Open Forum Infectious Diseases

MAJOR ARTICLE

Persistence of Robust Humoral Immune Response in Coronavirus Disease 2019 Convalescent Individuals Over 12 Months After Infection

Kei Miyakawa,[1,a] Sousuke Kubo,[1,2,a] Sundararaj Stanleyraj Jeremiah,[1,a] Hirofumi Go,[3] Yutaro Yamaoka,[1,4] Norihisa Ohtake,[5,6] Hideaki Kato,[7] Satoshi Ikeda,[8] Takahiro Mihara,[9] Ikuro Matsuba,[10] Naoko Sanno,[11] Masaaki Miyakawa,[12,13] Masaharu Shinkai,[14] Tomoyuki Miyazaki,[15] Takashi Ogura,[8] Shuichi Ito,[16] Takeshi Kaneko,[2] Kouji Yamamoto,[3] Atsushi Goto,[9] and Akihide Ryo[1]

[1]Department of Microbiology, Yokohama City University Graduate School of Medicine, Kanagawa, Japan, [2]Department of Pulmonology, Yokohama City University Graduate School of Medicine, Kanagawa, Japan, [3]Department of Biostatistics, Yokohama City University Graduate School of Medicine, Kanagawa, Japan, [4]Life Science Laboratory, Technology and Development Division, Kanto Chemical Co, Inc., Kanagawa, Japan, [5]Advanced Medical Research Center, Yokohama City University, Yokohama, Japan, [6]Bioscience Division, Research and Development Department, Tosoh Corporation, Tokyo Research Center, Kanagawa, Japan, [7]Infection Prevention and Control Department, Yokohama City University Hospital, Kanagawa, Japan, [8]Department of Respiratory Medicine, Kanagawa Cardiovascular and Respiratory Center, Kanagawa, Japan, [9]Department of Health Data Science, Yokohama City University Graduate School of Data Science, Kanagawa, Japan, [10]Matsuba Medical Clinic, Kanagawa, Japan, [11]Shinagawa Strings Clinic, Tokyo, Japan, [12]Miyakawa Internal Medicine and Pediatrics Clinic, Kanagawa, Japan, [13]Japan Medical Association, Tokyo, Japan, [14]Division of Internal Medicine, Tokyo-Shinagawa Hospital, Tokyo, Japan, [15]Department of Physiology, Yokohama City University Graduate School of Medicine, Kanagawa, Japan, [16]Department of Pediatrics, Yokohama City University Graduate School of Medicine, Kanagawa, Japan

Background. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection elicits varying degrees of protective immunity conferred by neutralizing antibodies (nAbs). In this study, we report the persistence of nAb responses over 12 months after infection despite their decreasing trend noticed from 6 months.

Methods. The study included sera from 497 individuals who had been infected with SARS-CoV-2 between January and August 2020. Samples were collected at 6 and 12 months after onset. The titers of immunoglobulin (Ig)G to the viral nucleocapsid protein (NP) and receptor-binding domain (RBD) of the spike protein were measured by chemiluminescence enzyme immunoassay. The nAb titer was determined using lentivirus-based pseudovirus or authentic virus.

Results. Antibody titers of NP-IgG, RBD-IgG, and nAbs were higher in severe and moderate cases than in mild cases at 12 months after onset. Although the nAb levels were likely to confer adequate protection against wild-type viral infection, the neutralization activity to recently circulating variants in some of the mild cases (~30%) was undermined, implying the susceptibility to reinfection with the variants of concerns (VOCs).

Conclusions. Coronavirus disease 2019 convalescent individuals have robust humoral immunity even at 12 months after infection albeit that the medical history and background of patients could affect the function and dynamics of antibody response to the VOCs.

Keywords. humoral immunity; neutralizing antibodies; SARS-CoV-2.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of the coronavirus disease 2019 (COVID-19) pandemic, has infected approximately 200 million people worldwide, causing over 4 million deaths, as of the end of July 2021 (https://coronavirus.jhu.edu/). Vaccines are the only limiting modality [1]. Because of the lack of equitable distribution and rapid mass vaccination, the virus is spreading almost unchecked and has led to the emergence of variant mutants [2–4]. Some of the variants pose significant epidemiological problems, and the World Health Organization has defined these strains as variants of concern (VOCs) or variants of interest (VOIs). Because both naturally acquired postinfection immunity and artificially acquired vaccine-mediated immunity produce neutralizing antibodies (nAbs), which are helpful in limiting the future course of the pandemic, it is important to know the long-term persistence and assess the neutralizing activity of infection and vaccine-derived immunity against VOCs and VOIs. Several studies have investigated the longevity of postinfection nAb titers, but only a few long-term follow-up studies have been conducted, especially against the VOCs and VOIs, 1 year after infection [5–7].

In this study, we collected blood samples from 497 patients diagnosed with COVID-19 in Japan at 6 and 12 months after the onset of the disease and performed a comprehensive analysis

Received 20 October 2021; editorial decision 2 December 2021; accepted 9 December 2021; published online 10 December 2021.

[a]K. M., S. K., and S. S. J. contributed equally to this work.

Correspondence: Akihide Ryo, MD, PhD, Department of Microbiology, Yokohama City University Graduate School of Medicine, 3-9 Fuku-ura, Kanazawa, Yokohama 236-0004, Japan (aryo@yokohama-cu.ac.jp).

Open Forum Infectious Diseases®2022

© The Author(s) 2021. Published by Oxford University Press on behalf of Infectious Diseases Society of America. This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (https://creativecommons.org/licenses/by-nc-nd/4.0/), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

https://doi.org/10.1093/ofid/ofab626

of humoral immunity. We examined levels of immunoreactive antibodies targeting the spike protein (SP)-receptor-binding domain (RBD) and nucleocapsid protein (NP), as well as nAbs against multiple VOCs and VOIs. We also investigated host factors that influence the persistence of antibody response.

METHODS

Patient Consent Statement

All participants provided written informed consent and the study was approved by the Institutional Review Board of Yokohama City University (Reference No. B200700023).

Data Sharing

The datasets presented in this article are not readily available because it is difficult to ensure deidentification of data. However, they can be available from the corresponding author on reasonable request.

Experimental Design

Patients with COVID-19 were recruited in the manner described earlier [8]. In brief, we used our institutional website (not operational at present), social network services, and general mass media to recruit recovering patients. Eligible participants were aged ≥20 years at study entry time, resided in Japan, and had a positive result in either reverse- transcription polymerase chain reaction (RT-PCR), reverse-transcription loop-mediated isothermal amplification (RT-LAMP), or antigen tests for SARS-CoV-2. For all participants, physicians at cooperating outpatient clinics confirmed the diagnosis of COVID-19 based on information provided by their hospitals, clinics, or public health centers. The inclusion and exclusion criteria are available at the University Hospital Medical Information Network-Clinical Trials Registry (UMIN-CTR), where this study was registered (Number UMIN000041227; UMIN Clinical Trials Registry, 2021). The primary endpoints of this study were neutralizing titer (NT_{50}) and titers of antibodies against NP and SP antigens at 20–32 weeks (visit 1) and 46–58 weeks (visit 2) after the first positive SARS-CoV-2 test results. In this report, participants who provided their serum samples from January 25 to July 17, 2021 at visit 2 were included. Between September 2020 and February 2021, among 562 volunteers participating in the study, blood samples were obtained from 498 patients at 6 and 12 months, and 497 patients were included in the study, except for 1 patient who was missing a key variable. There was 1 patient whose blood was drawn 3 days earlier than the lower limit of visit 2, but this was not treated as missing and was included in the analysis as a measurement of visit 2. Demographic data of the cohort are shown in Supplementary Table S1. Severity is defined based on the following 3 levels: (1) no oxygen administered (mild); (2) oxygen administered by mask or nasal prong (moderate); (3) the requirement for mechanical ventilation or extracorporeal membrane oxygenation (severe).

Serological Testing for Severe Acute Respiratory Syndrome Coronavirus 2 Nucleocapsid Protein-Immunoglobulin (Ig)G and Receptor-Binding Domain-IgG

The amounts of SARS-CoV-2 NP-immunoglobulin (Ig)G and RBD-IgG in sera were quantified using the automated immunoassay AIA-CL1200 (Tosoh). In this study, the threshold index was set to 1 based on previous validations [9], which has been shown to have high sensitivity and specificity. To detect IgG against variant RBD, the antigen with a single mutation (N501Y) or triple mutations (N501Y, E484K, K417N/T) was expressed in Expi293F cells (Thermo Fisher Scientific), according to the manufacturer's instructions. The supernatants, including recombinant proteins, were harvested and purified using a Strep-tag purification system (IBA Lifesciences). The conjugation of RBD proteins to micromagnetic beads and their attachment to the AIA-CL instrument have been described previously [9].

Neutralizing Assays

Neutralizing assay using pseudovirus and rapid qualitative neutralizing assay were performed as previously described [8, 10, 11]. Authentic SARS-CoV-2 was obtained from the National Institute of Infectious Diseases in Japan and handled at the biosafety level 3 facility. Details of the strains used in this study are listed in Supplementary Table S2. For the neutralizing assay, VeroE6/TMPRSS2 cells seeded in 96-well plates were washed and infected with 100 µL of medium containing authentic SARS-CoV-2 (multiplicity of infection = 0.05) and 5-fold serially diluted serum (1:50–1:31 250 dilution). Three days after infection, cells were washed and 40 µL CellTiter-Glo Substrate (Promega) was added. Cell viability was measured using the GloMax Discover System (Promega). The calculation method for NT_{50} was described above.

Statistical Analysis

All continuous variables were expressed as median. We conducted a multiple regression analysis to examine the association between the amount of NT_{50} at 12 months after onset and host factors. The dependent variable was the amount of NT_{50}, which was natural logarithm-transformed (log) to follow a normal distribution. In logarithmic transformation, if the NT_{50} was less than 50 and considered negative (=0), the transformation was performed as log(X + 1). The independent variables were age, sex, body mass index (BMI), smoking status, and severity of illness during the disease. All reported *P* values are 2-tailed and were considered to indicate statistical significance at $P < .05$. All statistical analyses were performed using Prism v8.3.1 (GraphPad) and R version 4.0.3 (R, Foundation for Statistical Computing). Other statistical tests for each analysis are described in each legend.

RESULTS

Study Cohort

The study included sera from 497 volunteers who had been infected with SARS-CoV-2 between January and August 2020,

confirmed either by RT-PCR, RT-LAMP, or antigen testing. The median age of the participants was 49.0 ± 13.2 years with a male to female ratio of 50.7% to 49.3%. Demographic data and acute-phase symptoms of the cohort are shown in Supplementary Table S1. The participants were assigned as asymptomatic/mild (79%; 391 of 497), moderate (16%; 80 of 497), or severe (5%; 26 of 497) (Figure 1A).

Severity of Coronavirus Disease 2019 and Persistence of Severe Acute Respiratory Syndrome Coronavirus 2 Antibody

The presence of serum IgG against the viral NP or RBD of the viral SP was quantitatively examined using the Tosoh AIA-CL chemiluminescence enzyme immunoassay (CLEIA) [9]. Median NP-IgG titer was 2.9 at 6 months and 1.1 at 12 months (Figure 1B), and that of RBD-IgG was 13.0 at 6 months and 9.4 at 12 months (Figure 1C). Both titers decreased significantly between 6 and 12 months, but the rate of decrease was higher for NP-IgG than for RBD-IgG. All participants who had an increase in NP-IgG levels at 12 months exhibited only a slight increase of less than twice the value at 6 months, suggesting that no participant got reinfected during this period.

Next, we examined the NT_{50} values of these sera using the well established human immunodeficiency virus-derived pseudovirus neutralization assay to assess the presence of nAbs [12, 13]. We observed that the nAbs were maintained after 12 months from their levels at 6 months (Figure 1D), which was similar to the trend observed for RBD-IgG (Figure 1C). The NT_{50} values showed a definite correlation with RBD-IgG titers, but not with NP-IgG titers (Figure 1E and F). When antibody titers of NP-IgG, RBD-IgG, and nAbs were classified according to symptoms, all of them were higher in severe and

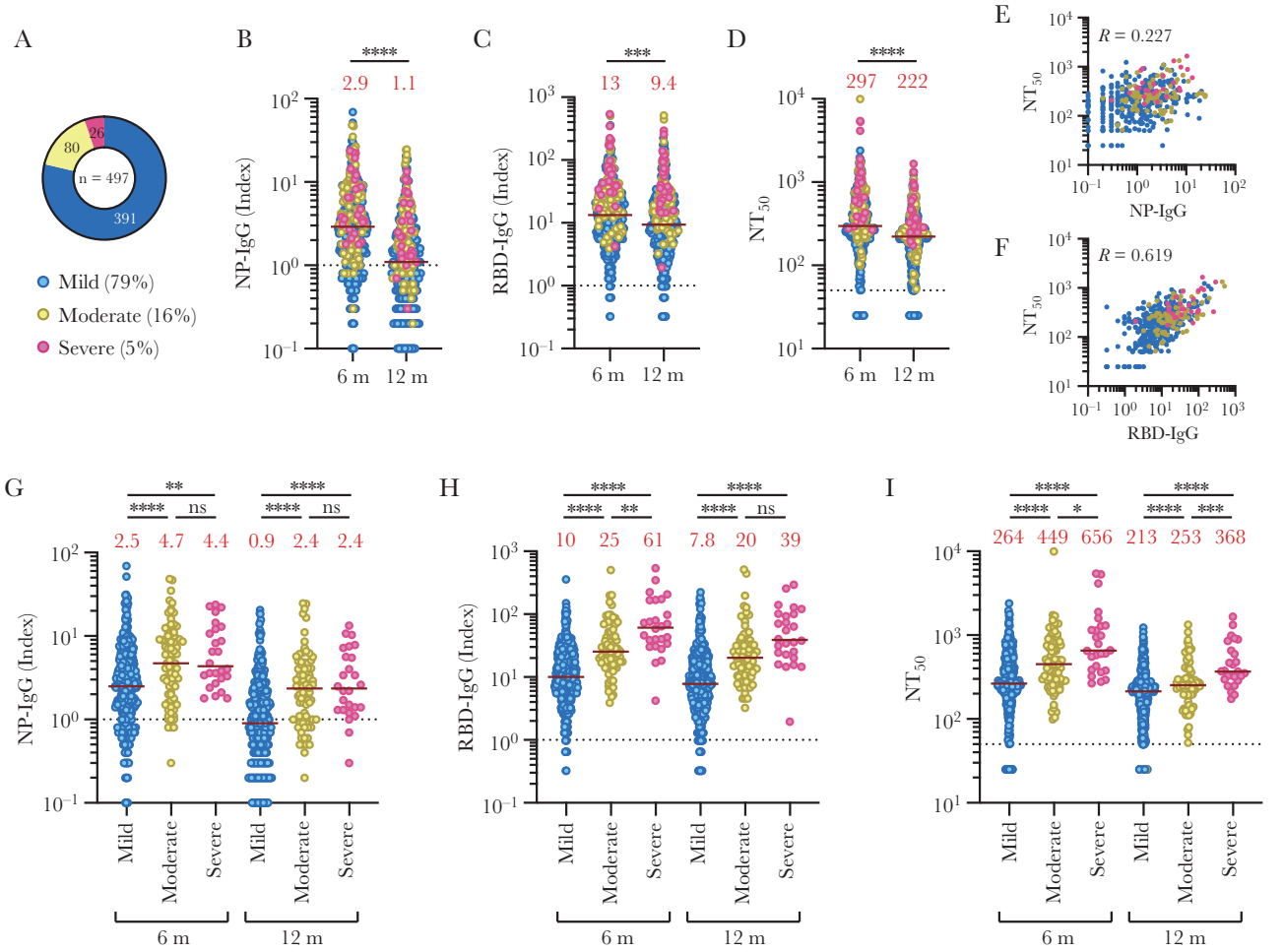

Figure 1. Severity of coronavirus disease 2019 (COVID-19) and persistence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibody. (A) Severity of acute phase in the study participants (total n = 497). (B–D) Amounts of SARS-CoV-2 nucleocapsid protein (NP)-immunoglobulin (Ig)G (B), receptor-binding domain (RBD)-IgG (C), and neutralizing activity (NT_{50}) (D) in COVID-19 convalescent sera at 6 and 12 months (m) after onset (total n = 497). Dotted lines indicate threshold values for each antibody. Blue, yellow, and pink indicate mild, moderate, and severe patients, respectively. Red letters on the graph indicate the median value of each antibody titer. ***, $P < .001$; ****, $P < .0001$; 2-tailed paired *t* test. (E and F) Correlation between NT_{50} and NP-IgG (E) or RBD-IgG (F) in COVID-19 convalescent sera at 12 months after onset. (G–I) Relationship between COVID-19 severity and the amounts of NP-IgG (G), RBD-IgG (H), or NT_{50} (I) in COVID-19 convalescent sera (total n = 497). *, $P < .05$; **, $P < .01$; ***, $P < .001$; ****, $P < .0001$; 2-tailed unpaired *t* test. ns, not significant.

moderate cases than in mild cases at both of the tested time periods (Figure 1G–I).

Host Factors Versus Persistence of Severe Acute Respiratory Syndrome Coronavirus 2 Antibodies

The study participants had a wide variation in age, ranging from the 20s to the 70s, and increasing rates of moderate and severe cases were observed starting from the 40s and above (Figure 2A). In the age-stratified cohort, the titers of NP-IgG, RBD-IgG, and NT_{50} after 12 months were higher in patients aged >50 years (Figure 2B–D). A decrease in nAbs over time was observed in all strata.

Coronavirus disease 2019 is known to be more severe in males and those with a higher BMI [14], and nAb antibody titers tended to be maintained in such individuals (Supplementary Figure S1A and B). Disease of increased severity occurred more commonly in smokers, but there were no significant differences in the 12-month nAb titers (Supplementary Figure S1B and C). The multiple regression analysis showed that the severity of the acute phase, BMI, and the age of onset were statistically

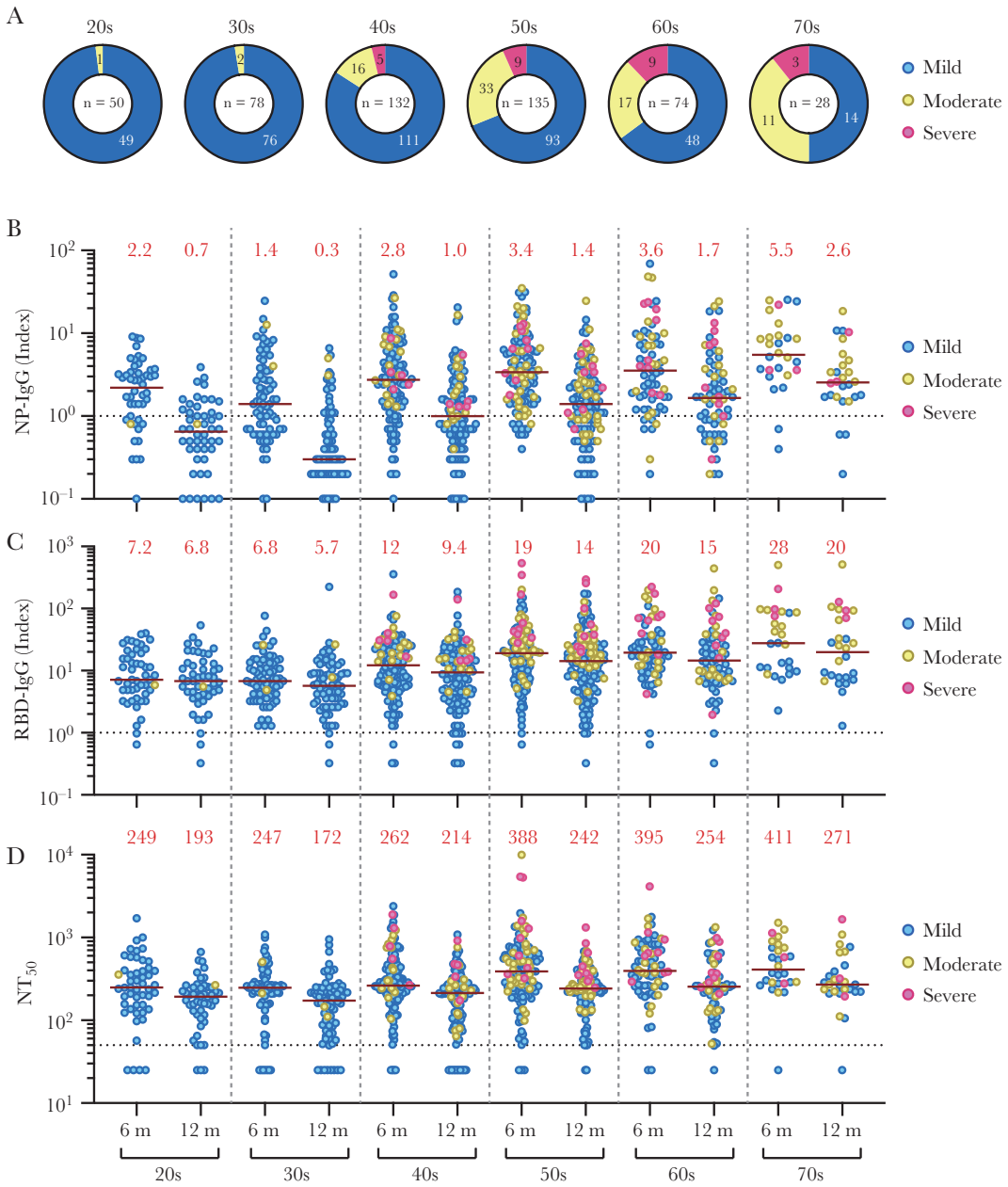

Figure 2. Onset age of coronavirus disease 2019 (COVID-19) and persistence of severe acute respiratory syndrome coronavirus 2 antibody. (A) Age and acute severity of the participants in this study (total n = 497). (B–D) Amounts of nucleocapsid protein (NP)-immunoglobulin (Ig)G (B), receptor-binding domain (RBD)-IgG (C), and neutralizing titer (NT_{50}) (D) by age groups in COVID-19 convalescent sera at 6 and 12 months (m) after onset. Blue, yellow, and pink indicate mild, moderate, and severe patients, respectively. Red letters on the graph indicate the median value of each antibody titer.

significantly associated with the amount of NT_{50} after 12 months (Supplementary Table S3).

Temporal Changes in Neutralizing Titer in Individual Subjects

Next, we classified the 497 subjects into 4 groups based on their nAb titer transition between 6 and 12 months, on the similar lines of Chia et al [15]: those whose nAb titers decreased by >50% (Group 1: Significant decrease), those whose titers decreased between 20% and 50% (Group 2: Gradual decrease), those whose titers remained between 20% increase or decrease (Group 3: Persistent), and those whose titers increased by >20% (Group 4: Slight increase) (Figure 3A). Of the 497 subjects, 38% (189 of 497) were in Group 1, 22% (111 of 497) were in Group 2, 23% (111 of 497) were in Group 3, and 17% (84 of 497) were in Group 4. A major proportion of the subjects (60%) showed a reduction in nAb titers (Groups 1 and 2) at 12 months (Figure 3B). Despite the reduction, effective neutralizing activity (NT_{50} > 50) was observed in most patients (468 of 497; 94%) after 12 months, with only a very few falling below the detection limit. When classified according to the symptoms presented during the acute period, most of the patients with severe disease belonged to Group 1, whereas those of mild cases were spread among all groups (Figure 3C). These results suggest that people presenting with severe disease form higher levels of nAbs, which also decrease at a faster rate. However, despite this decrease, the nAb titer may continue to be maintained at low functional levels.

Neutralizing Activity Against Severe Acute Respiratory Syndrome Coronavirus 2 Variants in Convalescent Sera

Next, we investigated whether nAbs of convalescents produced during past infections (possibly by the initial viral strains A or B.1) had the ability to neutralize the VOCs/VOIs of SARS-CoV-2, namely, alpha (B.1.1.7), beta (B.1.351), gamma (P.1), delta (B.1.617.2), and kappa (B.1.617.1) strains, which evolved later. These strains have multiple mutations throughout the length of the spike gene, of which the E484K/Q mutation is possessed by the beta, gamma, and kappa strains and causes immune evasion [16, 17]. The delta strain does not have an E484 mutation, but other mutations such as L452R and T478K accumulated in the RBD may confer this strain with the potential for immune evasion [18]. Using 20 randomly selected paired sera, we observed a significant decrease in neutralizing titer at 12 months against the beta, gamma, and kappa strains, which have an E484K/Q mutation, whereas the sera retained neutralizing efficacy for the alpha and the currently rampant delta strain (Figure 4A). It is notable that convalescents of severe cases had high neutralizing activity against all variants

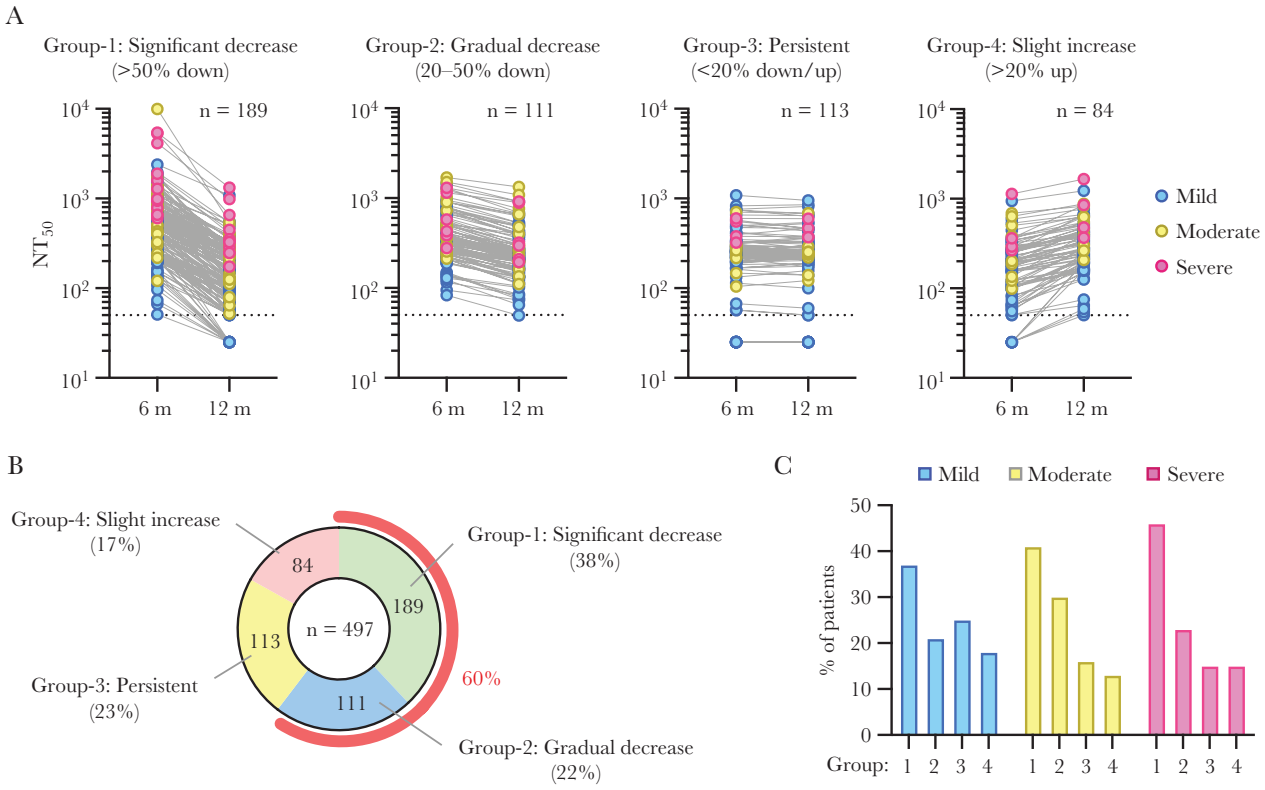

Figure 3. Temporal changes in neutralizing titer in the same patient. (A) Classification of 497 patients based on neutralizing activity (NT_{50}). Significantly decreased (Group 1), gradually decreased (Group 2), maintained (Group 3), and slightly increased (Group 4) between 6 and 12 months (m) of onset. Blue, yellow, and pink indicate mild, moderate, and severe patients, respectively. (B) Percentage of each group in all subjects. (C) Percentage of each group in indicated coronavirus disease 2019 severity.

compared to those of mild cases (Figure 4A). We also found that the RBD-IgG titer against the beta and gamma strains was also reduced compared to that against wild-type and alpha strains (Figure 4B), and NT_{50} correlated well with the RBD-IgG titer of each VOC (Supplementary Figure S2). These results together suggest that accumulated mutations favor the beta and gamma strains to escape humoral immunity by weakening the binding ability of nAbs. Although Moriyama et al [19] recently showed that the neutralization potency (NT_{50} divided by RBD-IgG) of the antibody increases over time as an indicator of the maturation of humoral immunity, we did not observe a robust increase in the neutralizing potency at 6 and 12 months (Supplementary Figure S3). We next examined the positivity rate of nAbs using the rapid qualitative neutralizing assay [10, 11], and we found that some convalescents of mild cases (~30%) lost the nAbs to the VOCs/VOIs with a greater proportion (Figure 4C). Furthermore, convalescents of moderate or severe cases showed a nAb positivity rate of more than 90% for all tested variants even 12 months postinfection (Figure 4C). These results indicate that VOCs/

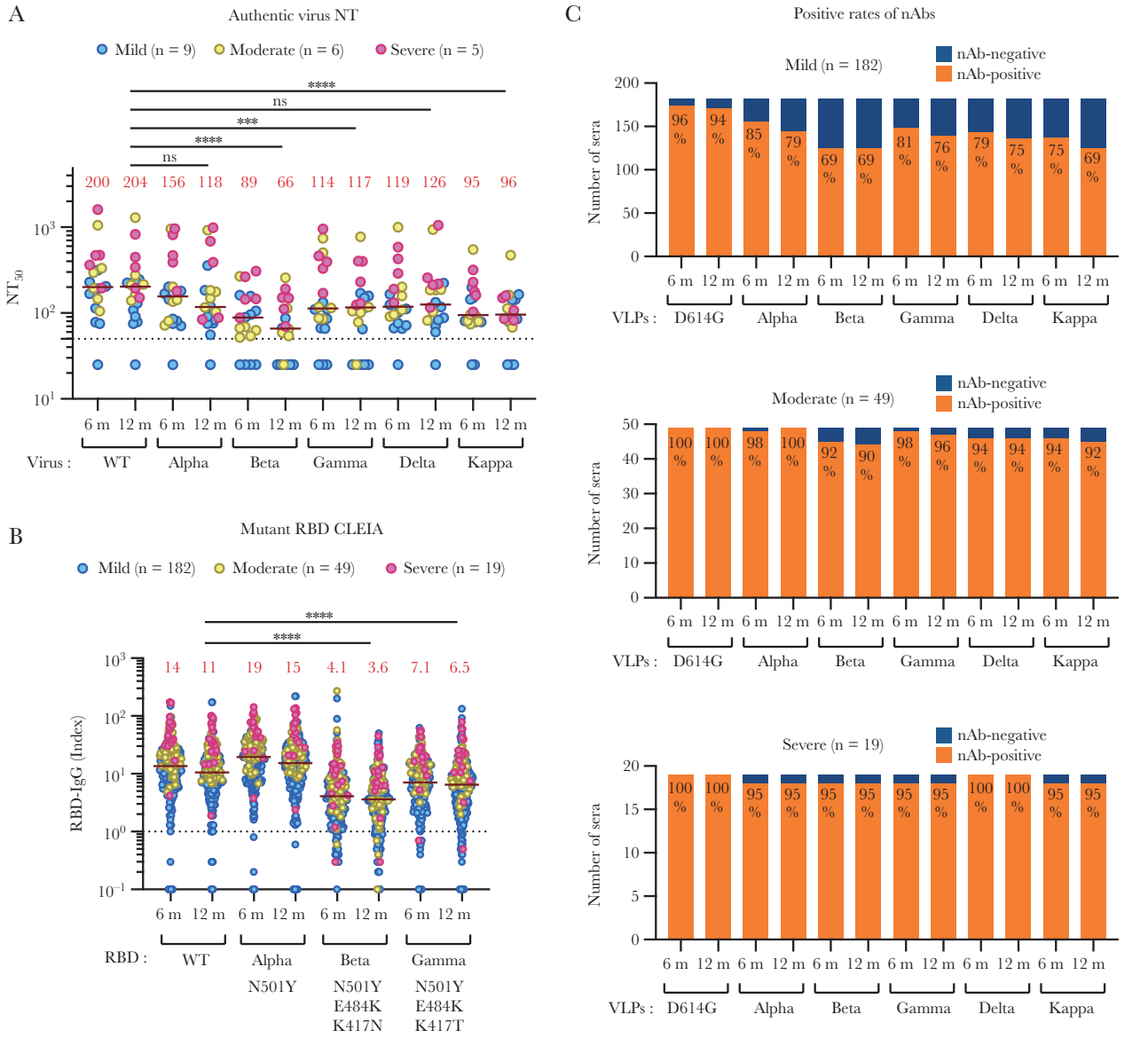

Figure 4. Neutralizing activity against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants in convalescent sera. (A) Neutralizing activity (NT_{50}) against wild-type (WT) SARS-CoV-2 and its variant strains (alpha, beta, gamma, delta, and kappa) in coronavirus disease 2019 convalescent sera at 6 and 12 months (m) after onset, as revealed by the authentic virus neutralizing test (total n = 20). Red letters on the graph indicate the median value of each antibody titer. ***, $P < .001$; ****, $P < .0001$; 2-tailed paired *t* test. (B) Amounts of immunoglobulin (Ig)G against mutated receptor-binding domain (RBD) antigen, as revealed by chemiluminescence enzyme immunoassay (CLEIA) (total n = 250). Red letters on the graph indicate the median value of each antibody titer. ****, $P < .0001$; ns, not significant, 2-tailed paired *t* test. (C) Positive rates of neutralizing antibodies (nAbs) against indicated variants, as revealed by qualitative neutralizing test (total n = 250, see Material and Methods). ns, not significant; VLPs, virus-like particles.

VOIs are particularly capable of evading humoral immunity acquired from earlier infection, but convalescent sera of moderate or severe cases have a higher ability to neutralize these strains than do those of mild cases.

DISCUSSION

In this study, we examined the persistence of serum nAbs and their effectiveness in neutralizing VOCs and VOIs at 12 months in 497 naturally infected individuals. Early reports have indicated that nAbs acquired upon infection could disappear within the following 3 months [6]; however, a series of recent reports indicate that they are maintained for longer durations [8, 20–23]. We reveal that nAbs persist at functionally effective titers at 1 year despite showing a declining trend in most study participants (60%). Our report supports a recent report that infection immunity is maintained for at least 1 year after natural infection [23], and it further suggests the possibility of nAbs persisting longer. In a cohort of 164 patients, Chia et al [15] demonstrated that more than 65% of subjects showed either a negative or a declining trend in nAbs at 6 months. We observed a similar trend, but at 12 months, which possibly indicates the better detection efficacy of the CLEIA used in our study than the methods used by Chia et al [15].

Several reports have suggested that overall antibody levels, including the nAb titer, correlate with the severity of infection [23, 24]. Severe patients have a much higher viral load, which may elicit a more robust humoral response than patients with mild or asymptomatic illness [25]. Consistent with these reports, we observed in our cohort that the individuals who recovered from severe disease possessed higher levels of all types of antibodies tested. These patients had high nAb titers and showed higher neutralizing capacity against VOCs, including the beta strain, notorious for immune escape, and the more rampant delta strain. These data may imply that those with severe disease, and thus higher antibody titers over time, might be more resistant to reinfection. In contrast, those who recovered from mild illness not only had lower nAb levels, but they also showed reduced neutralization against the VOCs. This may be because of nAbs having reduced binding activity against RBD of VOCs.

The duration and intensity of humoral immunity after SARS-CoV-2 infection have been shown to vary based on the severity of the clinical presentation [26]. Some host factors, such as sex, obesity, and smoking, have been reported to influence disease severity [14]. However, the effect of these host factors on the persistence of nAbs at 12 months after infection remains unknown. We reveal in this study that disease severity in the acute phase and the age of the patients correlate significantly with the magnitude of neutralization activity after 12 months. Muller et al [27] reported that older people have a weaker humoral response to vaccines characterized by lower titers of nAbs in the elderly cohort than in the younger cohort. In contrast, our findings suggest that in natural infection, the elderly possess more nAbs, probably because the elderly more often have serious diseases, which results in the production of more nAbs, which is not the scenario with a fixed dose of antigenic exposure in vaccination.

In this study, nAbs were detected in 94% of the total population, even after 12 months of infection. The majority showed a decreasing trend, whereas 40% showed a maintenance of neutralizing activity. Although the factors responsible for the maintenance of neutralizing activity remain unclear, recent reports suggest that B-cell clones expressing broad and potent nAbs are selectively retained in the repertoire over time [13, 28]. Moriyama et al [19] suggested that the neutralizing potency (NT_{50} divided by RBD-IgG) of the antibody increases over time. Although this seems to be an attractive indicator for the maturation of humoral immunity in convalescents, we did not observe a robust increase in the neutralizing potency between 6 and 12 months. This finding suggests that selection of B-cell clones expressing potent nAb may occur immediately after the acute phase, and selection between 6 and 12 months after onset may be rare [23]. Alternatively, the initial high-potency antibodies produced may continue to be produced, especially in those with severe disease. This study had limitations. We could only evaluate the persistence of humoral immunity and not that of cellular immunity. Therefore, it was not possible to discuss the risk of reinfection in previously infected patients. In addition, evaluation of antibody decline based on the severity of disease may be hindered by the lack of data at earlier time points than 6 months after infection.

CONCLUSIONS

This study indicated that individuals in the convalescent phase of COVID-19 were found to have robust humoral immunity even 12 months after infection, although the patient's history and background may affect the function and kinetics of antibody responses to an array of viral variants.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Author contributions. K. M., H. G., A. G., and A. R. contributed to conceptualization. K. M., S. K., Y. Y., N. O., and A. R. contributed to methodology. H. K., S. Ik., T. Mih., I. M., N. S., M. M., M. S., and A. G. contributed to research design and sample collection. K. M., S. K., S. S. J., H. G., Y. Y., N. O., H. K., T. Mih., T. Miy., S. It., T. K., and K. Y. contributed to data analysis. K. M. and S. S. J. contributed to writing the original draft. K. M., S. S. J., A. G., and A. R. contributed to writing, review, and editing.

Financial support. This research was funded by Japan Agency for Medical Research and Development (AMED) under Grant Numbers JP19fk0108110, JP20he0522001 and JP21fk0108104.

Potential conflicts of interest. Y. Y. is a current employee of Kanto Chemical Co., Inc., and N. O. is a current employee of Tosoh Corporation. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Acknowledgments

We acknowledge all patients and medical staff involved in this study. Cooperating clinics are listed in the Supplementary Appendix. We thank Kazuo Horikawa, Kenji Yoshihara, and Natsumi Takaira for their technical assistance.

References

1. Polack FP, Thomas SJ, Kitchin N, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. N Engl J Med **2020**; 383:2603–15.
2. Galloway SE, Paul P, MacCannell DR, et al. Emergence of SARS-CoV-2 B.1.1.7 lineage - United States, December 29, 2020-January 12, 2021. MMWR Morb Mortal Wkly Rep **2021**; 70:95–9.
3. Tegally H, Wilkinson E, Giovanetti M, et al. Detection of a SARS-CoV-2 variant of concern in South Africa. Nature **2021**; 592:438–43.
4. Fujino T, Nomoto H, Kutsuna S, et al. Novel SARS-CoV-2 variant in travelers from Brazil to Japan. Emerg Infect Dis **2021**; 27:1243–5.
5. Luchsinger LL, Ransegnola BP, Jin DK, et al. Serological assays estimate highly variable SARS-CoV-2 neutralizing antibody activity in recovered COVID-19 Patients. J Clin Microbiol **2020**; 58:e02005–20.
6. Seow J, Graham C, Merrick B, et al. Longitudinal observation and decline of neutralizing antibody responses in the three months following SARS-CoV-2 infection in humans. Nat Microbiol **2020**; 5:1598–607.
7. Wajnberg A, Amanat F, Firpo A, et al. Robust neutralizing antibodies to SARS-CoV-2 infection persist for months. Science **2020**; 370:1227–30.
8. Goto A, Go H, Miyakawa K, et al. Sustained neutralizing antibodies 6 months following infection in 376 Japanese COVID-19 Survivors. Front Microbiol **2021**; 12:661187.
9. Kubo S, Ohtake N, Miyakawa K, et al. Development of an automated chemiluminescence assay system for quantitative measurement of multiple anti-SARS-CoV-2 antibodies. Front Microbiol **2020**; 11:628281.
10. Miyakawa K, Jeremiah SS, Ohtake N, et al. Rapid quantitative screening assay for SARS-CoV-2 neutralizing antibodies using HiBiT-tagged virus-like particles. J Mol Cell Biol **2020**; 12:987–90.
11. Miyakawa K, Jeremiah SS, Kato H, et al. Rapid detection of neutralizing antibodies to SARS-CoV-2 variants in post-vaccination sera. J Mol Cell Biol **2021**; mjab050. doi:10.1093/jmcb/mjab050.
12. Schmidt F, Weisblum Y, Muecksch F, et al. Measuring SARS-CoV-2 neutralizing antibody activity using pseudotyped and chimeric viruses. J Exp Med **2020**; 217:e20201181. doi:10.1084/jem.20201181.
13. Gaebler C, Wang Z, Lorenzi JCC, et al. Evolution of antibody immunity to SARS-CoV-2. Nature **2021**; 591:639–44.
14. Williamson EJ, Walker AJ, Bhaskaran K, et al. Factors associated with COVID-19-related death using OpenSAFELY. Nature **2020**; 584:430–6.
15. Chia WN, Zhu F, Ong SWX, et al. Dynamics of SARS-CoV-2 neutralising antibody responses and duration of immunity: a longitudinal study. Lancet Microbe **2021**; 2:e240–9.
16. Greaney AJ, Loes AN, Crawford KHD, et al. Comprehensive mapping of mutations in the SARS-CoV-2 receptor-binding domain that affect recognition by polyclonal human plasma antibodies. Cell Host Microbe **2021**; 29:463–76.
17. Harvey WT, Carabelli AM, Jackson B, et al. SARS-CoV-2 variants, spike mutations and immune escape. Nat Rev Microbiol **2021**; 19:409–24.
18. Li Q, Wu J, Nie J, et al. The impact of mutations in SARS-CoV-2 spike on viral infectivity and antigenicity. Cell **2020**; 182:1284–94.e1289.
19. Moriyama S, Adachi Y, Sato T, et al. Temporal maturation of neutralizing antibodies in COVID-19 convalescent individuals improves potency and breadth to circulating SARS-CoV-2 variants. Immunity **2021**; 54:1841–52.e4.
20. Betton M, Livrozet M, Planas D, et al. Sera neutralizing activities against SARS-CoV-2 and multiple variants six month after hospitalization for COVID-19. Clin Infect Dis **2021**; 73:e1337–44.
21. L'Huillier AG, Meyer B, Andrey DO, et al. Antibody persistence in the first 6 months following SARS-CoV-2 infection among hospital workers: a prospective longitudinal study. Clin Microbiol Infect **2021**; 27:784.e1–8.
22. Yao L, Wang GL, Shen Y, et al. Persistence of antibody and cellular immune responses in COVID-19 patients over nine months after infection. J Infect Dis **2021**; 224:586–94.
23. Wang Z, Muecksch F, Schaefer-Babajew D, et al. Naturally enhanced neutralizing breadth against SARS-CoV-2 one year after infection. Nature. **2021**; 595:426–31.
24. Long QX, Tang XJ, Shi QL, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nat Med **2020**; 26:1200–4.
25. To KK, Tsang OT, Leung WS, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. Lancet Infect Dis **2020**; 20:565–74.
26. Chen X, Pan Z, Yue S, et al. Disease severity dictates SARS-CoV-2-specific neutralizing antibody responses in COVID-19. Signal Transduct Target Ther **2020**; 5:180.
27. Muller L, Andree M, Moskorz W, et al. Age-dependent immune response to the Biontech/Pfizer BNT162b2 COVID-19 vaccination. Clin Infect Dis **2021**;73:2065–72.
28. Muecksch F, Weisblum Y, Barnes CO, et al. Affinity maturation of SARS-CoV-2 neutralizing antibodies confers potency, breadth, and resilience to viral escape mutations. Immunity **2021**;54:1853–68.e7.