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Characterization of high-affinity antibodies against the surface Gc protein of *Dabie bandavirus* / severe fever with thrombocytopenia syndrome virus

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ABSTRACT

Severe fever with thrombocytopenia syndrome virus (SFTSV) or *Dabie bandavirus* is an emerging pathogen responsible for SFTS. It is considered a novel threat to human health, given the high associated fatality. SFTSV is a segmented negative-strand RNA virus containing three single-stranded RNAs, with the M segment encoding the glycoproteins Gn and Gc. Gc is vital for viral entry into the host cell surface, along with the Gn protein. As the Gc is the surface-exposable antigen from virions, it is a critical diagnostic marker of infection. Although various SFTSV Gn or N protein-based sero-diagnostic methods have been developed, there are no commercially available sero-diagnostic kits. Therefore, we generated monoclonal antibodies (mAbs) against SFTSV Gc and explored their application in serum diagnostic tests to develop sensitive serodiagnostic tools covering broad-range genotypes (A to F). First, 10 SFTSV Gc antibody-binding fragments (Fabs) were isolated using a phage display system and converted into human IgGs. Enzyme-linked immunosorbent assays (ELISA) of the SFTSV and Rift Valley fever virus (RVFV: same genus as SFTSV) Gc antigens showed that all antibodies attached to the SFTSV Gc protein had high affinity. An immunofluorescence assay (IFA), to verify the cross-reactivity of seven antibodies with high affinities for various SFTSV genotypes (A, B2, B3, D, and F) and detect mAb binding with intact Gc proteins, revealed that five IgG type mAbs were bound to intact Gc proteins of various genotypes. Six high-affinity antibodies were selected using ELISA and IFA. The binding capacity of the six antibodies against the SFTSV Gc antigen was measured using surface plasmon resonance. All antibodies had high binding capacity. Consequently, these antibodies serve as valuable markers in the serological diagnosis of SFTSV.

1. Introduction

Severe fever with thrombocytopenia syndrome (SFTS) is a viral disease caused by a phlebovirus from the family *Bunyaviridae* (the SFTS virus, SFTSV) and was discovered in China in 2009. Since 2013, approximately 2000 cases have been reported in South Korea (https://npt.kdca.go.kr). SFTSV can be transmitted to humans through tick bites. The most common signs of the syndrome include high fever, thrombocytopenia, leukopenia, and gastrointestinal symptoms, including vomiting and diarrhea (fatality rate, 5–20 %) [1,2]. In serious cases, it causes central nervous system impairment, hemorrhagic fever, and multiple organ (kidney, lung, and liver) dysfunction [3].

SFTSV is a single-stranded negative-RNA virus with three genomic segments: large (L), medium (M), and small (S). The L segment encodes an RNA-dependent RNA polymerase (RdRp) that functions as a viral transcriptase/replicase. The M segment encodes two glycoproteins, Gn and Gc, that constitute the envelope. These glycoproteins bind to receptors. The S segment encodes two proteins: nucleoprotein (NP), which functions in viral RNA encapsidation/formation of the RNA complex, and non-structural protein (NS), which interferes with host infection production [1].

The M segment encodes a glycoprotein localized in the Golgi

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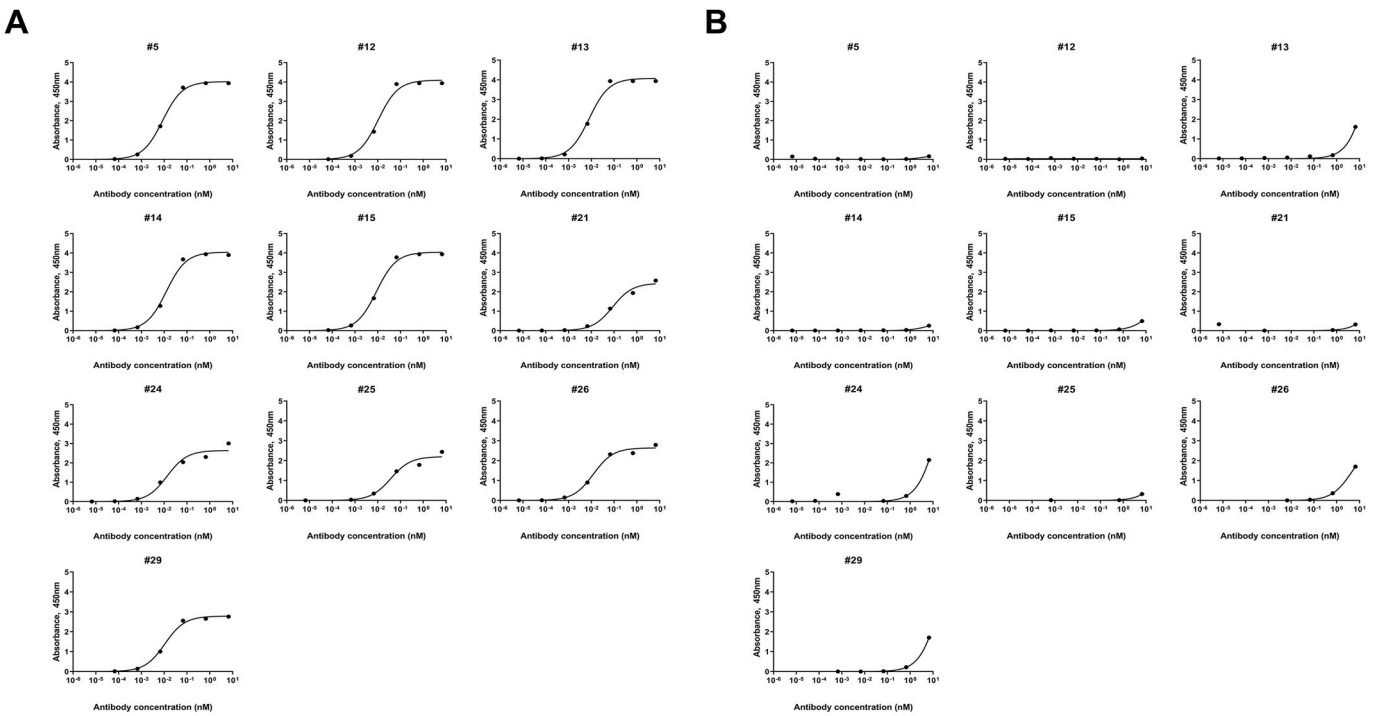

Fig. 1. Enzyme-linked immunosorbent assays confirm the affinity of the purified antibodies with SFTSV and RVFV Gc antigens. Affinity of antibodies against SFTSV Gc antigen (A) and RVFV Gc antigen (B). All antibodies exhibited high affinity for SFTSV Gc and no cross-reactivity to RVFV Gc.

apparatus, which is cleaved into Gn and Gc by proteolysis. Gn mediates the first step in the viral replication cycle of binding to host cell receptors, and Gc plays a role in virus entry into the host cell; thus, Gn and Gc are the primary targets for neutralizing antibodies [4]. During the membrane fusion process, a fusion peptide of the Gc protein is inserted into the cell membrane, followed by refolding from a dimeric to a trimeric structure, shortening the distance between the cell membrane and the viral envelope [5]. Furthermore, strong cell fusion activity is associated with an amino acid substitution (arginine [R] to tryptophan [W]) at position 624 in Gc. Position 624 is critical for low pH-dependent cell fusion [6].

Currently, there are no commercial therapeutics or vaccines for SFTSV, despite mortality rates of 23.3, 27, and 6.18 % in South Korea, Japan, and China, respectively [7]. With the development of therapeutics and vaccines against SFTSV, there is an increasing need for antibodies with a high affinity for the virus. The development of these antibodies is urgently needed for the rapid early diagnosis and treatment of patients with SFTSV infections.

In this study, an antibody library was constructed using phage display technology, and antibodies against Gc glycoproteins were selected to determine their role in serological diagnosis. Antibodies targeting SFTSV nucleocapsid protein have been generated for serological diagnosis [8,9]. We posited that the Gc antigen could be a suitable immunogenic protein due to its surface-exposable characteristics compared to intracellular nucleocapsid cells. In addition, Gc protein was well detected by commercial anti-SFTSV polyclonal antibodies in western blots (data not shown).

Our antibodies against Gc glycoproteins of SFTSV represent potential candidates against SFTS in infected patients and can be employed in serological diagnosis, including in rapid kits.

2. Materials and methods

2.1. Cells and viruses

Vero cells (Clone E6, ATCC, CRL-1586) and HEK293FT cells (Invitrogen, Grand Island, NY, USA) were maintained in Dulbecco's modified Eagle medium (DMEM) with 10 % fetal bovine serum (FBS), 1 % penicillin/streptomycin (P/S) at 37 °C in 5 % CO_2. SFTSV is a Biosafety Level 3 (BSL-3) pathogen; therefore, all experiments with live SFTSV were conducted in BSL-3 containment facilities at the Korea Disease Control and Prevention Agency (Cheongju, Korea).

2.2. Clinical specimens and ethics statement

Human sera were collected from convalesced SFTSV Korean patients. The participants provided their written informed consent to participate in this SFTSV research. The research protocols were reviewed and approved by the Korea Disease Control and Prevention Agency Institutional Review Board (IRB No. 2019-09-04).

2.3. Generation of SFTSV Gc protein

In the modified pFUSE2ss-CLIg-hk (Invivogen, San Diego, CA, USA), we inserted the SFTSV Gc 536–1035 region (Gene Accession No. KU507548.1) without the transmembrane site (ΔTM) and 6 × histidine (6 × His) and AVITAG (Avi) regions. HEK 293FT cells were transfected using the glycoprotein expression vector pKNIHss-SFTSV Gc-ΔTM6 × His-Avi and incubated at 37 °C for 4–5 days. The proteins were purified using a Ni-NTA column and dialysis with Slide-A-Lyzer™ dialysis cassettes (10K MWCO, Thermo Fisher Scientific, Waltham, MA, USA).

2.4. Enzyme-linked immunosorbent assay (ELISA) using SFTSV and Rift Valley fever virus (RVFV) Gc proteins

Ninety-six-well nickel-coated plates (Thermo Fisher Scientific) were coated with 100 ng of SFTSV Gc (Immune Tech., NY, USA) and RVFV Gc protein (Sino Biological, Beijing, China) in PBS overnight at 4 °C. Plates were blocked with 300 μL of phosphate-buffered saline using 0.05 % tween-20 (PBS-T) and 5 % bovine serum albumin (BSA; blocking solution) for 1 h at room temperature (RT, 20–25 °C). After removing the blocking solution, the plates were washed with PBS-T. Washed plates

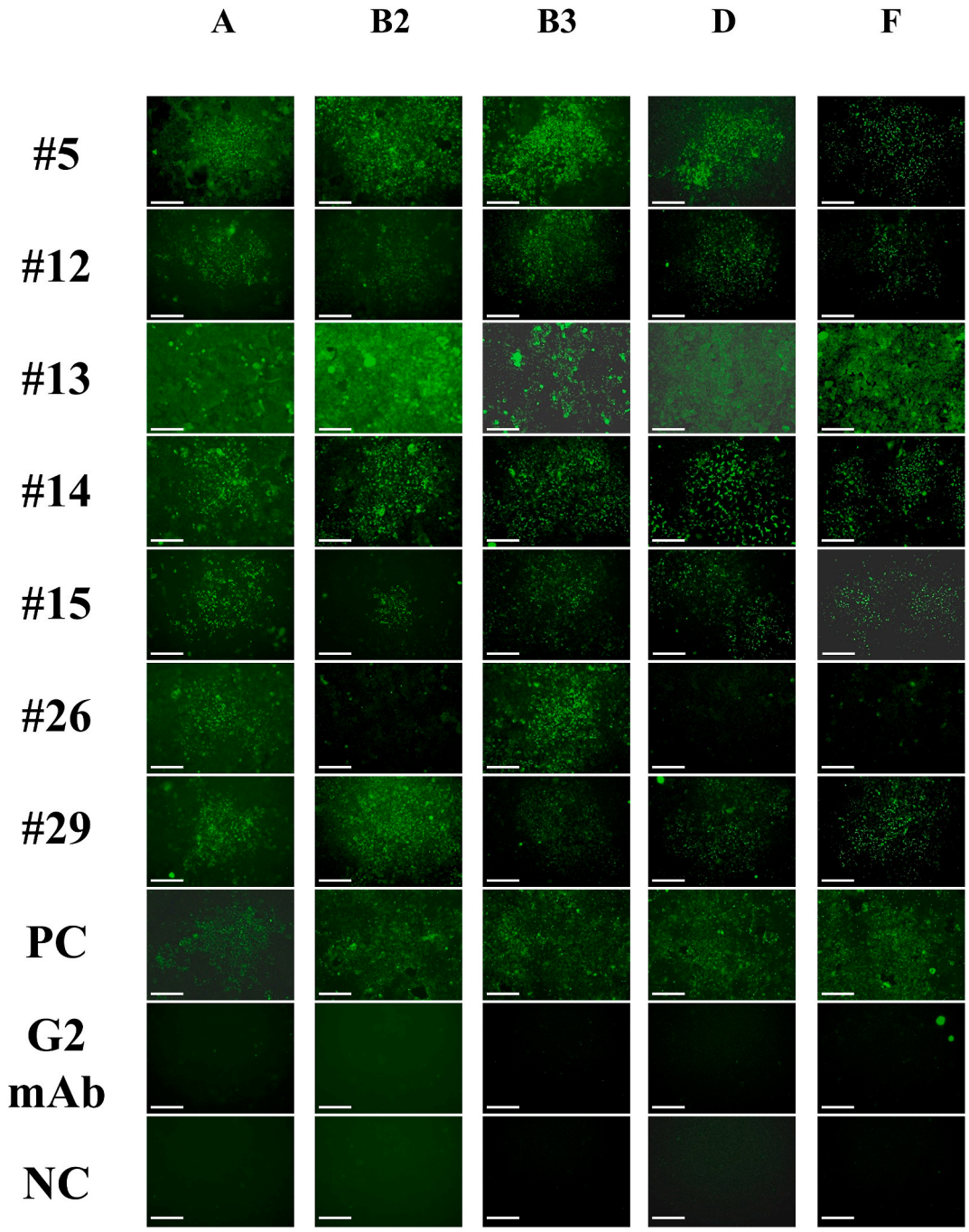

Fig. 2. Broad-range binding of human anti-SFTSV Gc IgGs to SFTSV with various genotypes (A, B2, B3, D, and F) in indirect immunofluorescence assays. Scale bars = 150 μm.

were incubated with 10 IgGs (100 μL/well) diluted 10-fold at 1 μg to 10^{-7} μg in the blocking solution for 1 h at RT. After washing the plates, samples were incubated for 1 h at RT with secondary antibodies (SFTSV: Rabbit anti-human IgG H&L [Abcam, Cambridge, UK], RVFV: Anti-6 × His-tag® [Abcam]). Tetramethylbenzidine (TMB; Thermo Fisher Scientific) substrate solution was added to each well, and the reaction was stopped using a stop solution (Thermo Fisher Scientific). Absorbance was measured using an ELISA microplate reader (VICTOR Nivo™; PerkinElmer, Waltham, MA, USA) at 450 nm. The dissociation constants (K_D values) between antibodies and antigens were calculated using the Prism 9 (GraphPad Software Inc., San Diego, CA, USA).

2.5. Indirect immunofluorescence assay (IFA) using SFTSV genotypes

SFTSV genotypes A, B2, B3, D, and F [10] were propagated in Vero E6 cells (ATCC, CRL-1586) and cultured in DMEM supplemented with 2 % FBS and P/S (100 U/mL). Vero E6 cells were infected with each SFTS virus genotype (A, B2, B3, D, and F) at a multiplicity of infection of 0.01 and incubated at 37 °C for 72 h. Subsequently, the cells were fixed with 4 % paraformaldehyde and permeabilized using 1 × DBPS with 0.5 % Triton X-100. The fixed cells in blocking solution (PBS supplemented with 1 % BSA) were treated with human monoclonal anti-SFTSV Gc IgGs at 1 μg/100 μL for 1 h. After washing the cells with 1 × DPBS, they were treated with rabbit anti-human IgG-FITC (Jackson ImmunoResearch, PA, USA) in a blocking solution for 1 h. Subsequently, the cells were

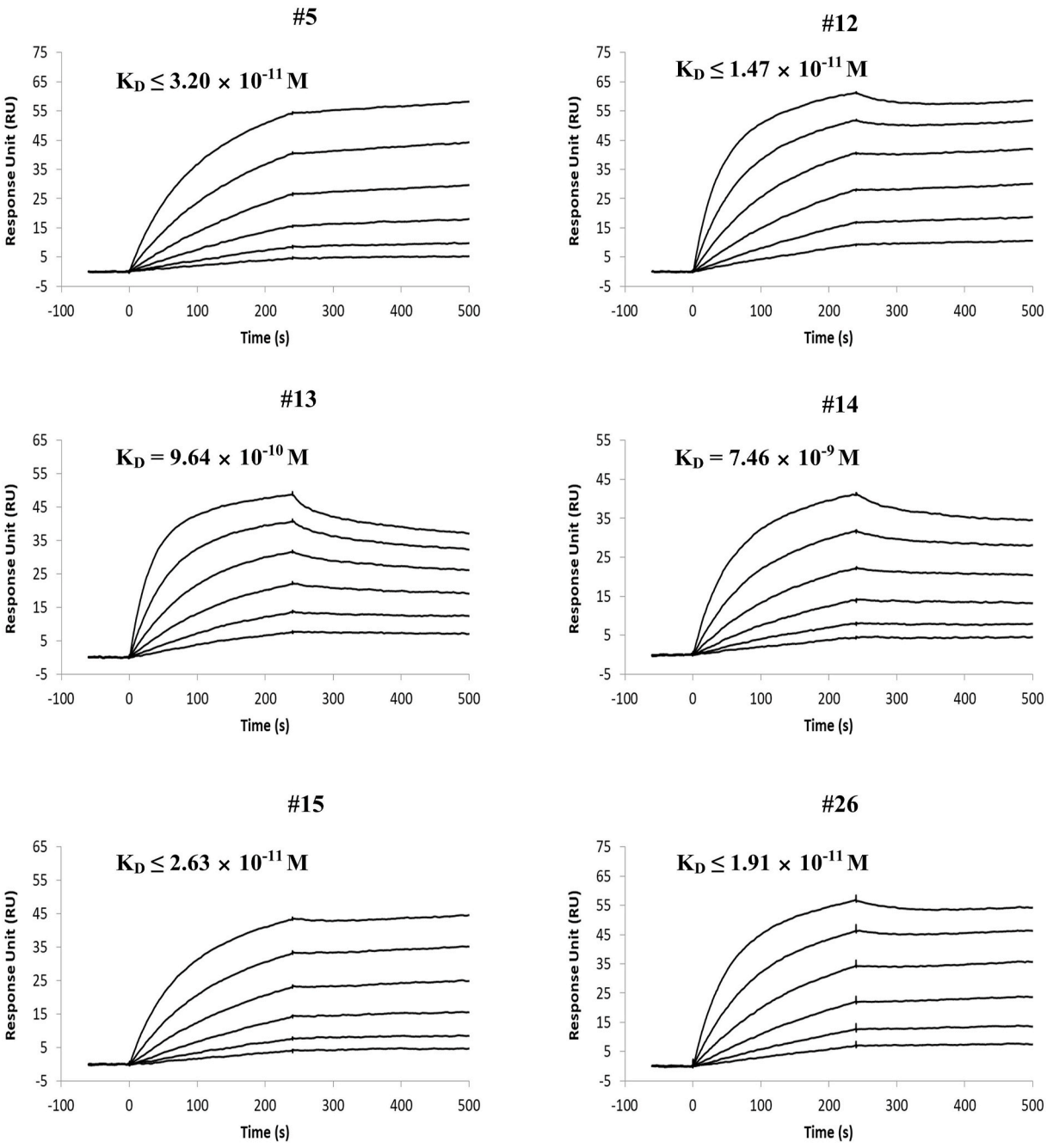

Fig. 3. Concentration-dependent binding of human anti-SFTSV Gc IgGs to SFTSV Gc recombinant protein in surface plasmon resonance analysis (SPR). SPR analysis of six antibodies was performed on the CM5 chip with an immobilized anti-histidine antibody binding to a poly-histidine-tagged SFTSV Gc antigen. The sensorgrams display the time course of the SPR signal in the response unit (RU, 1000 RU = 1 ng/mm^2) and were normalized to a zero baseline. K_{on} and K_{off} denote dissociation rate constants, and K_D denotes the equilibrium dissociation constant.

observed under an inverted fluorescence microscope (Thermo Fisher Scientific, EVOS M7000). In this experiment, human serum from a convalescent patient with SFTS was used as a positive control.

2.6. Surface plasmon resonance (SPR) measurement

Antibodies binding to SFTSV Gc were assayed using a Biacore™ T200 instrument (Cytiva, MA, USA). An anti-histidine antibody from the His Capture kit (Cytiva) was amine-coupled in the active and reference flow cells of Sensor Chip CM5, according to the manufacturer's instructions [11]. Immobilization levels in the 14,000–15,000 RU range were used, with similar levels in the active and reference flow cells. Histidine-tagged SFTSV Gc was injected for 30 s at 10 μL/min in the active flow cell. Then, diluted antibodies were injected at 30 μL/min for 4 min, and an HBS-EP + buffer (0.1 M HEPES, 1.5 M NaCl, 0.03 M EDTA, and 0.5 % v/v Surfactant P20) was injected for 5 min to monitor dissociation. After each binding cycle, a regeneration solution (10 mM glycine, pH 1.5) was injected for 1 min at 30 μL/min to remove proteins with non-covalent bonds. Signal detection was performed at a rate of 10 signals/s. The binding constants were determined using the 1:1 binding model of the Biacore™ T200 Evaluation software version 3.1 (Cytiva).

Table 1
Binding interactions between anti-SFTSV Gc IgGs and the SFTSV Gc recombinant protein[a].

	k_a (1/Ms)	k_d (1/s)[b]	K_D (M)	Chi^2 (RU^2)	SE (k_a)	SE (k_d)
#5	**3.13 × 10^5**	**≤ 1.00 × 10^{-5}**	**≤ 3.20 × 10^{-11}**	**0.8**	**258.3**	**7.5 × 10^{-8}**
#12	**6.82 × 10^5**	**≤ 1.00 × 10^{-5}**	**≤ 1.47 × 10^{-11}**	**1.7**	**543.6**	**2.9 × 10^{-7}**
#13	**7.59 × 10^5**	**7.31 × 10^{-4}**	**9.64 × 10^{-10}**	**1.1**	**859.9**	**3.2 × 10^{-6}**
#14	**5.61 × 10^4**	**4.19 × 10^{-4}**	**7.46 × 10^{-9}**	**0.4**	**46.1**	**2.2 × 10^{-6}**
#15	**3.80 × 10^5**	**≤ 1.00 × 10^{-5}**	**≤ 2.63 × 10^{-11}**	**0.4**	**295.6**	**4.4 × 10^{-8}**
#26	**5.25 × 10^5**	**≤ 1.00 × 10^{-5}**	**≤ 1.91 × 10^{-11}**	**0.8**	**329.9**	**2.4 × 10^{-7}**

Abbreviations: k_a, apparent association rate constant; k_d, apparent dissociation rate constant; K_D, apparent dissociation equilibrium constant; SE, standard error and M, molar.

[a] Determined using surface plasmon resonance (SPR) technology on a Biacore T200.

[b] The k_d values of #5, #12, #15, and #26 exceeded the instrumental capabilities of 10^{-5} s^{-1}.

2.7. Statistical analyses

Statistical analyses were performed on ELISA results using Student's two-tailed *t*-tests (Prism 9; GraphPad Software Inc.). All data are expressed as means ± standard error of the mean (SEM).

3. Results

3.1. Selection and characterization of anti-Gc glycoprotein antibodies using ELISA

After four rounds of panning, 94 phage clones were randomly picked from the third and fourth rounds to select the Fabs specific for SFTSV Gc (Fig. S1) [12–14]. Among them, 10 unique clones of Fab exhibited high affinity for SFTSV Gc, as evaluated using ELISA and SPR assay (Fig. S2). Subsequently, we examined the affinity and specificity of ELISA against SFTSV and RVFV Gc antigens using 10 antibodies converted to the human immunoglobulin G1 type (Fig. 1). The IgGs exhibited high affinity according to ELISA using the SFTSV Gc antigen (Fig. 1A). Moreover, the IgGs were bound to the SFTSV Gc antigen but not the RVFV Gc antigen, indicating that the IgGs had high specificity for SFTSV Gc.

3.2. Selected antibodies combined with SFTSV-infected cells in vitro

Immunofluorescence assays exploit the specificity of antibodies for antigens, allowing visualization of numerous components *in vitro* [15–17]

To confirm that the selected antibodies combined with cells infected with the SFTSV virus, we performed an IFA using six anti-Gc IgGs, considering the high affinities (K_D values) and optical densities of the 10 mAbs in the ELISA experiments, and Vero cells infected with different genotypes (A, B2, B3, D, and F) of the SFTS virus. Our mAbs bound to SFTSV-infected cells of all genotypes, except for #26 mAb, which bound to cells infected with genotypes A and B3 (Fig. 2). We observed that our mAbs had a broad-range binding capacity for Gc proteins from genotypes A to F. Therefore, our mAbs can be utilized to screen for SFTSV through sandwich ELISA or rapid lateral flow immunoassays.

3.3. Anti-Gc antibodies bind SFTS Gc antigen with high affinity

SPR was used to analyze antigen-antibody interactions in detail. It is a standard biophysical characterization tool for measuring a diverse range of protein–protein interactions [18–20]. SPR analysis was performed to confirm the interactions of the six IgGs binding to the SFTSV Gc antigen. One of the six IgGs (#12) bound to the Gc antigen with an equilibrium dissociation constant (K_D) of 15 pM, an association rate (K_{on}) of 6.820 × 10^5, and a dissociation rate (K_{off}) of ≤1.000 × 10^{-5} (Fig. 3, Table 1). All the other IgGs demonstrated high affinity and dissociation constants at the pM level, except for #14. Thus, the six IgGs demonstrate high affinity to the Gc antigen and can effectively detect the SFTSV.

4. Discussion

Severe fever with thrombocytopenia syndrome has spread to many countries, such as South Korea, Japan, and Vietnam, following its discovery. SFTSV was first reported in South Korea in 2013, causing significant morbidity and mortality annually, with approximately 2000 cases reported between 2013 and 2022 and a fatality rate of 18.7 % [3,21,22]. The Gc glycoprotein of the SFTSV is a target protein required to screen for the virus because it is inserted into the cell membrane and has a high binding capacity to the antigen epitope [5]. Here, we explored monoclonal antibodies to diagnose SFTSV infection in humans. In the ELISA using SFTSV and RVFV Gc antigens, 10 IgGs had a high affinity for the SFTSV Gc antigen. Moreover, the IFA of SFTSV genotypes revealed that five IgGs bound to the Gc proteins of various SFTS viruses. Six of the IgGs showed high affinity for SFTSV Gc antigen. Antibody #12 was bound with a higher K_D than the other IgGs. Therefore, monoclonal antibodies selected by phages that display a specific binding capacity are potential agents against SFTSV infection in humans. Further investigations should be performed to explore the domains of the Gc antibodies that affect the SFTS virus.

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Data statement

Not applicable.

CRediT authorship contribution statement

Pyeonghwa Jeon: Writing – original draft, Visualization, Validation. **Bin Yoo:** Writing – original draft, Visualization, Validation. **Yoonji Kim:** Resources, Formal analysis. **So-Young Lee:** Visualization, Resources. **Hye-Min Woo:** Visualization, Resources. **Hee-Young Lim:** Visualization, Project administration. **Joo-Yeon Lee:** Writing – review & editing, Investigation, Funding acquisition. **Sora Park:** Writing – review & editing, Investigation. **Hansaem Lee:** Writing – review & editing, Writing – original draft, Investigation.

Declaration of competing interest

We declare competing interests related to the patent titled "Monoclonal antibodies for Gc protein of Severe Fever Thrombocytopenia Syndrome Virus, and use thereof" identified by patent number No. 10-2021-0163,224 (Korean patent application).

Data availability

No data was used for the research described in the article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2024.101779.

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