ChAdOx1 nCoV-19 vaccine elicits monoclonal antibodies with cross-neutralizing activity against SARS-CoV-2 viral variants

Highlights

- AZD1222 vaccination elicits monoclonal antibodies with potent neutralization
- Vaccine antibodies target epitopes overlapping infection-induced antibodies
- Vaccine antibodies have broad neutralization activity against VOCs, including Omicron
- Despite low serum neutralization, Spike-reactive B cells are detected at 9 months

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In brief

Seow et al. isolate antibodies following double vaccination with AZD1222. Isolated antibodies target epitopes overlapping with the epitopes of infection-derived antibodies and show potent neutralization of SARS-CoV-2 variants of concern (VOCs), including Omicron. Despite low serum neutralization at 9 months post-vaccine, Spike-reactive B cells are detected.
ChAdOx1 nCoV-19 vaccine elicits monoclonal antibodies with cross-neutralizing activity against SARS-CoV-2 viral variants

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SUMMARY

Although the antibody response to COVID-19 vaccination has been studied extensively at the polyclonal level using immune sera, little has been reported on the antibody response at the monoclonal level. Here, we isolate a panel of 44 anti-SARS-CoV-2 monoclonal antibodies (mAbs) from an individual who received two doses of the ChAdOx1 nCoV-19 (AZD1222) vaccine at a 12-week interval. We show that, despite a relatively low serum neutralization titer, Spike-reactive IgG+ B cells are still detectable 9 months post-boost. Furthermore, mAbs with potent neutralizing activity against the current SARS-CoV-2 variants of concern (Alpha, Gamma, Beta, Delta, and Omicron) are present. The vaccine-elicited neutralizing mAbs form eight distinct competition groups and bind epitopes overlapping with neutralizing mAbs elicited following SARS-CoV-2 infection. AZD1222-elicited mAbs are more mutated than mAbs isolated from convalescent donors 1–2 months post-infection. These findings provide molecular insights into the AZD1222 vaccine-elicited antibody response.

INTRODUCTION

The SARS-CoV-2-encoded Spike glycoprotein is the key target for neutralizing antibodies (nAbs) generated in response to natural infection. The Spike trimer consists of two subunits: S1, which is required for interaction with the ACE2 receptor on target cells, and S2, which orchestrates membrane fusion. Many monoclonal antibodies (mAbs) have been isolated from SARS-CoV-2-infected individuals, allowing identification of key neutralizing epitopes on Spike (Andreano et al., 2021; Barnes et al., 2020; Brouwer et al., 2020; Graham et al., 2021; Piccoli et al., 2020; Robbiani et al., 2020; Rogers et al., 2020; Seydoux et al., 2020; Tortorici et al., 2020). Neutralizing epitopes are present on the receptor-binding domain (RBD) and the N-terminal domain (NTD) of Spike and S2. RBD-specific nAbs tend to be potently neutralizing and target four epitopes (Barnes et al., 2020; Dejnirattisai et al., 2021; Yuan et al., 2020), including the receptor-binding motif (RBM), which interacts directly with the ACE2 receptor. Furthermore, several non-overlapping neutralizing epitopes on NTD have been identified that are susceptible to sequence variation in this region (Cerutti et al., 2021; Graham et al., 2021; McCallum et al., 2021; Suryadvara et al., 2021). SARS-CoV-2 infection also generates a large proportion of non-neutralizing antibodies of which the biological function is not fully understood (Anderson et al., 2021; Beaudoin-Bussieres et al., 2022; Li et al., 2021). Combined, studying the antibody response to SARS-CoV-2 infection has generated an antigenic map of the Spike surface (Corti et al., 2021; Dejnirattisai et al., 2021).

Following the emergence of SARS-CoV-2 in the human population, vaccines against COVID-19 have been rapidly developed. Most licenced vaccines use, or encode, a SARS-CoV-2 Spike antigen to elicit both humoral and cellular responses, and many have shown remarkable efficacy in Phase III trials (Baden et al., 2021; Polack et al., 2020; Voysey et al., 2021). However, there are concerns that vaccine efficacy could be reduced against newly emerging SARS-CoV-2 variants of concern (VOCs), in particular against the Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2) and Omicron (B.1.1.529) variants, which harbor mutations throughout Spike. Serum-neutralizing activity against viral variants has been reported in many double-vaccinated individuals, albeit at a reduced potency (Alter et al., 2021; Collier et al., 2021; Edara et al., 2021; Monin et al., 2021; Alter et al., 2021; Collier et al., 2021; Edara et al., 2021; Monin et al., 2021).
been studied extensively at the polyclonal level using immune sera (Ater et al., 2021; Collier et al., 2021; Dejnirattisai et al., 2021; Edara et al., 2021; Emary et al., 2021; Monin et al., 2021; Supasa et al., 2021; Wall et al., 2021; Wang et al., 2021d; Zhou et al., 2021), little has been reported on the antibody response at the monoclonal level (Amanat et al., 2021; Andreano et al., 2021b; Cho et al., 2021; Turner et al., 2021; Wang et al., 2021d). To address this paucity of information, we isolated a panel of 44 anti-SARS-CoV-2 monoclonal antibodies (mAbs) from an individual (VA14) who had received two doses of the ChAdOx1 nCoV-19(AZD1222) vaccine at a 12-week interval (Figure 1A). The AZD1222 vaccine is a replication-defective chimpanzee adenovirus-vectored vaccine expressing the full-length Wuhan SARS-CoV-2 spike glycoprotein gene (Ramasamy et al., 2021; Voysey et al., 2021). Even though low serum neutralization titers (ID_{50} ~100) were detected in VA14 at 4 months post-vaccine booster, nAbs were isolated that displayed potent cross-neutralizing activity against SARS-CoV-2 VOCs (IC_{50} values as low as 0.003 μg/mL), including the highly mutated Omicron VOC. The AZD1222 vaccine elicited NTD- and RBD-specific nAbs that bind epitopes overlapping with nAbs generated following natural infection. Assessment at 9 months post-second-vaccine dose revealed the presence of Spike-reactive IgG+ B cells despite undetectable neutralization. These data suggest that, although plasma neutralization may be suboptimal for protection from infection, memory B cells may be sufficient to provide rapid-recall responses to protect from serious illness/hospitalizations upon re-infection.

RESULTS

Serum-neutralizing activity following AZD1222 vaccination

Plasma and peripheral blood mononuclear cells (PBMCs) were isolated from donor VA14 (23 years, white male) at 4 months (time point 1, TP1) and 9 months (time point 2, TP2) after receiving two doses of the AZD1222 vaccine at a 12-week interval (Figure 1A). VA14 reported no previous SARS-CoV-2 infection (based on regular PCR testing), did not have N-specific IgG in their plasma at the time of sampling, and was therefore presumed to be SARS-CoV-2 naive. Presence of IgG to Spike was determined by ELISA (Figure 1B), and a semi-quantitative ELISA measured 0.39 and 0.17 μg/mL of Spike IgG at TP1 and TP2, respectively.

Plasma-neutralizing activity was measured using an HIV-1 (human immunodeficiency virus type 1)-based virus particles, pseudotyped with the Spikes of SARS-CoV-2 VOCs, including AZD1222-matched Spike (Wuhan-1, WT), and VOCs Alpha, Gamma, Beta, and Delta, and a HeLa cell line stably expressing the ACE2 receptor (Graham et al., 2021; Seow et al., 2020). Overall, neutralization titers at 4 months post-vaccine boost (TP1) were low. ID_{50}s of ~100 were measured against WT and Gamma but were reduced against Alpha, Delta, and Beta (Figure 1C). Although weak binding to Spike was observed at TP2, neutralization was not detected at a serum dilution of 1:20 (Figure 1D). Similar neutralization levels were observed against SARS-CoV-2 live virus (England 02/2020/407,073) (Figure 1E).

Spike-reactive B cells detected up to 1 year following AZD1222 vaccination

Next, we determined the percentage of RBD or Spike-reactive IgG-expressing B cells at 4 and 9 months post-vaccine booster using flow cytometry (Figures 1F and S1A–S1D). A total of 0.25% of IgG+ B cells were Spike reactive, and 0.06% were RBD reactive at 4 months post-vaccine booster. Despite the undetectable neutralization by serum antibodies produced by plasma cells at 9 months post-vaccine booster, 0.27% of IgG + B cells were Spike reactive.

AZD1222 vaccination elicits antibodies targeting epitopes on NTD, RBD, S2, and Spike

RBD or Spike-reactive B cells at 4 months post-vaccine booster were sorted into individual wells, and the antibody heavy- and light-chain genes were rescued by reverse transcription followed by nested PCR using gene-specific primers (Graham et al., 2021). Variable regions were ligated into IgG1 heavy- and light-chain expression vectors using Gibson assembly and directly transfected into HEK293T/17 cells. Crude supernatants containing IgG were used to confirm specificity to Spike, and the variable heavy and light chain regions of Spike-reactive mAbs were sequenced. In total, 44 Spike-reactive mAbs were isolated from VA14.

Binding to Spike, S1, RBD, NTD, and S2 was determined by ELISA and used to identify the domain specificity of each mAb (Figure 2A). Of the 40 mAbs isolated using the stabilized Spike-sorting antigen, 45% (18/40) bound RBD, 35% (14/40) bound NTD, 17.5% (7/40) bound S2, and 2.5% (1/40) bound Spike only (Figure 2B). An additional four RBD-specific mAbs were isolated using the RBD-sorting probe. A similar distribution between mAbs targeting RBD, NTD, and S2 was seen for mAbs isolated from convalescent donors 6–8 weeks post-onset of symptoms (POS) (Graham et al., 2021).

AZD1222 vaccination elicits neutralizing and non-neutralizing antibodies against epitopes across the full Spike

Neutralizing activity of mAbs was initially measured using HIV-1 virus particles pseudotyped with SARS-CoV-2 Spike encoded by the AZD1222 vaccine. Twenty six of 44 mAbs (59.1%) displayed neutralizing activity of which 21/26 (80.8%) were RBD-specific, 4/26 (15.5%) were NTD-specific and 1/26 (3.8%) only bound Spike (Figure 2B) consistent with previous studies (Graham et al., 2021; McCullum et al., 2021). None of the S2-specific nAbs showed neutralizing activity. 95.5% of RBD-specific mAbs and 38.6% of NTD-specific mAbs had neutralizing activity (Figure 2B). Neutralization potency against wild-type Spike ranged from 0.01–7.3 μg/mL. As previously reported for natural infection, RBD-specific nAbs had a lower geometric mean IC_{50}
Figure 1. VA14 plasma neutralization and Spike-reactive B cells
(A) Timeline of AZD1222 vaccination, and blood sampling for donor VA14.
(B–D) Plasma IgG binding to Spike at TP1 (4 months post-booster) and TP2 (9 months post-booster). Plasma-neutralizing activity against HIV-1-based virus particles, pseudotyped with the Wuhan, B.1.1.7, P.1, B.1.351, or B.1.617.2 Spike at (C) TP1 and (D) TP2. Experiments were performed in duplicate and repeated twice. A representative dataset is shown. Error bars represent the range of the value for experiments performed in duplicate (not shown when smaller than symbol size).
(E) Plasma-neutralizing activity against neutralization of SARS-CoV-2 (England 02/2020/407,073) at TP1 and TP2. Experiments were performed in duplicate.
(F) Fluorescent-activated cell sorting (FACS) showing percentage of CD19+ IgG+ B cells binding to SARS-CoV-2 Spike at TP1 and TP2. A healthy control PBMC sample collected prior to the COVID-19 pandemic was used to measure background binding to Spike. The full gating strategy and sorting of RBD-specific B cells can be found in Figure S1.
compared with NTD-specific nAbs (Figure 2C) (Graham et al., 2021; Liu et al., 2020b). AZD1222-elicited mAbs are more highly mutated than mAbs from natural infection The heavy and light chain variable regions of Spike-reactive mAbs were sequenced, and the germline usage and level of somatic hypermutation (SHM) were determined using IMGT (Brochet et al., 2008). Averages of 4.9% and 2.8% divergence from VH and VL germlines, respectively, were observed at the nucleotide level for AZD1222-elicited mAbs (Figure 3A), which is higher than mAbs isolated in our previous study from convalescent individuals 3–8 weeks post-onset of symptoms (1.9% and 1.4% for VH and VL, respectively) (Graham et al., 2021). Three pairs of related clones were identified (Figure S2A).

Germline gene usage and divergence from germline of both neutralizing and non-neutralizing AZD1222 mAbs were compared with a database of SARS-CoV-2-specific mAbs isolated from convalescent individuals (n = 1,292) (Raybould et al., 2021) as well as paired heavy and light chains of IgG B cell receptors (BCRs) from blood of CD19+ B cells from healthy individuals, representative of circulating IgG-expressing B cell repertoire (n = 862) (Siu et al., 2022). Since the SARS-CoV-2 mAb database included amino acid sequences for only some mAbs, divergence from germline was determined at the amino acid level (which correlated well with nucleotide divergence [Figure S2B]). AZD1222-elicited mAbs from donor VA14 had a statistically higher amino acid mutation (VH 9.2% and VL 6.1%) compared with mAbs isolated from SARS-CoV-2 convalescent donors (VH 4.2% and VL 3.0%) but had a level similar to that of B cell receptors from healthy subjects (VH 10.9% and VL 8.0%) (Figures 3B and 3C). Similar differences in mutation levels were observed for both neutralizing and non-neutralizing antibodies (Figure S2C).

An enrichment in VH3-30 and VH3-53 germline gene usage was observed for both SARS-CoV-2 infection and AZD1222-elicited mAbs, similar to that seen for COVID-19 mRNA vaccine-elicited mAbs (Wang et al., 2021d) (Figure 3D). Three of 21 RBD-specific nAbs used the VH3-53/3-66 germlines that are common among nAbs that directly bind the ACE2-binding site on Spike (Barnes et al., 2020; Graham et al., 2021; Kim et al., 2021; Robbiani et al., 2020; Yuan et al., 2020c). An enrichment of VH3-15, VH3-48, VH4-34, and VH4-59 germline use was observed for AZD1222-elicited mAbs compared with mAbs isolated from
convalescent donors; 11/44 (25.0%) and 8/44 (18.2%) mAbs used VK3-20 and VK1-39 light chains, respectively (Figure 3E).

**AZD1222-elicited nAbs bind epitopes overlapping with nAbs generated in response to SARS-CoV-2 infection**

To gain insight into the epitopes targeted by the AZD1222-elicited nAbs, competition ELISAs with trimeric Spike and previously characterized nAbs isolated from SARS-CoV-2-infected individuals were performed. The panel of competing antibodies encompassed four RBD-, two NTD-, and one Spike-only competition groups (Graham et al., 2021) (Figures 4A and 4B). Additionally, the ability of nAbs to inhibit the interaction between Spike and the ACE2 receptor was determined by flow cytometry (Figure 4D).

Four RBD-neutralizing antibody classes have been previously identified and characterized (Figure 4E) (Barnes et al., 2020;
Figure 4. AZD1222 nAbs target epitopes overlapping with nAbs elicited following natural SARS-CoV-2 infection

(A and B) Competitive binding of AZD1222 and SARS-CoV-2 infection-elicited nAbs. Inhibition of IgG binding to SARS-CoV-2 Spike by F(ab')2 fragments was measured. The percentage of competition was calculated using the reduction in IgG binding in the presence of F(ab')2 (at 100 molar excess of the IC50) as a percentage of the maximum IgG binding in the absence of F(ab')2. Competition was measured between (A) RBD-specific and (B) NTD-specific/S-only nAbs. Competition groups were determined according to binding epitopes. Experiments were performed in duplicate. Competition <25% is in white.

(C) Neutralization potency (IC50) of mAbs targeting RBD, NTD, or non-S1 and/or in competition groups 1–8 against SARS-CoV-2 WT pseudotyped virus. Competition groups are color coded according to the key. The black lines represent the geometric mean IC50 for each group. Neutralization experiments were performed in duplicate and carried out at least twice.

(D) Ability of nAbs to inhibit the interaction between cell surface ACE2 and soluble SARS-CoV-2 Spike. nAbs (at 600 nM) were pre-incubated with fluorescently labeled Spike before addition to HeLa-ACE2 cells. The percentage reduction in mean fluorescence intensity is reported. Experiments were performed in duplicate. Bars are color coded based on the antibody competition group.

(E) Mapping of previously determined neutralizing and non-neutralizing epitopes on SARS-CoV-2 Spike (PDB: 6XM0) (Zhou et al., 2020). Cartoon representation of Spike showing antibody-binding footprint for nAbs used in competition ELISAs as colored spheres. Epitopes were previously determined using crystal structures or cryo-electron microscopy of RBD or Spike-Fab complexes: COVA2-04 (gold, group 2 [RBD Class 1], [PDB: 7JMO] [Wu et al., 2020]), COVA2-39 (gray, group 3 [RBD Class 2] [PDB: 7JMP] [Wu et al., 2020]), S309 (orange, group 4 [RBD Class 3] [PDB: 6WPS] [Pinto et al., 2020]), COVA1-16 and CR3022 (dark blue [PDB: 7JMW] [Liu et al., 2020a] and turquoise [PDB: 6W41] [Yuan et al., 2020c], respectively, group 1 [RBD Class 4]), and P008_056 (green, NTD group 6 [Rosa et al., 2021]). Structures were generated in Pymol using the referenced PBDs.
Yuan et al., 2020b). nAbs that neutralize by binding to the receptor-binding motif (RBM) (equivalent to RBD class 1) (Barnes et al., 2020; Dejnirattisai et al., 2021; Yuan et al., 2020a) commonly use the VH3-53 or VH3-66 germ lines. As expected, the three VH3-53/VH3-66 VA14 nAbs competed with the group 3 (RBD class 1) infection nAbs as well as competing strongly for ACE2 binding (Figure 4D). Group 3 nAbs were most potent at neutralizing the matched vaccine strain (Wuhan-1) (Figure 4C).

The majority of RBD-specific nAbs isolated from VA14 (13/20) competed with the group 4 (RBD class 3) RBD infection nAbs (Figure 4A) and included both potent and modest neutralizing Abs with varying degrees of ACE2 competition (Figures 4C and 4D). Five VA14 nAbs competed with group 1 (RBD class 4) RBD infection nAbs and showed a wide range of neutralization potencies and levels of ACE2 competition. Only one VA14 nAb (VA14_26) competed with group 2 (RBD class 2) RBD infection nAbs, which also competed strongly with ACE2.

NTD nAbs formed three competition groups (Figure 4B). Non-neutralizing nAbs VA14_25 and VA14_58 competed with NTD group 6 nAbs including P008_056, which has been shown to bind NTD adjacent to the β-sandwich fold (Figure 4E) (Rosa et al., 2021). These two nAbs did not inhibit Spike binding to ACE2 (Figure 4D). nAbs VA14_21 and VA14_22 competed with NTD group 5 nAbs and showed 51%–58% inhibition of Spike binding to ACE2. Two NTD nAbs (group 8) did not compete with any of the infection NTD-specific nAbs or prevent ACE2 binding.

The S-only-binding nAb VA14_47 competed with P008_060 (group 7) (Figure 4B), the only other S-only infection nAb, and showed 59% inhibition of Spike binding to ACE2 (Figure 4D). P008_060 has been shown to bind a neutralizing epitope on the SD1 domain of Spike (manuscript in preparation).

**AZD1222-elicited nAbs cross-neutralize SARS-CoV-2 variants of concern**

Assessing the cross-neutralizing activity of nAbs isolated from SARS-CoV-2 convalescent donors has revealed that Spike mutations in VOCs selectively hinder neutralizing activity of specific nAb classes (Graham et al., 2021; Wang et al., 2021a, 2021b, 2021c; Wibmer et al., 2021). Therefore, we measured the neutralization potency of AZD1222-elicited nAbs against SARS-CoV-2 variants of concern, including B.1.1.7 (Alpha), B.1.351 (Beta), B.1.617.2 (Delta), P.1 (Gamma), and B.1.1.529 (Omicron) and compared this with nAbs isolated following natural infection (Graham et al., 2021). Spike proteins from these VOCs encode mutations in RBD, NTD, and S2 (Figure 5A). Some RBD mutations are shared among multiple variants; e.g., Alpha, Gamma, Beta, and Omicron all share an N501Y mutation, and Gamma, Beta, and Omicron share a mutation at K417 and E484. In contrast, NTD mutations vary considerably among VOCs and include amino acid mutations as well as insertions and deletions. Omicron encodes >30 mutations in Spike and has been reported to evade neutralization by sera from individuals receiving two doses of a COVID-19 vaccine (Cele et al., 2021; Garcia-Beltran et al., 2022; Gruell et al., 2022; Wu et al., 2022) as well as many SARS-CoV-2-specific monoclonal antibodies (Cameroni et al., 2021; Cao et al., 2021; Planas et al., 2021; VanBlargan et al., 2022). Although a reduction in neutralization potency was observed for some AZD1222 nAbs, RBD- and NTD-specific nAbs with potent cross-neutralization against all VOCs, including Omicron, were identified (Figures 5B and 5C).

All group 3, several group 4 (including VA14_33, VA14_36, VA14R_38 and VA14_61), and one group 1 (VA14R_39) RBD-specific nAbs potently neutralized WT, Alpha, Beta, Gamma, and Delta variants at IC_{50}s below 0.09 μg/mL (Figure 5B), and all but two of these nAbs also neutralized Omicron at IC_{50}s below 0.16 μg/mL. VA14R_37 was most potent against Omicron, neutralizing with an IC_{50} of 0.002 μg/mL. Several nAbs showed enhanced neutralization of VOCs compared with WT (Figure 5E). When nAbs elicited following infection (Graham et al., 2021) and vaccination were compared, infection nAbs showed a greater sensitivity to Spike mutations in VOCs (Figure 5F). Cross-neutralization of nAbs in RBD groups 1, 2, and 3 was observed for AZD1222 nAbs, whereas some infection nAbs in these competition groups showed greatly reduced neutralization of VOCs Gamma, Beta, and Omicron, which share RBD mutations at positions K417, E484, and N501Y (Figure 5A).

RBD group 4 mAbs varied in their neutralization of VOCs. Six of 13 nAbs showed cross-neutralizing activity. The remaining seven showed a >3-fold reduction in neutralization against at least one VOC, with neutralizing activity against Beta, Delta, and Omicron being most greatly reduced. Despite some RBD nAbs showing a decreased neutralization against VOCs, binding to variant RBD in ELISA was retained for most nAbs except group 4 nAbs VA14_19 and VA14_50 (Figure S3A), indicating that binding to RBD does not always correlate with neutralization activity.

Considering the geometric mean IC_{50} values, VA14 NTD-specific nAbs were most potent at neutralizing the Alpha VOC. However, the three NTD-competition groups showed differential sensitivities toward the other four SARS-CoV-2 variants (Figure 5C). For example, group 5 VA14 NTD nAbs had either reduced or lacked neutralization toward Gamma and Delta, whereas group 8 NTD nAbs VA14_16 and VA14_68 maintained neutralizing activity.
activity against all variants. The NTD-specific nAb VA14_16 potently neutralized Gamma, Alpha, Delta, and Beta with an IC50 < 0.22 μg/mL and Omicron with an IC50 1.59 μg/mL, and is the only cross-neutralizing NTD-specific nAbs reported thus far (McCallum et al., 2021). Interestingly, two group 6 NTD-specific mAbs that had shown no neutralizing activity against WT pseudotyped virus neutralized both Alpha and Gamma VOCs (Figure 5C). Despite the lack of neutralizing activity, VA14_21 maintained binding to S1 of Gamma, Alpha, Beta, and Delta (Figure S3B). Since the differences in neutralization of VOCs by group 6 NTD-specific nAbs are not reflected in their binding to S1 by ELISA, these data indicate that NTD binding alone is not sufficient for neutralization by this class of mAbs. We have previously shown that Spike binding of the group 6 infection mAb P008_056 is dependent on the heme metabolite biliverdin, and although P008_056 does not neutralize WT pseudovirus, it can potently neutralize live virus (Rosa et al., 2021). Similarly to P008_056, VA14_21 and VA14_61 potently neutralized SARS-CoV-2 live virus (Figure S4A) and binding to Spike was inhibited by biliverdin (Figure S4B), strongly suggesting that these mAbs bind the NTD-neutralizing epitope adjacent to the β-sandwich fold (Rosa et al., 2021).

The S-only-reactive nAb elicited by vaccination (VA14_47) weakly neutralized all SARS-CoV-2 variants except Gamma (Figure 5D), whereas infection mAb P008_060 had modest neutralization against all variants.

Overall, AZD1222 vaccine-elicited nAbs showed greater resistance to Spike mutations in VOCs compared with infection-elicited nAbs (Figure 5E).

**Role of somatic hypermutation in neutralization breadth**

To probe the role of increasing somatic hypermutation in neutralization breadth, we selected four RBD-specific nAbs and expressed the reverted germline versions. Group 1 nAbs VA14_01 and VA14_04 both use VH3-13 (5.6% and 0.2% divergent from germline, respectively) and VK1-39 (1.4% and 6.4% divergent from germline, respectively) (Figures S5A and S5B). The reverted germline of VA14_01 did not bind to WT Spike in ELISA (Figure 6A). In contrast, VA14_04 reverted germline bound very weakly to WT Spike but did not neutralize WT or any VOCs, thus...
demonstrating the importance of SHM for antigen recognition and neutralization for these group 1 mAbs (Figure 6B).

Germline-reverted versions of two group 3 mAbs, VA14R_33 and VA14R_37, were also generated (Figures S5C and S5D). VA14R_33 is encoded by VH3-66 (8.4% mutated) and VK1-33 (3.9% mutated), and VA14R_37 is encoded by VH3-53 (2.4% mutated) and VK3-20 (6.0% mutated). VH3-53/VH3-66 are commonly used by RBM-targeted nAbs, and these germlines have been reported to have amino acid motifs that are pre-configured to recognize RBM (Clark et al., 2021; Yuan et al., 2020a), in particular Asn32-Tyr33 and Ser53-Gly54-Gly55-Ser56. The reverted germlines of both VA14R_33 and VA14_37 retained binding to WT Spike (Figure 6C). Reversion of VA14R_33 to germline reduced the neutralization potency against Alpha, Beta and Omicron (Figure 6D), but neutralization of WT and Delta was largely unaffected. The reverted germline of VA14R_37 was unable to neutralize Omicron (Figure 6E) and had reduced potency against Beta. Interestingly, neutralization potency of germline-reverted VA14R_37 was increased against WT and Delta. Overall, these results highlight the importance of SHM for neutralization breadth and potency against VOCs.

**DISCUSSION**

Efficacy of COVID-19 vaccines in the face of SARS-CoV-2 emerging viral variants will be critical for control of the current pandemic. Here, we studied the antibody response to two doses of the AZD1222 vaccine administered with a 12-week interval at the monoclonal antibody level. The majority of studies examining immune sera from AZD1222-vaccinated individuals have revealed a lower potency against Alpha (range 2.2- to 9.0-fold) (Dejnirattisai et al., 2021; Emary et al., 2021; Wall et al., 2021), Gamma (2.9-fold) (Dejnirattisai et al., 2021), Beta (range 4.0- to 9.0-fold) (Dejnirattisai et al., 2021; Madhi et al., 2021; Zhou et al., 2021) and Delta (range 4.3- to 9.0-fold) (Liu et al., 2021; Wall et al., 2021) compared with neutralization of Wuhan or D614G variants, and very limited neutralization against Omicron (Wu et al., 2022). Although VA14 had a low plasma-neutralizing activity (ID₅₀ ∼1:100) at 4 months post-vaccine booster, 59.1% of Spike-reactive mAbs isolated from antigen-reactive B cells had neutralizing activity against the matched vaccine strain, and many of these mAbs displayed potent cross-neutralizing activity against current SARS-CoV-2 VOCs. Similar to previous studies, RBD and NTD were the predominant targets for neutralizing antibodies (80.8% and 15.5% of nAbs, respectively) (Graham et al., 2021; McCallum et al., 2021). Importantly, we identified RBD-specific nAbs from competition groups 1, 3, and 4, as well as NTD-specific nAbs, that cross-neutralized all five VOCs, including the highly divergent Omicron. Therefore, the polyclonal nature of the nAb response elicited by AZD1222 vaccination will likely help limit full vaccine escape in the face of emerging Spike mutations.

Competition ELISAs revealed that nAbs elicited by AZD1222 vaccination target overlapping epitopes of nAbs elicited from natural SARS-CoV-2 infection. However, despite similar antibody footprints, vaccine-elicited nAbs from RBD competition groups 1 to 4 showed greater neutralization breadth than those elicited from natural infection. This was also apparent for some NTD-specific nAbs. This increased neutralization breadth is likely due to the increased divergence from germline in AZD1222-elicited nAbs (isolated 4 months post-booster) compared with nAbs isolated following natural infection (isolated 2–8 weeks post-onset of symptoms) leading to better tolerance of Spike mutations in VOCs. Somatic hypermutation was shown to be critical for antigen recognition by group 1 RBD mAbs VA14_01 and VA14_04, whose reverted germlines had low or undetectable Spike binding by ELISA and lacked neutralization activity. In contrast, the reverted germline of VA14R_37 and VA14R_33 retained Spike reactivity but had a reduced neutralization breadth against Omicron and Beta VOCs. Previous structural analysis of RBM mAbs CC12.1 and CC12.3 (also encoded by VH3-53) revealed that germline encoded amino acid motifs Asn32-Tyr33 and Ser53-Gly54-Gly55-Ser56 are critical for antigen recognition (Yuan et al., 2020a). A germline-reverted version RBM mAb CV30, also encoded by VH3-53, retained Spike-binding activity but had reduced neutralization potency (Hurbutt et al., 2020). The observation that mutation of the VA14R_37 germline VH and VK genes leads to potent neutralization of Beta and Omicron variants demonstrates the importance of somatic hypermutation for enhancing neutralization breadth. Indeed, several other studies have shown that increased somatic hypermutation enhances neutralization breadth against VOCs (Chen et al., 2021; de Mattos Barbosa et al., 2021; Gaebler et al., 2021; Goel et al., 2021; Muecksch et al., 2021). Analysis of the antibody-antigen interaction at the molecular level will give further insight into the specific mechanisms of increased neutralization breadth for AZD1222-elicited nAbs.

Although Spike-reactive mAbs generated following AZD1222 have not previously been reported, several studies report mAbs isolated following mRNA COVID-19 vaccination (Amanat et al., 2021; Andreano et al., 2021b; Cho et al., 2021; Turner et al., 2021; Wang et al., 2021). Comparison between epitopes targeted by mRNA- and AZD1222-elicited nAbs showed a higher proportion of RBM-targeted nAbs following mRNA vaccination (Gaebler et al., 2021; Hurbutt et al., 2020). A similar enrichment in VH3-53 and VH3-30 germline usage was also observed (Andreano et al., 2021b; Wang et al., 2021). Despite differences in the timing of mAb isolation across reported studies, the AZD1222 mAbs identified had a higher level of SHM compared with mRNA-elicited mAbs and showed greater cross-neutralizing activity (Scheid et al., 2009; Seydoux et al., 2020). Possible reasons for these differences include (1) timing of mAb isolation following vaccine booster, (2) timing of vaccine boosters (3 weeks for mRNA studies versus 12 weeks in this study), (3) a prolonged antigen persistence for ChAdOx1 vectored Spike (which may also be relevant to the Ad26 vectored Ad26.COV2.S vaccine [Sadoff et al., 2021]), or (4) differences in Spike antigen encoded by each vaccine (in particular, mRNA-1273 [Moderna] and BNT162b2 [Pfizer] vaccines encode Spike with stabilizing mutations and a mutation that prevents S1/S2 cleavage [Jackson et al., 2020; Walsh et al., 2020]). Understanding these factors will be important for optimizing vaccine strategies aimed at eliciting the broadest nAb response against both known and newly emerging VOCs.

Plasma was not available to determine the peak neutralizing response in VA14 and therefore the relative decline in
neutralization following AZD1222 vaccination. The neutralizing antibody titer was low 4 months post-vaccine boost, and it is not known whether this level would be sufficient to provide sterilizing or near-sterilizing immunity. However, the identification of B cells producing antibodies with potent cross-neutralizing activity against non-overlapping epitopes and the presence of Spike+ IgG+ B cells at ~1 year post-vaccine prime suggests that a rapid recall response will likely occur, which could be sufficient to protect against severe disease and/or hospitalization in the face of VOCs.

In summary, we show that AZD1222 vaccine administered at a 12-week interval can elicit nAbs with potent cross-neutralizing activity against current SARS-CoV-2 VOCs, including Omicron, that target non-overlapping epitopes on RBD and NTD. Despite undetectable plasma-neutralizing activity, Spike-reactive IgG+ B cells are detected up to 1 year following initial vaccine priming. These data provide important insights into long-term immunity and protection against SARS-CoV-2 emerging viral variants.

Limitations of the study
The main limitation of this study is that it examines mAbs isolated from only one AZD1222-vaccinated individual. How representative these mAbs are of the humoral immune response arising from AZD1222 needs to be investigated further by isolating mAbs from other AZD1222 vaccine recipients. We have not determined how neutralization breadth against VOCs is achieved by the reported mAbs. Further studies examining the epitopes recognized by neutralizing antibodies with broad and potent activity, particularly against Omicron, will be important for optimizing immunogens that elicit mAbs with broad activity. Finally, we have not studied the protective activity of these mAbs in vivo or the amount required to achieve sterilizing immunity.

STAR*METHODS
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SUPPLEMENTAL INFORMATION
Supplemental information can be found online at https://doi.org/10.1016/j.celrep.2022.110757.

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AUTHOR CONTRIBUTIONS

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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REFERENCES

Alter, G., Yu, J., Liu, J., Chandrashekar, A., Borducchi, E.N., Tostanoski, L.H., McMahan, K., Jacob-Dolan, C., Martinez, D.R., Chang, A., et al. (2021).
Immunogenicity of Ad26.COV2.S vaccine against SARS-CoV-2 variants in humans. Nature 596, 268–272.

Amanat, F., Thapa, M., Lei, T., Ahmed, S.M.S., Adamsberg, D.C., Carreno, J.M., Strohmeier, S., Schmitz, A.J., Zatar, S., Zhou, J.Q., et al. (2021). SARS-CoV-2 mRNA vaccination induces functionally diverse antibodies to NTD, RBD, and S2. Cell 184, 3936–3948.e3910.

Anderson, E.M., Goodwin, E.C., Verma, A., Arevalo, C.P., Bolton, M.J., Weirick, M.E., Gouna, S., McAllister, C.M., Christensen, S.R., Weaver, J., et al. (2021). Seasonal human coronavirus antibodies are boosted upon SARS-CoV-2 infection but not associated with protection. Cell 184, 1858–1864.e1810.

Andreao, E., Nicolastr, E., Paciello, I., Pileri, P., Manganaro, N., Piccini, G., Manenti, A., Pantano, E., Kabanova, A., Troisi, M., et al. (2021a). Extremely potent human monoclonal antibodies from COVID-19 convalescent patients. Cell 784, 1821–1835.e16.

Andreao, E., Paciello, I., Piccini, G., Manganaro, N., Pileri, P., Hyseni, I., Leonardi, M., Pantano, E., Abbitto, V., Benincasa, L., et al. (2021b). Hybrid immunity improves B cells and antibodies against SARS-CoV-2 variants. Nature 600, 530–533.

Aricescu, A.R., Lu, W., and Jones, E.Y. (2006). A time- and cost-efficient system for high-level protein production in mammalian cells. Acta Crystallogr. D Biol. Crystallogr. 62, 1243–1250.

Baden, L.R., El Sahly, H.M., Essink, B., Kotloff, K., Frey, S., Novak, R., Diemert, D., Spector, S.A., Rouphael, N., Creech, C.B., et al. (2021). Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. N. Engl. J. Med. 384, 403–416.

Barnes, C.O., Jette, C.A., Abernathy, M.E., Dam, K.A., Esswein, S.R., Gristick, H.B., Malyutin, A.G., Shafar, N.G., Huey-Tubman, K.E., Lee, Y.E., et al. (2020). SARS-CoV-2 neutralizing antibody structures inform therapeutic strategies. Nature 589, 682–687.

Beaudoin-Bussieres, G., Chen, Y., Ila, I., Prevost, J., Tolbert, W.D., Symmes, K., Ding, S., Benlarbi, M., Gong, S.Y., Tauzin, A., et al. (2022). A Fc-enhanced NTD-binding neutralizing antibody delays virus spread and synergizes with a nAb to protect mice from lethal SARS-CoV-2 infection. Cell Rep. 38, 110388.

Brochet, X., Lefranç, M.P., and Giudicelli, V. (2008). IGMT/V-QUEST: the highly customized and integrated system for Ig and TR standardized V-J and V-D-J sequence analysis. Nucleic Acids Res. 36, W503–W508.

Brouwer, P.J.M., Caniels, T.G., van der Straten, K., Snitselaar, J.L., Aldon, Y., Brouwer, P.J.M., Caniels, T.G., van der Straten, K., Snitselaar, J.L., Aldon, Y., et al. (2016). Potent neutralizing antibodies from COVID-19 patients define multiple targets of vulnerability. Science 369, 643–650.

Cameroni, E., Bowen, J.E., Rosen, L.E., Saliba, C., Zepeda, S.K., Culap, K., Pinto, D., VanBlargan, L.A., De Marco, A., di Iulio, J., et al. (2021). Broadly antibodies directed against spike N-terminal domain target a single supersite. Cell Host Microbe 29, 819–833.e817.

Chen, E.C., Gilchuk, P., Zost, S.J., Suryadevara, N., Winkler, E.S., Cabel, C.R., Binshtein, E., Chen, R.E., Sutton, R.E., Rodriguez, J., et al. (2021). Convergent antibody responses to the SARS-CoV-2 spike protein in convalescent and vaccinated individuals. Cell Rep. 36, 109624.

Cho, A., Muecksh, F., Schaefer-Babajew, D., Wang, Z., Finkin, S., Gaebler, C., Ramos, V., Cipolla, M., Mendoza, P., Agudelo, M., et al. (2021). Anti-SARS-CoV-2 receptor-binding domain antibody evolution after mRNA vaccination. Nature 600, 517–522.

Clark, S.A., Clark, L.E., Pan, J., Coscia, A., McKay, L.G.A., Shankar, S., John-son, R.I., Brusic, V., Choudhary, M.C., Regan, J., et al. (2021). SARS-CoV-2 evolution in an immunocompromised host reveals shared neutralization escape mechanisms. Cell 184, 2605–2617.e2618.

Collier, D.A., De Marco, A., Ferreira, I., Meng, B., Datir, R., Walls, A.C., Kern, S.S., Bassi, J., Pinto, D., Fregni, C.S., et al. (2021). Sensitivity of SARS-CoV-2 B.1.1.7 to mRNA vaccine-elicited antibodies. Nature 593, 136–141.

Corti, D., Purcell, L.A., Snell, G., and Veesler, D. (2021). Tackling COVID-19 with neutralizing monoclonal antibodies. Cell 184, 3086–3108.

de Mattos Barbosa, M.G., Liu, H., Huynh, D., Shelley, G., Keller, E.T., Emmer, B.T.,Sherin, E., Ginsburg, D., Kennedy, A.A., Tai, A.W., et al. (2021). IgV somatic mutation of human anti-SARS-CoV-2 monoclonal antibodies governs neutralization and breadth of reactivity. JCI Insight 6, e147386.

Dejirattisai, W., Zhou, D., Ginn, H.M., Duyvesteyn, H.E.M., Supasa, P., Case, J.B., Zhao, Y., Walter, T.S., Mentzer, A.J., Liu, C., et al. (2021). The antigenic anatomy of SARS-CoV-2 receptor binding domain. Cell 184, 2183–2200.e2122.

Dixon, E.V. (2014). Mechanisms of immunoglobulin deactivation by Strepto- coccus pyogenes (University of Oxford). PhD Thesis.

Dupont, L., Snell, L.B., Graham, C., Seow, J., Merrick, B., Lechmere, T., Ma-guire, T.J.A., Hallett, S.R., Pickering, S., Charalampous, T., et al. (2021). Neutralizing antibody activity in convalescent sera from infection in humans with SARS-CoV-2 and variants of concern. Nat. Microbiol. 6, 1433–1442.

Edara, V.V., Pinsky, B.A., Suthar, M.S., Lai, L., Davis-Gardner, M.E., Floyd, K., Flowers, M.W., Wrammert, J., Hussaini, L., Ciric, C.R., et al. (2021). Infection and vaccine-induced neutralizing-antibody responses to the SARS-CoV-2 B.1.617.1 variants. N. Engl. J. Med. 385, 664–666.

Emary, K.R.W., Golubchik, T., Aley, P.K., Ariani, C.V., Angus, B., Bibi, S., Blane, B., Borsali, D., Cicconi, P., Charlton, S., et al. (2021). Efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine against SARS-CoV-2 variant of concern 202012/01 (B.1.1.7): an exploratory analysis of a randomised controlled trial. Lancet 397, 1351–1362.

Gaebler, C., Wang, Z., Zorenco, J.C.C., Muecksh, F., Finkin, S., Tokuyama, M., Cho, A., Jankovic, M., Schaefer-Babajew, D., Oliveira, T.Y., et al. (2021). Evolution of antibody immunity to SARS-CoV-2. Nature 591, 639–644.

Garcia-Beltran, W.F., St Denis, K.J., Hoelzemer, I., Larm, E.C., Nitido, A.D., Sheehan, M.L., Berrios, C., Otoman, O., Chang, C.C., Hauser, B.M., et al. (2022). mRNA-based COVID-19 vaccine boosters induce neutralizing immunity against SARS-CoV-2 Omicron variant. Cell 185, 457–466.e4.

Goel, R.R., Painter, M.M., Apostolidis, S.A., Mathew, D., Meng, W., Rosenfeld, A.M., Lundgreen, K.A., Reynald, A., Khoury, D.S., Pattakear, A., et al. (2021). mRNA vaccination induces durable immune memory to SARS-CoV-2 with continued evolution to variants of concern. Preprint at bioRxiv. https://doi. org/10.1101/2021.08.23.457229.

Graham, C., Seow, J., Huetten, I., Khan, H., Koupouh, N., Acors, S., Winstone, H., Pickering, S., Galao, R.P., Dupont, L., et al. (2021). Neutralization potency of monoclonal antibodies recognizing dominant and subdominant epitopes on SARS-CoV-2 Spike is impacted by the B.1.1.7 variant. Immunity 54, 1276–1289.e6.

Grehan, K., Ferrara, F., and Temperton, N. (2015). An optimised method for the production of MERS-CoV spike expressing viral pseudotypes. MethodsX 2, 379–384.

Gruell, H., Vanshylla, K., Tober-Lau, P., Hils, D., Schommer, P., Lehmann, C.K., Fand, S.L., and Klein, F. (2022). mRNA booster immunization elicits potent neutralizing serum activity against the SARS-CoV-2 Omicron variant. Nat. Med. 28, 477–480.
Gu, Z., Eils, R., and Schlesner, M. (2016). Complex heatmaps reveal patterns and correlations in multidimensional genomic data. Bioinformatics 32, 2847–2849.

Howarth, M., Liu, W., Puthenveetil, S., Zheng, Y., Marshall, L.F., Schmidt, M.M., Wittrup, K.D., Bawendi, M.G., and Ting, A.Y. (2008). Monovalent, reduced-size quantum dots for imaging receptors on living cells. Nat. Methods 5, 397–399.

Hurlburt, N.K., Seydoux, E., Wan, Y.H., Edara, V.V., Stuart, A.B., Feng, J., Suthar, M.S., McGuire, A.T., Stamatatos, L., and Pancera, M. (2020). Structural basis for potent neutralization of SARS-CoV-2 and role of antibody affinity maturation. Nat. Commun. 11, 5413.

Jackson, L.A., Anderson, E.J., Rouphael, N.G., Roberts, P.C., Makhen, M., Coler, R.N., McCullough, M.P., Chappell, J.D., Denison, M.R., Stevens, L.J., et al. (2020). An mRNA vaccine against SARS-CoV-2 - preliminary report. N. Engl. J. Med. 383, 1920–1931.

Kim, S.I., Noh, J., Kim, S., Choi, Y., Yoo, D.K., Lee, Y., Lee, H., Jung, J., Kang, C.K., Song, K.H., et al. (2021). Stereotypic neutralizing VH antibodies against SARS-CoV-2 spike protein receptor binding domain in patients with COVID-19 and healthy individuals. Sci. Transl Med. 13, eabd9990.

Lefranc, M.P., Giudicelli, V., Ginestoux, C., Bodmer, J., Muller, W., Bontrop, R., Lemaitre, M., Malik, A., Barbie, V., and Chaume, D. (1999). IMGT, the international ImMunoGeneTics database. Nucleic Acids Res. 27, 209–212.

Li, D., Edwards, R.J., Manne, K., Martinez, D.R., Schafer, A., Alam, S.M., Wiehe, K., Lu, X., Parks, R., Sutherland, L.L., et al. (2019). Structural immunogenic mapping reveals a site of vulnerability for SARS-CoV-2. Cell Reports 27, 2187–2201.

M.M., Wittrup, K.D., Bawendi, M.G., and Ting, A.Y. (2008). Monovalent, reduced-size quantum dots for imaging receptors on living cells. Nat. Methods 5, 1853–1869.e1857.

Nicola, L., Park, Y.J., Tortorici, M.A., Czudnochowski, N., Walls, A.C., Beltramello, M., Silacci-Fregnì, C., Pinto, D., Rosen, L.E., Bowen, J.E., et al. (2020). Mapping neutralizing and immunodominant sites on the SARS-CoV-2 spike receptor-binding domain by structure-guided high-resolution serology. Cell 183, 1024–1042.e21.

Pickering, S., Betancor, G., Galao, R.P., Merrick, B., Signell, A.W., Wilson, H.D., Xia Ik, M.T., Seow, J., Graham, C., Acors, S., et al. (2020). Comparative assessment of multiple COVID-19 serological technologies supports continued evaluation of point-of-care lateral flow assays in hospital and community healthcare settings. PLoS Pathog. 16, e1008817.

Pinto, D., Park, Y.J., Beltramello, M., Walls, A.C., Tortorici, M.A., Bianchi, S., Jaconi, S., Culap, K., Zatta, F., De Marco, A., et al. (2020). Cross-neutralization of SARS-CoV-2 by a human monoclonal SARS-CoV antibody. Nature 583, 290–295.

Platas, D., Saunders, N., Maes, P., Guivel-Benzahine, F., Planhais, C., Buchrieser, J., Bolland, W.H., Porrot, F., Staropoli, I., Lemoine, F., et al. (2021). Considerable escape of SARS-CoV-2 Omicron to antibody neutralization. Nature 602, 671–675.

Polack, F.P., Thomas, S.J., Kitchin, N., Ahsan, J., Gurtman, A., Lockhart, S., Perez, J.L., Perez Marc, G., Moreira, E.D., Babbage, G., et al. (2020). Safety and efficacy of the BNT162b2 mRNA covid-19 vaccine. N. Engl. J. Med. 383, 2603–2615.

Ramosamy, M.N., Minassian, A.M., Ewer, K.J., Flaxman, A.L., Folegatti, P.M., Owens, D.R., Voysey, M., Aley, P.K., Angus, B., Babbage, G., et al. (2021). Safety and immunogenicity of ChAdOx1 nCoV-19 vaccine administered in a prime-boost regimen in young and old adults (COV002): a single-blind, randomised, controlled, phase 2/3 trial. Lancet 396, 1795–1993.

Raybould, M.I.J., Kovaltsuk, A., Marks, C., and Deane, C.M. (2021). Cov-AbD: the coronavirus antibody database. Bioinformatics 37, 734–735.

Rees-Spear, C., Muir, L., Griffith, S.A., Heaney, J., Aldon, Y., Shintseva, L.L., Thomas, P., Graham, C., Seow, J., Lee, N., et al. (2021). The effect of spike mutations on SARS-CoV-2 neutralization. Cell Rep. 34, 108890.

Robbiani, D.F., Gaebler, C., Muecksch, F., Lorenzi, J.C.C., Wang, Z., Cho, A., Agudelo, M., Barnes, C.O., Gazumyan, A., Finkin, S., et al. (2020). Convergent antibody responses to SARS-CoV-2 in convalescent individuals. Nature 584, 437–442.

Rogers, T.F., Zhao, F., Huang, D., Beutler, N., Burns, A., He, W.T., Limbo, O., Smith, C., Song, G., Woeihl, J., et al. (2020). Isolation of potent SARS-CoV-2 neutralizing antibodies and protection from disease in a small animal model. Science 369, 956–963.

Rosa, A., Pye, V.E., Graham, C., Muir, L., Seow, J., Ng, K.W., Cook, N.J., Rees-Spear, C., Parker, E., Silva Dos Santos, M., et al. (2021). SARS-CoV-2 can reassemble into a haem metabolite to evade antibody immunity. Sci. Adv. 7, eabg7867.

Sadoff, J., Gray, V., and Deane, C. (2021). CoV-AbD: the coronavirus antibody database. Bioinformatics 37, 734–735.

Scheid, J.F., Mouquet, H., Feldhahn, N., Seow, J., Graham, C., Pickering, S., et al. (2021). Structural immunogenicity of the SARS-CoV-2 Spike protein revealed by global analysis of lymphoid tissues and cells. Nat. Immunol. 22, 450–456.

Seow, J., Graham, C., Merrick, B., Acors, S., Pickering, S., Steel, K.J.A., Hemmings, O., O’Byrne, A., Koupou, N., Galao, R.P., et al. (2020). Longitudinal observation and decline of neutralizing antibody responses in the three months following SARS-CoV-2 infection in humans. Nat. Microbiol. 5, 1598–1607.

Seydoux, E., Homad, L.J., MacCamy, A.J., Parks, K.R., Hurlburt, N.K., Jennewein, M.F., Akins, N.R., Stuart, A.B., Wan, Y.H., Feng, J., et al. (2021). Analysis of a SARS-CoV-2-infected individual reveals development of potent neutralizing antibodies with limited somatic mutation. Immunity 53, 98–105.e105.

Siu, J.H.Y., Pitcher, M.J., Tull, T.J., Velounias, R.L., Guesdon, W., Montorsi, L., Mahlubani, K.T., Ellis, R., Dhami, P., Todd, K., et al. (2022). Two subsets of human marginal zone B cells resolved by global analysis of lymphoid tissues and blood. Sci. Immunol. 7, eabm9660.

Supasa, P., Zhou, D., Dejnirattisai, W., Liu, C., Mentzer, A.J., Ginn, H.M., Zhao, Y., Duyvesteyn, H.M.E., Natali, R., Tuekprakhon, A., et al. (2021). Reduced...
neutralization of SARS-CoV-2 B.1.1.7 variant by convalescent and vaccine sera. Cell 184, 2201–2211.e2207.
Suryadvara, N., Shrirari, S., Gilchuk, P., VanBlargan, L.A., Binshtein, E., Zost, S.J., Nargi, R.S., Sutton, R.E., Winkler, E.S., Chen, E.C., et al. (2021). Neutralizing and protective human monoclonal antibodies recognizing the N-terminal domain of the SARS-CoV-2 spike protein. Cell 184, 2316–2331.e2315.

Ter Meulen, J., van den Brink, E.N., Poon, L.L., Marissen, W.E., Leung, C.S., Cox, F., Cheung, C.Y., Bakker, A.O., Bogaards, J.A., van Deventer, E., et al. (2006). Human monoclonal antibody combination against SARS coronavirus: synergy and coverage of escape mutants. PLoS Med. 3, e237.
Thompson, C.P., Grayson, N.E., Paton, R.S., Bolton, J.S., Lourenco, J., Pennman, B.S., Lee, L.N., Odon, V., Mongkolsapaya, J., Chinnakannan, S., et al. (2020). Detection of neutralising antibodies to SARS-CoV-2 to determine population exposure in Scottish blood donors between March and May 2020. Euro Surveill. 25, 2000885.
Tiller, T., Meffre, E., Yurasov, S., Tsuji, M., Nussenzweig, M.C., and Warde-Agrau, W. (2006). Human monoclonal antibody combination against SARS coronavirus: cleavage site in the SARS-CoV-2 spike modulates viral sensitivity to Type I interferon and IFITM2. J. Virol. 90, e02422–20.
Wu, M., Wall, E.C., Carr, E.J., Harvey, R., Townsley, H., Mears, H.V., Adams, L., Kjaer, S., Kelly, G., Warchal, S., et al. (2022). Three-dose vaccination elicits neutralising antibodies against omicron. Lancet 399, 715–717.
Wu, N.C., Yuan, M., Liu, H., Lee, C.D., Zhu, X., Bangaru, S., Torres, J.L., Caniels, T.G., Brouwer, P.J.M., van Gils, M.J., et al. (2020). An alternative binding mode of IGHV3-53 antibodies to the SARS-CoV-2 receptor binding domain. Cell Rep. 33, 108274.
Yuan, M., Liu, H., Wu, N.C., Lee, C.D., Zhu, X., Zhao, F., Huang, D., Yu, W., Hu, Y., Tien, H., et al. (2020a). Structural basis of a shared antibody response to SARS-CoV-2. Science 369, 1119–1123.
Yuan, M., Liu, H., Wu, N.C., and Wilson, I.A. (2020b). Recognition of the SARS-CoV-2 receptor binding domain by neutralizing antibodies. Biochem. Biophys. Res. Commun. 538, 192–203.
Yuan, M., Wu, N.C., Zhu, X., Lee, C.D., So, R.T.Y., Lv, H., Mok, C.K.P., and Wilson, I.A. (2022). A highly conserved cryptic epitope in the receptor binding domain of SARS-CoV-2 and SARS-CoV. Science 368, 630–633.
Zhou, D., Dejnirattisai, W., Supasa, P., Liu, C., Mentzer, A.J., Ginn, H.M., Zhao, Y., Duyvesteyn, H.M.E., Tukeprakhon, A., Nuttall, R., et al. (2021). Evidence of escape of SARS-CoV-2 variant B.1.351 from natural and vaccine-induced sera. Cell 174, 2348–2361.e6.
Zhou, D., Tsybovsky, Y., Gorman, J., Rapp, M., Cerutti, G., Chuang, G.Y., Katamba, P.S., Sampson, J.M., Schon, A., Bimela, J., et al. (2020). Cryo-EM structures of SARS-CoV-2 spike without and with ACE2 reveal a pH-dependent switch to mediate endosomal positioning of receptor-binding domains. Cell Host Microbe 28, 867–879.e865.
Zufferey, R., Nagy, D., Mandel, R.J., Naidini, L., and Trono, D. (1997). Multiply attenuated lentiviral vector achieves efficient gene delivery in vivo. Nat. Biotechnol. 15, 871–875.
### Key Resources Table

| REAGENT or RESOURCE          | SOURCE | IDENTIFIER                  |
|------------------------------|--------|-----------------------------|
| Antibodies                   |        |                             |
| Goat-anti-human-Fc-AP        | Jackson| RRID: AB_2337608 Cat#: 109-055-098 |
| horse-anti-mouse-IgG-HRP     | Cell Signaling Technology | Cat#: S7076                  |
| Mouse-anti-human IgG Fc-PE   | Biolegend| RRID: AB_10895907 Cat#: 409304 |
| anti-CD3-APC/Cy7             | Biolegend| RRID: AB_10644011 Cat#: 344817 |
| anti-CD8-APC-Cy7             | Biolegend| RRID: AB_2044005 Cat#: 344713 |
| anti-CD14-BV510              | Biolegend| RRID: AB_2561379 Cat#: 301841 |
| anti-CD19-PerCP-Cy5.5        | Biolegend| RRID: AB_2275547 Cat#: 302229 |
| anti-IgM-PE                  | Biolegend| RRID: AB_493006 Cat#: 314507 |
| anti-IgD-Pacific Blue        | Biolegend| RRID: AB_2561596 Cat#: 348823 |
| Streptavidin-Alexa-488       | Thermofisher Scientific| RRID: AB_2315383 Cat#: S32354 |
| Streptavidin-APC             | Thermofisher Scientific| Cat#: S32362                  |
| Streptavidin-PE              | Thermofisher Scientific| Cat#: S21388                  |
| Murinized mAb CR3009         | This manuscript (van den Brink et al., 2005) N/A |
| mAb CR3022                   | This manuscript (ter Meulen et al., 2006) N/A |
| SARS-CoV-2 specific nAbs and non-nAbs | This manuscript and (Graham et al., 2021) N/A |
| Bacterial and virus strains  |        |                             |
| NEB® Stable Competent E. coli| New England Biolabs| Cat#: C3040H                   |
| SARS-CoV-2 Strain England 2 (England 02/2020/407073) | Public Health England (PHE) N/A |
| Biological samples           |        |                             |
| PBMC and plasma from AZD1222 vaccinated individual | This manuscript N/A |
| Chemicals, peptides, and recombinant proteins |        |                             |
| Polyethylenimine, Linear, MW 25000 (PEI Max) | Polysciences, Inc| Cat#: 23966                  |
| Polyethylenimine Hydrochloride, Linear, MW 4,000 | Polysciences, Inc| Cat#: 24885                  |
| Recombinant S1 (WT, B.1.1.7, B.1.351, B.1.617.2) | Peter Cherepanov (Crick) (Rosa et al., 2021) and this manuscript N/A |
| Recombinant NTD              | Peter Cherepanov (Crick) (Rosa et al., 2021) N/A |
| Recombinant SARS-CoV-2 RBD ((WT, B.1.1.7, B.1.351, B.1.617.2) | (Seow et al., 2020) and this manuscript N/A |
| Recombinant Stabilized SARS-CoV-2 Spike | Marit van Gils (Amsterdam) (Brouwer et al., 2020) N/A |

(Continued on next page)
| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Recombinant SARS-CoV-2 Spike (biotinylated) | This manuscript | N/A |
| IdeS | Max Crispin (University of Southampton) (Dixon, 2014) | N/A |
| Recombinant S2 protein | SinoBiological | Cat#: 40590-V08B |
| Protein G agarose | GE Healthcare | Cat#: Cytiva 17-0618-02 |
| HiTrap IMAC columns | GE Healthcare | Cat#: Cytiva 17-0921-04 |
| HILOAD 16/600 SUPERDEX 200 PG | GE Healthcare | Cat#: 28989335 |
| Strep-TactinXT Superflow 50% Suspension | IBA | Cat#: 2-4010-002 |
| BioLock blocking solution | IBA | Cat#: 2-0205-050 |
| Ni Sepharose® 6 Fast Flow | Cytiva | Cat#: GE17-5318-06 |
| Bright-Glo Luciferase Assay System | Promega | Cat#: E2610 |

**Critical commercial assays**

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| QS® Site-Directed Mutagenesis Kit | New England Biolabs | Cat#: E0554 |
| Bright-Glo luciferase kit | Promega | Cat#: E2610 |
| Qiagen Multiplex PCR kit | Qiagen | Cat#: 206145 |
| Phusion High-Fidelity DNA Polymerase | NEB | Cat#: E2611L |
| SuperScript III RT | Thermofisher Scientific | Cat#: 18080085 |
| LIVE/DEAD Fixable Aqua Dead Cell Stain Kit | Thermofisher Scientific | Cat#: L34957 |
| 1-Step™ Ultra TMB-ELISA Substrate Solution | Thermofisher Scientific | Cat#: 34028 |
| Phosphatase substrate | Sigma Aldrich | Cat#: S0942-200TAB |

**Deposited data**

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| mAb sequence data | This manuscript | Accession numbers Genbank: ON088359–ON088446 |

**Experimental models: Cell lines**

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| FreeStyle™ 293F Cells | Thermofisher Scientific | Cat#: R79007 |
| HEK293T/17 | ATCC | ATCC® CRL-11268™ |
| HeLa-ACE2 | James Voss (Scripps) (Rogers et al., 2020) | N/A |
| Vero-E6 TMRPSS2 cells | Stuart Neil | N/A |
| HEK293T | ATCC | ATCC® CRL-3216™ |

**Oligonucleotides**

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Heavy, kappa and Lambda PCR1 and 2 primers | (Scheid et al., 2009; Tiller et al., 2008; von Boehmer et al., 2018) | N/A |
| Spike mutagenesis primers | This manuscript | N/A |

**Recombinant DNA**

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Biotinylated Spike (pHLSec) | This manuscript | N/A |
| Pre-fusion, stabilized and uncleaved SARS-CoV-2 Spike (pcDNA3.1+) | Marit van Gils (Amsterdam) (Brouwer et al., 2020) | N/A |
| Full length SARS-CoV-2 Spike (pcDNA3.1+) | Nigel Temperton (Seow et al., 2020) | N/A |
| Full length B.1.1.7 variant Spike (pcDNA3.1+) | Laura Mccoy (UCL) (Rees-Spear et al., 2021) | N/A |
| Full length P.1 variant Spike (pcDNA3.1+) | (Dupont et al., 2021) | N/A |
| Full length B.1.351 variant Spike (pcDNA3.1+) | (Dupont et al., 2021) | N/A |
| Full length B.1.617.2 variant Spike (pcDNA3.1+) | Wendy Barclay and (Dupont et al., 2021) | N/A |

(Continued on next page)
Continued

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Full length B.1.1.529 variant Spike (pcDNA3.1+) | Wendy Barclay | N/A |
| BirA | Addgene (Howarth et al., 2008) | Cat#: 20856 |
| pHIV-Luc (constructed by replacing GFP in pHIV'SIN-SEW (PMID: 11975847) with HA-luciferase) | Luis Apolonia (KCL) | N/A |
| HIV 8.91 gag/pol packaging construct | p8.91 (Zufferey et al., 1997) | N/A |
| Heavy/Kappa/Lambda human IgG1 expression vectors | M. Nussenzweig (Rockefeller University) | von Boehm et al., 2016 |

Software and algorithms

| FlowJo | Tree Star | https://www.flowjo.com |
| Prism | Graphpad | https://www.graphpad.com/scientific-software/prism/ |
| Tableau | TABLEAU SOFTWARE, LLC | https://www.tableau.com/ |
| IMGT/V-QUEST | IMGT (Lefranc et al., 1999) | http://www.imgt.org/IMGT_vquest/vquest |
| R statistical programming environment | R Foundation for Statistical Computing | https://www.r-project.org |
| R studio | RStudio | https://www.rstudio.com/ |
| ggplot2 | (Wickham, 2016) | https://ggplot2.tidyverse.org |
| PyMol | The PyMOL Molecular Graphics System, Version 2.0 Schroedinger, LLC | https://www.pymol.org/ |

Other

| FACS Melody | BD Biosciences | N/A |
| Victor™ X3 multilabel reader | Perkin Elmer | 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, Katie J Doores (katie.doores@kcl.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

The antibody sequences generated during this study are available at GenBank (accession numbers Genbank: ON088359–ON088446).

This paper does not report original code.

Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Ethics

This study used human samples from one donor collected as part of a study entitled “Antibody responses following COVID-19 vaccination”. Ethical approval was obtained from the King’s College London Infectious Diseases Biobank (IBD) (KDJF-110121) under the terms of the IDB’s ethics permission (REC reference: 19/SC/0232) granted by the South Central – Hampshire B Research Ethics Committee in 2019. VA14 is male and 23 aged years.

Bacterial strains and cell culture

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). Small and large scale expression of monoclonal antibodies was performed in HEK293T/17 (ATCC; ATCC® CRL-11268™) and 293 Freestyle cells (Thermofisher Scientific), respectively. Bacterial transformations were performed with NEB® Stable Competent E. coli.
METHOD DETAILS

Protein expression and purification
Recombinant Spike and RBD for ELISA were expressed and purified as previously described (Pickering et al., 2020; Seow et al., 2020). Recombinant S1 (residues 1-530) and NTD (residues 1-310) expression and purification was described in Rosa et al. (Rosa et al., 2021). S2 protein was obtained from SinoBiological (Cat number: 40590-V08B).

For antigen-specific B cell sorting, Spike glycoprotein consisted of the pre-fusion S ectodomain (residues 1–1138) with a GGGG substitution at the furin cleavage site (amino acids 682–685), proline substitutions at amino acid positions 986 and 987, and an N-terminal T4 trimerization domain. RBD consisted of amino acids 331-533. Spike and RBD were cloned into a pHLsec vector containing Avi and 6xHis tags (Aricescu et al., 2006). Biotinylated Spike or RBD were expressed in 1L of HEK293F cells (Invitrogen) at a density of 480 μg/mL in 2% casein, and then 25 μl of 2% casein in PBS was added per well. This solution was supplemented with biotinylatin at 10 μM where indicated. Serial dilutions of IgGs were prepared in separate 96-well plates in 2% casein in PBS for 1 h at room temperature. The wells were emptied and 25 μl of 2% casein in PBS was added per well. This was followed. The presence of IgG in supernatants was detected using Goat-anti-human-Fc conjugated to alkaline phosphatase (1:1,000) (Jackson: 109-055-098). Wells were blocked with 5% milk in PBS/T for 2 hr at room temperature. Serial dilutions of IgGs were prepared in separate 96-well plate in 2% casein, and then 25 μl of each serial dilution were added to the ELISA assay plates and incubated for 2 h at room temperature. Wells were washed with PBS-T. IgG binding was detected using goat-anti-human-Fc, goat-anti-human-IgM-HRP (horseradish peroxidase) (1:1,000) (Sigma: A6907) and IgG was detected using Goat-anti-human-Fc-AP (alkaline phosphatase) (1:1,000) (Jackson: 109-055-098). Plates were washed (5 times with PBS-T) and developed with either AP substrate (Sigma) and read at 405 nm (AP) or 1-step TMB (3,3′,5,5′-Tetramethylbenzidine) substrate (Thermo Scientific) and quenched with 0.5 M H2SO4 before reading at 450 nm (HRP).

For IgG digestion to generate F(ab’)2
IgG were incubated with IdeS (Dixon, 2014) (4 μg of IdeS per 1 mg of IgG) in PBS for 1 hour at 37°C. The Fc and IdeS A were removed using a mix of Protein A Sepharose® Fast Flow (250 μL per 1 mg digested mAb; GE Healthcare Life Sciences) and Ni Sepharose™ 6 Fast Flow (50 μL per 1 mg digested mAb; GE Healthcare Life Sciences) which were washed twice with PBS before adding to the reaction mixture. After exactly 10 minutes the beads were removed from the F(ab’)2-dilution by filtration in Spin-X tube filters (Costar) and the filtrate was concentrated in Amicon® Ultra Filters (10k, Millipore). Purified F(ab’)2 fragments were analysed by SDS-PAGE.

ELISA (S, RBD, NTD, S2 or S1)
96-well plates (Corning, 3690) were coated with S, S1, NTD, S2 or RBD at 3 μg/mL overnight at 4°C. The plates were washed (5 times with PBS/0.05% Tween-20, PBS-T), blocked with blocking buffer (5% skimmed milk in PBS-T) for 1 hr at room temperature. Serial dilutions of plasma, mAb or supernatant in blocking buffer were added and incubated for 2 hr at room temperature. Plates were washed (5 times with PBS-T) and secondary antibody was added and incubated for 1 hr at room temperature. IgM was detected using Goat-anti-human-IgM-HRP (horseradish peroxidase) (1:1,000) (Sigma: A6907) and IgG was detected using Goat-anti-human-Fc-AP (alkaline phosphatase) (1:1,000) (Jackson: 109-055-098). Plates were washed (5 times with PBS-T) and developed with either AP substrate (Sigma) and read at 405 nm (AP) or 1-step TMB (3,3′,5,5′-Tetramethylbenzidine) substrate (Thermo Scientific) and quenched with 0.5 M H2SO4 before reading at 450 nm (HRP).

Fab/Fc ELISA
96-well plates (Corning, 3690) were coated with goat anti-human Fc IgG antibody at 3 μg/mL overnight at 4°C. The antibody protocol was followed. The presence of IgG in supernatants was detected using Goat-anti-human-Fc (alkaline phosphatase) (1:1,000) (Jackson: 109-055-098).

Biliverdin competition ELISA
ELISA plates were coated with 3 μg/ml (25 μl per well) SARS-CoV2 WT S1 antigen in PBS overnight at 4°C. Wells were blocked with 100 μl 2% casein in PBS for 1 h at room temperature. The wells were emptied and 25 μl of 2% casein in PBS was added per well. This was followed. The presence of IgG in supernatants was detected using Goat-anti-human-Fc conjugated to alkaline phosphatase (1:1,000; Jackson, product code 109-055-098). Wells were washed with PBS-T and alkaline phosphatase substrate (Sigma-Aldrich) was added and read at 405 nm.

IgG digestion to generate F(ab’)2
IgG were incubated with IdeS (Dixon, 2014) (4 μg of IdeS per 1 mg of IgG) in PBS for 1 hour at 37°C. The Fc and IdeS A were removed using a mix of Protein A Sepharose® Fast Flow (250 μL per 1 mg digested mAb; GE Healthcare Life Sciences) and Ni Sepharose™ 6 Fast Flow (50 μL per 1 mg digested mAb; GE Healthcare Life Sciences) which were washed twice with PBS before adding to the reaction mixture. After exactly 10 minutes the beads were removed from the F(ab’)2-dilution by filtration in Spin-X tube filters (Costar®) and the filtrate was concentrated in Amicon® Ultra Filters (10k, Millipore). Purified F(ab’)2 fragments were analysed by SDS-PAGE.

F(ab’)2 and IgG competition ELISA
96-well half area high bind microplates (Corning®) were coated with S-protein at 3 μg/mL in PBS overnight at 4°C. Plates were washed (5 times with PBS/0.05% Tween-20, PBS-T) and blocked with 5% milk in PBS/T for 2 hr at room temperature. Serial dilutions (5-fold) of F(ab’)2, starting at 100-molar excess of the IC50 of S binding, were added to the plates and incubated for 1 hr at room temperature. Plates were washed (5 times with PBS-T) before competing IgG was added at their IC50 of S binding and incubated at room temperature. Plates were washed (5 times with PBS-T) and AP substrate (Sigma) was added. Optimal density was measured at 405 nm in 5-minute intervals. The percentage competition was calculated as the reduction in IgG binding in the presence of F(ab’)2 at (100-molar excess of the IC50) as a percentage of the maximum IgG binding in the absence of F(ab’)2. Competition groups were determined using Ward2 clustering (R, Complex Heatmap package (Gu et al., 2016)) for initial analysis and Groups were then arranged by hand according to binding epitopes.
Semi-quantitative ELISA
In 96-well plates (Corning, 3690), 10 columns were coated with SARS-CoV-2 Spike at 3 μg/mL in PBS, with the remaining 2 columns coated with Goat anti-Human IgG F(ab')₂ at 1:1000 dilution, and incubated overnight at 4 °C. The plates were washed (5 times with PBS/0.05% Tween-20, PBS-T) and blocked with blocking buffer (5% skimmed milk in PBS-T) for 1 h at room temperature. Serial dilutions of serum and a known concentrations of IgG standard (in blocking buffer) were added to the Spike coated and standard curve columns, respectively. After 2 h incubation at room temperature, plates were washed 5 times with PBS-T. Secondary antibody, goat-anti-human-Fc-AP, was added at 1:1000 dilution in blocking buffer and incubated for 1 h at room temperature. Plates were washed 5 times with PBS-T and developed with AP substrate (Sigma). Absorbance was measured at 405 nm. Antigen-specific serum IgG was quantified by averaging values interpolated from a standard curve of IgG standard using four-parameter logistic regression curve fitting (Rees-Spear et al., 2021).

SARS-CoV-2 pseudotyped virus preparation
Pseudotyped HIV-1 virus incorporating either the SARS-CoV-2 Wuhan, B.1.1.7, P.1, B.1.351, B.1.617.2, B.1.1.529 full-length Spike were produced in a 10 cm dish seeded the day prior with 5x10⁶ HEK293T/17 cells in 10 mL of complete Dulbecco’s Modified Eagle’s Medium (DMEM-C, 10% fetal bovine serum (FBS) and 1% Pen/Strep (100 IU/mL penicillin and 100 mg/mL streptomycin)). Cells were transfected using 90 μg of PEI-Max (1 mg/mL, Polysciences) with: 15 μg of HIV-luciferase plasmid, 10 μg of HIV 8.91 gag/pol plasmid (Zufferey et al., 1997) and 5 μg of SARS-CoV-2 spike protein plasmid (Grehan et al., 2015; Thompson et al., 2020). Pseudotyped virus was harvested after 72 h, filtered through a 0.45μm filter and stored at -80°C until required.

Neutralization assay with SARS-CoV-2 pseudotyped virus
Neutralization assays were conducted as previously described (Carter et al., 2020; Monin et al., 2021; Seow et al., 2020). Serial dilutions of serum samples (heat inactivated at 56 °C for 30 mins) or mAbs were prepared with DMEM-C media and incubated with pseudotyped virus for 1-hour at 37 °C in 96-well plates. Next, HeLa cells stably expressing the ACE2 receptor (provided by Dr James Voss, Scripps Research, La Jolla, CA) were added (12,500 cells/50μL per well) and the plates were left for 72 hours. The amount of infection was assessed in lysed cells with the Bright-Glo luciferase kit (Promega), using a Victor™ X3 multilabel reader (Perkin Elmer). Measurements were performed in duplicate and duplicates used to calculate the ID₅₀.

Infectious virus strain and propagation
Vero-E6 TMPRSS2 cells (Winstone et al., 2021) (Cercopithecus aethiops derived epithelial kidney cells) cells were grown in Dulbecco’s Modified Eagle’s Medium (DMEM, Gibco) supplemented with GlutaMAX, 10% fetal bovine serum (FBS), 20 μg/mL gentamicin, and incubated at 37 °C with 5% CO₂. SARS-CoV-2 Strain England 2 (England 02/2020/407073) was obtained from Public Health England. The virus was propagated by infecting 60-70% confluent Vero-E6 TMPRSS2 cells in T75 flasks, at an MOI of 0.005 in 3 mL of media (2% FBS and 1% Pen/Strep) and allowed to adhere overnight. Serial dilutions of mAbs were prepared with DMEM media (10% FBS and 1% Pen/Strep) and incubated with replication competent live SARS-CoV-2 for 1 hour at 37 °C. The media was removed from the pre-plated Vero-E6 TMPRSS2 cells and the serum-virus mixtures were added to the cells and incubated at 37 °C for 24 h. The virus/serum mixture was aspirated, and each well was fixed with 150μL of 4% formalin at 4 °C overnight and then topped up to 300μL using PBS. The cells were washed once with PBS and permeabilized with 0.1% Triton-X in PBS at room temperature for 15 min. The cells were washed twice with PBS and blocked using 3% milk in PBS at room temperature for 15 min. The blocking solution was removed and an N-specific mAb (murinized-CR3009 (van den Brink et al., 2005)) was added at 1:2000 in 1% milk in PBS, A2554-1mL, Sigma-Aldrich) at room temperature for 15 min. The cells were washed twice with PBS, developed using TMB substrate for 30 min and quenched using 2M H₂SO₄ prior to reading at 450 nm. Measurements were performed in duplicate and duplicates used to calculate the ID₅₀.

Infectious virus neutralization assay
Vero-E6 TMPRSS2 cells (Winstone et al., 2021) were seeded at a concentration of 20,000 cells/100μL per well in 96-well plates in DMEM media (10% FBS and 1% Pen/Strep) and allowed to adhere overnight. Serial dilutions of mAbs were prepared with DMEM media (2% FBS and 1% Pen/Strep) and incubated with replication competent live SARS-CoV-2 for 1 hour at 37 °C. The media was removed from the pre-plated Vero-E6 TMPRSS2 cells and the serum-virus mixtures were added to the cells and incubated at 37 °C for 24 h. The virus/serum mixture was aspirated, and each well was fixed with 150μL of 4% formalin at 4 °C overnight and then topped up to 300μL using PBS. The cells were washed once with PBS and permeabilized with 0.1% Triton-X in PBS at room temperature for 15 min. The cells were washed twice with PBS and blocked using 3% milk in PBS at room temperature for 15 min. The blocking solution was removed and an N-specific mAb (murinized-CR3009 (van den Brink et al., 2005)) was added at 1:2000 in 1% milk in PBS, A2554-1mL, Sigma-Aldrich) at room temperature for 15 min. The cells were washed twice with PBS, developed using TMB substrate for 30 min and quenched using 2M H₂SO₄ prior to reading at 450 nm. Measurements were performed in duplicate and duplicates used to calculate the ID₅₀.

Antigen-specific B cell sorting
Fluorescence-activated cell sorting of cryopreserved PBMCs was performed on a BD FACs Melody as previously described (Graham et al., 2021). Sorting baits (SARS-CoV-2 Spike and RBD) were pre-complexed with the streptavidin fluorophore at a 1:4 molar ratio prior to addition to cells. PBMCs were stained with live/dead (fixable Aqua Dead, Thermofisher), anti-CD3-APC/Cy7 (Biolegend), anti-CD8-APC-Cy7 (Biolegend), anti-CD14-BV510 (Biolegend), anti-CD19-PerCP-Cy5.5 (Biolegend), anti-IgM-PE (Biolegend), anti-IgD-Pacific Blue (Biolegend) and anti-IgG-PeCy7 (BD) and Spike-Alexa488 (Thermofisher Scientific, S32354) and Spike-APC (Thermofisher Scientific, S32362) or RBD-Alexa488 and RBD-APC. Live CD3/CD8 CD14 CD19*IgM* IgD*IgG*Spike*Spike or CD3/CD8/CD14/CD19*IgM* IgD*IgG*RBD*RBD cells were sorted using a BD FACs Melody into individual wells containing RNase
OUT (Invitrogen), First Strand SuperScript III buffer, DTT and H₂O (Invitrogen) and RNA was converted into cDNA (SuperScript III Reverse Transcriptase, Invitrogen) using random hexamers (Bioline Reagents Ltd) following the manufacturer’s protocol.

**Full-length antibody cloning and expression**

The human Ab variable regions of heavy and kappa/lambda chains were PCR amplified using previously described primers and PCR conditions (Scheid et al., 2009; Tiller et al., 2008; von Boehmer et al., 2016). PCR products were purified and cloned into human-IgG (Heavy, Kappa or Lambda) expression plasmids (von Boehmer et al., 2016) using the Gibson Assembly Master Mix (NEB) following the manufacturer’s protocol. Gibson assembly products were directly transfected into HEK-293T cells and transformed under ampicillin selection. Ab supernatants were harvested 3 days after transfection and IgG expression and Spike-reactivity determined using ELISA. Ab variable regions of heavy-light chain pairs that generated Spike reactive IgG were sequenced by Sanger sequencing.

**IgG expression and purification**

Ab heavy and light plasmids were co-transfected at a 1:1 ratio into HEK-293F cells (Thermofisher) using PEI Max (1 mg/mL, Polysciences, Inc.) at a 3:1 ratio (PEI Max:DNA). Ab supernatants were harvested five days following transfection, filtered and purified using protein G affinity chromatography following the manufacturer’s protocol (GE Healthcare).

**ACE2 competition measured by flow cytometry**

To prepare the fluorescent probe, Streptavidin-APC (Thermofisher Scientific, S32362) was added to biotinylated SARS-CoV-2 Spike (3.5 times molar excess of Spike) on ice. Additions were staggered over 5 steps with 30 min incubation times between each addition. Purified mAbs were mixed with PE conjugated SARS-CoV-2 S in a molar ratio of 4:1 in FACS buffer (2% FBS in PBS) on ice for 1 h. HeLa-ACE2 and HeLa cells were washed once with PBS and detached using PBS containing 5mM EDTA. Detached cells were washed and resuspended in FACS buffer. 0.5 million HeLa-ACE2 cells were added to each mAb-Spike complex and incubated on ice for 30 m. The cells were washed with PBS and resuspended in 1 mL FACS buffer with 1 µL of LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (Invitrogen). HeLa-ACE2 cells alone and with SARS-CoV-2 Spike only were used as background and positive controls, respectively. The geometric mean fluorescence for PE was measured from the gate of singlet and live cells. The ACE2 binding inhibition percentage was calculated as described previously (Graham et al., 2021; Rogers et al., 2020).

**Monoclonal antibody sequence analysis**

The heavy and light chain sequences of SARS-CoV-2 specific mAbs were examined using IMGT/V-QUEST (http://www.imgt.org/IMGT_vquest/vquest) to identify the germline usages, percentage of SHM and CDR region lengths. To remove variation introduced through cloning using mixture of forward primers, 5 amino acids or 15 nucleotides were trimmed from the start and end of the translated variable genes. D’Agostino & Pearson normality test, Kruskal-Wallis test with Dunn’s multiple comparisons post hoc test, Ordinary one-way ANOVA with Tukey’s multiple comparisons post hoc test and two-sided binomial tests) were performed using GraphPad Prism software. Significance defined as p < 0.0332 (*), 0.0021 (**), 0.0002 (***), and >0.0001 (****).

**QUANTIFICATION AND STATISTICAL ANALYSIS**

All neutralization and ELISA experiments were performed in duplicate. The 50% inhibitory concentrations/dilutions (IC/ID₅₀) were calculated using GraphPad Prism software. Statistical analysis in Figures 3 and S2 (D’Agostino & Pearson tests was performed to determine normality and Kruskal-Wallis with Dunn’s multiple comparison test post hoc test or an ordinary one-way ANOVA with Turkey’s multiple comparison post hoc) were performed using GraphPad Prism software, significance defined as p<0.05. Linear correlations (Figure S2, Spearman correlation) were also calculated using GraphPad Prism. The fold decrease in mAb IC₅₀ was calculated by dividing the average IC₅₀ value for a given mAb against the indicated VOC by the IC₅₀ value for that mAb against the WT (Figure 6).