Short Communication

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Cross-Reactivity of Neutralizing Antibodies Against SARS-CoV-2 in Patients Infected by Various Clades (S, L, V, GR, G, GH and GV)

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ABSTRACT

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsive for the most recent worldwide pandemic since December 2019. The present study aimed to determine the characteristics of neutralizing antibody cross-reactions of coronavirus disease 2019 (COVID-19)-causing variant viruses compared with those of non-variant viruses. Seven virus clades (S, L, V, GR, G, GH, and GV) were isolated and confirmed in the sera of 19 patients diagnosed with COVID-19. Among the patient, ten, five, and four cases were positive for S, V, and GH clades, respectively, according to full-length genomic analysis. Plaque reduction neutralizing antibody test was performed using seven types of non-variant virus isolates. Noteworthy, the neutralizing antibodies against the same virus clade in one V clade and one GH serum samples exhibited more than 4-fold higher neutralizing ability against viruses of different clades (i.e., GR, G, or GV). Although the neutralizing ability differed for viruses in the same or different clades, similar neutralizing antibody cross-reactivity was observed in all 19 samples. These findings suggest that people with existing infection are protected from re-infection through neutralizing antibody cross-reactivity against COVID-19, although re-infection can occur in some cases.

Keywords: Cross-Reactivity; Neutralizing Antibody; Re-Infection; Severe Acute Respiratory Syndrome Coronavirus 2; Virus Clade

Introduction

The coronavirus disease-19 (COVID-19), which is caused by the severe acute respiratory virus syndrome coronavirus 2 (SARS-CoV-2), has reached the status of global pandemic since its initial outbreak of unidentified pneumonia in Wuhan, China, in December 2019. In the Republic of Korea, the first confirmed case was reported on January 20, 2020, and a total of 3,691,488 cases of COVID-19 were reported until March 2, 2022. In the first stage of the COVID-19 pandemic, the World Health Organization Global Initiative on Sharing All Influenza Data classified different types of SARS-CoV-2 as S, V, and G clades, which were later on expanded to S, V, G, GH, and GR clades. In November 2020, the GV clade was added as a subdivision of the G clade [1]. In the Republic of Korea, the S and V clades were identified until March 2020, the GH clade was detected in early April 2020, and the G and GR clades have also been detected. The GV clade was detected in October 2020. Passive immunization using monoclonal antibodies is vital to tackle COVID-19. The early antibody response towards COVID-19 is highly convergent and can be mined for therapeutic candidates with broad neutralization potential against widely circulating

SARS-CoV-2 strains. Previous studies have published experimental results on cross-reactivity of neutralizing antibodies responses detected to SARS-CoV-2 and SARS-CoV using the sera from infected human and mice [2], and detected to D614 and G614 clades using the pseudoviruses of COVID-19 [3]. In addition, the recent emergence of viral variants with reduced sensitivity to some current antibodies and vaccines highlights the importance of broad cross-reactivity [4]. Thus, the present study aimed to evaluate serum samples from patients infected with the S, V, and GH genotypes and assess their potential for re-infection with non-variant coronavirus.

Material and Methods

Sample Collection and SARS-CoV-2 Detection

A total of 19 patients with confirmed detection of SARS-CoV-2 were enrolled in the study, among whom 12 were females. The participants were aged from 27 to 76 years (mean age: 47 years). Nasopharyngeal and oropharyngeal swab and/or sputum samples were collected at the time of admission from patients suspected of having COVID-19. To confirm the diagnosis, SARS-CoV-2 RdRP and E gene were evaluated by real-time reverse transcription polymerase chain reaction (RT-PCR) as described previously [5]. Blood samples were collected during hospitalization. The study was approved by the Institutional Review Board at the Korea Centers for Disease Control and Prevention (2020-03-01-P-A). Written informed consent was waived by the Institutional Review Board for COVID-19 cases.

Plaque Reduction Neutralization Test

Neutralizing antibodies against SARS-CoV-2 were detected in the serum samples by plaque reduction neutralization tests (PRNT). Probit analysis was used to determine the serum titre required to reduce SARS-CoV-2 virus plaques by 50% (PRNT50) compared with that of the control, as previously described [6-9]. Briefly, Vero E6 cells (American Type Culture Collection, Manassas, VA, USA) were cultured (1 × 105 cells per mL) in Gibco minimum essential media (Thermo Fisher Scientific, Waltham, MA, USA) containing 10% heat-inactivated foetal bovine serum, 1% glutamine, and 100 U of penicillin and streptomycin (Thermo Fisher Scientific) at 37°C in a 5% CO2 incubator. Test sera were heat-inactivated at 56°C for 30 min, serially diluted (1:10; 1:50; 1:250; 1:1,250; 1:6,250; and 1:31,250) with SARS-CoV-2 virus diluent (100 plaque forming units/mL), and then incubated for 1 h at 37°C. The virus-serum mixture was then inoculated into the Vero E6 cell culture (0.1 mL per well) and incubated for 1 h at 37°C. Next, overlay medium containing agarose (SeeKem LE Agarose, Lonza, Basel, Switzerland), heat-inactivated calf serum, L-glutamine, and 100 U of penicillin and streptomycin was added to the cell culture (1.5 mL per well) and allowed to solidify for 15 min. The plates were incubated at 37 °C with 5% CO2 for 2 days. After fixing the cells in 4% paraformaldehyde overnight, 4% Neutral Red solution (Sigma-Aldrich, St. Louis, MO, USA) was added, and the plaques were counted. Positive control wells (virus without sera) were established for each assay to ensure infectivity of the cell monolayer (formation of approximately 50 plaques). Antibody levels ≥ 1:10 relative to the PRNT50 were considered to indicate neutralizing antibody-positive results. To establish experimental conditions for neutralization analysis, the incubation time at which cytopathic cell effects occurred after cell infection with non-variant virus isolates was determined. Briefly, seven types of non-variant virus isolates (at the same dose) were mixed with patient sera and then incubated with infected cells. Five virus clades (S, L, GR, G, and GV) showed cytopathic effects within 44 h after cell infection and two clades (V and GH) showed cytopathic effects within 52 h. Each type of coronavirus induced cytopathic cell effects at different times. Subsequent experiments were conducted using the optimized cell infection and culture times for neutralization analysis of each virus type.

Virus Isolation in Cell Culture

For viral cell culture, Vero E6 cells were inoculated with nasopharyngeal or oropharyngeal samples and cultured for 5 days as previously described [10]. After one additional passage for sub-culturing, cytopathic effects were observed. Cell proliferation was evaluated by real-time RT-PCR.

Whole-Genome Sequencing, Genome Assembly, and Phylogenetic Analysis

cDNA was synthesized from the extracted RNA using random primers followed by gene-specific multiplex PCR according to the ARTIC V.3 protocol (https://www.protocols.io/view/covid-19-artic-v3-illumina-library-construction-an-bibtkann). Briefly, extracted RNA was converted to cDNA using the Life Technologies Superscript IV First Strand synthesis system (Thermo Fisher Scientific) and random hexamer primers. SARS-CoV-2 whole-genome amplification was performed by multiplex PCR using primers designed through the DNASTAR Lasergene software (Madison, WI, USA). PCR products were purified using QIAquick PCR purification kit (Qiagen, Hilden, Germany) and quantified using the Qubit dsDNA High Sensitivity assay on a Qubit 4.0 instrument (Thermo Fisher Scientific). The Nextera Flex DNA Library Prep kit (Illumina, San Diego, CA, USA) was used according to the manufacturer's protocol to prepare indexed paired-end libraries of gDNA. Sequencing libraries were normalized to 4 nM, pooled, and denatured with 0.2 N sodium acetate. A 12-pM sample library was spiked with 1% PhiX (a PhiX Control v.3 adaptor-ligated library was used as a control) and sequenced using 500-cycle v.2 MiSeq Reagent Kit and Illumina MiSeq instrument [11]. Paired-end fastq reads were assembled using CLC Genomics Workbench (Qiagen). MEGA 6 software (https://www.megasoftware.net) was used for genomic sequence alignment and phylogenetic analysis using the maximum-likelihood method.

Statistical Analysis

Two-sided Friedman test with Dunn's multiple comparison was performed between each of the viral strains. $P > 0.05$ was deemed statistically significant.

Results and Discussion

Sample Characteristics Concerning Seven Clades of SARS-CoV-2

Seven clades of isolated viruses from SARS-CoV-2-positive patients (four males and three females, ages ranging from 16 to 61 years) were evaluated. The S clade inflowed from foreign countries in the early stages of the pandemic, such as from Wuhan residents, Guro call centres, and overseas immigrants, and the V clade was first identified in the Daegu Sincheonji Church and Daenam Hospital in Cheongdo. The G clade was first detected among incoming travellers to the Republic of Korea, and the GR clade was identified among a Russian ship crew and arrivals at Gamcheon Port in Busan. The GV clade was first confirmed in Deji High School/Jukjeon High School in Yongin-si, Gyeonggi Province. Four clades (S, L, GR, and G) were isolated from foreign cases (China, Japan, England, and Spain), and three clades were isolated from domestic cases.

Genetic Sample Characterization

The serum used to analyse the neutralizing ability was obtained from 19 patients whose upper respiratory tract and serum samples were obtained. Full-length genomic analysis was conducted on the upper respiratory tract samples to confirm the clade of the infected sample. Antibodies formed during the ongoing infection with a specific clade were used for the neutralizing ability test to determine their protective effects against other clades. Seven clades were evaluated: S, L, V, GR, G, GH, and GV. The sequences of the samples were confirmed by comparative analysis with reference sequences available in the Global Initiative on Sharing All Influenza Data. Ten participants were infected with the S clade, five with the V clade, and four with the GH clade, which protected against the infecting clade in the serum of the patient. According to the phylogenetic tree, various clusters (≥ 4) were formed by 10 cases of S clade introduced mainly from China in the early days of the pandemic. In contrast, five cases of V clade identified in an outbreak at a hospital in Gyeongsangbuk-do and four cases of GH clade from an outbreak in a pub in Seoul formed different clusters; each cluster had the same infection source (Figure 1), supporting the results of epidemiological investigation.

Neutralizing Antibody Cross-Reactivity Against SARS-CoV-2 by Clade

To confirm that patients infected with one SARS-CoV-2 type could exhibit neutralizing ability against viruses from other clades, sera samples from 19 patients infected with the S, V, and GH clades were evaluated further.

Seventeen serum samples, including eight, five, and four cases of S, V, and GH clade serum, respectively, showed similar levels of neutralizing antibodies against seven viruses ($P > 0.05$), thereby indicating neutralizing antibody cross-reactivity (Figure 2). Based on these results, if the patient is exposed to another COVID-19 clade other than the initial infecting clade, the risk of re-infection may be low. The neutralizing antibody levels against seven viruses in two S clade sera were similar and less than 1:10, and thus did not contain neutralizing antibodies against other clades, including the S clade. In addition, neutralizing antibodies against the same virus clade in one V and one GH genotype serum samples exhibited more than 4-fold higher neutralizing ability against viruses of different clades (i.e., GR, G, and GV). Further studies are needed to investigate why the GR, G, and GV clade exhibit high neutralization and if differences in amino acid sequences can be used to distinguish virus genotypes. Although serum of patients infected with viruses from the L, GR, G, and GV clades were not evaluated, infected people may be protected against re-infection because of the presence of cross-reactive neutralizing antibodies against SARS-CoV-2. Taken together, the present analysis of the protective effect of non-variant virus clades against infection by SARS-CoV-2 in patients diagnosed with COVID-19 suggest that infected people may be protected from re-infection because of the neutralizing antibody cross-reactivity against SARS-CoV-2 among the S, L, V, GR, G, GH, and GV clades. As the sample size of the present study was small and some virus clades were not evaluated, further studies are still warranted to confirm these findings. Nevertheless, this study may pave the way for the development of effective immunotherapies and universal vaccines against emerging variants of SARS-CoV-2 based on antigenic cross-reactivity.

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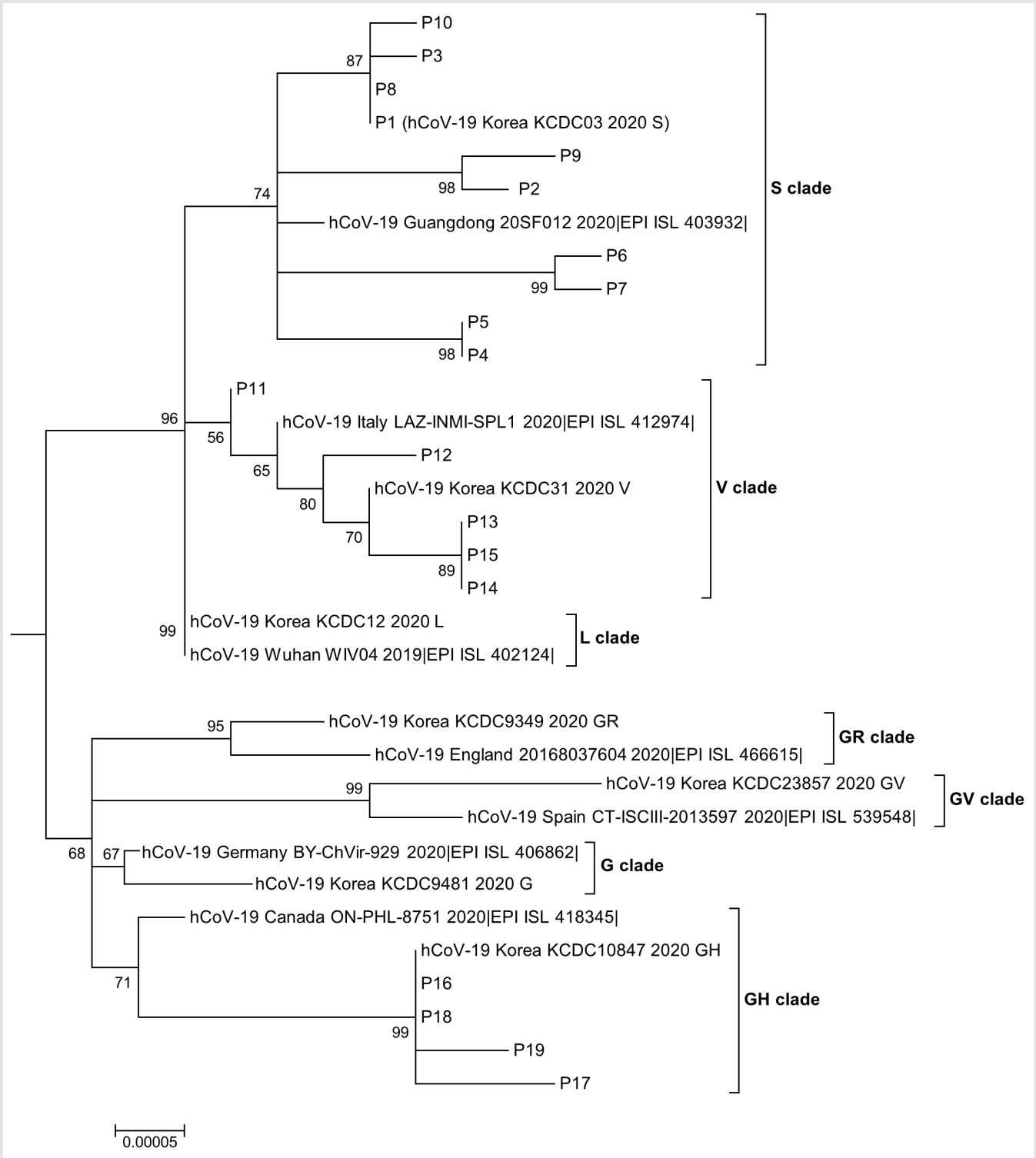

Figure 1: Phylogenetic analysis based on full nucleotide sequences of SARS-CoV-2 strains using the maximum likelihood (ML) method and Kimura 2-parameter model. Numbers on branches indicate bootstrap percentages based on 1,000 replications. The scale bar indicates nucleotide substitutions per site.

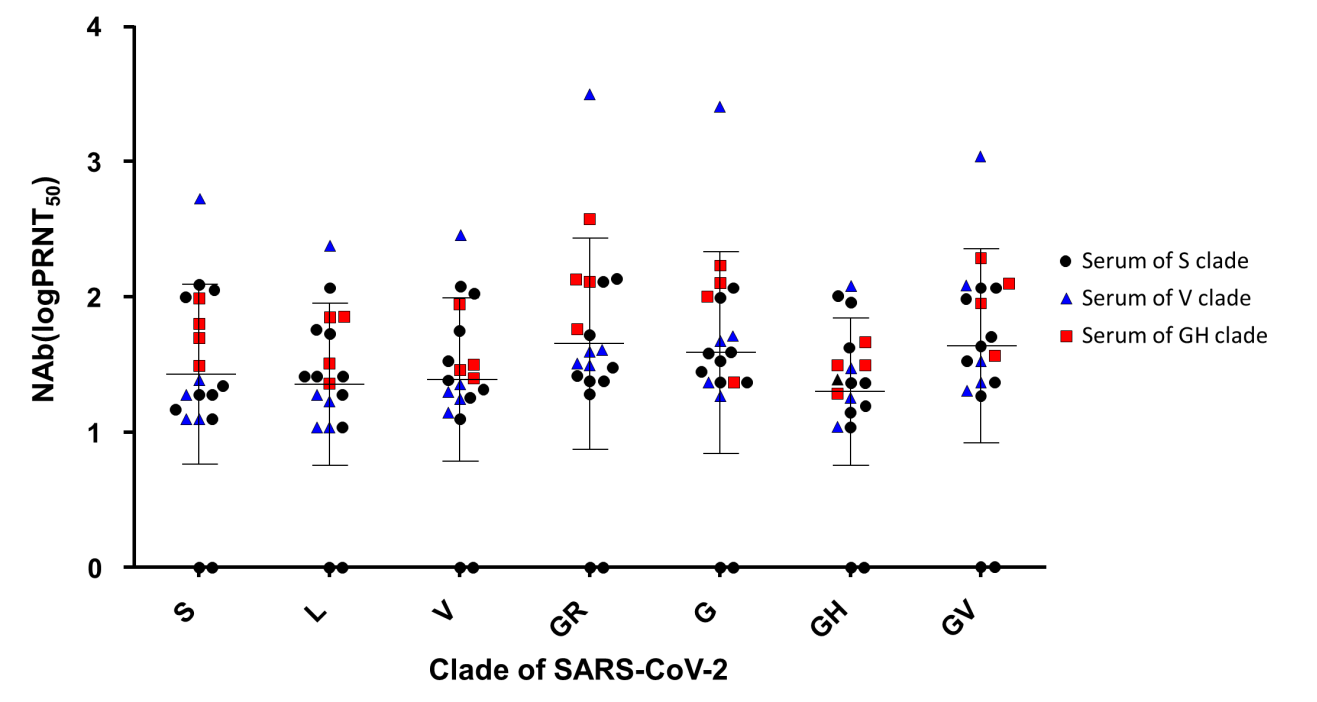

Figure 2: Scatter plot of neutralizing antibody for SARS-CoV-2 by clades. Two-sided Friedman test with Dunn's multiple comparison was performed between each of the viral strains. P > 0.05 was deemed statistically significant.

Author Contributions

HL,EL, SO, JK, NL, HJ, and SW performed the experiment. HK carried out the samples of patient. HL, JR, and EK wrote paper. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no competing interests.

Ethics Approval and Consent to Participate

The study was approved by the Institutional Review Board at the Korea Centers for Disease Control and Prevention (2020-03-01-P-A). Written informed consent was waived by the Institutional Review Board for COVID-19 cases.

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