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Response kinetics of different classes of antibodies to SARS-CoV2 infection in the Japanese population: The IgA and IgG titers increased earlier than the IgM titers

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ABSTRACT

To better understand the immune responses to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in individuals with COVID-19, it is important to investigate the kinetics of the antibody responses and their associations with the clinical course in different populations, since there seem to be considerable differences between Western and Asian populations in the clinical features and spread of COVID-19. In this study, we serially measured the serum titers of IgM, IgG and IgA antibodies generated against the nucleocapsid protein (NCP), S1 subunit of the spike protein (S1), and receptor-binding domain in the S1 subunit (RBD) of SARS-CoV-2 in Japanese individuals with COVID-19. Among the IgM, IgG, and IgA antibodies, IgA antibodies against all of the aforementioned viral proteins were the first to appear after the infection, and IgG and/or IgA seroconversion often preceded IgM seroconversion. In regard to the timeline of the antibody responses to the different viral proteins (NCP, S1 and RBD), IgA against NCP appeared than IgA against S1 or RBD, while IgM and IgG against S1 appeared earlier than IgM/IgG against NCP or RBD. The IgG responses to all three viral proteins and responses of all three antibody classes to S1 and RBD were sustained for longer durations than the IgA/IgM responses to all three viral proteins and responses of all three antibody classes to NCP, respectively. The seroconversion of IgA against NCP occurred later and less frequently in patients with mild COVID-19. These results suggest possible differences in the antibody responses to SARS-CoV-2 antigens between the Japanese and Western populations.

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1. Introduction

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was declared a worldwide pandemic in early 2020, and morbidity and mortality associated with the disease continues to be encountered throughout the world. The coronavirus genome encodes four major proteins, namely, the spike protein (S), the nucleocapsid protein (NCP), the envelope protein, and the membrane protein. The spike protein consists of two subunits, S1 and S2, and the S1 subunit contains the receptor-binding domain (RBD) [1,2]. To obtain a clearer understanding of the immune responses to SARS-CoV-2 in patients with COVID-19, it is important to investigate the kinetics of the antibody responses to SARS-CoV-2 infection. Although numerous studies have investigated the kinetics of the antibody responses to SARS-CoV-2 infection, few studies have conducted serial and simultaneous measurements of the IgM, IgG, and IgA responses to various SARS-CoV-2 antigens in the same patients [3–6].

It has become apparent over time since the start of the pandemic that the clinical features and spread of COVID-19 may differ considerably among races: people in Western countries appear to be more susceptible to severe disease caused by the virus than people in East Asia, where the disease was originally identified [7]. It is considered that analysis of the kinetics and associations of the antibody responses to SARS-CoV-2 in relation to the clinical course, especially in different populations, may offer some clue(s) to the reason(s) for these ethno-racial differences. In general, in infectious diseases, at the first infection, the IgM response precedes the IgG and IgA responses. However, several studies have reported that SARS-CoV-2 IgG is often detected a little earlier, or almost at the same time during the clinical course of COVID-19, as SARS-CoV-2 IgM [3,8–14], although it still remains unclear if the IgG response actually precedes the IgM response, because of the limited number of samples examined and lack of serial measurement data. Furthermore, several reports from China have also indicated that IgG seroconversion preceded IgM seroconversion [15,16], suggesting the possibility that the antibody response patterns differ among populations of different countries.

To compare the timing of seroconversion for different antibody classes and verify the possible existence of cross-immunity between SARS-CoV-2 and other coronaviruses, frequent and serial measurements of antibodies against specific antigens of SARS-CoV-2, especially in the early phase after symptom onset diagnosis of COVID-19, are necessary; however, most studies until date have measured the antibody responses only once a week or so. Although several studies have investigated the antibody response kinetics in Japanese patients with COVID-19, they measured the responses of only limited antibody types within a limited time frame, which is not sufficient to precisely compare the differences in the timings of the seroconversions for various antibody types [6,17–20]. In particular, few studies have investigated the time-course of the IgA and other antibody responses to RBD by serial measurements in the Japanese population.

Under these circumstances, in the present study, we attempted to answer the following questions as they pertain to the Japanese population. Are there any differences in the responses of different antibody classes to different viral antigens, especially in the timing of seroconversion, in Japanese patients with SARS-CoV-2 infection?

2. Methods

2.1. Samples

We collected residual serum samples (samples remaining after routine clinical testing) of 58 subjects who had been diagnosed as having COVID-19 by RT-PCR. The subjects were classified into three groups according to the disease severity: severity group 1 (mild disease; did not require oxygen therapy), severity group 2 (moderately severe disease; required oxygen therapy, but not mechanical ventilatory support), and severity group 3 (severe disease; required mechanical ventilatory support). The characteristics of the subjects are described in Supplemental Table S1.

The current study was performed in accordance with the ethical guidelines of the Declaration of Helsinki. Participants were given an explanation about the study and informed consent was obtained in the form of an opt-out on the website. Patients who expressed their unwillingness to be enrolled in our study were excluded. The study design was approved by The University of Tokyo Medical Research Center Ethics Committee, which waived the need for obtention of written informed consent, because archived specimens were used and the data for this retrospective study were retrieved from medical records (2019300NI-3 and 2019300NI-4).

2.2. Measurements of antibodies against SARS-CoV2

Antibody testing was performed using an iFlash3000 fully automated chemiluminescence immunoassay analyzer (Shenzhen YHLO Biotech Co., Ltd., China). The assay procedure described by Qian C, et al. [21] was adopted, with minor modifications. Briefly, magnetic beads in these chemiluminescence immunoassay kits were coated with each of the major SARS-CoV-2 antigens (NCP, S1, RBD), and 5-μL serum samples were added to the beads. Then, acridinium-labeled anti-human IgM, IgG, or IgA conjugated antibody was used to detect the antibodies bound to the beads. The SARS-CoV-2 IgM, IgG, or IgG antibody titers were calculated as relative light units (RLU) and described in arbitrary units per milliliter (AU/mL), by comparing the RLU detected by the iFlash optical system with the cutoff calculated from the calibrators containing anti-SARS-CoV-2 IgM, IgG, or IgA chimeric antibody.

2.3. Statistical analysis

Locally weighted scatter plot smoothing (LOESS), which shows smooth lines through a timepoint or scatter plot based on regression analysis, were fitted to visualize the time-course of changes of the antibody titers from day 0 to day 50 (Supplemental Figure S2) or day 0 to day 210 (Fig. 1) after symptom onset, using the results of subjects whose antibody titers were monitored until at least 45 days after the symptom onset of COVID-19. To analyzes the time-course of the antibody responses in further detail, we constructed two sets of curves. The number of the samples used for describing these lines are listed in Supplemental Table S2.

All the data were statistically analyzed using SPSS (Chicago, IL). The results were expressed as dot plots, with the bar representing the median. We performed non-parametric analyses, since normality or equality of variance was rejected by the Kolmogorov-Smirnov test or the Levene test for most of the parameters or analyses. The antibody titers among three independent groups were compared using an independent Kruskal-Wallis test, followed by the Games Howell test for post hoc analysis, and the antibody titers, antibody titers adjusted to the maximum titers, days of peak titers, and days of intervals from diagnosis to seroconversion in three paired groups were compared using the Friedman rank test, followed by Bonferroni test for post hoc analysis. To visually understand whether the different antibody classes exhibit viral antigen-specific behaviors, we compared the antibody titers for all pairwise combinations and viral antigens at each timepoint (Fig. 3 and Supplemental Figure S3). We used the Wilcoxon signed rank test [22] to identify any differences between pairs of antibody data for individuals that did not show normal distribution. We adopted the Benjamini and Hochberg procedure [23] to reduce the false discovery rate for multiple comparisons. $P < 0.05$ was regarded as denoting statistical significance in all the analyses.

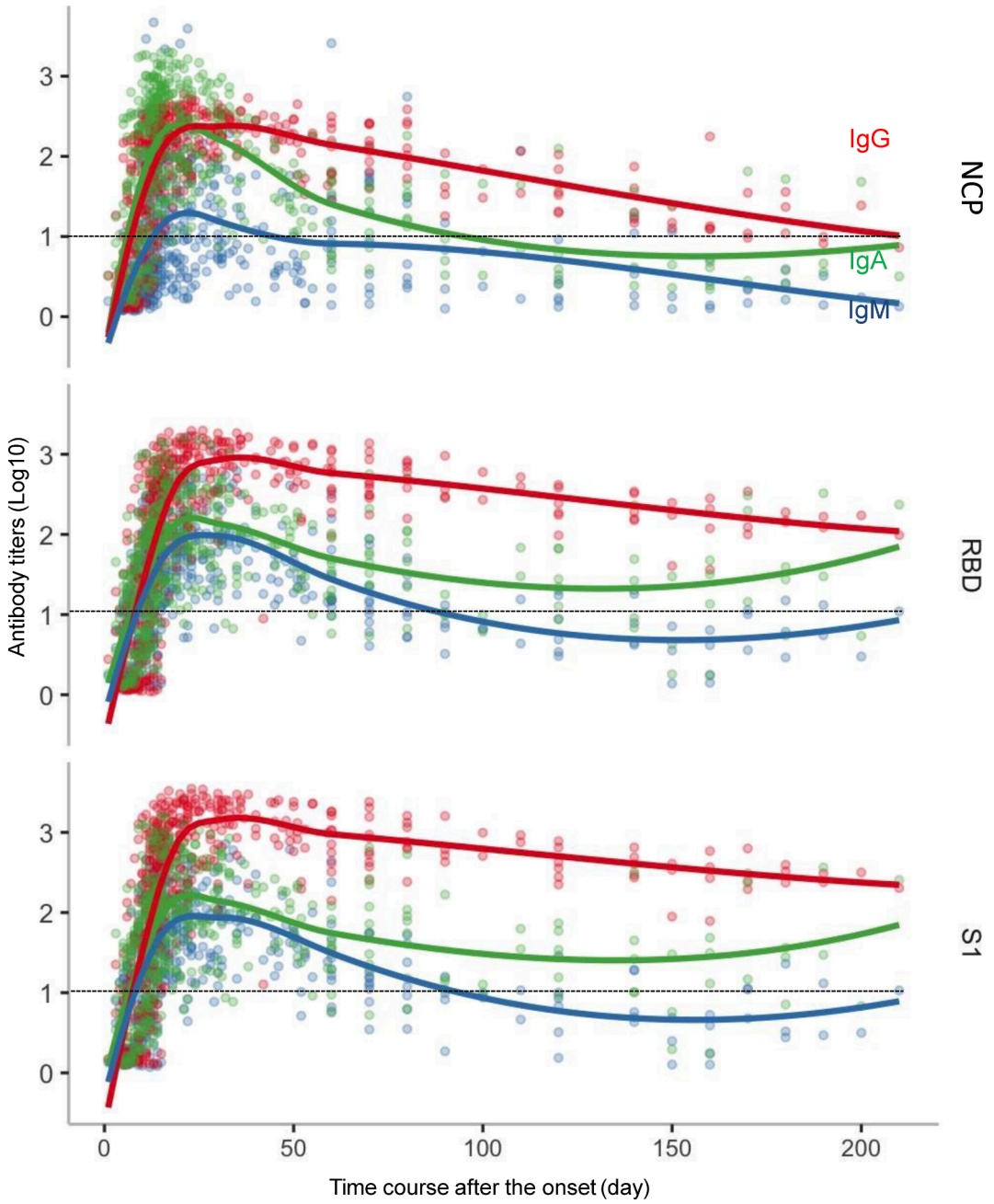

Fig. 1. Serial response curves of antibodies in patients with COVID-19. Local polynomial regression curves were fitted to indicate the antibody responses over time to the COVID-19 antigens, using the results of subjects whose antibody titers were monitored at least until day 45 after the onset of COVID-19 (n = 35). The dotted lines represent the cutoff levels.

3. Results

3.1. The IgG responses to all three viral proteins and responses of all three antibody classes to S1 and RBD were sustained for relatively long durations

We measured the serum titers of IgM, IgG, and IgA against NCP (IgM (NCP), IgG(NCP), and IgA(NCP)), S1 (IgM(S1), IgG(S1), and IgA(S1)), and RBD (IgM(RBD), IgG(RBD), and IgA(RBD)) in the residual samples of 58 patients with COVID-19. The cutoff titer for a positive response was set at 10 AU/mL for all the three antibody classes, which was determined by the productor and judged as being appropriate from the antibody titers in the sera collected from PCR-negative and PCR-positive COVID-19 patients (Supplemental Figure S1).

The approximate curves from 0 to 210 days (Fig. 1) and 0 to 50 days (Supplemental Figure S2), drawn from the results in the subjects whose antibody titers were monitored until at least 45 days after the onset of COVID-19 symptoms, are shown. Elevated IgG(S1) and IgG(RBD) titers were well sustained for more than 6 months, followed in duration by elevated IgA(S1) and IgA(RBD) titers.

3.2. The IgA response was seen earlier than the IgM and IgG responses

Next, we investigated the differences in the response kinetics of the different antibody classes in response to each of the major SARS-CoV-2 proteins (NCP, S1, RBD) (Fig. 2A and Fig. 3). In regard to the antibody responses to NCP, the IgA and IgG titers increased more rapidly than the IgM titers until day 34, and the IgG titers were higher than the IgA and

A

days after onset		day5-7	day8-10	day11-13	day14-16	day17-19	day20-24	day25-29	day30-34	day35-39	day40-44	day45-54	day55-64	day65-84	day85-104	day105-124	day125-144	day145-174	day175-214
n		21	31	45	40	26	33	15	17	9	5	17	13	16	4	7	5	7	4
NCP	IgM	1.1	1.16	1.09	1.15	1.08	1.24	1	1.06	1	1.2	1.12	1.36	1.31	1.5	1.57	1.6	1.14	1.25
	IgG	2.19	2.29	2.36	2.35	2.42	2.45	2.4	2.59	2.89	3	3	2.85	2.88	3	2.71	2.8	2.71	2.75
	IgA	2.71	2.55	2.56	2.5	2.5	2.3	2.6	2.35	2.11	1.8	1.88	1.77	1.81	1.5	1.71	1.6	2.14	2
p-value		***	***	***	***	***	***	***	***	***	*	***	**	***				*	
post hoc		†, ‡	†, ‡	†, ‡	†, ‡	†, ‡	†, ‡	†, ‡	†, ‡	†	†	†, §	†, §	†, §				†	
S1	IgM	1.95	1.81	1.51	1.38	1.38	1.3	1.33	1.29	1.44	1.4	1.29	1.38	1.06	1	1	1	1	1
	IgG	1.67	1.79	2.47	2.72	2.85	2.88	3	3	3	2.6	3	3	3	3	3	2.8	2.86	2.75
	IgA	2.38	2.4	2.02	1.9	1.77	1.82	1.67	1.71	1.56	2	1.71	1.62	1.94	2	2	2.2	2.14	2.25
p-value			*	***	***	***	***	***	***	**		***	***	***	*	**	*	**	*
post hoc			§	†, ‡	†, ‡	†, §	†, §	†, §	†, §	†, §		†, §	†, §	†, ‡, §	†	†	†	†	†
RBD	IgM	1.98	1.97	1.62	1.45	1.5	1.33	1.4	1.29	1.44	1.2	1.24	1.31	1.19	1.25	1	1	1	1
	IgG	1.52	1.68	2.27	2.56	2.73	2.76	2.8	2.88	2.89	2.6	3	3	3	3	3	2.8	2.86	2.75
	IgA	2.5	2.35	2.11	1.98	1.77	1.91	1.8	1.82	1.67	2.2	1.76	1.69	1.81	1.75	2	2.2	2.14	2.25
p-value		**	*	**	***	***	***	***	***	**		***	***	***	*	**	*	**	*
post hoc		§	§	†	†, §	†, §	†, §	†, §	†, §	†, §		†, §	†, §	†, §	†	†	†	†	†

rank: 1 low, 2, 3 high — p-value: *P < 0.05, **P < 0.01, ***P < 0.001 — post hoc: † P < 0.05 IgM vs. IgG, ‡ P < 0.05 IgM vs. IgA, § P < 0.05 IgG vs. IgA

B

days after onset		day5-7	day8-10	day11-13	day14-16	day17-19	day20-24	day25-29	day30-34	day35-39	day40-44	day45-54	day55-64	day65-84	day85-104	day105-124	day125-144	day145-174	day175-214
n		21	31	45	40	26	33	15	17	9	5	17	13	16	4	7	5	7	4
IgM	NCP	1.38	1.19	1.22	1.4	1.27	1.33	1.8	1.06	1.22	1	1	1.69	1.62	1.5	1.43	1.4	1.21	1.25
	S1	2.48	2.58	2.7	2.52	2.5	2.33	2.27	2.59	2.44	3	2.88	2.31	2.31	2.5	2.14	2.2	2.07	2.25
	RBD	2.14	2.23	2.08	2.08	2.23	2.33	2.13	2.35	2.33	2	2.12	2	2.06	2	2.43	2.4	2.71	2.5
p-value		**	***	***	***	***	***		***	*	**	***						*	
post hoc		†, ‡	†, ‡	†, ‡, §	†, ‡	†, ‡	†, ‡		†, ‡	†	†	†, ‡						‡	
IgG	NCP	2	2.19	1.76	1.4	1.08	1.12	1	1.12	1.22	1.4	1	1	1.12	1	1	1	1	1
	S1	2.43	2.29	2.64	2.82	3	2.94	3	3	2.78	2.8	3	3	2.84	3	3	3	3	3
	RBD	1.57	1.52	1.6	1.78	1.92	1.94	2	1.88	2	1.8	2	2	1.94	2	2	2	2	2
p-value		*	**	***	***	***	***	***	***	**		***	***	***	*	**	**	**	*
post hoc		§	‡, §	†, §	†, §	†, ‡, §	†, ‡, §	†, ‡, §	†, §	†		†, ‡, §	†, ‡, §	†, §	†	†	†	†	†
IgA	NCP	2.36	2.45	2.47	2.35	2.38	2.12	2.47	2.24	2.33	2	1.53	1.73	1.44	1.5	1.29	1.4	1.57	1.5
	S1	2.02	2.08	2.04	2.15	1.92	2	1.8	2	2.11	2.2	2.47	2.08	2.19	2.75	2.57	2.2	2.43	2.25
	RBD	1.62	1.47	1.49	1.5	1.89	1.88	1.73	1.76	1.56	1.8	2	2.19	2.38	1.75	2.14	2.4	2	2.25
p-value			***	***	***	*						*		*		*			
post hoc			†, §	†, §	†, §	‡						†		‡		†			

rank: 1 low, 2, 3 high — p-value: *P < 0.05, **P < 0.01, ***P < 0.001 — post hoc: † P < 0.05 NCP vs. S1, ‡ P < 0.05 NCP vs. RBD, § P < 0.05 S1 vs. RBD

Fig. 2. Ranking of the titers of the three antibody classes and of antibodies against the three viral antigens. We compared the titers of the antibody classes generated against each of the viral proteins (A) and the titers of each of the antibody classes generated against the three viral proteins (B) in all the subjects (n = 58) using the Friedman rank test, followed by Bonferroni's test for post hoc analyses. The data are shown are the average titers of the three antibody classes. (A, B) *P < 0.05, **P < 0.01, ***P < 0.001, (A)† P < 0.05 IgM vs. IgG, ‡ P < 0.05 IgM vs. IgA, §P < 0.05 IgG vs. IgA, (B) † P < 0.05 N vs. S1, ‡ P < 0.05 N vs. RBD, §P < 0.05 S1 vs. RBD.

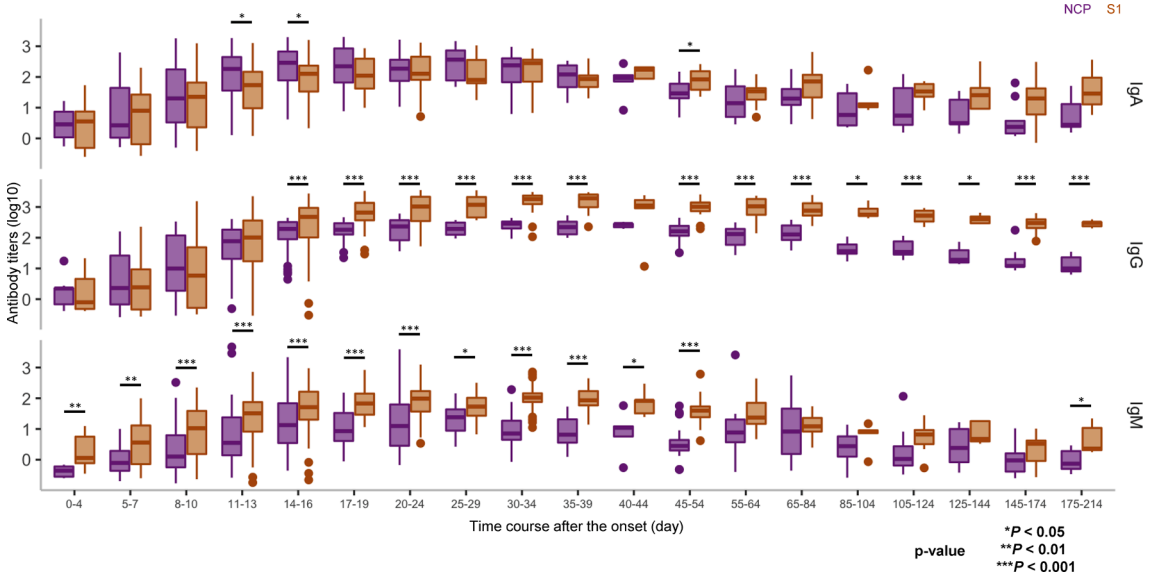

Fig. 3. Antibody titers by number of days after the symptom onset of COVID-19. We compared the titers of each of the antibodies generated against NCP and S1 in all the subjects (n = 58) , using the Wilcoxon matched-pairs signed rank test with multiple testing correction by the Benjamini-Hochberg method. *P < 0.05, **P < 0.01, ***P < 0.001.

IgM titers in the late phase. As for the antibody responses to S1 protein and RBD, the IgA titers increased more rapidly than the IgM and IgG titers until day 10, but thereafter, the IgG titers were the highest, followed by the IgA and IgM titers.

3.3. The IgM and IgG responses to S1 occurred earlier, while the IgA response to NCP occurred earlier

Comparison of the responses of each of the antibody classes to the three viral proteins also revealed differences in the response kinetics (Fig. 2B and Supplemental Figure S3). For the case of IgM, the IgM(S1) titers increased faster, followed by IgM(RBD) and IgM(NCP) until day

13, after which the IgM(S1) titers were almost similar to those of IgM (RBD). For the case of IgG, almost throughout the course, the IgG(S1) titers were the highest, followed by the IgG(RBD) and IgG(NCP) titers. For IgA, the IgA(NCP) titers remained higher until day 19, and from day 45, the IgA(NCP) titers tended to become lower than the IgA(S1) and IgA (RBD) titers.

To eliminate the confounding influence of differing detection ability of the antibody kits for the antibody classes or antibodies for specific antigens, we also analyzed the time-courses of the antibody titers adjusted to the maximum titers in subjects whose antibody titers were monitored until at least day 45 after the diagnosis and in whom the maximum titers of all the measured antibodies were over the cutoff level. As shown in Supplemental Figure S4, the elevated IgG titers were well sustained, followed by elevated IgA and IgM titers, and the elevated antibody titers against S1 and RBD were sustained for longer periods of time than those against NCP. The time-courses of the antibody responses in representative subjects are shown in Fig. 4.

3.4. The maximum titers of the antibodies against the different viral proteins differed among the antibody classes

Next, we compared the maximum titers and intervals from diagnosis to peak titer for the different antibody classes to the three viral proteins. In regard to the antibody responses to NCP, the maximum titers of IgA and IgG were higher than the maximum titer of IgM, and the peak IgG (NCP) titer was reached later than the peak IgA(NCP) and IgM(NCP)

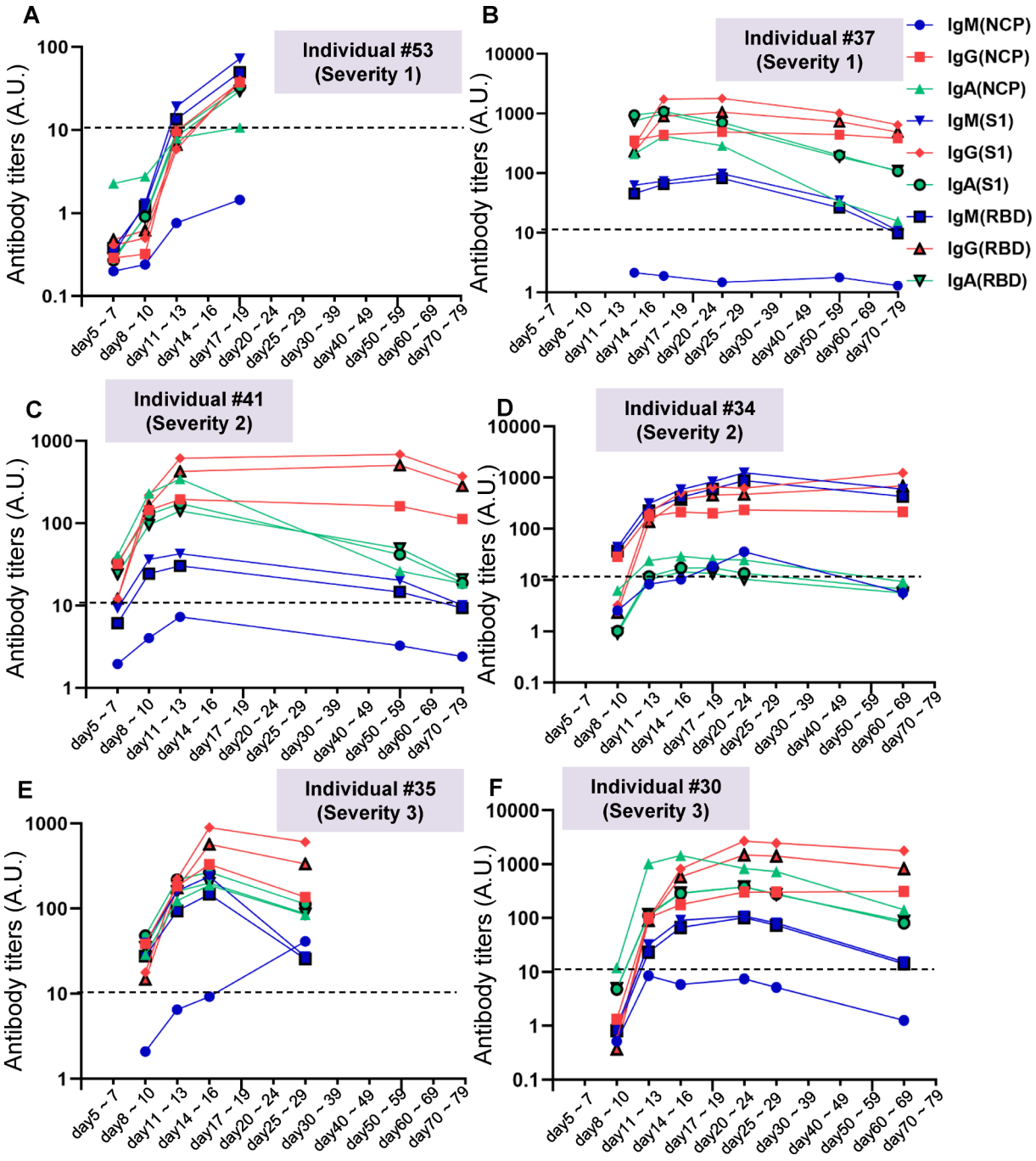

Fig. 4. Time-course of antibody responses in representative COVID-19 subjects. Time-courses of the antibody responses in two subjects each with mild COVID-19 (A, B), moderate COVID-19 (C, D), and severe COVID-19 (E, F) are shown. The dotted lines represented the cutoff titers.

titers (Fig. 5A). As for the antibody responses to S1 and RBD, the maximum IgG(S1, RBD) titers were higher than those of the IgM(S1, RBD) or IgA(S1, RBD) titers, and the IgG(S1, RBD) titers reached their peak later than the IgA(S1, RBD) and IgM(S1, RBD) titers (Fig. 5A). When we compared the peak titers among the antibody classes, the peak IgM(S1) and IgM(RBD) titers were higher that the peak IgM(NCP) titer, and the order in which the maximum IgG titers were reached was IgG (S1), followed by IgG(RBD), followed by IgG(NCP) (Fig. 5B). For the case of IgA, the maximum IgA(NCP) titer was higher than the maximum IgA(S1) and IgA(NCP) titers, and the IgA(NCP) titer reached its peak earlier than the IgA(S1) titer (Fig. 5B). These results suggest that the maximum antibody titers and the time-points at which the antibody titers reached their peaks differed, especially between the antibodies against NCP vs. antibodies to S1 or RBD, and between IgA vs. IgM or IgG.

3.5. IgM(NCP) seroconversion was the last to occur and was less frequent

Next, we compared the time-point of seroconversion for each of the antibodies. Comparison of the seroconversions for the antibody classes to a specific viral protein revealed that IgM(NCP) seroconversion was the last to occur and also relatively less frequent (Supplemental Figure S5A). Furthermore, IgM(NCP) seroconversion also occurred later/less frequently as compared to IgM(S1) and IgM(RBD) seroconversion (Supplemental Figure S5D). No significant difference in the timing of seroconversion was observed between antibodies to S1 and RBD (Supplemental Figure S5B, C), or between antibodies of the IgG and IgA classes (Supplemental Figure S5E, F).

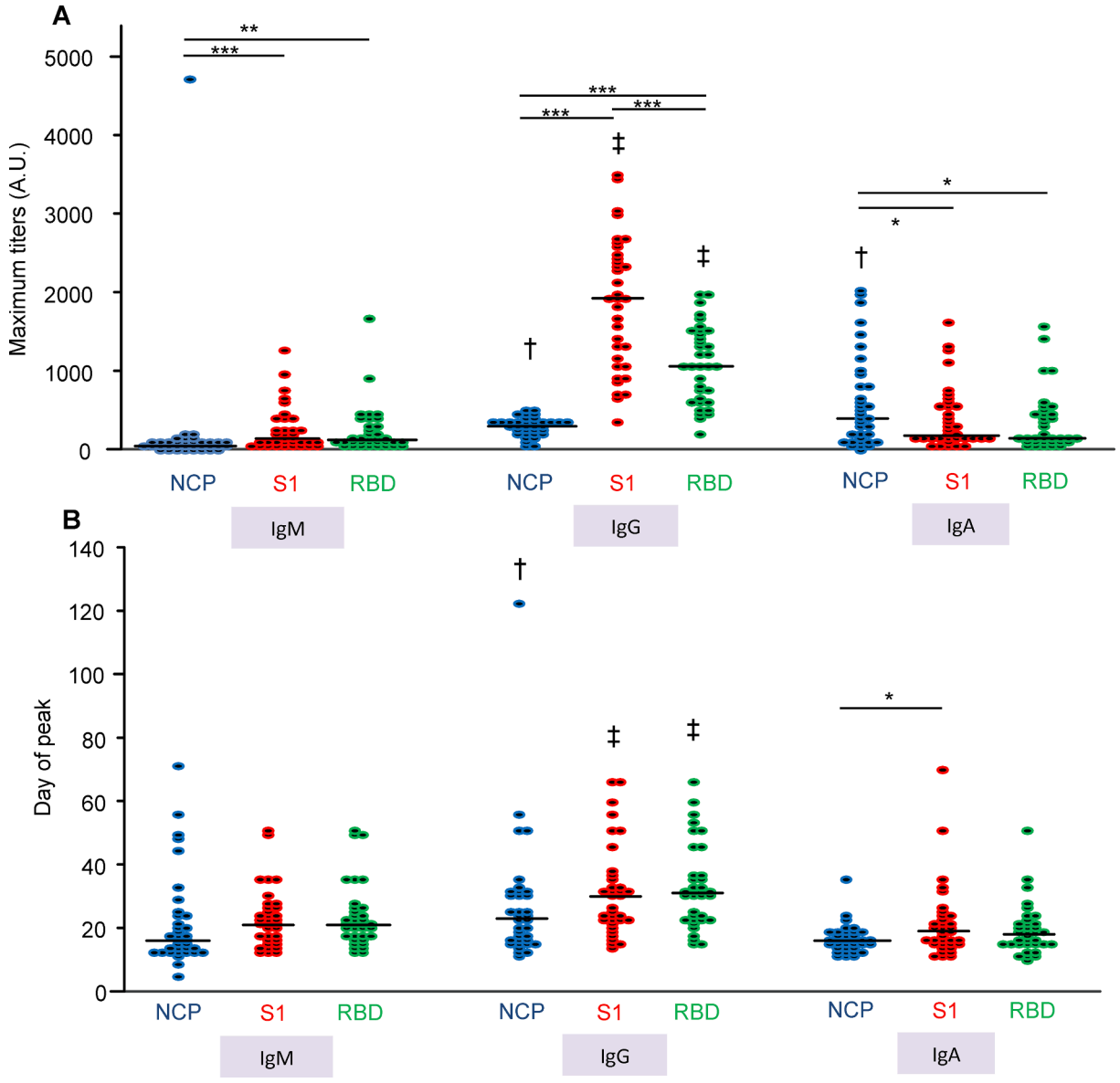

Fig. 5. Maximum titers and the interval from the onset of COVID-19 to detection of the peak titers for the different classes of antibodies. We compared the maximum titers (A) and the interval from the onset of COVID-19 to detection of the peak titers (B) in subjects whose antibody titers were monitored until at least day 45 after the onset of COVID-19 (n = 35), using the Friedman rank test, followed by Bonferroni's test as post hoc analysis. *P < 0.05, **P < 0.01, ***P < 0.001 for comparison of antibody responses to the three viral proteins. (A) † P < 0.001 vs. IgM (NCP), ‡ P < 0.001 vs. IgM(NCP) and IgA(NCP) or IgM(RBD) and IgA(RBD), (B) † P < 0.05 vs. IgM(NCP) and P < 0.01 vs. IgA(NCP), ‡ P < 0.05 vs. IgM(S1) or IgM (RBD) and P < 0.001 vs. IgA(NCP) or IgA(RBD), for the comparison of the response of each antibody subclass to the three viral proteins. The black bars represent the median values.

3.6. The maximum antibody titers and the time-points of seroconversion were associated with the severity of COVID-19

Comparison of the maximum titers among patient groups with different levels of disease severity revealed that the maximum IgA(NCP), IgM(S1), and IgM(RBD) titers were higher in severity group 3 group than in severity group 1 (Fig. 6). IgA(NCP) seroconversion occurred later and less frequently in severity 1 group than in severity group 2, and the subjects who did not show seroconversion also appeared to be well observed in IgM(NCP), IgM(S1), IgM(RBD), IgG(NCP), IgG(S1), and IgG (RBD) responses (Fig. 7).

4. Discussion

The present study revealed differing kinetics of different antibody responses to SARS-CoV-2 antigens in COVID-19 patients. Among the

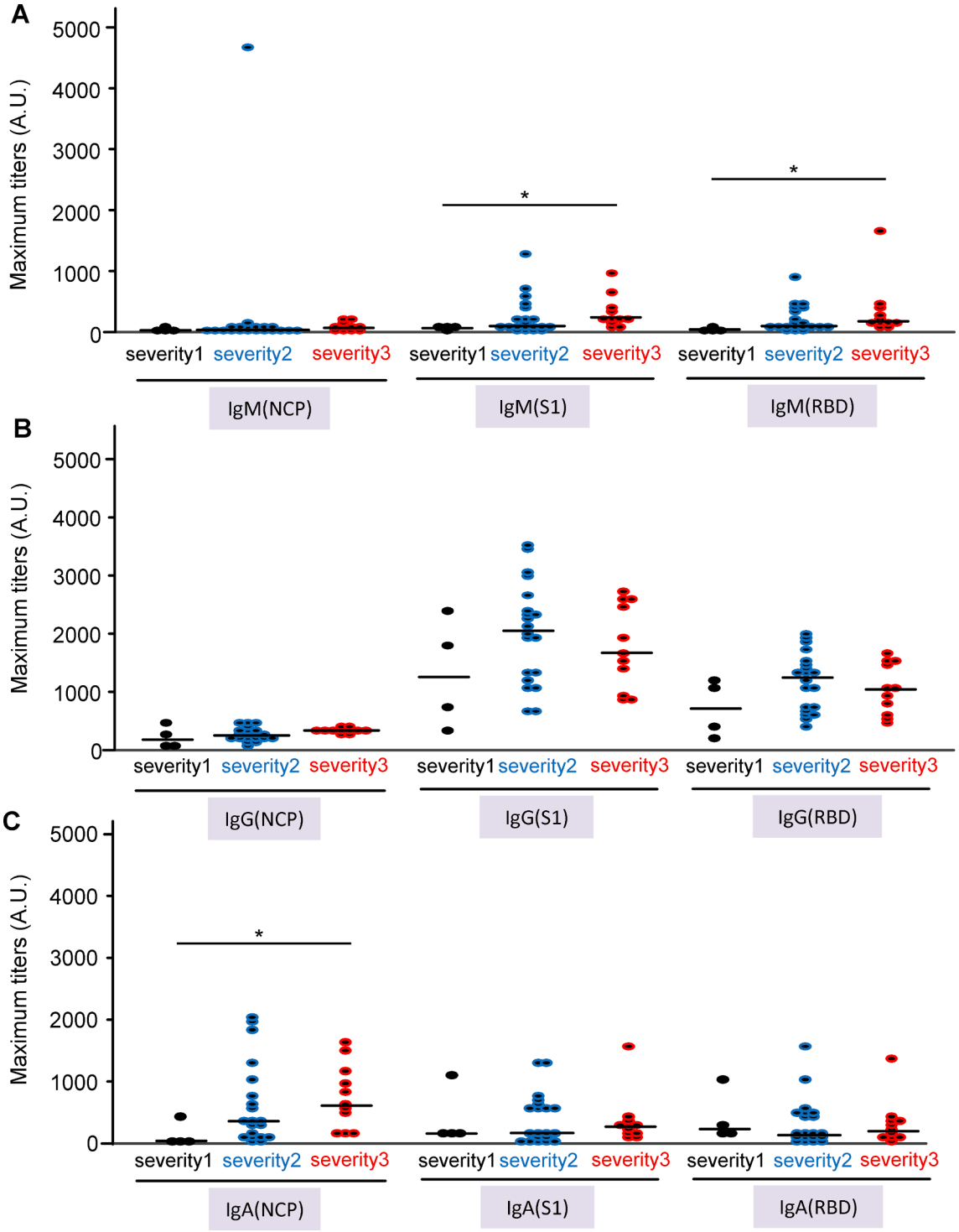

Fig. 6. Maximum titers of the different antibody classes in COVID-19 patients classified by the disease severity. We compared the maximum titers of different types of antibodies in patients with COVID-19 classified by the disease severity (severity group 1, n = 4; severity group 2, n = 20; severity group 3, n = 11) whose antibody titers were monitored until at least day 45 after the diagnosis of COVID-19. *P < 0.05, **P < 0.01.

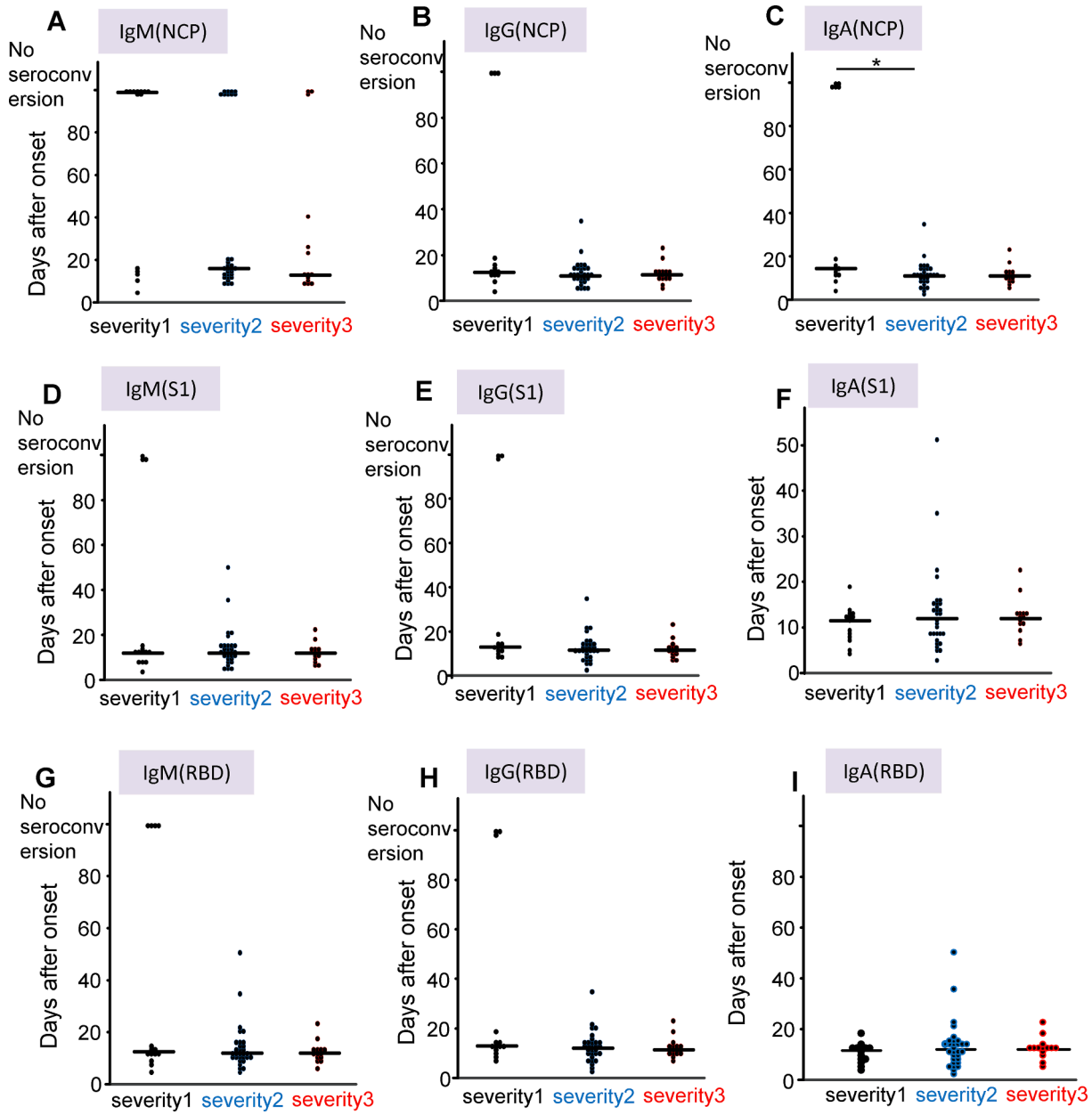

Fig. 7. Interval from symptom onset to seroconversion for different antibody classes in COVID-19 patients classified by the disease severity. We compared the day from disease onset to seroconversion for the different classes of antibodies in patients with COVID-19 classified by the disease severity (severity group 1, n = 14; severity group 2, n = 30; severity group 3, n = 14). *P < 0.05, **P < 0.01.

IgM, IgG, and IgA antibody classes, IgA was generated rapidly against all the viral proteins. In regard to the responses of the three antibody classes to the three major viral proteins, unique characteristics of the IgA responses were observed; while the IgM(S1) and IgG(S1) responses were seen earlier than the response of either class of antibodies to NCP and RBD, the IgA(NCP) response occurred earlier than the IgA response to S1 or RBD. In regard to the responses of the antibody classes to each specific antigen, the IgM(S1) and IgG(S1) titers, and the IgA(NCP) titers increased relatively early. The earlier elevation of the IgA(NCP) titer than the IgA(S1) titer was consistent, while that of IgM(S1) as compared to IgM(NCP) was inconsistent with a previous report from Europe [24].

The unique characteristics of the IgA responses observed in the present study might be due to the different pathway of production of IgA vs the IgG and IgM antibodies; IgA antibody repertoires are generated mainly in the mucosa-associated lymphoid tissue [25]. While some IgA clones are derived from a germinal center (GC)-independent pathway, others are derived from the GC pathway. Outside the GCs, antigen-specific activated B cells undergo immunoglobulin class switching without somatic mutation to differentiate into short-lived plasma cells, leading to low-affinity antibody production of short durations [26,27]. Therefore, the IgA clones formed via the GC-independent pathway are not associated with prior appearance of IgM antibodies, resulting in rapid, but short-lived IgA antibody responses to the antigens.

In regard to the different antibody response kinetics to NCP versus the S protein, this difference could arise from the different forms in which these proteins exist *in vivo*. NCP forms a complex with the viral RNA, while S does not form complexes with high-molecular-weight substances, such as nucleic acids. Therefore, the complex formed by NCP with the viral RNA might potentially lead to cross-linking of the B-cell receptors (BCRs) or dual signaling by the BCRs and Toll-like receptors (TLRs) for RNA, resulting in antigen-specific B-cell activation, independently of follicular helper T cells outside the GCs [28,29]. In this

case, the N antigen-reactive B cells undergo immunoglobulin class switching without somatic mutation to differentiate into short-lived plasma cells. Other N antigen-reactive B cells enter the GCs and undergo both immunoglobulin class switching and affinity maturation through somatic hypermutation to differentiate into memory B cells or long-lived plasma cells [30]. On the contrary, the S protein, which is not bound to viral RNA, cannot lead to cross-linking of the BCRs or dual signaling by the BCRs and TLRs. Therefore, S antigen-reactive B cells are thought to require helper signals arising in the GCs, and S antigen-reactive B cells might be activated exclusively in the GCs.

Although elevation of IgA could precede that of IgM as described above, considering the established concept of class switching, our finding that the IgG responses appeared earlier than the IgM responses at least suggests that a substantial proportion of Japanese subjects might possess cross- immunity for SARS-CoV-2, which might be associated with the disease severity of COVID-19 and explain the different susceptibilities to COVID-19 between the Japanese and Western populations. Existence of cross-immunity between SARS-CoV-2 and other coronaviruses has been suggested previously [8]. Grifoni, et al. reported that the NCP and S proteins of SARS-CoV-2 show high degrees of homology of 90% and 76%, respectively, to the corresponding proteins of severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) [31]. In fact, evidence of humoral immunity to SARS-CoV-2 has been reported in subjects without COVID-19 [32]. Presence of cross-immunity might be one of the reasons for the differences in clinical phenotypes between the west and Asia, since people in East Asia might have been exposed to viruses similar to SARS-CoV-2 even before the appearance of SARS-CoV-2. Actually, reports of earlier IgG seroconversion as compared to IgM seroconversion were published from China [15,16]. In a recent study conducted in the Japanese population, the IgG seroconversion rates were higher than the IgM seroconversion rates for NCP and S [20], which is concordant with our present results.

While the existence of cross-immunity might protect persons from severe COVID-19, no significant association was observed between the timing of seroconversions for the various antibody classes and the severity of COVID-19 (Supplemental Table S3), whereas IgA(NCP) seroconversion occurred later and less frequently in severity group 1 than in severity group 2, and there were several subjects who did not show either IgM(NCP) or IgG(NCP) response (Fig. 7). In a recent study conducted in the Japanese population, IgM(NCP) and IgG(NCP) seroconversions between day 11 and day 21 from symptom onset were observed less frequently in patients with mild COVD-19 than in those with moderate or severe COVID-19 [20]. Considering the proposed hypothesis from experimental studies of SARS-CoV-1 that antibodies to S1 have beneficial properties, while antibodies to NCP protein have harmful properties [33,34], early IgG seroconversion to SARS-CoV-2 might not necessarily be associated with better outcomes of COVID-19, whereas later/less frequent antibody responses to the NCP protein might be associated with protection against COVID-19 in Japan. Further basic and clinical studies are necessary to investigate this hypothesis.

A major limitation of the present study is that we did not directly prove the presence of cross-immunity. Comparison of the timings of seroconversion alone cannot confirm the presence of cross immunity, since previous reports have also shown earlier IgG and/or IgA seroconversions after symptom onset in COVID-19 patients than in patients with other infections caused by coronaviruses, which suggest that the early appearance of IgG and/or IgA responses might be characteristic of SARS-CoV-2 infection [8–11,15,16]. However, we believe that the results of the present study, in which we serially and simultaneously measured various antibody classes, would help us better understand the characteristics of the humoral immunity against SARS-CoV2 in Japanese subjects, who have been proposed as possessing some degree of resistance to the virus as compared to western populations [7].

In regard to the antibody response kinetics in the intermediate and later part of the course, as expected, the maximum titers of IgG were higher, the peak IgG titers were reached later, and the elevated titers were sustained for longer periods of time than the IgM and IgA responses (Figs. 1–3), which are consistent with previous reports [3,5,35,36]. In regard to the association of the antibody responses with the disease severity, the results of the present study demonstrating an association between the maximum antibody titers and the disease severity are also consistent with previous reports [3,5,6,37].

In summary, the present study revealed differing response kinetics of different classes of antibodies to the major SARS-CoV-2 antigens in patients with COVID-19. Among the IgM, IgG, and IgA antibody classes, IgA was generated most rapidly against all the viral proteins. In regard to the responses of the antibody classes to the different viral proteins, while IgM(S1) and IgG(S1) occurred earlier than the response of either class of antibodies to NCP or RBD, the IgA(NCP) response occurred earlier than the IgA response to S1 or RBD. The earlier IgG and/or IgA seroconversion as compared to IgM seroconversion suggests the possible existence of cross-immunity for SARS-CoV-2 in the Japanese population, which might be associated with the lower severity of COVID-19 in Japan.

CRediT authorship contribution statement

Makoto Kurano: Conceptualization, Investigation, Formal analysis, Writing – original draft, Supervision, Funding acquisition. **Yoshifumi Morita:** Investigation. **Yuki Nakano:** Investigation. **Rin Yokoyama:** Investigation. **Takuya Shimura:** Investigation. **Chungen Qian:** Methodology. **Fuzhen Xia:** Methodology. **Fan He:** Methodology. **Liang Zheng:** Methodology. **Hiroko Ohmiya:** Methodology. **Yoshiro Kishi:** Methodology. **Jun Okada:** Methodology. **Naoyuki Yoshikawa:** Investigation. **Kazuki Nakajima:** Investigation. **Yutaka Nagura:** Investigation. **Hitoshi Okazaki:** Writing – review & editing. **Daisuke Jubishi:** Writing – review & editing. **Kyoji Moriya:** Writing – review & editing. **Yasuyuki Seto:** Writing – review & editing. **Fumihiko Yasui:** Writing – review & editing. **Michinori Kohara:** Writing – review & editing. **Masatoshi Wakui:** Writing – review & editing. **Takeshi Kawamura:** Writing – review & editing. **Tatsuhiko Kodama:** Conceptualization, Writing – review & editing, Supervision. **Yutaka Yatomi:** Conceptualization, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Availability Statement

The datasets generated or analyzed in the current study will be made available upon reasonable request.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2021.108491.

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