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Human B Cell Clonal Expansion and Convergent Antibody Responses to SARS-CoV-2

Highlights

- Human IGH repertoire sequencing identifies SARS-CoV-2-specific B cell clones
- Convergent virus-specific antibody sequences are shared between COVID-19 patients
- SARS-CoV antibodies are detected by sequence in COVID-19 patients

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In Brief

B cells produce antibodies and provide protective immunity to viruses. In longitudinal data from COVID-19 patients, Nielsen et al. analyze the development of antibody gene repertoires responding to SARS-CoV-2. COVID-19 patients share subsets of similar antibody sequences that bind SARS-CoV-2 antigens, with rare antibodies that also recognize SARS-CoV.
SUMMARY

B cells are critical for the production of antibodies and protective immunity to viruses. Here we show that patients infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) who develop coronavirus disease 2019 (COVID-19) display early recruitment of B cells expressing a limited subset of IGHV genes, progressing to a highly polyclonal response of B cells with broader IGHV gene usage and extensive class switching to IgG and IgA subclasses with limited somatic hypermutation in the initial weeks of infection. We identify convergence of antibody sequences across SARS-CoV-2-infected patients, highlighting stereotyped naive responses to this virus. Notably, sequence-based detection in COVID-19 patients of convergent B cell clonotypes previously reported in SARS-CoV infection predicts the presence of SARS-CoV/SARS-CoV-2 cross-reactive antibody titers specific for the receptor-binding domain. These findings offer molecular insights into shared features of human B cell responses to SARS-CoV-2 and SARS-CoV.

INTRODUCTION

The novel human severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the etiological agent of the coronavirus disease 2019 (COVID-19) pandemic (Huang et al., 2020; Zhu et al., 2020). Prior to the emergence of SARS-CoV-2, six human coronaviruses (hCoVs) were known; four seasonal hCoVs (hCoV-229E, -NL63, -HKU1, and -OC43) (Su et al., 2016) causing usually mild upper respiratory illness. and the more recently discovered are SARS-CoV (Peiris et al., 2003) and MERS-CoV (Zaki et al., 2012), viruses that arose from spillover events of virus from animals into humans. Humans are expected to be naive to SARS-CoV-2 and display a primary immune response to infection. Humoral immune responses will likely be critical for the development of protective immunity to SARS-CoV-2. Recently, SARS-CoV-2 neutralizing antibodies from convalescent COVID-19 patients have been reported (Cao et al., 2020; Ju et al., 2020; Robbiani et al., 2020; Wu et al., 2020), offering an important resource of potential protective or therapeutic antibodies. Further analysis of the B cell antigen receptors (BCRs) specific for this virus is needed to define the shared or distinct features of humoral responses elicited in comparison with other viral infections and to assess the extent to which responses to SARS-CoV-2 have breadth extending to other coronaviruses within the subgenus Sarbecovirus.
RESULTS

SARS-CoV-2 Infection Causes Global Changes in the Antibody Repertoire

High-throughput DNA sequencing of BCR heavy chain genes defines clonal B cell lineages based on their unique receptor sequences and captures the hallmarks of clonal evolution, such as somatic hypermutation (SHM) and class-switch recombination in the humoral response (Zhou and Kleinstein, 2019). To study SARS-CoV-2-specific humoral responses, we collected a total of 38 peripheral blood specimens from 13 adult patients, sampled at a median of three time points (range 1–5) each, admitted to Stanford Healthcare with COVID-19 confirmed by quantitative reverse transcription PCR (RT-qPCR) testing. Sampling time points were measured as days post-symptom onset (DPSO). All patients showed SARS-CoV-2 receptor-binding domain (RBD)-specific IgA, IgG, and IgM antibodies (Table 1). Immunoglobulin heavy chain (IGH) repertoires were sequenced and compared to those from a healthy human control (HHC) dataset of 114 adults (Nielsen et al., 2019). Data from a HHC individual matched to the COVID-19 cohort by mean sequencing depth and to a lesser extent IgA, with little to no SHM. Data from a patient prior to and after seroconversion shows an increase in the proportion of class-switched low-SHM clones (bottom panels in Figure 1, top panel). In HHC at baseline, IgM and IgD are primarily derived from naïve B cells with unmutated IGHV genes, whereas class-switched cells expressing IgA or IgG subclasses show IGH SHM. In contrast, SARS-CoV-2 seroconverted patients (blue labels in Figure 1) show a highly polyclonal burst of B cell clones expressing IgG, and to a lesser extent IgA, with little to no SHM. Data from a patient prior to and after seroconversion shows an increase in the proportion of class-switched low-SHM clones (bottom panels in Figure 1). Seronegative samples (red labels in Figure 1) had IGH repertoires similar to uninfected HHC, suggesting an earlier stage in the immune response to infection at these time points.

The increased fraction of unmutated (<1% SHM in IGHV gene) clones in the IgG subclasses in seroconverted COVID-19 patients in comparison with HHC was statistically significant (IgG1: p value = 1.884e-08; IgG2: 1.554e-08; IgG3: 3.754e-08; IgG4: 0.00044) (Figures 2A and 2B). SHM frequencies and proportions of unmutated clones in each sample are in Figure S1. Most B cells in COVID-19 patients prior to seroconversion showed IgG SHM comparable to HHC, but the proportion of IgG-expressing (IgG+) B cells with low SHM increased rapidly over time (Figure 2A, lower panels). Notably, prior to seroconversion, B cells expressing some IGHV genes, particularly IGHV3-30-3 and IGHV1-2, showed earlier changes than the rest of the repertoire, with increased IgG class-switched low-SHM clones (Figures 2C and 2D). IGHV3-9 showed a similar trend but was not significant (Figures 2C and 2D).

We previously reported a similar influx of low-mutation clones into the IgG compartment in acute Ebola virus (EBOV) infection (Davis et al., 2019), but there was a prolonged delay, lasting months, in accumulation of SHM in those clones. In contrast, clones detected at two or more time points in COVID-19 patients for whom the longest time courses were available (patients 7453 and 7455; Figure 2B) show that after the initial appearance of low-SHM clone members, the proportion of IgG+ B cells with intermediate SHM frequencies (2%–5%) increases in the first three weeks post-onset of symptoms. Examination of the total clones within each isotype shows the appearance of low-SHM clones post-seroconversion within the first two weeks post-onset of symptoms and subsequent increases in SHM over the following two weeks (Figure 2E). In further contrast to EBOV, COVID-19 primary infection stimulated polyclonal B cell responses with both IgG and, in some patients, IgA subclasses, rather than predominantly IgG (Figure 1). Overall, among IgG+ B cells in COVID-19 patients, the proportion of IgG1+ cells was increased, with decreases in IgG2 and IgG3. Median usage of IgG1 was 1.7-fold greater than that seen in HHC B cells (Table S1).

Comparison of the IGHV genes used by COVID-19 and HHC individuals (Figure S2) revealed COVID-19 skewing of the responding IGHV1-24, IGHV3-9, IGHV3-13, and IGHV3-20 in IgG+ B cells. IGHV3-53 is reported to be enriched in RBD-binding B cells. Most B cells in COVID-19 patients prior to seroconversion show low median IgG1 SHM (range 2.4%–7.5%), compared to higher median IgA1 SHM (range 5.8%–9.5%) (Figure 2C).

In COVID-19 patients, expanded clones detected in two or more replicate IGH sequence libraries showed increased proportions of low-SHM members (Figures S3A and S3B; IgG1, p values = 0.005; IgG2, 0.014, IgG3, 0.036). Expanded clones had longer and more hydrophobic IGH complementarity-determining region-3 (CDR-H3) sequences in class-switched isotypes in comparison with HHC (Figure 2F), consistent with a rapid proliferation of cells recently differentiated from naïve B cells (Grimshoim et al., 2020). CDR-H3 charge and aromaticity showed modest differences in COVID-19 patients in comparison with HHC, including more negative charge in non-expanded clones (Figure S3C). Notably, the IGHV gene usage frequencies in expanded clones in comparison with non-expanded clones of COVID-19 patients showed a different pattern than overall IGHV gene usage, with IGHV1-24, IGHV3-13, and IGHV3-20 frequencies increased in the total repertoire but used less often in expanded clones (Figures S2 and S3D), suggesting that the B cells expressing these IGHV genes are highly polyclonal with small clone sizes. Eleven IGHV genes were significantly enriched in expanded clones versus non-expanded clones (Figure S3D), suggesting preferential recruitment and viral epitope binding of B cells by using these germline IGHV.

B Cells Expressing Convergent IGH Are Detected in COVID-19 Patients

Despite the diversity of antigen-driven antibody responses, we and others have previously identified patterns of highly similar, “convergent” antibodies shared by different individuals in response to pathogens such as EBOV (Davis et al., 2019), dengue virus (Parameswaran et al., 2013), and influenza virus (Jackson et al., 2014). Such convergent antibodies make up a small proportion of the total virus-specific B cell response in each individual (Davis et al., 2019). To identify putative SARS-CoV-2-specific antibody signatures, we analyzed clones with shared IGHV, IGHJ, and CDR-H3 region length and clustered
the CDR-H3 sequences at 85% amino acid identity to find clusters spanning two or more COVID-19 patients and absent from the 114 HHC individuals. One thousand two hundred and thirty-six convergent clusters met these criteria and showed SHM frequencies averaging 1.7% (range 0.5%–5.5%). An average of 196 convergent clusters were found per patient, ranging from 69 clusters in patient 7485 to 477 clusters in patient 7455. One thousand one hundred and seventy-one clusters were shared pairwise between two patients, 53 clusters spanned three patients, nine clusters spanned four patients, and three clusters spanned five patients (Figure 3A). To assess the significance of these convergent clones in COVID-19 patients, we undertook repeated resampling of 13 randomly selected HHC samples with the same parameters for 100 iterations. The number of

Table 1. Individual COVID-19 Patient Sample Information in DPSO

| Patient ID | Age | Sex | Status | DPSO | IgA | IgG | IgM | gDNA Library | cDNA Library |
|------------|-----|-----|--------|------|-----|-----|-----|--------------|--------------|
| 7450       | 73  | F   | ICU    | 9    | yes | yes | yes | yes          | yes          |
|            |     |     |        | 22   | yes | yes | yes | yes          | yes          |
|            |     |     |        | 25   | yes | yes | yes | yes          | no           |
|            |     |     |        | 27   | yes | yes | yes | yes          | yes          |
| 7451       | 62  | M   | inpatient | 8   | no  | no  | no  | yes          | yes          |
| 7452       | 61  | F   | inpatient | 18  | yes | yes | yes | yes          | yes          |
| 7453       | 64  | M   | ICU    | 8    | no  | no  | no  | yes          | yes          |
|            |     |     |        | 11   | no  | no  | no  | yes          | yes          |
|            |     |     |        | 15   | yes | yes | yes | yes          | yes          |
|            |     |     |        | 18   | yes | yes | yes | yes          | yes          |
|            |     |     |        | 20   | yes | yes | yes | yes          | no           |
| 7454       | 42  | M   | ICU    | 16   | yes | yes | yes | yes          | yes          |
| 7455       | 36  | M   | ICU    | 9    | yes | yes | yes | yes          | yes          |
|            |     |     |        | 11   | yes | yes | yes | yes          | yes          |
|            |     |     |        | 12   | yes | yes | yes | yes          | yes          |
|            |     |     |        | 16   | yes | yes | yes | yes          | yes          |
|            |     |     |        | 19   | yes | yes | yes | yes          | yes          |
| 7480       | 40  | F   | ICU    | 11   | yes | yes | yes | yes          | yes          |
|            |     |     |        | 14   | yes | yes | yes | yes          | yes          |
|            |     |     |        | 17   | yes | yes | yes | yes          | no           |
| 7481       | 66  | M   | ICU    | 32   | yes | yes | yes | yes          | yes          |
|            |     |     |        | 35   | yes | yes | yes | yes          | yes          |
| 7482       | 88  | F   | inpatient | 5   | no  | no  | no  | yes          | yes          |
|            |     |     |        | 8    | no  | no  | no  | yes          | yes          |
|            |     |     |        | 10   | no  | no  | no  | yes          | yes          |
|            |     |     |        | 12   | no  | yes | yes | yes          | yes          |
| 7483       | 77  | F   | inpatient | 35   | yes | yes | yes | yes          | yes          |
|            |     |     |        | 40   | yes | yes | yes | yes          | yes          |
| 7484       | 36  | M   | ICU    | 11   | yes | yes | yes | yes          | yes          |
|            |     |     |        | 14   | yes | yes | yes | yes          | yes          |
|            |     |     |        | 17   | yes | yes | yes | yes          | yes          |
| 7485       | 45  | F   | ICU    | 8    | no  | yes | yes | no           | yes          |
|            |     |     |        | 10   | yes | yes | yes | yes          | yes          |
|            |     |     |        | 12   | yes | yes | yes | yes          | yes          |
| 7486       | 69  | F   | inpatient | 14   | no  | yes | yes | yes          | yes          |
|            |     |     |        | 17   | yes | yes | yes | yes          | yes          |
|            |     |     |        | 19   | yes | yes | yes | yes          | yes          |

Seroconversion was determined by ELISA to the SARS-CoV-2 RBD antigen (STAR Methods).

aAll patients were inpatients; eight patients required ICU care during their hospital stay.
bIgA seroconversion.
cIgG seroconversion.
dIgM seroconversion.
Figure 1. COVID-19 Patient IGH Repertoires Show Early and Extensive Class Switching to IgG and IgA Subclasses without Significant Somatic Mutation

Points indicate B cell clonal lineages. Panel rows are different blood samples (HHC or patient ID), and panel column indicates the clone member’s isotype. Within panels, IGHV gene is indicated on the x axis, in the same order and position in each panel, not listed by name due to space constraints, and CDR-H3 length in amino acids (AAs) is indicated on the y axis. Point color indicates median IGHV SHM frequency for each clone, and point size indicates the number of unique reads in the clone. Points are jittered to decrease over-plotting. Patient label colors indicate IgG seroconversion status (red is seronegative, and blue is seropositive) and the number following the patient ID corresponds to days post-symptom onset. The final four rows of panels show the IGH repertoire changes within a single participant (7455) prior to and after seroconversion.
convergent clones shared by COVID-19 patients greatly exceeded the mean convergent clone counts from the HHC resampling (Figure 3B), consistent with antigen-driven shared selection of the convergent clones identified in COVID-19 patients. To directly test the antigen specificity of these convergent clones, we expressed human IgG1 monoclonal antibodies (mAbs 2A and 4A; Table S2) from two COVID-19 convergence groups in two patients after paired immunoglobulin heavy and light chain sequencing of single B cells with the 10x Genomics platform. mAb2A had a single nonsynonymous mutation at the beginning of the CDR-H2 of IGHV3-30-3, whereas mAb4A expressed germline IGHV3-15. In enzyme-linked immunosorbent assay (ELISA) testing, both mAbs bound SARS-CoV-2 spike and S1 domain but not the RBD or nucleocapsid (Figure 3C). We further identified 10 sequence clusters shared with independent external COVID-19 patient datasets and reported to be specific for SARS-CoV-2 RBD (Cao et al., 2020; Noy-Porat et al., 2020; Shi et al., 2020). Nine of 13 COVID-19 patients (69%) in our study (7450, 7452, 7454, 7455, 7480, 7483, 7484, 7485, and 7486) had these RBD-specific clonotypes (Figures S4A and S4B), although they make up only a small fraction of each individual’s responding BCR repertoire. RBD-specific antibodies are primary candidates for virus-neutralizing, potentially protective antibodies (Ju et al., 2020; Robbiani et al., 2020). With IGH from two COVID-19 patients (7455 and 7480), we expressed four mAbs (CoV.1–CoV.4) from a cluster that is convergent with BD-494 (Yuan et al., 2020), pairing these with the germline-reverted BD-494 light chain and reported CDR-L3. All four mAbs bound SARS-CoV-2 spike protein, S1, and RBD but not nucleocapsid (Figure 3C). BD-494 belongs to a cluster of SARS-CoV-2 RBD-specific antibodies (BD-500, BD-506, and BD-507) using IGHV3-53 and IGHJ6 with CDR-H3 length of 11 amino acid residues (Figure 3D). IGHV3-53 antibodies are reported to contact virus-neutralizing, potentially protective antibodies (Ju et al., 2020; Robbiani et al., 2020). With IGH from two COVID-19 patients (7455 and 7480), we expressed four mAbs (CoV.1–CoV.4) from a cluster that is convergent with BD-494 (Yuan et al., 2020), pairing these with the germline-reverted BD-494 light chain and reported CDR-L3. All four mAbs bound SARS-CoV-2 spike protein, S1, and RBD but not nucleocapsid (Figure 3C). BD-494 belongs to a cluster of SARS-CoV-2 RBD-specific antibodies (BD-500, BD-506, and BD-507) using IGHV3-53 and IGHJ6 with CDR-H3 length of 11 amino acid residues (Figure 3D). IGHV3-53 antibodies are reported to contact RBD with several germline-encoded residues in CDR-H1 and CDR-H2, as well as residues in CDR-H3 (Yuan et al., 2020). Contact residues in CDR-H1 and CDR-H2 had the germline IGHV-encoded sequence in CoV.2-CoV.4, whereas CoV.1 had a threonine to isoleucine change in CDR-H1 and an overall IGHV SHM frequency of 4.4% (Figure 3E). CoV.1-CoV.4 each differed from BD-494 by one amino acid residue in CDR-H3.

We hypothesized that in addition to sharing common RBD-binding antibody types, some COVID-19 patients might also demonstrate breadth in their antibody responses and recognize antigens from the distinct but related sarbecovirus, SARS-CoV that was responsible for SARS. Comparison with published SARS patient IGH data (Coughlin et al., 2007; Pinto et al., 2020; Robbiani et al., 2020) revealed convergent SARS-CoV RBD-specific antibodies in two COVID-19 patients, 7453 and 7455 (Figures S4B and S4C). These SARS-CoV-specific convergent IGH sequences were not detected in the 114 HHC samples. To evaluate whether such in silico IGH sequence comparisons could predict the serological responses of patients, we tested the plasma samples from COVID-19 patients in SARS-CoV RBD ELISAs and detected cross-reactivity in five of the 13 patients (Figure 3F). Strikingly, the two patients with the highest ELISA OD450 values for SARS-CoV RBD were those who had demonstrated convergent IGH sequences specific for SARS-CoV RBD.

**DISCUSSION**

In these initial months of the COVID-19 pandemic, understanding human antibody responses to SARS-CoV-2 has become a global priority. Our results provide several key findings that could lend some support for vaccine strategies currently under development and suggest that most individuals convalescent from SARS-CoV-2 infection could be, at least for some time, protected against reinfection by commonly elicited RBD-specific antibodies. The IGH repertoires of patients with diagnostically confirmed SARS-CoV-2 infection reveal robust polyclonal responses with early class switching to IgG, and to a lesser extent, IgA isotypes, as well as evidence of accumulating SHM in responding clones within the first month after onset of symptoms, rather than the delayed SHM seen in Ebola patients (Davis et al., 2019). We note that the current COVID-19 study and prior analysis of EBOV infection are among very few published studies of human IGH repertoire longitudinal responses to primary infections; examples from acute dengue virus infection (Appanna et al., 2016; Godoy-Lozano et al., 2016) or H5N6 avian influenza virus (Peng et al., 2019) either had few patients with true primary infection or did not analyze SHM development in responding B cells. Additional follow up of convalescent patients should further clarify the rates of SHM accumulation in SARS-CoV-2-specific BCRs.

Nine of 13 COVID-19 patients (69%) demonstrated convergent IGH specific for the viral RBD, a major target for potentially
Figure 3. Convergent IGH Sequences among COVID-19 Patients and to Reported Antigen-Specific IGH for SARS-CoV-2 and SARS-CoV

(A) Convergent IGH clusters among patient samples (top panel). The sample distribution is indicated by the lines and dots with the number of clusters sharing that sample distribution indicated by the vertical histogram bars. The total number of convergent clusters identified in each sample is indicated in the histogram to the left of the plot.

(Expanded panel) Lineages belonging to convergent clusters (y axis) shared across four or five patients (light green or dark green brackets, respectively) are plotted by the expressed isotype (x axis). Point color indicates SHM and size shows the number of unique reads.

(B) Distribution of the number of convergent clones shared in two to eight HHC subjects, determined by using 100 resamplings of 13 HHC individuals from the 114 total HHCs. The histogram shows the distribution of convergent clones shared by HHC samples over these trials. The black dashed line is the mean value, and the wide red dashed line is the number of convergent clones shared among the 13 COVID-19 participants.

(C) ELISA results for mAb binding to SARS-CoV-2 spike protein (Spike), S1 domain (S1), RBD, and nucleocapsid. Purified mAbs (mAb2A, mAb4A, B8, C11, D6, E7, F6, F10, mAb CR3022) or HEK293 cell supernatants (CoV.1–CoV.4) were tested. mAbs generated from COVID-19 patient convergent clones (red) or clones convergent with BD-494 (green) were compared to negative control mAbs B8, C11, D6, E7, F6, and F10 (black). The SARS-CoV-2 RBD-binding mAb CR3022
neutralizing antibodies. SARS-CoV-2 neutralizing serum antibodies are reported to be present in 67%–90% of patients post-infection, depending on the severity of disease, neutralization assay, and threshold for positive results (Robbiani et al., 2020; Suthar et al., 2020; Wu et al., 2020a). It seems reasonable to predict that vaccines based on spike or RBD antigens will also stimulate B cells expressing these common antibody types in a significant fraction of the human population. A subset of patients also had B cell clones expressing convergent IGH to previously described SARS-CoV RBD antibodies; strikingly, the patients with these SARS-CoV-2/SARS-CoV clonotypes also had the highest SARS-CoV RBD binding serum antibody IgG levels. This association suggests that it could become possible to predict the fine specificity of human serological responses from IGH sequence data, as the number of documented antigen-specific clonotypes in public databases increases. This example also highlights the possibility that common modes of human antibody response could enable some breadth of protection or humoral memory against other sarbecoviruses in the future. Longitudinal tracking of IGH repertoires in larger patient cohorts; further investigation into the binding properties, functional activity, and serum antibody levels produced by convergent responding clones in patients; and assessment of clinical outcomes under conditions of exposure to infection will be important next steps toward determining the immunological correlates of protection against SARS-CoV-2 infection.

**STAR METHODS**

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**SUPPLEMENTAL INFORMATION**

Supplemental Information can be found online at https://doi.org/10.1016/j.chom.2020.09.002.

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**AUTHOR CONTRIBUTIONS**

C.A.B. and S.D.B. conceived the project. C.A.B., K.C.N., A.R., and A.J.R. recruited, enrolled, and consented patients and contributed to clinical sampling and processing and support of clinical sample collection. S.C.A.N., R.A.H., K.R., G.H.J., B.S., J.-Y.L., A.E.P., M.H., J.N., A.R.O.-C., K.E.Y., B.D., and B.A.P. performed the experiments. M.H., H.Y.C., A.T.S., K.C.N., T.S.J., P.S.K., and T.T.W. contributed to serological or 10x assays and analysis. S.C.A.N., F.Y., K.J.L., R.A.H., K.R., G.H.J., S.D.B., and B.A.P. contributed to data analysis. S.C.A.N., F.Y., K.J.L., R.A.H., K.R., K.E.Y., C.A.B., and S.D.B. wrote the manuscript. All authors edited the manuscript.

**DECLARATION OF INTERESTS**

A.T.S. is a scientific founder of Immunai and receives research funding from Arsenal Biosciences not related to this study. M.H. is an employee of ATUM. S.D.B. has consulted for Regeneron, Sanofi, and Novartis on topics unrelated to this study. S.D.B., K.R., P.S.K., and A.E.P. have filed provisional patent applications related to serological tests for SARS-CoV-2 antibodies. K.C.N. reports grants from National Institute of Allergy and Infectious Diseases (NIAID), Food Allergy Research & Education (FARE), and End Allergies Together (EAT);
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### Key Resources Table

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**       |        |            |
| Goat anti-Human IgM-HRP | Sigma  | Cat#A6907; lot#SLCD6915; RRID: AB_258318 |
| Goat anti-Human IgG-HRP | Thermo Fisher | Cat#62-8420; lot#VC298928; RRID: AB_88136 |
| Rabbit anti-Human IgA-HRP | Dako  | Cat#P0216 |
| Rabbit anti-Human IgG-HRP | Agilent | Cat#P0214 |
| mAb CR3022           | Expressed for this paper | N/A |
| mAb2A                | This paper | N/A |
| mAb4A                | This paper | N/A |
| CoV.1                | This paper | N/A |
| CoV.2                | This paper | N/A |
| CoV.3                | This paper | N/A |
| CoV.4                | This paper | N/A |
| **Biological Samples** |        |            |
| COVID-19 patient samples | Stanford Healthcare | N/A |
| Healthy adult control samples | Stanford Blood Center | N/A |
| **Chemicals, Peptides, and Recombinant Proteins** |        |            |
| SARS-CoV RBD protein | This paper | N/A |
| SARS-CoV-2 RBD protein | ATUM  | Cat#CoV2-RBD-His |
| SARS-CoV-2 spike ectodomain protein | ATUM  | Special request |
| SARS-CoV-2 spike S1 protein | ATUM  | Cat#CoV2-S1-Ctag |
| SARS-CoV-2 nucleocapsid | ATUM  | Special request |
| **Critical Commercial Assays** |        |            |
| AllPrep DNA/RNA kit | QIAGEN  | Cat#80204 |
| MiSeq Reagent Kit v3 for isotype library sequencing | Illumina | Cat#MS-102-3003 |
| MiSeq Reagent Kit v2 for gDNA library sequencing | Illumina | Cat#MS-102-2003 |
| MiSeq Reagent Kit v3 for 10x library sequencing | Illumina | Cat#MS-102-3001 |
| 10x Single Cell Immune Profiling Solution Kit (v1.1) | 10x Genomics | Cat#1000127; Cat#1000167; Cat#1000016; Cat#1000213 |
| HiTrap Protein A HP columns for antibody purification | GE healthcare | Cat#29-0485-76 |
| **Deposited Data** |        |            |
| COVID-19 IGH repertoire data (Illumina NGS) | This paper | BioProject: PRJNA628125 |
| Healthy adult control IGH repertoire data (Illumina NGS) | Nielsen et al., 2019 | BioProject: PRJNA491287 |
| **Experimental Models: Cell Lines** |        |            |
| HEK293-EBNA1-6E | National Research Council, Canada | RRID: CVCL_HF20 |
| Exp293F | Thermo Fisher | Cat#A14528; RRID: CVCL_D615 |
| **Oligonucleotides** |        |            |
| Primers for isotype/gDNA libraries (PCR1), see Table S3 | Integrated DNA Technologies | N/A |

(Continued on next page)
**RESOURCE AVAILABILITY**

**Lead Contact**
Further information and requests for resources and reagents should be directed to the Lead Contact, Dr. Scott D. Boyd (sboyd1@stanford.edu).

**Materials Availability**
Unique reagents generated in this study will be provided upon reasonable request.

**Data and Code Availability**
All data is available in the main text or the extended materials. Code will be provided to readers upon request. The accession number for the 10x Genomics data used for monoclonal antibody expression and the IGH repertoire data reported in this paper is BioProject: PRJNA628125. The IGH repertoire data for the healthy human control (HHC) dataset is in BioProject: PRJNA491287.

**EXPERIMENTAL MODELS AND SUBJECT DETAILS**

**COVID-19 Patient Samples**
Patients admitted to Stanford Hospital with signs and symptoms of COVID-19 and confirmed SARS-CoV-2 infection by reverse-transcription quantitative polymerase chain reaction (RT-qPCR) of nasopharyngeal swabs were recruited. Demographic and clinical data for patients are provided in Table 1. Venipuncture blood samples were collected in dipotassium ethylenediaminetetraacetic acid (K2EDTA)- or sodium heparin-coated vacutainers for peripheral blood mononuclear cell (PBMC) isolation or serology on plasma, respectively. Recruitment of COVID-19 patients, documentation of informed consent, collections of blood samples, and experimental measurements were carried out with Institutional Review Board approval (IRB-55689 and IRB-48973) at Stanford University.

**Healthy Human Control Samples**
The dataset containing healthy adult control immunoglobulin heavy chain (IGH) repertoires has been described previously (Nielsen et al., 2019). In summary, healthy adults with no signs or symptoms of acute illness or disease were recruited as volunteer blood donors at the Stanford Blood Center. Pathogen diagnostics were performed for cytomegalovirus, human immunodeficiency virus,
hepatitis C and B virus, West Nile virus, human T cell leukemia virus, TPPA (Syphilis), and Trypanosoma cruzi. Volunteer age range was 17-87 with median and mean of 52 and 49, respectively.

**Cell Lines**

HEK293-EBNA1-6E cells were from National Research Council (NRC), Canada; RRID:CVCL_HF20. Expi293F cells were from Thermo Fisher (cat. A14528; RRID:CVCL_D615). Cell lines were cultured using manufacturer’s guidelines and used as described in Method Details below.

**METHOD DETAILS**

**Viral and Serological Testing**

SARS-CoV-2 infection in patients was confirmed by RT-qPCR testing of nasopharyngeal swab specimens, using the protocols described in (Corman et al., 2020; Hogan et al., 2020). An enzyme-linked immunosorbent assay (ELISA) based on a protocol described in (Stadlbauer et al., 2020) was performed to detect anti-SARS-CoV and anti-SARS-CoV-2 spike receptor-binding domain (RBD) antibodies in plasma samples from COVID-19 patients. Briefly, 96-well high binding plates (Thermo Fisher) were coated with either SARS-CoV or SARS-CoV-2 spike RBD protein (0.1 μg per well) overnight at 4°C. After blocking plates with 3% non-fat milk in phosphate-buffered saline (PBS) containing 0.1% Tween 20 (PBST), plasma samples were incubated at a dilution of 1:100 and bound antibodies were detected with goat anti-human IgM/horseradish peroxidase (HRP) (Sigma: cat. A6907; 1:6,000 dilution), goat anti-human IgG/HRP (Thermo Fisher: cat. 62-8420, 1:6,000 dilution), or rabbit anti-human IgA/HRP (Dako: cat. P0216, 1:5,000 dilution). Assays were developed by addition of 3,3’5,5’-tetramethylbenzidine (TMB) substrate solution. After stopping the reaction with 0.16 M sulfuric acid, the optical density (OD) at 450 nanometers was read using an EMax Plus microplate reader ( Molecular Devices).

The cut-off value for seroconversion was calculated as OD450 = 0.2 for the anti-SARS-CoV IgG assay and as OD450 = 0.3 for anti-SARS-CoV-2 IgM, IgG, and IgA assays after analyzing SARS-CoV-2 pre-pandemic negative control samples from healthy blood donors.

**IGH Library Prep and Sequencing**

The AllPrep DNA/RNA kit (QIAGEN) was used to extract genomic DNA (gDNA) and total RNA from PBMCs. For each blood sample, six independent gDNA library PCRs were set up using up to 100 ng template/library. Multiplexed primers to IGHJ and the IGHV FR1 or FR2 framework regions (3 FR1 and 3 FR2 libraries), per the BIOMED-2 design were used (van Dongen et al., 2003) with additional sequence representing the first part of the Illumina linkers (Table S3). In addition, for each sample, total RNA was reverse-transcribed to cDNA using Superscript III RT (Invitrogen) with random hexamer primers (Promega). Total RNA yield varied between patients and between 6 ng-100 ng was used for each of the isotype PCRs using IGHV FR1 primers based on the BIOMED-2 design (van Dongen et al., 2003) with additional six independent gDNA library PCRs were set up using up to100 ng template/library. Multiplexed primers to IGHJ and the IGHV FR1 or FR2 framework regions (3 FR1 and 3 FR2 libraries), per the BIOMED-2 design were used (van Dongen et al., 2003) with additional sequence representing the first part of the Illumina linkers (Table S3). The different isotypes were amplified in separate reaction tubes. Eight-nucleotide barcode sequences were included in the primers to indicate sample (isotype and gDNA libraries) and replicate identity (gDNA libraries). Four randomized bases were included upstream of the barcodes on the IGHU primer (gDNA libraries) and constant region primer (isotype libraries) for Illumina clustering (Table S3). PCR was carried out with AmpliTaq Gold (Applied Biosystems) following the manufacturer’s instructions, and used a program of: 95°C 7 min; 3 cycles of 94°C 30 s, 58°C 45 s, 72°C 60 s; and final extension at 72°C for 10 min. A second round of PCR using QIAGEN’s Multiplex PCR Kit was performed to complete the Illumina sequencing adapters at the 5-prime and 3-prime ends of amplicons; cycling conditions were: 95°C 15 min; 12 cycles of 95°C 30 s, 60°C 45 s, 72°C 60 s; and final extension at 72°C for 10 min. Products were subsequently pooled, gel purified (QIAGEN), and quantified with the Qubit fluorometer (Invitrogen). Samples were sequenced on the Illumina MiSeq using 600 cycle kits for isotype libraries (PE300) and 500 cycle kits for gDNA libraries (PE250).

**IGH Sequence Analysis**

Paired-end reads were merged using Fast Length Adjustment of Short reads software (Magoč and Salzberg, 2011), demultiplexed (100% barcode match), and primer trimmed. The IGHV, IGHD, and IGJH gene segments and IGHV-IGHD (N1), and IGHJ-IGHJ (N2) junctions were identified using the IgBLAST alignment program (Ye et al., 2013). Quality filtering of sequences included keeping only productive reads with a CDR-H3 region, and minimum IGHV-gene alignment score of 200. Sample cDNA or gDNA libraries with poor read coverage were excluded from further analysis (Table 1). For cDNA-templated IGH reads, isotypes and subclasses were called by exact matching to the constant region gene sequence upstream of the primer. Clonal identities within each subject were inferred using single-linkage clustering and the following definition: same IGHV and IGHJ usage (disregarding allele call), equal CDR-H3 length, and minimum 90% CDR-H3 nucleotide identity. A total of 1,259,882 clones (per sample, mean number of clones: 33,154; median number of clones: 18,503) were identified. A total of 24,888,790 IGH sequences amplified from cDNA were analyzed for the COVID-19 subjects (mean: 754,205 per sample; median: 650,812) and 68,831,446 sequences from healthy adult controls (mean: 603,785 per individual; median: 637,269). Each COVID-19 patient had on average 372,304 in-frame gDNA sequences and each adult control had an average of 8,402 in-frame gDNA sequences.

For each clone, the median somatic mutation frequency of reads was calculated. Mean mutation frequencies for all clonal lineages from a sample for each isotype were calculated from the median mutation frequency within each clone, and so represent the mean of
the median values. Clones with < 1% mutation were defined as unmutated and clones with greater or equal to 1% mutation were defined as being mutated. Subclass fractions were determined for each subject by dividing the number of clones for a given subclass by the total number of clones for that isotype category. Expanded clones within each sample were defined as clones that were present in two or more of the gDNA replicate libraries. Clonal expansion in the isotype data was inferred from the gDNA data. Analyses were conducted in R (Team, 2017) using base packages for statistical analysis and the ggplot2 package for graphics (Wickham, 2016).

To identify convergent rearranged IGH among patients with SARS-CoV-2 infection, heavy-chain sequences annotated with the same IGHV and IGHJ segment (not considering alleles) and the same CDR-H3 length were clustered based on 85% CDR-H3 amino acid sequence similarity using CD-HIT (Fu et al., 2012). To exclude IGH that are generally shared between humans and to enrich the SARS-CoV-2-specific IGH that are likely shared among the patients, clusters were selected as informative if (1) they contained at least five IGH sequences from each COVID-19 patient and were present in at least two subjects; (2) no IGH sequences from SARS-CoV-2-specific IGH were present in COVID-19 patients and previously reported IGH sequences to SARS-CoV and SARS-CoV-2. Convergent IGH sequences between the deeply sequenced COVID-19 patients and 10x Genomics single B cell immune profiling on two COVID-19 patients were selected for mAb expression. To assess whether the number of convergent clones identified among COVID-19 patients was significantly greater than the number expected by chance in HHC repertoires, we randomly selected with replacement sets of 13 HHC individuals from the 114 available, determining the number of convergent clones detected in two, three, four or larger numbers of participants, and repeated this process 100 times to generate the distribution of convergent clone counts shown in Figure 3B.

**Single-Cell Immunoglobulin Analysis**

Single-cell immunoglobulin (Ig) libraries were prepared using the 10x Single Cell Immune Profiling Solution Kit (v1.1 Chemistry), according to the manufacturer’s instructions. Briefly, cells were washed once with PBS + 0.04% BSA. Following reverse transcription and cell barcoding in droplets, emulsions were broken and cDNA purified using Dynabeads MyOne SILANE (Thermo Fisher) followed by PCR amplification (98 °C for 45 s; 15 cycles of 98 °C for 20 s, 67 °C for 30 s, 72 °C for 1 min; 72 °C for 1 min). For targeted Ig library construction, 2 μL of amplified cDNA was used for target enrichment by PCR (Human B cell primer sets 1 and 2: 98 °C for 45 s; 6 and 8 cycles of 98 °C for 20 s, 67 °C for 30 s, 72 °C for 1 min; 72 °C for 1 min). Following Ig enrichment, up to 50 ng of enriched PCR product was fragmented and end-repaired, size selected with SPRISelect beads (Beckman Coulter), PCR amplified with sample indexing primers (98 °C for 45 s; 9 cycles of 98 °C for 20 s, 54 °C for 30 s, 72 °C for 20 s; 72 °C for 1 min), and size selected with SPRISelect beads. Targeted single-cell Ig libraries were sequenced on an Illumina MiSeq to a minimum sequencing depth of 5,000 reads/cell using the read lengths 26bp Read1, 8bp i7 Index, 91bp Read2 and reads were aligned to the prebuilt GRCh38/Ensembl/10x reference dataset and consensus Ig annotation was performed using Cell Ranger (10x Genomics, version 3.1.0).

**IGH Sequence Analysis for mAb Expression**

IGH sequences from single cells with paired productive heavy and light chains were searched against COVID-19 patient bulk IGH repertoires to identify convergent sequences according to the following criteria: utilization of the same IGHV and IGHJ genes; same CDR-H3 lengths; and CDR-H3 amino acid sequences that were within a Hamming distance cutoff of 15% of the length of the CDR-H3. Two native heavy and light chain pairs, designated mAb2A and mAb4A, which were found in convergent clusters characterized by low- to mid-SHM frequencies and included at least one class-switched member, were selected for cloning and expression. Additionally, four IGHs were selected from clones that clustered with SARS-CoV-2 RBD-specific antibody BD-494. Full-length light chain sequence for the BD-494 was not available so to produce mAbs for CoV.1-CoV.4, the four IGH were paired with a germ-line-reverted light chain that utilizes IGKV1-9, IGKJ3, and the published CDR-L3 sequence for BF-494, ‘QQLNSYPFT’.

**mAb Cloning**

Heavy and light chain sequences were synthesized by Integrated DNA Technologies as gBlocks encoding full-length heavy and light chain V(D) regions. gBlocks were resuspended at 50 ng/μL and amplified with AmpliTaQ Gold (Applied Biosystems) following the manufacturer’s instructions, using a program of: 95 °C 7 min; 30 cycles of 94 °C for 30 s, 55 °C for 45 s, 72 °C for 60 s; final extension at 72 °C for 10 min. Products were gel purified (QIAGEN) and cloned as in-frame fusions to human IgG1, IgK or IgL constant regions into the pTT5 vector (Durocher et al., 2002) (National Research Council (NRC), Canada) using Gibson Assembly Master Mix (New England Biolabs) for 45 min at 50 °C. Assembled constructs were verified by Sanger sequencing.

**mAb Expression and Purification**

Constructs were transiently transfected in HEK293-EBNA1-6E cells (NRC) or Exp293F cells (Thermo Fisher) at a density of 1.2-1.6 million cells/mL or 3 million cells/mL, respectively, using 25 kDa linear polyethylenimine (PEI) at a 3:1 PEI:DNA ratio in OptiMEM reduced serum medium (GIBCO), with a heavy chain: light chain ratio of 1:1. HEK293-EBNA1-6E cells were maintained in Freestyle 293 Expression Medium (GIBCO) and were supplemented with 0.5% tryptone 24-36 h after transfection. Exp293F cells were maintained in Exp293 Expression Medium (Thermo Fisher). Cell supernatants were harvested after 96 h and filtered through 0.45-μM filters (Millipore). Antibodies were purified via HiTrap Protein A HP columns (GE Healthcare) run at a flow rate of 0.5-1 mL/min on Äkta Start protein purification system (GE Healthcare). Antibodies were eluted using 0.1M glycine pH 2, dialyzed with 3 changes
of PBS pH 7.4 using Slide-A-Lyzer-G2 10K dialysis cassettes (Thermo Fisher), and concentrated using 30,000 kDa molecular weight cutoff polyethersulfone membrane spin columns (Pierce). Final concentrations of purified antibodies were quantified with Nanodrop 2000 (Thermo Fisher).

**ELISA Testing of mAbs**

ELISA conditions for mAbs were as described for COVID-19 plasma samples with the following modifications. Purified mAbs were tested at 50 \( \mu \text{g/mL} \) for intra-COVID-19 convergent antibodies or peanut-specific negative mAb controls, or at 0.506 \( \mu \text{g/mL} \) for mAb CR3022; antibody supernatants were diluted 1:1 with PBST plus 1% milk; plates were coated overnight with RBD (0.1 \( \mu \text{g per well} \)), S1 (0.5 \( \mu \text{g per well} \)), spike protein (0.1 \( \mu \text{g per well} \)), or nucleocapsid (0.1 \( \mu \text{g per well} \)); and bound mAbs were detected with rabbit anti-human IgG gamma chain-specific/HRP (Agilent: cat. P0214, 1:15,000 dilution).

**QUANTIFICATION AND STATISTICAL ANALYSIS**

Statistical tests were performed in R (Team, 2017) using base packages for statistical analysis and the ggplot2 package was used for graphics (Wickham, 2016). Box-whisker plots show median (horizontal line), interquartile range (box), and 1.5 times the interquartile range (whiskers). Linear regression lines were plotted with 95% confidence intervals calculated using the function `stat_smooth`. In all analyses where statistical significance was tested, significance was defined as: ***p value < 0.001; **p value < 0.01; *p value < 0.05; not significant (NS): p value > 0.05. Differences between the seropositive group and HHC (Figure 2A) was tested using two-sided Wilcoxon–Mann-Whitney (for patients with more than one sample, the mean value of these was used). Patient IGHV gene SHM for seroconverted and non-seroconverted samples were compared to HHC (Figure 2D) using paired Wilcoxon tests with Bonferroni correction for multiple hypothesis testing. Differences between the expanded/non-expanded groups and HHC (Figure 2F) were tested using one-way ANOVA with Tukey’s HSD test.