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Persistence of the neutralizing antibody response after SARS-CoV-2 infection

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Abstract

Objective: Neutralizing antibodies are among the factors used to measure an individual’s immune status for the control of infectious diseases. We aimed to confirm the persistence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) neutralizing antibody levels in patients who had recovered from coronavirus disease 2019 (COVID-19).

Methods: Plasma donors in South Korea who had completely recovered from SARS-CoV-2 infection had follow-up testing to determine the persistence of neutralizing antibodies using a plaque-reduction neutralization test and ELISA.

Results: Of the 111 participants aged 20–29 years, 37/111 (33.3%); 30–39 years, 17/111 (15.3%); 40–49 years, 23/111 (20.7%); 50–59 years, 21/111 (18.9%); 60–65 years, 13/111 (11.7%); male, 43/111 (38.7%); female, 68/111 (61.3%) still had neutralizing antibodies approximately 9 months (range 255–302 days) after confirmation of the diagnosis.

Conclusions: In this study we analysed the titre of neutralizing antibodies associated with predicting immune status in individuals with natural infection. Information about the persistence and change in levels of neutralizing antibodies against SARS-CoV-2 can be utilized to provide evidence for developing vaccination schedules for individuals with previous infection. Sang-Mu Shim, Clin Microbiol Infect 2022;28:614.e1–614.e4 © 2022 The Authors. Published by Elsevier Ltd on behalf of European Society of Clinical Microbiology and Infectious Diseases. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

It is important to confirm the change in antibody levels and the persistence of neutralizing antibodies in individuals who recover from natural infections in order to determine their infection status, predict prevention of reinfection, and establish vaccination policies in the context of a pandemic [1,2]. In this study we aimed to confirm the development and maintenance of neutralizing antibodies in South Korean patients who had had coronavirus disease 2019 (COVID-19) during the early phase of the pandemic and had recovered completely.

Methods

Blood collection

Blood samples were collected from healthy individuals who had fully recovered from COVID-19 approximately 3 months (140 days), 6 months (181 days), and 9 months (271 days) after the confirmation of COVID-19 in February or March 2020. The participants were aged ≥19 years, lived in South Korea, and had agreed to become plasma donors. The participants were recruited through the plasma donation recruitment notice and consented for their plasma specimens to be used for research.
Plaque-reduction neutralization tests (PRNT) and enzyme-linked immunosorbent assay (ELISA)

PRNTs were performed as previously described [3–5] using severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (clade S; hCoV-19/South Korea/KCDC03/2020, EPI_ISL_407,193) obtained from the National Culture Collection for Pathogens in South Korea. PRNT titres ≥1:20 were considered positive for SARS-CoV-2 neutralizing antibodies [6]. Neutralizing antibodies (nAbs) were also tested using the SARS-CoV-2 Surrogate Virus Neutralization Test (sVNT) Kit (GeneScript), and total antibodies (IgG, IgM and IgA) were measured using the STANDARD E COVID-19 Total Ab Kit (SD Corporation). The nAb ELISA used a competitive ELISA detection method involving protein–protein interaction between human angiotensin-converting enzyme 2 (ACE2) receptors attached to the surface of the plate in competition with a SARS-CoV-2 receptor binding domain fragment conjugated with horse-radish peroxidase and neutralizing antibodies in plasma samples. The recombinant COVID-19 antigens, containing nucleocapsids and Spike-1:20 within 8 months following SARS-CoV-2 infection [9,10]. Many studies have also shown nAb measured using ELISA (which may replace the PRNT method even though it has lower sensitivity than the PRNT method). In this study, 63.4% of the blood donors had sustained nAb responses 9 months after infection detected using ELISA. The difference in the sensitivity of the ELISA and PRNT methods for detecting SARS-CoV-2 nAb may be attributable to differences in the detection system. Generally, nAb ELISA for SARS-CoV-2 targets the protein of human ACE2 receptors for binding neutralizing antibodies in plasma, whereas PRNT uses living cells with ACE receptors and other factors. Furthermore, recent studies have reported that coreceptors or cofactors may influence SARS-CoV-2 infection [11–13]. These factors may also have contributed to the differences in the results of nAb testing between PRNT and nAb ELISA.

It may be useful to evaluate the duration of neutralizing antibodies using nAb ELISA using a large number of samples during the pandemic period. However, comparison of nAb titres, positivity rates, and changes in titres over time after infection measured using the two methods must be interpreted carefully, considering the differences between the PRNT and nAb ELISA methods and their limitations.

Currently, COVID-19 vaccines are being rolled out in stages in many countries, including South Korea. The analysis of neutralizing antibodies against SARS-CoV-2 in this study could inform public health policies against COVID-19, including the need for COVID-19 vaccination in individuals with a history of COVID-19 due to natural SARS-CoV-2 infection.

This study has some limitations. First, the information on the clinical features of COVID-19 (including disease severity) in the participants could not be obtained from the medical institutions in which the blood was collected. Second, the main purpose of this study was to compare the results of antibodies measured using the PRNT and ELISA methods. However, a reference standard for serological methods of antibody measurement could not be defined between the two analysis methods. For more detailed information, long-term follow-up studies should be conducted to confirm the duration of nAb levels. Moreover, further research should be conducted to determine the mechanisms for the different patterns of development and maintenance of nAb levels.
Fig. 1. Neutralizing antibody responses against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). (A) Levels of neutralization antibodies in all participants, (B) antibody levels by gender, and (C) antibody levels by response pattern. (D) Correlation between the neutralizing antibody enzyme-linked immunosorbent assay (nAb ELISA) and plaque-reduction neutralization test (PRNT) results. *p < 0.05, statistically significant difference; ns, not significant.
Neutralizing antibody response (plaque-reduction neutralization test (PRNT50) and ELISA) in patients with coronavirus disease 2019 (COVID-19) according to time from diagnosis

| 140 d | 187 d | 271 d |
|-------|-------|-------|
| 40    | 16    | 12    | 9    |
| 80    | 20    | 16    | 15   |
| 160   | 26    | 24    | 18   |
| 320   | 32    | 28    | 22   |
| 640   | 48    | 40    | 34   |
| 1280  | 64    | 56    | 46   |
| Negative (<20) | 64% | 56% | 46% |
| Positive | 36% | 44% | 54% |

PRNT titre

| 140 d | 187 d | 271 d |
|-------|-------|-------|
| nAb on ELISA |
| Negative | 10 | 16 | 19 |
| Positive  | 33 | 49 | 47 |

ELISA, enzyme-linked immunosorbent assay; nAb, neutralizing antibody.

Table 1

Author contributions

SMS, JWK, JSY, KCK and JYL were involved in the design of this study. JWK, SJ, YJ and HMW performed the experiments. SMS, WJK, JSY and KCK assembled the data. SMS, JWK, JSY, KCK and JYL were involved in writing and all authors approved the manuscript.

Transparency declaration

The authors declare that they have no conflict of interest regarding the publication of this research note. This study was supported by intramural funds (2019-NI-039-00) from the Korea National Institute of Health.

Acknowledgements

The authors would like to thank the blood donors and Dae Seong Kim (Korean Red Cross) for organizing the collection of blood samples.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2021.12.012.

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