Fourth mRNA vaccination increases cross-neutralizing antibody titers against SARS-CoV-2 variants, including BQ.1.1 and XBB, in a very elderly population

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学 位 論 文 の 内 容 要 旨

Fourth mRNA vaccination increases cross-neutralizing antibody titers against SARS-CoV-2 variants, including BQ.1.1 and XBB, in a very elderly population

超高齢者は新型コロナウイルス mRNA ワクチンの四回接種により

変異株 BQ.1.1 および XBB にも有効な交差性中和抗体を獲得する

神戸大学大学院医学研究科医科学専攻

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1. Introduction

Infection by Severe Acute Respiratory Syndrome - Coronavirus 2 (SARS-CoV-2) can cause Coronavirus Disease 2019 (COVID-19). COVID-19 has been a pandemic that had caused over 6.7 million deaths worldwide. Vaccination is the most reliable measure to prevent infection and to reduce the morbidity and mortality of COVID-19. Since Omicron variants with immune evasion ability had emerged, they have mutated rapidly and raised concerns about the weakening of vaccine efficacy, and the very elderly populations are vulnerable to severe COVID-19. To assess whether 3rd and 4th vaccinations can induce neutralizing antibodies against the newly appeared variants for the elderly, we analyzed the cross-neutralizing antibodies for several SARS-CoV-2 variants (D614G, Delta, Omicron BA.2, BA.5, BA.2.75, BQ.1.1, and XBB) after 3rd and 4th mRNA vaccinations in a very elderly population.

2. Materials and Methods

Blood samples were taken from elderly residents at four long-term care facilities in Hyogo prefecture, Japan (Koyukai Nishi Hospital, Subaru Uozaki-no-sato, Subaru Rokko, and Carehome Subaru) (median age, 91 years). Participants were divided into two groups based on the total number of vaccination doses they had received, namely three (n=67) or four (n=48). Underlying medical conditions of participants were also documented. The mRNA vaccines administered were Comirnaty (BNT162b2, Pfizer-BioNTech) for the 1st to 3rd doses, and either Comirnaty or Spikevax (mRNA-1273, Moderna) for the 4th vaccination. Blood samples were taken at two time points, i.e., 103 days after 3rd vaccination and 48 days after 4th vaccination. A live virus microneutralization assay was performed. Two-fold serial dilution of serum samples were done to determine neutralizing antibody titers against SARS-CoV-2 variants.

The enzyme-linked immunosorbent assay (ELISA) method was done to detect anti-SARS-CoV-2-Spike (S) of conventional (D614G) virus, Omicron BA.2, BA.5, and BA.2.75 variants or -Nucleocapsid (N) immunoglobulin G (IgG) antibodies in participants' sera. Samples from participants who had a history of COVID-19 infection (confirmed by polymerase-chain reaction) or high serum titers of anti-N antibody were analyzed separately from the main group.

3. Results

<u>Neutralizing antibody titers increased after 4th vaccination</u>

After the 4th vaccination, the neutralizing antibody titers increased significantly compared to 3rd vaccination: 2.6-fold, 2.0-fold, 4.2-fold, 3.5-fold, 2.7-fold, 1.9-fold,

and 2.0-fold, for D614G, Delta, Omicron BA. 2, BA.5, BA.2.75, BQ.1.1, and XBB, respectively. The neutralizing antibodies potency decreased with each emerging variant. Although the neutralizing antibody positivity rate increased with 4 doses of vaccine, the titers of those against BQ.1.1 and XBB was still low.

We compared neutralizing antibody titers of participants aged under 90 years old and 90 years or more. Neutralizing antibody positivity rates and titers were similar between the participants aged less than- and over 90 years, at both time points.

Participants' medical conditions were disclosed during the recruitment process; these included hypertension, hyperlipidemia, diabetes mellitus, chronic heart disease, chronic respiratory disease, cerebrovascular disease, and malignancy. There was no significant difference found in neutralizing antibody titers after the 3rd or 4th vaccination among the various medical conditions.

Anti-SARS-CoV-2-S antibody titers were correlated with neutralizing antibody titers

By ELISA, anti-S IgG was detected in all participants' samples. The binding affinity of anti-S IgG was significantly increased for the SARS-CoV-2 S protein of the D614G, Omicron BA.2, BA.5, and BA.2.75 variants, respectively, after the 4th vaccination. We found a moderate-to-strong positive correlation of anti-S titers and neutralizing antibody titers, with correlation coefficients (r) of 0.55, 0.77, 0.73, and 0.74 for D614G, BA.2, BA.5, and BA.2.75, respectively.

Reactivity to N protein alone was not enough to screen for past infection

During participant recruitment, we excluded some participants from the 3rd vaccination (n=5) and 4th vaccination (n=6) groups based on their COVID-19 history and sera reactivity towards the N protein. An excluded participant who was infected in April 2021 (before the emergence of Delta variant) had a high titer of anti-N IgG at both time points, i.e., after the 3rd and 4th vaccinations. But two other excluded participants who were infected after the emergence of Delta and Omicron variants, i.e., in 2022, displayed low titer of anti-N (below cut-off value).

Excluded participants had higher positivity rates of cross-neutralizing antibodies

Excluded participants (3rd vaccination group, n=5; 4th vaccination group, n=6), i.e., who have either history of COVID-19 or high reactivity to N protein had higher positivity rates and titers of cross-neutralizing antibodies against SARS-CoV-2 variants. However, even after 4th vaccination, cross-neutralizing antibody titers against BQ.1.1 and XBB were lower than that against the other variants.

4. Discussion

Since the Comirnaty and Spikevax mRNA vaccines used for 3rd and 4th vaccination were made based on wild type SARS-CoV-2 S, the numerous mutations in Omicron variants result in immune escape. Omicron BQ.1.1 is a descendant of BA.5, while XBB is a mixture product of BA.2.10.1 and BA.2.75. Specifically, the Spike proteins of BQ.1.1 and XBB have the same R346T, N460K, and F486X mutations, with additional K444T mutation in BQ.1.1 and V445P, G446S, and F490S mutations in XBB, which conferred resistance to many monoclonal antibodies. Our result showed that after 3rd vaccination, the positivity rates and titers of neutralizing antibody against D614G and Delta variant were higher than those against Omicron variants. Adequate neutralizing antibodies for Omicron BA.2 were induced after 3rd vaccination. Those for BA.5 and BA.2.75 were also induced, albeit at lower levels than BA.2. After 4th vaccination, positivity rates and titers of neutralizing antibody against all tested variants, including BQ.1.1 and XBB, were increased, indicating that the 4th vaccination is important for the elderly. The cross-neutralizing antibody positivity rates and titers induced by 4th vaccination in the elderly population aged under 90 years old were similar to those aged 90 years old or more. Neutralizing assay results of the excluded participants' sera showed higher positivity rates of cross-neutralizing antibody against all tested variants compared to naïve vaccinated individuals, indicating the immune booster effects by previous infection, although cross-neutralizing antibody titers against BQ.1.1 and XBB were still low.

Antibodies against the N protein may be elicited after infection with SARS-CoV-2, but not after COVID-19 mRNA vaccination. The discrepancy of N protein titers between previously and recently infected individuals may need further investigation. Had we not used past COVID-19 history as another exclusion criteria, we would have incorrectly grouped some of the participants who had hybrid immunity (i.e., immunity conferred by past infection and vaccinations). We reported that the use of N protein-based serology tests to determine history of infection may not be accurate in elderly populations who have received multiple vaccinations.

We reported that the 4th mRNA vaccination can readily induce cross-neutralizing antibodies against many SARS-CoV-2 variants in the very elderly population. However, it may not be enough to protect them from newer variants, e.g., BQ.1.1 and XBB. Considering the rapid mutation of viruses and the efficacy of vaccines, it may be necessary to create a system that can develop vaccines suitable for each epidemic in consideration of the epidemic of the virus.

神戸大学大学院医学(系)研究科（博士課程）

論文審査の結果の要旨

受付番号	甲 第 3322 号	氏 名	SILVIA SUTANDHIO
論文題目 Title of Dissertation	超高齢者は新型コロナウイルス mRNA ワクチンの四回接種により変異株 BQ.1.1 および XBB にも有効な交差性中和抗体を獲得する Fourth mRNA vaccination increases cross-neutralizing antibody titers against SARS-CoV-2 variants, including BQ.1.1 and XBB, in a very elderly population		
審査委員 Examiner	主 査 Chief Examiner 勝二郁夫 副 査 Vice-examiner 宮良高維 副 査 Vice-examiner 野津寛大		

（要旨は１，０００字～２，０００字程度）

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The candidate, having completed studies on COVID-19, with a specialty in the mRNA vaccine and cross-neutralizaing antibody titers against SARS-CoV-2 variants, and having advanced the field of knowledge in the area of Clinical Virology, is hereby recognized as having qualified for the degree of Ph.D. (Medical).