infection participated in this study by providing recovery phase blood samples. Serum was separated and inactivated at 56 °C for 30 min