LETTER TO THE EDITOR

mRNA vaccines expressing homo-prototype/Omicron and hetero-chimeric RBD-dimers against SARS-CoV-2

Cell Research (2022) 0:1–4; https://doi.org/10.1038/s41422-022-00720-z

Dear Editor,

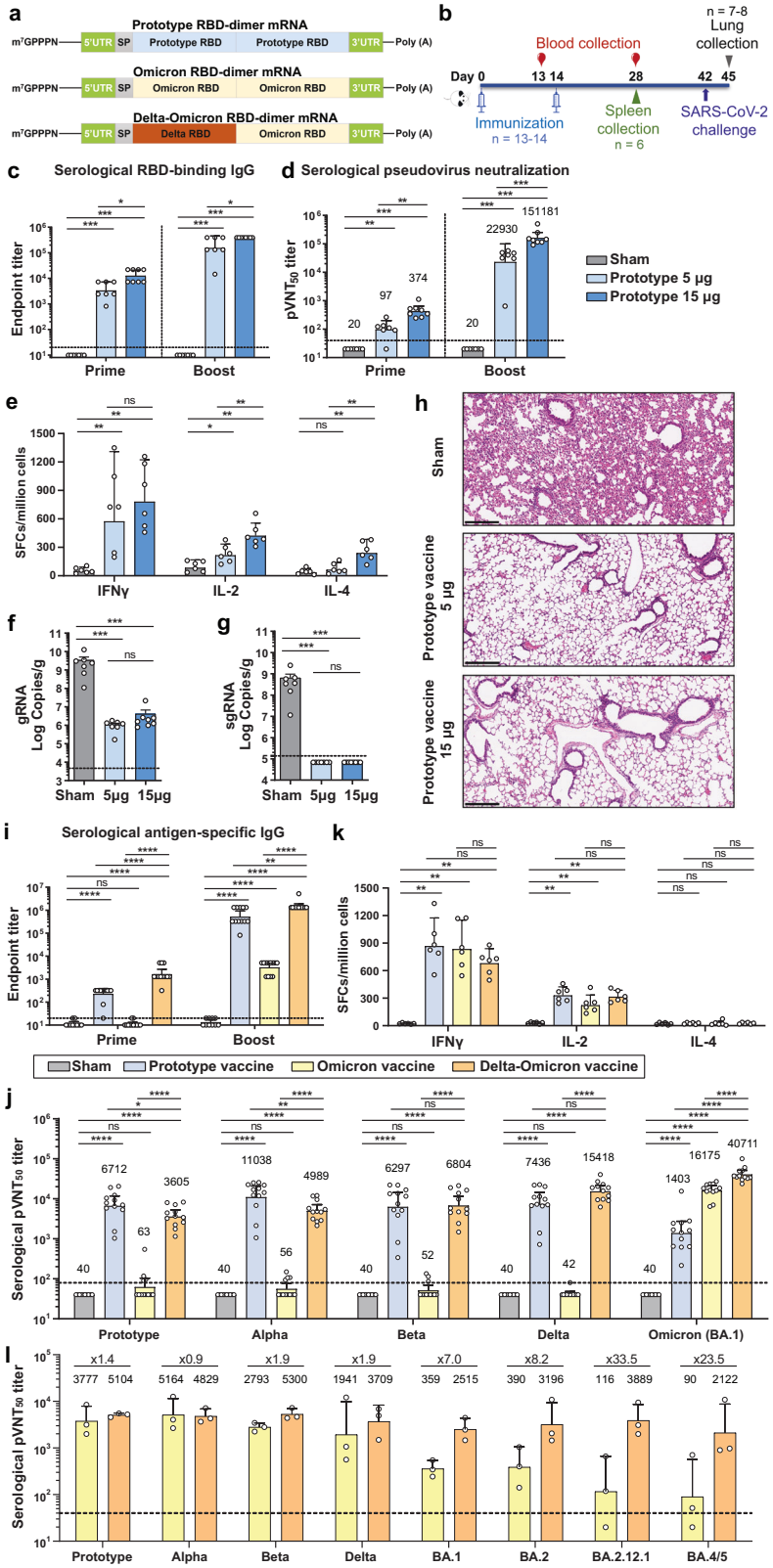
Since emerging in 2019, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused an ongoing human pandemic of coronavirus disease 2019 (COVID-19).[1] Vaccines against COVID-19 were developed and rolled out for large-scale vaccination, including two mRNA vaccines (BNT162b2 and mRNA-1273) encoding SARS-CoV-2 pre-fusion spike protein.[2,3] These mRNA vaccines have demonstrated strong immunogenicity and high efficacy, and offer the additional advantage of rapid development.[4,5] Further mRNA vaccine candidates include those targeting the receptor-binding domain (RBD), including one from our group,[6,7] which mediates engagement of the viral spike protein with its cellular receptor human angiotensin-converting enzyme 2 (hACE2). Previously, we reported the development of a protein subunit COVID-19 vaccine, ZF2001, based on the dimeric RBD of viral spike protein as immunogen.[8,9] This vaccine showed an efficacy of 81.4% against symptomatic COVID-19 in the Phase 3 clinical trial,[10] and has been approved in China, Uzbekistan, Indonesia and Colombia. To leverage advantages of the mRNA platform, we applied the RBD-dimer immunogen design as an mRNA vaccine. Here, we report the immunogenicity and efficacy of the RBD-dimer mRNA vaccine in mice. We also demonstrate that the flexibility of mRNA platform can be highly advantageous for rapid immunogen updates according to SARS-CoV-2 variants, exemplified by Delta and Omicron.

The vaccine mRNA transcript contains a 5′ untranslated region (UTR), the RBD-dimer coding sequence (HB-01 strain, prototype) (Supplementary information, Ref. 9), a 3′ UTR, and a poly-adenyl tail generated from a linear DNA template (Fig. 1a). In vitro immunogen expression was verified by detection of RBD-dimers in the supernatant of mRNA-transfected HEK 293 T cells via Western Blot (Supplementary information, Fig. S1). Thereafter, the mRNA was encapsulated into lipid nanoparticles (LNP) as mRNA vaccine, with an average diameter of 97 nm (Supplementary information, Fig. S2). To evaluate the immunogenicity of the prototype RBD-dimer mRNA vaccine, groups of BALB/c mice were immunized with two doses of 5 μg or 15 μg mRNA vaccine, at a 14 days interval (Fig. 1b). Another group of mice was immunized with LNP-GFP as negative control. After first and second immunization, the serological RBD-binding IgG and neutralizing antibodies were measured. The results showed that the first dose induced significant binding and neutralizing antibody production, and the second dose boosted the antibody titers to high levels (binding antibody titer $>10^5$; neutralizing antibody titer $>10^4$) (Fig. 1c, d). Of note, in a head-to-head comparison with the RBD-trimer mRNA vaccine candidate BNT162b1 (from BioNTech),[11] the RBD-dimer mRNA vaccine elicited comparable levels of antibodies at different time points after priming at higher or equal doses (Supplementary information, Fig. S3). Moreover, immunization of mice with the prototype RBD-dimer mRNA vaccine elicited a strong cellular immune response as demonstrated by ELISpot assays and intracellular cytokine staining (ICS), which showed a marked increase of IFNγ-secreting T cells, whereas the Th2 cytokine (IL-4) was only moderately induced in high dose (15 μg), but not low dose (5 μg) group (Fig. 1e; Supplementary information, Fig. S4). Hence, these data indicate that the RBD-dimer mRNA vaccine induced Th1-biased cytokine production.

To evaluate the efficacy of prototype RBD-dimer mRNA vaccine in vivo, the immunized BALB/c mice were challenged with a high dose (5×10^5 $TCID_{50}$) of SARS-CoV-2 (hCoV-19/China/CAS-B001/2020 strain) via intranasal route at 28 days post second dose (Fig. 1b). Mice were transduced with recombinant type 5 adenovirus expressing hACE2 5 days prior to SARS-CoV-2 infection. Lung tissues were collected for virus titration and pathological examination 3 days post challenge. High viral loads were detected in the lungs of mice within the sham-treated group, with a genomic RNA (gRNA) burden of average 1.68×10^9 copies/g and subgenomic RNA (sgRNA) of average 2.97×10^8 copies/g (Fig. 1f, g). For mice immunized with either low dose (5 μg) or high dose (15 μg) of RBD-dimer mRNA vaccine, the viral gRNA titers significantly decreased to 8.99×10^5 (5 μg) and 2.14×10^6 (15 μg) copies/g, respectively (Fig. 1f). The viral sgRNA was below detection limit in all immunized mice, indicating the complete inhibition of virus replication by the vaccine-induced immune response (Fig. 1g). In addition, the pathologic analysis of lung tissues revealed that vaccination relieved the pathological damage and inflammatory response caused by SARS-CoV-2 infection, such as thickened alveolar walls, vascular congestion, and inflammatory cell infiltration (Fig. 1h). These results demonstrate that the RBD-dimer mRNA vaccine was immunogenic in mice, induced a strong humoral as well as Th1-skewed cellular immune response, and caused protection against SARS-CoV-2 infection in the lung.

Since the beginning of the pandemic outbreak, new SARS-CoV-2 variants rapidly emerged. Different variants including Beta, Delta as well as the currently circulating Omicron variants have acquired the ability to evade immune responses induced by COVID-19 vaccines. With more than 30 amino acid mutations in the spike protein, Omicron variants are currently the most resistant to COVID-19 neutralizing antibodies and vaccine-elicited sera.[12] To combat the Omicron variants, mRNA vaccine candidates encoding Omicron-matched spike proteins were developed by several independent research groups and studies with these have shown immunogenicity and the feasibility of adapting vaccines to new variants (Supplementary information, Ref. 10). Sera of mice vaccinated with mRNA vaccines targeting Omicron BA.1 RBD showed strong neutralizing activity against BA.1, but displayed weak or even no neutralization to ancestral variants such as D614G, Beta and Delta (Supplementary information, Ref. 11). Since the composition of circulating variants changes over time, it would be useful to have vaccines that are effective against a broad spectrum of different SARS-CoV-2 variants.

Received: 12 May 2022 Accepted: 24 August 2022
Published online: 14 September 2022



Based on the RBD-dimer strategy, we designed a chimeric tandem Delta-Omicron (BA.1) protein subunit vaccine.[13] Compared to the prototype RBD-dimer vaccine ZF2001, the chimeric vaccine elicited broader responses against SARS-CoV-2 and its variants, and provided better protection against both Delta and Omicron.[13] In this study, we applied the chimeric Delta-Omicron (BA.1) design in the mRNA vaccine platform, verified the antigen expression in vitro (Supplementary information, Figs. S5 and S6), and evaluated its immunogenicity together with two homologous RBD-dimer mRNA vaccines (prototype-prototype and Omicron (BA.1)-Omicron (BA.1)) (Fig. 1a). BALB/c mice were immunized with two doses of each vaccine, 14 days apart. The Delta-Omicron

Fig. 1 Immunogenicity and efficacy of dimeric RBD-based mRNA vaccines. a Illustration of mRNA constructs of prototype RBD-dimer, Omicron RBD-dimer, and Delta-Omicron chimeric RBD-dimer. **b** Immunization and challenge schedule. Female BALB/c mice ($n = 13$–14) were immunized i.m. with two doses of mRNA vaccines, 14 days apart. Sera were collected on days 13 and 28. Spleens were collected on day 28 after first immunization ($n = 6$). The remaining animals ($n = 7$–8) were challenged with SARS-CoV-2 on day 42 and sacrificed to collect lung tissues. **c** Prototype SARS-CoV-2 RBD-binding IgG endpoint titers in the sera of immunized or control mice. **d** Prototype SARS-CoV-2 S protein pseudovirus neutralization titers in the sera of immunized or control mice. **e** Measurement of the IFNγ, IL-2 and IL-4 secretion of mouse splenocytes after stimulation with a SARS-CoV-2 prototype RBD peptide pool by ELISpot assays. SARS-CoV-2 titration from lung tissues by qRT-PCR probing virus gRNA (**f**) and sgRNA (**g**). **h** Histological pathology of lung sections of mice. Shown are the representative lung sections by H&E staining. Scale bars, 200 μm. **i**–**k** Female BALB/c mice ($n = 12$) were immunized i.m. with two doses of mRNA vaccines, at a 14-day interval. Sera were collected on days 7 and 21 after the first dose. Spleens were collected at Day 28 from immunized mice ($n = 6$). Measurement of the serological antigen-specific IgG titers (**i**). Measurement of neutralization titers against pseudoviruses (pVNT) of prototype SARS-CoV-2 and variants of mouse sera elicited by two-dose immunization (**j**). Measurement of the IFNγ, IL-2 and IL-4 secretion of mice splenocytes after stimulation with peptide pool by ELISpot assays (SFCs: spot-forming cells) (**k**). **l** Female BALB/c mice were immunized i.m. with two doses (10 μg/dose) of adjuvanted prototype RBD-dimer-based protein subunit vaccine, 21 days apart. Serum samples were collected and tested for RBD-binding antibodies on day 330 after priming (Supplementary information, Fig. S7). Mice were distributed into two groups ($n = 3$ each) with similar average titers of binding antibodies between groups. Each group of mice was boosted with either the Delta-Omicron chimeric RBD-dimer or Omicron RBD-dimer mRNA vaccine on day 335 after priming. Seven days later, serum samples were collected to measure the neutralizing activity. For (**c**, **d**, **f**, **g**, **i**, **k**, **l**), the values are the GMT ± 95% confidence interval (CI). *P* values were determined with two-tailed Mann–Whitney test (ns, $P > 0.05$; *,$P < 0.05$; **,$P < 0.01$; ***,$P < 0.001$; ****,$P < 0.0001$). The horizontal dashed line indicates the limit of detection (LOD). LOD in (**c**) is 20; in (**d**) is 40; in (**i**) is 20; in (**j**) is 80. For (**e**) and (**k**), data indicate means ± SEM (standard errors of means). *P* values were analyzed with two-tailed Mann–Whitney test (ns, $P > 0.05$; *,$P < 0.05$; **,$P < 0.01$).

RBD-dimer mRNA vaccine induced a higher geometric mean titer (GMT) of RBD-binding antibodies than the prototype and Omicron vaccines (Fig. 1i). The neutralizing activity of mouse sera was analyzed against pseudotyped virus expressing SARS-CoV-2 prototype, Alpha, Beta, Delta or Omicron (BA.1) spike. Analysis of sera from prototype vaccine-immunized mice revealed that the neutralizing GMT was preserved against Alpha (11,038), Beta (6297), and Delta (7436) variants. However, compared to the prototype pseudovirus (6712), the neutralizing capacity against Omicron (1403) was strongly reduced. The lower titers of neutralizing antibodies against the prototype pseudovirus compared to the Alpha and Delta VOCs are likely due to the lower binding affinities of RBD-targeting antibodies to prototype RBD, as shown recently.[14] Sera from mice immunized with the Omicron BA.1 vaccine strongly neutralized BA.1 pseudovirus, but displayed little cross-neutralization of prototype, Alpha, Beta, or Delta pseudoviruses (Fig. 1j), consistent with previous observations.[15] In the sera of mice immunized with the Delta-Omicron vaccine, broader neutralizing activity was observed, with comparable GMTs against prototype (3605), Alpha (4989), and Beta (6804), and high titers especially against Delta (15,418) and Omicron (40,711) pseudoviruses (Fig. 1j). In addition, a strong increase in Th1 cytokine (IFNγ and IL-2) secreting lymphocytes was detected 14 days after the second dose of prototype, Omicron or Delta-Omicron mRNA vaccine, without significant difference between groups (Fig. 1k). In contrast, no substantial Th2 cytokine (IL-4) production was observed in all the groups.

Antigen expression of the different mRNA vaccines was evaluated. After transfection of cells, protein expression was detected for all candidates. Compared to prototype RBD-dimer, Omicron RBD-dimer was detected in the supernatant to a lesser extent (Supplementary information, Fig. S5). This yield reduction of Omicron RBD-dimer may be due to the decreased stability of Omicron RBD as observed by others (Supplementary information, Ref. 12). By contrast, the Omicron RBD fused to a Delta RBD as the chimeric RBD-dimer largely increased the antigen expression (Supplementary information, Fig. S5). The results suggested another benefit of using chimeric RBD-dimer as the antigen to overcome the poor antigen expression of Omicron variant. Different expression patterns of the three dimers could partially explain the differential immunogenicity results that Delta-Omicron mRNA vaccine induced higher neutralizing antibody titer against Omicron pseudovirus than Omicron mRNA vaccine as we have demonstrated (Fig. 1j).

To test a heterologous boosting scenario, prototype protein subunit vaccine-experienced mice were boosted with either the Omicron vaccine or the Delta-Omicron vaccine. Before heterologous boost, animals had comparable RBD-binding IgG titers against the prototype (Supplementary information, Fig. S7). The heterologous booster with the Delta-Omicron vaccine induced high neutralizing activity against all previously tested variant pseudoviruses, whereas neutralization of Omicron BA.1 was substantially lower after the Omicron booster (Fig. 1l). Given the recent surge of Omicron BA.2 and BA.2.12.1, and the current surge of BA.4/5, we also tested the vaccine-elicited serum neutralization of pseudoviruses expressing spikes from these sub-lineage variants. In addition, GMTs against the Omicron sub-lineage variants BA.2, BA.2.12.1, and BA.4/5 were comparable in sera from mice that received the Delta-Omicron booster. In contrast, GMTs against these Omicron variants were markedly lower in sera from Omicron-boosted mice.

Our data support that a chimeric Delta-Omicron immunogen can improve the vaccine-elicited serum cross-neutralization of SARS-CoV-2 variants, including the currently circulating Omicron sub-lineage variants BA.4/5. In summary, the data of a Delta-Omicron RBD mRNA vaccine tested in mice indicated the feasibility of a rapid RBD-dimer immunogen update using the mRNA vaccine technology platform. The variant-adapted multivalent mRNA vaccine strategy outlined above could accelerate the development of vaccines that address circulating and emerging SARS-CoV-2 variants.

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ACKNOWLEDGEMENTS

This work is supported by the National Key R&D Program of China (2020YFA0907100), the National Natural Science Foundation of China (NSFC) (82041048 and 81991494) and a grant from the Bill & Melinda Gates Foundation (INV-027420). L.D. is supported by the Excellent Young Scientist Program from NSFC (82122031) and the Chinese Academy of Sciences (YSBR-010). We thank Minrun Duan (Yunnan University), Tianyi Zheng (Zhejiang University), Sheng Liu (Southern University of Science and Technology) and Pei Du (Institute of Microbiology, CAS) for their assistance in the experiments and helpful suggestions. We thank Yuhai Bi, Ning Zhang, and all the staff of ABSL-3 at Institute of Microbiology, CAS for their assistance with the experiments performed in ABSL-3. We are grateful to Tong Zhao in Institute of Microbiology, CAS for her technical assistance with flow cytometry experiments.

AUTHOR CONTRIBUTIONS

G.F.G., L.D., and Q.W. conceived and coordinated the project. Y.H., Y.A., Q.C., X.L., S.X., and H.D. conducted the experiments. A.B.V. and U.Ş. coordinated the comparison of the immunogenicity of RBD-dimer and RBD-trimer vaccines. K.X. and Y.H. wrote the manuscript. G.F.G., L.D., and Q.W. revised the manuscript.

COMPETING INTERESTS

Y.A., K.X., L.D, and G.F.G. are listed in the patent as the inventors of the prototype RBD-dimer as coronavirus vaccines. K.X., T.Z., L.D., and G.F.G. are listed in the patent as the inventors of chimeric Delta-Omicron RBD-dimer as coronavirus vaccine. U.Ş. is a management board member at BioNTech SE (Mainz, Germany) and U.Ş and A.B.V. are employees at BioNTech SE. A.B.V. and U.Ş. are inventors on patents and patent applications related to RNA technology and COVID-19 vaccines having securities from BioNTech SE. All other authors declare no competing interests.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41422-022-00720-z.

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