Antibodies induced by an ancestral SARS-CoV-2 strain that cross-neutralize variants from Alpha to Omicron BA.1

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Neutralizing antibodies that recognize the SARS-CoV-2 spike glycoprotein are the principal host defense against viral invasion. Variants of SARS-CoV-2 bear mutations that allow escape from neutralization by many human antibodies, especially those in widely distributed (“public”) classes. Identifying antibodies that neutralize these variants of concern and determining their prevalence are important goals for understanding immune protection. To determine the Delta and Omicron BA.1 variant specificity of B cell repertoires established by an initial Wuhan strain infection, we measured neutralization potencies of 73 antibodies from an unbiased survey of the early memory B cell response. Antibodies recognizing each of three previously defined epitopic regions on the spike receptor binding domain (RBD) varied in neutralization potency and variant-escape resistance. The ACE2 binding surface (“RBD-2”) harbored the binding sites of neutralizing antibodies with the highest potency but with the greatest sensitivity to viral escape; two other epitopic regions on the RBD (“RBD-1” and “RBD-3”) bound antibodies of more modest potency but greater breadth. The structures of several Fab:spike complexes that neutralized all five variants of concern tested, including one Fab each from the RBD-1, -2, and -3 clusters, illustrated the determinants of broad neutralization and showed that B cell repertoires have specificities that avoid immune escape driven by public antibodies. The structure of the RBD-2 binding, broad neutralizer shows why it retains neutralizing activity for Omicron BA.1, unlike most others in the same public class. Our results correlate with real-world data on vaccine efficacy, which indicate mitigation of disease caused by Omicron BA.1.

INTRODUCTION

The high global rate of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections leads to regular emergence of previously unidentified variants (1). Both transmissibility and immune evasion appear to determine fitness of variant phenotypes. Strength of attachment to the viral receptor, angiotensin-converting enzyme 2 (ACE2), is one determinant of transmissibility, restricting the variability of the receptor binding surface. However, this restriction also limits the potential for escape from the most potent neutralizing antibodies (Abs), which share contacts with ACE2 and neutralize by blocking cell entry. That is, immune escape and receptor binding exert competing selective pressures on the evolution of the SARS-CoV-2 spike protein, which is both the viral receptor binding and fusion protein and the sole known target of protective Abs. Abs that maintain affinity to variants reflect the outcome of these countervailing selective forces.

We recently characterized cross-competition for spike binding by 73 monoclonal Abs (mAbs) in an epitope-unbiased set representing sequences of memory B cell receptors from 19 coronavirus disease 2019 (COVID-19) convalescent donors (2). Clustering analysis of those competition data defines seven principal clusters corresponding to distinct epitopic regions on the SARS-CoV-2 spike.

Three of those regions are on the receptor binding domain (RBD), two on the N-terminal domain (NTD), and the rest on the S2 fragment. The panel of 73 mAbs is thus a broad sampling of components of the human polyclonal response to infection with a Wuhan-like (i.e., early pandemic) SARS-CoV-2.

The most potent neutralizing Abs, from many studies, fall into the RBD-2 competition group, which includes all that recognize epitopes overlapping the ACE2 footprint on the spike protein RBD (2). Many of the RBD-2 Abs belong to well-studied public classes (3–12)—that is, they have V(D)J recombinase gene segments for both heavy and light chains and heavy-chain complementarity-determining regions (HCDR3s) of similar length and sequence in common with others found in many unrelated donors from diverse cohorts. Although more neutralizing mAbs in our unbiased set fall into the RBD-1 competition group than into RBD-2, their activity is generally weaker (2). Because it contains the target sites for potent neutralization, the RBD-2 epitopic region has varied more than RBD-1 and RBD-3 despite strong constraints on the receptor binding surface. Most of the RBD-2 Abs maintain affinity and in vitro pseudotype neutralization potency for the Alpha variant, but not for Beta, or Gamma.

We report here an extension of our previous analysis to the Delta and Omicron BA.1 variants. We identified one mAb in each group that binds and neutralizes all five variants tested here (Alpha, Beta, Gamma, Delta, and Omicron BA.1), as well as a fourth (in RBD-2) that neutralized all variants tested except Omicron BA.1. Each of the two RBD-2 competition-group members belonged to a public class. Most known members of one of these two public classes fail to neutralize Beta, Gamma, and Omicron BA.1; the RBD-bound
structure of the exception described here suggests the basis for its broader neutralizing activity. The results provide a molecular rationale for flexibility in the Ab system to extend neutralizing recognition breadth from an ancestral strain across evolving viral variants (13–15).

RESULTS

Memory B cell repertoire contribution to SARS-CoV-2 variant neutralization

The left-hand side of Fig. 1 illustrates our published characterization of the early memory B cell repertoires from 19 convalescent donors during the first 6 to 8 months of the pandemic and hence from infection with virus closely related to the original Wuhan isolate (2). The colored blocks in the schematic diagonal plot represent the seven clusters from our all-against-all competition Ab-binding assays—three RBD-binding clusters, two NTD clusters, and two S2 clusters. We mapped four of the corresponding epitopic regions (RBD-1, -2, and -3 and NTD-1) by determining representative structures and by including Abs for published structures in the competition experiments. We also determined neutralization profiles of all 73 Abs for the Wuhan isolate and for the Alpha, Beta, and Gamma variants.

In the work reported here, represented by the schematics on the right-hand side of Fig. 1, we examined binding and neutralization of the Delta and Omicron BA.1 variants by mAbs in our panel. We characterized three Abs (G32R7, C98C7, and G32Q4) that neutralized all five variants of concern tested C98C7, one from each of the RBD clusters and two others from RBD-2 (one that neutralized all variants except Omicron BA.1; the other, only Alpha and Delta). Figure 2 (A to D) shows the binding of each of the 73 mAbs to the SARS-CoV-2 spike and their neutralization potencies for the D614G variant of the original Wuhan isolate and for the five variants of concern derived from it. Mutations in NTD-1 occur readily (16); they eliminated neutralization of all the variants by the NTD-1 Abs in our panel (Fig. 2B) and also substantially lowered affinity (Fig. 2A). The binding assay was more sensitive than the neutralization assay, accounting for the instances of detectable binding but undetectable neutralization. Three of the 11 RBD-2 competition-group mAbs in our panel (G32A4, C98C7, and G32C4) retained strong binding to all five variants of concern tested (Fig. 2A and fig. S1) but only one of these (C98C7) neutralized all five, whereas a second (G32A4) neutralized all but Omicron BA.1. Like the majority of our RBD-2 mAbs, most clinical-stage mAbs compete with ACE2 and lose neutralization potency for Omicron BA.1 (17). Loss of the RBD-2 contribution to Beta and Omicron BA.1 variant neutralization in our assay correlated with the escape of these variants from immune control of infection as monitored in studies (see Materials and Methods for specific references) of real-world vaccine effectiveness (Fig. 2E).

Affinity of broad mAbs for variants of concern

Table 1 shows affinities of the antigen-binding fragments (Fabs) from mAbs G32R7, C98C7, G32A4, and G32Q4 for the RBDs of the D614G Wuhan-isolate mutant and of the variants of concern. Measuring the monomer–monomer interaction between a Fab and the monomeric RBD avoided format- and epitope-dependent avidity effects that might have confounded comparisons of specific mutations. The quantitative measurements correlated with neutralizing potency. Although all four Fabbs bound the RBDs of variants Alpha, Gamma, and Delta, as well as that of the unmutated Wuhan isolate, all but G32R7 lost affinity for the Omicron BA.1 RBD. The equilibrium dissociation constants ($K_d$) for the C98C7 and G32Q4 Fabs (22 and 1.8 μM, respectively) were consistent with the diminished, but measurable, neutralizing potencies of the corresponding mAbs. The Omicron BA.1 mutations eliminated G32A4 binding completely. The effects of mutations on affinity were consistent with the Ab footprints, as shown by the structures described below.

Cryo-EM structures of broadly neutralizing mAbs

We determined structures for prefusion stabilized spike ectodomain trimer bound with Fab fragments of three mAbs (G32A4, C98C7, and G32Q4), two of which (C98C7 and G32Q4) neutralized all variants, whereas the third (G32A4) neutralized all except Omicron BA.1 (Figs. 3 and 4). Local reconstructions enabled us to obtain resolutions sufficient to model the Ab–antigen interaction (figs. S2 and S3 and table S2). The epitopes of C98C7 and G32A4, both in the RBD-2 group, overlapped the ACE2 footprint (Fig. 3, A and B); related Abs compete with ACE2 (2). G32Q4, in the RBD-3 cluster, bound the face of the RBD that contacts an adjacent RBD in the “down” conformation; it competed with CR3022, an Ab originally isolated from a convalescent SARS-CoV–infected donor [Fig. 3C and (18)].

G32A4 and C98C7 belong to well-studied public classes, which dominate the RBD-2 cluster [table S3 and (21)]; G32Q4 showed a convergence of structure and recognition with a published Ab encoded by completely different $V_H$ and $V_L$ gene segments. C98C7, a
product of V(D)J recombination of IGVH3-53/66 with IGHD3-22 and IGJH6, is nearly identical in the sequences of both variable domains to Ab P2C-1F11 (19). Despite this similarity, the structure showed that the pose of the C98C7 Fv module with respect to the RBD deviated slightly from that of P2C-1F11, perhaps because of a glycine residue rather than a serine at position 30 of the light chain (fig. S4A). Its heavy-chain contacts were almost identical to those of P2C-1F11, but the light chain contacted the RBD more intimately, especially near residue 501, correlating with different main-chain configurations for LCDR2 (fig. S4A). G32A4, with a heavy chain encoded by IGVH1-58, bound the RBM with contacts like those of other members of its public class (fig. S4B) (9–12). The G32Q4 heavy chain derives from V(D)J recombination of IGVH3-30, one of the most prevalent heavy-chain V gene segments in humans (2). Although there are published structures for several spike-directed IGVH3-30 Abs (5, 20, 21), none reported so far share an epitope with G32Q4. Instead, we found the HCDR3 loop of G32Q4 to be nearly identical to that of COVA1-16 (22), both from recombination of gene segments IGH3-22 and IGJH1, whereas the COVA1-16 heavy chain has an otherwise very different sequence encoded by IGHV1-46. The two Abs have nearly identical RBD recognition patterns [fig. S4C and (22)].

**Variant mutations and breadth of neutralization and binding**

One Ab from each of the three RBD epitopic regions (G32R7, C98C7, and G32Q4, in competition clusters RBD-1, -2 and -3, respectively) neutralized all five variants of concern in a pseudovirus assay (Fig. 2); the same three also neutralized authentic SARS-CoV-2 Omicron BA.1 (fig. S6). The spike-bound structure of the G32R7 Fab showed binding to the face of the RBD that is adjacent to the NTD when the RBD is in the down conformation (2). Its footprint included none of the Omicron BA.1 RBD mutations. G32R7 does not belong to a recognized public class, and its contacts with the RBD included many from its long (24-residue) HCDR3. Three other RBD-1 class mAbs also had detectable neutralization activity for all variants we tested (Fig. 2B and table S1), but their potency was low, even for the D614G parent strain, despite relatively high apparent affinities.
The observed footprint of Ab G32Q4 avoided any of the Omicron BA.1 variant mutations (Fig. 4A). In addition to the convergence of its HCDR3 sequence and RBD interface with that of COVA1-16, the divergent LCDR2 loops of both these Abs contacted the RBD between residues 405 and 410. For G32Q4 to bind, both the targeted RBD and the adjacent RBD need to be in the “up” configuration. The immune-escape mutations in the Beta, Gamma, and Omicron BA.1 variants fell within the footprint of RBD-2-cluster Ab C98C7. Nonetheless, unlike other Abs with heavy chains encoded by IGHV1-53/66, such as C93D9 [see (2)], C98C7 retained some binding and neutralizing potency for all the variants, including Omicron BA.1 (Figs. 2B and 4B).

VH1-58 Abs, including G32A4, bind and potently neutralize all the variants of concern except Omicron BA.1 (23–26). Several Omicron BA.1 mutations fell within the epitope of G32A4 (Fig. 4C). One of them, Q493R, which contributes to ACE2 affinity through a salt bridge (27, 28), appeared as an escape mutant in a selection for escape from neutralization by Ab B1-182.1, a member of the VH1-58 public class (9, 29, 30). Two others are S477N and T478K. The latter, also present in Delta, had no measurable effect on binding and neutralization of Delta by the four Abs studied here and thus could not alone explain the loss of affinity for Omicron BA.1. To assess the effects of the other RBM mutations, we measured binding of the S477N/T478K and Q493R mutant RBDs with Fab G32A4 and found ratios, to binding the Wuhan-isolate RBD, of 15 and 70 for their equilibrium dissociation constants (fig. S5 and table S4). The structure of the G32A4 complex showed that, barring local main-chain rearrangements, the arginine side

### Table 1. Equilibrium dissociation constants for broad mAbs and RBDs and pseudovirus neutralization potency for coordinate variants of concerns. Values of $K_d$ reported in nanomolar. n.b., no detectable binding. Values of IC$_{50}$ are reported in micrograms per milliliter with 50 &mu;g/ml as the upper limit. IC$_{50}$ of original, Alpha, and Gamma were reported previously (2).

| mAb    | $K_d$ | IC$_{50}$ | $K_d$ | IC$_{50}$ | $K_d$ | IC$_{50}$ | $K_d$ | IC$_{50}$ |
|--------|-------|-----------|-------|-----------|-------|-----------|-------|-----------|
| Original | Alpha | Gamma | Delta | Omicron BA.1 |
| G32R7  | <1 0.109 | 3 0.080 | 22 0.375 | 50 1.851 | 68 0.162 |
| G32Q4  | 57 0.578 | 75 1.476 | 13 0.089 | 53 0.316 | 1800 6.666 |
| C98C7  | 3 0.013 | 6 0.015 | 1 0.012 | 3 0.023 | 22,000 1.067 |
| G32A4  | 53 0.014 | 60 0.007 | 360 0.003 | 290 0.003 | n.b. >50 |

Fig. 3. RBD-bound structures of cross-neutralizing antibodies. In all panels, the view is of the RBD in the up configuration on a spike trimer with the threefold axis vertical. The RBD from each local reconstruction is aligned to the up RBD from the full spike (7krr) for reference, which is shown as a transparent surface. (A) Structure of C98C7 (blue, with light chain in a lighter shade than the heavy chain and only the Fv module shown) bound with RBD (dark gray). Light surface representation shows the outline of the spike. (B) G32A4 [red, with lighter shade for light chain and other details as in (A)]. The view is rotated with respect to all the other panels by about 60° counterclockwise (ccw) about the vertical. (C) As in (A), for G32Q4 (gold). (D) Cartoon representation of the RBD. The footprints of C98C7, G32A4, G32Q4, and G32R7 (including side chains) are in blue, red, gold, and green, respectively. Residues identified by PISA at the interface of the RBD and Fab are shown as sticks, and their labels are underlined.
chain of the mutant would overlap the Ab HCDR2. Loss of interaction between D100c in VH1-58 mAbs and S477 (Fig. 4B) may also contribute to reduced affinity of the G32A4 Fab for the Omicron BA.1 RBD.

IGHV1-2 encodes the heavy chains of another public Ab class whose members lose affinity for Beta and Gamma (Fig. 4B) (31). These Abs contact the side chain of E484 (31), mutated to lysine in the Beta and Gamma variants; these two variants lose sensitivity to IGHV1-2 Abs and to certain IGHV3-53/66 mAbs that bind with a different footprint from the majority (32). The E484A mutation in Omicron BA.1 apparently also compromises binding of IGHV1-2 Abs, such as C12A2 (Figs. 2A and 4B), by loss of either bulk or negative charge, even without the charge reversal found in Beta and Gamma. Abs from each of the well-characterized public classes thus contact at least one of the few sites of mutation—K417 and E484 in particular—found in multiple variants of independent origin.

**DISCUSSION**

Three VH gene segments, IGHV1-2, -1-58, and -3-53/66, account for 7 of the 11 RBD-2 mAbs in our unbiased sampling of 19 convalescent donors (2). The structures we report here and in our previous work (2) confirm that their interactions conform to the stereotypical contacts seen in published structures of Abs in the corresponding public classes. The positions of mutations leading to resistance that have appeared in distinct viral lineages are primarily within the footprints of immunoglobulin Gs (IgGs) such as C98C7, G32A4, and C12A2, consistent with the expectation that widespread occurrence of such Abs will likely give them a prominent role in selecting for viral immune escape mutations. The recurring substitution K417N/T, present in the RBDs of the Beta, Gamma, and Omicron BA.1 variants, greatly reduces the affinity of most IGHV3-53/66 Abs for the SARS-CoV-2 RBD, and the E484K substitution, also present in Beta, Gamma, and Omicron BA.1, reduces the affinity of most IGVH1-2 Abs. No repeatedly found mutations lie within the footprints of the more “idiiosyncratic” G32R7 and G32Q4, in the RBD-1 and RBD-3 competition groups, respectively. The several other Abs reported to neutralize all five variants of concern (17, 33) are not members of any identified public class. Together with G32R7 and G32Q4, they may represent responses not prevalent enough to have been major determinants of antigenic drift. Collectively, such broadly protective Abs might be relatively widespread.

C98C7 is unusual among RBM-binding, IGHV3-53/66 Abs in retaining affinity for the Omicron BA.1 variant spike protein. A characteristic of this public class is a short HCDR3 loop projecting toward Lys417, the site of the K417N/T mutation common to Beta, Gamma, and Omicron BA.1. Third hypervariable loops longer than about 11 to 12 residues would probably collide with the RBD if the Ab docked as defined by its germline encoded HCDR1 and HCDR2. Spike-binding, IGVH1-53/66 Abs bearing a nonpolar residue at the tip of HCDR3 frequently pair with light chains encoded by IGVK3-20; those with an acidic residue at the tip of HCDR3, which can salt-bridge with Lys417, tend to pair with IGVK3-20 (8). The HCDR3 of C98C7 is nonpolar, but it pairs with a light chain encoded by IGVK3-20; Ab P2C-1F11, which, like C98C7, has a nonpolar residue at the apex of HCDR3 but pairs with IGVK3-20 (8), is also insensitive to the mutations in the Beta and Gamma variants (2, 8). Their structures suggest that interactions of the IGVK3-20 LCDR1 with the RBD loop that surrounds residue 501 (mutated from Asn to Tyr in all five variants) might compensate for any loss of affinity from heavy-chain contacts due to the K417N mutation, consistent with a published light-chain swap experiment with IGHV3-53 Abs (34). G32A4, like other IGVH1-58 Abs, is insensitive to the K417N/T and E484K mutations in the Beta and Gamma variants and to T478K in Delta, but it fails to neutralize Omicron BA.1 probably because of the S477N and Q493R substitutions. It nonetheless has detectable binding to Omicron BA.1, suggesting that just a few amino acid differences could restore neutralizing potency. The sequence variations seen in clonal lineages of human Abs elicited by exposure to influenza virus (35, 36) suggest that the amino acid differences required might be present in the memory B cell repertoires of many individuals. Reactivation of that B cell memory could have a role in protection against developing severe disease.

The N501Y mutation, common to the Alpha, Beta, Gamma, and Omicron BA.1 variants, but absent in Delta, increases ACE2 affinity. The K417N/T mutation, present in Beta, Gamma, and Omicron BA.1, decreases it (37). Thus, RBM mutations in the Beta and Gamma variants allowed them to escape neutralization by Abs common to the repertoires of donors in many cohorts, such as those specified by IGHV3-53/66 and IGHV1-2, but at the cost of affinity for ACE2. Alpha and Delta spread rapidly and remained until displaced by other variants: They were thus in evolutionary terms more successful than Beta or Gamma (38), which appeared locally but failed to spread. Omicron BA.1 contains both mutations apparently driven by immune escape, K417N/T and E484A, with others, Q493R and N501Y, that compensate for loss of affinity due to the escape
The effects of Q493R combine enhanced affinity for ACE2 with escape from neutralization by IGHV1-58 Abs such as G32A4. Whether the RBD mutations in Omicron BA.1 also contribute to other phenotypic changes in that variant, including tissue tropism and generally milder disease course (39), is not yet clear. If so, they would represent a different optimum in the evolution of the virus, potentially of advantage both to virus and to host.

The structures, binding, and neutralization data presented here show that just two or three mutations at key positions in the SARS-CoV-2 spike protein can strongly diminish the capacity of RBD-binding Abs from an unbiased panel to neutralize variants such as Beta, Gamma, and Omicron BA.1. Nonetheless, the example of C98C7 shows that some public-class, RBD-2 binding Abs can retain neutralizing activity for those variants, and the data in Fig. 2 show that several more of the RBD-2 directed mAbs in the panel retained detectable binding. The mutations in the Omicron BA.1 variant that have restored ACE2 affinity loss from K417N/T and E484K/A have apparently allowed it to supplant the previously prevalent Delta variant and to cause breakthrough infections in SARS-CoV-2–immune experienced individuals. In such cases, reactivation of B cell memory, for which relatively weak residual affinity appears to be sufficient, could then, in principle, update the repertoire by affinity maturation against the mutated antigen, as suggested by recently published studies (40, 41).

Localized point mutations, which are the sources of immune escape in the Beta, Gamma, and Omicron BA.1 variants of SARS-CoV-2, as illustrated by the structures described here, are also responsible for immune escape of drifted influenza hemagglutinin variants (42). In the sequence of events that has characterized successive variation of influenza virus subtypes over the course of the past century (43–45), recalled memory, rather than activation of naive, mature B cells, has been the primary component of the response to antigenically drifted strains ((46–48). Although both memory and naive cells are present in secondary germinal centers (49), the reactivated and affinity-matured memory cells appear to dominate the output. Whether, upon recall, somatic hypermutation will be able to adapt Abs in widely prevalent classes to an emerging variant or to expand and affinity mature those exemplified by the non–public-class Abs in our panel may determine how frequently one will need to update spike-based vaccines.

**MATERIALS AND METHODS**

**Study design**

The aim of this study was to perform an unbiased analysis of the early memory B cell repertoire specific for SARS-CoV-2 Delta and Omicron BA.1 variants. We tested 73 previously charted mAbs from COVID-19 convalescents (2) and determined their binding and neutralizing potency against SARS-CoV-2 variants of concern. We further characterize some of the broadly neutralizing mAbs from three key epitopic regions using cryogenic electron microscopy (cryo-EM).

**Summary of real-world mRNA vaccine effectiveness**

Data for Fig. 2E came from the following references. A clinical trial of mRNA vaccine showed an efficacy of 94 to 95% in preventing infection after two doses (50, 51), confirmed in real-world settings (52–54). Real-world effectiveness against the Alpha variant was shown to be similar to clinical trials (13, 54, 55). Direct comparison of BNT162b2 mRNA vaccine efficacy against Alpha and Beta variant infection in Qatar during a time of about 50% prevalence of each (55) showed 89.5 and 75% efficacy against infection by Alpha and Beta, respectively, and over 90% for each for protection from severe outcomes (55). A comparison in the United Kingdom of the protective efficacy of two doses of the BNT162b2 mRNA vaccine for infection by Alpha and Delta variants showed similar results (93.7 and 88%, for Alpha and Delta, respectively) (13, 14). Data for the Omicron BA.1 variant indicate 70% protection from severe disease after two doses of the BNT162b2 mRNA vaccine (14). Real-world studies allowing direct comparison of mRNA vaccine efficacy for the Gamma variant with direct reference to another variant of concern are not yet available.

**Protein expression and purification**

The expression plasmids for SARS-CoV-2 spike hexapro and RBD were gifts from J. McLellan (56) and A. Schmidt (57), respectively. RBD and spike hexapro were expressed in Expi293F cells (Thermo Fisher Scientific, catalog no. A14527) by transfecting cells at a density of 3.0 × 10^6 cells/ml with 1 μg of DNA complexed with 3 μg of polyethylenimine per milliliter of culture. After 24 hours, glucose was added to 3 g/liter and valproic acid was added to a final concentration of 3.5 mM. The conditioned medium was harvested 6 days after transfection by pelleting the cells and passing the clarified sample over Talon resin (Takara). Eluted protein was concentrated and applied to an S200 size exclusion chromatography column. RBD eluting at about 16 ml or spike eluting at about 9 ml was concentrated and used without further purification. Spike hexapro was treated with 3C protease and separated from protease and any fragments on Talon resin. Fab was obtained as previously described (2).

**Cell surface binding assays**

Assays for Ab binding to spike variants were described previously (2). Briefly, human embryonic kidney (HEK) 293T cells were cotransfected with plasmids encoding SARS-CoV-2 spike (HDM-SARS2-spike-D614G–Δ21, Addgene, catalog no. 158762; or HDM-SARS2-Delta variant spike–Δ21 or HDM-SARS2–Omicron BA.1 variant spike–Δ21) and green fluorescent protein (GFP) (pmaxGFP, Lonza) using Lipo-ectamine 3000 (Thermo Fisher Scientific, catalog no. L3000015). Delta variant spike modifications are T19R, G142D, 156–157 deletion, R158G, L452R, T478K, D614G, P681R, and D950N. Omicron BA.1 variant spike modifications are A67V, 69–70 deletion, T95I, G142D, 143–145 deletion, 211 deletion, L212I, 214 insertion EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, Q498R, Q498R, n501Y, n501Y, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, and L981F. At 24 hours after transfection, supernatant was replaced with fresh culture medium. At 48 hours, cells were detached with phosphate-buffered saline (PBS) supplemented with 2 mM EDTA. Cells were stained with 10 μg/ml of each mAb on ice for 1 hour, then washed with fluorescence-activated cell sorting buffer [PBS with 2% fetal bovine serum (FBS)] twice. Goat anti-human IgG (Alexa Fluor 647; Thermo Fisher Scientific, catalog no. A21445) was the secondary Ab for detection by flow cytometry (BD Canto II). 4′,6-Diamidino-2-phenylindole (DAPI) was used to distinguish dead and live cells. Spike” cells were gated on DAPI/GFP. Data were analyzed by FlowJo 10.7.1. The relative binding intensities of the tested mAbs for each spike variant were calculated as follows: log10(mAb MFI) – log10(background
Supernatant was collected and clarified by centrifugation. Stocks infected cells were observed daily for progression of cytopathic effect. Infection of 0.001 plaque-forming units (PFU) per cell in Dulbecco’s (BEI Resources) by infection at an approximate multiplicity of infection (m.o.i.) of 0.7 mg/ml, applied to thick C-flat 1.2/1.3 400-mesh Cu grids, and cryo-plunged with a Gatan CP3. Grids were imaged with a Titan Krios 300-keV microscope equipped with a Gatan K3 direct electron detector by automated low-dose imaging with SerialEM (59). Details of data collection are in table S4.

Cryo-EM structure determination

Details of data collection are in table S2.

Software and hardware used to process micrographs were configured and maintained by SBGrid. All data processing was performed in RELION (60, 61). Beam-induced motion correction of the reference micrographs was achieved using SBGrid software. The tilt axis was determined using Amoeba (62) and corrected using Relion tools. The cryo-EM structure was refined at 5.8 Å with RELION (60, 61) and cryoSPARC (46, 63). The final model was validated using MolProbity (64). The model is available in the Protein Data Bank with accession code 6OYI.
micrograph movies were performed with UCSF MotionCor2 (62) followed by contrast transfer function estimation with CTFFIND-4.1 (63), both implemented in RELION. Particles were picked from motion-corrected micrographs by cryolo using a general model (64). Particles were extracted with fourfold down-sampling and subjected to two-dimensional (2D) and 3D classification in RELION. We obtained 3D classes that reached the down-sampled Nyquist limited resolution, re-extracted these subsets of particles at the original pixel size, and subjected them to 3D autorefinement in RELION. Complexes of G98C7 and G32Q4 were C3 symmetric with the three Fab-bound RBD in an up configuration; sparseness in complex with G32A4 had one RBD each in “up,” “down,” and “out” conformations and lacked overall threefold symmetry.

In all cases, the Fab was poorly resolved at high resolution in the full particle reconstructions, necessitating local refinement. C3-symmetric, full-particle stacks were symmetry expanded, and a new particle stack was extracted centered on the Fab-epitope interface and with a smaller box size. In the case of G32A4, only the Fab bound to the RBD in the down configuration was well resolved. A particle stack, centered on this Fab-epitope interface, was extracted, and 3D autorefinement carried out; in some cases, there were several rounds with progressively tighter masks. We then used 3D classification to remove both noisy subparticles and RBD without bound Fab. Subsequent rounds of 3D autorefinement with a tighter mask followed by sharpening with RELION yielded ~4-Å reconstructions for each Fab-RBD complex. Maps involved in key 3D processing decisions are in fig. S2, and details of image processing are in table S2. The angular distribution plots in fig. S7 show sampling of most of the angular space, despite varying degrees of preferential orientation.

Models of the Fab-RBD complexes were prepared by identifying the most similar heavy and light chain in the Protein Data Bank (PDB IDs: for C98C7, 7CH4, and 7CDI; for G32A4 and 7MLZ; and for G32Q4, 7BEL, and 7JE). The coordinates were docked as rigid bodies into cryo-EM densities along with an RBD fragment (6YZ5). Residues in the template structures were altered to the actual sequences and with a smaller box size. In the case of G32A4, only the Fab bound to the RBD in the down configuration was well resolved. A particle stack was extracted centered on the Fab-epitope interface, and subjected to two-dimensional (2D) and 3D classification in RELION. The angular distribution plots in fig. S7 show sampling of most of the angular space, despite varying degrees of preferential orientation.

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