The effect of spike mutations on SARS-CoV-2 neutralization

Graphical Abstract

Highlights

- SARS-CoV-2 pseudotypes produced by amino acids substitutions in SARS-CoV

- SARS-CoV-2 pseudotyped virus that encodes the B.1.1.7 variant spike

- Amino acid changes and B.1.1.7 can decrease monoclonal antibody neutralization

- Minimal effect on sera with only 10% losing potency against the B.1.1.7 pseudotype

Authors

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In brief

This study describes neutralization by antibodies and convalescent sera of SARS-CoV-2 spike mutants. Rees-Spear et al. show that SARS-CoV amino acid substitutions and the B.1.1.7 variant can block monoclonal antibody neutralization and that serum samples collected following mild illness are less resilient to spike variation than those following severe illness.

Rees-Spear et al., 2021, Cell Reports 34, 108890
March 23, 2021 © 2021 The Authors.
https://doi.org/10.1016/j.celrep.2021.108890
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SUMMARY

Multiple severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccines show protective efficacy, which is most likely mediated by neutralizing antibodies recognizing the viral entry protein, spike. Because new SARS-CoV-2 variants are emerging rapidly, as exemplified by the B.1.1.7, B.1.351, and P.1 lineages, it is critical to understand whether antibody responses induced by infection with the original SARS-CoV-2 virus or current vaccines remain effective. In this study, we evaluate neutralization of a series of mutated spike pseudotypes based on divergence from SARS-CoV and then compare neutralization of the B.1.1.7 spike pseudotype and individual mutations. Spike-specific monoclonal antibody neutralization is reduced dramatically; in contrast, polyclonal antibodies from individuals infected in early 2020 remain active against most mutated spike pseudotypes, but potency is reduced in a minority of samples. This work highlights that changes in SARS-CoV-2 spike can alter neutralization sensitivity and underlines the need for effective real-time monitoring of emerging mutations and their effect on vaccine efficacy.

INTRODUCTION

Serum neutralization activity is a common correlate of protection against viral infection following vaccination or natural infection (Plotkin, 2008). However, effective protection from viral infection can also require a sufficient breadth of serum neutralization rather than potency alone. This is because of the high levels of variation observed in major antigens across some viral populations (Burton et al., 2012). For example, in the response against influenza, the majority of neutralizing serum antibodies target only a particular set of influenza strains as a result of antigenic drift of the immunodominant hemagglutinin head (Zost et al., 2019). Because of this, an annual vaccine is required and must be matched to the most probable circulating strain in any given year to ensure protection from infection. Data emerging from human vaccine trials and challenge studies in animal models suggest that neutralizing antibodies can prevent disease caused by infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes coronavirus disease 2019 (COVID-19) (McMahan et al., 2021; Polack et al., 2020). However, new variants of SARS-CoV-2 have begun to emerge (Kemp et al., 2020; Oude Munnink et al., 2021; Tegally et al., 2020; Welkers et al., 2021). These variants include mutations in the major neutralizing antigen, the spike glycoprotein, and raises the question of whether neutralizing serum responses induced by early circulating strains or by vaccines based on the spike sequence of these early strains can neutralize the recently emerged virus variants.

Prior to emergence of multiple mutations in spike in the human population, we reasoned that a logical way to identify potential escape mutations was to look at sites of amino acid variation relative to the most closely related human betacoronavirus, SARS-CoV, which caused the original SARS outbreak (CDC, 2003). These two closely related viruses are characterized by notable differences in transmission dynamics and disease outcomes (Cevik et al., 2020; Lipsitch et al., 2003; Petersen et al., 2020), but use the human ACE2 protein as a viral entry receptor (Li et al., 2003) and share approximately 75% similarity overall in
**A**

![Graphs showing the effect of spike mutations on SARS-CoV-2 neutralization](Image)

**B**

<table>
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<tr>
<th>RBD</th>
<th>Cluster I</th>
<th>Cluster III</th>
<th>Cluster VI</th>
<th>Cluster VII</th>
<th>Cluster IX</th>
<th>Cluster XI</th>
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</table>

**KEY**

- <0.01 µg/ml
- 0.1-0.01 µg/ml
- >1 µg/ml

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spike at the amino acid level (Gralinski and Menachery, 2020). Both viruses use the same region of their respective spikes to bind ACE2, the receptor binding domain (RBD; found in the S1 subunit of spike). There is considerable amino acid variation between the two RBDs despite their conserved binding to ACE2, which explains why the majority of COVID-19 sera have weaker or no neutralizing activity against SARS-CoV, but cross-neutralizing monoclonal antibodies (mAbs) have been isolated (Brouwer et al., 2020).

Since the start of the pandemic, sequencing of virus populations has been deployed to enable detection of individual mutations in SARS-CoV-2. Recently, a new variant, B.1.1.7, has emerged in the United Kingdom (Kemp et al., 2020; Rambaut et al., 2020) that includes multiple mutations in the RBD and the N-terminal domain (NTD) of spike, targets for neutralizing antibodies. Similarly, additional variants have been identified in South Africa (B.1.351) and Brazil (P.1) (Faria et al., 2021; Tegally et al., 2020). The B.1.351 and P.1 variants share a deletion of three amino acids in Orf1ab and key mutations in the RBD (E484K and N501Y); data so far consistent with convergent evolution and recombination (Varabyou et al., 2020). Early reports indicated that, although the RBD mutation N501Y in the B.1.1.7 strain does not compromise post-vaccine serum neutralization (Xie et al., 2021), the additional changes in the B.1.351 strain do impair neutralization (Greaney et al., 2021; Wibmer et al., 2021).

In this study, we evaluated the potential role of individual amino acids in facilitating escape from neutralizing antibodies. First, we made a series of point mutations to change the amino acids in SARS-CoV-2 to those found at the analogous position in SARS-CoV. Second, we made individual point mutations emerging in real-world populations and generated a pseudotype virus using the B.1.1.7 variant spike sequence. We identify multiple mutations that can abrogate neutralization by some mAbs targeting the RBD of spike. However, in contrast, we show that serum responses are more resilient to these mutations, especially following severe illness, where the antibody response is characterized by increased breadth.

RESULTS

Generation of potential escape mutants by SARS-CoV amino acid substitution

There are 56 individual amino acid changes between the RBD of SARS-CoV-2 and SARS-CoV (Ortega et al., 2020). We prioritized 15 of the 56 changes by considering which mutations resulted in amino acids of substantially different biochemical character and which changes occurred in sequential positions. These sites were mutated in the SARS-CoV-2 spike to match SARS-CoV (Figure S1) and used to produce pseudotyped viruses (Seow et al., 2020). Twelve of the 15 mutated pseudotypes gave virus titers and were then screened for any alternation in neutralization against a panel of human mAbs (Brouwer et al., 2020) isolated after SARS-CoV-2 infection. These mAbs have been mapped previously into 11 binding clusters, where mAbs within a cluster compete reciprocally for binding to spike. Representatives of each neutralizing cluster were selected for evaluation against the spike mutant pseudotypes.

Effect of SARS-CoV spike substitutions on SARS-CoV-2 mAb neutralization

Initial screening assays showed no major effect on neutralization by any of the mAbs against pseudotypes encoding RFA_{346\rightarrow KFP}, S_{459\rightarrow G}, and ST_{477\rightarrow GK}. In contrast, the remaining nine viral pseudotype mutants diminished neutralization for at least one mAb, as described below (Figures 1A and 1B).

P_{384\rightarrow A}

The P_{384\rightarrow A} substitution resulted in complete loss of neutralization by COVA1-16, a cluster III RBD-specific mAb that allosterically competes with ACE2 rather than directly blocking the binding site (Liu et al., 2020). This mutation has been described and characterized structurally elsewhere (Wu et al., 2020), revealing that this proline-to-alanine change results in a relatively small alteration in protein structure that can enable SARS-CoV mAbs to neutralize SARS-CoV-2 P_{384\rightarrow A}. However, P_{384\rightarrow A} does not weaken neutralization by any other mAbs, including another mAb in the cluster III competition group.

K_{417\rightarrow V}

The K_{417\rightarrow V} mutation results in a pseudotyped virus that is less susceptible to COVA2-07-mediated RBD-specific neutralization. That this mutation should affect this mAb, which competes directly with ACE2 for binding, is not unexpected because the lysine at position 417 forms a hydrogen bond with ACE2 (Lan et al., 2020) that is likely disrupted by this substitution. We then evaluated an additional mAb, COVA2-04, from the same competitive binding cluster as COVA2-07. This is because COVA2-04 is representative of a class of SARS-CoV-2-neutralizing antibodies that use the VH3-53 gene (Cao et al., 2020; Mor et al., 2020; Robbiani et al., 2020). COVA2-04 was not able to neutralize the K_{417\rightarrow V} pseudotype (data not shown).

KVG_{444\rightarrow G}

This multiple substitution, which is a substantial change between SARS-CoV-2 and SARS-CoV, results in a 3.7-fold drop in neutralization potency for COVA2-29, which is a cluster I RBD-specific antibody. This is the largest effect of this mutation; the neutralization activity of the other mAbs is largely unaffected despite alteration of three sequential amino acids. This may be explained by the relatively minor differences in the amino acid side chains at the mutated residues.

L_{452\rightarrow K}

This mutation is situated directly in the receptor binding motif (RBM) of the RBD. It renders pseudotyped virus resistant to
neutralization by COVA2-29 but does not affect the other cluster I mAb COVA1-18 or any other mAb tested. 

**LF**<sub>455-4YL</sub> 
This double substitution reduces neutralization by RBD-specific mAbs from different clusters; specifically, the cluster I mAb COVA2-29, cluster III mAb COVA2-07, and cluster VI mAb COVA1-12. For COVA1-12, all neutralization activity is abolished, whereas COVA2-07 activity is just below the level required to calculate a 50% inhibitory concentration (IC<sub>50</sub>). **TEI**<sub>470-2NVP</sub> 
This triple mutation is located in a loop within the RBM where other substitutions have been reported to abolish ACE2 binding (Xu et al., 2021; Yi et al., 2020). This mutation prevents neutralization by COVA2-29 (cluster I), COVA2-07 (cluster III), and COVA2-02 (cluster VII). It also reduces the activity of the most potent mAb, COVA1-18 (cluster I), whereas this mAb is only minimally affected by other mutations. Moreover, TEI<sub>470-2NVP</sub> lowers the potency of the structurally un mapped non-RBD cluster XI mAb, COVA1-21, to the limit of detection.

**S<sub>494</sub>D** 
This single substitution toward the end of the RBM destroys neutralization activity by COVA2-29 (cluster I) and COVA1-12 (cluster VI) but does not have a major effect on the other cluster I mAbs tested or those from other epitope clusters.

In summary, different mAbs can lose their neutralization activity when confronted with different spike mutations, and the effects are not delineated strictly by binding clusters, so mAbs in the same competition cluster are frequently affected differentially. The triple substitution TEI<sub>470-2NVP</sub> has the most detrimental effect on different antibodies and affects mAbs from nearly all binding clusters, and S<sub>494</sub>D also affects many different clusters.

**Effect of spike mutations on serum neutralization**
Following identification of seven spike mutations that can limit or abrogate neutralizing activity of mAbs (Figure 2A), the next step was to assess the effect of these mutations on serum neutralization. Samples were tested following two different scenarios: from a previously characterized cohort of healthcare workers who experienced mild COVID-19 (Houlihan et al., 2020) and sera from a cohort of hospitalized individuals who experienced severe COVID-19. Samples from both cohorts were collected between March and July 2020. Eighteen samples were chosen from both cohorts to obtain representatives with intermediate (1:100–1,000) and potent (>1:1,000) neutralizing 50% inhibitory dilution (ID<sub>50</sub>) values (Figure 2B). Strikingly, serum samples from both cohorts are less affected by spike mutations than individual mAbs in terms of fold decrease in neutralization potency (Figures 2C and 2D). Only one of 36 serum samples lost all neutralizing activity, in contrast to the five mAbs from five different epitope clusters where neutralization was abrogated completely by a single spike mutation (Figure 1C). Moreover, the fold decrease in neutralization potency was more modest for sera than mAbs, with an average 3-fold decrease across all sera for the most disadvantageous mutation, TEI<sub>470-2NVP</sub>, compared with a more than 100-fold decrease observed for several of the mAbs (Figures 2C and 2D). Interestingly, only one of the 36 serum samples lost more than 3-fold potency against the other triple substitution, KVG<sub>444-6TST</sub>, which is consistent with recent data showing that a single mutation at G<sub>446</sub> caused a major loss of neutralization in one sample (Greaney et al., 2021). Importantly, there was a notable difference between the resilience of serum samples from severely ill, hospitalized individuals and those who experienced mild illness. Only three serum samples from hospitalized individuals lost more than 3-fold potency against any individual mutant (Figure 2D), whereas half of the mild illness serum samples showed a 3-fold drop in potency against at least one spike mutant (Figure 2D).

**Greater levels of spike-reactive antibodies in sera after severe illness**
The differences in resilience to spike mutations seen in the neutralizing sera from these two infection scenarios is plausibly due to greater polyclonality arising from greater antigenic stimulation during severe illness. To assess the serological profiles of these two cohorts, we compared the ID<sub>50</sub> values across 192 samples and measured the binding titers by semiquantitative ELISA for 199 samples, as described previously (Ng et al., 2020; O’Nions et al., 2021). There are significantly higher median immunoglobulin G (IgG) binding titers and median ID<sub>50</sub> in the severe illness cohort compared with the mild illness group (Figures 3A and 3B; Figure S2), in line with previous observations (Seow et al., 2020). However, when considering how the IgG binding titer from each individual relates to the neutralization titer, it became clear that there was a discrepancy (Figures 3C and 3D). Most hospitalized individuals required a binding titer of more than 10 µg/mL to achieve strong neutralization (ID<sub>50</sub> > 100). Moreover, mild infection could lead to potent neutralization (ID<sub>50</sub> > 1,000) at binding titers of less than 10 µg/mL (Figure 3D), whereas this was observed for only two individuals following severe illness (Figure 3C). In fact, the amount of specific IgG present at the serum ID<sub>50</sub> is significantly higher with severe illness compared with mild disease (Figure 3E).

**Effect of spike variants on mAb and serum neutralization**
Investigating the ability of post-SARS-CoV-2 infection mAbs and serum to cope with mutations based on differences with SARS-CoV was a rational first approach to study escape because these mutations were likely to form viable spike proteins. However, to date, none of the mutations engineered in our study have been observed more than 20 times in global SARS-CoV-2 sequences, although other amino acid substitutions have occurred at these positions, including one change (L<sub>452</sub>R) that has been observed more than 1,000 times. However, additional viral variants have started to emerge on a significant scale (Li et al., 2020; Weisblum et al., 2020), such as the D<sub>614G</sub> mutation, observed in western Europe in February 2020 and now dominant across the globe (Korber et al., 2020). More recently, a new variant, B.1.1.7, has emerged in England and is associated with a rapid rise in case numbers (Kemp et al., 2020; Rambaut et al., 2020). B.1.1.7 encodes nine sites of change in spike relative to the original Wuhan strain. Of these, the most likely candidates to alter neutralization sensitivity are the deletion in the NTD (∆H<sub>552</sub>V<sub>392</sub>) and the N<sub>501</sub>Y substitution in the RBM (Kemp et al., 2020; Rambaut et al., 2020). Therefore, we introduced these changes into the
Wuhan-strain spike in the presence of D614G. We found that D619/H69/V70 did not affect the neutralization potency of most of the mAbs tested, including COVA2-17 (Figure 4A), which binds the RBD and NTD (Rosa et al., 2021). The exception was the structurally unmapped COVA1-21, as reported previously (Kemp et al., 2021). Similarly, no major drop in serum neutralization was observed against D619/H69/V70 (Figure 4B). In contrast, introduction of the N501Y substitution dramatically lowered the neutralization potency of COVA1-12 with a fold decrease in IC50 of more than 40 (Figures 4A and 4C). Moreover, a 5-fold decrease in IC50 values for each mAb against each mutant pseudotype relative to the SARS-CoV-2 wild-type pseudotype; the group of affected individuals is indicated above each graph.

(C and D) The dotted horizontal lines indicate a 3-fold drop in neutralization potency.

Figure 2. Neutralization by serum is affected less adversely by SARS-CoV amino acid substitutions in SARS-CoV-2 spike
(A) Representation of the SARS CoV-2 spike trimer (blue) in complex with ACE-2 (pink) (PDB: 7DF4). The magnified image shows mutated amino acid side chains at residues of interest.
(B) Thirty-six serum samples were assessed by pseudotyped neutralization assay. Average ID50 values for 3 independent repeats are linked by horizontal bars for each individual sample.
(C) Fold decrease in IC50 values for each mAb against each mutant pseudotype relative to the SARS-CoV-2 wild-type pseudotype. Competitive binding clusters of each mAb that loses more than 3-fold neutralization activity are labeled.
(D) The y axis shows the fold decrease in ID50 values for each serum sample against each mutant pseudotype relative to the SARS-CoV-2 wild-type pseudotype; the group of affected individuals is indicated above each graph.

Kemp et al., 2021.
decrease in COVA2-17 potency was observed against the N501Y pseudotype. However, as seen for the other mutations that abrogate mAb function, the N501Y change had less of an effect on sera obtained after severe and mild infection (Figures 4B and 4C).

Effect of B.1.1.7 spike on mAb and serum neutralization

Finally, a B.1.1.7 spike pseudotyping plasmid was synthesized to incorporate the mutations observed in this new variant \((\Delta H69/V70, \Delta Y144, N501Y, A570D, D614G, P681H, T716I, S982A, \text{and } D1118H)\). This showed that, similar to the individual N501Y and \(\Delta H69/V70\) mutants, B.1.1.7 can lessen the potency of three mAbs: COVA2-17, COVA1-12, and COVA1-21 (Figure 4A). These belong to distinct clusters and so do not compete for binding to the same epitope. First, COVA2-17 showed an approximate 5-fold drop in potency against the N501Y single mutant and the B.1.1.7 pseudotype, implying that this loss of potency is primarily N501Y driven. In contrast, the decrease in COVA1-12 potency noted with the single N501Y change was less profound against B.1.1.7. Furthermore, COVA1-21 experienced a substantial drop in potency against B.1.1.7. This mAb, which does not bind to RBD or S1 subunits, lost potency by more than 100-fold. The B.1.1.7 pseudotype was then tested against the 36 serum samples (Figures 4B and 4C). The maximum fold decrease in potency for the serum samples from mild illness was 10, but the majority of samples showed less than a 3-fold change. Similarly, the maximum decrease seen for samples from hospitalized individuals was 10-fold, but most of the samples showed a minimal change in neutralization potency. Ten samples (28%) showed a 3- to 10-fold reduction, but because they were potently neutralizing sera, the reduced ID\(_{50}\) values were still more than 1:100 with an average reduced ID\(_{50}\) of 1:523, with only two samples having an ID\(_{50}\) of less than 1:200.

DISCUSSION

This study demonstrates that spike mutations can diminish or abolish neutralizing activity by individual mAbs, but that serum neutralization is affected less strongly. Notably, no serum sample failed to neutralize B.1.1.7, and only one engineered mutation resulted in complete escape from neutralizing activity from just one serum sample. The spike mutants evaluated comprise seven substitutions designed to mimic possible escape changes based on homology with SARS-CoV, two observed high-frequency mutations, and the B.1.1.7 spike variant. The observation of a modest reduction in neutralization potency against B.1.1.7 by convalescent sera is consistent with concurrent reports (Collier et al., 2021; Hu et al., 2021; Shen et al., 2021). The most likely explanation for the greater effect on mAbs compared with sera is the inherent polyclonality underlying serum neutralization. This concept is supported by the observation that single spike mutations can weaken neutralization for a particular mAb but
not for other mAbs in the same binding cluster. This highlights that different antibodies use distinct molecular contacts within shared epitopes so that a single mutation may not be detrimental to all antibodies in the same binding cluster. Thus, because polyclonal sera contain multiple antibodies that target the major neutralizing sites in subtly different ways, they are less sensitive to spike mutations.

The spike mutations studied here were designed to identify potential escape variants by mimicking in part the natural variation observed between SARS-CoV and SARS-CoV-2 and are focused mainly on the RBD as the major site of neutralizing antibody activity. Therefore, it was not surprising that many of the RBD-specific mAbs evaluated here lost neutralization activity against one or more of these mutations. For example, COVA2-07 and COVA2-04 lose potency against the K417V pseudotyped virus. COVA2-04 belongs to the VH3-53 "public" B cell receptor against SARS-CoV-2 identified from multiple human infections. Thus, COVA2-04-like antibodies are thought to be widespread among the seropositive population, but despite this, serum samples from mild infection showed very little change in neutralization potency with K417V pseudotyped virus. Interestingly, the strongest effect on serum samples from mild infection was mediated by the TEI 470-2NVP substitution, which is part of what has been termed the RBD binding ridge (Greaney et al., 2021). Any mutation in this zone should be monitored closely in virus populations because of the potential for escape. Notably, the mutations that most substantially decrease serum neutralization are those that negatively affect mAb activity against the widest range of clusters (I, III, XI, IX, and VI), suggesting that mAb screening is a useful proxy for potential serum effects when a range of antibody clones is used. However, the capacity to predict the in vivo effect of a drop in neutralization potency requires correlation of in vitro serum neutralization ID_{50} values with protection, which so far has only been achieved in animal models where, encouragingly, an ID_{50} value of 1:50 was found to be protective (McMahan et al., 2021).

Figure 4. Variant B.1.1.7 SARS-CoV-2 spike effect on mAb and serum neutralization
(A) The indicated mAbs were assessed by pseudotype neutralization assay. Data are representative of three independent repeats. The horizontal dotted line in each graph indicates 50% neutralization.
(B) Thirty-six serum samples (mild illness, left; severe illness, right) were assessed by pseudotype neutralization assay. ID_{50} values are linked by horizontal bars for each individual sample.
(C) Fold decrease in average ID_{50} values from 3 repeats for each serum sample against each mutant pseudotype versus D614G. The dotted horizontal line indicates a 3-fold drop in neutralization potency.

Please cite this article in press as: Rees-Spear et al., The effect of spike mutations on SARS-CoV-2 neutralization, Cell Reports (2021), https://doi.org/10.1016/j.celrep.2021.108890
A caveat of the first part of this study is that only RBD substitutions were considered. Further studies to assess potential mutations before they arise should include those in the NTD, given the emerging importance of the NTD as a site for neutralizing antibodies (Andreano et al., 2020; Rosa et al., 2021). A further limitation of our original approach is that the exact mutations evaluated have not yet been found to any great degree in circulating virus populations. To understand whether the conclusions from studying the effect of the SARS-CoV-2/SARS-CoV substitutions on neutralization parallel those of real-world spike mutations, we examined the responses to the newly emerged B.1.1.7 variant (Kemp et al., 2020; Rambaut et al., 2020). The RBD mutation N501Y, shared between B.1.1.7, B.1.351, and P.1, did remove almost all neutralizing activity for one mAb, but, in a pattern similar to other substitutions, this did not translate into any large effect on serum potency. We did not study the changes at position 484 that have been observed in B.1.351 and P.1. Recently, pseudotyped and live B.1.351 have been shown to be resistant to neutralization by a large proportion of convalescent plasma samples (Cele et al., 2021; Wibmer et al., 2021).

Theoretically, it is likely that combinations of mutations have more potential to lead to loss of serum activity than single amino acid changes by destroying multiple parts of key epitopes. This has been observed partially in terms of the B.1.1.7 spike pseudotype analyzed here. Only one mAb was affected more dramatically by the full set of B.1.1.7 mutations compared with the ΔH69/V70 and N501Y individual mutations. However, serum samples with reduced neutralization were affected more strongly by B.1.1.7 (Figures 4B and 4C). Importantly, these samples were collected prior to July 2020 and therefore are highly unlikely to be derived from B.1.1.7 infection. However, all of the affected samples were still able to neutralize B.1.1.7, and the average reduced serum ID50 value was 1:523. This is 10 times higher than the reported serum ID50 correlate of protection in animal studies (McMahan et al., 2021) and suggests that these responses would likely still be effective against infection with B.1.1.7.

This study underlines the potential for escape from neutralizing antibodies because of mutations in spike and the relative resilience of serum responses compared with individual mAbs. This difference likely derives from the breadth inherent in polyclonal sera compared with the precision interaction of a given mAb. Our results suggest that the majority of vaccine responses should be effective against the B.1.1.7 variant because the sera evaluated were obtained after infection early in 2020, when the commonly circulating virus was highly similar in sequence to the vaccines now being deployed. These findings are in agreement with concurrent studies that have reported a minimal drop in neutralization potency against B.1.1.7 in vaccinee and/or convalescent sera (Muik et al., 2021; Shen et al., 2021; Wang et al., 2021a, 2021b; Wu et al., 2021). Finally, because SARS-CoV-2 seropositivity will increase across the human population (because of vaccination efforts and natural infection), there may be selection for spike mutations that result in substantial antigenic drift. Recent data showing limited serum neutralization against B.1.351 (Cele et al., 2021; Wibmer et al., 2021) suggest that major antigenic drift has already occurred. Vaccine-induced responses appear to be more resilient to the mutations in B.1.351, in part because of higher initial titers (Collier et al., 2021; Wang et al., 2021a, 2021b; Wu et al., 2021). However, that this level of antigenic change has already occurred in SARS-CoV-2 suggests that, with increasing seroprevalence, additional potential neutralization escape mutations will emerge and require scrutiny. The data here suggest that evaluation of neutralizing mAbs from non-overlapping binding clusters can highlight which spike mutations will most affect serum neutralization. Our findings stress the importance of continuous monitoring of variants and in vitro assessment of their effect on neutralization. This is particularly relevant for use of convalescent plasma and development of therapeutic mAbs as well as vaccine development and implementation.

CONSORTIA


STAR★METHODS

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

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
  - Mild illness serum samples
  - Severe illness serum samples
  - Bacterial Strains and Cell Culture
- METHOD DETAILS
  - Spike mutant generation
  - Neutralization assay
  - Semiquantitative ELISA
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.celrep.2021.108890.

ACKNOWLEDGMENTS

The authors would like to thank James E. Voss for the gift of HeLa ACE2-expressing cells and George Kassiotis, Dan Frampton, Ann-Kathrin Reuschl, and Joe Grove for critical feedback. We are indebted to the Biobank staff.
and study participants and their families at the Royal Free Hospital and the UCLH SAFER study recruitment team and study participants. L.E.M is supported by a Medical Research Council career development award (MR/R008688/1). M.J.v.G is a recipient of an AMC fellowship, and R.W.S is a recipient of a Vici grant from the Netherlands Organization for Scientific Research (NWO). C.G is supported by the MRC-KCL Doctoral Training Partnership in Biomedical Sciences (MR/N013700/1). This work was also funded by the UCL Coronavirus Response Fund, made possible through generous donations from UCL’s supporters, alumni, and friends (to L.E.M.); the King’s Together Rapid COVID-19 Call fund (to K.J.D.); the Hsu Family Foundation (to K.J.D.); the Royal Free Charity; and the UK Coronavirus Immunology Consortium. This work was also supported by National Institutes of Health grant P01 AI110657 and Bill and Melinda Gates Foundation grant (1U01GM122202) (to R.W.S.). The work in laboratory of P.C. was supported by the Francis Crick Institute (FC001061), which receives its core funding from Cancer Research UK, the UK Medical Research Council, and the Wellcome Trust. The SAFER study was funded by MRC UKRI (MC_PC_19082) and supported by the UCLH/UCL NIHR BRC.

AUTHOR CONTRIBUTIONS


DECLARATION OF INTERESTS

Amsterdam UMC submitted a patent application on SARS-CoV-2 monoclonal antibodies, some of which were used in this study.

Received: January 15, 2021
Revised: February 17, 2021
Accepted: March 1, 2021
Published: March 6, 2021

REFERENCES


### STAR METHODS

#### KEY RESOURCES TABLE

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| HIV p8.91 packaging construct | Zufferey et al., 1997 | p8.91 |

**Software and Algorithms**

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RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Laura McCoy (l.mccoy@ucl.ac.uk).

Materials availability
Reagents generated in this study are available from the Lead Contact with a completed Materials Transfer Agreement.

Data and code availability
This study did not generate datasets/code. Original source data for SARS-CoV-2 spike structure used in Figure 2 is available at https://doi.org/10.2210/pdb7DF4/pdb, and in Figure S1 at https://doi.org/10.2210/pdb6VXX/pdb.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Mild illness serum samples
These samples are part of the UCLH SAFER study and were collected as previously described (Houlihan et al., 2020). Briefly, samples are from 81 seropositive individuals previously identified (Houlihan et al., 2020) who donated blood at monthly intervals from March to July 2020 as well as undergoing regular PCR testing. Informed consent was obtained from all participants. The median age of participants was 81 (interquartile range 70-87), 43% were female and 57% male. The study protocol was approved by the NHS Health Research Authority (ref 20/SC/0147) on 26 March 2020. Ethical oversight was provided by the South- Central Berkshire Research Ethics Committee.

Severe illness serum samples
These samples are from patients hospitalized for COVID-19 between March and July 2020 and were obtained during their hospital stay through the Tissue Access for Patient Benefit (TAPb) scheme at The Royal Free Hospital (approved by UCL–Royal Free Hospital BioBank Ethical Review Committee Reference number: NC2020.24 NRES EC number: 16/WA/0289). Informed consent was obtained from all participants and a single blood sample was taken without interfering with normal clinical care. The median age of participants was 34 years (interquartile range 29–44), 62% were female and 38% male.

Bacterial Strains and Cell Culture
Bacterial transformations were performed with XL1-Blue Supercompetent Cells (Agilent). SARS-CoV-2 pseudotypes were produced by transfection of HEK293T/17 cells and neutralization activity assayed using HeLa cells stably expressing ACE2 (Kind gift James E Voss).

METHOD DETAILS

Spike mutant generation
QuikChange Lightening Site-Directed Mutagenesis kit was used to generate amino acid substitutions in the SARS-CoV-2 Wuhan spike expression vector (Seow et al., 2020) or the D614G pCDNA spike plasmid (Kemp et al., 2021) following the manufacturer’s instructions (Agilent Technologies, Inc., Santa Clara, CA). Spike B.1.1.7 (ΔH69/ΔV70, ΔY144, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H) was synthesized by Genewiz, Inc. and cloned into the pCDNA3.1+ expression vector using BamHI and EcoRI restriction sites.

Neutralization assay
HIV-1 particles pseudotyped with SARS-CoV-2 spike were produced in a T75 flask seeded the day before with 3 million HEK293T/17 cells in 10 ml complete DMEM, supplemented with 10% FBS, 100 IU/ml penicillin and 100 μg/ml streptomycin. Cells were transfected using 60 μg of PEI-Max (Polysciences) with a mix of three plasmids: 9.1 μg HIV-1 luciferase reporter vector (Seow et al., 2020), 9.1 μg WT SARS-CoV-2 spike expression vector (Seow et al., 2020). Supernatants containing pseudotyped virions were harvested 48 h post-transfection, filtered through a 0.45-μm filter and stored at −80 °C. Neutralization assays were conducted by serial dilution of monoclonal IgG at the indicated concentrations in DMEM (10% FBS and 1% penicillin–streptomycin) and incubated with pseudotyped virus for 1 h at 37 °C in 96-well plates. HeLa cells stably expressing ACE-2 (provided by J.E. Voss, Scripps Institute) were then added to the assay (10,000 cells per 100 μl per well). After 48–72 h luminescence was assessed as a proxy of infection by lysing cells with the Bright-Glo luciferase kit (Promega), using a Glomax plate reader (Promega). Measurements were performed in duplicate and used to calculate 50% inhibitory dilutions/concentration (ID50 values in GraphPad Prism software.

Semi-quantitative ELISA
As described previously (O’Nions et al., 2021) nine columns of a half-well 96-well MaxiSorp plate were coated with purified SARS-CoV-2 spike S1 protein in PBS (3 μg/ml per well in 25 μL) and the remaining three columns were coated with 25 μL goat anti-human F(ab)2 diluted 1:1000 in PBS to generate the internal standard curve. After incubation at 4 °C overnight, the ELISA plate was blocked
for 1 h in assay buffer (PBS, 5% milk, 0.05% Tween 20). Sera was diluted in assay buffer at dilutions from 1:50 to 1:5000 and 25 μL added to the ELISA plate. Serial dilutions of known concentrations of IgG standards were applied to the three standard curve columns in place of sera. The ELISA plate was then incubated for 2 h at room temperature and then washed 4 times with PBS-T (PBS, 0.05% Tween 20). Alkaline phosphatase-conjugated goat anti-human IgG at a 1:1000 dilution was then added to each well and incubated for 1 h. Following this, plates were washed 6 times with PBS-T and 25 μL of colorimetric alkaline phosphatase substrate added. Absorbance was measured at 405 nm. Antigen-specific IgG concentrations in serum were then calculated based on interpolation from the IgG standard results using a four-parameter logistic (4PL) regression curve fitting model.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

All neutralization measurements were performed in duplicate and 50% inhibitory concentrations/dilutions (IC/ID<sub>50</sub>) were calculated using GraphPad Prism software. ID<sub>50</sub> values calculated as indicated in the relevant Figure legends. Statistical analysis in Figure 3 (non-parametric Mann-Whitney U test) was performed using GraphPad Prism software, significance defined as ****p < 0.05. Fold decrease in serum ID<sub>50</sub> was calculated by dividing the average ID<sub>50</sub> value for a given sample against SARS-CoV-2 or SARS-CoV-2<sub>D614G</sub> (as indicated) by the average ID<sub>50</sub> value for that sample against the indicated mutant or variant pseudotype. Fold decrease in mAb IC<sub>50</sub> was calculated by dividing the average IC<sub>50</sub> value for a given mAb against the indicated mutant or variant pseudotype by the average IC<sub>50</sub> value for that mAb against the SARS-CoV-2 or SARS-CoV-2<sub>D614G</sub> (as indicated).