COVID-19 Special Report

Cross-reactivity of neutralizing antibodies against SARS-CoV-2 patients by classification of clade

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Abstract

Variant viruses or mutant viruses are neither new nor unexpected. The nature of RNA viruses, such as the SARS CoV-2 coronavirus, is to evolve and change over time. In addition to the coronavirus disease-19 (COVID-19) virus first detected in China, the Republic of Korea has recorded multiple variants.

The aim of this study was to analyze the characteristics of the neutralization antibody cross-reaction of various COVID-19 variant viruses through an analysis of the neutralization antibody cross-reaction of the COVID-19 non-variant virus. Furthermore, by examining the neutralizing ability of variant viruses, this study aimed to analyze whether the neutralizing ability also acts on different viruses.

Seven clades (S, L, V, GR, G, GH, GV) were isolated. The clades were confirmed by analyzing the sera (i.e., upper respiratory tract tissue samples) of 19 confirmed COVID-19 patients. The sera were analyzed using Real-time RT-PCR and full-length genomic analysis, and the cross-reactivity of neutralizing antibodies was confirmed using the non-variant viruses of the seven clades.

A plaque reduction neutralization antibody test was performed using seven types of non-variant virus isolates. Among the sera samples, there were ten cases of S clade, five cases of V clade, and four cases of GH clade identified through a full-length genomic analysis. Results showed that neutralizing antibodies against the same clade virus in one case of V clade serum and one GH genotype serum exhibited more than four times the neutralizing ability against viruses of different clades (i.e., GR, G, and GV). Although differences in the neutralizing ability of viruses of the same or different clades were found, this study confirmed that a similar level of neutralizing antibody cross-reactivity was observed in all 19 sera.

In this study, we confirmed the possibility of protecting existing infected people from exposure to the risk of reinfection because of the existence of neutralizing antibody cross-reactivity against COVID-19. Nevertheless, we still cannot rule out the risk of reinfection completely. Further study needs to inform strategies for developing effective immunotherapies and universal vaccines against emerging variant COVID-19 viruses because antigenic cross-reactivity is a key scientific question that needs to be addressed.

Keywords: Coronavirus Disease-19 (COVID-19), Whole Genome Sequencing (WGS), Cross-reactions of neutralizing antibodies

Introduction

From 2019-2021, the coronavirus disease-19 (COVID-19) spread across the world. It started with an outbreak among unidentified pneumonia patients in Wuhan, Hubei Province, China, in December 2019. In the Republic of Korea, the first confirmed case was reported on January 20, 2020. Subsequently, a total of 180,481 COVID-19 infection cases were reported by July 20, 2021.

In the first stage of the COVID-19 pandemic, the World Health Organization's (WHO) global initiative on sharing all influenza data (GISAID) classified types of COVID-19 into S clade, V clade, and G clade. Following that, the clades of COVID-19 were expanded to S, V, G, GH, and GR. In November 2020, the G clade was subdivided into GV clade [1], and this classification has been used until now (Table 1). In the Republic of Korea, the S and V clades were identified until March 2020 after the COVID-19 outbreak, the GH clade was detected in early April 2020, and then the G and GR clades were also detected. In addition, the detection of the GV clade was confirmed in October 2020. The S clade includes inflow from foreign countries in the pandemic's early stages, Wuhan residents, Guro call centers, and overseas immigrants, and the V clade includes Daegu Sincheonji Church and the Daenam Hospital in Cheongdo. The G clade was first detected among incoming travelers to the Republic of Korea, and the GR clade was identified among the Russian ship crew and arrivals at Gamcheon Port in Busan [2]. The GV clade was first confirmed in Deji High School/Jukjeon High School in Yongin-si, Gyeonggi Province. The world is facing another pandemic due to the mutated virus.

After the alpha variant virus infection was reported on September 20, 2020 [3], various variant viruses from South Africa and Brazil have been spreading to many countries, and the induction of variant viruses from international arrivals has also been observed [4]. Beta and gamma variant viruses derived from South Africa and Brazil have been reported to have a high level of transmission power and the possibility of antibody avoidance [5-8]. It is necessary to continuously pay attention to cases of reinfection.

Results

1. General characteristic of samples

The serum used to analyze the neutralizing ability was obtained from 19 patients whose upper respiratory tract and

Table 1. Classification of clades for SARS-CoV-2 (WHO)

Clade		Target genome for classification of clade	Target amino acid for classification of clade
S clade		ORF8	L84S
V clade		NS3	G251V
G group	G clade	S	D614G
	GH clade	S	D614G
		NS3	Q57H
	GR clade	S	D614G
		N	G204R
	GV clade	S	D614G, A222V
L clade		Genetic correlation with WIV04 strain (Isolate of Wuhan)	

serum samples were obtained simultaneously. Full-length genomic analysis was conducted on the upper respiratory tract samples to confirm the infected sample's clade. Ten people were infected with the S clade, five people with the V clade, and four people with the GH clade, which protects the infected clade in the patient's serum. The antibodies formed by infection with a specific clade are used for the neutralizing ability test, which analyzes the level of protective ability to other clades. There were seven clade types: S, L, V, GR, G, GH, and GV.

2. Pre-experiment

In this study, the plaque reduction neutralization test (PRNT) was used to characterize and quantify the level of neutralizing antibodies. In the PRNT method, serum and virus were reacted, and then the mixture was injected into the cell. Cell cytopathic effect of the cell was measured, and if the cytopathic effect level was high, it was considered as a high level of neutralizing ability. To establish experimental conditions for neutralization analysis, the incubation time that causes cytopathic cell effects after cell infection of seven types of non-variant virus isolates was measured.

Briefly, the same dose of seven types of non-variant virus isolates were reacted with patient serums and then incubated in infected cells. The virus's five clades (S, L, GR, G, GV) confirmed the cytopathic cell effect within 44 to 48 hours after cell infection, but the virus's two clades (V, GH) confirmed the cytopathic cell effect within up to 56 hours. Pre-experiment, each type of coronavirus showed the time difference to induce cytopathic cell effect, and the experiment was conducted by applying optimized cell infection and culture time for accurate neutralization analysis for each type of virus.

3. Results of neutralization analysis

To confirm that patients infected with the existing COVID-19 virus may exhibit neutralizing ability against viruses of other clades, 19 serum cases of patients with identified S, V, and GH clade for seven types of viruses were used for neutralization analysis (Figure 1). The neutralized antibody levels against seven viruses in the two S clade serums were similar and less than 1:10. As a result, the two S clade serums did not contain any neutralizing antibodies against other clades, including the S clade. It indicated that neutralizing antibodies after COVID-19

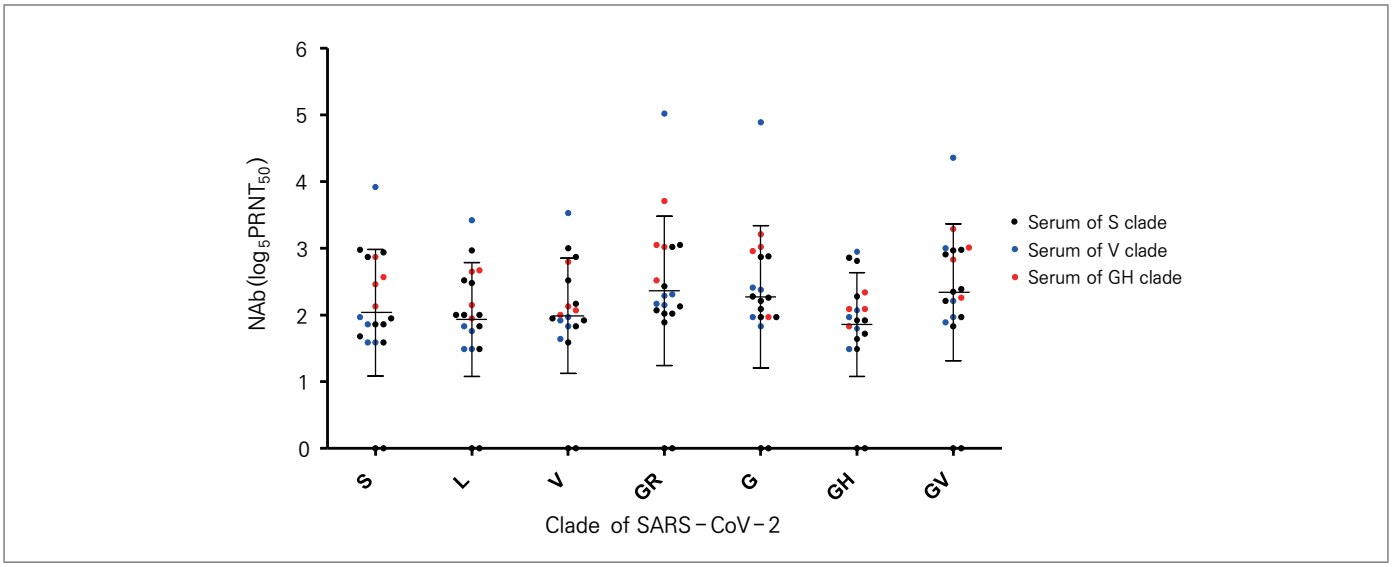

Figure 1. Scatter plot of neutralizing antibody for SARS-CoV-2 by clade

infection may have been maintained for a short period or not generated initially. Therefore, further analysis such as T-cell immune response will be required to analyze immunological characteristics caused by COVID-19 infection. The rest of the 17 serums cases, including 8 cases of S clade serum, 5 cases of V clade serum, and 4 cases of GH clade serum, showed similar levels of neutralizing antibodies against seven viruses. Based on these results, if the patient is exposed to another COVID-19 clade except initial infection clade, the risk of reinfection may be low. In addition, neutralizing antibodies against the same clade virus in one case of V clade serum and one GH genotype serum exhibited more than four times the neutralizing ability against viruses of different clades (i.e., GR, G, and GV). Further study is needed to investigate why GR, G, and GV clade exhibit high neutralization, whether differences in amino acids are the basis for distinguishing genotypes or differences in other amino acids. Although there are limitations in that this study could not include L, GR, G, and GV clade serums, the possibility of protecting existing infected people from exposure to the risk of reinfection because of the existence of neutralizing antibody cross-reactivity against COVID-19 was confirmed.

Conclusion

This study examined the neutralization capacity of seven viruses (S, L, V, GR, G, GH, and GH) in serums (S, V, and GH clade) from patients infected with COVID-19. Nineteen patient serum samples (10 cases of S clade, 5 cases of V clade, 4 cases of GH clade) demonstrated similar or high neutralizing ability, and it was confirmed the possibility of protecting existing infected people from exposure to the risk of reinfection because of the existence of neutralizing antibody cross-reactivity against COVID-19. A high level of neutralization was observed in the G family group (GR, G, and GV) to V clade and GH clade infected serums, and further study is needed to explore the underlying mechanism.

This study is important in that it examined the possibility of reinfection in existing infections with various clades, although patient serums infected with the rest of the clades have not been added, and the target sample was small.

Several studies have reported the possibility of antibody avoidance of mutating viruses, along with the outbreak of mutating COVID-19 virus in the Republic of Korea and the introduction of new mutate viruses into the country. Therefore, further study is needed to analyze various immunological characteristics, including cross-reactivity of neutralization in existing virus-infected or mutant virus-infected patients and vaccinated persons, against the mutant virus.

① What was known?

Characteristic analysis of mutant viruses worldwide suggests the possibility of reducing virus neutralization or immune avoidance due to mutations in specific proteins.

② What is newly learned?

Cross-reaction analysis of neutralizing antibodies between viruses confirmed that the neutralizing antibodies were high in G-type clade for some group serums and generally showed neutralization.

③ What are the implications?

Further research is needed to analyze immunological characteristics after COVID-19 infection, including studies of neutralizing antibody cross-reactions following non-variant and mutant virus cross-reactions for vaccinators.

Acknowledgment

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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References

1. GISAID (Global Initiative on Sharing All Influenza), https://www.gisaid.org
2. KDCA (Korea Disease Control and Prevention Agency), coronavirus disease 19 (COVID-19) state in the Republic of Korea (July 6, 2021, Regular briefing)
3. WHO (World Health Organization). COVID-19 Weekly Epidemiological Update, 2021.3.14.
4. Kim Ii-Hwan, Park Ae Kyung, et al., COVID-19 Variant surveillance study in the Republic of Korea. Public Health Weekly Report. 2021;14(13):724-733.
5. Emanuele A, Giulia P, Danilo L, et al. SARS-CoV-2 escape in vitro from a highly neutralizing COVID-19 convalescent plasma. bioRxiv. 2020.12.28. doi:https://doi.org/10/1101/2020.12.28.424451.
6. Gard N, Oleksandr B, Patricia S, et al. Molecular dynamic simulation reveals E484K mutation enhances spike RBD-ACE2 affinity and the combination of E484K, K417N, and N501Y mutations (501Y.V2 variant) induces conformational change greater than N501Y mutant alone, potentially resulting in a escape mutant. bioRxiv. 2021.01.13. doi:https://doi.org/10/1101/2021.01.13.426558.
7. Constantinos W, Frances A, Tandile H, et al. SARS-CoV-2 501Y.V2 escapes neutralization by South African COVID-19 donor plasma. nature medicine. 2021.3.2. doi:https://doi.org/10.1038/s41591-021-01285-x
8. Sandile C, Inbal G, Laurelle J, et al. Escape of SARS-CoV-2 501Y.V2 from neutralization by convalescent plasma. 2021.3.29. doi:https://doi.org/10.1038/s41586-021-03471-w

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