





SARS-CoV-2-Neutralizing Humoral IgA Response Occurs Earlier but Is Modest and Diminishes Faster than IgG Response

 Yuki Takamatsu,^a Kazumi Omata,^b Yosuke Shimizu,^b Noriko Kinoshita-Iwamoto,^c Mari Terada,^{b,c} Tetsuya Suzuki,^c Shinichiro Morioka,^c Yukari Uemura,^b Norio Ohmagari,^c Kenji Maeda,^a  Hiroaki Mitsuya^{a,d,e}

^aDepartment of Refractory Viral Infections, National Center for Global Health and Medicine Research Institute, Tokyo, Japan

^bCenter for Clinical Sciences, National Center for Global Health and Medicine, Tokyo, Japan

^cDisease Control and Prevention Center, Center Hospital of the National Center for Global Health and Medicine, Tokyo, Japan

^dExperimental Retrovirology Section, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA

^eDepartment of Clinical Sciences, Kumamoto University School of Medicine, Kumamoto, Japan

ABSTRACT Secretory immunoglobulin A (IgA) plays a crucial role in mucosal immunity for preventing the invasion of exogenous antigens; however, little is understood about the neutralizing activity of serum IgA. Here, to examine the role of IgA antibodies against COVID-19 illnesses, we determined the neutralizing activity of serum/plasma IgG and IgA purified from previously SARS-CoV-2-infected and COVID-19 mRNA vaccine-receiving individuals. We found that serum/plasma IgA possesses substantial but rather modest neutralizing activity against SARS-CoV-2 compared to IgG with no significant correlation with the disease severity. Neutralizing IgA and IgG antibodies achieved the greatest activity at approximately 25 and 35 days after symptom onset, respectively. However, neutralizing IgA activity quickly diminished to below the detection limit approximately 70 days after onset, while substantial IgG activity was observed until 200 days after onset. The total neutralizing activity in sera/plasmas of those with COVID-19 largely correlated with those in purified IgG and purified IgA and levels of anti-SARS-CoV-2-S1-binding IgG and anti-SARS-CoV-2-S1-binding IgA. In individuals who were previously infected with SARS-CoV-2 but had no detectable neutralizing IgA activity, a single dose of BNT162b2 or mRNA-1273 elicited potent serum/plasma-neutralizing IgA activity, but the second dose did not further strengthen the neutralization antibody response. The present data show that the systemic immune stimulation with natural infection and COVID-19 mRNA-vaccines elicits both SARS-CoV-2-specific neutralizing IgG and IgA responses in serum, but the IgA response is modest and diminishes faster than the IgG response.

IMPORTANCE Secretory dimeric immunoglobulin A (IgA) plays an important role in preventing the invasion of foreign objects by its neutralizing activity on mucosal surfaces, while monomeric serum IgA is thought to relate to the phagocytic immune system activation. Here, we report that individuals with the novel coronavirus disease (COVID-19) developed both systemic neutralizing IgG (nIgG) and IgA (nIgA) active against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Although the nIgA response was quick and reached the highest activity earlier than the nIgG response, nIgA activity was modest and diminished faster than nIgG activity. In individuals who recovered from COVID-19 but had no detectable nIgA activity, a single dose of COVID-19 mRNA vaccine elicited potent nIgA activity, but the second dose did not further strengthen the antibody response. Our study provides novel insights into the role and the kinetics of serum nIgA against the pathogen in both naturally infected and COVID-19 mRNA vaccine-receiving COVID-19-convalescent individuals.

KEYWORDS COVID-19, SARS-CoV-2, humoral immunity, neutralizing antibodies, immunoglobulin A, immunoglobulin G, anti-SARS-CoV-2 immunoglobulin, COVID-19 mRNA vaccine

Editor Takamasa Ueno, Kumamoto University

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Address correspondence to Hiroaki Mitsuya, hmitsuya@hosp.ncgm.go.jp.

The authors declare no conflict of interest.

Received 15 July 2022

Accepted 13 September 2022

Published 11 October 2022

Immunoglobulin A (IgA) is the most abundant type of antibody in the body (1), comprising most of the immunoglobulin in secretions primarily in the gut, milk, and bronchial secretions as a noninflammatory antibody against microbes (2). Such secretory-IgA plays a crucial role in neutralizing the viruses, toxins, and inflammatory microbial molecules invading the mucosal epithelial cells (3) and exerts greater efficacy in preventing infections than serum IgG (4). Thus, selective IgA deficiency, the most common immunologic defect in humans (5), causes recurrent sinopulmonary infections, autoimmune disorders, and allergic disorders. However, most individuals with selective IgA deficiency are asymptomatic, and serum IgA levels in patients do not necessarily correlate with the occurrence or severity of these disorders (6). Serum IgA is the second most abundant isotype following IgG (7), and the functions of serum IgA appear to be related to the phagocytic system activation mediated through the Fc-alpha-RI (CD89) (8), although it is not fully understood. In this regard, it had been recognized that immunization via mucosal routes can elicit robust mucosal immune responses, while the systemic vaccination approach (e.g., administered intramuscularly or intradermally) mainly induces IgG and apparently induces, in part, protective mucosal IgA responses (9).

In terms of the novel coronavirus disease (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), we previously reported that highly neutralizing activity-confirmed COVID-19 convalescent plasma and purified-IgG block the Syrian hamster disease progression with limited viral antigen-positive cells in terminal bronchioles and alveolar regions (10). Sterlin et al. reported that mucosal IgA produced shortly after symptom onset plays a crucial role in the early stage of the disease (11). It has also been reported that COVID-19 mRNA vaccines elicit high titers of anti-SARS-CoV-2-S1-binding IgG (S1-binding IgG) and IgA (S1-binding IgA) antibodies in serum (12–14). In this regard, while systemic neutralizing IgG (nIgG) antibodies induced by COVID-19 and mRNA vaccines are thought to be responsible for the protection against symptomatic infection, further evaluation of the role of IgA in COVID-19 infection and COVID-19 vaccines, especially the evaluation of the neutralizing activity of such natural infection- or vaccine-induced IgA, are needed.

Here, we report that individuals with COVID-19 developed both systemic nIgG and nIgA irrespective of the severity of the disease; however, even though the nIgA response was quick, the activity was modest and diminished faster than that of nIgG. We also report that the COVID-19 mRNA vaccines elicit highly neutralizing serum IgA in COVID-19-experienced individuals.

RESULTS

Clinical characteristics of the participants. A total of 14 individuals who were confirmed to have SARS-CoV-2 infection with positive RNA-quantitative-PCR (RNA-qPCR) results and were admitted to the Center Hospital of the National Center for Global Health and Medicine in Tokyo, Japan, from February to April 2020 (COVID-19 group) (Table 1) and 8 individuals who received a COVID-19 mRNA vaccine (either BNT162b2 or mRNA-1273) from April to July 2021 after recovery from COVID-19 (convalescent-vaccine group) (Table 2) were enrolled. These individuals agreed to participate in the present clinical studies. All the individuals were Japanese, and 2 out of 14 (14.3%) in the COVID-19 group and 2 out of 8 (25.0%) in the convalescent-vaccine group were female (Tables 1 and 2). The median (range) ages were 53 (37 to 68) and 53 (35 to 61) years in the COVID-19 group and convalescent-vaccine group, respectively (Tables 1 and 2). In the COVID-19 group, seven individuals (50%) had moderate symptoms of lower respiratory disease or imaging with no oxygen requirement, while seven individuals (50%) had severe symptoms and required oxygen treatment during the clinical course without any sequential organ failure. There were no significant differences in the age, sex, or sample collection dates between the moderate and severe symptom groups (Table 1). Individuals in the COVID-19 group received experimental therapeutic agents, which are now mostly considered to be ineffective (see Table S1 in the supplemental material) (15). The convalescent-vaccine group received the primary

TABLE 1 Characteristics of COVID-19-experienced individuals (COVID-19 group) in the study

Characteristic	All patients (n = 14)	Moderate (n = 7)	Severe (n = 7)
Age, yrs (median)	37–68 (53)	47–62 (53)	37–68 (63)
Sex [n (%)]			
Male	12 (85.7)	6 (85.7)	6 (85.7)
Female	2 (14.3)	1 (14.3)	1 (14.3)
Oxygen requirement			
Days [median (%)]		NA ^a	2–15 (6.5)
None	7 (50.0)	7 (100)	0 (0)
Nasal canula	6 (42.9)	0 (0)	6 (85.7)
High-flow nasal canula	1 (7.1)	0 (0)	1 (14.3)
Sample collection, days (median)			
Acute phase	3–15 (9)	3–13 (8)	4–15 (9)
Convalescent phase	17–201 (86)	19–201 (88)	17–196 (74)

^aNA, not applicable.

series of COVID-19 mRNA vaccine 70 to 458 (median, 306) days after disease onset (Table 2).

SARS-CoV-2-neutralizing serum/plasma IgA response occurs earlier and diminishes faster than the IgG response. We previously described the kinetics of neutralizing activity of immunoglobulin G (IgG) fractions purified from plasmas of 43 SARS-CoV-2-infected individuals using cell-based assays (16). In the current study, we chose 14 individuals with moderate to severe COVID-19 symptoms and evaluated the neutralizing activity of whole sera/plasmas and purified-IgG and -IgA fractions against wild-type SARS-CoV-2^{05-2N} (PANGO lineage B). As shown in Fig. 1a, whole sera/plasmas from all 14 SARS-CoV-2-infected individuals had significantly high titers of SARS-CoV-2-neutralizing activity by 30 days after symptom onset, and thereafter their neutralizing activity gradually decreased, but the decay became slower after around 50 days (Fig. 1a). Significant levels of neutralizing activity persisted in sera/plasmas in all participants as examined on up to day 200 (Fig. 1a). Purified-IgG from sera/plasmas also exerted neutralizing activity expressed as a 50% effective concentration (EC₅₀) of up to 1.0 μg/mL (Fig. 1b) and showed substantial neutralizing activity by around 200 days postonset. Substantial amounts of S1-binding IgG antibodies were also seen by around 200 days after the onset (Fig. 1e). We also identified good immune response to pro-

TABLE 2 Characteristics of COVID-19 mRNA-vaccinees after recovery from the disease (convalescent-vaccine group) in the study

Characteristic	All participants (n = 8)	mRNA-1273 (n = 3)	BNT162b2 (n = 5)
Age, yrs (median)	35–61 (53)	55–61 (55)	35–56 (44)
Sex [n (%)]			
Male	6 (75.0)	2 (66.6)	4 (80.0)
Female	2 (25.0)	1 (33.3)	1 (20.0)
Severity [n (%)]			
Mild	5 (62.5)	2 (66.6)	3 (60.0)
Moderate	2 (25.0)	0 (0)	2 (40.0)
Severe	1 (12.5)	1 (33.3)	0 (0)
Days to vaccination after onset (median)	70–458 (306)	173–458 (436)	70–335 (286)
Sample collection, day (median)			
Before 1st vaccination (median)	17–298 (209)	97–249 (209)	17–298 (219)
After 1st vaccination (median)	4–103 (56)	4–91 (57)	8–103 (55)

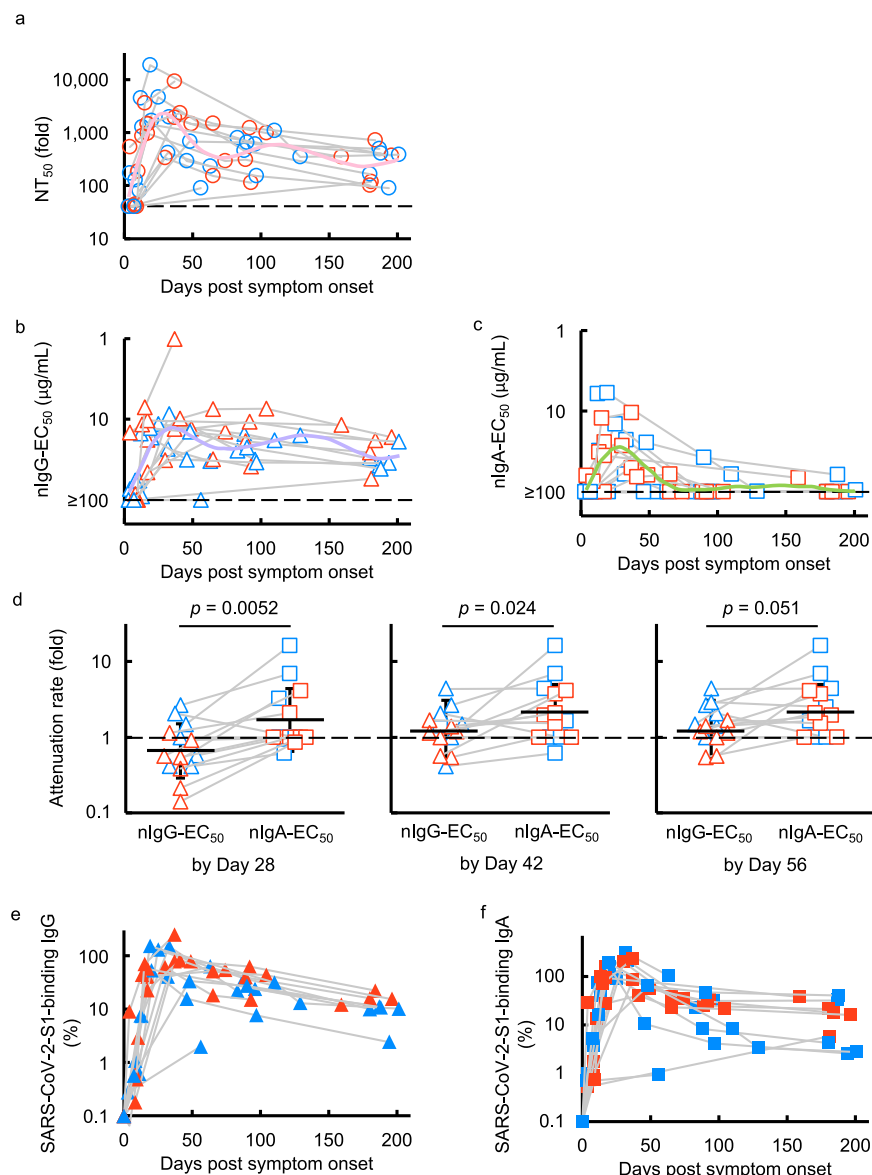


FIG 1 Kinetics of SARS-CoV-2-neutralizing activity and S1-binding antibodies. (a to c) VeroE6TMPRSS2 cells were exposed to wild-type SARS-CoV-2^{95-2N} with or without various concentrations of diluted sera/plasmas (a), purified IgG (b), or purified IgA (c), and the neutralizing activity and the amounts of S1-binding antibodies were determined. The dashed line denotes the assay limit values (≤ 40 -fold for the panel a and ≥ 100 $\mu\text{g/mL}$ for panels b and c). Note that the highest viral neutralizing activity of purified IgG and IgA was seen around 35 and 25 days, respectively, after onset. Furthermore, the neutralizing activity of serum IgA diminished much quicker than that of IgG. The colored lines (pink, NT_{50} for pink; purple, nIgG-EC_{50} ; light green, nIgA-EC_{50}) denote the fitted curve. (d) Attenuation rate of the nIgG-EC_{50} and nIgA-EC_{50} between the highest neutralizing activity of purified IgG and IgA by day 28, 42, and 56 postonset and neutralizing activity determined latest in the study. (e and f) The kinetics of the amount of S1-binding IgG (e) and IgA (f) are also shown. The amount of S1-binding IgG and IgA increased by approximately day 21 post-symptom onset, followed by a gradual decrease. Note that in contrast, substantial amounts of S1-binding IgG and IgA persisted around 200 days after onset, while the decay occurred more rapidly in IgA. Blue symbols denote the samples collected from individuals with moderate symptoms, while red symbols are those from individuals with severe symptoms.

duce nIgA following the emergence of COVID-19 symptoms; however, the decay of the IgA neutralizing activity occurred much earlier than that of nIgG, and by 129 days after onset, 12 of the 14 individuals (85.7%) had an EC₅₀ value of ~ 100 $\mu\text{g/mL}$ or undetectable (> 100 $\mu\text{g/mL}$) neutralizing activity (Fig. 1c). Of the 14 individuals, 3 (21.4%) showed no detectable nIgA activity throughout the study. In contrast to the early

decay in nIgA activity, substantial amounts of S1-binding IgA persisted for up to 200 days after the onset (Fig. 1f).

To quantify and compare the time-dependent kinetics of serum/plasma, nIgG, and nIgA activity, we generated fitted curves by using the generalized additive model (17, 18), which showed that the nIgA response occurred significantly earlier than the nIgG response; it took 25 days postonset for the nIgA response to reach its peak but 35 days for the nIgG response to reach its peak (Fig. 1b and c). It was also noted that the nIgA response diminished faster than the nIgG response; the average nIgA-EC₅₀ value virtually reached the detection limit ($\geq 100 \mu\text{g/mL}$) approximately 70 days postonset (Fig. 1c), while substantial nIgG activity persisted until ~ 200 days postonset (Fig. 1b). We also attempted to quantify the time-dependent reduction of the nIgG-EC₅₀ and nIgA-EC₅₀ by calculating the attenuation rate between the highest neutralizing activity of purified IgG and IgA by days 28 (range, 3 to 25), 42 (range, 3 to 41), and 56 (range, 3 to 56) postonset and neutralizing activity determined in the latest in the study (Fig. 1d). As shown in Fig. 1d, the attenuation rates of nIgA-EC₅₀ were significantly greater than those of nIgG-EC₅₀ by 28 days (4 weeks; Fig. 1d, left panel) and 42 days (6 weeks; Fig. 1d, middle panel) postonset, with *P* values of 0.0052 and 0.024, respectively. The same trend was seen when the attenuation rates of nIgG-EC₅₀ and nIgA-EC₅₀ were determined by 56 days (8 weeks; Fig. 1d, right panel; *P* = 0.051).

Sterlin and his colleagues previously reported that IgA dominates the early neutralizing antibody response to SARS-CoV-2 in patients with COVID-19 (11). Thus, we attempted to examine whether the amounts of S1-binding IgA produced predominated timewise over those of S1-binding IgG by using the slope indexes determined with the initial (first) value determined and the following (second) value determined for S1-binding IgA and IgG amounts in each individual. Then, the slope indexes of S1-binding IgA and IgG amounts were compared using the Wilcoxon signed-rank test. We found that the slope indexes made with the first and second S1-binding IgA were significantly greater than those made with S1-binding IgG, suggesting that the amount of S1-binding IgA produced significantly predominated over the amount of S1-binding IgG at the early phase of the antibody response after symptom onset (*P* = 0.009) (Fig. 1e and f).

Neutralizing IgG activity is greater in the COVID-19-convalescent phase than in the acute phase regardless disease severity. We next asked if higher neutralization activity is seen in the acute (less than 14 days postonset or when oxygen treatment was required) or convalescent (14 days postonset and when no oxygen was required) phase or in patients with moderate or severe COVID-19. A significant increase was seen in 50% neutralizing titers (NT₅₀) of sera/plasmas in the convalescent phase compared to the acute phase in both moderate and severe symptom groups (*P* = 0.02 and 0.03, respectively) (Fig. 2a). There was also a significant increase in nIgG activity in the convalescent phase in both moderate and severe symptom groups (*P* = 0.03 and 0.03, respectively) (Fig. 2b). The same pattern was seen in S1-binding IgG amounts (Fig. 2d). In contrast, there was no significant difference in nIgA activity between acute and convalescent phases in either the moderate or severe symptom group (Fig. 2c). The amounts of S1-binding IgA were higher in the convalescent than in the acute phase in the moderate symptom group, although the difference in the severe symptom group was not significant (Fig. 2e).

The contribution of neutralizing IgG antibody to serum/plasma neutralizing activity is greater than that of neutralizing IgA. The amounts of S1-binding IgG antibodies in sera from patients with COVID-19 correlate highly with SARS-CoV-2-specific neutralizing activity levels in serum IgG fraction (16, 19). However, the role of SARS-CoV-2-specific humoral IgA antibodies in protecting against SARS-CoV-2 infection remains to be clarified. Thus, we asked whether S1-binding IgA antibody amounts correlate with nIgA activity. The NT₅₀ values of sera/plasmas from patients with COVID-19 proved to correlate well with nIgG activity (nIgG-EC₅₀ values) with the rho (ρ) value of -0.72 (95% confidence interval [CI], -0.84 to -0.54) (Fig. 3a), in line with our previous observations (16). In the case of purified-IgA from patients with COVID-19, a high

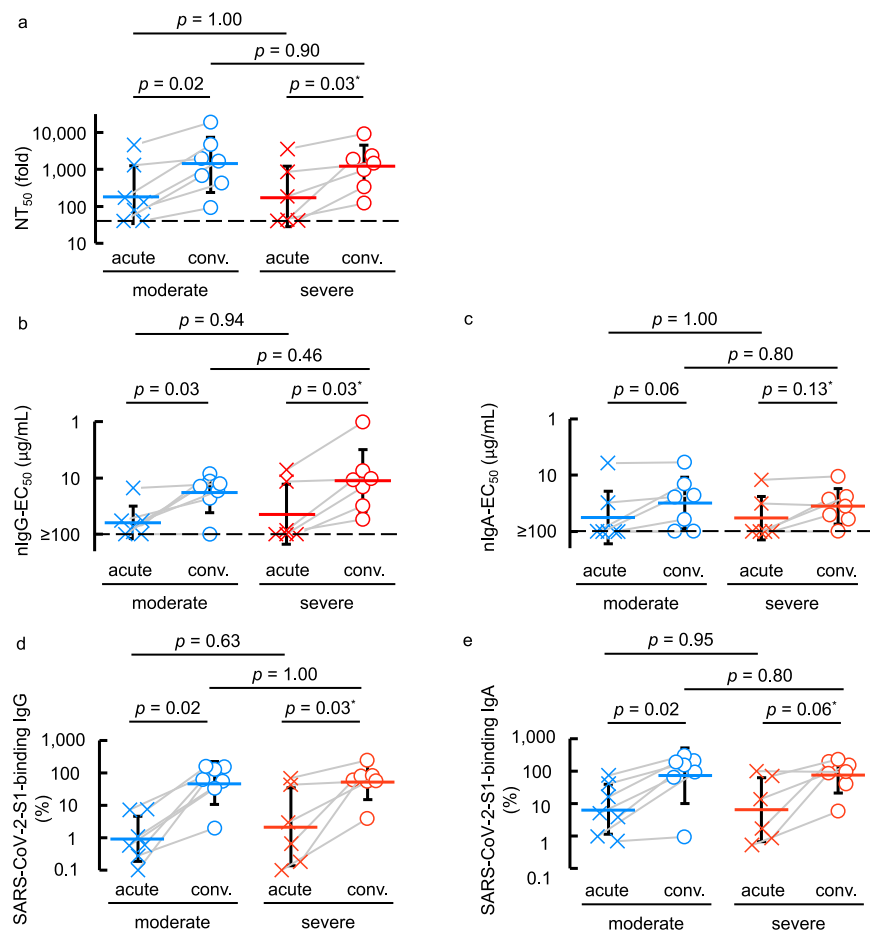


FIG 2 COVID-19-convalescent individuals possess greater neutralizing activity and SARS-CoV-2-S1-binding antibody levels than those at the acute phase. (a to e) The neutralizing activity of sera/plasmas, purified IgG, and purified IgA (a, b, and c, respectively) and the amounts of S1-binding IgG and IgA (d and e, respectively) were compared between the acute phase (less than 14 days post-symptom onset or when the individual required oxygen treatment) and the convalescent phase (14 days post-symptom onset and beyond which no oxygen was required). Blue symbols denote the samples collected from individuals with moderate symptoms, while red symbols are those from individuals with severe symptoms.

correlation was also observed with nIgA activity (nIgA-EC₅₀ values), with a ρ value of -0.78 (95% CI, -0.88 to -0.62), although 31 of 56 IgA samples had very low or undetectable (≥ 100 $\mu\text{g/mL}$) neutralization activity (Fig. 3b). Between nIgG-EC₅₀ and nIgA-EC₅₀ values, however, a moderate correlation was seen, with a ρ value of 0.42 (95% CI, 0.13 to 0.65) (Fig. 3c). The NT₅₀ values of sera/plasmas and nIgG-EC₅₀ values also had a high correlation with S1-binding IgG amounts (Fig. S2a and b). There was also a high correlation between the NT₅₀ values of sera/plasmas and nIgA-EC₅₀ values with S1-binding IgA amounts (Fig. S2c and d). S1-binding IgA in nasopharyngeal swab samples collected at the earliest point of the infection (fewer than 20 days post-symptom onset) tend to have a higher amount as time progresses (Fig. S3a). Further, the nasal S1-binding IgA was highly correlated with serum S1-binding IgA, with a Spearman's ρ value of 0.73 (95% CI, 0.40 to 0.89) (Fig. S3b). On the other hand, the serum total human IgG and IgA were consistent during the study period (Fig. S3c and d), with a low correlation (Fig. S3e).

The present data suggest that the neutralizing activity seen in sera/plasmas of patients with COVID-19 is largely composed of the neutralizing activity of serum IgG (Fig. 3a) but also of that of IgA (Fig. 3b). Moreover, as has been seen in the case of neutralizing activity of sera/plasmas that are in large part correlated with the amount of

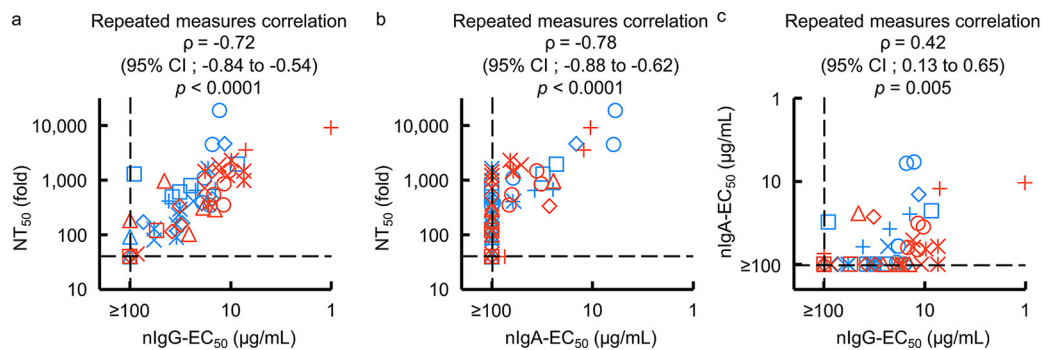


FIG 3 Correlations of purified IgG and IgA neutralizing activities with serum/plasma neutralizing titers. (a and b) The neutralizing activity of purified-IgG (a) (nlgG-EC₅₀) and -IgA (b) (nlgA-EC₅₀) against serum/plasma neutralizing activity (NT₅₀) values are plotted. (c) The nlgA-EC₅₀ values are plotted against the nlgG-EC₅₀ values. Note that a high correlation is observed between NT₅₀ values and nlgG-EC₅₀ values (repeated measures correlation $\rho = -0.72$ [95% CI, -0.84 to -0.54]) (a) and between the NT₅₀ values and nlgA-EC₅₀ values ($\rho = -0.78$ [95% CI, -0.88 to -0.62]) (b), while moderate correlation was observed between nlgA-EC₅₀ and nlgG-EC₅₀ (repeated measures correlation $\rho = 0.42$ [95% CI, 0.13 to 0.65]) (c). Each symbol denotes the sample from one individual. Blue symbols denote the samples collected from individuals with moderate symptoms, while red symbols denote those from individuals with severe symptoms.

S1-binding IgG (16, 20), the neutralizing activity of serum IgA is correlated with the amounts of S1-binding IgA (Fig. S2d), while the neutralizing activity of IgA was modest compared to that of IgG (Fig. 1b and c, and Fig. 3c).

mRNA-COVID-19 vaccine induces high-level neutralizing activity in COVID-19 convalescent individuals. We next examined the SARS-CoV-2-specific IgG and IgA neutralizing activity elicited with the primary series of mRNA vaccine administration (BNT162b2 or mRNA-1273) in eight individuals who had experienced quantitative PCR (qPCR)-confirmed symptomatic COVID-19 70 to 458 days before the first immunization (Table 2). All eight individuals had low but detectable to moderate levels of neutralizing activity in sera/plasmas before vaccination (Fig. 4a). Most of these individuals had significantly high titers of neutralizing activity within 28 days after the first vaccination. The high NT₅₀ titers were not further boosted following the second dose, which is a quite different pattern of NT₅₀ values from the patterns seen in those who were SARS-CoV-2-naïve and received the first and second doses of vaccine (20–22). A similar pattern was seen when nlgG-EC₅₀ values were determined in the same participants (Fig. 4b). In the case of nlgA-EC₅₀ values, none of the participants had detectable neutralizing activity ($\geq 100 \mu\text{g/mL}$) before the COVID-19 mRNA-vaccination, but 3 of the 8 participants had a substantial increase in the nlgA-EC₅₀ values before the second dose of mRNA vaccine (Fig. 4c). In contrast, these COVID-19-experienced individuals had moderate to high levels of S1-binding IgG and IgA before the first dose, and greater levels of S1-binding IgG and IgA were documented following the first dose, although no further increase was seen after the second dose (Fig. 4d and e). It was noted, however, that the nlgA activity rapidly decreased (Fig. 4c) compared to nlgG activity (Fig. 4b), although such rapid decay was not seen in the amounts of S1-binding IgA antibodies (Fig. 4e).

All of the NT₅₀, nlgG-EC₅₀, nlgA-EC₅₀, percent S1-binding IgG, and percent S1-binding IgA values proved to have significantly increased following primary series administration (Fig. S4a to e). These data demonstrate that COVID-19 mRNA vaccines induce high titers of nlgA in previously COVID-19-experienced individuals after a single dose of vaccine. However, such nlgA activity apparently diminished faster than the nlgG activity, while the difference was not statistically significant ($P = 0.069$) (Fig. 4b and c). Such an early decay of nlgA activity had been seen in those with symptomatic infection with SARS-CoV-2 (Fig. 1c). The second-dose vaccination did not significantly slow the speed of decay (Fig. 4c). We also examined whether there are correlations among NT₅₀, nlgG-EC₅₀, nlgA-EC₅₀, and S1-binding IgG and IgA values. As we have seen that nlgG activity greatly contributes to serum/plasma SARS-CoV-2-neutralizing activity

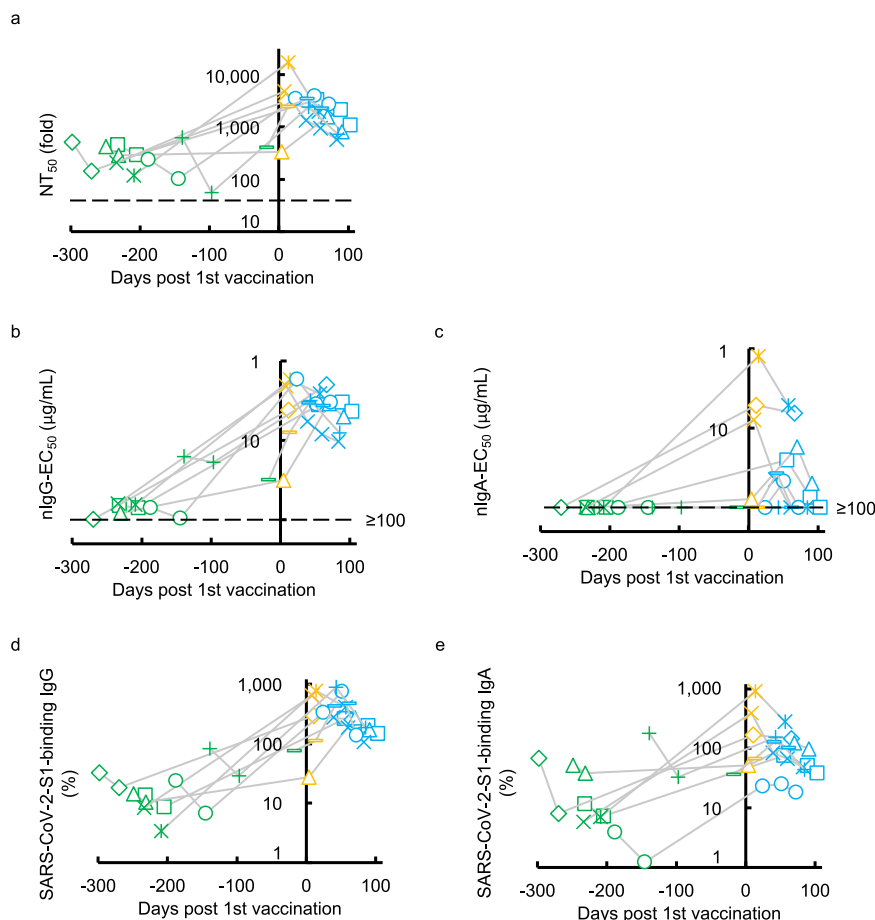


FIG 4 Kinetics of neutralizing activity and S1-binding antibody levels before and after COVID-19 mRNA vaccination. (a to e) The kinetics of neutralizing activity (a, b, and c) and S1-binding antibody levels (d and e) in eight previously COVID-experienced individuals who received the COVID-19 mRNA vaccine are shown. Note that all the values significantly rose after the first dose of the vaccine. Also note that none of the participants had detectable nIgA-EC₅₀ values ($\geq 100 \mu\text{g/mL}$) before vaccination (c). On the other hand, all of them had low to high levels of nIgA-EC₅₀ after a single dose of the vaccine, and such levels quickly decreased (c), and their S1-binding IgA levels persisted after the two doses of vaccine in the study period (e). The dashed line denotes the assay detection limit (≤ 40 -fold dilution for NT₅₀ and $\geq 100 \mu\text{g/mL}$ for nIgG-EC₅₀ and nIgA-EC₅₀). Green symbols denote the samples collected before COVID-19 mRNA vaccination, while yellow and light-blue symbols denote samples collected after the first and second doses, respectively. Each symbol denotes the sample from one individual.

compared to that of nIgA activity in individuals with COVID-19 (Fig. 3a to c and Fig. S2a to d), similar profiles were identified in previously COVID-19-contracted individuals following COVID-19 mRNA vaccination (Fig. S5a to g).

DISCUSSION

In respiratory tract infections such as influenza virus infection, natural infection induces systemic IgG responses (23, 24) and mucosal secretory-IgA responses (25). In terms of COVID-19, Sterlin et al. reported that IgA-expressing-circulating plasmablasts detected shortly after symptom onset have a consistent phenotype with that found in lung and eventually produced mucosal IgA (11). Our present data also showed that the amounts of nasal S1-binding IgA antibody and the amounts of serum S1-binding IgA are highly correlated (Pearson's $\rho = 0.73$, Fig. S3b), suggesting the serum S1-binding IgA and mucosal S1-binding IgA share similar, albeit not the same, antigenic determinants or immunological repertoire in response to SARS-CoV-2-S1. In this regard, it is of note that Wang et al. have suggested that serum IgA monomers are produced by the

same cells that produce secretory dimers (19). Of note, Sterlin et al. reported that serum IgA specific to the receptor-binding domain (RBD), which represents a critical target for neutralization, was detected earlier than anti-RBD IgG as assessed with a photonic ring immunoassay (11). In the present study, we also showed that the nIgA response occurred significantly earlier than the nIgG response; it took 25 days postonset for the nIgA response to reach its peak and 35 days for the nIgG response to reach its peak (Fig. 1b and c). Moreover, S1-binding IgA production significantly predominated over S1-binding IgG production (Fig. 1d and e). These data are in line with the observations by Sterlin et al. (11).

Although the neutralization of pathogens is attributed to the neutralizing activity of IgG, providing long-term immunity for as long as decades in cases such as mumps, varicella-zoster virus (VZV), and Epstein-Barr virus (EBV) (26), protective immunity to seasonal coronaviruses (27), SARS-CoV, and Middle East respiratory syndrome (MERS)-CoV (28) is known to be short-lived. Vanshilla et al. reported that the neutralizing activity in serum waned quickly (half-life, 3.6 months) compared to the neutralizing activity of purified-IgG (half-life, 7.8 months), and such a short half-life of activity of serum is thought to be partially attributed to the presence of S-binding IgA and IgM in serum (29). Moreover, Iyer et al. reported that RBD-binding IgA antibodies are short-lived compared to RBD-binding IgG antibodies (30). In the present study, we extended the observations by Vanshilla et al. and Iyer et al. and demonstrated that SARS-CoV-2-neutralizing activity of serum/plasma IgA was identified earlier and diminished faster than that of IgG as assessed in 14 individuals with COVID-19 (Fig. 1b and c). Moreover, when such activity was determined following mRNA vaccination in eight COVID-19-experienced individuals, SARS-CoV-2-neutralizing activity in serum/plasma IgA also quickly diminished compared to that in serum/plasma IgG (Fig. 4b and c). However, there were no significant differences in the decay rate of SARS-CoV-2-S1-binding IgG and IgA levels (Fig. 1d and e and Fig. 4d and e). The faster decay in the nIgA activity compared to that in nIgG may derive from the difference in half-lives of serum IgA and IgG (i.e., 3 to 5 and 21 days, respectively). Also, it is possible that since the total amount of serum IgG in the body is greater than that of IgA, the consumption and absorption of nIgA by the viral antigens could be more apparent than in the case of IgG.

While the 1st-dose vaccination in the COVID-19-experienced individuals elicited a good response comparable to the response seen in COVID-19-unexperienced individuals following the 2nd dose (20, 31), the response following the 2nd dose was comparable to or even less than the response after the 1st dose in those COVID-19-experienced individuals (Fig. 4a to e). In this respect, it is noteworthy that the intervals following the 1st dose until the 2nd dose was administered were 3 or 4 weeks. These intervals were probably too short for eliciting the otherwise boosted immune response. In fact, there are several published articles that describe the antibody responses after COVID-19 vaccination in previously COVID-19-experienced individuals (21, 22, 32–34). In such articles, a single dose of COVID-19 mRNA vaccine (whether BNT162b2 or mRNA1273) or adenoviral vector-based vaccine (AZD1222) induced substantial neutralizing antibodies and T cells responses against the virus that are compatible with the responses seen after two doses of vaccine in individuals without prior SARS-CoV-2 infection. While the mechanism of such robust immunogenicity seen with a single dose of vaccine in COVID-19-experienced individuals remains to be clarified, it is presumed that the prior COVID-19 infection served as the primary immunization.

It has been reported that the neutralizing activity of IgM and IgA are dramatically greater than that of IgG when the activity of recombinant monoclonal antibodies, which share the same anti-SARS-CoV-2 spike protein Fab region, was examined using a pseudotyped lentivirus coated with the SARS-CoV-2 spike protein and angiotensin-converting enzyme 2 (ACE2)-transfected Crandell-Rees feline kidney cells as the host cell line (35). In the current study, unlike their findings, we observed that the neutralizing activity of purified-IgA is modest compared to that of purified-IgG (Fig. 1b and c, and Fig. 3c). In this regard, we used a cell-based neutralization assay using IgA fractions

purified from sera/plasmas, which are of polyclonal nature. Thus, our data should possibly represent the more comprehensive protective effect of serum-derived IgA, although more studies are needed.

There are reports that individuals with selective IgA deficiency tend to have higher risks of severe COVID-19 (36, 37). Thus, we initially hypothesized that individuals with moderate symptoms would possess greater neutralizing activity of serum IgA than those with severe COVID-19. However, there were no significant differences in nIgA-EC₅₀ values between those with moderate and severe disease (Fig. 2d and e). In this regard, we have lately shown that patients with severe COVID-19 had greater nIgG levels in serum than those with mild COVID-19 (16), and we reasoned that the exposure to larger amounts of SARS-CoV-2 over the long term in those with severe COVID-19 resulted in greater nIgG activity (29).

The kinetics of S-binding IgG, IgA, and IgM upon natural SARS-CoV-2 infection has been well described during the 2 years of the COVID-19 pandemic (11, 30, 38, 39). There is a good body of literature regarding SARS-CoV-2-neutralizing IgG antibody responses, yet there are only a few reports on the nIgA antibody. However, those reports describe the neutralizing activity of monoclonal IgA antibodies produced by peripheral (35) or mucosal (40) memory B cells. There are also a number of reports describing immune responses elicited by various types of vaccines, such as mRNA (BNT162b2 or mRNA1273), nonreplicating adenoviral vector (AZD1222, Sputnik V, or Ad26.COV2.S) and inactivated (BBIBP-CorV) vaccines (41, 42); however, such articles have only described S-binding IgA antibodies, and no neutralizing activity of such IgA antibodies has been evaluated. Thus, our present report, which describes the nIgA activity in detail together with the nIgG activity in individuals with COVID-19, should shed light on the understanding of the immune response upon SARS-CoV-2 infection.

It should be noted that the limitation of the present work is that we did not systematically characterize SARS-CoV-2-specific secretory IgA antibodies, which represent the dominating immunoglobulins in exocrine secretions. In the literature, there are currently only a few reports documenting the role of secretory IgA antibodies in protection against SARS-CoV-2 infection. Studies to elucidate the protective effect of secretory IgA upon SARS-CoV-2 infection and anti-COVID-19 vaccination remain to be conducted.

In conclusion, the present data showed that the SARS-CoV-2-neutralizing serum/plasma IgA response is seen earlier than the nIgG response, suggesting that the humoral IgA plays a critical role in the acute phase of the infection, although that nIgA response diminishes faster than the nIgG response, which should in turn play a role in the later phase of infection. Further, in previously SARS-CoV-2-infected individuals, the first (initial) administration of COVID-19 mRNA vaccines induces high titers of nIgG and nIgA; however, the neutralizing activity of IgA also diminishes faster than that of IgG.

MATERIALS AND METHODS

Participants. A total of 14 individuals who were diagnosed with COVID-19 based on positive RNA-quantitative-PCR (RNA-qPCR) results from February to April 2020 and another 8 individuals who received a COVID-19 mRNA vaccine (either BNT162b2 or mRNA-1273) from April to July 2021 after recovery from COVID-19 and agreed to participate in the clinical studies (Certified Review Board of National Center for Global Health and Medicine approval numbers NCGM-G-003472 and NCGM-G-003536) for specimen collection and convalescent plasma donation (10, 43) were enrolled in the present work. The data were analyzed anonymously. Nasopharyngeal swab samples were collected at early time points after admission and stored at -80°C until use. Sera or plasmas were obtained intermittently and stored at -20°C until use.

Cells, viruses, and immunoglobulin purification. Transmembrane protease serine 2 (TMPRSS2)-overexpressing VeroE6 (VeroE6^{TM_{PRSS2}}) cells (RRID CVCL_YQ49) were obtained from the Japanese Collection of Research Bioresources (JCRB) Cell Bank (Osaka, Japan). VeroE6^{TM_{PRSS2}} cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 $\mu\text{g}/\text{mL}$ penicillin, 100 $\mu\text{g}/\text{mL}$ kanamycin, and 1 mg/mL G418 under a humidified atmosphere containing 5% CO_2 at 37°C . A SARS-CoV-2 strain, SARS-CoV-2^{05-2N} (PANGO lineage B) was isolated in March 2020 in Tokyo, Japan, as previously described (16). IgG fractions were obtained from SARS-CoV-2-infected individuals' sera or plasmas using spin column-based antibody purification kit (Protein G) (Cosmo Bio, Tokyo, Japan). IgA fractions were purified from the IgG purification flowthrough using Pierce Jacalin agarose (Thermo Fisher Scientific, Waltham, MA) and eluted in phosphate-buffered saline (PBS) using Zeba spin desalting columns, 40K MWCO (Thermo Fisher Scientific). The total human IgG

and IgA concentrations were determined using the human IgG enzyme-linked immunoassay (ELISA) kit and human IgA ELISA kit, respectively (abcam, Cambridge, UK). The purity of the IgG and IgA was determined using the capillary electrophoresis Simple Western Jess apparatus and the total protein detection module (Protein Simple, San Jose, CA), anti-human IgA, alpha-chain specific, horseradish peroxidase (HRP)-linked antibody no. 80403, and anti-human IgG, Fc gamma fragment-specific, HRP-linked antibody no. 32935 (Cell Signaling Technology, Danvers, MA). The purities of the IgG and IgA were approximately 85% (84.0 ± 2.4) and 75% (75.2 ± 1.6), respectively, as four representative IgG and IgA samples were examined.

Antiviral assays. The SARS-CoV-2 neutralizing activity of donated plasma and purified immunoglobulin against the wild-type SARS-CoV-2 (PANGO lineage B) was determined as previously described (10, 16, 20). In brief, VeroE6^{TM_{PRSS2}} cells were seeded in 96-well flat-bottom microtiter culture plates at a density of 1×10^4 cells/well. On the following day, the virus (SARS-CoV-2^{25-2N}) was mixed with the various concentrations of the serum/plasma or purified immunoglobulin fractions and incubated for 20 min at 37°C. The preincubated mixture was inoculated to the cells at a multiplicity of infection (MOI) of 0.01. The cells were cultured for 3 days, and the number of viable cells in each well was measured using cell counting kit 8 (Dojindo, Kumamoto, Japan). The potency of SARS-CoV-2 inhibition by sera/plasmas or purified immunoglobulin was determined based on its inhibitory effect on virally induced cytopathicity in VeroE6^{TM_{PRSS2}} cells. The amounts of S1-binding antibodies in each plasma sample were determined using anti-SARS-CoV-2 ELISA (IgG) and (IgA) (Euroimmun, Lübeck, Germany). The serially diluted donor 84 (D84) plasma (10) was used as a reference (100%) for quantification with a four-parameter logistic curve calculated using ImageJ (Fiji) (Fig. S1) (44).

Statistical analysis. The 50% neutralizing titers of sera/plasmas (NT₅₀), 50% effective concentration of purified IgG and IgA (nIgG-EC₅₀ and nIgA-EC₅₀, respectively), and the amounts of anti-SARS-CoV-2-S1-binding IgG and anti-SARS-CoV-2-S1-binding IgA (S1-binding IgG and S1-binding IgA, respectively) were determined and compared between the acute and convalescent phases of COVID-19 and between the moderate and severe symptoms using a Wilcoxon signed-rank test and Wilcoxon rank sum test, respectively. The attenuation rates of nIgG-EC₅₀ and nIgA-EC₅₀ were calculated by dividing the nIgG-EC₅₀ or nIgA-EC₅₀ values determined to be the latest in the study with the highest neutralizing activity (lowest nIgG-EC₅₀ or nIgA-EC₅₀ values) by days 28, 42, and 56 postonset. To examine which of the nIgG-EC₅₀ and nIgA-EC₅₀ values diminished faster in the convalescent-vaccine group, the values obtained by subtracting the lowest EC₅₀ values from the highest EC₅₀ values post-1st vaccine administration were compared. Then, the attenuation rates of nIgG-EC₅₀ and nIgA-EC₅₀ and the differences after vaccination were compared by the Wilcoxon signed-rank test. To compare the amounts of S1-binding IgG and IgA timewise, the slope indexes were determined with the initial (first) value obtained and the following (second) value obtained for S1-binding IgA and IgG amounts in each individual. Then, the slope indexes of S1-binding IgA and IgG amounts were compared using the Wilcoxon signed-rank test. The correlations and corresponding 95% confidence intervals of NT₅₀, IgG-EC₅₀, IgA-EC₅₀, S1-binding IgG, and S1-binding IgA were determined using the repeated measures correlation method to consider the within-individual association (45) using rmcrr (R package v. 0.4.6) (46). The computed correlation coefficients were considered high if the absolute value was above 0.7, moderate if the absolute value was between 0.4 and 0.7, and low if the absolute value was below 0.4, according to Guilford's rule of thumb. The nIgG-EC₅₀ and nIgA-EC₅₀ kinetics were fitted with a generalized additive model (17) with mgcv (R package v. 1.8-40). The fitting was implemented for superimposed data of all samples. All the analyses were performed using R statistical software v. 4.1.3 (47). Statistical significance was defined as $P < 0.05$.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

ACKNOWLEDGMENTS

This work was supported in part by the Japan Agency for Medical Research and Development (AMED) (grant no. JP20fk0108160 and JP20fk0108502 to K.M. and JP20fk0108502, JP20fk0108257, and JP20fk0108510 to H.M.), by MHLW Research on Emerging and Re-emerging Infectious Diseases and Immunization Program (grant no. JPMH20HA1006 to K.M.), by a grant from National Center for Global Health and Medicine Research Institute (grant no. 20A2003D to K.M.), and by the Intramural Research Program of the Center for Cancer Research, National Cancer Institute, National Institutes of Health (H.M.). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

We thank Mariko Kato for technical assistance.

We thank all the patients who participated in the clinical trial for the collection of convalescent plasma and staff of the Diseases Control and Prevention Center, Center for Clinical Sciences, Department of Hematology, Clinical Laboratory Department, Department of Clinical Engineering, and Nursing Department at the Central Hospital of the National Center for Global Health and Medicine.

Conceptualization: Y.T. and H.M.; methodology: Y.T., K.M., and H.M.; formal analysis: K.O., Y.S., and Y.U.; investigation: Y.T., K.O., and N.K.-I.; data curation: Y.T., Y.S., N.K.-I., M.T., and T.S.; resources: N.K.-I., M.T., T.S., and S.M.; writing-original draft: Y.T. and H.M.; writing-review and editing: K.O., Y.S., N.K.-I., Y.U., and K.M.; visualization: Y.T.; supervision: N.O., K.M., and H.M.; project administration: H.M.; funding acquisition: K.M. and H.M.

We have declared that no competing interests exist.

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