

## Persistent Antibody Responses up to 18 Months after Mild SARS-CoV-2 Infection

Pyoeng Gyun Choe,<sup>1,a</sup> Jisu Hong,<sup>2,3,a</sup> Jiyoung Park,<sup>2,3</sup> Euijin Chang,<sup>1</sup> Chang Kyung Kang,<sup>1</sup> Nam Joong Kim,<sup>1</sup> Chang-Han Lee,<sup>2,3,4,5,b</sup> Wan Beom Park,<sup>1,b</sup> and Myoung-don Oh<sup>1</sup>

<sup>1</sup>Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Republic of Korea

<sup>2</sup>Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul, Republic of Korea

<sup>3</sup>Department of Pharmacology, Seoul National University College of Medicine, Seoul, Republic of Korea

<sup>4</sup>BK21 FOUR Biomedical Science Project, Seoul National University College of Medicine, Seoul, Republic of Korea

<sup>5</sup>Wide River Institute of Immunology, Seoul National University, Hongcheon, Republic of Korea

<sup>a</sup>P.G.C. and J.H. contributed equally to this work

<sup>b</sup>C.-H.L. and W.B.P. contributed equally to this work as senior authors

**Correspondence:**

Wan Beom Park, MD, PhD

Department of Internal Medicine

Seoul National University College of Medicine

103 Daehak-ro, Jongro-gu, Seoul, 03080, Republic of Korea

E-mail: wbpark1@snu.ac.kr

Accepted Manuscript

## **ABSTRACT**

### ***Background***

Humoral immunity to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) may wane rapidly in persons recovered from mild coronavirus disease 2019 (COVID-19), but little is known about the longevity.

### ***Methods***

Serum samples were obtained 8, 12, and 18 months after infection from 20 patients with mild COVID-19. The binding activities of serum antibodies (IgA, IgG, and IgM) against SARS-CoV-2 antigens of the Wuhan-1 reference strain (wild-type) and the B.1.1.7, P.1, B.1.167.2, and B.1.1.529 variants were measured by enzyme-linked immunosorbent assays. Neutralizing antibody titers were measured using a cytopathic effect-based live virus neutralization assay.

### ***Results***

Serum IgA and IgG antibodies against spike or receptor-binding domain (RBD) protein of wild-type SARS-CoV-2 were detected for up to 18 months, and neutralizing antibodies persisted for 8 to 18 months after infection. However, any significant antibody responses against RBD proteins of SARS-CoV-2 variants were not observed, and median neutralizing antibody titers against the Delta variant at 8, 12, and 18 months were 8–11 fold lower than against wild-type viruses ( $P < .001$ ).

### ***Conclusions***

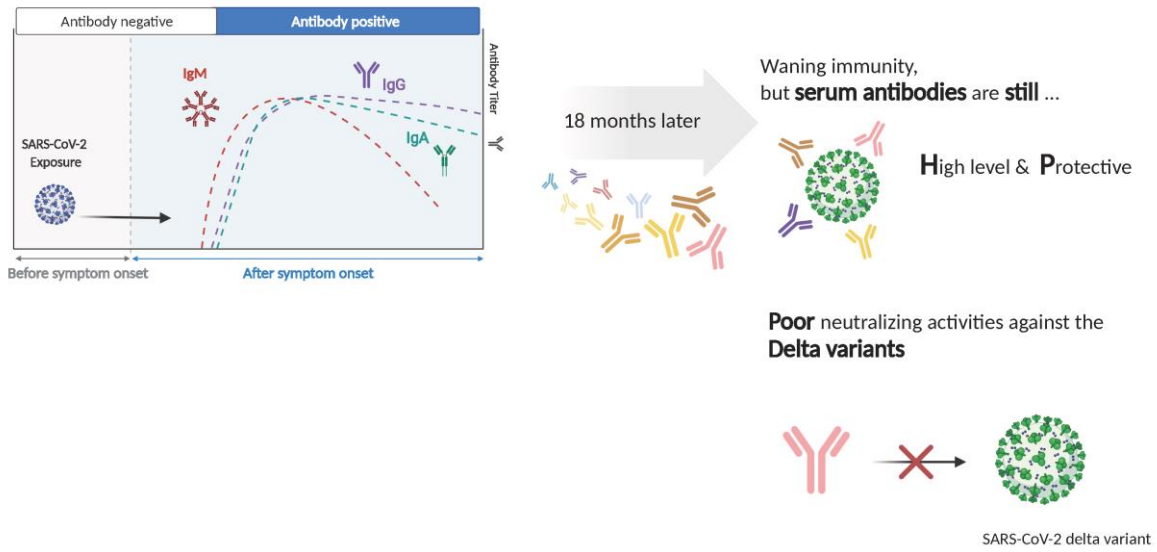
Humoral immunity persisted for up to 18 months after SARS-CoV-2 infection in patients with mild COVID-19. Humoral immune activity against more recently circulating variants, however, was reduced in this population.

**Keywords:** SARS-CoV-2; COVID-19; neutralizing antibodies; ELISA; serological response

**Key points:**

Despite decreasing over time, humoral immunity persisted for up to 18 months after SARS-CoV-2 infection in persons who had recovered from mild COVID-19. Humoral immune activity against more recently circulating viral variants, however, was reduced in this population.

# Graphical\_Abstract



Accepted Manuscript

## INTRODUCTION

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection induces humoral immune responses, including the production of neutralizing antibodies, even in patients with asymptomatic or mild coronavirus disease 2019 (COVID-19) [1]. Neutralizing antibodies play an essential role in virus clearance and have been considered a critical component of the immune system for protection against viral diseases. The persistence of these antibodies is, therefore, clinically meaningful. The initial humoral response is not as strong in patients with mild than with more severe COVID-19, raising concerns about waning immunity in patients with mild disease [2, 3]. In addition, the duration of the humoral responses was found to vary more in patients with mild COVID-19 than in those with severe disease [4].

In response to the COVID-19 epidemic, the Korean government established non-hospital facilities called “community treatment centers (CTCs)” for the isolation of patients with mild diseases [5, 6]. Individuals were admitted to the CTCs if they were alert, age < 65 years, had no or well-controlled underlying disease, and did not have dyspnea. Therefore, the CTCs provided a unique opportunity to study patients with mild COVID-19, including determinations of cross-sectional serologic responses 8 and 12 months after infection [7, 8]. The present study comprehensively analyzed humoral immunity 18 months after infection in persons who had recovered from mild COVID-19. In addition, antibody responses against the B.1.1.7 (Alpha), P1 (Gamma), B.1.167.2 (Delta), and B.1.1.529 (Omicron) variants were assessed in these patients infected with the Wuhan-1 reference strain (wild-type) at the beginning of the COVID-19 pandemic.

## **MATERIALS AND METHODS**

### ***Study Participants and Design***

Patients were eligible for this study if they were previously healthy; were diagnosed with mild COVID-19, as confirmed by real-time reverse transcription-PCR assays; and had been isolated in a CTC operated by Seoul National University Hospital (SNUH) from March 5 to April 22, 2020 [5]. During their stay in a CTC, all patients were comprehensively evaluated twice daily by physicians and nurses using a video consultation system and were confirmed as not having shortness of breath, dyspnea on exertion, or abnormalities on chest radiography [6]. Asymptomatic infection was defined as those who had no symptoms for the duration of their CTC stay, and mild illness was defined as those who had COVID-19 symptoms but did not have shortness of breath, dyspnea, or abnormal chest imaging, according to the World Health Organization's definition of COVID-19 severity [9]. These patients did not receive specific treatments, such as antiviral agents, anti-SARS-CoV-2 monoclonal antibodies, or convalescent plasma.

Blood samples were prospectively collected 8, 12, and 18 months after SARS-CoV-2 infection, and serum was separated within 6 h after blood collection and stored at -80°C. At each time of blood sampling, infectious disease specialists interviewed the participants to obtain clinical information, including re-exposure to SARS-CoV-2, vaccination against SARS-CoV-2, and morbidity or medication potentially affecting immune responses. Serum samples were also collected from healthy volunteers who participated in a SARS-CoV-2 vaccination study [10].

## ***Ethical Approval***

The Institutional Review Board of SNUH approved this study (IRB no. H-2009-168-1160), and all participants provided written informed consent in accordance with the Declaration of Helsinki.

## ***Preparation of Recombination SARS-CoV-2 Antigens***

R619-M64-303: CMV51p> SARS-CoV-2 S(1-1208)-2P-T4f-3C-His8-Strep2x2 D614G was a gift from Dominic Esposito (Addgene plasmid # 166011 ; <http://n2t.net/addgene:166011> ; RRID:Addgene\_166011) [11]. Genes encoding the receptor-binding domain (RBD) of SARS-CoV-2 Wild-type, Alpha, Gamma, Delta, and Omicron variants were cloned in-frame into the pcDNA3.4-SARS-CoV-2-spike RBD-his using Gibson Assembly cloning (NEB, Ipswich, MA, USA). SARS-CoV-2 antigens were produced in Expi293 cells (Thermo Fisher Scientific, Waltham, MA, USA) and his-tagged SARS-CoV-2 proteins were purified according to the manufacture's instructions using Ni-NTA agarose resin (Thermo Fisher Scientific) affinity chromatography, as described [12]. A commercially available SARS-CoV-2 nucleocapsid recombinant protein was also used (Thermo Fisher Scientific, Waltham, MA, USA).

Five days after transfection, each supernatant was collected and passed over a Ni-NTA agarose resin column three times. Each column was initially washed with 100 mL of 1× PBS to remove nonspecific bound proteins, followed by passage of 3 mL elution buffer (50 mM sodium phosphate, pH 8.0; 300 mM NaCl; 250 mM imidazole) to elute the bound proteins. The samples buffer was subsequently exchanged PBS, pH 7.4 using Amicon Ultra-4 spin column with a 10 kDa cutoff



(Merck Millipore, Burlington, MA, USA). The purity of purified samples was assessed by 14% SDS-PAGE gel.

### ***Binding Antibody Enzyme-linked Immunosorbent Assay (ELISA)***

To measure the binding activity of serum antibodies against each SARS-CoV-2 antigen (spike protein D614G, RBD wild-type [WT], RBD Alpha, RBD Gamma, RBD Delta, RBD Omicron, and nucleocapsid protein), each well of a 96-well polystyrene ELISA plate (Thermo Fisher Scientific) was coated with 100 ng of antigen, and the plates were incubated overnight at 4°C. After blocking with 1× PBS (pH 7.4) containing 3% bovine serum albumin for 1 h at room temperature, the plate was washed four times with PBST (PBS containing 0.05% Tween 20). Serum samples diluted at 1:50 were added to each well, and the plates were incubated at room temperature for 1 h.

The plates were washed with PBST four times, followed by the addition of anti-human immunoglobulin M (IgM, 1:5,000) or immunoglobulin A (IgA, 1:100) antibodies, and were incubated for 1 h. After four washes with PBST, horseradish peroxidase (HRP)-conjugated anti-mouse IgG (H+L) antibody (1:20,000; Thermo Fisher Scientific) was added, and the plates were incubated for 1 h. After washing four times with PBST, 50 µL of 3,3',5,5'-tetramethylbenzidine substrate was added to each well (Thermo Fisher Scientific), followed by the addition of 50 µL of 2 M H<sub>2</sub>SO<sub>4</sub>. The absorbance of each well at 450 nm was measured using an Infinite 200 PRO NanoQuant microplate reader (Tecan Trading AG, Männedorf, Switzerland). For detecting antigen-reactive total IgG level, HRP-conjugated anti-human IgG Fc

(1:12,000; Arigobio, Hsinchu, Taiwan) was added and incubated for 1 h at room temperature.

Anti-S1 (spike subunit) IgG antibodies were semi-quantitatively measured using a commercial ELISA kit (Euroimmun, Lübeck, Germany) according to the manufacturer's instructions. Optical density ratios (sample/calibrator) of  $\geq 1.1$ ,  $\geq 0.8$  to  $< 1.1$ , and  $< 0.8$  were regarded as positive, borderline, and negative, respectively, according to the manufacturer's instructions.

### ***Neutralizing Antibody Live Virus Assay***

Neutralization assays based on cytopathic effect (CPE) were performed as previously described [13]. Briefly, 50  $\mu$ L serum samples diluted from 2- to 4,096-fold were added to 50  $\mu$ L of cell culture medium containing 50 times the tissue culture infective dose of the WT virus without the D614G mutation (BetaCoV/Korea/SNU01/2020) [14] or the Delta variant virus (isolated from SNUH, GenBank accession no.MZ853946) in each of a 96-well plate, and the plates were incubated at 37 °C for 2 h in CO<sub>2</sub> 5% vol/vol. Virus antibody mix was added to these wells, and the plates were incubated at 37°C for 5 days. CPE was assessed after 5 days by microscopic examination. The highest dilution of each serum sample that inhibited SARS-CoV-2 activity was defined as the neutralizing antibody titer. Each assay was performed in duplicate with negative control samples.

## ***Graphs and Statistical Analysis***

Data were analyzed and were visualized using GraphPad Prism ver. 9.2.0 software (San Diego, CA, USA). Immune responses were reported as geometric mean titers (GMT) with 95% confidence intervals (CIs) for neutralizing antibody titers at each timepoint. Differences between GMTs at various timepoint differences were analyzed by Wilcoxon matched-pairs signed-ranks test with two-sided  $P$  values  $< .05$  considered statistically significant.

## **RESULTS**

### ***Study Participants***

Twenty patients, 14 women (70%) and six men (30%, of median age 24 years (range, 19–46 years), participated in this study. All 20 participants had asymptomatic/mild COVID-19. None received the COVID-19 vaccine during the study period, and all denied re-exposure to SARS-CoV-2 after the initial infection. At the time of blood collection, none of these participants had a condition or were taking medication that potentially affected immune responses. Serum samples were obtained from 18 patients 8 months after infection and from all 20 patients 12 and 18 months after infection.

The healthy control group consisted of three healthy female volunteers, of median age 38 years (range 22–48 years). None had been diagnosed with COVID-19 or had received a COVID-19 exposure notification until the time of blood donation.

## ***Binding Antibody ELISA***

Serologic responses to SARS-CoV-2 antigens over time were assessed using the spike protein D614G and RBD proteins of SARS-CoV-2 WT and the Alpha, Gamma, Delta, and Omicron variants (Supplementary Figure 1). None of the COVID-19 patients group showed significant IgM responses to the spike protein and the RBD proteins of the SARS-CoV-2 variants, similar to the healthy control group. Also, neither COVID-19 patients nor healthy control groups showed significant binding activities against SARS-CoV-2 nucleocapsid proteins, indicating that reinfection did not occur (Supplementary Figure 2). The reactivity of IgG and IgA antibodies to each antigen did not change or decrease significantly over time, although the level of IgG against RBD WT tended to decrease between 8 and 12 months after infection (Figure 1). Similarly, semi-quantitative antibody assays using a commercial ELISA kit developed against the Wuhan-Hu-1 virus showed that, although the level of IgG against S protein subunit 1 including RBD significantly decreased from 8 months to 12 months after infection ( $P = .006$ ), these levels did not differ significantly between 12 and 18 months after infection ( $P = .099$ ) (Supplementary Figure 3).

Unlike against RBD WT, the serum samples from the patients with mild COVID-19 infected at the beginning of the pandemic did not show any significant antibody responses against RBD proteins of the SARS-CoV-2 variants except for IgA against RBD Gamma. Serum antibodies demonstrated no significant binding activities to RBD omicron, which was prevalent globally in early 2022 (Figure 1). The levels of both IgG and IgA against RBD WT were significantly higher than those against RBD of the variants, including RBD omicron, 18 months after infection (Figure 2).

### ***Neutralizing Antibody Live Virus Assay***

Neutralizing antibody titers against SARS-CoV-2 WT were slightly lower at 12 months than at 8 months after infection (GMT, 92 versus 73;  $P = .088$ ), but were comparable at 12 and 18 months (GMT, 73 versus 78;  $P = .678$ ) (Figure 3). The GMT of neutralizing antibody against the Delta variant was 11 (95% CI, 6–22) at 8 months, 10 (95% CI, 6–18) at 12 months, and 9 (95% CI, 5–16) at 18 months after infection. Neutralizing antibody titers against the Delta variant were lower than those against WT virus by a median 8–11 fold at 8, 12, and 18 months after infection ( $P < .001$ ) (Figure 4).

### **DISCUSSION**

Recent reports on COVID-19 vaccines with strong efficacy provide hopes for pandemic control [15, 16], and many nations are pursuing the rollout of SARS-CoV-2 vaccination as an exit strategy from unprecedented social distancing [4]. However, achieving herd immunity among the population depends critically on the duration of protective immunity resulting from natural infection and vaccination. Therefore, determining the longevity of humoral immunity against SARS-CoV-2 among individuals who have recovered from COVID-19 is essential for predicting herd immunity. The present study describes binding and neutralizing antibody responses against WT and variant viruses for up to 18 months after infection in individuals who have recovered from mild COVID-19, a group more susceptible to waning immunity than those who have recovered from more severe COVID-19.

Early reports have shown that the antibody titers of patients with mild SARS-CoV-2 infection declined more rapidly than those reported for patients infected with SARS-CoV-1 [17], and neutralizing antibodies acquired upon natural infection could disappear within 3 months [18]. Antibody titers in patients recovering from Middle East respiratory syndrome (MERS) were found to decrease rapidly during the first 6 months after infection, but to decrease less steeply between 6 months and 1 year after infection and to be maintained over a longer period [19]. Antibody kinetics profiles in patients recovering from COVID-19 were similar to those in patients recovering from MERS [20], with humoral immunity against SARS-CoV-2 remaining relatively stable for up to 8–12 months [21–24].

This study found that antibody titers against spike and RBD WT proteins persisted for 18 months in this patient cohort without significant change. These results indicate that patients recovering from COVID-19 have long-lived plasma cells producing anti-spike and RBD antibodies, as reported previously [25]. Although mucosal IgA level was not directly measured in the present study, the persistence of the serum IgA response to S protein suggests that these antibodies contribute to viral clearance in mucosal membranes for up to 18 months after infection [26]. The present study also found that neutralizing antibody activities against SARS-CoV-2 persisted for up to 18 months. This finding is consistent with recent reports that humoral immunity to SARS-CoV-2 is maintained for at least 1 year after infection, and that primary SARS-CoV-2 infection decreased the risk of subsequent infection by 77–83% for at least 12 months [27]. Our results suggest that humoral immunity and protection from reinfection with the original virus causing the primary infection may persist for over 18 months, even after mild infection.

Serum antibodies in our cohort were generated by infection with SARS-CoV-2 WT, but the current circulating SARS-CoV-2 variants of concern have several mutations in their spike and RBD proteins, resulting in high transmissibility and potential immune escape [28]. Thus, we tested the binding activities of serum antibodies in these patients against the RBD proteins of the SARS-CoV-2 Alpha, Gamma, Delta, and Omicron variants. These serum samples rarely showed significant antibody responses against the RBD proteins of the SARS-CoV-2 variants and barely neutralized the Delta variant, implying that these patients are susceptible to reinfection by these variant viruses.

Although these SARS-CoV-2 variants of concern have been reported to be less susceptible to neutralization by serum from vaccinated individuals [29], the effectiveness of two-dose AstraZeneca and Pfizer vaccines have been reported to be 60% and 88%, respectively [30]. Furthermore, although pre-vaccination sera from previously infected persons rarely neutralized the Beta variant *in vitro*, a single dose of vaccine boosted neutralizing antibody titers against all variants by up to 1000-fold [31]. Vaccination of persons previously diagnosed with COVID-19 was found to increase anti-RBD IgG to levels that strongly neutralize the Alpha and Beta variants regardless of pre-vaccine IgG levels and vaccine type [32]. These results suggest that vaccination augments immune responses, even to variants to which infected individuals had not been previously exposed. Indeed, a recent study showed that unvaccinated individuals were 2.34 times likely to be reinfected with COVID-19 than those who were fully vaccinated after initial SARS-CoV-2 infection [33]. These findings, together with our results, indicate that persons who have recovered from mild SARS-CoV-2 infection should be offered the COVID-19 vaccine.

The limitations of the present study include relatively small sample size and the predominance of healthy young individuals, reducing the ability to generalize from our results. However, the substantial heterogeneity in antibody responses among individuals with mild SARS-CoV-2 infection [34] suggests that results obtained from our study cohort, with relatively homogenous age and disease severity, may help to determine the longevity of immune responses to SARS-CoV-2 infection.

In conclusion, humoral immunity in persons who had recovered from mild COVID-19 was found to persist for up to 18 months after SARS-CoV-2 infection. Humoral immune activity against recently circulating viral variants was significantly blunted, however, underscoring the need for vaccination of this population.

Accepted Manuscript



## NOTES

### ***Acknowledgments***

R619-M64-303: CMV51p> SARS-CoV-2 S(1-1208)-2P-T4f-3C-His8-Strep2x2 D614G was a gift from Dominic Esposito (Addgene plasmid # 166011 ; <http://n2t.net/addgene:166011> ; RRID:Addgene\_166011) [11]. We thank Kyung Sook Ahn, MD, Director of the Gyeongsan City Health Center, for administrative support; and Areum Jo and Su Jin Choi of the Seoul National University Hospital Biomedical Research Institute for technical support.

### ***Financial Support***

This work was supported in part by the Creative-Pioneering Researchers Program through Seoul National University (to C.-H.L.) and the Bio and Medical Technology Development Program of the National Research Foundation (2021M3A9I2080496 to C.-H. L. and W.B. P.). The funding agencies had no role in the design and conduct of the study; in the collection, management, analysis, and interpretation of the data; in the preparation, review, or in the approval of the manuscript; and decision to submit the manuscript for publication.

### ***Potential Conflicts of Interest***

The No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

## REFERENCES

1. Choe PG, Kang CK, Suh HJ, et al. Antibody responses to SARS-CoV-2 at 8 weeks postinfection in asymptomatic patients. *Emerg Infect Dis* **2020**; 26(10): 2484-7.
2. Wang K, Long QX, Deng HJ, et al. Longitudinal Dynamics of the Neutralizing Antibody Response to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Infection. *Clin Infect Dis* **2021**; 73(3): e531-e9.
3. Choe PG, Kang CK, Suh HJ, et al. Waning antibody responses in asymptomatic and symptomatic SARS-CoV-2 infection. *Emerg Infect Dis* **2021**; 27(1): 327-9.
4. Milne G, Hames T, Scotton C, et al. Does infection with or vaccination against SARS-CoV-2 lead to lasting immunity? *Lancet Respir Med* **2021**.
5. Choe PG, Kang EK, Lee SY, et al. Selecting coronavirus disease 2019 patients with negligible risk of progression: early experience from non-hospital isolation facility in Korea. *Korean J Intern Med* **2020**; 35(4): 765-70.
6. Kang E, Lee SY, Jung H, Kim MS, Cho B, Kim YS. Operating Protocols of a Community Treatment Center for Isolation of Patients with Coronavirus Disease, South Korea. *Emerg Infect Dis* **2020**; 26(10): 2329-37.
7. Choe PG, Kim KH, Kang CK, et al. Antibody Responses 8 Months after Asymptomatic or Mild SARS-CoV-2 Infection. *Emerg Infect Dis* **2021**; 27(3): 928-31.
8. Choe PG, Kim KH, Kang CK, et al. Antibody Responses One Year after Mild SARS-CoV-2 Infection. *J Korean Med Sci* **2021**; 36(21): e157.
9. A minimal common outcome measure set for COVID-19 clinical research. *Lancet Infect Dis* **2020**; 20(8): e192-e7.

10. Hwang YH, Song KH, Choi Y, et al. Can reactogenicity predict immunogenicity after COVID-19 vaccination? *Korean J Intern Med* **2021**; 36(6): 1486-91.
11. Esposito D, Mehalko J, Drew M, et al. Optimizing high-yield production of SARS-CoV-2 soluble spike trimers for serology assays. *Protein Expr Purif* **2020**; 174: 105686.
12. Lee CH, Kang TH, Godon O, et al. An engineered human Fc domain that behaves like a pH-toggle switch for ultra-long circulation persistence. *Nat Commun* **2019**; 10(1): 5031.
13. Shen C, Wang Z, Zhao F, et al. Treatment of 5 Critically Ill Patients With COVID-19 With Convalescent Plasma. *JAMA* **2020**; 323(16): 1582-9.
14. Park WB, Kwon NJ, Choi SJ, et al. Virus isolation from the first patient with SARS-CoV-2 in Korea. *J Korean Med Sci* **2020**; 35(7): e84.
15. Polack FP, Thomas SJ, Kitchin N, et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N Engl J Med* **2020**; 383(27): 2603-15.
16. Voysey M, Clemens SAC, Madhi SA, et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. *Lancet* **2021**; 397(10269): 99-111.
17. Ibarondo FJ, Fulcher JA, Goodman-Meza D, et al. Rapid Decay of Anti-SARS-CoV-2 Antibodies in Persons with Mild Covid-19. *N Engl J Med* **2020**; 383(11): 1085-7.
18. 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(12): 1598-607.

19. Choe PG, Perera R, Park WB, et al. MERS-CoV Antibody Responses 1 Year after Symptom Onset, South Korea, 2015. *Emerg Infect Dis* **2017**; 23(7): 1079-84.
20. Choe PG, Kang CK, Kim KH, et al. Persistence of Neutralizing Antibody Response up to One Year after Asymptomatic or Symptomatic SARS-CoV-2 infection. *J Infect Dis* **2021**; 224(6): 1097-9.
21. Dan JM, Mateus J, Kato Y, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. *Science* **2021**; 371(6529).
22. Feng C, Shi J, Fan Q, et al. Protective humoral and cellular immune responses to SARS-CoV-2 persist up to 1 year after recovery. *Nat Commun* **2021**; 12(1): 4984.
23. Zhang J, Lin H, Ye B, et al. One-year sustained cellular and humoral immunities of COVID-19 convalescents. *Clin Infect Dis* **2021**.
24. Glöckner S, Hornung F, Baier M, et al. Robust Neutralizing Antibody Levels Detected after Either SARS-CoV-2 Vaccination or One Year after Infection. *Viruses* **2021**; 13(10).
25. Turner JS, Kim W, Kalaidina E, et al. SARS-CoV-2 infection induces long-lived bone marrow plasma cells in humans. *Nature* **2021**; 595(7867): 421-5.
26. Sterlin D, Mathian A, Miyara M, et al. IgA dominates the early neutralizing antibody response to SARS-CoV-2. *Sci Transl Med* **2021**; 13(577).
27. Hansen CH, Michlmayr D, Gubbels SM, Mølbak K, Ethelberg S. Assessment of protection against reinfection with SARS-CoV-2 among 4 million PCR-tested individuals in Denmark in 2020: a population-level observational study. *Lancet* **2021**; 397(10280): 1204-12.
28. Singh J, Rahman SA, Ehtesham NZ, Hira S, Hasnain SE. SARS-CoV-2 variants of concern are emerging in India. *Nat Med* **2021**; 27(7): 1131-3.

29. Liu C, Ginn HM, Dejnirattisai W, et al. Reduced neutralization of SARS-CoV-2 B.1.617 by vaccine and convalescent serum. *Cell* **2021**; 184(16): 4220-36.e13.
30. Lopez Bernal J, Andrews N, Gower C, et al. Effectiveness of Covid-19 Vaccines against the B.1.617.2 (Delta) Variant. *N Engl J Med* **2021**; 385(7): 585-94.
31. Stamatatos L, Czartoski J, Wan YH, et al. mRNA vaccination boosts cross-variant neutralizing antibodies elicited by SARS-CoV-2 infection. *Science* **2021**.
32. Gallais F, Gantner P, Bruel T, et al. Evolution of antibody responses up to 13 months after SARS-CoV-2 infection and risk of reinfection. *EBioMedicine* **2021**; 71: 103561.
33. Cavanaugh AM, Spicer KB, Thoroughman D, Glick C, Winter K. Reduced Risk of Reinfection with SARS-CoV-2 After COVID-19 Vaccination - Kentucky, May-June 2021. *MMWR Morb Mortal Wkly Rep* **2021**; 70(32): 1081-3.
34. Manisty C, Treibel TA, Jensen M, et al. Time series analysis and mechanistic modelling of heterogeneity and sero-reversion in antibody responses to mild SARS-CoV-2 infection. *EBioMedicine* **2021**; 65: 103259.

## FIGURE LEGENDS

**Figure 1. Antibody responses to SARS-CoV-2 antigens over time following recovery from mild COVID-19.** Binding activities of each Ig isotype to SARS-CoV-2 D614G spike protein and the RBD proteins of SARS-CoV-2 wild-type; the Alpha variant (B.1.1.7) with a N501Y mutation; the Gamma variant (P.1) with K417T, E484K, and N501Y mutations; the Delta variant (B.1.617.2) with L452R and T478K mutations; the Omicron variant (B. 1.1.529) with G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, and Y505H. Binding activities of serum antibody to SARS-CoV-2 antigens were determined using anti-IgM, anti-IgG, and anti-IgA antibodies. HC (2021): Healthy control (n = 3), COVID-19 patients (8 months: n = 15 [n=12 for Omicron]; 12-months: n = 20; 18 months: n = 20). Statistical analyses were performed using the Mann–Whitney test in GraphPad Prism (n.s. :  $P > .05$ , \* :  $P < .05$ , \*\* :  $P < .01$ , \*\*\* :  $P < .001$ ).

**Figure 2. Antibody responses against SARS-CoV-2 variants 18 months after diagnosis of mild COVID-19.** HC (2021): Healthy control (n = 3), COVID-19 patients 18 months after infection (n = 20). Statistical analyses were performed using one-way ANOVA multiple comparison in GraphPad Prism (n.s. :  $P > .05$ , \* :  $P < .05$ , \*\* :  $P < .01$ , \*\*\* :  $P < .001$ ).

**Figure 3. Changes over time in neutralizing antibody titers against wild-type virus.** Neutralizing antibody titers measured by live virus neutralization assay using cytopathic effect. Geometric mean titer with 95% CI was shown. Data from two patients lacking serum samples 8 months after infection were excluded.

**Figure 4. Difference in neutralizing antibody titers against SARS-CoV-2 wild-type and Delta variant.** Neutralizing antibody titers against wild-type and Delta variant virus of each patient 8 months (n = 18), 12 months (n = 20), and 18 months (n = 20) after SARS-CoV-2 infection.

Accepted Manuscript

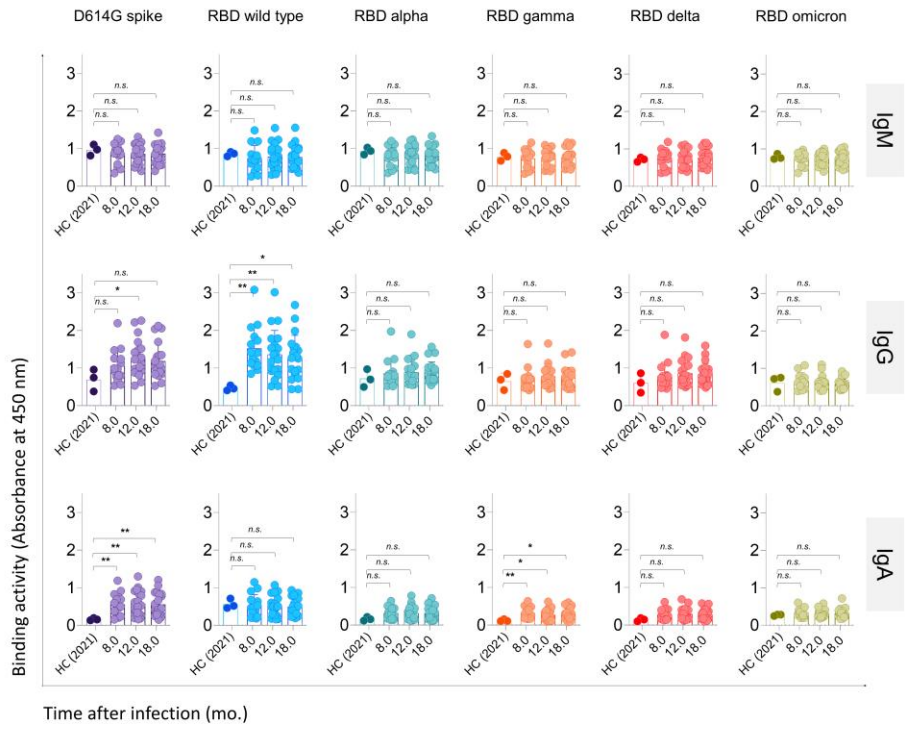
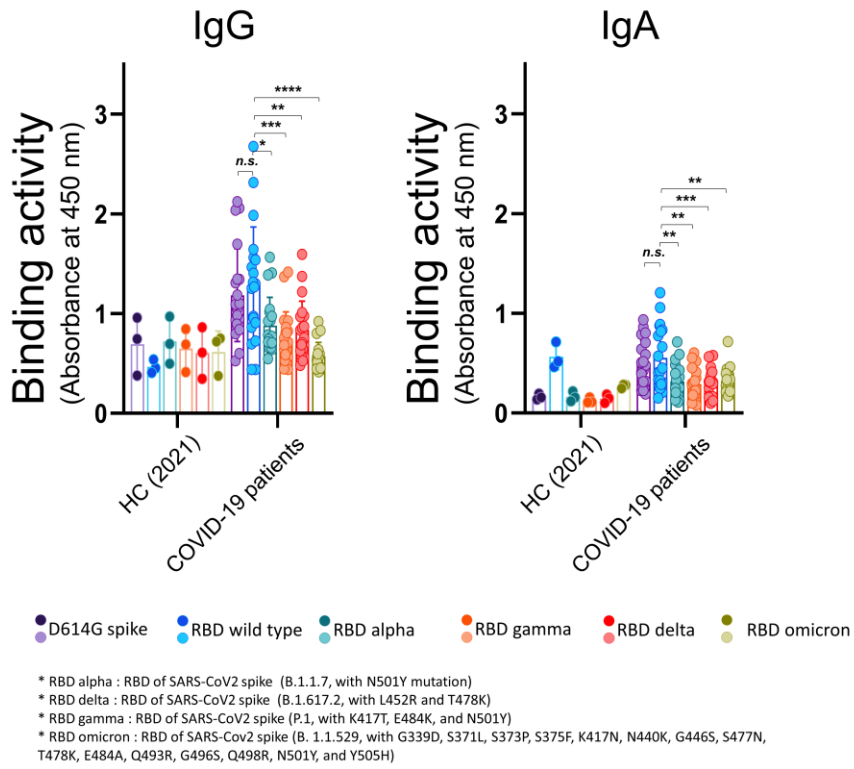


Figure 1

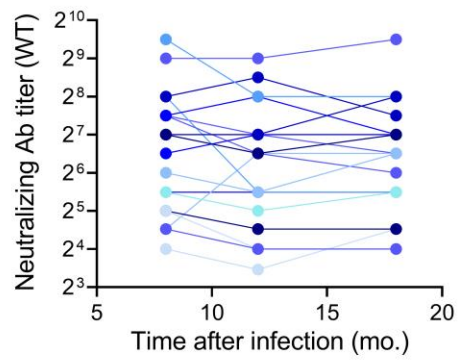
ACCEPTED





**Figure 2**

Accel



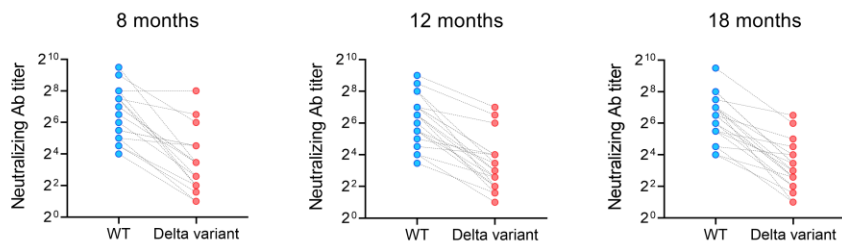


Figure 4

Accel