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One-year antibody durability induced by EuCorVac-19, a liposome-displayed COVID-19 receptor binding domain subunit vaccine, in healthy Korean subjects

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ABSTRACT

Objective: EuCorVac-19 (ECV-19), an adjuvanted liposome-displayed receptor binding domain (RBD) COVID-19 vaccine, previously reported interim Phase 2 trial results showing induction of neutralizing antibodies 3 weeks after prime-boost immunization. The objective of this study was to determine the longer-term antibody response of the vaccine.
Methods: To assess immunogenicity 6 and 12 months after vaccination, participants in the Phase 2 trial (NCT04783311) were excluded if they: 1) withdrew, 2) reported COVID-19 infection or additional vaccination, or 3) exhibited increasing Spike (S) antibodies (representing possible non-reported infection). Following exclusions, of the 197 initial subjects, anti-S IgG antibodies and neutralizing antibodies were further assessed in 124 subjects at the 6-month timepoint, and 36 subjects at the 12-month timepoint.
Results: Median anti-S antibody half-life was 52 days (interquartile range [IQR]:42-70), in the "early" period from 3 weeks to 6 months, and 130 days (IQR:97-169) in the "late" period from 6 to 12 months. There was a negative correlation between initial antibody titer and half-life. Anti-S and neutralizing antibody responses were correlated. Neutralizing antibody responses showed longer half-lives; the early period had a median half-life of 120 days (IQR:81-207), and the late period had a median half-life of 214 days (IQR:140-550).
Conclusion: These data establish antibody durability of ECV-19, using a framework to analyze COVID-19 vaccine-induced antibodies during periods of high infection.

Introduction

The rapid development and deployment of vaccines against SARS-CoV-2, the etiological agent of the COVID-19 pandemic, has been remarkable and has led to large-scale testing of numerous new vaccine technologies, notably including viral vectors and messenger ribonucleic acid (mRNA) vaccines [1]. While most approved vaccines have focused on vaccine systems that express the entire large Spike (S) surface protein of SARS-CoV-2, its compact receptor binding domain (RBD) is the target of most neutralizing antibodies [2], and thus the RBD has also been the target of extensive vaccine efforts [3]. We previously found that the RBD could be displayed

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on immunogenic liposomes to enhance functional immunogenicity in preclinical testing [4–6]. Stable display of recombinant proteins on the surface of immunogenic liposomes is enabled by the inclusion of cobalt-porphyrin-phospholipid (CoPoP) which sequesters the His-tag of His-tagged proteins and can improve the delivery of antigens to antigen-presenting cells in draining lymph nodes [7]. The display of antigens on adjuvanted liposome surfaces is an emerging concept that holds potential for improved antigen delivery to immune cells, co-delivery of antigen and adjuvant, and arrayed display of antigens to the immune system [8]. More recently, this approach was translated into human clinical testing in the form of EuCorVac-19 (ECV-19), which safely induced neutralizing antibody responses in recently reported interim Phase 2 trial results (NCT04783311) [9]. ECV-19 also includes a unique *Escherichia coli*-derived monophosphoryl lipid A (MPLA) termed EcML, which is produced in genetically engineered *E. coli,* thereby bypassing some of the challenges of MPLA production [10]. MPLA is a detoxified form of lipid A and an immunostimulatory toll-like receptor 4 agonist that has a long history of use as a human vaccine adjuvant, frequently in liposome format [11]. Liposomes have attracted considerable interest in vaccinology and have been used to formulate immunostimulatory molecular adjuvants that exert immunological effects through engagement with specific targets such as toll-like receptors, depending on the adjuvant which is used [12]. Liposomes can also influence antigen delivery to immune cells depending on liposome size, charge, and composition [13].

COVID-19 vaccine development during the pandemic has been challenging due to several factors including conversion of populations from naïve to vaccine- and virus-exposed, difficulty in confirming infections in participants (including asymptomatic cases), emergence of variants of concern resistant to initial vaccines, and declining levels of antibodies in immunized subjects. Although the immune response to SARS-CoV-2 vaccination is multifaceted, involving both humoral (antibody-mediated) and cellular immune responses, the ability of vaccine-induced antibodies to neutralize SARS-CoV-2 has emerged as the major correlate of protection [14]. As SARS-CoV-2 transitions from a pandemic virus to an endemic one, vaccination strategies must consider the durability of vaccine-induced immunity, which is essential in evaluating the long-term effectiveness of vaccination strategies and informing public health policies for considering booster injections.

Several factors could contribute to the durability of SARS-CoV-2 vaccine-induced immunity. Individual factors, such as age, sex, underlying health conditions, and previous COVID-19 infection, may impact the durability of vaccine-induced immune responses. Vaccine characteristics, such as the platform used, dosage, immunization schedule, and adjuvants, would also influence the magnitude and persistence of immune responses. As a novel vaccine system comprising nanoliposome-displayed RBD adjuvanted with integrated EcML, the durability of ECV-19-induced immune responses has not yet been assessed in humans. In this study, we report on the follow-up 6- and 12-month data obtained in the Phase 2 trial in South Korea for which only interim results were previously reported which analyzed serology up to 3 weeks following booster immunization [9]. During this 1-year period, the prevalence of SARS-CoV-2 infection increased in South Korea from approximately 0.5% to 40% [9]. Furthermore, the Wuhan strain was supplanted first by the Delta variant and then by the RBD sequence-divergent Omicron variant in this time frame [15]. Since the vaccine made use of the original Wuhan strain RBD antigen, the clinical effectiveness of this vaccine technology against later variants (that were circulating when the study was conducted) would be difficult to meaningfully assess in this small Phase 2 trial. However, meta-analyses have suggested that waning antibody responses of SARS-CoV-2 vaccines can inform design of vaccination programs [16], and therefore we set out to assess the durability of ECV19 antibody responses. In this study, we use a methodology to account for likely unreported COVID-19 cases in trial participants to determine durability of ECV-19-induced antibody response.

Methods

Study design and participants

A Phase 2 randomized, observer-blind, placebo-controlled study in healthy adults between 19 and 67 years old, who did not have known previous COVID-19 infection or vaccination, was carried out between July 2021 to September 2022 at five hospital sites in South Korea. Written informed consent was obtained from all participants and the trials were done according to the principles of the Declaration of Helsinki and Good Clinical Practice. The study was registered with *ClinicalTrials.gov* (NCT04783311). Details of study design, safety, and immunological results up to 3 weeks after the second immunization (median of 21 days with interquartile range [IQR] of 21-23 days; until October 2021; referred to as 3W timepoint herein) were reported previously [9]. In brief, the high-dose group (n = 100) received 20 μg of recombinant SARS-CoV-2 RBD (Wuhan-Hu-1 strain, GenBank: MT380724.1) on immunogenic liposomes that contained 20 μg of MPLA derived from genetically engineered EcML and 40 μg CoPoP twice at 3-week interval. The low-dose group (n = 100) received 10 μg RBD with 10 μg EcML and 20 μg CoPoP with the same schedule.

Serum sample collections and immunological assessment

Serum was collected at ~6 months (median of 179 days with IQR of 174-186 days; February-March 2022) and ~12 months (median of 352 days with IQR of 344-359 days; July-September 2022) after the second immunization. These are referred to as 6M and 12M timepoints, respectively, herein. Anti-S titers were assessed via enzyme-linked immunosorbent assay (ELISA) using recombinant Wuhan-Hu-1 S glycoprotein as a plate coating agent. Virus neutralization was assessed against the Wuhan strain using focus reduction neutralization test (FRNT). Refer to the Supporting Information for the methods of determination for anti-S titers and neutralizing antibody titers. Antibody responses to the His-tag of the RBD were assessed as previously described [9].

Exclusion criteria for the immunological assessment

Participants in the placebo group (n = 29), were injected saline twice with the same schedule and subsequently received other SARS-CoV-2 vaccines approved by the Korean government before the 6M timepoint. Therefore, their data are not considered here. For 200 people who received at least one ECV-19 immunization, two participants withdrew from the study by 3W timepoint as reported previously [9], and one volunteer received a vaccine (other than ECV-19) during the period. These three subjects were excluded. Of the 197 subjects who received two doses of ECV-19 vaccines, serum samples were collected from 186 (at 6M) and 173 (at 12M) individuals. During the ~12-month follow-up, participants were asked by the study investigators (or self-reported voluntary) whether they received a COVID-19 vaccine other than the study vaccine (ECV-19) or experienced COVID-19 infection (without specific diagnostic criteria). If a participant reported such event(s), any immunological data collected after the event were excluded from the analysis. In addition, if a person's paired sample showed an increase in anti-S titers over time (i.e., 6M titer was higher than 3W titer, or 12M titer was higher than 6M titer in the same individual), which was indicative of a non-reported (possibly asymptomatic) COVID-19 infection(s), his/her data were excluded. As a

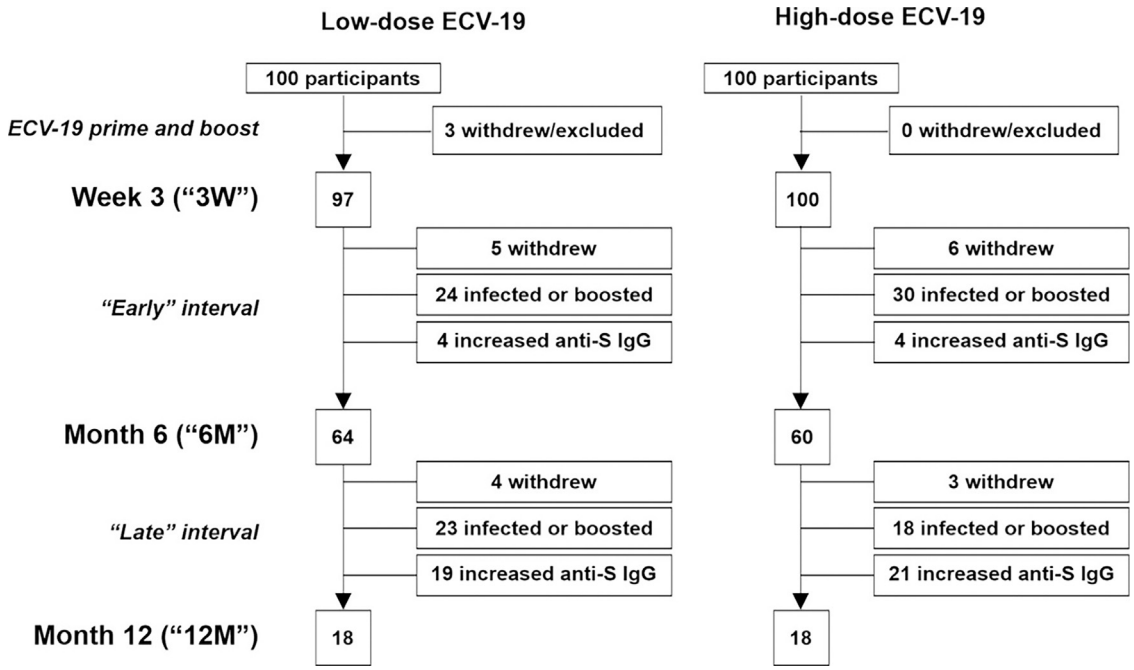

Figure 1. Subject selection for the analysis of ECV-19 antibody durability. ECV-19, EuCorVac-19;Ig, Immunoglobulin; M, month; W, week.

result, anti-S titer data were analyzed in 124 subjects at 6M and 36 at 12M timepoints. The number and reason for the exclusion are summarized in Figure 1. For $FRNT_{50}$ analysis, further exclusions were performed if subjects showed increased $FRNT_{50}$ over time. $FRNT_{50}$ data from a total of 104 subjects at 6M and 24 subjects at 12M timepoints were analyzed, and the number and reason for exclusion in the $FRNT_{50}$ analysis are summarized in Supplementary Figure 1. To estimate the half-life of $FRNT_{50}$ values, to avoid a risk of overestimation, subjects whose $FRNT_{50}$ level went down to 10 (less than minimum detection level) at 6M or 12M timepoints were excluded, ended up with n = 98 and 17 subjects at 6M and 12M, respectively (Supplementary Figure 1).

Statistical analyses

Statistical analysis is described throughout the text and figure captions. Refer to the Supporting Information for additional information on the statistical methods used.

Role of funding source

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Results

A randomized Phase 2 trial was carried out after enrolling 229 healthy adults and the initial vaccine or placebo injection was carried out between July 7 and August 24, 2021. The safety and immunogenicity results up to 3 weeks after second immunization (3W timepoint) have been reported [9]. During the 1-year follow-up period, serious adverse events (SAEs) and adverse events of special interests (AESIs, as defined by "Ministry of Food and Drug Safety, considerations for the COVID-19 Vaccine Development [September 28, 2020]", such as cardiovascular, hematological AEs) were monitored in 64 (low-dose) and 72 (high-dose) participants who did not receive other COVID-19 vaccinations nor withdrew from the study. There was only one SAE, a tendon rupture, in the high-dose group, and it was considered unrelated to ECV-19. There were no AESIs in either high-dose or low-dose groups.

For the placebo group (n = 29), since all participants showed only background levels of anti-S titers and virus neutralization activity 3 weeks after second placebo immunization [9], this group is not considered further. Of the 197 subjects (97 in low-dose group and 100 in high-dose group) who received two doses of ECV-19 vaccines and enrolled for the immunogenicity analysis at 3W timepoint, 54 subjects reported either receiving COVID-19 vaccinations (other than ECV-19) or reported COVID-19 infections before the 6M timepoint. In addition, 11 subjects withdrew from the immunological assessments (no serum samples were collected at 6M). From 6M to 12M (~12 months after second immunization) period, an additional 42 individuals reported either vaccination or infection, and seven subjects withdrew. Anti-S titers at 3W, 6M, and 12M for the subjects who did not have reported vaccination/infection events or withdrew from the study during the follow-up are shown in Supplementary Figure 2a. While median titers decreased in both dose groups from 3W to 6M as expected, the median titers at 12M were higher than that at 6M. When the changes in anti-S titers were examined individually, ~6% of subjects showed higher titers at 6M compared to 3W, and ~50% of subjects demonstrated higher titers at 12M compared to 6M (Supplementary Figure 2b). The results strongly suggest that a significant proportion of study participants had unreported infections (possibly asymptomatic infections) and/or vaccinations during the 12M follow-up period. Therefore, to account for this possibility, any participants who showed any increase in anti-S titers over time were further excluded. As a result, anti-S titer data from 124 and 36 subjects were utilized for the analysis at 6M and 12M timepoints, respectively. The flowchart for the exclusions is presented in Figure 1.

Table 1
Participant demographic characteristics

	Lo dose			Hi dose		
Time	3W	6M	12M	3W	6M	12M
Characteristics: N	97	64	18	100	60	18
Sex						
Male: no. (%)	60 (62)	41 (64)	13 (72)	44 (44)	32 (53)	12 (67)
Female: no. (%)	37 (38)	23 (36)	5 (28)	56 (56)	28 (47)	6 (33)
Age years (y)						
All: Mean (SD)	41 (12)	40 (12)	39 (11)	42 (13)	42 (14)	44 (11)
19-42 y: no. (%)	51 (53)	34 (53)	10 (56)	49 (49)	32 (53)	9 (50)
43-67 y: no. (%)	46 (47)	30 (47)	8 (44)	51 (51)	28 (47)	9 (50)

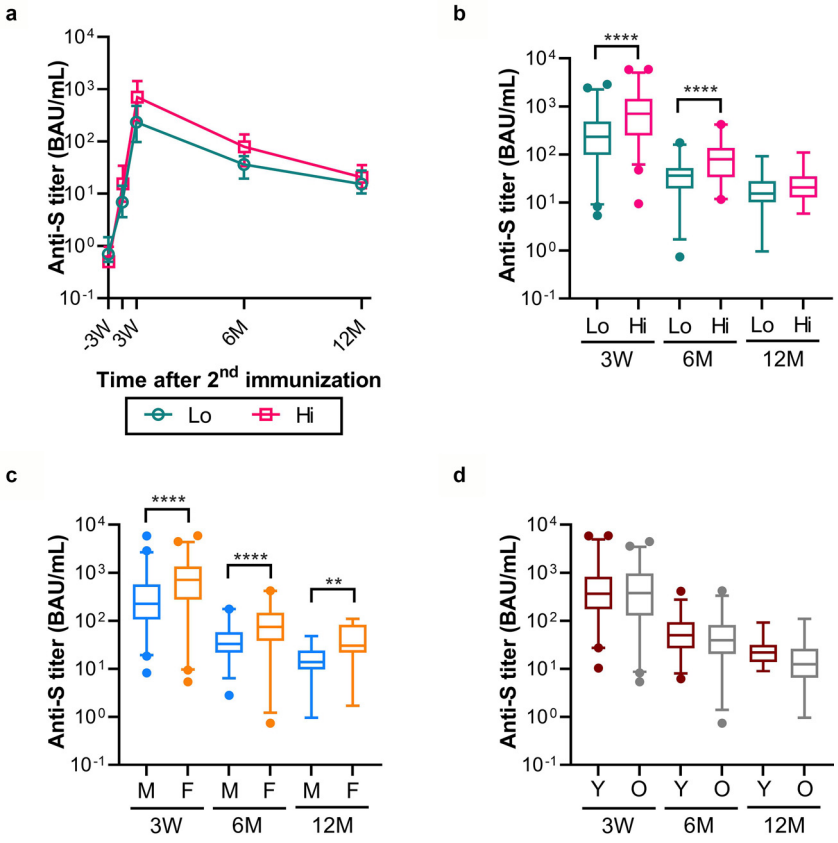

Figure 2. Change in anti-S antibody titers over one year. Subjects were immunized twice at low (Lo) or high (Hi) doses of EuCorVac-19 vaccines twice at 3-week interval. Serum samples were collected on the day of each immunization, then at 3-week (3W), 6-month (6M) and 12-month (12M) after the second immunization. (a) Anti-S titers (median and interquartile range, IQR) for each group at each timepoint are shown. The x-axis is proportional to time. The subjects were grouped by dose (b), sex (M, male; F, female) (c) or age (Y, 19-42 y; O, 43-67 y) (d), then the box plot (25/50/75 percentiles) with 2.5/97.5 percentiles (error bars) in each group are shown. The difference between two groups at each timepoint was assessed by a Mann-Whitney test, and indicated **, *** and **** correspond to *P*-values of <0.01, <0.001, and <0.0001, respectively.
M, month; W, week.

The demographic characteristics and anti-S titers for the original 197 subjects and selected 124 subjects at 3W were compared (Table 1 and Supplementary Figure 3). There was no significant difference in age and sex distributions ($p > 0.25$ by Fisher's exact tests), and anti-S titers were similar ($p > 0.79$ by Mann-Whitney tests) between the two data sets in each dose group. The results indicate there was no selection bias in the analysis. In addition, there was no difference in age and sex distributions in each dose group between 6M and 12 M ($p > 0.41$ by Fisher's exact tests).

Overall changes in anti-S titers from all (up to 3W) or selected subjects (at 6M and 12M) are shown in Figure 2a. In both dose groups, 12M titers remained higher than before vaccination (-3W timepoints; $P < 0.0001$ for both). As reported previously (up to the 3W timepoint) [9], there were significant impacts of dose (Figure 2b) and sex (Figure 2c) on anti-S titers at 6M, and the difference between men and women still reached significance at 12M. In this study population (only 4 of 197 subjects were within 65-67-year-old age range, and all others were less than 65 years old), age did not have a significant impact (Figure 2d).

For subjects with both 3W and 6M data (n = 124) or both 6M and 12M data (n = 36), half-life of anti-S titers was calculated using an exponential decay model during each observation period (Figure 3). The period between 3W and 6M is referred to as the "early" decay period and between 6M and 12M is the "late" decay period hereafter. The median (and interquartile range; [IQR]) half-life was 51.8 (41.9 to 70.3) days in early decay period, and 130.0 (96.8 to 168.5) days in late decay period, and the early decay half-lives were significantly shorter than the late decay half-lives ($P < 0.0001$ by a Mann-Whitney test, Figure 3a). When the half-life data were stratified by the dose (Figure 3b), sex (Figure 3c), and age (Figure 3d), significant differences were seen

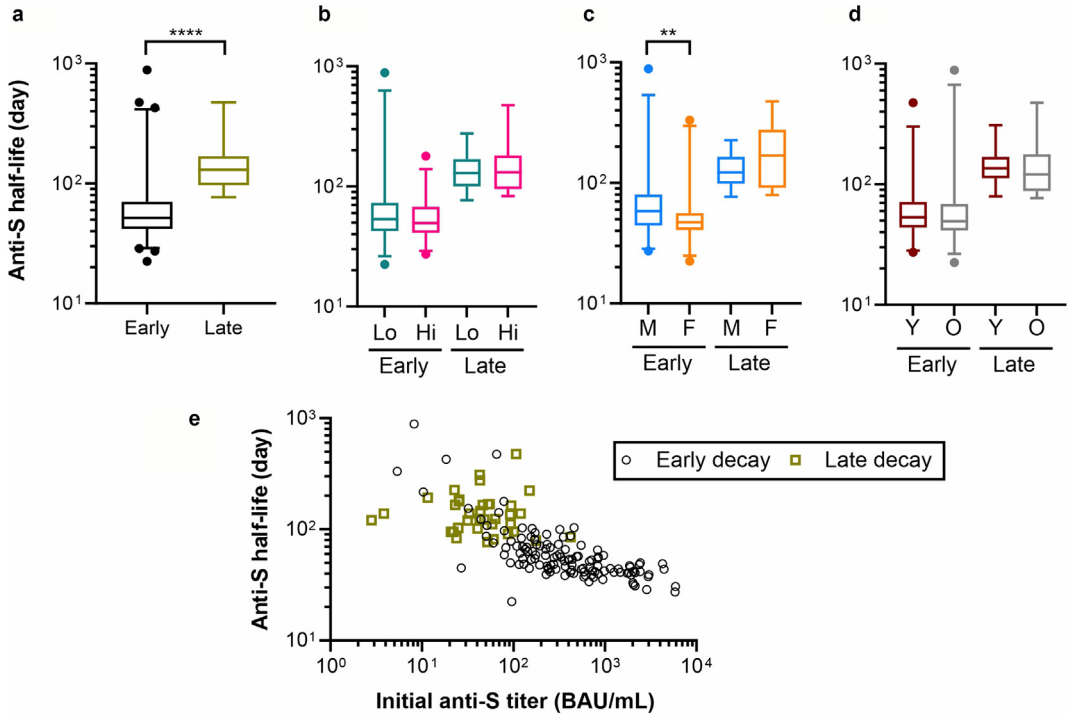

Figure 3. Half-life of anti-S antibody titers. (a) For individuals who had both 3W and 6M data (n = 124) or both 6M and 12M data (n = 36), half-life of anti-S titers was calculated using an exponential decay model during 3W-6M (early) or during 6M-12M (late) decay period. The data were grouped by dose (b), sex (M, male; F, female) (c), or age (Y, 19-42 years; O, 43-67 years) (d). The box plot (25/50/75 percentiles) with 2.5/97.5 percentiles (error bars) in each group is shown. Significant differences by a Mann-Whitney test between two groups are indicated ** and ****, which correspond to *P*-values of <0.01 and <0.0001, respectively. (e) The correlation between initial anti-S titers (anti-S titers at 3W for early decay data, and those at 6M for late decay data) and their half-life are shown. Each dot represents data from each individual. When both early and late decay data were combined (n = 124 + 36), the Spearman coefficient was -0.805 (95% confidence interval, 95% CI, -0.854 to -0.740; P <0.0001).
M, month; W, week

only between men (median of 58.3 days) and women (47.1 days) in the early decay period (P = 0.009).

When the antibody half-lives were plotted against corresponding initial anti-S titers (anti-S titers at 3W for early decay half-live data, and those at 6M for late decay half-life data) individually, there was a negative correlation between the two readouts in early decay period (Figure 3e). The Spearman coefficient was -0.700 (95% CI, -0.782 to -0.594) and the correlation was significant (P <0.0001). Data from the late decay period was more scattered, and the correlation was not significant (P = 0.524). However, when both early and late decay data were combined (n = 124 + 36), the Spearman coefficient became -0.805 (95% CI, -0.854 to -0.740; P <0.0001). For subjects with initial anti-S titers of > ~200 BAU/mL, most showed half-lives of ~50 ± 10 days. Subjects with an initial anti-S titer of < ~50 BAU/mL tended to show half-life of >100 days. When initial anti-S titers were between 10-200 BAU/mL, regardless of early or late decay periods, subjects were likely to demonstrate similar half-lives at the same initial anti-S titer levels. To determine the impact of each factor on half-life, a multiple linear regression analysis was performed using combined data (n = 160). In the model, log-transformed half-life was entered as a dependent variable, and log-transformed initial anti-S titer, dose, sex, age group (19-42 years or 43-67 years), and observation period (early or late) as explanatory variables. The initial anti-S titers had the strongest impact (P <0.0001), followed by observation period (P = 0.013) and age group (P = 0.032). The effects of dose (P = 0.066) and sex (P = 0.163) were insignificant.

6M and 12M serum samples were tested by FRNT assay with the Wuhan strain of SARS-CoV-2, matching the ancestral viral strain of the antigen. There were significant correlations between anti-S titers and $FRNT_{50}$ values both at 6M (n = 104) and 12M (n = 24) timepoints (P <0.0001 for both by a Spearman rank test) (Figure 4a). While 6M and 12M data points shifted to the left, indicative of functional maturation compared to 3W data points, 6M and 12M data were also more scattered (Spearman coefficients were 0.94 (95% CI: 0.92-0.96), 0.72 (0.60-0.80), and 0.81 (0.60-0.92) at 3W, 6M and 12M, respectively) and more deviated from a linear correlation (R^2 of linear fits in log-transformed anti-S and log-transformed $FRNT_{50}$ values were 0.87, 0.45 and 0.58 at 3W, 6M and 12M, respectively). Thus, with the limited data set, it is difficult to judge whether there was functional antibody maturation over time. The change in $FRNT_{50}$ values over time is seen in Figure 4b.

As predicted from the strong correlation between anti-S titers and $FRNT_{50}$ values, when FRNT data at 3W, 6M, and 12M were stratified by dose, sex, or age (Supplementary Figure 4), results were similar to those shown in Figure 2.

Half-life in $FRNT_{50}$ values were also individually calculated in each observation period (Figure 4c). The median (IQR) half-life was 120.3 (81.1 to 207.1) days in early decay period, and 213.5 (139.6 to 549.7) days in late decay period, and the early decay half-lives were again significantly shorter than the late decay half-lives (P = 0.002). There was a strong negative correlation between initial values and the half-live data (Figure 4d). The Spearman coefficient was -0.663 (95% CI; -0.757 to -0.542) with P <0.0001 when both early and late decay data were combined (n = 115).

ECV19 contains a six-residue His-tag, thus there was theoretical risk that the vaccine could induce anti-His-tag antibody responses. When a fraction of -3W (before immunization) and 3W (peak of immune response) samples (n = 21 for each) were tested previously for anti-His-tag antibody responses using an anti-His-tag chimeric human monoclonal antibody as a standard, ~10% were

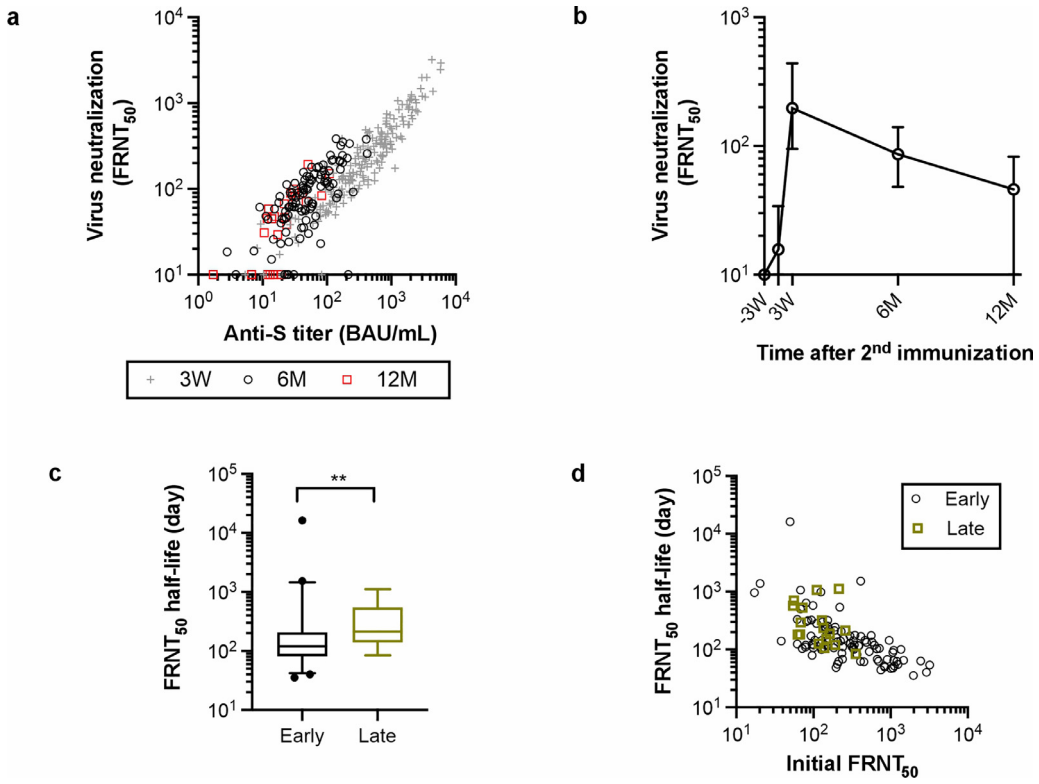

Figure 4. Analysis of neutralizing antibodies with $FRNT_{50}$ characterization. (a) the correlation between anti-S titers and $FRNT_{50}$ values is shown. Each dot represents data from each individual at 3W (n = 197), 6M (n = 104), and 12M (n = 24). There was a significant correlation at each timepoint (P <0.0001 for all three timepoints by a Spearman rank test). (b) $FRNT_{50}$ values (median and IQR) at each timepoint are shown. The x-axis is proportional to time. (c) For each individual, half-life of $FRNT_{50}$ values was calculated using an exponential decay model during 3W-6M (early, n = 98) or during 6M-12M (late, n = 17) decay period. The box plot (25/50/75 percentiles) with 2.5/97.5 percentiles (error bars) in each period is shown. ** indicates P-value of <0.01 by a Mann-Whitney test. (d) The correlation between initial $FRNT_{50}$ values and their half-life is shown. Each dot represents data from each individual. When both early and late decay data were combined, the Spearman coefficient was -0.663 (95% CI, -0.757 to -0.542; P <0.0001).
FRNT, focus reduction neutralization test; M, month; W, week.

sero-positive (more than mean plus 2 SD of ELISA absorbance units for blank wells) at both timepoints, but all of the positive signals were lower than 4 ng/mL of the standard [9]. When anti-His antibody responses were evaluated at 12M (n = 87) in this study, again only ~7% (6 of 87) of participants had detectable responses, and their ELISA absorbance values were lower than that at 16 ng/mL of standard (Supplementary Figure 5).

Discussion

The COVID-19 pandemic has led to rapid development of vaccine countermeasures. Two associated challenges are evolving SARS-CoV-2 variants that escape immunity and waning immunity. With respect to the former point, this small trial was not designed to assess efficacy in preventing COVID-19, and several participants developed it. ECV-19 used an RBD antigen derived from the ancestral Wuhan strain, and by the time the 12M serum was collected around September 2022, 8 months had passed since the Omicron variant supplanted the Delta strain in South Korea [15]. ECV-19-induced antibodies were 6-fold less effective at neutralizing the Omicron strain relative to the ancestral Wuhan strain [9]. With respect to waning immunity, understanding vaccine-induced neutralizing antibody response decay is important, yet relatively under-reported [17]. Through measuring of the long-term half-lives of COVID-19 vaccines, planning of boosting regimes could be facilitated in the future. Another factor to consider is that this study involved vaccination of participants who had not been exposed to COVID-19 infection or vaccination, whereas in the future that will no longer be the case.

When a vaccine trial is conducted in an area where the pathogen is highly endemic, it is challenging to distinguish immune responses induced by the vaccination and by natural infection(s). In this study, we used the exclusion criteria of any increase in anti-S titers overtime, but other studies used different criteria, such as >2-fold increase in titers [18] or presence of anti-nucleocapsid IgGs [19,20]. Whichever criteria are selected, unless all participants are carefully examined (such as by RT-PCR-based testing) with high frequency, there are risks of misclassification. To evaluate the robustness of anti-S half-life values reported in this manuscript, the half-life values were calculated using different exclusion criteria (Supplementary Figure 6). The early decay half-lives were between 51 and 55 days when 2- to 0.5-fold titer increase exclusion criteria were used. Similarly, in the same fold-increase range, the late decay half-lives were between 109 and 137 days. Therefore, we believe the exclusion criteria for this study were adequate.

After immunization or infection, without re-immunization or re-infection, antibody titers usually show two-phase kinetics, rapid decay followed by slower decay. To describe the two-phase change by a single mathematical model, different approaches have been utilized [21–26]. However, to describe the half-life for first 3 to 12M after the last immunization (rapid decay phase), a one-phase exponential decay model is common [27–35]. Therefore, this model was applied to this study. The half-life in the early decay period (between 3W and 6M) was shorter than in the late decay period (between 6M and 12M). The early decay half-life of 52 days for ECV-19 vaccines is similar to the ones reported in several other studies evaluating widely used mRNA-1273 or BNT162b2 vaccines;

45 to 53 days. A summary of immunological half-life for COVID-19 vaccines is shown in Supplementary Table 1. While dose and sex had an effect on anti-S titers, the impact of those factors on half-life was minimal. Our results are in line with other studies where there was no significant impact of sex and age on decay kinetics [26,28,36].

This study revealed a strong negative correlation between initial anti-S titers and the half-life; higher titers were associated with shorter half-life. While such association has been reported for COVID-19 [31,32] and influenza [37,38] vaccines, this study evaluated the correlation on individual basis, not by a group comparison. Furthermore, subjects with the same level of initial anti-S titers (at least 10-200 BAU/mL level) demonstrated similar half-life, regardless of whether the data were obtained from early or late decay period. To our knowledge, this study is the first to report such phenomenon, at least for COVID-19 vaccines. The result of multiple linear regression analysis suggests that the observation period had a significant impact on half-life ($P = 0.013$) even after adjusting the initial anti-S titers and other factors. However, the impact was much smaller than that for the initial titers ($P < 0.0001$).

Certain limitations should be pointed out with this analysis. This study included a limited number of subjects, especially in the late decay period, after exclusion of subjects with reported and suspected COVID-19 infections and vaccinations. In addition, no biospecimen samples were collected to uncover cellular mechanisms, such as quantifying short-lived and long-lived plasma cells, and memory B cells. Previous preclinical studies showed that a similar adjuvant system induced antigen-specific long-lived plasma cells in mice [7]. Additionally, T cell response durability was not assessed in this study, which is an important component of immunity including for COVID-19 vaccines [39]. Thus, the immunological assessment of vaccine durability in this study was restricted to antibody titer and neutralizing antibody levels in subjects of limited sample size. A larger study is required to confirm the impact of initial titers and observation period on half-life, and additional analysis could assess a mechanistic explanation of the phenomenon if confirmed. Currently, a Phase 3 trial is being conducted in the Philippines (NCT05572879), and this should provide further insight into 6M and 12M antibody durability, albeit in a population that has been exposed to SARS-CoV-2 virus and vaccines to a greater extent.

Conclusion

Understanding COVID-19 vaccine durability is relevant for developing countermeasures to control the disease. In this study, we demonstrate a methodology to analyze ECV-19 vaccine-induced antibody durability over a 12M period, via exclusion of participant data that may have unreported COVID-19 infection. ECV-19-induced long-lived antibody responses, with lower titers being associated with longer half-lives. In the earlier period, median antibody half-life was 52 days (similar to mRNA vaccines) and median neutralizing antibody half-life was 130 days. In the later period, ECV-19 antibody half-life increased to 130 days and neutralizing antibody half-life to 215 days. Further testing is required to determine how the magnitude of the observed antibody responses would afford protection against disease and to assess antibody durability in a larger study. An ongoing Phase 3 trial (NCT05572879) should shed further light on ECV-19-induced antibody durability.

Declaration of Competing Interest

JFL holds interest in POP Biotechnologies. YOB and CL hold interest in and are employees of Eubiologics. JYL is an employee of Eubiologics. The remaining authors have no competing interests to declare.

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Ethical approval

This study was approved in South Korea by the Korea Ministry of Food and Drug Safety (approval #33475) and Institutional Review Board at five sites; Catholic University of Korea, Eunpyeong St. Mary's Hospital (approval #PC21BDDF0015), Soonchunhyang University Bucheon Hospital (approval #SCHBC 2021-03-017), Hanyang University College of Medicine (approval #GURI 2021-03-044), Catholic University of Korea, Bucheon St. Mary's Hospital (approval #HC21BDDS0027) and Ewha Womans University Seoul Hospital (approval #SEUMC 2021-03-038). The study was registered with *ClinicalTrials.gov* (NCT04783311).

Author contributions

Writing and analysis: JFL and KM. Conceptualization and funding acquisition: YOB. Investigation: CL, J-YL, Y-SP, IH, JHL, TK, SHS, J-OK, MS, C-JK, J-KC, JK, EJC, and J-HC.

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Consent for publication

Written informed consent was obtained from all participants.

Availability of data and materials

All data are available from the authors upon reasonable request.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2023.11.004.

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