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Short communication

# The effect of the E484K mutation of SARS-CoV-2 on the neutralizing activity of antibodies from BNT162b2 vaccinated individuals

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ABSTRACT

The reduced vaccine efficacy against the SARS-CoV-2 variant lineage B. 1.351 (beta variant) containing the E484K and N501Y mutations is well known. The E484K mutation in SARS-CoV-2 is thought to be responsible for weakened humoral immunity. Vaccine efficacy against the R.1 lineage, which contains the E484K mutation but not the N501Y mutation, is uncertain. Serum samples were collected from 100 healthy Japanese participants three weeks after receiving the second dose of the BNT162b2 vaccine, and serum neutralization antibody titers were measured against five SARS-CoV-2 variants. The geometric mean neutralization titers measured for the original and R.1 lineages were equivalent (91.90 ± 2.40 and 102.67 ± 2. 28, respectively), whereas a low titer was measured for the beta variant (18.03 ± 1.92). Although further investigations with other variant strains and serum samples are essential, our results imply that the weakened humoral response is not caused solely by the E484K mutation. (UMIN000043340).



## 1. Introduction

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is threatening public health and society worldwide. The spike protein of SARS-CoV-2 plays an important role in viral pathogenesis and host defenses. In particular, neutralizing antibodies against the receptor binding domain (RBD) of the spike protein can be generated in an individual as a response to the virus through natural infection or vaccination [1]. Several types of vaccines have been developed against SARS-CoV-2 based on the original strain originating from Wuhan, some of which have been used to control the disease [2].

Recently, the emergence of SARS-CoV-2 variants has become a global concern because certain mutations of the spike protein have been shown to confer resistance against neutralizing antibodies [1]. The alpha variant (lineage B. 1.1.7), which possesses the N501Y mutation, is known to be highly contagious compared to conventional strains [3], and was responsible for a wave of new infections globally, including in Japan. Although the BNT162b2 mRNA SARS-CoV-2 vaccine (Pfizer, New York, NY, USA), one of the most widely used vaccines worldwide, has been shown to be effective against the alpha variant, reduced efficacy was reported against the beta variant (lineage B. 1.351), which possesses both the N501Y and E484K mutations [4]. E484K substitutions in the B. 1.1.7 background were thought to be the cause of reduced vaccine efficacy [5]; in fact, studies using a pseudo-typed virus with the E484K mutation [6] or recombinant virus with the E484K mutation [7] demonstrated weaker neutralizing activity of BNT162b2-vaccinated sera. However, it is unclear whether the E484K mutation affects the BNT162b2 vaccine efficacy independently of the N501Y mutation against naturally derived virus variants. The dominant variant in Tokyo, Japan in early 2021 was from the R.1 lineage and possessed the E484K mutation, but not the N501Y mutation [8]. Knowledge about vaccine efficacy against the R.1 lineage is limited so far. To determine whether the E484K mutation reduces the efficacy of the BNT162b2 vaccine independently of the N501Y mutation, we measured neutralizing antibody titers against several major SARS-CoV-2 variants, including those of the R.1 lineage, using

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serum samples from Japanese university staff who were immunized with two doses of BNT162b2 vaccine.

## 2. Materials and methods

Participants were recruited from 16 February 2021 to 9 March 2021 from Keio University, Shinanomachi Campus (Tokyo, Japan) for a prospective observational study on BNT162b2 vaccine efficacy, directly before a mass vaccination drive at the university hospital. The study protocol was approved by the institutional review board (20200330) and written informed consent was obtained from all the participants. Serum samples were collected from 673 participants approximately three weeks after the administration of the second dose of the BNT162b2 vaccine. The participants were categorized into four groups based on age (aged above or below 45 years old, which was the median age of the participants) and sex. From each group, 25 participants were randomly selected and a total of 100 samples were analyzed against five SARS-CoV-2 variants.

The five strains used in this study included the original Wuhan strain (original), R.1 lineage (R.1) strain, alpha variant (Alpha), beta variant (Beta), and delta variant (lineage B. 1.617.2, which possesses the L452R and E484Q mutations) (Delta) strains. The original (EPI_ISL_408667), Alpha (EPI_ISL_768526), and Beta (EPI_ISL_1123289) strains were provided by the National Institute of Infectious Diseases (Tokyo, Japan), whereas the Delta and R.1 strains were clinically isolated at Keio University Hospital.

Mutations in the five virus preparations used in the experiments were detected by whole genome sequencing. RNA extracted from the virus preparations was reverse- transcribed to cDNA, amplified, and used to prepare a library for sequencing using the Twist Total Nucleic Acid Library Preparation Kit for Viral Pathogen Detection and Characterization and SARS-CoV-2 Research Panel (Twist bioscience, San Francisco, CA, USA). Paired-end sequencing was performed on the NovaSeq 6000 platform (Illumina, San Diego, CA, USA) (Supplementary Table S1). The neutralizing antibodies were measured using VeroE6/TMPRSS2 cells [9], which constitutively express transmembrane protease serine 2 (TMPRSS2). First, 96-well plates were prepared by plating VeroE6/TMPRSS2 cells at $1 \times 10^4$ cells/well in 100 μL of medium. To measure the neutralizing antibody activity, the serum was serially diluted two-fold, beginning with a 1:10 initial dilution, in 96-well plates. Then, $2 \times 10^2$ median tissue culture infectious dose ($TCID_{50}$) of each SARS-CoV-2 variant was mixed with an equal volume of serum (the final dilutions were 1:20 to 1:640) and incubated at 37 °C for 1 h in a 96-well plate. The mixture was then added to the prepared VeroE6/TMPRSS2 96-well plates. The cells were cultured for 7 days and monitored for cytopathic effects (CPE).

The geometric mean titer (GMT) of neutralizing antibodies was determined for each variant, along with the standard deviation (SD). For calculations, the neutralizing antibody titers of samples without any neutralization effect at 1:20 dilutions were set as 10. Furthermore, the neutralization antibody was compared between age groups (<45 years-old or ≥ 45 years-old), sex, and history of COVID-19, which was determined from the questionnaire administered during the study entry.

Additionally, IgG antibody titers for the RBD of the SARS-CoV-2 spike protein were measured by commercially available chemiluminescent enzyme immunoassay (CLEIA)-based SARS-CoV-2 antibody reagents (SARS-CoV-2 IgG II Quant reagents, Abbott Laboratories, Illinois, USA) according to the manufacturer's instructions [10].

## 3. Results

The neutralization observed in serum samples at 1:20 dilutions for the original, R.1, Alpha, Beta, and Delta strains was 97%, 97%, 98%, 53%, and 87%, respectively; the GMT ± SD was 91.90 ± 2.40, 102.67 ± 2.28, 97.81 ± 2.48, 18.03 ± 1.92, and 42.87 ± 2.38, respectively (Fig. 1). No significant difference in GMT of the neutralizing antibody was observed based on age, sex, and history of COVID-19 (Supplementary Table S2).

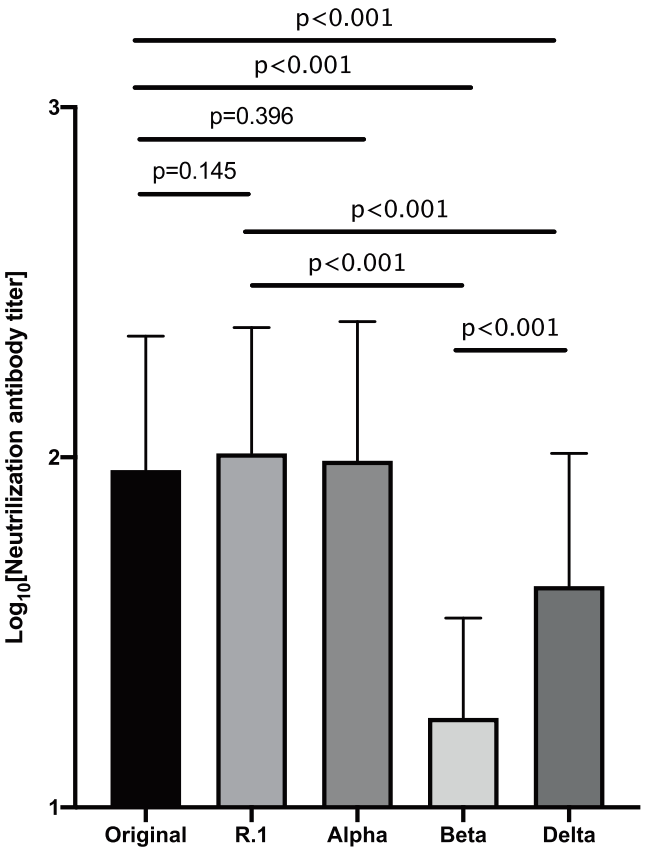


**Fig. 1. Geometric means of neutralizing antibody titers for SARS-CoV-2 variants.** Neutralizing antibody titers against the original SARS-CoV-2 (original), R.1 lineage (R.1), B. 1.1.7 strain (Alpha), B. 1.351 strain (Beta), and B. 1.617.2 (Delta) strains were measured using serum samples from 100 participants who received two doses of the BNT162b2 vaccine. Geometric means of neutralizing antibody titers were calculated. The neutralization antibody titer of samples without a neutralizing effect at 1:20 dilutions was treated as 10. Results are displayed as the logarithms of the geometric mean. Standard deviation and *p*-values of paired *t*-test are displayed.

Antibody titers for RBD using Abbott's reagents ranged from 260.9 AU/mL to 55135.4 AU/mL. Thus, all the samples were antibody-positive (manufacturers cut off; 50 AU/mL), as determined by the reagents, whereas three of them did not exhibit neutralization effects against the original strain within our dilution ranges. The logarithm of the antibody titer for RBD was correlated with the logarithm of neutralizing antibody titer for the original, R.1, Alpha, Beta, and Delta strains. (r = 0.684, 0.674, 0.614, 0.520, and 0.710, respectively) (Fig. 2).

## 4. Discussion

Although several clinical trials have already demonstrated sufficient neutralizing antibody titers after two doses of the BNT162b2 vaccine, the number of reports on neutralizing antibody titers against SARS-CoV-2 from Asian countries, including Japan, is limited, particularly for variant strains. Our data demonstrated that the participants were able to obtain sufficient humoral immunity against the original and Alpha variants, while a weak humoral immune response was observed against the Delta variant, which is currently the dominant strain in Japan.

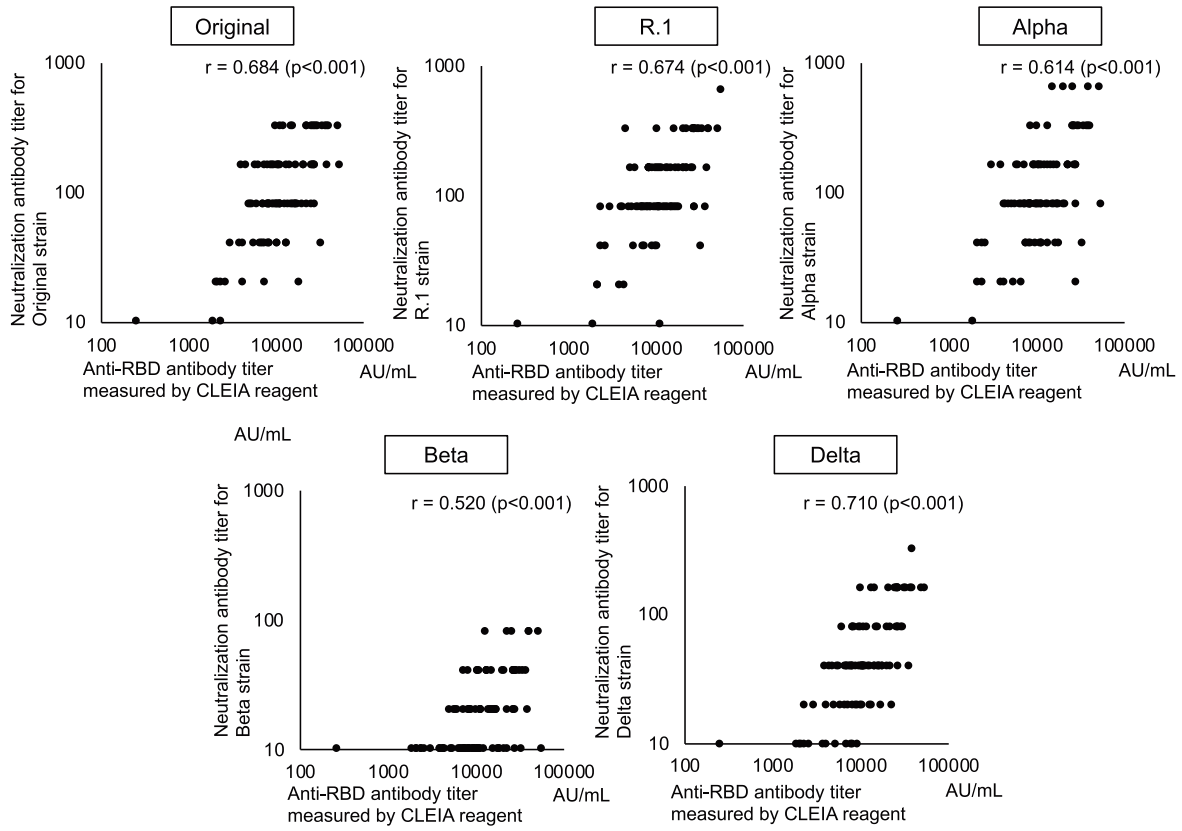


**Fig. 2. Relationship between antibody titers measured by commercially available CLEIA based SARS-CoV-2 IgG assay and neutralizing antibody titers for variants.** Logarithm of neutralizing antibody titers against the original SARS-CoV-2 (original), R.1 lineage (R.1), B. 1.1.7 strain (Alpha), B. 1.351 strain (Beta), and B. 1.617.2 (Delta) strains was compared to the logarithm of anti-RBD IgG antibody measured by SARS-CoV-2 IgG II Quant reagents (Abbott Laboratories, Illinois, USA). The neutralizing antibody titer of samples that did not exhibit the neutralization effect at 1:20 dilutions was treated as 10. Pearson's correlation coefficient was calculated.

Additionally, our data demonstrated that a lower antibody response was observed against the Beta variant, and not against the R.1 lineage variant, which indicates that the weak antibody response against the Beta variant was not solely due to the E484K mutation. Previous reports demonstrated that although the E484K mutation of Beta variants might cause humoral immunity escape, the combination of N501Y and K417N was further associated with a weakened response; this enhances the binding of the spike protein to ACE2 and reduces the binding to antibodies [11,12]. Therefore, the E484K mutation of R.1 alone did not have a sufficient impact in reducing vaccine response, but the combination of E484K, N501Y, and K417N of Beta strains caused severe depletion of the neutralization effect of vaccinated sera in our study. Although further *in vitro* and *in vivo* research is essential, to reinforce humoral immunity against beta variants, the development of an mRNA vaccine against the two or three-point mutations could be effective.

Moreover, it has been reported that the Delta variant, which harbors the L452R mutation, causes a lower cellular immunity response among Asians who have human leukocyte antigen HLA-A24 [13]. Similar to previous reports [14], the weak humoral immune response observed against the Delta variant in this study involving Asian participants supports the reason why Asian countries that were not severely affected by waves of the Alpha variant suffered from the big wave of SARS-CoV-2 infections due to the Delta variant, even after completion of universal vaccination.

The comparison with commercially available CLEIA-based anti-RBD IgG assay demonstrated that the IgG titer was correlated with the neutralizing antibodies regardless of the types of variants. The measurement of CLEIA-based antibody titers is a useful tool for assessment in a clinical laboratory. Furthermore, the combinational use of a neutralization test and a CLEIA-based anti-RBD IgG assay is necessary to evaluate vaccine efficacy against the novel variant strains of SARS-CoV-2.

Although further studies involving more clinical strains and serum samples, especially using a panel of viruses within the same lineage to determine the impact of virus variants on neutralization as performed by Lu et al. [15], are essential to obtain a firm conclusion, this study assessed neutralizing antibody titers using authentic SARS-CoV-2 variants. A majority of the studies in the literature employ pseudo-virus-mediated systems [16,17] because of the difficulty in handling SARS-CoV-2 with regards to the regulations and infectivity. Humoral immunity against SARS-CoV-2 infection might not be monoclonal but rather polyclonal; therefore, neutralizing antibody titers measured using authentic viruses are a more reliable indicator than those obtained using pseudo-virus systems.

## 5. Conclusions

In conclusion, sufficient neutralizing antibodies in Japanese participants after BNT162b2 vaccination were produced against the original, R.1 lineage, and Alpha variants of SARS-CoV-2, while an insufficient humoral immunity was observed against the Beta and Delta variants, indicating that the E484K mutation is not the sole factor contributing toward a weakened humoral response.

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## Authors' contribution

All authors attest they meet the ICMJE criteria for authorship. YU and TY conceived and designed the study. YU and MW recruited the participants. TY, TS and KK collected the data. YU, TY, and YS analyzed and interpreted the data. YU and TY wrote the manuscript. TN, YS, MW, NH, and MM discussed the data and critically reviewed and revised the manuscript. All authors approved the final version of the manuscript for publication.

## 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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## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2022.02.047.

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