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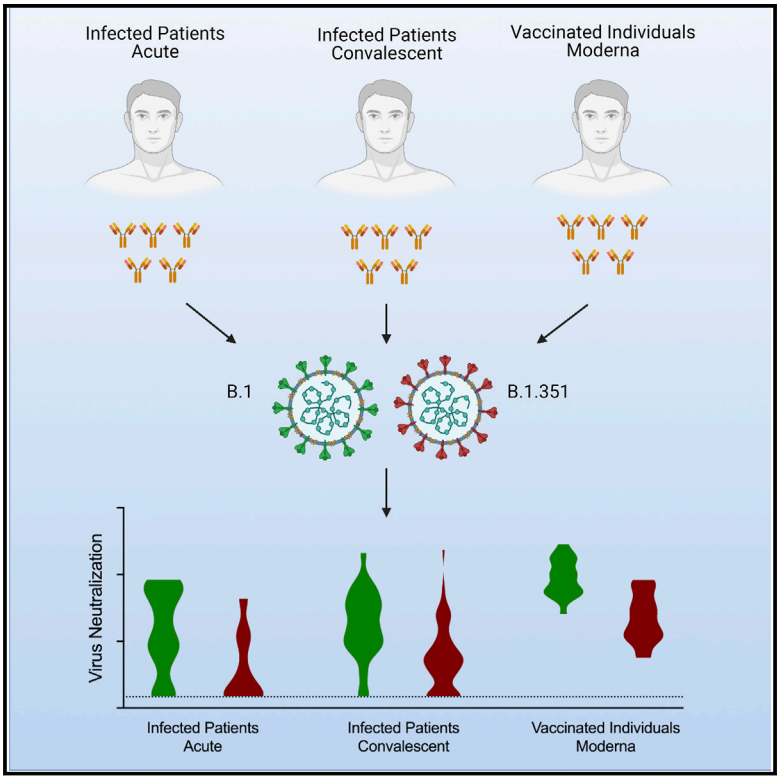
Brief Report

Cell Host & Microbe

Infection- and vaccine-induced antibody binding and neutralization of the B.1.351 SARS-CoV-2 variant

Graphical abstract



Highlights

- Antibodies from infected and vaccinated individuals bind to the B.1.351 RBD
- Convalescent sera through eight months can neutralize the B.1.351 variant
- Serum from vaccinated individuals retains neutralization against the B.1.351 variant

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In brief

In this study, Edara et al. (2021) report that, despite reduced antibody binding to the B.1.351 RBD, sera from infected (acute and convalescent) and Moderna (mRNA-1273)-vaccinated individuals were still able to neutralize the SARS-CoV-2 B.1.351 variant, suggesting that protective humoral immunity may be retained against this variant.

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Brief Report

Infection- and vaccine-induced antibody binding and neutralization of the B.1.351 SARS-CoV-2 variant

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SUMMARY

The emergence of SARS-CoV-2 variants with mutations in the spike protein is raising concerns about the efficacy of infection- or vaccine-induced antibodies. We compared antibody binding and live virus neutralization of sera from naturally infected and Moderna-vaccinated individuals against two SARS-CoV-2 variants: B.1 containing the spike mutation D614G and the emerging B.1.351 variant containing additional spike mutations and deletions. Sera from acutely infected and convalescent COVID-19 patients exhibited a 3-fold reduction in binding antibody titers to the B.1.351 variant receptor-binding domain of the spike protein and a 3.5-fold reduction in neutralizing antibody titers against SARS-CoV-2 B.1.351 variant compared to the B.1 variant. Similar results were seen with sera from Moderna-vaccinated individuals. Despite reduced antibody titers against the B.1.351 variant, sera from infected and vaccinated individuals containing polyclonal antibodies to the spike protein could still neutralize SARS-CoV-2 B.1.351, suggesting that protective humoral immunity may be retained against this variant.

SARS-CoV-2 is the causative agent of Coronavirus Disease 2019 (COVID-19), which has resulted in a devastating global pandemic with over 100 million cases and 2.4 million deaths worldwide (WHO, 2021). As SARS-CoV-2 has spread across the world, there has been a dramatic increase in the emergence of variants with mutations in the nonstructural and structural proteins (Plante et al., 2021). The viral spike protein is found on the outside of the virion and binds to the ACE2 receptor expressed on cells within the respiratory tract (Walls et al., 2020). As compared to the Wuhan-Hu-1 reference genome, several mutations within the spike protein have been identified over the past year. The first major spike protein variant to emerge was a mutation at position 614 from an Aspartic acid (D) to a Glycine (G). This mutation led to an increase in viral fitness, replication in the respiratory tract, binding to the ACE2 receptor, and confirmational changes within the spike protein (Gobeil et al., 2021; Plante et al., 2020; Ozono et al., 2021). Over the past few months, there has been a surge in the emergence of novel SARS-CoV-2 variants, raising significant concerns about alterations to viral fitness, transmission, and disease. In particular, the emergence of the B.1.351 variant, which was originally identified in South Africa, includes several mutations within the structural and nonstructural proteins (Tegally et al., 2020).

Following SARS-CoV-2 infection in humans, antibody responses are rapidly generated against the viral spike protein (Suthar et al., 2020). The receptor-binding motif within the spike protein interacts with the ACE2 receptor and is a major target of antibody-mediated neutralization (Shrock et al., 2020). Longitudinal and cross-sectional studies have estimated that antibodies to the spike protein can last for at least a year following infection (Dan et al., 2021; Sherina et al., 2021; Pradenas et al., 2021; Anand et al., 2021). The mRNA-1273 vaccine encodes the viral spike protein and elicits a potent neutralizing antibody response to SARS-CoV-2 that is durable at least for several months (Anderson et al., 2020; Jackson et al., 2020; Widge et al., 2021). Mutations within the viral spike protein, in particular the receptor-binding domain (RBD), could influence viral binding and

neutralization. The emerging B.1.351 SARS-CoV-2 variant includes three mutations within the receptor-binding domain (K417N, E484K, and N501Y) and several additional mutations within the spike protein. Two of these mutations are located at putative contact sites for the ACE2 receptor (amino acid positions 417 and 501) (Shrock et al., 2020). It is likely that these mutations within the spike protein can influence viral binding to the ACE2 receptor, antibody binding, and resistance to neutralization by human immune sera.

In this study, we compared antibody binding and viral neutralization against two variants that have emerged in various parts of the world. EHC-083E (herein referred to as the B.1 variant) is within the B.1 PANGO lineage and was isolated from a residual nasopharyngeal swab collected from a patient in Atlanta, GA in March 2020 (SARS-CoV-2/human/USA/GA-EHC-083E/2020). This variant contains the D614G mutation within the spike protein. The B.1.351 variant was isolated from an oropharyngeal swab from a patient in Ugu district, KwaZulu-Natal, South Africa in November 2020. The B.1.351 viral variant contains the following amino acid mutations within the viral spike protein: L18F, D80A, D215G, deletion at positions 242–244 (L242del, A243del, and L244del), K417N, E484K, N501Y, and D614G. This virus was isolated as described by Sigal and colleagues (Wibmer et al., 2021). We subsequently plaque purified the virus, a step followed by a single round of propagation in VeroE6 cells. Relative to the deposited sequence on GISAID (EPI_ISL_678615), we identified two additional mutations within the spike protein at positions Q677H and R682W (Figure S1).

Here, we analyzed a cohort of acutely infected COVID-19 patients (n = 19) enrolled at Emory University Hospital between 5 and 19 days after symptom onset (Table S1). To measure IgG antibody binding, we utilized an electrochemiluminescence-based multiplex immune assay provided by Mesoscale Discovery (MSD). As compared to the B.1-lineage, RBD-specific IgG responses (GMT: 4,829; range: <239–168,890), we found that all of the patients had significantly reduced IgG binding to the B.1.351 RBD (GMT: 1,081; range: <239–20,254). We next determined the impact on the neutralization capacity of these samples using a live virus neutralization assay. In comparison to the D614G variant (GMT: 135; range: <20–836), we observed a significant reduction in the neutralization capacity of samples from the acutely infected cohort against the B.1.351 variant (GMT: 40; range: <20–433). Of the samples that exhibited neutralization against the B.1 variant, we found that 4/15 samples (26%) failed to neutralize the B.1.351 variant. While there was a range of RBD-specific and neutralizing antibody responses across this cohort of acutely infected COVID-19 patients, we observed a positive correlation of the B.1-lineage RBD-specific IgG titers against the B.1 variant neutralization titers ($r = 0.9209$; $p < 0.0001$; Figure 1C) as well as the B.1.351 RBD-specific IgG titers against the B.1.351 variant neutralization titers ($r = 0.8020$; $p < 0.0001$). These findings suggest that, during the acute phase of SARS-CoV-2 infection, mutations within the B.1.351 RBD account for the loss of neutralizing acitivity against the B.1.351 variant.

Recent studies have found that binding and neutralizing antibodies are maintained for at least 8 months following SARS-CoV-2 infection (Dan et al., 2021; Pradenas et al., 2021; Sherina et al., 2021). To understand how antibody breadth is impacted during convalescence, we performed a longitudinal analysis of RBD binding and viral neutralization in 30 convalescent COVID-19 individuals across two longitudinally sampled time points through 8 months (Table S1). We observed a significant reduction in IgG binding to the B.1.351 RBD at the 1–3 month time point (B.1: GMT: 24,000; range: 1,856–320,059; B.1.351: GMT: 4,792; range: <239–32,158) and the 3–8 month time point (B.1: GMT: 8,314; range: 527–94,643; B.1.351: GMT: 1,946; range: <239–18,544; Figure 1E). We observed similar reductions in IgG binding titers to the B.1 and B.1.351 RBD across these two time points (Figures 1F and 1G). We next determined the impact on the neutralization capacity of these samples across the two time points. At the 1- to 3-month time point, we observed a 4.8-fold reduction ($p < 0.0001$) in neutralization capacity between the B.1 variant (GMT: 288; range: 29–2,117) and the B.1.351 variant (GMT: 59; range: < 20–2,363). At the 3- to 8-month time point, we observed a 2.1-fold reduction ($p < 0.0001$) in neutralization capacity between the B.1 variant (GMT: 107; range: < 20–836) and the B.1.351 variant (GMT: 50; range: <20–627). Of the samples that exhibited neutralization against the B.1 variant, 7 of 30 samples (23%) at the 1- to 3-month time point and 4 of 26 samples (15%) at the 3- to 8-month time point failed to neutralize the B.1.351 variant. Statistical analysis showed a significant correlation between B.1-lineage RBD-specific IgG and neutralization titers against the B.1 variant ($r = 0.8584$; $P < 0.0001$; Figure 1K). Similarly, sera from convalescent COVID-19 individuals showed a statistically significant correlation between the B.1.351 RBD-specific IgG and neutralization titers against the B.1.351 variant ($r = 0.7459$; $p < 0.0001$; Figure 1L). Taken together, these data demonstrate that antibody titers are reduced through 8 months following SARS-CoV-2 infection; however, there is a modest impact on the neutralization potency against the B.1.351 variant during this time period.

The messenger RNA vaccine mRNA-1273 generates durable neutralizing antibodies against SARS-CoV-2 (Anderson et al., 2020). We examined binding and neutralizing antibody titers in 19 healthy adult participants that received two injections of the mRNA-1273 vaccine at a dose of 100 μg (age >56 years; 14 days post-2nd dose; Table S2). We found that all vaccinated individuals had significantly reduced IgG binding to the B.1.351 RBD (GMT: 83,909; range: 2,588–333,451) compared to the B.1-lineage RBD-specific IgG responses (GMT: 316,554; range: 7,313–975,553; Figure 2A). Similarly, we observed a 3.8-fold reduction ($p < 0.0001$) in neutralization capacity between the B.1. variant (GMT: 734; range: 256–2,868) and the B.1.351 variant (GMT: 191; range: 61–830; Figure 2B). In contrast to the infected individuals, all vaccinated individuals retained neutralization capacity against the B.1.351 variant. Further, we observed a strong correlation between the corresponding RBD-specific IgG titers to the B.1 variant neutralization titers ($r = 0.7912$; $P < 0.0001$; Figure 2C) and the B.1.351 variant neutralization titers ($r = 0.9386$; $P < 0.0001$). These findings demonstrate that the antibodies elicited by the mRNA-1273 vaccine are effective at neutralizing the B.1.351 variant.

This study examined the impact of infection- and vaccine-induced antibody responses against two SARS-CoV-2 variants. We observed reduced antibody binding to the B.1.351-derived RBD of the spike protein and neutralization potency against the B.1.351 variant virus in sera from SARS-CoV-2-infected

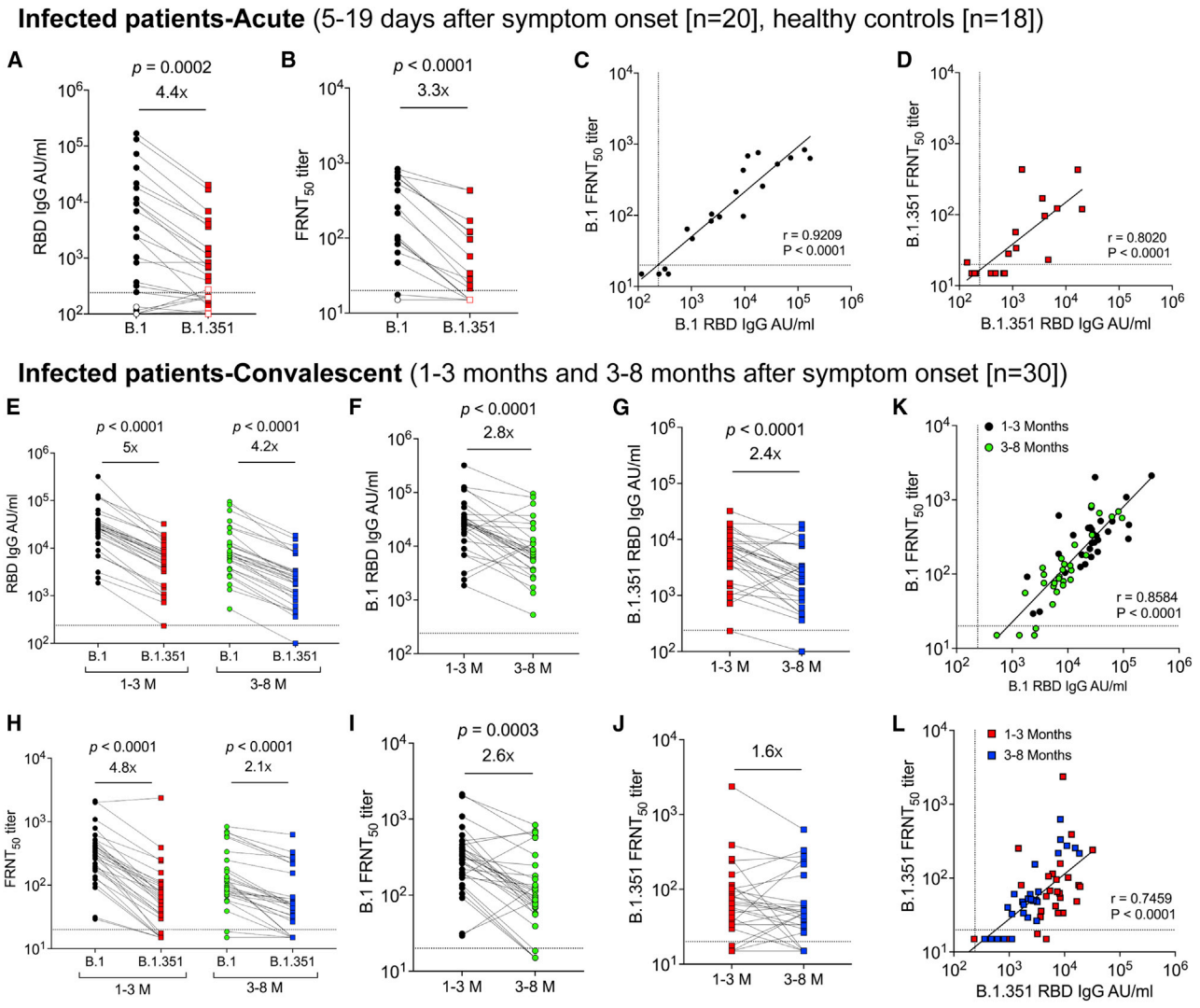

Figure 1. RBD binding and neutralizing antibody response against SARS-CoV-2 B.1.351 variant in SARS-CoV-2-infected individuals

Shown are data from the following cohorts based on natural infection: 19 acutely infected COVID-19 patients (5–19 days PSO; closed symbols), 30 convalescent COVID-19 individuals (1–3 months and 3–8 months PSO, closed symbols), and 18 healthy controls (open symbols).

(A) IgG antibody responses against SARS-CoV-2 receptor-binding domain (RBD) were measured by an electrochemiluminescent multiplex immunoassay and reported as arbitrary units per ml (AU/mL) as normalized by a standard curve for the B.1 (black) and B.1.351 (red) SARS-CoV-2 variants.

(B–D) (B) The 50% inhibitory titer ($FRNT_{50}$) on the focus reduction neutralization test (FRNT) for the B.1 (black) and B.1.351 (red) SARS-CoV-2 variants and correlations plots between the corresponding RBD and $FRNT_{50}$ for the (C) B.1 variant and (D) B.1.351 variant are shown for the acutely infected COVID-19 patients.

(E) Comparison of IgG antibody responses between the B.1 (black) and B.1.351 (red) SARS-CoV-2 variants at 1-3 month and the B.1 (**green**) and B.1.351 (**blue**) SARS-CoV-2 variants at 3–8 month time points are shown for the convalescent COVID-19 patients.

(F and G) Changes in IgG antibody responses over two time points through 8 months for the (F) B.1 (1–3 months [black] and 3-8 months [**green**]) and (G) B.1.351 (1–3 months [red] and 3–8 months [**blue**]) are shown for the convalescent COVID-19 patients.

(H) Comparison of $FRNT_{50}$ titer between the B.1 (black) and B.1.351 (red) SARS-CoV-2 variants at 1–3 month time points and the B.1 (**green**) and B.1.351 (**blue**) SARS-CoV-2 variants at 3–8 month time points are shown for the convalescent COVID-19 patients.

(I and J) Changes in $FRNT_{50}$ titers over two time points through 8 months for the (I) B.1 (1–3 months [black] and 3–8 months [**green**]) and (J) B.1.351 (1–3 months [red] and 3–8 months [**blue**]) are shown for the convalescent COVID-19 patients.

(K and L) Spearman correlation plots between the corresponding RBD and $FRNT_{50}$ for the (K) B.1 variant (1–3 month [black] and 3–8 month [**green**]) and (L) B.1.351 variant (1–3 month [red] and 3–8 month [**blue**]) are shown for the convalescent COVID-19 patients.

The dotted line in the RBD-binding assays represents the limit of detection (239 IgG AU/mL). The dotted line in the FRNT assays represents the maximum concentrations of the serum tested (1/20). Normality of the antibody binding and neutralization titers was determined using a Shapiro Wilk normality test. A non-parametric pairwise analysis for RBD-specific IgG titers and neutralization titers was performed by a Wilcoxon matched-pairs signed rank test. A Spearman rank test was used for the correlation analysis of the RBD-specific IgG AU/mL values against $FRNT_{50}$ titers.

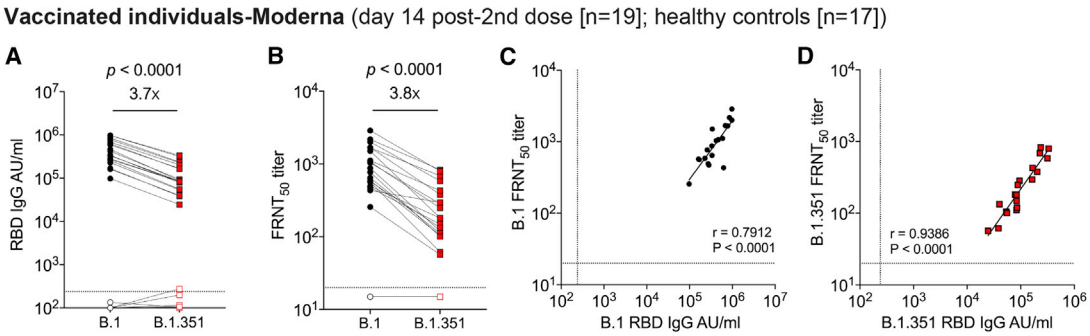

Figure 2. RBD binding and neutralizing antibody response against SARS-CoV-2 B.1.351 viral variant among mRNA-1273-vaccinated individuals

Shown are data from the individuals that received 100 μg of mRNA-1273 on day 14 post-2nd dose (>56 years or older, 19 participants; closed symbols) and 18 healthy controls (open symbols).

(A) IgG antibody responses against SARS-CoV-2 receptor-binding domain (RBD) were measured by an electrochemiluminescent multiplex immunoassay and reported as arbitrary units per ml (AU/mL) as normalized by a standard curve for the B.1 (black) and B.1.351 (red) SARS-CoV-2 variants.

(B–D) (B) The 50% inhibitory titer ($FRNT_{50}$) on the focus reduction neutralization test (FRNT) for the B.1 (black) and B.1.351 (red) SARS-CoV-2 variants and correlation plots between the corresponding RBD and $FRNT_{50}$ for the (C) B.1 variant and (D) B.1.351 variant are shown.

The dotted line in the RBD binding assays represents the limit of detection (239 AU/mL). The dotted line in the FRNT assays represents the maximum concentrations of the serum tested (1/20). The GMT fold change for the respective isolates relative to B.1 is shown in each of the plots. Normality of the antibody binding and neutralization titers was determined using a Shapiro Wilk normality test. Non-parametric pairwise analysis for RBD specific IgG titers and neutralization titers was performed by a Wilcoxon matched-pairs signed rank test. A Spearman rank test was used for correlation analysis of the RBD-specific IgG AU/mL values against $FRNT_{50}$ titers.

and -vaccinated individuals. Using our longitudinal convalescent COVID-19 cohort, we examined the impact on antibody binding to the RBD and viral neutralization across the SARS-CoV-2 variants. One of the interesting findings is that, in most convalescent COVID-19 individuals, we observed that neutralization potency against the B.1.351 variant was retained through 8 months after symptom onset. This suggests that antibodies capable of neutralizing the B.1.351 variant are generated early during infection and are durable for several months.

The immune correlates of protection against SARS-CoV-2 are not yet known. We and others have previously shown that IgG antibody responses to the RBD can serve as a surrogate of viral neutralization in infected individuals (Piccoli et al., 2020; Greaney et al., 2021; Suthar et al., 2020). The B.1.351 RBD contains three mutations (K417N, E484K and N501Y) which reduces antibody binding. In turn, we found that these mutations accounted for the resistance to antibody neutralization against the B.1.351 variant in sera from infected and vaccinated individuals. However, at later times after infection (3–8 months), non-RBD binding antibodies appears to mediate viral neutralization against the B.1.351 variant (Figures 1E and 1H; 4.2-fold loss in RBD binding compared to 2.1-fold loss in neutralization, respectively). Further studies are needed to better understand the cellular dynamics of B-cell responses following SARS-CoV-2 infection and vaccination.

Within the the RBD, we and others have shown that the presence of N501Y mutation found in the B.1.1.7 UK variant does not affect the neutralizing ability of serum from either naturally infected or mRNA-1273 vaccinated individuals (Edara et al., 2021; Johnson et al., 2021; Rathnasinghe et al., 2021; Shen et al., 2021; Wu et al., 2021). The substitution at position E484, located in the receptor-binding ridge epitope (Greaney et al., 2021), shows resistance to the neutralization of convalescent human sera (Chen et al., 2021; Wu et al., 2021; Xie et al., 2021, Wibmer et al., 2021). Single-point mutant pseudoviruses, chimeric viruses, or recombinant infectious clone-derived SARS-CoV-2 have demonstrated that this mutation displays resistance to neutralization by infection- and vaccine-induced antibodies (Johnson et al., 2021; Xie et al., 2021; Shen et al., 2021). This suggests that a majority of individuals develop antibodies that target this region within the RBD. However, it is still unclear how mutations within the RBD, as well as mutations within the N- and C-terminal domains, impact antibody neutralization, viral fitness, pathogenesis, or transmission.

We observed that most of the sera samples from acute and convalescent COVID-19 individuals showed antibody binding to the B.1.351-dervied RBD. In addition to most of these samples showing capacity to neutralize the B.1.351 variant, the effector functions of these antibodies could also contribute to controlling SARS-CoV-2 infection. Recent studies have shown that antibody Fc effector functions are important for optimal therapeutic intervention, and perhaps in mediating antibody prophylactic protection, against SARS-CoV-2 in mouse and hamster models (Schäfer et al., 2021; Winkler et al., 2020). Future studies should evaluate the contribution of neutralizing potency and Fc effector functions in promoting viral control and protective immunity following infection or vaccination against SARS-CoV-2 within the respiratory tract.

Our results show that despite a few-fold decrease, most infected individuals showed binding and neutralizing titers against the B.1.351 variant in acute sera and in convalescent sera through 8 months; further, all mRNA-1273-vaccinated individuals still maintained neutralization. These findings support the notion that, in the context of the B.1.351 variant, infection- and vaccine-induced immunity can provide protection against COVID-19.

Limitations

One of the limitations of our study is that it focused on antibody binding to the RBD of the spike protein. It is becoming

increasingly clear that monoclonal antibodies targeting the N-terminal domain and other regions of the spike protein outside of the RBD can neutralize SARS-CoV-2 (Xie et al., 2021; Greaney et al., 2021; Suryadevara et al., 2021). Examining binding to the full-length B.1.351 spike protein as well as to individual point mutations will provide important insight into the breadth of the antibody response to the viral spike protein following virus infection and vaccination. In addition, the recombinant RBD fragment used for the binding studies might be different from the native confirmation found within the spike protein, which may influence antibody binding and neutralization. It is possible that mutations outside the RBD could alter the confirmation of the spike, resulting in increased or decreased sensitivity to neutralizing antibodies. Another limitation is that the B.1.351 virus that we used in our study contains two substitutions within the spike protein that were not reported in the reference sequence deposited into GISAID (EPI_ISL_678615). One of these is a substitution of a Q677H, which has now been reported in multiple lineages of circulating variants of SARS-CoV-2 in the US population as early as mid-August 2020 (Hodcroft et al., 2021). The other is a substitution (R682W) within the furin cleavage motif (PRRAR) located between the S1/S2 regions of the spike protein.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.chom.2021.03.009.

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AUTHOR CONTRIBUTIONS

V.E., M.S., C.N., K.F., L.L., and M.D.G. contributed to the acquisition, analysis and interpretation of the data. W.H.H., G.M., L.E.N., M.W.A., R.F., S.P., R.B., D.N.G., G.M., H.A., N.B., B.P., N.M., K.H., J.P., J.W., J.K., J.U., J.B.O., A.P., J.J.W., and A.B. contributed to the acquisition, analysis, and interpretation of the data. S.E. served as the principal investigator of the clinical protocol for acquisition of patient samples and contributed to interpretation of the data. E.J.A. and N.R. contributed to the acquisition, analysis, and interpretation of the data. R.A., J.W., V.E., and M.S. contributed to the acquisition, analysis, and interpretation of the data as well as the conception and design of the work and writing of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
CR3022-biotin	Dr. Jens Wrammert Emory University	N/A
Virus Strains		
SARS-CoV-2/human/USA/GA-EHC-083E/2020 (EHC-083E)	residual nasopharyngeal swab	N/A
N501Y HV2 (B.1.351)	BEI Resources	hCoV-19/South Africa/KRISP-K005325/2020
Biological Samples		
Acute and Convalescent human Serum/Plasma samples	Emory University Hospital	N/A
mRNA-1273 Phase-1 study samples	Division of Microbiology and Infectious Diseases, NIAID	N/A
Chemicals, Peptides, and Recombinant Proteins		
Methylcellulose	Sigma-Aldrich	Cat. #: M0512-250G
TrueBlue Peroxidase Substrate	KPL	Cat. #: 5510-0050
Experimental Models: Cell Lines		
VeroE6 C1008 cells	ATCC	Cat# CRL-1586, RRID:CVCL_0574
Software and Algorithms		
GraphPad Prism (v7 and v8)	N/A	N/A
Viridot	Katzelnick et al.	https://github.com/leahkatzelnick/Viridot
Deposited data		
Additional Supplemental items are available from Mendeley Data: https://doi.org/10.17632/b2jm65g69n.1	N/A	N/A

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact Author Mehul Suthar (mehul.s.suthar@emory.edu).

Materials availability

All unique/stable reagents generated in this study are available from the Lead Contact with a completed Materials Transfer Agreement.

Data and code availability

The datasets supporting the current study are available from the corresponding author on request. Original data have been deposited to Mendeley Data: https://doi.org/10.17632/b2jm65g69n.1.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Ethics statement

For samples Emory University, collection and processing were performed under approval from the University Institutional Review Board (IRB #00001080 and #00022371). Adults $\geq$ 18 years were enrolled who met eligibility criteria for SARS-CoV-2 infection (PCR or rapid antigen test confirmed by a commercially available assay) and provided informed consent. For the mRNA-1273 phase 1 clinical trial, the neutralization assays were conducted on deidentified specimens, as protocol-defined research. The mRNA-1273 phase 1 clinical trial (NCT04283461) was reviewed and approved by the Advarra institutional review board, which functioned as a single board. The trial was overseen by an independent safety monitoring committee. All participants provided written informed consent before enrollment. The trial was conducted under an Investigational New Drug application submitted to the Food and Drug Administration. The NIAID served as the trial sponsor and made all decisions regarding the study design and implementation.

Serum samples

For Emory University, acute peripheral blood samples were collected from hospitalized patients at the time of enrollment. Infected patients were randomly selected from a convenience sample and no data was collected on the number of patients that were pre-screened or declined participation. All patients enrolled in July 2020 and had a mean age of 57 (range: 26-85; 50% male). Samples were collected in the first 9 days (range: 2-9) of their hospital stay (range: 3-33 days) and mostly 1-2 weeks after symptom onset (range 5-19 days), the majority of the patients had comorbid conditions (n = 16) with 19 out of 20 having severe disease and one patient had moderate disease. All of these patients had radiological evidence of pneumonia; 19 out of the 20 patients required supplemental oxygen, and 4 out of 20 patients were admitted to the intensive care unit (ICU). Three enrolled patients died of COVID-19. Convalescent samples were a convenience sample of individuals that had recovered from mild, moderate or severe COVID-19. The patients had a mean age of 52 (range: 28-77; 53% male). Patients had PCR or rapid antigen-test confirmed COVID-19 between March-August 2020 and samples were collected between April 2020 to February 2021. These individuals were recruited using multiple methods, including advertisements on the university campus, primary care clinics and at COVID-19 testing sites. Interested participants contacted the clinical research site and underwent a phone screening to assess if they met eligibility criteria. In addition, primary care clinic patients who were being managed for COVID-19 were contacted to see if they are interested in participating in this study. At the time of study enrollment, some of the participants had residual symptoms but others had recovered with no residual symptoms. No data was collected on the number of patients that were pre-screened or declined participation. Convalescent samples from COVID-19 patients were collected when the patients were able to return for a visit to the clinical research site at the next study visit. Convalescent samples were collected at a range of times (1-8 months) post symptom onset. Serum samples for the mRNA-1273 phase 1 study were obtained from the Division of Microbiology and Infectious Diseases, National Institute of Allergy and Infectious diseases for the mRNA-1273 phase 1 study team and Moderna Inc. Study protocols and results were previously reported (Anderson et al., 2020). Samples tested were collected from 19 healthy individuals on day 14 post-2nd dose of the mRNA-1273 vaccine (56 years of age and older).

Cells

VeroE6 cells were obtained from ATCC (clone E6, ATCC, #CRL-1586) and cultured in complete DMEM medium consisting of 1x DMEM (VWR, #45000-304), 10% FBS, 25mM HEPES Buffer (Corning Cellgro), 2mM L-glutamine, 1mM sodium pyruvate, 1x Non-essential Amino Acids, and 1x antibiotics.

METHOD DETAILS

Virus isolation and sequencing

EHC-083E (herein referred to as the B.1 variant) was derived from a residual nasopharyngeal swab collected from an Emory Healthcare patient in March 2020, as part of a study approved by the institutional review board at Emory University. As described previously (Babiker et al., 2020), the primary sample underwent RNA extraction, DNase treatment, random primer cDNA synthesis, Nextera XT tagmentation, Illumina sequencing, and reference-based viral genome assembly. Results were confirmed by sequencing of an independent library. A total of 47,542,787 reads were derived from this sample, leading to 100% SARS-CoV-2 genome coverage with a mean depth of 488X. All sequencing reads (cleaned of human reads) are available on NCBI under BioProject PRJNA634356, and the consensus SARS-CoV-2 genome is available under GenBank accession number MW008579.1. Following virus isolation, culture supernatant underwent metagenomic sequencing as described above. A total of 836,424 paired-end 150bp reads were generated by Illumina MiSeq, and reference-based SARS-CoV-2 genome assembly was performed using viral-ngs v.2.1.7 (https://github.com/broadinstitute/viral-ngs) with reference sequence NC_045512.2. The resulting consensus sequence had 100% coverage with a mean depth of 750X and was identical to the consensus sequence from the primary sample. The B.1 variant was plaque purified on VeroE6 cells propagated two times on VeroE6 cells (MOI 0.01), aliquoted to generate a working stock and sequenced. The B.1.351 variant was isolated as previously described (Tegally et al., 2020). Our laboratory plaque isolated the virus on VeroE6 cells followed by a single round of propagation on VeroE6 cells (MOI 0.05), aliquoted to generate a working stock and sequenced. Additional Supplemental items are available from Mendeley Data: https://doi.org/10.17632/b2jm65g69n.1. The primary sample underwent RNA extraction and cDNA synthesis was performed with random primers followed by pooling non-overlapping amplicons and Barcoding and library prep with ONT Ligation protocol and 96 PCR Barcoding expansion. Quality check was performed by excluding reads that are not in 200-800 base range. The resulting sequences were mapped to Wuhan reference with minimap2. Soft clip primer regions were identified using BAMClipper based on mapping position. Consensus variants were identified using ONT Medaka software and the variants were filtered with < 30 g score. Finally, variants and masking were applied to the reference sequence. Viral titers were determined by focus-forming assay on VeroE6 cells. Viral stocks were stored at −80°C until use.

RBD-binding assay

Plasma from acute and convalescent COVID-19 patients, mRNA-1273 vaccine recipients (14 days post-dose 2), and healthy controls was tested for IgG antibody binding against SARS-CoV-2 reference RBD (herein referred to as the B.1-lineage RBD), and B.1.351 RBD using an electrochemiluminescent-based multiplex immunoassay (kindly provided by Mesoscale Discovery (MSD)). Plates pre-coated with the RBD antigens were blocked for 30 min at room temperature, shaking at a speed of 700 rpm, with 150 uL per well of MSD Blocker A. To assess IgG binding, plasma samples were diluted 1:5000 and MSD Reference Standard-1 was diluted

per MSD instructions in MSD Diluent 100. 50 uL of each sample and Reference Standard-1 dilution was added to the plates and incubated for two h at room temperature, shaking at a speed of 700 rpm. Following this, 50 uL per well of 1X MSD SULFO-TAG Anti-Human IgG Antibody was added and incubated for one h at room temperature, shaking at a speed of 700 rpm. Following the detection reagent step, 150 uL per well of MSD Gold Read Buffer B was added to each plate immediately prior to reading on an MSD plate reader. Plates were washed three times with 300 uL PBS/0.05% Tween between each step. Data was analyzed using Discovery Workbench and GraphPad Prism software. Plasma antibody concentration in arbitrary units (AU) was calculated relative to Reference Standard 1.

Focus reduction neutralization assay

FRNT assays were performed as previously described (Vanderheiden et al., 2020). Briefly, samples were diluted at 3-fold in 8 serial dilutions using DMEM (VWR, #45000-304) in duplicates with an initial dilution of 1:10 in a total volume of 60 μl. Serially diluted samples were incubated with an equal volume of SARS-CoV-2 (100-200 foci per well) at 37°C for 1 h in a round-bottomed 96-well culture plate. The antibody-virus mixture was then added to Vero cells and incubated at 37°C for 1 h. Post-incubation, the antibody-virus mixture was removed and 100 μl of prewarmed 0.85% methylcellulose (Sigma-Aldrich, #M0512-250G) overlay was added to each well. Plates were incubated at 37°C for 24 h. After 24 h, methylcellulose overlay was removed, and cells were washed three times with PBS. Cells were then fixed with 2% paraformaldehyde in PBS (Electron Microscopy Sciences) for 30 min. Following fixation, plates were washed twice with PBS and 100 μl of permeabilization buffer (0.1% BSA [VWR, #0332], Saponin [Sigma, 47036-250G-F] in PBS), was added to the fixed Vero cells for 20 min. Cells were incubated with an anti-SARS-CoV spike primary antibody directly conjugated to biotin (CR3022-biotin) for 1 h at room temperature. Next, the cells were washed three times in PBS and avidin-HRP was added for 1 h at room temperature followed by three washes in PBS. Foci were visualized using TrueBlue HRP substrate (KPL, # 5510-0050) and imaged on an ELISPOT reader (CTL).

QUANTIFICATION AND STATISTICAL ANALYSIS

Antibody neutralization was quantified by counting the number of foci for each sample using the Viridot program (Katzelnick et al., 2018). The neutralization titers were calculated as follows: 1 - (ratio of the mean number of foci in the presence of sera and foci at the highest dilution of respective sera sample). Each specimen was tested in duplicate. The FRNT-50 titers were interpolated using a 4-parameter nonlinear regression in GraphPad Prism 8.4.3. Samples that do not neutralize at the limit of detection at 50% are plotted at 15 and was used for geometric mean calculations. Normality of the antibody binding and neutralization titers were determined using Shapiro Wilk normality test. Non-parametric pairwise analysis for RBD specific IgG titers and neutralization titers were performed by Wilcoxon matched-pairs signed rank test. Spearman rank test was used for the correlation analysis. The SARS-CoV-2 Spike structure was visualized with PyMOL (Schrödinger, Inc.).