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RBD-mRNA vaccine induces broadly neutralizing antibodies against Omicron and multiple other variants and protects mice from SARS-CoV-2 challenge



JUAN SHI[#], JIAN ZHENG[#], XIUJUAN ZHANG, WANBO TAI, ABBY E. ODLE, STANLEY PERLMAN, and LANYING DU

ATLANTA, GEORGIA; NEW YORK, NEW YORK; AND IOWA CITY, IOWA

Multiple SARS-CoV-2 variants are identified with higher rates of transmissibility or greater disease severity. Particularly, recent emergence of Omicron variant with rapid human-to-human transmission posts new challenges to the current prevention strategies. In this study, following vaccination with an mRNA vaccine encoding SARS-CoV-2 receptor-binding domain (RBD-mRNA), we detected serum antibodies that neutralized pseudoviruses expressing spike (S) protein harboring single or multiple mutations, as well as authentic SARS-CoV-2 variants, and evaluated its protection against SARS-CoV-2 infection. The vaccine induced durable antibodies that potently neutralized prototypic strain and B.1.1.7 lineage variant pseudoviruses containing N501Y or D614G mutations alone or in combination with a N439K mutation (B.1.258 lineage), with a L452R mutation (B.1.427 or B.1.429 lineage), or a L452R-E484Q double mutation (B.1.617.1 variant), although neutralizing activity against B.1.1.7 lineage variant containing 10 amino acid changes in the S protein was slightly reduced. The RBD-mRNA-induced antibodies exerted moderate neutralization against authentic B.1.617.2 and B.1.1.529 variants, and pseudotyped B.1.351 and P.1 lineage variants containing K417N/T, E484K, and N501Y mutations, the B.1.617.2 lineage variant harboring L452R, T478K, and P681R mutations, and the B.1.1.529 lineage variant containing 38 mutations in the S protein. Particularly, RBD-mRNA vaccine completely protected mice from challenge with a virulent mouse-adapted SARS-CoV-2 variant. Among these lineages, B.1.1.7, B.1.351, P.1, B.1.617.2, and B.1.1.529 belong to Alpha, Beta, Gamma, Delta, and Omicron variants, respectively. Our observations reveal that RBD-mRNA vaccine is promising and highlights the need to design novel vaccines with improved neutralization against current and future pandemic SARS-CoV-2 variants. (*Translational Research* 2022; 248:11–21)

Abbreviations: ACE2 = angiotensin converting enzyme 2; COVID-19 = Coronavirus Disease 2019; E = envelope; I.D. = intradermally; LNPs = lipid nanoparticles; N = nucleocapsid; NTD = N-terminal domain; M = membrane; PFU = plaque-forming unit; PRNT = plaque reduction neutral-

[#]These authors contributed equally.

From the Institute for Biomedical Sciences, Georgia State University, Atlanta, Georgia; Lindsley F. Kimball Research Institute, New York Blood Center, New York, New York; Department of Microbiology and Immunology, and Department of Pediatrics, University of Iowa, Iowa City, Iowa. Submitted for Publication January 2, 2022; revision submitted April 19, 2022; Accepted for Publication April 21, 2022.

Reprint requests: Lanying Du, Institute for Biomedical Sciences, Georgia State University, Atlanta, GA, 30303. e-mail: stanley-perlman@uiowa.edu.

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ization assay; RBD = receptor-binding domain; S = spike; SARS-CoV-2 = severe acute respiratory syndrome coronavirus-2; VLPs = virus-like particles; VOCs = variants of concern; VOIs = variants of interest

At a Glance Commentary

Juan Shi, et al.

Background

SARS-CoV-2, the causative agent of COVID-19, has mutated frequently since its first emergence, leading to a number of variants of concern (VOCs), including Alpha, Beta, Gamma, Delta, and Omicron variants.

Translational Significance

An mRNA vaccine encoding SARS-CoV-2 receptor-binding domain (RBD) induced antibodies that broadly neutralized all VOCs tested, albeit the activity in neutralizing Beta, Gamma, Delta, and Omicron was moderately reduced than the prototypic strain. It also completely protected immunized mice against a virulent SARS-CoV-2 variant. These findings suggest an urgent need to develop novel and effective vaccines with improved efficiency against current and future pandemic SARS-CoV-2 variants.

INTRODUCTION

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), first reported in 2019,¹ causes the global pandemic of Coronavirus Disease 2019 (COVID-19). This pandemic has led to millions of deaths with devastating damages, significantly affecting the public health, and global economy. The genome of SARS-CoV-2 encodes 4 structural proteins, including spike (S), nucleocapsid (N), membrane (M), and envelope (E); among them, the S protein plays an important role in virus infection and pathogenesis.^{2,3} Similar to other coronavirus S proteins, the S protein of SARS-CoV-2 consists of S1 and S2 subunits.³ During SARS-CoV-2 infection, the virus first binds a cellular receptor, angiotensin converting enzyme 2 (ACE2), through the receptor-binding domain (RBD) in the S1 subunit, followed by virus-cell membrane fusion through the S2 subunit, mediating the virus entry into host cells.^{4,5} Therefore, the S protein, including the RBD, is a critical target for the development of SARS-CoV-2 vaccines and therapeutic antibodies.^{4,6,7}

SARS-CoV-2 has mutated frequently since its first emergence, and increasing numbers of variants have been identified with mutations in the S, M, N, and E structural proteins and several non-structural proteins. Notably, mutation(s) in the S1 subunit of SARS-CoV-2, including RBD, generally have more significant effects than the non-S mutations.^{8,9} SARS-CoV-2 variants belonging to the B.1.1.7 (Alpha) (United Kingdom), B.1.351 (Beta) (South Africa), P.1 (Gamma) (Brazil), B.1.427, B.1.429 (Epsilon) (USA), B.1.617.1 (Kappa) (India), and B.1.617.2 (Delta) (India) lineages have been efficiently transmitted to other regions and are therefore the variant strains of concern (VOCs) or variants of interest (VOIs).^{10–13} In addition, recent emergence of a new SARS-CoV-2 variant, B.1.1.529 (Omicron), which was first reported in South Africa and spread rapidly to other countries,^{14–16} has posed new challenges to the current prevention strategies. All of these variant strains carry key mutations (substitutions, insertions, or deletions) in the S protein, particularly S1 subunit (N-terminal domain: NTD or RBD), including 69del, 70del, S371L, S373P, S375F, K417T/N, N440K, G446S, L452R, S477N, T478K, E484A, E484K, Q493R, Q498R, N501Y, D614G, P681H, and P681R, either alone or in combination (Table 1). Among variant SARS-CoV-2 strains, the B.1.617.2, B.1.1.7, B.1.427, and B.1.429 lineages have demonstrated higher transmissibility and increased disease severity; the B.1.1.529 variant has spread rapidly among humans.^{17–22} The emergence of such mutant SARS-CoV-2 strains decreases the efficacy of current COVID-19 vaccines and therapeutic agents, potentially allowing the virus to evade vaccine-induced immune responses or antibody neutralization.^{23–25} Therefore, development of vaccines with broad-spectrum activity against multiple SARS-CoV-2 strains is critically important to prevent infection by current mutant strains and future variants.

In this study, we evaluated the broad-spectrum activity of antibodies induced by an mRNA vaccine encoding SARS-CoV-2 RBD (RBD-mRNA) to neutralize these SARS-CoV-2 variant strains, including B.1.1.529 (Omicron) and B.1.617.2 (Delta) variants. We also demonstrated that this vaccine induced complete protection of mice from challenge of a virulent mouse-adapted SARS-CoV-2.

MATERIALS AND METHODS

Construction of recombinant plasmids. Recombinant plasmids expressing S protein of prototypic (GenBank accession number: QHR63250.1) and B.1.1.7 variant (GISAID accession number: EPI_ISL_718813, which contains all 10 amino acid substitutions or deletions) SARS-CoV-2 strains were constructed by inserting each codon-optimized S gene into pcDNA3.1/V5-His-TOPO vector (Thermo Fisher Scientific, Waltham, MA). B.1.1.529 (GISAID accession number: EPI_ISL_6795835) and other recombinant plasmids expressing the S protein with single or multiple amino acid substitutions were constructed using multi-site-directed mutagenesis kit (Agilent Technologies, Santa Clara, CA), and confirmed for correct mutation(s) by sequencing analysis. The constructed plasmids were used to generate pseudoviruses as described below.

Pseudovirus neutralization assay. The mouse serum samples were detected for neutralizing antibody activity against infection of SARS-CoV-2 prototypic strain and mutant pseudoviruses using our established pseudovirus neutralization assay with some modifications.^{7,26} Briefly, a plasmid encoding the S protein of SARS-CoV-2 prototypic strain or each of the variants containing single or multiple amino acid mutations was transfected into 293T cells in the presence of 2 additional plasmids (psPAX2 and pLenti-CMV-luciferase encoding luciferase protein) (Addgene, Watertown, MA). Pseudovirus-containing culture supernatants were collected at 72 hours after transfection, incubated with serially diluted mouse sera for 1 hour at 37°C, and the virus-serum mixture was added into 293T cells expressing SARS-CoV-2 receptor human ACE2 (hACE2/293T). After being cultured at 37°C for 72 hours, the cells were lysed using cell lysis buffer (Promega, Madison, WI), and detected for relative luciferase activity using Infinite 200 PRO Luminometer (Tecan, Morrisville, NC). Neutralizing activity of serum antibodies against SARS-CoV-2 was calculated using the CalcuSyn computer program and expressed as 50% pseudovirus neutralizing antibody titer (NT₅₀).

Plaque reduction neutralization assay (PRNT). Sera from immunized mice were detected for their neutralizing activity against authentic SARS-CoV-2 original strain, as well as B.1.617.2 and B.1.1.529 variants, using a PRNT assay as described before.²⁷ Specifically, sera were serially diluted in DMEM cell culture media and mixed with SARS-CoV-2 (40-80 plaque-forming unit: PFU/well) for 1 hour at 37°C. The serum-virus mixture was then added into Vero E6 (for original strain and B.1.617.2 variant) or ACE2 + TMPRSS2 + Vero E6 (for B.1.1.529 variant) cells for

an additional 45 minutes at 37°C. After removing the culture medium, cells were overlaid with 0.6% agarose and cultured for 3 days. Plaques were visualized by 0.1% crystal violet staining. The neutralizing antibody titer was calculated as the highest serum dilution capable of reducing the number of virus plaques by 50% (ie, NT₅₀).

Ethics statement. Male and female BALB/c mice were used in the study. The animal protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the Georgia State University, New York Blood Center, and University of Iowa. All animal studies were carried out in strict accordance with the guidance and recommendations in the Guide for the Care and Use of Laboratory Animals (National Research Council Committee).

Mouse immunization and serum collection. Six- to 8-week-old BALB/c mice were intradermally (I.D.) immunized with lipid nanoparticle (LNP)-encapsulated SARS-CoV-2 RBD-mRNA (10 μg/mouse), or empty LNPs (control), and boosted with the same immunogen at 4 weeks and 8 months, respectively.²⁸ Sera were collected at 10 days post-last dose, and detected for neutralizing antibodies against pseudotyped SARS-CoV-2 expressing S protein of prototypic strain or each of the mutant variants as described above.

Mouse challenge study. Six- to 8-week-old BALB/c mice were I.D. immunized with SARS-CoV-2 RBD-mRNA or empty LNPs (control) as described above (for mouse immunization), and boosted with the same immunogen at 4 weeks. At 70 days post-2nd immunization, the vaccinated mice were intranasally challenged with SARS2-N501Y_{MA30} (a virulent mouse-adapted strain of SARS-CoV-2, 5 × 10³ PFU/mouse, in 50 μL DMEM), and observed for 2 weeks for survival and body weight changes.^{27,29,30} Mouse sera collected at 10 days post-last immunization or before virus challenge were assessed for their activity to neutralize pseudotyped or authentic SARS-CoV-2 prototypic strain, B.1.1.529, or B.1.617.2 variant.

RESULTS

RBD-mRNA vaccine elicited long-term neutralizing antibodies against SARS-CoV-2 B.1.1.7 lineage variants with or without mutations of B.1.258 lineage variants

We generated a series of mutant pseudoviruses (Table 1) by transfecting plasmids expressing SARS-CoV-2 S protein with single or combined substitutions, deletions, or insertions in the S1 (NTD and RBD) and S2 subunits. For comparison, we also constructed a pseudovirus containing all 10 mutations (substitutions

or deletions) in the S protein of SARS-CoV-2 B.1.1.7 lineage variant. Because the resultant pseudoviruses lack the ability to replicate, they undergo single-cycle infection, and the experimental results reflect the ability of antibodies to block S protein-mediated cell entry. Sera collected at 8 months from SARS-CoV-2 RBD-mRNA-immunized mice were tested for neutralizing antibodies against the SARS-CoV-2 B.1.1.7 lineage variants with or without mutations of B.1.258 lineage variants.

The results revealed that SARS-CoV-2 RBD-mRNA induced long-term antibodies that potently neutralized infection by pseudovirus expressing S protein of SARS-CoV-2 (prototypic strain), with neutralizing antibody titer reaching $\sim 1:44,000$ (Fig 1A). These antibodies had similar or higher potency in neutralization of pseudoviruses containing single (N501Y or D614G) (Fig 1B and C) or combined (N501Y-D614G, N439K-

D614G, 69-70del-N501Y-D614G, and 69-70del-N439K-D614G) mutations (Fig 1D–G). By comparison, these antibodies had less neutralizing activity against pseudoviruses containing deletions of amino acids 69 and 70 (69-70del) (Fig 1H) or these deletions in combination with other single mutations (69-70del-N501Y and 69-70del-N439K) (Fig 1I and J). Although neutralizing activity was slightly reduced against a B.1.1.7 lineage variant containing 10 amino acid changes in the S protein, the neutralizing antibody titer was still greater than 1:10,000 (Fig 1K). In contrast, the control (empty LNPs) only induced a background level of neutralizing antibodies against the aforementioned pseudoviruses (Fig 1). These data indicate that SARS-CoV-2 RBD-mRNA vaccine elicited durable neutralizing antibodies against SARS-CoV-2 B.1.1.7 lineage variants harboring single or multiple mutations in the S protein, as well as combinations of these

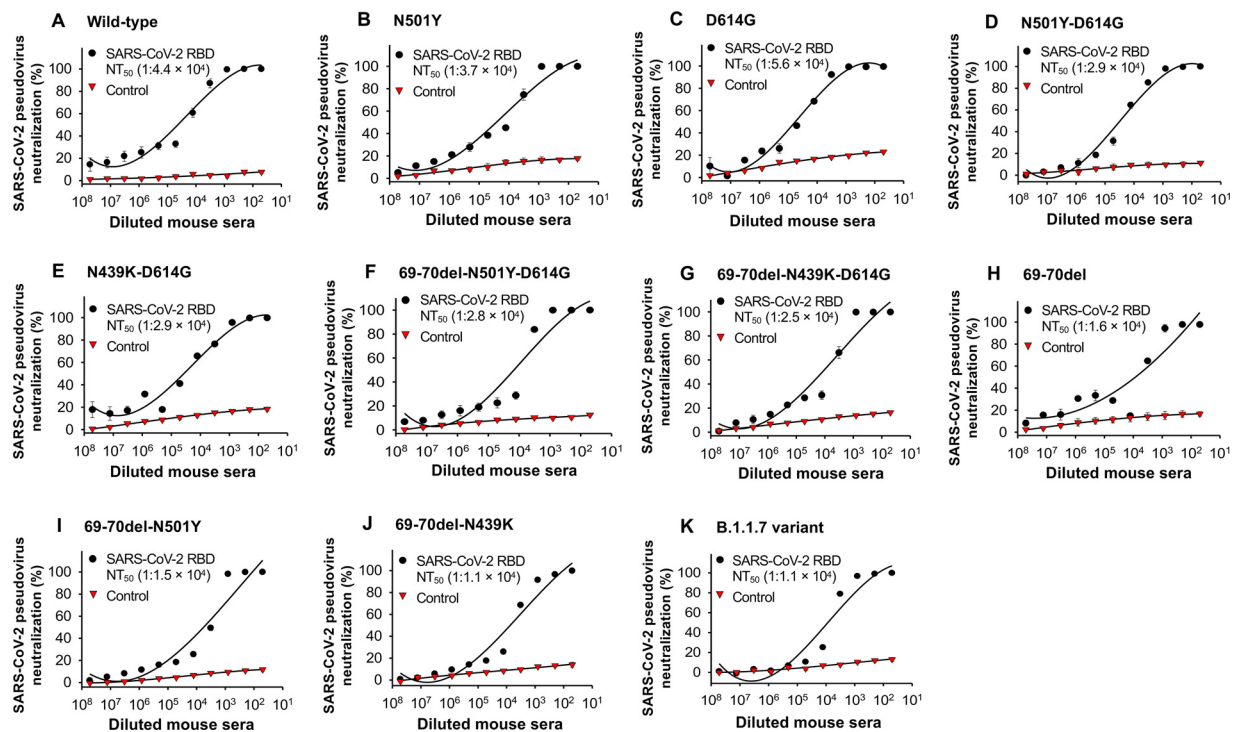


Fig 1. SARS-CoV-2 RBD-mRNA induced neutralizing antibodies against SARS-CoV-2 B.1.1.7 variant and its combinations with other lineages. BALB/c mice were immunized with SARS-CoV-2 RBD-mRNA (eg, RBD) or empty LNPs (control), boosted twice at 4 weeks and 8 months, respectively, and then the sera were collected at 10 days post-last immunization to assess neutralizing antibodies against the SARS-CoV-2 prototypic (wild-type) or mutant pseudoviruses. (A) Neutralizing antibodies against SARS-CoV-2 pseudovirus expressing prototypic S protein was constructed as comparison. Neutralizing antibodies against pseudovirus variants containing single or multiple key substitutions or deletions, including N501Y (B), D614G (C), and 69-70del (H) in the S1 subunit of SARS-CoV-2 S protein, as well as their combinations with or without N439K mutation (D–G, I, J). (K) Neutralizing antibodies against SARS-CoV-2 pseudovirus containing all 10 mutations (69-70 deletion, 145 deletion, N501Y, A570D, D614G, P681H, T716I, S982A, and D1118H) in the S protein of SARS-CoV-2 B.1.1.7 lineage variant. 50% neutralizing antibody titer (NT_{50}) was calculated against each pseudovirus infection in hACE2-293T cells, and the data are presented as mean \pm standard deviation (s.e.m) of 4 wells/dilution of pooled sera from each group ($n = 4$). Experiments were repeated twice, yielding similar results.

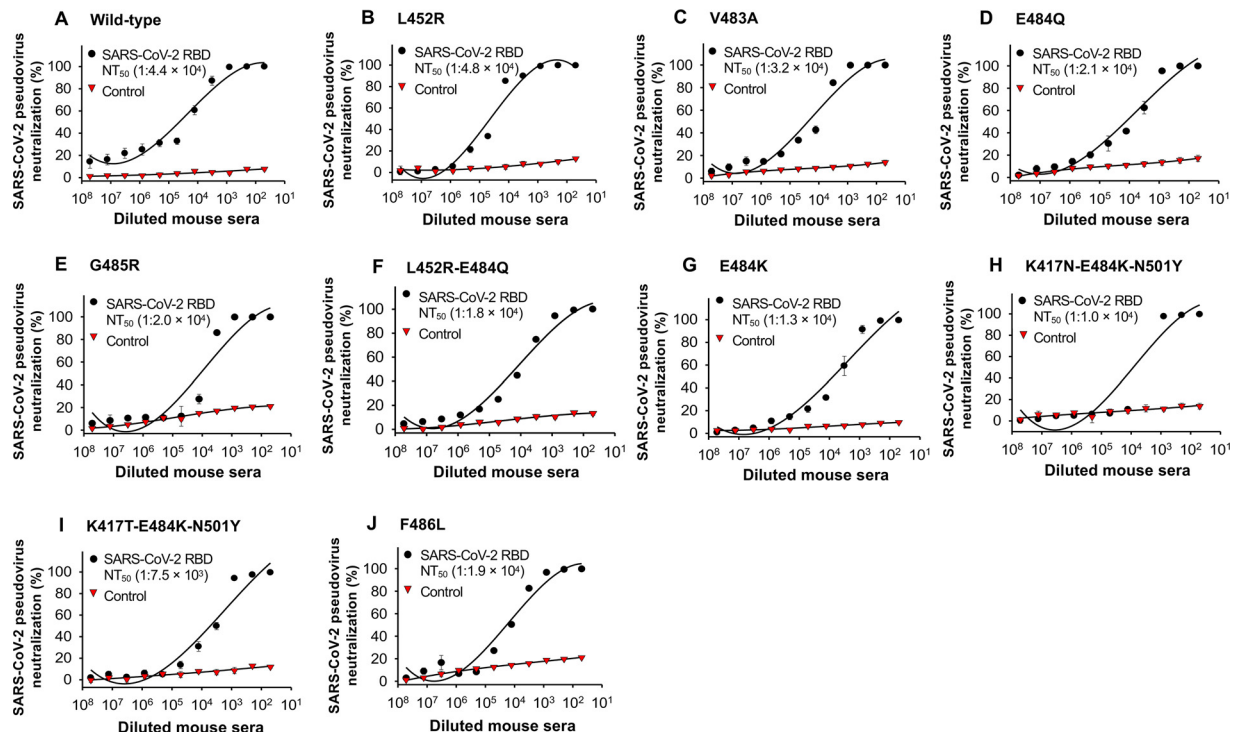


Fig 2. SARS-CoV-2 RBD-mRNA induced neutralizing antibodies against SARS-CoV-2 B.1.351, P.1, B.1.617.1, B.1.427, and B.1.429 lineage variants. Same mouse sera described in Fig 1 were tested for neutralizing antibodies against pseudoviruses expressing SARS-CoV-2 prototypic (wild-type) S protein control (A), or containing single substitutions of L452R (B), V483A (C), E484Q (D), G485R (E), E484K (G), and F486L (J), dual mutations of L452R and E484Q (F), or the combinations of E484K and N501Y with K417N/T (H, I) in the S1 subunit of SARS-CoV-2 S protein. NT_{50} was calculated against each pseudovirus infection in hACE2-293T cells, and the data are presented as mean \pm s.e.m of 4 wells/dilution of pooled sera from each group ($n = 4$). Experiments were repeated twice, yielding similar results.

mutations with the B.1.258 lineage variant containing the N439K mutation at the RBD region.

RBD-mRNA vaccine elicited long-term neutralizing antibodies against SARS-CoV-2 B.1.427, B.1.429, B.1.617.1, B.1.351, and P.1 lineage variants

We further evaluated the neutralizing activity of SARS-CoV-2 RBD-mRNA vaccine against other SARS-CoV-2 variants, including B.1.427, B.1.429, B.1.617.1, B.1.351, and P.1 lineages. As such, sera collected above were tested for the neutralizing activity against pseudoviruses harboring the respective mutations in the RBD of SARS-CoV-2 (Table 1).

The results indicated that RBD-mRNA elicited durable and potent neutralizing antibodies against pseudovirus containing the L452R mutation in the RBD (B.1.427 or B.1.429 lineage), with the antibody titer (1:48,000) (Fig 2B) higher than that against the prototypic pseudovirus (Fig 2A). These antibodies neutralized pseudoviruses containing a single substitution (V483A, E484Q, or G485R), or L452R-E484Q double mutations (B.1.617.1 lineage) in the RBD (Fig 2C–F), with slightly lower neutralizing antibody titers than

those against the prototypic pseudovirus. These serum antibodies also neutralized pseudoviruses containing the E484K single mutation (Fig 2G), or its combination with the K417N/T and N501Y mutations (Fig 2H and I), albeit the neutralizing antibody titers were slightly lower than the titers of antibodies to neutralize other mutant pseudoviruses. Notably, K417N, particularly K417T, mutation potentially increased resistance to vaccine-induced neutralizing antibodies, with the neutralizing antibody titers against K417N-E484K-N501Y (B.1.351 lineage) and K417T-E484K-N501Y (P.1 lineage) variants were reduced to about 1:10,000 and 1:7500, respectively (Fig 2H and I). In addition to human strains, the vaccine-induced antibodies also neutralized a pseudovirus expressing S protein (F486L) of mink SARS-CoV-2, and the neutralizing titer was slightly reduced than the titer of antibodies to neutralize the SARS-CoV-2 prototypic strain (Fig 2J). In contrast, sera from mice immunized with the control (empty LNPs) only had a background level of neutralizing antibodies against these pseudoviruses (Fig 2). The above data suggest that neutralizing antibodies

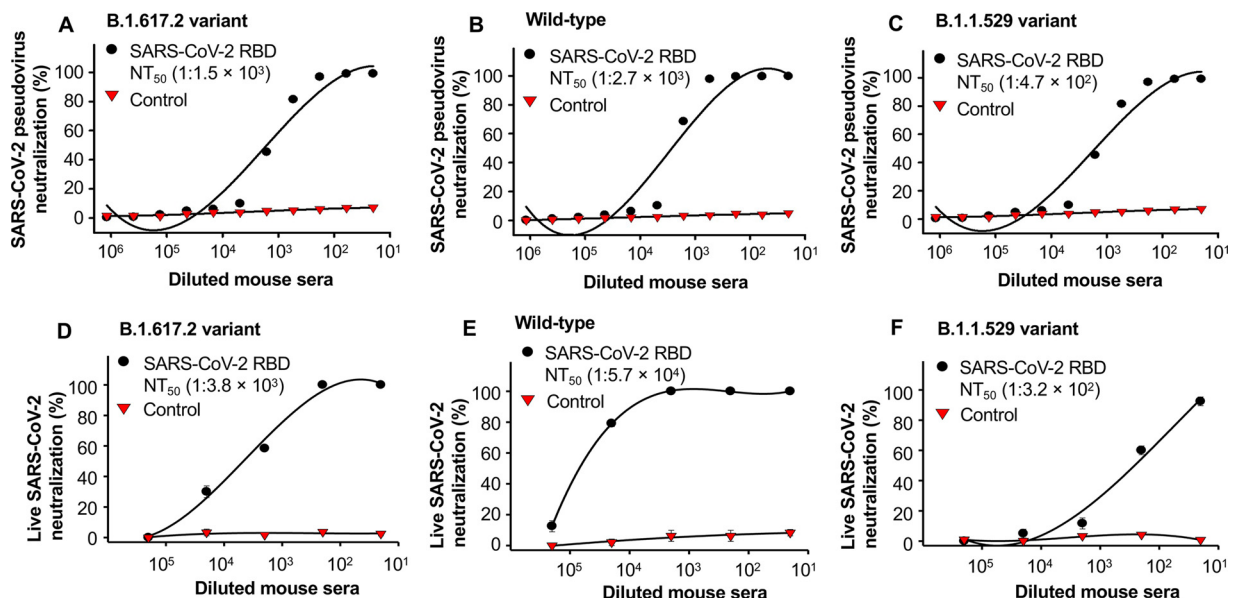


Fig 3. SARS-CoV-2 RBD-mRNA-induced neutralizing antibodies against SARS-CoV-2 B.1.617.2 and B.1.1.529 lineage variants. BALB/c mice were immunized with SARS-CoV-2 RBD-mRNA (eg, RBD) or empty LNPs (control), and boosted once at 4 weeks. Sera were collected at 10 days post-2nd immunization, and tested for neutralizing antibodies against the following SARS-CoV-2: pseudotyped SARS-CoV-2 expressing S protein of B.1.617.2 lineage variant containing L452R, T478K, and P681R mutations in the S1 subunit (A), prototypic (wild-type) virus strain (B), and B.1.1.529 lineage variant containing 38 mutations in the full-length S (C), as well as authentic SARS-CoV-2 B.1.617.2 variant (D), prototypic (wild-type) virus strain (E), and B.1.1.529 variant (F). NT₅₀ was calculated against each pseudovirus or live virus infection, and the data are presented as mean \pm s.e.m of 5 mouse sera in each group (n = 5). Experiments were repeated twice, yielding similar results.

induced by RBD-mRNA vaccine potently neutralized B.1.427 and B.1.429 lineage variants containing L452R mutation and B.1.617.1 lineage variant containing L452R-E484Q mutations in the RBD, whereas their neutralizing activity against B.1.351 and P.1 variant pseudoviruses, which contain the K417N/T, E484K, and N501Y substitutions, respectively, was moderately reduced.

RBD-mRNA vaccine elicited durable neutralizing antibodies against SARS-CoV-2 B.1.617.2 and B.1.1.529 variants with complete protection against SARS-CoV-2 infection.

To determine the protective efficacy of SARS-CoV-2 RBD-mRNA vaccine against SARS-CoV-2 infection, BALB/c mice were immunized with this vaccine, and challenged with SARS2-N501Y_{MA30}, a virulent mouse-adapted SARS-CoV-2 variant 70 days post-2nd immunization. SARS-CoV-2 B.1.617.2, particularly B.1.1.529, variants have become important variants of concern (VoCs), among which B.1.617.2 variant has shown increased infectivity and transmissibility, and B.1.1.529 variant has demonstrated rapid human-to-human spread.^{31,32} Therefore, sera collected at 10 days post-2nd immunization were assessed for their neutralizing activity against B.1.617.2 and B.1.1.529 variant

pseudoviruses. The results demonstrated that RBD-mRNA induced neutralizing antibodies against pseudotyped SARS-CoV-2 B.1.617.2 variant carrying L452R-T478K-P681R mutations (Fig 3A), albeit the neutralizing antibody titer was \sim 2 times lower than that against the pseudotyped SARS-CoV-2 prototypic strain (Fig 3B). Notably, the RBD-mRNA vaccine-induced antibodies also neutralized pseudotyped B.1.1.529 variant, although its neutralizing antibody titer was \sim 6 and 3 times lower than the neutralizing antibody titers against the pseudotyped prototypic strain and B.1.617.2 variant, respectively (Fig 3C). These antibodies were able to neutralize authentic SARS-CoV-2 prototypic, B.1.617.2 and B.1.1.529 variant strains, respectively, with a trend of neutralizing antibody titers similar to that against pseudotyped SARS-CoV-2 (Fig 3D–F). Sera collected before SARS-CoV-2 challenge also neutralized pseudotyped B.1.617.2 variant, with a reduced neutralizing antibody titer (\sim 3.6 times lower) than that against the prototypic pseudovirus (Fig 4A and B). In contrast, sera of mice injected with the control (empty LNPs) only elicited a background level of neutralizing antibodies against these pseudotyped and authentic SARS-CoV-2 viruses (Fig 3 and 4A and B). Data from the challenge studies revealed

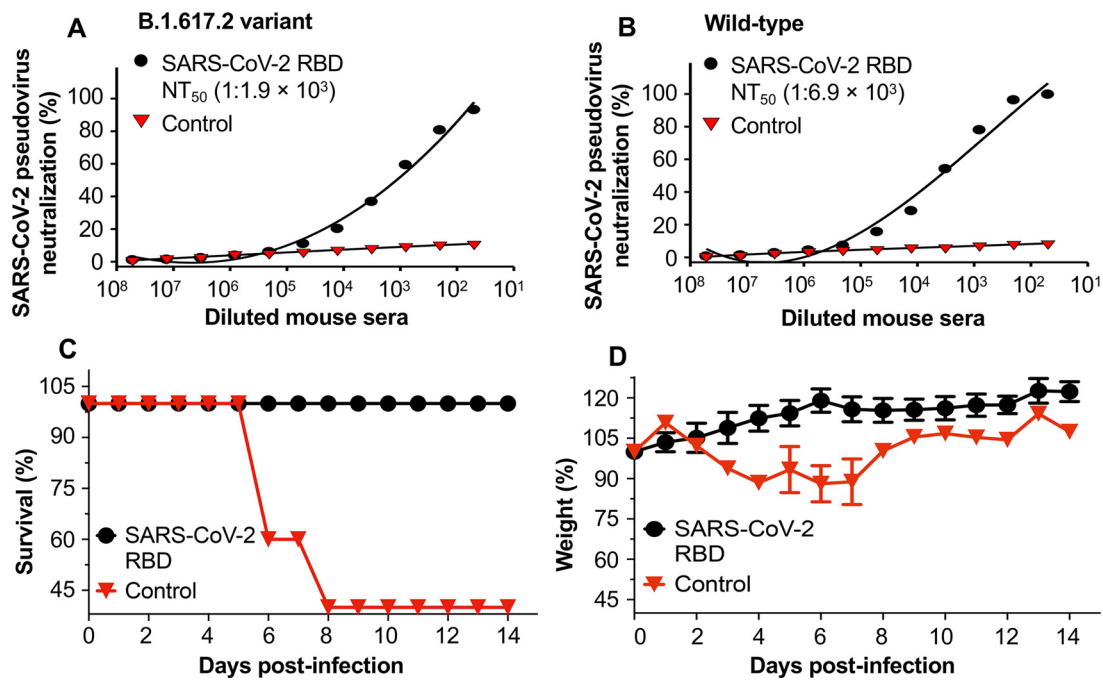


Fig 4. SARS-CoV-2 RBD-mRNA protected mice against a virulent SARS-CoV-2 challenge. BALB/c mice were immunized and boosted with SARS-CoV-2 RBD-mRNA (eg, RBD) or empty LNPs (control) (as described in Fig 3), and challenged with a virulent mouse-adapted SARS-CoV-2 variant (SARS2-N501Y_{MA30}) at 70 days post-2nd immunization. Sera collected before challenge were tested for neutralizing antibodies against pseudotyped SARS-CoV-2 B.1.617.2 lineage variant (A) and prototypic (wild-type) strain (B). NT₅₀ was calculated against each pseudovirus infection. Experiments were repeated twice, yielding similar results. Mouse survival (C) and body weight changes (D) were observed for 14 days after challenge. The data (in A, B, and D) are presented as mean ± s.e.m. of 5 mice in each group (n = 5).

that 100% of RBD-mRNA-immunized mice survived SARS2-N501Y_{MA30} infection (Fig 4C), and body weight continuously increased during 14 days after virus infection (Fig 4D). In contrast, the control mice receiving empty LNPs significantly lost weight, and 60% of them died at day 8, after virus challenge (Fig 4C and D). The above data indicate that antibodies induced by RBD-mRNA vaccine were able to neutralize B.1.617.2 and B.1.1.529 lineage variants. Importantly, these neutralizing antibodies were sufficient to protect immunized mice against infection of a virulent mouse-adapted SARS-CoV-2.

DISCUSSION

SARS-CoV-2 has continually mutated since it was first detected in 2019. Multiple mutant residues have been identified in the S protein, including RBD, of SARS-CoV-2. Almost all of the currently developed therapeutic antibodies and vaccines, including the approved or authorized 2 mRNA vaccines (BNT162b2 and mRNA-1273) and one viral vector vaccine (Ad26.COV2), target the S protein.^{33–35} These vaccines have

shown reduced neutralizing activity or protective efficacy against the variant SARS-CoV-2 strains.^{9,36–38} Indeed, decreased neutralization of B.1.351, B.1.617.1, and B.1.617.2 variants compared to the original strains has been reported following immunization of BNT162b2 and ChAdOx1 vaccines.^{37,39,40} Reports also showed that sera induced by vaccines, such as BNT162b2, mRNA-1273 and NVX-CoV2373, had reduced neutralizing activity against different SARS-CoV-2 variants, such as B.1.1.7, B.1.351, P.1, and B.1.617.2 lineages.^{24,41–43}

The recent emergence of the new variant B.1.1.529 has spread globally. This new variant appears to have even higher transmissibility than the previous variants, and it contains more diverse mutations, particularly in the S protein, than any other variants identified so far (Table 1).^{44–46} Posting a new-round of challenges to the already developed COVID-19 vaccines that are based on the original or previous variant strains. Therefore, there is an urgent need to develop effective vaccines with ability to neutralizing multiple variants, including B.1.1.529 lineage variant.

Several RBD-based COVID-19 vaccines have been developed and/or tested preclinically or clinically

against infection of SARS-CoV-2 variants, in addition to the prototypic strain. For example, a SARS-CoV-2 RBD displayed on hepatitis B surface antigen (HBsAg)-virus-like particles (VLPs) induced neutralizing antibodies against SARS-CoV-2 B.1.1.7 and B.1.351 variants and protective efficacy in non-human primates, with reduced viral titers in bronchoalveolar lavage and nasal mucosa.⁴⁷ Similarly, a RBD displayed on Cucumber mosaic virus incorporated tetanus toxin (CuMV_{TT}) VLPs elicited neutralizing antibodies in mice against SARS-CoV-2 B.1.351, P.1, and B.1.617.2 variants.⁴⁸ In addition, a lumazine synthase VLP vaccine displaying SARS-CoV-2 RBD induced potent neutralizing antibodies against SARS-CoV-2 B.1.1.7, B.1.351, and P.1 variants, protecting immunized mice from SARS-CoV-2 challenge, with reduced clinical signs and pathological changes in the lung.²⁹ Moreover, a yeast cell-expressed Kappa (B.1.617.1)-RBD elicited neutralizing antibodies in mice against SARS-CoV-2 B.1.351, B.1.617.2, and B.1.1.529 variants.⁴⁹ Overall, the neutralizing antibody titers induced by RBD vaccines are generally lower against these SARS-CoV-2 variants than against the prototypic virus strain.^{29,47–49}

In this study, we found that RBD-mRNA vaccine, which contains the RBD sequence of the prototypic SARS-CoV-2, induced durable neutralizing antibodies that broadly neutralized B.1.1.7, B.1.351, P.1, B.1.617.2, B.1.617.1, B.1.427, B.1.429, and B.1.258 lineage variants. Notably, slightly or moderately reduced neutralizing activity was identified against B.1.351, P.1, and B.1.617.2 variants, which harbor one or several key mutant residues (eg, K417N/T, L452R, E484K, T478K, and N501Y) in the RBD of SARS-CoV-2 S protein. In addition, these RBD-mRNA vaccine-induced antibodies can neutralize B.1.1.529 variant, albeit the antibody titer against this new variant was lower than the titers to neutralize the prototypic SARS-CoV-2 strain, B.1.617.2, and other variants. Among the tested SARS-CoV-2 lineage variants, B.1.1.7, B.1.351, P.1, B.1.617.2, B.1.1.529, B.1.617.1, and B.1.427/B.1.429 are labeled by the World Health Organization (WHO) as Alpha, Beta, Gamma, Delta, Omicron, Kappa, and Epsilon variants, respectively, whereas Alpha, Beta, and Gamma variants are previously circulating VOCs, and Delta and Omicron are currently circulating VOCs.⁵⁰ Of note, since SARS-2-N501Y_{MA30} contains several mutations found in the Omicron strain, our results suggest that the RBD-mRNA vaccine will have some efficacy against this newly emergent virus strain.

Further studies are needed to design novel and effective universal vaccines with improved broad-spectrum neutralizing activity and protective efficacy against

multiple current pandemic SARS-CoV-2 variants and future variant virus strains which have pandemic potential. In particular, vaccines with strong efficiency against Omicron and other SARS-CoV-2 variants which present high transmissibility and/or infectivity would be a priority for development. While a 3rd dose of prototypic BNT162b2 or mRNA-1273 mRNA vaccine may induce moderate neutralizing antibodies against Omicron variant,^{51–53} other strategies, such as T-cell-inducing vaccines based on the conserved viral sequences, will have potential to enhance the potency against not only the prototypic strain but also Omicron and other SARS-CoV-2 variants.

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Conflicts of Interest: The authors have read the journal's policy on disclosure of potential conflicts of interest and agreed to the journal's authorship statement. The authors declare no conflicts of interest.

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