CORRESPONDENCE

Recombinant vaccine containing an RBD-Fc fusion induced protection against SARS-CoV-2 in nonhuman primates and mice

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The novel coronavirus SARS-CoV-2 has infected more than 104 million individuals and resulted in more than 2.2 million deaths worldwide as of February 7, 2021 (https://covid19.who.int). The COVID-19 pandemic highlights the need for safe and effective vaccines against SARS-CoV-2 infection. Several licensed vaccines and multiple vaccine candidates currently in clinical trials have shown different strengths and weaknesses (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/covid-19-vaccines).

Herein, we report the pilot-scale production of a recombinant subunit vaccine (RBD-Fc Vacc) with the receptor-binding domain of the SARS-CoV-2 S protein fused with the Fc domain of human IgG1. The immunogenicity of the SARS-CoV-2 RBD, which is located in the S1 subunit and is crucial in mediating viral entry into host cells by binding to the ACE2 receptor, has been determined to induce neutralizing antibodies without evident antibody-dependent enhancement effects1 and can protect animals against SARS-CoV-2 infection2. The Fc fusion protein has been recently used as an important backbone for drug development due to its advantages of rapid purification, a relatively long half-life, and the ability to increase the immunogenicity of target antigens3. In addition, Fc promotes the correct folding of the fusion protein and enhances binding to antigen-presenting cells4. We have previously developed Fc-fused protein vaccines against MERS, SARS-CoV, and H5N1 influenza and found that Fc-fused proteins are more immunogenic than those lacking fused Fc5,6. The advantages of recombinant protein vaccines, including safety (no viral genome integration, which ensures safe handling) and higher cost efficiency than other types of vaccines7, make them competitive vaccine candidates. In the present study, we developed a recombinant vaccine containing an RBD-Fc fusion (RBD-Fc Vacc), which is currently being assessed in randomized controlled phase I/II human clinical trials. In this study, the data showed the efficacy of RBD-Fc Vacc in protecting against SARS-CoV-2 infection in nonhuman primates and mice.

In this study, the dimer-based RBD-Fc Vacc against SARS-CoV-2 was first developed by fusing the RBD (aa 331-524) with the human IgG1 Fc fragment. The dimer structure predicted by protein structure prediction server version 3.0 is shown in Fig. 1a (left panel). Two RBD domains were fused through the Fc fragment to form the Y-shaped structure. The RBD-Fc fusion protein was expressed in mammalian CHO cells, and the antigenicity and dimer conformation were identified by western blot analysis using antiserum from recovered COVID-19 patients and a commercial antibody, respectively (Fig. 1a, right panel). The RBD-Fc Vacc bound to the hACE2 receptor with much higher avidity than the RBD-His monomer (74.13 nM vs. 8.26 nM), indicating the improved conformation of our RBD-Fc protein (Supplementary Fig. S1a). The immunogenicity of the RBD-Fc and RBD-His proteins was evaluated in BALB/c mice after two immunizations using aluminum as the adjuvant. Compared to serum from RBD-His-vaccinated mice, serum from RBD-Fc vaccinated mice showed significantly higher RBD-specific IgG antibody titers and live virus-neutralizing antibody titers (Supplementary Fig. S1b). These data suggested that the RBD-Fc fusion protein was more antigenic than the monomeric RBD protein.

We then evaluated the immunogenicity of RBD-Fc Vacc in Macaca fascicularis (n = 5/group) via intramuscular administration of 20 µg or 40 µg RBD-Fc Vacc or PBS control. The schedules for sample collection and examination are shown in Fig. 1c. High levels of RBD-specific immunoglobulin G (IgG) were rapidly induced in the serum of RBD-Fc Vacc-immunized Macaca fascicularis macaques, with median peaks of 1/17399 in the 20 µg group and 1/41900 in the 40 µg group on day 28 (Fig. 1d). There was a much higher NT50 in sera from Macaca fascicularis macaques immunized with three doses of RBD-Fc Vacc (day 42)

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than in sera from macaques immunized with two doses of RBD-Fc Vacc (d28) (Fig. 1e), which indicated that immunization with three doses may induce much better protective responses. When the cross-neutralizing activity was analyzed, *Macaca fascicularis* sera post RBD-Fc vaccination showed similar neutralizing capability against three SARS-CoV-2 strains with the same RBD sequence as the one in our RBD-Fc vaccine (Supplementary Fig. S2a). More importantly, immunization with the RBD-Fc fusion protein protected BALB/c mice against infection with the SARS-CoV-2 adapted virus MASCp6, which contains the N501Y mutation, a
key mutation that increases the binding affinity to human and murine ACE2 and has been found in the epidemic strain B.1.1.7.10 These data suggested that the RBD-Fc-based vaccine could induce broader neutralizing activity than current vaccines. In addition, the NT50 in sera from nonhuman primates receiving RBD-Fc Vacc immunization was comparable to that in sera from 23 convalescent COVID-19 patients (Supplementary Fig. S2b). Collectively, these results demonstrated that three immunizations with RBD-Fc Vacc induced high levels of neutralizing antibodies against SARS-CoV-2 in nonhuman primates.

To explore the cellular immune response induced by RBD-Fc Vacc, an IFN-γ ELISPOT assay and flow cytometry were performed in Macaca fascicularis on d42. There were comparable S1- or RBD-specific IFN-γ responses in splenocytes from nonvaccinated and RBD-Fc Vacc-vaccinated Macaca fascicularis macaques (Supplementary Fig. S3a), and the percentages of S1-specific IL-4+CD4+ T cells, IL-4+CD8+ T cells, TNF-α+CD4+ T cells, and TNF-α+CD8+ T cells were comparable (Supplementary Fig. S3b), suggesting that RBD-Fc Vacc mainly induced a humoral immune response in vaccinated animals.

We next evaluated whether RBD-Fc Vacc induces protective immunity against SARS-CoV-2 infection in Macaca fascicularis macaques. Four weeks after the third injection of 40 µg RBD-Fc Vacc (d56), macaques were challenged with 10^7 TCID50/mL SARS-CoV-2 as follows: inoculation of 2 mL by the intratracheal route, 1 mL by the intranasal route and 0.2 mL by the intraocular route. The substantial fraction of viral RNA, which represents the input challenge virus, in nasal, throat, and anal swabs was examined at different times by quantitative reverse transcription PCR. Peak viral loads were observed at various time points post challenge. All control Macaca fascicularis macaques showed excessive copy numbers of viral genomic RNA in nasal (Fig. 1f), throat, anal (Supplementary Fig. S4) and lung swabs by days 2-6 post inoculation (Fig. 1g and Supplementary Table S1), along with more severe interstitial pneumonia than vaccinated macaques (Fig. 1h). In contrast, all vaccinated Macaca fascicularis macaques were protected against SARS-CoV-2 infection, with much lower or no viral RNA copies and very mild histopathological changes in a few lobes of the lung. These data demonstrated that RBD-Fc Vacc induced a potent immune response, which protected Macaca fascicularis macaques against SARS-CoV-2 infection.

The protective efficacy of RBD-Fc Vacc against SARS-CoV-2 infection was also confirmed in hACE2-Tg mice using the same protocols as those used in Macaca fascicularis (Fig. 1c). In hACE2-Tg mice, RBD-Fc Vacc immunization contributed to median reductions of 2.10 Log_{10}RNA copies/g and 2.13 Log_{10}RNA copies/g in the 10 µg and 20 µg groups, respectively (Fig. 1i). All vaccinated animals showed very mild histopathological changes, while moderate interstitial pneumonia was observed in nonvaccinated controls (Fig. 1j). Finally, we analyzed the live virus neutralization antibody titers, pseudovirus neutralization antibody titers and IgG titers in hACE2-Tg mice on day 49. The results showed that all antibody titers were inversely correlated with the viral RNA titer in the lungs of hACE2-Tg mice (Fig. 1k). The vaccine-induced immune response and protection also showed an inverse correlation in Macaca fascicularis macaques, although statistical analysis could not be performed due to the limitation of sample size. These data demonstrated that the serum IgG titers and neutralizing antibody titers elicited by RBD-Fc may correlate with protection against SARS-CoV-2 infection.

In summary, we generated a new recombinant vaccine by fusing the SARS-CoV-2 RBD with the Fc fragment of human IgG1 and assessed the protective efficacy of this vaccine against SARS-CoV-2 challenge in nonhuman primates (Macaca fascicularis) and mice. To the best of our knowledge, this Fc fusion protein-based vaccine is the first to be tested in clinical trials.

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AUTHOR CONTRIBUTIONS
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ADDITIONAL INFORMATION
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REFERENCES

1. Quinlan, B. D. et al. The SARS-CoV-2 receptor-binding domain elicits a potent neutralizing response without antibody-dependent enhancement. 2020.04.2010.036418, https://doi.org/10.1101/2020.04.10.036418%JbioRxiv (2021).
2. Yang, J. et al. A vaccine targeting the RBD of the S protein of SARS-CoV-2 induces protective immunity. Nature 586, 572–577 (2020).
3. Wieland, A. & Ahmed, R. Fc receptors in antimicrobial protection. Curr. Top. Microbiol. Immunol. 423, 119–150 (2019).
4. Martyn, J. C. et al. Surface display of IgG Fc on baculovirus vectors enhances binding to antigen-presenting cells and cell lines expressing Fc receptors. Arch. Virol. 154, 1129–1138 (2009).
5. Li, Y. et al. A recombinant protein containing highly conserved hemagglutinin residues 81-122 of influenza H5N1 induces strong humoral and mucosal immune responses. Biosci. Trends 7, 129–137 (2013).
6. Du, L. et al. Receptor-binding domain of SARS-CoV spike protein induces long-term protective immunity in an animal model. Vaccine 25, 2832–2838 (2007).
7. Kaur, S. P. & Gupta, V. COVID-19 vaccine: a comprehensive status report. Virus Res. 288, 198114 (2020).
8. Zhang, N. N. et al. A thermostable mRNA vaccine against COVID-19. Cell 182, 1271–1283 e1216 (2020).
9. Gu, H. et al. Adaptation of SARS-CoV-2 in BALB/c mice for testing vaccine efficacy. Science 369, 1603–1607 (2020).
10. McCallum, M. et al. N-terminal domain antigenic mapping reveals a site of vulnerability for SARS-CoV-2. bioRxiv, https://doi.org/10.1101/2021.01.14.426475 (2021).