Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus responsible for the coronavirus disease 2019 (COVID-19) pandemic, has caused serious health problems worldwide since its emergence in 2019 in Wuhan, China; indeed, it remains an ongoing health issue in every country on the globe [1]. Since the most effective way to combat virus infection is vaccination, there was a rush to develop effective vaccines against SARS-CoV-2 soon after its identification [2-4]. With unprecedented speed, several vaccines were developed; these include mRNA-based vaccines (BNT162b2 and mRNA-1273), virus-vector vaccines (ChAdOx-1, Ad26COVs1, and Sputnik V), recombinant protein-based vaccines (NVX-CoV373 and Co-VLP), and inactivated virus-based vaccines (BBIBP-CorV and CVAXIN) [5]. After emergency approval was granted, these vaccines were quickly introduced into the field. More vaccines are on the way. The degree of protection afforded by these vaccines varied, but BNT162b2 and mRNA-1273 provided >90% protection against SARS-CoV-2 [6,7]. However, one continuing challenge is the emergence of new variants, particularly vaccine-resistant variants [8]. After identification of the original SARS-CoV-2 strain in Wuhan, China, in 2019, numerous variants have emerged, including B.1.1.7 (Alpha), B.1.351 (Beta), P1 (Gamma), B.1.617.2 (Delta), and Omicron (B.1.1.529) [9]. These variants harbor a different number of mutations from the original SARS-CoV-2 strain. Omicron harbors 34 mutations compared to the Wuhan strain. Omicron has emerged as a major concern due to its high transmissibility and potential to escape immunity induced by previous infections or vaccinations. Therefore, understanding the immune responses induced by Omicron is crucial for developing effective vaccines and strategies to combat it.
mutations within the spike protein. Those mutations occur in the S1 subunit, in particular the receptor-binding domain (RBD). These variants show varying degrees of interaction with neutralizing antibodies induced by current vaccines [10,11]. Indeed, the new variants can cause breakthrough infections in people who have been vaccinated or infected before [12]. Therefore, a new vaccine targeting new variants has to be developed.

In this study, we aimed to investigate the characteristics of antibodies induced by an S protein containing the RBD of the Omicron variant. First, we generated a recombinant construct comprising the S protein (amino acids 14–1162) from the D614G variant of SARS-CoV-2 and the RBD of the Omicron variant. We did this by introducing 15 Omicron-specific mutations into the RBD (Fig. 1A). In addition, we introduced three additional mutations (A942P, K986P, and V987P) to increase the stability of the S protein, and deleted the furin cleavage site (∆PRRA) to maintain the S protein in its pre-fusion form. To express the S protein as a trimer, we fused the foldon motif of T4 fibrin to the C-terminus [13]. Additionally, we added histidine and HDEL residues to the C-terminus as an affinity tag (for purification) and an endoplasmic reticulum (ER) retention signal, respectively (Fig. 1A). Finally, a leader sequence (NB) from Arabidopsis BiP1 was added to the N-terminus (NB:S(rOm3P)delFnL::Fd:7H:HDEL, referred to as pSrOm) for ER targeting. Thus, recombinant protein pSrOm was designed to be produced as a trimer in the ER of Nicotiana benthamiana. The construct pSrOm was introduced into the leaf tissues of N. benthamiana via Agrobacterium-mediated infiltration, and leaf tissues were harvested 4 days later [14,15]. First, we examined expression of pSrOm in the plants. Total soluble protein extracts were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS/PAGE) and analyzed by western blotting using anti-His antibody. His-tagged S1 protein (100 ng) was used as a loading control. The immunoblot was stained with Coomassie brilliant blue (CBB). As a reference, we loaded 100 ng of the recombinant D614G variant S1 protein tagged with a His tag; this was
done to estimate the expression level of \( \text{pSrOmi} \). The results showed that 40 μg of protein was produced by 1 g of leaf tissue (Fig. 1B). Thus, expression was considered to be very high. Next, we purified pSrOmi from total soluble protein extracts of \( N. \text{benthamiana} \) to use to induce immune responses in mice. pSrOmi was purified from total soluble protein extracts using an Ni\(^{2+}\)-IDA affinity column followed by further purification using a prepacked Q-HP column. The purity and quantity of the protein were examined by SDS/PAGE and Coomassie brilliant blue staining. The purity was high, with almost no visible contaminating proteins (Fig. 1C).

We examined the immunogenicity of pSrOmi in mice. Mice (BALB/c, female, 16–20 g, 5 weeks old) were intramuscularly injected 2 times at an interval of 3 weeks with pSrOmi proteins (0.1, 1, 5, or 10 μg) and CIA09A as an adjuvant [16,17]. All animal experiments were performed in compliance with the Pohang Technopark IACUC (approval no., ABCC2022004). We included 10 μg of pSrOmi alone or phosphate-buffered saline as controls. Serum was obtained at 14 days after the second immunization. Antigen-specific antibody titers in sera were measured by enzyme-linked immunosorbent assay after serial dilution (from 1:150 to 1:11,718,750). The antibody titers induced by 1, 5, or 10 μg of pSrOmi in the presence of CIA09A were similar (Fig. 2A), indicating that CIA09A increases the immunogenicity of pSrOmi. Mice that received 10 μg of pSrOmi alone also showed strong immune response. However, the immune response to 10 μg of pSrOmi alone was weaker than that to 0.1 μg pSrOmi plus CIA09A. Next, we examined the end-point titer of these vaccines doses. The end-point titer ranged from 4.41 log\(_{10}\) for 10 μg pSrOmi alone to 5.53 log\(_{10}\) for 10 μg pSrOmi plus CIA09A (Fig. 2B).

To access the degree of protection against SARS-CoV-2 infection afforded by pSrOmi-induced neutralizing antibodies, we examined the extent to which the neutralizing antibodies inhibit the interaction between the RBD and human angiotensin-converting enzyme 2 (hACE2), the human receptor for SARS-CoV-2 [18]. Current COVID-19 vaccines are thought to provide a certain degree of protection against newly emerged variants, including the Omicron variant [19,20]. Therefore, we set the cutoff value at 20%. Neutralizing antibodies in sera obtained from mice injected with 1–10 μg pSrOmi plus CIA09A inhibited interaction between the RBD of Omicron and hACE2 by >90% (Fig. 3A). However, this fell to 79% at a dose of 0.1 μg pSrOmi plus CIA09A, and to 33% when using 10 μg pSrOmi alone, indicating that the titer must be >5 log\(_{10}\) to effectively inhibit interaction between RBD and hACE2 by >90%. Next, we accessed the cross-reactivity of pSrOmi-induced neutralizing antibodies with other variants of SARS-CoV-2. mRNA-based vaccines such as BNT162b2 and mRNA-1273 provide >90% protection against serious disease after infection by the Delta variant [9,21]. However, other reports show that the majority of the current vaccines cannot neutralize the Omicron variant effectively [10,21]. Only 20% and 24% of BNT162b2 recipients had detectable neutralizing antibodies against Omicron variants HKU691 and HKU344-R346K, respectively [9]. Therefore, we next measured the ability of pSrOmi-induced neutralizing antibodies to inhibit the interaction between the RBD of variants and hACE2. The percentage inhibition of the RBD of the Beta and Delta variants was close to the cutoff value (20%), or

![Fig. 2](https://www.ecevr.org/)

Fig. 2. Analysis of antigen (Ag)-specific serum immunoglobulin G (IgG) from immunized mice at 14 dpi. (A) Antibody titration curve based on sera obtained from mice at 14 dpi (diluted from 1:150 to 1:11,718,750). The optical density (OD) value of the lowest three dilutions of serum from phosphate-buffered saline (PBS)-injected mice was multiplied by 4 to yield the titration cutoff threshold. (B) The end-point titration values. The end-point titer of IgG antibodies was measured in enzyme-linked immunosorbent assay coated with recombinant pSrOmi. The end-point titration value was expressed as log\(_{10}\) values.
slightly higher (Fig. 3C, D); however, inhibition of the RBD of the Wuhan strain ranged from 60% to 70% (Fig. 3B). These data suggest almost no inhibition of the Beta and Delta variants. Thus, pSrOmi-induced neutralizing antibodies are highly specific for the Omicron RBD and may not provide any protection against other variants. One possible explanation for this would be that the heavily mutated RBD of the Omicron variant induces Omicron RBD-specific antibodies that do not react with the RBD of other variants. However, this does not agree with data showing that three doses of mRNA-based vaccines provide a high level of protection against the Omicron variant [19,20]. In animal models and humans, neutralizing antibodies generated by mRNA vaccines appear to be the primary correlate of COVID‐19 protection [22].

In conclusion, we observed that antibodies raised by S proteins with RBD of the Omicron variant can inhibit interaction of hACE2 with the RBD of Omicron variant but not with that of other variants. These results suggest that vaccine generated using the Omicron variant may be specific for the protection of Omicron variant, but not other variants.

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References

1. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature 2020; 579:265-9.
2. Anderson EJ, Rouphael NG, Widge AT, et al. Safety and immunogenicity of SARS-CoV-2 mRNA-1273 vaccine in older adults. N Engl J Med 2020;383:2427-38.
3. Chen WH, Strych U, Hotez PJ, Bottazzi ME. The SARS-CoV-2 vaccine pipeline: an overview. Curr Trop Med Rep 2020;7:61-4.
4. Forni G, Mantovani A; COVID-19 Commission of Accademia Nazionale dei Lincei, Rome. COVID-19 vaccines: where we stand and challenges ahead. Cell Death Differ 2021;28:626-39.
5. Awadasseid A, Wu Y, Tanaka Y, Zhang W. Current advances in the development of SARS-CoV-2 vaccines. Int J Biol Sci 2021;17:8-19.
6. Haas EJ, Angulo FJ, McLaughlin JM, et al. Impact and effectiveness of mRNA BNT162b2 vaccine against SARS-CoV-2 infections and COVID-19 cases, hospitalisations, and deaths following a nationwide vaccination campaign in Israel: an observational study using national surveillance data. Lancet 2021;397:1819-29.
7. Kashte S, Gulbake A, El-Amin III SF, Gupta A. COVID-19 vaccines: rapid development, implications, challenges and future prospects. Hum Cell 2021;34:711-33.
8. Wang P, Nair MS, Liu L, et al. Antibody resistance of SARS-CoV-2 variants B.1.351 and B.1.1.7. Nature 2021;593:130-5.
9. Araf Y, Akter F, Tang YD, et al. Omicron variant of SARS-CoV-2: genomics, transmissibility, and responses to current COVID-19 vaccines. J Med Virol 2022;94:1825-32.
10. Liu J, Liu Y, Xia H, et al. BNT162b2-elicited neutralization of B.1.617 and other SARS-CoV-2 variants. Nature 2021;596:273-5.
11. Liu C, Ginn HM, Dejirattisai W, et al. Reduced neutralization of SARS-CoV-2 B.1.617 by vaccine and convalescent serum. Cell 2021;184:4220-36.
12. Bergwerk M, Gonen T, Lustig Y, et al. Covid-19 break-through infections in vaccinated health care workers. N Engl J Med 2021;385:1474-84.
13. Hsieh CL, Goldsmith JA, Schaub JM, et al. Structure-based design of prefusion-stabilized SARS-CoV-2 spikes. Science 2020;369:1501-5.
14. Marillonnet S, Thoeringer C, Kandzia R, Klimyuk V, Gleba Y. Systemic agrobacterium tumefaciens-mediated transfection of viral replicons for efficient transient expression in plants. Nat Biotechnol 2005;23:718-23.
15. Islam MR, Son N, Lee J, Lee DW, Sohn EJ, Hwang I. Production of bacteriophage-encoded endolysin, LysP11, in Nicotiana benthamiana and its activity as a potent antimicrobial agent against Erysipelothrix rhusiopathiae. Plant Cell Rep 2019;38:1485-99.
16. Wui SR, Kim KS, Ryu JI, et al. Efficient induction of cell-mediated immunity to varicella-zoster virus glycoprotein E co-lyophilized with a cationic liposome-based adjuvant in mice. Vaccine 2019;37:2131-41.
17. Wui SR, Ko A, Ryu JI, et al. The Effect of a TLR4 agonist/cationic liposome adjuvant on varicella-zoster virus glycoprotein E vaccine efficacy: antigen presentation, uptake, and delivery to lymph nodes. Pharmaceutics 2021;13:390.
18. Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 2020;181:271-80.
19. Pfizer. Pfizer and BioNTech provide update on Omicron variant [Internet]. New York (NY): Pfizer; 2021 [cited 2021 Dec 16]. Available from: https://www.pfizer.com/news/press-release/press-release-detail/pfizer-and-biontech-provide-update-omicron-variant.
20. Garcia-Beltran WF, St Denis KJ, Hoelzemer A, et al. mRNA-based COVID-19 vaccine boosters induce neutralizing immunity against SARS-CoV-2 omicron variant. Cell 2022;185:457-66.
21. Hoffmann M, Kruger N, Schulz S, et al. The Omicron variant is highly resistant against antibody-mediated neutralization: implications for control of the COVID-19 pandemic. Cell 2022;185:447-56.
22. Mudd PA, Minervina AA, Pogorelyy MV, et al. SARS-CoV-2 mRNA vaccination elicits a robust and persistent T follicular helper cell response in humans. Cell 2022;185:603-13.