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Short communication

Homologous and heterologous booster vaccinations of S-268019-b, a recombinant S protein-based vaccine with a squalene-based adjuvant, enhance neutralization breadth against SARS-CoV-2 Omicron subvariants in cynomolgus macaques

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Abstract

SARS-CoV-2 Omicron subvariants such as BA.2.12.1, BA.4 and BA.5 have been spreading rapidly and become dominant worldwide. Here we report the homologous or heterologous booster effects of S-268019-b, a recombinant spike protein vaccine with the squalene-based adjuvant A-910823 in cynomolgus macaques. In macaques which had been primed with S-268019-b or mRNA vaccines, boosting with S-268019-b enhanced neutralizing antibodies (NAb) against ancestral SARS-CoV-2. Since boosting with the antigen without adjuvant did not efficiently restore NAb titers, adjuvant A-910823 was essential for the booster effect. Importantly, boosting with S-268019-b enhanced NAb against all of the Omicron subvariants we tested, including BA.2.12.1, BA.4 and BA.5, in comparison to two vaccine doses. Additionally, expansion of Omicron-specific B cells was confirmed after boosting with S-268019-b. These results indicate that a booster dose of S-268019-b with the adjuvant enhances the neutralization breadth.

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1. Introduction

Severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) emerged at the end of 2019 in Wuhan, China, and spread globally [1]. Around autumn 2020, Alpha (B.1.1.7) and Beta (B.1.351) variants emerged in the United Kingdom and South Africa, respectively [2,3]. Subsequently, Delta (B.1.617.2) emerged in April 2021 and rapidly spread worldwide [4]. In November 2021, Omicron (B.1.1.529) appeared and rapidly rose to global prevalence [5]. The emergence of SARS-CoV-2 variants raised the issue of antibody escape, as the variants bear multiple amino acid changes in the receptor binding domain (RBD) of the spike (S) protein, the main target for potently neutralizing antibodies (NAb) [6,7]. Among the variants of concern that have emerged to date, the Omicron variants can evade the NAb in humans who have received the priming series of vaccine [8,9]. Despite the antigenic changes in the Omicron variants, a third dose of booster vaccine prominently elicits NAb against Omicron variants [10–12]. However, SARS-CoV-2 is still continuously evolving, and Omicron subvariants, such as BA.2.12.1, BA.4 and BA.5, have recently emerged and surged worldwide due to their higher transmission advantage [13,14]. Information on vaccine efficacy against these Omicron subvariants is still limited.

COVID-19 vaccine S-268019-b consists of a recombinant protein S-910823 combined with the squalene-based adjuvant A-910823 and is currently being developed in Phase 3 clinical trials [15–17]. Although most relevant cell lines for a baculovirus expression system (BEVS) are known to be persistently infected with various adventitious viruses, the S-910823 protein is generated in rhabdovirus-free insect cells. It has been demonstrated that S-268019-b can elicit NAb with Th1-skewed cellular immunity with two vaccination doses in cynomolgus macaques and humans.
Although two doses of vaccine with S-268019-b resulted in reduced NAb titers against Omicron variant BA.1 [15], a third dose as a booster elicited robust NAB against BA.1, consistent with approved vaccines [11,17]. However, the neutralization breadth has not been assessed against Omicron subvariants such as BA.2,12.1, BA.4 and BA.5. In this study, we report NAB titers and their breadth elicited by a third dose of S-268019-b, including homologous and heterologous boost regimens, against recently spreading SARS-CoV-2 Omicron subvariants in cynomolgus macaques.

2. Methods

2.1. Preparation of vaccines

The S-268019-b vaccine (Shionogi & Co., Ltd., Osaka, Japan) consists of the recombinant S protein S-910823 based on sequences from Pango lineage A and the squalene-based adjuvant A-910823. The recombinant S-910823 protein was expressed using BEVS as described previously [15]. Lipid nanoparticle-formulated mRNA vaccine (mRNA-LNP) was prepared with the ionicizable lipid (Lipid8, manufactured in-house) and mRNA encoding the full-length S protein from Pango lineage A. Briefly, Lipid8, dioleoylphosphatidylcholine (DSPC, Nippon Fine Chemical Co., Ltd., Osaka, Japan), cholesterol (Nippon Fine Chemical Co., Ltd., Osaka, Japan) and 1,2-dimyristoyl-sn-glycerol, methoxyethylpolyethylene glycol 2000 (DMG-PEG, NOF Corporation, Tokyo, Japan) were dissolved in ethanol. The lipid solution and mRNA in acetate buffer (pH 4.0) were mixed at a flow ratio of 3:1 (v/v) and a total flow rate of 12 mL/min. The mixture was dialyzed with PBS (pH 7.4) at 4°C, and then passed through a 0.22 μm filter. The mRNA was synthesized and characterized as described previously [18]. The particle size and mRNA encapsulation of mRNA-LNP was verified as reported previously [19,20]. The mean particle size (Z-average) and polydispersity index (PDI) were determined by Zetasizer Nano ZS (Malvern, Worcestershire, U.K.), ranging 79.21–84.02 nm for Z-average and 0.029–0.097 for PDI. Encapsulation efficiency (%) of mRNA was determined by Quant-iT Ribogreen RNA assay Kit (ThermoFisher Scientific). Briefly, the total and free mRNA concentrations in LNP preparation were measured by fluorescence intensity (excitation/emission: 480/520 nm) of Ribogreen dye in the presence and absence of 2% Triton X-100, resulting %EE was 94.1–96.1 %.

2.2. Vaccination of cynomolgus macaques

Sixteen female Cambodian cynomolgus macaques (Macaca fascicularis), 4 years of age and weighing 2.4 to 3.6 kg at the beginning of a 3-day acclimation period, were obtained from the stock colony of Drug Safety Research Laboratories of Shin Nippon Biomedical Laboratories, Ltd. (Kagoshima, Japan). The overall study design is shown in Fig. 1A. The macaques were randomly assigned to four experimental groups (n = 4/group) and received intramuscular vaccination of 12 mL/m2. The day prior to the first dosing was day 1. The second vaccine dose was administered 4 weeks later (day 29), and the third vaccine dose was given at weeks from the second vaccination (day 78), followed by monitoring until day 105. According to the experimental group (Fig. 1A), the animals received either S-910823 (10 μg) plus A-910823 (50 μg/v), or mRNA-LNP (30 μg). S-910823 without adjuvant was included in the third dose. Blood samples were drawn from the femoral veins at the volumes and time points specified for the indicated analyses. Details are described in the Supplementary Methods.

2.3. Cells, authentic SARS-CoV-2, and pseudoviruses

Transmembrane serine protease 2 (TMPRSS2)-expressing VeroE6/VeroE6/TMPRSS2 cells [21] were described previously [15]. Authentic SARS-CoV-2 strains were isolated and provided by the National Institute of Infectious Diseases, Japan. Authentic SARS-CoV-2 was propagated and titrated by tissue culture infectious dose 50 (TCID_{50}) assays with VeroE6/TMPRSS2 cells. Lentivirus-based pseudoviruses bearing the S protein were generated in Lenti-X 293 T cells (Takara Bio, Shiga, Japan) [15]. Details are provided in the Supplementary Methods.

2.4. Neutralizing and IgG antibody titer testing

Neutralization assays with authentic SARS-CoV-2 and pseudoviruses were conducted as described previously [15]. Anti-S and anti-receptor-binding domain (RBD) IgG titers were measured as described previously [15]. Details are described in the Supplementary Methods.

2.5. ELISpot assay with B cells

Frozen PBMC samples from individual cynomolgus macaques were prepared as described previously [15]. The PBMCs were cultured and stimulated for 4 h with the S protein from the ancestral strain (Pango lineage A, Wuhan-Hu-1) or Omicron BA.1.1.529, and the frequencies of IgG secreting cells were evaluated by ELISpot assay. Details are provided in the Supplementary Methods.

3. Results

3.1. Immunogenicity of a third dose of S-268019-b in cynomolgus macaques

We immunized 16 adult cynomolgus macaques with homologous and heterologous regimens with S-268019-b or mRNA-LNP (n = 4/group). Groups of macaques were primed with either vaccine at days 1 and 29. On day 78, the animals received a homologous or heterologous booster with mRNA-LNP or S-268019-b (Fig. 1A). NAB against authentic SARS-CoV-2 was induced on day 28 after priming immunization and was slightly higher in the mRNA-LNP primed animals compared to the S-268019-b ones (Fig. 1B). After the second dose, the NAB titers reached peak levels around days 36 and 43. The NAB titers in the mRNA-LNP vaccinated groups declined more than 4.8-fold at day 77, consistent with immune kinetics following BNT162b2 vaccination in rhesus macaques [22]. A similar decline was observed in the S-268019-b vaccinated groups. After the third dose on day 78, the NAB titer was restored in both mRNA-LNP and S-268019-b immunized groups, except for antigen S-910823-boosted animals (Fig. 1B). Similarly, both anti-S protein and anti-RBD IgGs were elevated following a third dose of mRNA-LNP or S-268019-b, whereas the anti-S protein and anti-RBD IgGs were not markedly elevated after administration of S-910823 alone as the third booster (Fig. 1C and 1D). These data suggest that boosting with S-268019-b elicited NAB against ancestral SARS-CoV-2.

3.2. Neutralization breadth against SARS-CoV-2 Omicron subvariants

We next evaluated NAb in sera at days 57 and 105 against pseudoviruses bearing the S protein from ancestral D614G and Omicron subvariants (Fig. 2A). The NAB titer against Omicron subvariants declined compared to that against D614G prior to the booster, but markedly increased after homologous or heterologous boosting with mRNA-LNP or S-268019-b. GMTs at day 105 increased 4.3–
7.2, 4.9–7.7 and 3.5–13.2-fold compared to those at day 57 in the groups of mRNA-LNP homologous-boosted, heterologous-boosted, and S-268019-b homologous-boosted groups, respectively (Fig. 2B). We next assessed NAb titers in sera against authentic viruses (Fig. 2C). Consistent with the NAb titers against pseudoviruses, NAb against Omicron variants were lower than that of the Pango lineage A in all groups prior to the boost, but rose after the homologous and heterologous boosting. Fold-changes of increase of GMTs at day 105 compared to those at day 57 were 2.3–4.0, 4.0–6.7 and 5.7–9.6-fold in the mRNA-LNP homologous-boosted, heterologous-boosted, and S-268019-b homologous-boosted groups, respectively. These results indicate that booster vaccination of S-268019-b enhances the neutralization breadth against SARS-CoV-2 Omicron subvariants.

Fig. 1. Immunogenicity of homologous and heterologous boosting with S-268019-b. (A) Schematic overview of the vaccination schedule and experimental groups. Cynomolgus macaques (n = 4/group) were vaccinated on day 1 and again 4 weeks later on day 29 with vaccine S-268019-b or mRNA-LNP. The third dose was given at 7 weeks from the second vaccination (day 78). Vaccinations were performed intramuscularly with a total volume of 500 µL. The animals were monitored for weight and clinical signs, and blood was collected as indicated for analysis of antibody content. (B to D) anti-SARS-CoV-2 antibody levels in cynomolgus macaques immunized with vaccine S-268019-b. Sera were subjected to (B) neutralizing antibody titer testing with ancestral strain and ELISA for measuring (C) total IgG titers of anti-S protein and (D) anti-RBD IgG titers. The horizontal dotted lines represent the limit of detection.
Neutralizing antibody levels in cynomolgus macaques boosted by S-268019-b or mRNA-LNP against Omicron subvariants. (A) Amino acid changes on the spike protein of SARS-CoV-2 variants used in this study. Amino acids that are not identical to the spike from Pango lineage A (WK-521) are filled in with black. Schematic representation of domains of the spike protein is shown above. NTD, N-terminal domain; RBD, receptor binding domain; CTD, C-terminal domain. (B) Neutralizing antibody titers against the indicated spike protein-bearing pseudoviruses were assessed in sera collected from vaccinated cynomolgus macaques at days 57 and 105. The 50% pseudovirus-neutralization titers (pVNT50) are shown. Each bar represents the geometric mean titer (GMT) with error bars indicating 95% confident interval. (C) Sera at days 57 and 105 were subjected to neutralizing antibody titer testing with the indicated authentic SARS-CoV-2 variants. Each bar represents the GMT with error bars indicating 95% confident intervals. (D) Antigen specific IgG-secreting B cells were assessed in PBMCs collected from vaccinated cynomolgus macaques at days 57 and 105 by ELISpot assay. Results are shown as antigen specific spots per 1 x 10^6 PBMCs. Each bar represents mean values with error bars indicating standard error of the mean. Data at day 57 are represented by circle plot and blank bar, and data at day 105 are shown by square plot and filled bar. Each color bar corresponds to respective SARS-CoV-2 variants.


3.3. Ancestral and Omicron S protein specific B cells in PBMCs

To further explore the mechanism of enhancement of the neutralization breadth by booster vaccination, we employed ELISpot assay to measure the S protein-specific B cell responses to ancestral Wuhan-Hu-1 or Omicron B.1.1.529 by means of the number of IgG secreting cells with the PBMCs on days 57 and 105 (Fig. 2D). The ancestral S-specific spots on day 105 increased by 1.0, 1.5, and 3.4-fold compared to those on day 57 in the mRNA-LNP homologous-boosted, heterologous-boosted, and S-268019-b homologous-boosted groups, respectively. On the other hand, the ratios of day 105 to 57 for Omicron B.1.1.529 S-specific spots were 1.1, 2.3, and 3.7-fold in the mRNA-LNP homologous-boosted, heterologous-boosted, and S-268019-b homologous-boosted groups, respectively. This observation indicates that booster vaccination with S-268019-b expands the neutralization breadth to include Omicron B.1.1.529 as well as ancestral S protein-specific B cells in the PBMCs.

4. Discussion

We have evaluated the NAb titers against SARS-CoV-2 Omicron subvariants in cynomolgus macaques immunized with homologous or heterologous boosting with S-268019-b. Following a third dose of S-268019-b, NAb against ancestral viruses were restored to the peak response after a third dose in the macaques primed with S-268019-b or mRNA-LNP. Intriguingly, a third dose of antigen S-910823 without adjuvant induced less NAb titer, showing that A-910823 is essential for the efficient booster effect. However, the decline of NAb stopped after a third dose of antigen S-910823, suggesting that a certain level of immunity had been conferred. Evaluation of affinity maturation of B-cell receptor and antibody avidity would be important for better understanding of A-910823 function and the efficacy of third dose. Given that recent clinical trials revealed that the mRNA vaccine BNT162b2 provided 70 % protection against hospitalization with Omicron infection [23] and the levels of NAb elicited by S-268019-b were comparable to mRNA-LNP, similar protective efficacy could be anticipated, although further clinical evidence is required regarding levels of NAb that can offer protection from infection with Omicron subvariants in humans.

Although homologous and heterologous boosting of S-268019-b elicited NAb titers to not only ancestral viruses but also Omicron subvariants, NAb against BA.4/5 was approximately 4-fold lower than that against the Omicron BA.1 variant. In the homologous booster setting, S-268019-b exhibited approximately 3-fold higher NAb titers at the peak levels compared to mRNA-LNP, with comparable overall trends of NAb titers against Omicron subvariants. Homologous boosting of S-268019-b elicited comparable levels of NAb titers against BA.1, BA.2 and BA.3. The spike protein of BA.3 differs to that from BA.1 by amino acid deletion of EPE at 214–216 amino acids and substitutions of L371F, D405N, S496G, K547T, K856N and F981L. These differences may cause the lesser impact on NAb titers. BA.2.10 exhibited slightly reduced sensitivity to NAb compared to BA.2, and this might be caused by the absence of deletions of amino acids 24 to 26. The Omicron BA.2.12, BA.2.12.1 and BA.4/5 variants displayed the lowest susceptibility to the homologous boosted sera. BA.2.12 and BA.2.12.1 bear unique substitutions at L452Q and S704L in addition to known mutations in BA.2, while BA.4/5 harbors deletion at 69–70, L452R, F486V, and R493Q, a reversion substitution, compared to BA.2, indicating these mutations might affect NAb titers. With heterologous boosting overall trends of NAb titers against Omicron subvariants were comparable to those in the homologous booster setting. Since the antigens of S-268019-b and mRNA-LNP are fundamentally identical and based on the S protein from ancestral SARS-CoV-2, the difference of modality does not seem to impact the breadth of NAb as far as tested in this study.

Limitations of this study include i) the lack of longitudinal observation of NAb titers and their breadth after a third dose of S-268019-b, ii) the lack of challenge experiments with SARS-CoV-2 Omicron subvariants in macaques, iii) the presence of a gap between mRNA-LNP used in this study and approved mRNA vaccines, iv) the number of cynomolgus macaques in each group was limited, and our results are qualitative in nature, and v) the existence of species-related difference between macaques and humans.

In summary, our findings showed that a third vaccination of S-268019-b given as a booster, regardless of homologous and heterologous immunization, could effectively elicit broadly neutralizing antibodies against Omicron subvariants. Better understanding of the breadth expansion of neutralizing activity should support the clinical use of S-268019-b.

Data availability

No data was used for the research described in the article.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Masayuki Hashimoto, Naomi M. Seki, Kumi Hashimoto, Ken Yoshihara, Tomoyuki Homma, Takuhiro Sonoyama, and Shinya Omoto are employees of Shionogi & Co., Ltd. Shinpei Aoe and Yusuke Kawazu are employees of Shionogi TechnoAdvance Research Co., Ltd.

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Ethical Approval

All procedures using authentic SARS-CoV-2 were conducted in a BSL3 laboratory. Experiments using recombinant DNA and pathogens were approved by the Committee for Experiments using Recombinant DNA and Pathogens at Shionogi & Co., Ltd., Japan. For the immunogenicity studies, the animal protocols were approved by the Institutional Animal Care and Use Committee (IACUC 055-912) of Shin Nippon Biomedical Laboratories in Japan, which is accredited by AAALAC International. All animal protocols
were in accordance with the animal welfare by-laws of Shin Nippon Biomedical Laboratories.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2022.10.092.

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