

CORONAVIRUS

A broadly cross-reactive antibody neutralizes and protects against sarbecovirus challenge in mice

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Severe acute respiratory syndrome coronaviruses 1 (SARS-CoV) and 2 (SARS-CoV-2), including SARS-CoV-2 variants of concern, can cause deadly infections. The mortality associated with sarbecovirus infection underscores the importance of developing broadly effective countermeasures against them, which could be key in the prevention and mitigation of current and future zoonotic events. Here, we demonstrate the neutralization of SARS-CoV; bat coronaviruses WIV-1 and RsSHC014; and SARS-CoV-2 variants D614G, B.1.1.7, B.1.351, P.1, B.1.429, B.1.526, B.1.617.1, and B.1.617.2 by a receptor binding domain (RBD)-specific human antibody, DH1047. Prophylactic and therapeutic treatment with DH1047 was protective against SARS-CoV, WIV-1, RsSHC014, and SARS-CoV-2 B.1.351 infection in mice. Binding and structural analysis showed high affinity binding of DH1047 to an epitope that is highly conserved among sarbecoviruses. Thus, DH1047 is a broadly protective antibody that can prevent infection and mitigate outbreaks caused by SARS-related strains and SARS-CoV-2 variants. Our results also suggest that the conserved RBD epitope bound by DH1047 is a rational target for a universal sarbecovirus vaccine.

INTRODUCTION

The emergence of severe acute respiratory syndrome coronavirus (SARS-CoV) in 2003 led to more than 8000 infections and 800 deaths (1, 2). In 2019, SARS-CoV-2 emerged in Wuhan, China (3). The spread of SARS-CoV-2, the virus that causes coronavirus disease 2019 (COVID-19) was rapid. By March 2020, the World Health Organization had declared the SARS-CoV-2 outbreak a global pandemic. By October 2021, more than 200 million people had been infected globally, resulting in more than 4.5 million deaths. This was associated with the documented emergence of several SARS-CoV-2 variants, which can partially evade host immunity. Therefore, there is a need to develop safe and effective broad-spectrum countermeasures that can prevent the rapid spread and attenuate the severe disease outcomes associated with current and future SARS-related virus emergence events.

Highly pathogenic coronavirus outbreaks in humans are likely of bat origin (4), and there is great genetic diversity among bat SARS-related viruses (5). Zoonotic coronaviruses of bat origin, such as RsSHC014 and WIV-1, can use the human angiotensin-converting enzyme 2 (ACE2) receptor for cell entry and can infect human airway cells (6, 7), highlighting their potential for emergence in naïve

human populations. Moreover, existing therapeutic monoclonal antibodies (mAbs) against SARS-CoV and SARS-CoV-2 mRNA vaccines do not fully protect against zoonotic SARS-related virus infection *in vivo* (6–8). Given the pandemic potential of SARS-related viruses, the development of broadly effective countermeasures, such as universal vaccination strategies (8–10) and coronavirus cross-reactive mAbs, is a global health priority. Moreover, given the emergence of the SARS-CoV-2 variants that are partially or fully resistant to some neutralizing antibodies authorized for COVID-19 treatment (11–13), there is a need to develop mAb therapies that are broadly effective against existing SARS-CoV-2 variants and zoonotic SARS-related viruses that may emerge in the future.

The receptor binding domain (RBD) of SARS-CoV-2 is one of the targets for highly potent neutralizing antibodies. Despite the high degree of genetic diversity within the RBD in SARS-related viruses (5), antibodies can be engineered to recognize diverse SARS-related viruses. Rappazzo *et al.* (14) recently reported that an engineered RBD-directed antibody, ADG-2, neutralized SARS-related viruses and protected against SARS-CoV and wild-type SARS-CoV-2. Therefore, the RBD of sarbecoviruses contains conserved epitopes that are the target of broadly neutralizing antibodies. In agreement with the notion that the RBD contains a conserved epitope shared among SARS, SARS-related, SARS-CoV-2, and the variants, we have identified a coronavirus cross-reactive RBD protective antibody: DH1047 (15).

RESULTS

The identification of broadly cross-binding and neutralizing antibodies

We previously isolated 1737 mAbs from a convalescent patient with SARS-CoV 17 years after infection and from a convalescent patient with SARS-CoV-2 36 days after infection (15). From this large panel

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of mAbs, previously described by Li *et al.* (15), we focused on 50 cross-reactive antibodies that bound to SARS-CoV, SARS-CoV-2, and other human and animal coronavirus antigens from genetically distinct sarbecoviruses. Sarbecoviruses vary in their ability to use human ACE2 and exhibit substantial genetic diversity in their RBDs (Fig. 1A). To examine whether these 50 cross-neutralizing mAbs neutralized pandemic and zoonotic sarbecoviruses, we measured neutralizing activity against a mouse-adapted (MA) SARS-CoV-2 virus, SARS-CoV, bat CoV WIV-1, and bat CoV RsSHC014 using live viruses. We identified four broadly cross-reactive antibodies, DH1235, DH1073, DH1046, and DH1047 (Fig. 1, B to E). We focused our analyses on these four antibodies because they had broad neutralization. DH1235 neutralized SARS-CoV-2 2AA MA, SARS-CoV, and bat coronavirus WIV-1 with half-maximal inhibitory concentration (IC_{50}) of 0.122, 0.0403, and 0.060 $\mu\text{g/ml}$, respectively (Fig. 1B and table S1). DH1073 neutralized SARS-CoV-2 2AA MA, SARS-CoV, and bat coronaviruses WIV-1 and RsSHC014 with IC_{50} of 0.808, 0.016, 0.267, and 1.32 $\mu\text{g/ml}$, respectively (Fig. 1C and table S1). DH1046 neutralized SARS-CoV-2 2AA MA, SARS-CoV, and bat coronaviruses WIV-1 and RsSHC014 with IC_{50} of 2.85, 0.103, 0.425, and 1.27 $\mu\text{g/ml}$, respectively (Fig. 1D and table S1). DH1047 was the most potent and neutralized SARS-CoV-2 2AA MA, SARS-CoV, and bat coronaviruses WIV-1 and RsSHC014 with IC_{50} of 0.397, 0.028, 0.191, and 0.200 $\mu\text{g/ml}$, respectively (Fig. 1E and table S1). As our study goal was to characterize the protective efficacy of cross-reactive antibodies, we compared the neutralization activity of DH1047 to two other mAbs currently in clinical trials, the cross-reactive ADG-2 (Adagio Therapeutics Inc., Adimab) and the potent REGN10933 (Regeneron Pharmaceuticals), against SARS-CoV-2 B.1.351, SARS-CoV urbani, and RsSHC014 (fig. S1). ADG-2

was engineered for increased potency, whereas DH1047 was not (14). REGN10933 had reduced neutralizing activity against SARS-CoV-2 B.1.351 compared to both ADG-2 and DH1047 (fig. S1). Similar to DH1047, ADG-2 effectively neutralized SARS-CoV urbani, whereas REGN10933 did not. Last, both ADG-2 and DH1047 neutralized RsSHC014, whereas REGN10933 did not (fig. S1).

To focus our screen on the four broadly neutralizing mAbs, we measured binding responses for DH1235, DH1073, DH1046, and DH1047 against other zoonotic bat spike proteins including RaTG13-CoV and bat RsSHC014 and against the spike protein of pangolin GXP4L-CoV. DH1235, DH1073, DH1046, and DH1047 mAbs showed strong binding to bat RaTG13-CoV, bat RsSHC014, and pangolin GXP4L-CoV spike proteins in addition to SARS-CoV and SARS-CoV-2 (Fig. 2, A to D). DH1235, DH1073, DH1046, and DH1047 bound to SARS-CoV-2 RBD and did not bind to the SARS-CoV-2 N-terminal domain (NTD). Although DH1235, DH1073, DH1046, and DH1047 were cross-reactive against epidemic, pandemic, and zoonotic sarbecovirus spike proteins, they did not bind to Middle East Respiratory Syndrome coronavirus (MERS-CoV), human coronavirus (HuCoV) OC43, HuCoV NL63, or HuCoV 229E spike proteins (fig. S2), suggesting that these mAbs recognize a conserved epitope found only in group 2B betacoronaviruses.

To examine whether these cross-reactive mAbs shared any overlap in their epitopes, we examined their binding footprint by both negative stain electron microscopy (NSEM) and surface plasmon resonance (SPR). DH1047 had an overlap with DH1235 but not with DH1073 and had different angles of approach to the SARS-CoV-2 spike protein (fig. S3, A to C). Moreover, from SPR competition experiments, DH1047, DH1046, and DH1235 were outcompeted by one another (fig. S3D), whereas DH1073 was not, indicating that DH1073 targets a distinct non-cross-competing epitope. Because DH1047 was the most potent mAb of the four cross-reactive mAbs in our screen, we also performed NSEM and observed binding of DH1047 to the RBD of bat RsSHC014 and SARS-CoV spike ectodomains, with overall similar orientations, as was observed for DH1047 binding to the SARS-CoV-2 spike ectodomain (fig. S4) (15). A 3.20-Å cryo-electron microscopy (cryo-EM) structure of DH1047 bound to the SARS-CoV spike protein showed three DH1047 Fab bound to each of the three RBD of the ectodomain in the “up” position (1:1 Fab/RBD ratio) (Fig. 2E, fig. S5, and table S2). DH1047 binding to the SARS-CoV RBD involved interactions between the antibody heavy chain complementarity-determining region 3 (HCDR3) and residues 356 to 372 of the RBD and of the light chain complementarity-determining region 1 (LCDR1) and LCDR3 regions with the RBD region spanning residues 390 to 404. The LCDR3 also interacted with receptor binding motif (RBM) residues 488 to 492. The angle of approach and footprint of DH1047 on the SARS-CoV RBD closely resembled that in the SARS-CoV-2 complex (15), with steric

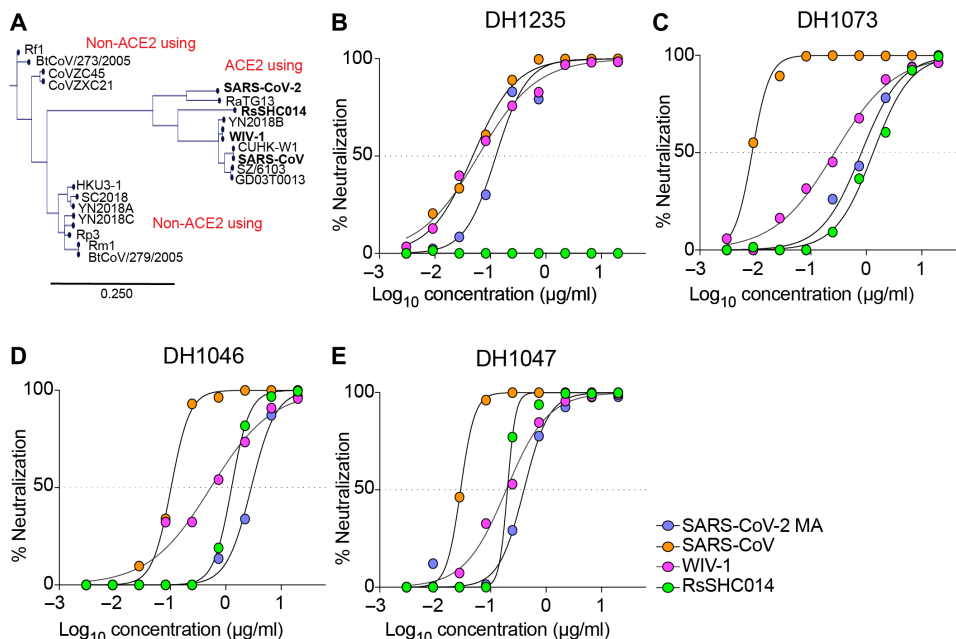


Fig. 1. The identification of broadly neutralizing antibodies. (A) The genetic relationships of ACE2 and non-ACE2 using sarbecovirus RBDs is shown. SARS-CoV-2 2AA MA is shown in purple, SARS-CoV is shown in orange, WIV-1 is shown in pink, and RsSHC014 is shown in green. The scale bar indicates the genetic distance scale among sarbecoviruses of 25%. The neutralization activity against sarbecoviruses is shown for DH1235 (B), DH1073 (C), DH1046 (D), and DH1047 (E). Data are representative of two technical replicates.

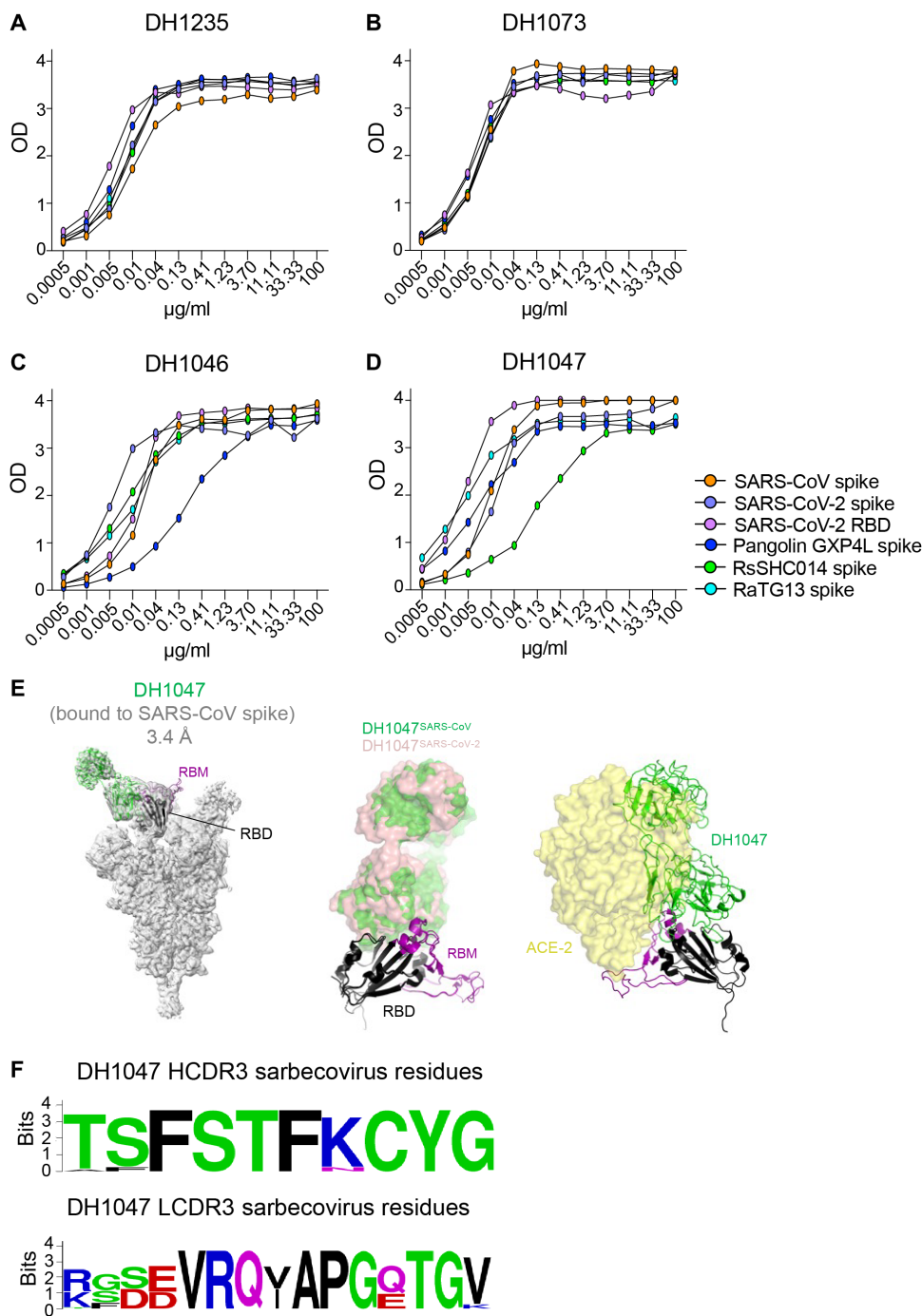


Fig. 2. The binding breadth and structural determinants of broad neutralization. (A to D) The optical density (OD) of cross-reactive antibodies against SARS-CoV spike protein, SARS-CoV-2 spike protein, SARS-CoV-2 RBD, pangolin GXP4L spike protein, RaTG13 spike protein, and RsSHC014 spike protein. The antibodies tested were DH1235 (A), DH1073 (B), DH1046 (C), and DH1047 (D). (E) A cryo-EM reconstruction of DH1047 Fab bound to SARS-CoV spike protein is shown. Spike protein is shown in gray, with the underlying fitted model shown in cartoon representation. DH1047 is colored green, and the RBD it is bound to is colored black, with the RBM within the RBD colored purple. Middle: An overlay of DH1047 bound to SARS-CoV and SARS-CoV-2 (PDB ID: 7LDI) spike proteins. Overlay was performed with the respective RBDs. DH1047 bound to SARS-CoV and SARS-CoV-2 spike protein are shown in green and salmon, respectively. ACE2 (yellow surface representation; PDB 6VW1) binding to RBD is sterically hindered by DH1047. The views in (B and C) are related by a 180° rotation about the vertical axis. (F) Sequence conservation within the DH1047 HCDR3 and LCDR3s is shown among 23 sarbecoviruses.

overlap predicted with ACE2 binding (Fig. 2E). These results demonstrate that DH1047 binds to SARS-CoV and SARS-CoV-2 spike ectodomains by involving homologous interactions (Fig. 2F), consistent with our analysis of RBD sequence variability that showed a high degree of convergence of the DH1047 epitope (Fig. 2F) (9), thereby defining an RBD conserved site of vulnerability in sarbecoviruses.

We also defined the binding affinity of DH1047 against epidemic and zoonotic spike proteins. We measured binding on and off rates against both SARS-CoV and RsSHC014-CoV spike proteins by SPR. DH1047 bound to the SARS-CoV and RsSHC014-CoV spike proteins with high affinity, association rates ($>8.60 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$), and dissociation rates ($<1.0 \times 10^{-5} \text{ s}^{-1}$) (fig. S6), demonstrating that DH1047 binds tightly to both the epidemic SARS-CoV and premergent bat coronavirus spike proteins. Last, DH1235, DH1073, DH1046, and DH1047 exhibited medium to long HCDR3 lengths and variable nucleotide somatic mutation rates in the heavy chain genes. DH1235, DH1073, and DH1046 had HCDR3 lengths of 21, 15, and 24 and nucleotide somatic hypermutation (SMH) rates of 1.7, 9.0, and 4.7, respectively (table S3). The most potent neutralizing antibody DH1047 had HCDR3 lengths and SMH rates of 24 and 8.05, respectively (table S3).

The protective activity of DH1235, DH1073, DH1046, and DH1047 against SARS-CoV

To define the protective efficacy of these four cross-reactive RBD-specific immunoglobulin G (IgG) broadly neutralizing antibodies, we passively immunized aged mice with DH1235, DH1073, DH1046, and DH1047 and a negative control influenza mAb, CH65 (16), at 10 mg/kg 12 hours before infection with SARS-CoV MA passage 15 (MA15) and evaluated lung viral titer replication. Demonstrating a disconnect between in vitro and in vivo protection, neither DH1235, DH1073, nor DH1046 protected SARS-CoV MA15 challenge in mice and all had lung viral replication comparable to that of control mice (Fig. 3A). In contrast, prophylactic administration of DH1047 fully protected mice from lung viral titer replication

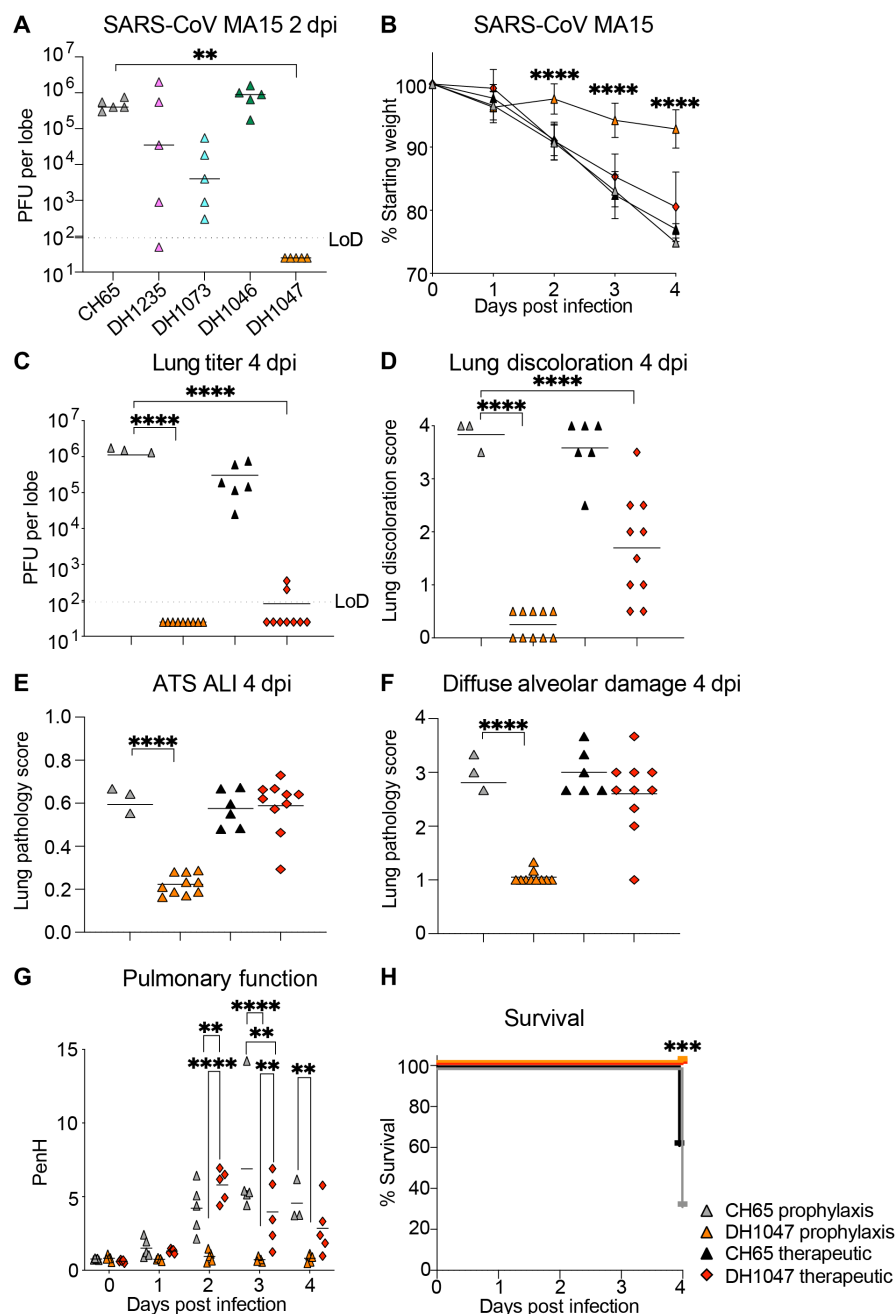


Fig. 3. Prevention and therapy of DH1047 against SARS-CoV in aged mice. (A) SARS-CoV MA15 lung viral replication is shown. Mice ($n=5$ per group) treated 12 hours before infection with a control influenza mAb CH65 or one of the four broadly neutralizing antibodies DH1235, DH1073, DH1046, and DH1047. (B) The percentage of starting weight is shown for mice ($n=10$ per group) treated prophylactically (12 hours before infection) or therapeutically (12 hours after infection) with DH1047 or control and challenged with SARS-CoV MA15. Data are presented as means \pm SEM. (C) Lung viral replication of SARS-CoV MA15 is shown for mice treated prophylactically or therapeutically with DH1047 and control at 4 dpi. (D) Macroscopic lung discoloration scores are shown for mice treated with DH1047 or control antibody prophylactically and therapeutically. (E) Lung pathology at 4 dpi was measured by ALI scores in mice treated with DH1047 or control prophylactically and therapeutically. (F) Lung pathology at 4 dpi was measured by DAD in mice treated prophylactically and therapeutically with DH1047 and control. (G) Pulmonary function was measured by whole-body plethysmography in mice treated with DH1047 or control mAb prophylactically or therapeutically. (H) Percent survival is shown for mice treated with control or DH1047 prophylactically or therapeutically and challenged with SARS-CoV MA15. Horizontal bars in (A) and (C to G) indicate means. P values shown in (B and G) were calculated using a two-way ANOVA followed by Tukey's multiple comparisons test. P values are for (A) and (C to E) were calculated using a one-way ANOVA followed by Dunnett's multiple comparisons. P values for (H) were calculated using a Mantel-Cox test. ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$.

(Fig. 3A). Given the prophylactic potential of DH1047, we sought to also evaluate its therapeutic potential in this mouse model. We treated mice with control mAb or DH1047 at 10 mg/kg either at 12 hours before or 12 hours after infection with SARS-CoV MA15 and monitored mice for signs of clinical disease, including weight loss and pulmonary function, which was measured by whole-body plethysmography (Buxco) through day 4 post infection (4 dpi). In agreement with the earlier SARS-CoV MA15 experiment, prophylactic treatment with DH1047 protected mice from weight loss through 4 dpi (Fig. 3B) and lung viral replication (Fig. 3C). Similarly, DH1047 therapy at 12 hours after infection resulted in reduced lung viral titers (Fig. 3C and fig. S7) and the macroscopic lung damage measured by the lung discoloration score (Fig. 3D and fig. S7). We also evaluated whether the prophylactic and therapeutic administration of DH1047 protected against lung pathology as measured by lung discoloration, which is a visual metric of gross lung damage taken at the time of the necropsy, microscopic evaluation as measured by an acute lung injury (ALI) scheme, and a diffuse alveolar damage (DAD) scheme. ALI and DAD, which are characterized by histopathologic changes including alveolar septal thickening, protein exudate in the air-space, hyaline membrane formation, and neutrophils in the interstitium or alveolar sacs, were both blindly evaluated by a board-certified veterinary pathologist blinded to the groups. The prophylactic administration of DH1047 resulted in complete protection from macroscopic lung discoloration (Fig. 3D) and microscopic lung pathology as measured by ALI (Fig. 3E and fig. S7) and DAD (Fig. 3F and fig. S7). In contrast to the prophylactic treatment condition, therapeutic administration of DH1047 did not reduce microscopic lung pathology compared to control mice as measured by ALI (Fig. 3E and fig. S7) and DAD (Fig. 3F and fig. S7) in this highly vulnerable model for SARS-CoV pathogenesis. The prophylactic and therapeutic administration of DH1047 prevented degradation of pulmonary function compared to controls (Fig. 3G). Last, both the prophylactic and therapeutic administration of DH1047 resulted in complete survival of infected mice, whereas only 50% of control mice

survived (Fig. 3H). Thus, DH1047 can prevent SARS-CoV disease when administered prophylactically and has measurable therapeutic benefits in a highly susceptible aged mouse model.

DH1047 has prophylactic and therapeutic activity against bat preemergent coronaviruses and neutralizes SARS-CoV-2 variants in vitro

Because DH1047 neutralized both WIV-1 and RsSHC014 (Fig. 1), we sought to define whether DH1047 had prophylactic and therapeutic efficacy in mice against these preemergent bat coronaviruses. We administered DH1047 prophylactically 12 hours before infection and therapeutically 12 hours after infection at 10 mg/kg in mice infected with bat coronaviruses. Prophylactic administration of DH1047 completely protected mice from WIV-1 lung viral replication and reduced lung viral titers in therapeutically treated mice compared to control mice (Fig. 4A). Similarly, prophylactic administration of DH1047 completely protected mice from RsSHC014 lung viral replication and reduced viral replication to near undetectable concentrations in therapeutically treated mice (Fig. 4B). Although we previously demonstrated the prophylactic and therapeutic efficacy of DH1047 against the SARS-CoV-2 Wuhan isolate in cynomolgus macaques (15), which exhibit mild SARS-CoV-2 disease (17), it was not known whether the mutations present in the newly emerging SARS-CoV-2 variants would ablate the neutralizing activity of DH1047. We therefore evaluated whether DH1047 could neutralize the prevalent variants of concern using both pseudovirus and live virus neutralization assays. DH1047 neutralized all tested variants of concern including SARS-CoV-2 D614G, B.1.1.7 (alpha), B.1.351 (beta), P.1 (gamma), B.1.429, B.1.526, B.1.617.1 (kappa), and B.1.617.2 (delta) (Fig. 4, C and D). The pseudovirus 50% neutralization assay (PsVNA₅₀) values against all the variants ranged

between 0.1214 and 0.1609 µg/ml, confirming broad neutralization of SARS-CoV-2 variants of concern by DH1047 (Fig. 4D). Live virus neutralization also demonstrated the broadly neutralizing activity of DH1047, with IC₅₀ values against D614G, B.1.1.7, and B.1.351 measured at 0.059, 0.081, and 0.111 µg/ml, respectively.

The prophylactic and therapeutic activity of DH1047 against SARS-CoV-2 B.1.351 in mice

Given that the B.1.351 variant is more resistant to both vaccine-elicited neutralizing antibodies (11, 18) and completely ablates the neutralizing activity of the Eli Lilly therapeutic mAb, LY-CoV555 (13), we also sought to evaluate whether DH1047 had prophylactic or therapeutic efficacy against SARS-CoV-2 B.1.351 in mice. We again used a highly susceptible and vulnerable aged mouse model in the SARS-CoV-2 B.1.351 protection experiments. Consistent with the SARS-CoV, WIV-1, and RsSHC014 in vivo data, prophylactic administration of DH1047 mediated complete protection against severe weight loss after SARS-CoV-2 B.1.351 challenge in aged mice (Fig. 5A). In contrast, we did not observe differences in weight loss after the therapeutic administration of DH1047 as compared to controls (Fig. 5A). Mice prophylactically treated with DH1047 had undetectable SARS-CoV-2 B.1.351 lung viral replication (Fig. 5B) and were also completely protected from macroscopic lung pathology compared to controls (Fig. 5C). Therapeutic administration of DH1047 resulted in a reduction in lung viral titers compared to control (Fig. 5B). We also evaluated the microscopic lung pathology as measured by ALI (Fig. 5D) and DAD scoring (Fig. 5E) schemes in this susceptible aged model for SARS-CoV-2 B.1.351 pathogenesis. Prophylactic administration of DH1047 protected mice from lung histopathology, as measured by ALI and DAD compared to control mice. In addition, we observed a reduction in ALI by the therapeutic administration

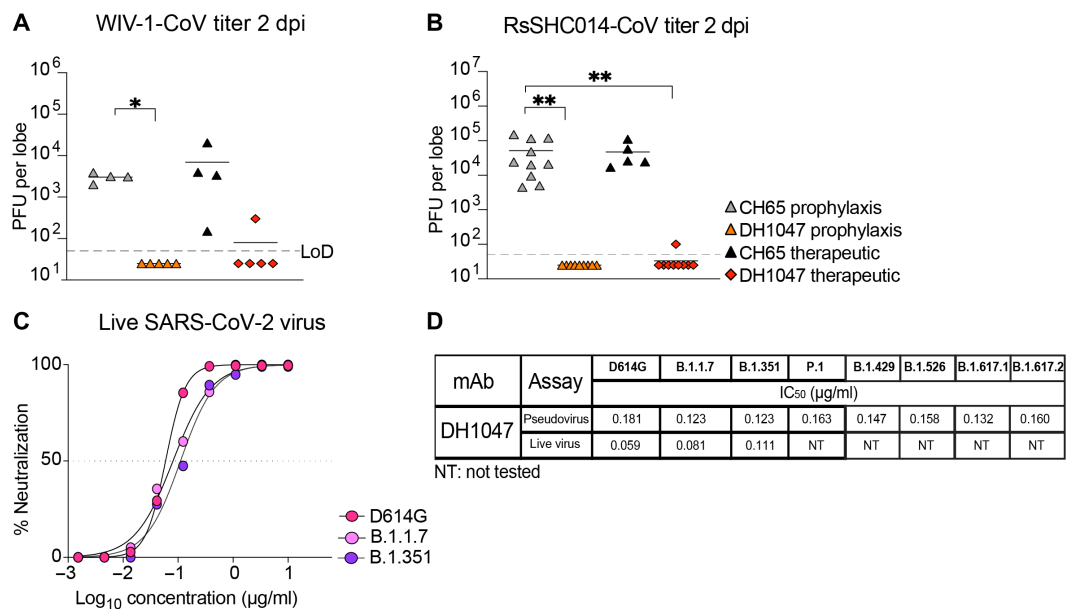
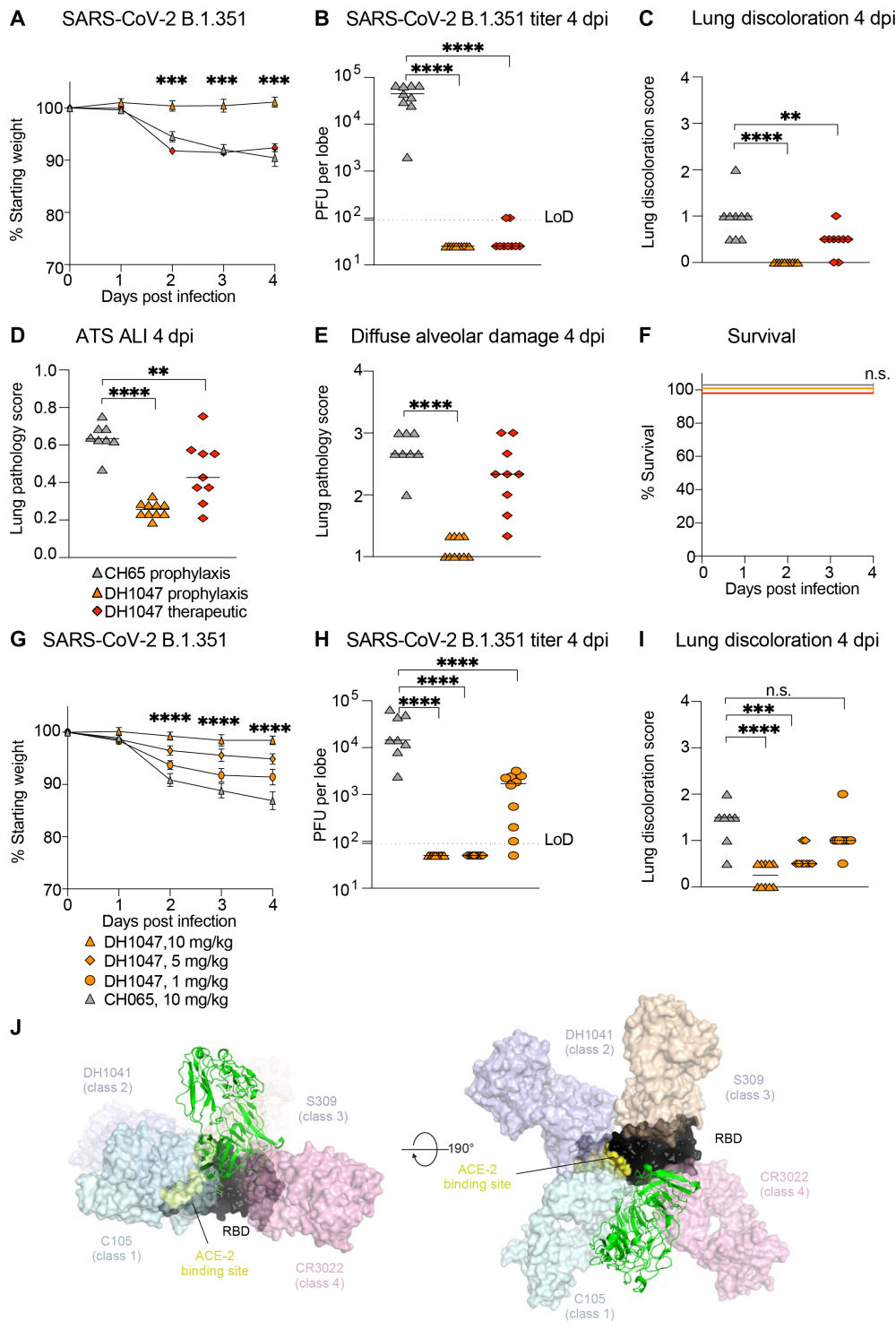


Fig. 4. Prophylactic and therapeutic activity of DH1047 against SARS-related bat coronaviruses and the in vitro neutralization against the SARS-CoV-2 variants. (A) Lung viral replication of WIV-1 is shown for mice ($n = 4$ to 5 per group) treated prophylactically or therapeutically with DH1047 or control at 2 dpi. Horizontal bars indicate mean. (B) Lung viral replication of RsSHC014 is shown for mice ($n = 5$ to 10 per group) treated prophylactically or therapeutically with DH1047 or control at 2 dpi. Horizontal bars indicate mean. (C) Live virus neutralization of SARS-CoV-2 D614G, B.1.1.7, and B.1.351 variants by DH1047 is shown. Data are representative of two technical replicates. (D) The comparison of the DH1047 neutralization activity against SARS-CoV-2 variants is shown for pseudovirus and live virus neutralization assays. NT, not tested. P values in (A) and (B) were calculated using a one-way ANOVA followed by Dunnett's multiple comparisons test. $*P < 0.05$ and $**P < 0.01$.

Fig. 5. Prophylaxis and therapy of DH1047 against SARS-CoV-2 B.1.351 in mice. (A) Percent of starting weight is shown for mice ($n = 10$ per group) treated prophylactically (12 hours before infection) or therapeutically (12 hours after infection) with DH1047 or control antibody and challenged with SARS-CoV-2 B.1.351. (B) Lung viral replication is shown at 4 dpi for mice infected with SARS-CoV-2 B.1.351 and treated prophylactically or therapeutically with DH1047 or control. (C) Macroscopic lung discoloration scores are shown at 4 dpi for mice infected with SARS-CoV-2 B.1.351 and treated prophylactically or therapeutically with DH1047 or control. (D) Lung pathology measured by ALI scores is shown at 4 dpi for mice infected with SARS-CoV-2 B.1.351 and treated prophylactically or therapeutically with DH1047 or control. (E) Lung pathology measured by DAD is shown at 4 dpi for mice infected with SARS-CoV-2 B.1.351 and treated prophylactically or therapeutically with DH1047 or control. (F) Percent survival is shown for SARS-CoV-2 B.1.351-infected mice treated with DH1047 prophylactically or therapeutically with DH1047 or control. (G) Percent of starting weight is shown for mice ($n = 10$ per group) treated prophylactically with DH1047 (12 hours before infection) at de-escalating doses of 10, 5, and 1 mg/kg or with control mAb (10 mg/kg), CH065. (H) Lung viral titers in control and DH1047-treated mice at 10, 5, and 1 mg/kg. (I) Lung discoloration in control and DH1047-treated mice at the various mAb doses. Data in (A and G) are presented as means \pm SEM. Horizontal bars in (B to E) and (H and I) represent means. P values shown in (A and G) are from a two-way ANOVA followed by Tukey's multiple comparisons test, and P values shown in (B to E) and (H and I) are from a one-way ANOVA followed by Dunnett's multiple comparisons test. Differences in survival were measured by a Mantel-Cox test. $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$; ns, not significant. (J) DH1047 binding is shown relative to binding of other known antibody classes that bind the RBD. RBD is shown in black with the ACE2 footprint on the RBD colored yellow. DH1047 is shown in cartoon representation and colored green. The other antibodies are shown as transparent surfaces: C105 (pale cyan, class 1; PDB ID: 6XC� and 6XCA), DH1041 (light blue, class 2; PDB ID: 7LAA), S309 (wheat, class 3; PDB ID: 6WS6 and 6WPT), and CR3022 (pink, class 4; PDB ID: 6YLA).



of DH1047, as measured by macroscopic lung pathology (Fig. 5C) and lung histopathology (Fig. 5D). Last, there was no difference in mortality in the SARS-CoV-2 B.1.351 challenge model in the control and DH1047-treated mice (Fig. 5F). Therefore, DH1047 can prevent and treat SARS-CoV-2 infections with the B.1.351 variant of concern in vivo. To determine the concentration at which we observe

breakthrough infection in vivo, we performed a dose de-escalation study and treated mice prophylactically at 10, 5, and 1 mg/kg. We observed full protection in mice treated with the dose of 10 mg/kg but not in mice treated with the dose of 5 mg/kg, measured by weight loss and lung discoloration, despite full protection lung viral replication compared to control (Fig. 5, G to I). In contrast, incomplete

protection from weight loss, breakthrough viral lung replication, and lung pathology was observed at the prophylaxis dose (1 mg/kg) of DH1047 (Fig. 5, G to I).

The structural comparison of DH1047 to other antibodies targeting the RBD

Comparing the DH1047 epitope with that of representative RBD-directed antibodies from classes 1, 2, 3, and 4 (19), we found that the DH1047 footprint overlapped with antibody C105 (class 1) and CR3022 (class 4) (Fig. 5J and fig. S8). The DH1047 footprint on the RBD showed similarity with antibody H014 (20) and overlapped with the footprint of other antibodies whose epitopes overlap with that of CR3022 (21–23). The classification of RBD-directed antibodies has recently been redefined to include a larger set of antibodies and finer epitope binning (24). DH1047 classifies in the RBD-6 category in this new system based on overlap of its footprint with CR3022, proximity to RBD-2a antibodies (such as REGN10933), and its ability to compete with ACE2 binding (15). Single domain antibodies, or nanobodies, that potentially neutralize SARS-CoV-2 show a close overlap with the DH1047 variable heavy (VH) domain (fig. S8). Nanobody VHH V shows the closest overlap with its elongated HCDR3 loop binding to a similar RBD region as the HCDR3 of DH1047. We performed alanine scanning mutagenesis of the DH1047 HCDR3 residues proximal to the RBD epitope (fig. S9). Mutating residue Trp^{100B} to Ala did not alter its binding to the ectodomain of SARS-CoV-2 spike protein stabilized with the two prolines (S-2P), consistent with the orientation of the Trp side chain away from the epitope. Similarly, mutating residues Ser^{100A} and Ser^{100C} to Ala did not alter binding. Ala mutants of residues Leu^{100G} and Asp^{100F} notably reduced DH1047 binding to the spike protein, although these residues did not directly contact the RBD, possibly by disrupting the conformation of the long HCDR3 loop. Mutagenesis of the LCDR3 similarly resulted in notable reduction in binding observed for Tyr⁹¹Ala and Arg⁹⁶Ala substitutions, although neither of these residues directly contacted the RBD. Reduction in binding was also observed for Ala substitution of residue Leu⁹⁴ where the Leu side chain occupied a hydrophobic pocket formed by the heavy chain residues Leu^{100G} and Ile⁵⁸ and RBD residue Val³⁹⁴.

The DH1047 epitope also overlaps with that of antibody ADG-2, which was previously shown to neutralize SARS-related viruses and protecting against SARS-CoV and SARS-CoV-2 (14). The approach angles of DH1047 and ADG-2 differ, with a rotation about the Fab longitudinal axis pivoting the ADG-2 antibody more toward the ACE2 binding region compared to DH1047 (fig. S10, A and B). In contrast to ADG-2, which uses VH3-21 for its heavy chain and has a 17-amino acid long HCDR3, DH1047 uses VH1-46 and has a 24-amino acid long HCDR3 (table S3) (14). To compare the therapeutic efficacy of DH1047 against ADG-2, we performed a head-to-head comparison study. Aged mice were therapeutically treated with DH1047, ADG-2, or CH65 control mAbs at 10 mg/kg at 6 hours after infection with SARS-CoV-2 B.1.351. Mice treated with either DH1047 or ADG-2 were completely protected from weight loss, lung viral replication, and lung damage compared to controls (fig. S10C), suggesting that DH1047 has similar in vivo therapeutic profiles to ADG-2, which is another potent cross-protective mAb (14).

DISCUSSION

The emergence of SARS-CoV and SARS-CoV-2 in the last two decades underscores a critical need to develop broadly effective countermeasures

against sarbecoviruses. Moreover, with the recent emergence of the more transmissible (25), modestly more virulent (26) B.1.1.7 variant (alpha), B.1.351 (beta), B.1.1.28 (gamma), and the highly transmissible B.1.617.2 variant (delta), all of which can partially evade existing countermeasures (11, 13, 27), there is a need to develop next-generation mAb therapeutics that can broadly neutralize these variants, as well as future variants of concern. For example, the SARS-CoV-2 B.1.351 variant completely ablates the neutralization activity of the mAb LY-CoV555 (13, 28). As a result, the emergency use authorization of LY-CoV555 was recently rescinded by the U.S. Food and Drug Administration. In addition, the presence of the E484K mutation in many variants of concern severely dampens the neutralization activity by more than sixfold of the AstraZeneca COV2-2196 mAb, Bria Biosciences Brii-198 mAb, and the Regeneron REGN10933 mAb (11, 28). In addition to evading currently mAb therapeutics, some of the variants including B.1.351 can diminish the efficacy of clinically approved vaccines, including the Johnson & Johnson single-dose vaccine and the AstraZeneca ChAdOx1 nCoV-19 vaccine (29, 30). Furthermore, some mAbs isolated from vaccine recipients of the Moderna mRNA-1273 and Pfizer/BioNTech BNT162b2 vaccines also demonstrated reduced efficacy against mutations present in the variants (31). Therefore, current vaccine and mAb therapies must be monitored in real time to define the performance of existing therapies against newly emerging and spreading variants. In the setting of reduced vaccine efficacy, the deployment of mAb therapies that are effective against variants, such as DH1047, could be a strategy to help control the COVID-19 pandemic.

The development of universal vaccination strategies against sarbecoviruses will be improved by the identification and characterization of broadly protective and conserved epitopes across SARS-related virus strains. Several SARS-related cross-reactive antibodies were recently described. For example, RBD-specific antibody, S2X259, neutralized SARS-CoV-2 variants, and zoonotic SARS-related viruses, as measured by pseudovirus neutralization (32), and another RBD-specific antibody had broad protection against the SARS-CoV-2 variants (33). Similarly, a recent subset of RBD-specific cross-reactive mAbs also showed in vitro activity (34), although their in vivo breadth and protective efficacy against divergent sarbecoviruses remains unconfirmed. In contrast to DH1047, neither DH1235, DH1073, nor DH1046 fully protected against SARS-CoV infection in aged mice, possibly because these cross-reactive mAbs may have differences in their mechanisms of protection in vivo. Although it is not clear why these mAbs have in vivo protection differences, it is possible that they have differences in their angle of approach to the spike protein, require optimal Fc effector functions (35), or may have stability differences in vivo.

DH1047 had broad protective in vivo efficacy against preemerging SARS-related viruses, epidemic SARS-CoV, and SARS-CoV-2 B.1.351 variant and neutralized all tested SARS-CoV-2 variants including the delta variant, underscoring that DH1047 recognizes a pan-sarbecovirus neutralizing epitope. Consistent with this notion, we have described a SARS-CoV-2 RBD-ferritin nanoparticle vaccine that elicited neutralizing antibodies against preemerging SARS-related viruses and protected against SARS-CoV-2 challenge in nonhuman primates (9). The serum antibody responses in these SARS-CoV-2 RBD-ferritin nanoparticle-vaccinated monkeys could block DH1047 binding responses against SARS-CoV-2 spike proteins, suggesting that SARS-CoV-2 RBD vaccines elicit DH1047-like antibody responses and could potentially protect against the future emergence of

SARS-CoV or SARS-CoV-2–related viruses. As other broad sarbecovirus vaccine formulations are developed and after a recent study that reported broad neutralization of sarbecoviruses in convalescent patients with SARS fully immunized with SARS-CoV-2 mRNA vaccines (8, 36), it will be interesting to examine whether these vaccines and individuals generate DH1047-like neutralizing antibodies.

Although we demonstrate the broad protective efficacy of DH1047 in mice, our study has limitations. First, our study does not evaluate the protective breadth of DH1047 against SARS-related viruses in larger animal models, such as nonhuman primates. Second, our study does not evaluate the role of DH1047 Fc region modifications, such as those that more favorably interact with activating Fc receptors, in mediating protection against SARS-related viruses. Future studies should examine whether the protective efficacy of DH1047 can be amplified by modulating Fc receptors *in vivo*.

Moving forward, it will be critical to closely monitor SARS-CoV and SARS-CoV-2–related viruses of zoonotic origin and actively monitor whether broad-spectrum antibodies such as ADG-2, DH1047, and S2X259 retain their inhibitory activity against preeminent viruses. We envision a system in which broad-spectrum antibodies such as DH1047 could be tested for safety in small phase 1 clinical trials so that if a future SARS-related virus emerges, then DH1047 could immediately be tested in larger efficacy trials at the site of an outbreak to potentially prevent the rapid spread of an emergent coronavirus. Moreover, given that DH1047 neutralized all tested SARS-CoV-2 variants including the highly transmissible delta variant, this mAb could also be deployed now to help curb the ongoing COVID-19 pandemic. Similar to other therapeutic mAbs evaluated against COVID-19, our data suggest that early administration will prove critical for protecting against severe disease outcomes (37). We conclude that DH1047 is a broadly protective mAb that has efficacy against preeminent, zoonotic SARS-related viruses from different clades, neutralizes highly transmissible SARS-CoV-2 variants of concern, and protects against SARS-CoV-2 B.1.351.

MATERIALS AND METHODS

Study design

The study objective was to discover and characterize broadly neutralizing antibodies that are protective against divergent SARS-related viruses. To this end, mAbs were tested *in vitro* to examine their binding and neutralizing activity and *in vivo* to measure their protective efficacy against multiclade sarbecoviruses. mAbs were tested in duplicate in neutralization assays, and experiments were repeated independently for rigor and reproducibility. No statistical methods were used to predetermine mouse sample size. All mouse experiments used 5 to 10 mice per group. A control mAb-treated group was included in each independent experiment for each different virus. No data points were excluded as outliers in any experiment. Naïve BALB/c mice (Envigo, #047) were the same age (around 12 months) and sex (female) for the SARS-CoV MA15, RsSHC014, and SARS-CoV-2 B.1.351 challenge experiments and were randomly assigned to the different mAb versus control groups. Male and female 8- to 10-week-old human ACE2–transgenic mice [B6].Cg-Tg(FOXJ1-ACE2)1Rba/Mmnc] bred at University of North Carolina (UNC) at Chapel Hill were equally distributed to the DH1047 and control mAb treatment groups for the WIV-1 challenge experiments. Active animal biosafety level 3 (BSL-3) experiments were not performed blinded. After the completion of animal BSL-3 experiments and sample collection,

all immunological, virological, and histopathological measurements were performed blinded. Immunological and virological samples were assigned new numbers to blind researchers during readouts. Mice were euthanized at 2 dpi, which is peak lung viral replication, to rigorously assess mAb-mediated protection against lung viral infection. Mice were euthanized at 4 dpi, a time where signs of coronavirus disease are set in these mouse models, to measure weight loss, lung viral replication, lung pathology, pulmonary function, and mortality. All active mouse experiments were carried to completion through 2 or 4 dpi except when mice reached below 70% of their starting weight, a point in which mice were humanely euthanized in accordance with UNC Chapel Hill Institutional Animal Care and Use Committee (IACUC)–approved protocols. Lung microscopic pathology readouts were performed by a board-certified veterinary pathologist who was blinded to the treatment groups. Neutralization and SPR experiments for DH1047 and control were repeated twice independently with technical replicates in each experiment. DH1047 and CH65 control *in vivo* protection experiments against SARS-CoV MA15, RsSHC014, WIV-1, and SARS-CoV-2 B.1.351 were repeated twice independently.

Biocontainment and biosafety

Studies were approved by the UNC Institutional Biosafety Committee approved by animal and experimental protocols in the Baric laboratory. All work described here was performed with approved standard operating procedures for SARS-CoV-2 in a BSL-3 facility, conforming to requirements recommended in the Microbiological and Biomedical Laboratories by the U.S. Department of Health and Human Service, the U.S. Public Health Service, the U.S. Center for Disease Control and Prevention, and the National Institutes of Health (NIH).

Antibody isolation and measurement of coronavirus spike protein binding by enzyme-linked immunosorbent assay

mAbs were isolated from antigen-specific single B cells from an individual who had recovered from SARS-CoV infection 17 years prior and from a SARS-CoV-2 convalescent individual 36 dpi as previously described (15). mAbs were screened by binding enzyme-linked immunosorbent assays (ELISAs). Indirect binding ELISAs were conducted in 384-well ELISA plates (Costar, #3700) coated with antigen (2 µg/ml) in 0.1 M sodium bicarbonate overnight at 4°C and washed and blocked with assay diluent [1× phosphate-buffered saline (PBS) containing 4% (w/v) whey protein, 15% normal goat serum, 0.5% Tween 20, and 0.05% sodium azide]. mAbs were incubated for 60 min in threefold serial dilutions beginning at 100 µg/ml followed by washing with PBS/0.1% Tween 20. Horseradish peroxidase-conjugated goat anti-mouse IgG secondary antibody (SouthernBiotech, 1030-05) was diluted to 1:10,000 in assay diluent without azide, incubated at for 1 hour at room temperature, washed, and detected with 20 µl of SureBlue Reserve (KPL, 53-00-03) for 15 min. Reactions were stopped via the addition of 20 µl of HCl stop solution. Plates were read at 450 nm. Area under the curve measurements were determined from binding of serial dilutions.

Measurement of neutralizing antibodies against live viruses

Full-length SARS-CoV-2 Seattle, an MA SARS-CoV-2 2AA Q498Y/P499T, SARS-CoV-2 D614G, SARS-CoV-2 B.1.351, SARS-CoV-2 B.1.1.7, SARS-CoV, WIV-1, and RsSHC014 viruses were designed to express nanoluciferase (8). Virus titers were measured in Vero E6

(C1008) cells, as defined by plaque-forming units (PFU) per ml, in a six-well plate format in quadruplicate technical replicates for accuracy. For the 96-well neutralization assay, Vero E6 (C1008) cells were plated at 20,000 cells per well the day prior in clear bottom black-walled plates. Cells were inspected to ensure confluency on the day of assay. mAbs were serially diluted threefold to nine dilution spots at specified concentrations. Serially diluted mAbs were mixed in equal volume with diluted virus. Antibody-virus and virus-only mixtures were then incubated at 37°C with 5% CO₂ for 1 hour. After incubation, serially diluted mAbs and virus-only controls were added in duplicate to the cells at 75 PFU at 37°C with 5% CO₂. After 24 hours, cells were lysed, and luciferase activity was measured via Nano-Glo Luciferase Assay System (Promega) according to the manufacturer's specifications. Luminescence was measured using a SpectraMax M3 plate reader (Molecular Devices). Virus neutralization titers were defined as the sample dilution at which a 50% reduction in relative light units was observed relative to the average of the virus control wells.

Lentivirus PsVNA

The PsVNA using the 293-ACE2-TMPRSS2 cell line was described previously (38, 39). Antibody preparations were evaluated by SARS-CoV-2 PsVNA₅₀ using WA-1 strain, B.1.1.7 (with spike protein mutations H69-V70del, Y144del, N501Y, A570D, D614G, P681H, T716I, S982A, and D1118H), B.1.429 (with spike protein mutations S13I, W152C, L452R, and D614G), B.1.526 (with spike protein mutations L5F, T95I, D253G, E484K or S477N, D614G, and A701V), P.1 (with spike protein mutations L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, H655Y, T1027I, D614G, and V1176F), B.1.351 (with spike protein mutations L18F, D80A, D215G, L242-244del, K417N, E484K, N501Y, D614G, and A701V), B.1.617.1 (with spike protein mutations G142D, E154K, V382L, L452R, E484Q, P681R, Q1071H, and D1153Y), and B.1.617.2 (with spike protein mutations T19R, G142D, E156del, F157del, R158G, L452R, T478K, D614G, P681R, and D950N). Controls included cells only, virus without any antibody, and positive serum samples. The cutoff value or the limit of detection for the neutralization assay was 1:10 dilution.

Surface plasmon resonance

Kinetic measurements of the DH1047 Fab binding to SARS-CoV and RsSHC014 spike proteins were obtained using a Biacore S200 instrument (Cytiva, formerly GE Healthcare) in HEPES-buffered saline-EP (HBS-EP) + 1× running buffer. The spike proteins were first captured onto a Series S Streptavidin chip from 300 to 400 response units (RUs) for the SARS-CoV spike proteins and 850 to 1000 RUs for the RsSHC014 spike protein. The DH1047 Fab was diluted from 2.5 to 200 nM and injected over the captured coronavirus spike proteins using the single-cycle kinetics injection type at a flow rate of 50 µl/min. There were five 120-s injections of the Fab at increasing concentrations followed by a dissociation of 600 s after the final injection. After dissociation, the spike proteins were regenerated from the streptavidin surface using a 30-s pulse of glycine (pH 1.5). Results were analyzed using the Biacore S200 Evaluation software (Cytiva). A blank streptavidin surface along with blank buffer binding was used for double reference subtraction to account for non-specific protein binding and signal drift. Subsequent curve fitting analyses were performed using a 1:1 Langmuir model with a local R_{max} for the DH1047 Fab. The reported binding curves are representative of two datasets.

Biolayer interferometry

Binding assays were performed by biolayer interferometry in an Octet RED384 instrument (FortéBio). Anti-human IgG Fc Capture (AHC) biosensor tips were dipped into 200 µl of filtered supernatant DH1047 wild-type or mutant proteins for 5 min at 1000 rpm followed by 1 min in 1× PBS. Next, the tips were dipped into 100 nM purified SARS-CoV-2 2P spike ectodomain, and binding response was measured. Nonspecific binding was accounted for by using reference subtraction of signal on a sensor tip captured with control mock transfection. Analysis was performed on Octet Data Analysis HT software (FortéBio), and visualization and normalization were performed using Prism 9.

Protein expression and purification for EM studies

The SARS-CoV spike ectodomain construct comprised the residues 1 to 1190 (UniProt P59594-1) with proline substitutions at 968 to 969, a C-terminal T4 fibrin trimerization motif, a C-terminal HRV3C protease cleavage site, a Twin-Strep-tag, and an 8xHisTag. The construct was cloned into the mammalian expression vector pαH (40). The RsSHC014 spike ectodomain construct was prepared similarly, except that it also contained the 2P mutations that placed two consecutive prolines at the HR1-CH junction at residue positions 986 and 987. FreeStyle 293-F cells (Thermo Fisher Scientific) were used for the spike ectodomain production. Cells were maintained in FreeStyle 293 Expression Medium (Gibco) at 37°C and 9% CO₂, with agitation at 120 rpm in a 75% humidified atmosphere. Transfections were performed as previously described (41, 42) using Turbo293 (SPEED Biosystems). Sixteen to 18 hours after transfection, HyClone CDM4HEK293 (Cytiva) medium was added. On 6 dpi, spike ectodomain was harvested from the concentrated supernatant. The purification was performed using Strep-Tactin resin (IBA Lifesciences) and size exclusion chromatography on a Superose 6 10/300 GL Increase column (Cytiva) in 2 mM Tris (pH 8.0), 200 mM NaCl, and 0.02% NaN₃. All steps were performed at room temperature, and the purified spike proteins were concentrated from 1 to 5 mg/ml, flash frozen in liquid nitrogen, and stored at −80°C until further use.

DH1047 IgG was produced in Expi293F (Thermo Fisher Scientific) cells maintained in Expi293 Expression Medium (Gibco) at 37°C, 120 rpm, 8% CO₂, and 75% humidity. Plasmids were transfected using the ExpiFectamine 293 Transfection Kit and protocol (Gibco) (41, 43) and purified by Protein A affinity (EMD Millipore). The IgG was digested to the Fab state using Lys-C (Thermo Fisher Scientific).

Negative stain electron microscopy

NSEM was performed as described previously (15). Briefly, Fab-spike protein complexes were prepared by mixing Fab and spike to give a 9:1 molar ratio of Fab to spike protein. After an incubation for 1 hour at 37°C, the complex was cross-linked by diluting to a final spike protein concentration of 0.1 mg/ml into room temperature buffer containing 150 mM NaCl, 20 mM Hepes (pH 7.4), 5% glycerol, and 7.5 mM glutaraldehyde and incubating for 5 min. Excess glutaraldehyde was quenched by adding sufficient 1 M Tris (pH 7.4) stock to give a final concentration of 75 mM Tris and incubated for 5 min. Carbon-coated grids Electron Microscopy Sciences (EMS, CF300-cu-UL) were glow-discharged for 20 s at 15 mA, after which a 5-µl drop of quenched sample was incubated on the grid for 10 to 15 s, blotted, and then stained with 2% uranyl formate. After air drying, grids were imaged with a Philips EM420 electron microscope operated at 120 kV at ×82,000 magnification, and images

were captured with a 2000 × 2000 charge-coupled device camera at a pixel size of 4.02 Å.

The RELION 3.0 program was used for all negative stain image processing. Images were imported and contrast transfer function–corrected with CTFFIND, and particles were picked using a spike protein template from previous two-dimensional (2D) class averages of spike protein alone. Extracted particle stacks were subjected to two to three rounds of 2D class averaging and selection to discard junk particles and background picks. Cleaned particle stacks were then subjected to 3D classification using a starting model created from a bare spike protein model, Protein Data Bank (PDB) 6vsb, and low-pass–filtered to 30 Å. Classes that showed clearly defined Fabs were selected for final refinements followed by automatic filtering and B-factor sharpening with the default RELION postprocessing parameters.

Cryo–electron microscopy

Purified SARS-CoV spike ectodomain was incubated for about 2 hours with a sixfold molar equivalent of the DH1047 Fab in a final volume of 10 µl. The sample concentration was adjusted to spike protein at about 1.5 mg/ml in 2 mM tris (pH 8.0), 200 mM NaCl, and 0.02% NaN₃. Before freezing, 0.1 µl of glycerol was added to the 10 µl of sample. A 2.4-µl drop of protein was deposited on a QUANTIFOIL-1.2/1.3 grid (Electron Microscopy Sciences) that had been glow discharged for 10 s using a PELCO easiGlow Glow Discharge Cleaning System. After a 30-s incubation in greater than 95% humidity, excess protein was blotted away for 2.5 s before being plunge frozen into liquid ethane using a Leica EM GP2 plunge freezer (Leica Microsystems). Frozen grids were imaged using a Titan Krios (Thermo Fisher Scientific) equipped with a K3 detector (Gatan). Data processing was performed using cryoSPARC (44). Model building and refinement was done using Phenix (45, 46), Coot (47), PyMOL (48), Chimera (49), ChimeraX (50), and ISOLDE (51). Similar to what we had observed for the DH1047 complex with the SARS-CoV-2 spike ectodomain (15), there was considerable heterogeneity in the RBD region. Further classification of particles was performed to better resolve the antibody binding interface, resulting in an asymmetric reconstruction of a population refined to a resolution of 3.4 Å that was used for model fitting.

Animals and challenge viruses

Eleven-month-old female BALB/c mice were purchased from Envigo (#047) and were used for the SARS-CoV, SARS-CoV-2 B.1.351, and RsSHC014-CoV protection experiments. Eight- to 10-week-old hACE2 transgenic mice [B6J.Cg-Tg(FOXJ1-ACE2)1Rba/Mmnc] were bred at UNC Chapel Hill and were used for WIV-1–CoV protection experiments. The study was carried out in accordance with the recommendations for care and use of animals by the Office of Laboratory Animal Welfare, NIH, and the IACUC of UNC (UNC permit no. A-3410-01). Animals were housed in groups of five and fed standard chow diets. Virus inoculations were performed under anesthesia, and all efforts were made to minimize animal suffering. All mice were anesthetized by injecting xylazine and ketamine intraperitoneally and infected intranasally with SARS-CoV MA15 (1 × 10⁴ PFU/ml), SARS-CoV-2 B.1.351-MA10 (1 × 10⁴ PFU/ml), RsSHC014 (1 × 10⁴ PFU/ml), or WIV-1 (1 × 10⁴ PFU/ml), which have been described previously (6, 52, 53). Mice were weighed daily and monitored for signs of clinical disease, and selected groups were subjected to daily whole-body plethysmography. For all mouse studies, groups of *n* = 10 mice were included per arm of the study

except for the hACE2 transgenic mice, which included *n* = 5 mice per group because of a limited availability of these mice. Viral titers, weight loss, and histology were measured from individual mice per group.

Lung pathology scoring

ALI was quantified by two separate lung pathology scoring scales: Matute-Bello and DAD scoring systems. Analyses and scoring were performed by a board-certified veterinary pathologist who was blinded to the treatment groups as described previously (54). Lung pathology slides were read and scored at ×600 total magnification.

The lung injury scoring system used is from the American Thoracic Society (Matute-Bello) to help quantitate histological features of ALI observed in mouse models to relate this injury to human settings. In a blinded manner, three random fields of lung tissue were chosen and scored for the following: (A) neutrophils in the alveolar space (none, 0; one to five cells, 1; and greater than five cells, 2), (B) neutrophils in the interstitial septa (none, 0; one to five cells, 1; and greater than five cells, 2), (C) hyaline membranes (none, 0; one membrane, 1; and greater than one membrane, 2), (D) proteinaceous debris in air spaces (none, 0; one instance, 1; and greater than one instance, 2), and (E) alveolar septal thickening (less than 2× mock thickness, 0; 2 to 4× mock thickness, 1; and greater than 4× mock thickness, 2). To obtain a lung injury score per field, A to E scores were put into the following formula score = [(20 × A) + (14 × B) + (7 × C) + (7 × D) + (2 × E)]/100. This formula contains multipliers that assign varying degrees of importance for each phenotype of the disease state. The scores for the three fields per mouse were averaged to obtain a final score ranging from 0 to, and including, 1. The second histology scoring scale to quantify ALI was adopted from a lung pathology scoring system from lung respiratory syncytial virus (RSV) infection in mice (55). This lung histology scoring scale measures DAD. Similar to the implementation of the American Thoracic Society (ATS) histology scoring scale, three random fields of lung tissue were scored for the following in a blinded manner: 1, absence of cellular sloughing and necrosis; 2, uncommon solitary cell sloughing and necrosis (one to two foci per field); 3, multifocal (3 + foci) cellular sloughing and necrosis with uncommon septal wall hyalinization; or 4, multifocal (greater than 75% of field) cellular sloughing and necrosis with common or prominent hyaline membranes. The scores for the three fields per mouse were averaged to get a final DAD score per mouse. The microscope images were generated using an Olympus Bx43 light microscope and cellSens Entry v3.1 software.

WebLogo

As the interface in the SARS-CoV-2/DH1047 complex was better resolved (15), we used this complex for visualizing epitope details and analyzing extent of conservation of the epitope residues in different sarbecoviruses. The WebLogo tool from University of California Berkeley was used to generate the consensus epitopes of the DH1047, HCDR3, and LCDR3 epitopes (<https://weblogo.berkeley.edu/logo.cgi>). The following 23 sarbecovirus genomes were used for the WebLogo analysis: KT444582, AY278554, AY278741, AY151512, AY525636, KC881005, KC881006, KF367457, MK211376, MN908947, MN996532, MG772933, MG772934, MK211374, DQ412042, DQ648856, MK211378, DQ022305, DQ412043, DQ648857, DQ071615, MK211375, and MK211377.

Statistics

A two-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test to adjust for false discovery rates, a one-way ANOVA followed by Dunnett's multiple comparisons to adjust for false discovery rates, or a Mantel-Cox test were used in mouse experiment measurements. See figure legends for details. All statistical analyses were performed using GraphPad Prism 9.

SUPPLEMENTARY MATERIALS

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Figs. S1 to S10

Tables S1 to S3

Data file S1

[View/request a protocol for this paper from Bio-protocol.](#)

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A broadly cross-reactive antibody neutralizes and protects against sarbecovirus challenge in mice

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Broad sarbecovirus neutralization

Although monoclonal antibodies (mAbs) targeting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have emerged as an essential component of coronavirus disease 2019 (COVID-19) treatment, these therapeutics are highly specific to SARS-CoV-2. Thus, they may not be as effective against other sarbecoviruses or against variants of SARS-CoV-2. Here, Martinez *et al.* identified a mAb, DH1047, which binds to the receptor-binding domain (RBD) of multiple sarbecoviruses. DH1047 neutralized SARS-CoV, SARS-CoV-2, and multiple bat coronaviruses in vitro and protected mice against infection with sarbecoviruses in vivo. Together, these findings support further development of broadly cross-reactive sarbecovirus-targeting antibodies.

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