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Maturation and persistence of the anti-SARS-CoV-2 memory B cell response.

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Summary.

Memory B cells play a fundamental role in host defenses against viruses, but to date, their role has been relatively unsettled in the context of SARS-CoV-2. We report here a longitudinal single-cell and repertoire profiling of the B cell response up to 6 months in mild and severe COVID-19 patients. Distinct SARS-CoV-2 spike-specific activated B cell clones fueled an early antibody-secreting cell burst as well as a durable synchronous germinal center response. While highly mutated memory B cells, including pre-existing cross-reactive seasonal Betacoronavirus-specific clones, were recruited early in the response, neutralizing SARS-CoV-2 RBD-specific clones accumulated with time and largely contributed to the late remarkably stable memory B-cell pool. Highlighting germinal center maturation, these cells displayed clear accumulation of somatic mutations in their variable region genes over time. Overall, these findings demonstrate that an antigen-driven activation persisted and matured up to 6 months after SARS-CoV-2 infection and may provide long-term protection.

Introduction

The new emerging coronavirus, SARS-CoV-2, has infected 88 million people and killed over 1.9 million individuals worldwide since the beginning of the pandemic. Understanding the mechanisms underlying the establishment of protective immune memory in recovering individuals is a major concern for public health and for anticipating vaccination outcomes.

In response to viral infection, virus-specific T and B cells are activated, expand and differentiate into effector cells (Wherry and Ahmed, 2004). At the early phase of COVID-19 infection, most infected individuals generate antibodies targeting the viral nucleocapsid (N) and the spike (S) proteins of SARS-CoV-2, including spike receptor binding domain (RBD) specific antibodies with strong neutralizing potential (Wajnberg et al., 2020). COVID-19 patients also develop potent early CD8⁺ and CD4⁺ T cell specific responses (Braun et al., 2020; Ferretti et al., 2020; Grifoni et al., 2020, 2020; Le Bert et al., 2020; Meckiff et al., 2020; Peng et al., 2020; Sekine et al., 2020; Swadling and Maini, 2020)

Studies have documented that B cells from patients with severe COVID-19 harbored low mutations frequencies in their heavy-chain variable region (V_H) genes, notably those producing anti-RBD antibodies (Nielsen et al., 2020). This suggested an active early extrafollicular response in which naïve unmutated B cells first engage in cognate interactions with T cells and become fully activated to divide and differentiate into plasma cells (Jenks et al., 2019). Along with limited somatic hypermutation (SHM) and selection, cells derived from extrafollicular responses are usually considered as short-lived (Mac Lennan et al., 2003).

Long-term humoral immunity following infection relies on two types of cells derived from germinal center responses: long-lived plasma (LLPC), which continuously secrete antibodies (Slifka et al., 1998) and memory B cells (MBCs), which can expand and differentiate into antibody-secreting cells (ASC) upon a new antigenic challenge (Yoshida et al., 2010). MBCs can also adapt to cope with antigenic changes linked to virus mutations, as exemplify by the coevolution of neutralizing antibodies against mutating epitopes during HIV infection (Escolano et al., 2016; Liao et al., 2013; Wu et al., 2011). Assessing whether a MBC response, with the ability to rapidly produce SARS-CoV-2 neutralizing antibodies upon a new infectious challenge months or years after initial infection, is indeed established in convalescent COVID-19 patients is thus of immediate relevance for ongoing modeling of herd immunity and the formulation of vaccine strategies.

Serological studies have so far reported contradictory results on the persistence of humoral immunity in asymptomatic, mild and severe patients (Iyer et al., 2020; Long et al., 2020; Luchsinger et al., 2020; Pierce et al., 2020; Reynolds et al., 2020; Ripperger et al., 2020; Röltgen et al., 2020; Wajnberg et al., 2020; Weisberg et al., 2021). Furthermore, although memory B cells have been observed up to 6 months after infection in an increasing number of recent publications (Chen et al., 2020; Dan et al., 2021; Gaebler et al., 2020; Hartley et al., 2020; Nguyen-Contant et al., 2020; Rodda et al., 2020; Vaisman-Mentesh et al., 2020), a recent study suggested that severe SARS-CoV-2 infection may blunt the germinal center (GC) response and subsequently compromise the generation of long-lived, affinity-matured memory B cells (Kaneko et al., 2020). It was also suggested that the strong extra-follicular B cell activation observed after symptomatic SARS-CoV-2 infection could prevent or delay a germinal center-dependent immune response (Woodruff et al., 2020).

In this context, analysis of the longevity and functionality of the anti-SARS-CoV-2 memory B cell response becomes a major issue, notably to evaluate the protection afforded by a prior infection to subsequent viral challenges.

In the present study, we present a longitudinal deep profiling of the anti-SARS-CoV-2 memory B cell response in two parallel cohorts of patients with severe (S-CoV) and mild (M-CoV) COVID-19. We combined single cell transcriptomics, single cell culture and IgH VDJ sequencing to track and characterize the cellular and molecular phenotype and clonal evolution of spike-specific MBC clones from early time points after SARS-CoV-2 infection up to 6 months after the initial symptoms. Our results provide new insights on the origin, magnitude and stability of the anti-SARS-CoV-2 MBC response and revealed in most patients the robust acquisition of a GC-derived immune memory.

Results

Investigating anti-SARS-CoV-2 humoral response in mild and severe COVID-19 patients.

To characterize the longitudinal evolution of the humoral response against SARS-CoV-2, we set-up a prospective cohort of patients with severe COVID-19 requiring oxygen (S-CoV, n=21, median age: 57 years [40-78], corresponding to WHO progression scale > 4 and ≤ 8) and mild ambulatory forms of COVID-19 (M-CoV, n=18, median age 35.5 years [28-64], corresponding to WHO progression scale < 4 (Marshall et al., 2020)) (MEMO-COV, NCT04402892, see **Methods and Table S1**). Initial samples were collected from patients in median 18.8 days (\pm SD: 8.8) and 35.5 days (\pm SD: 12.8) after disease onset for S-CoV and M-CoV respectively (M0). Two additional blood samples were collected at 3 (M3), and 6 months (M6), at a stage when all patients had fully recovered. All sera were first examined for SARS-CoV-2-specific IgG antibodies against the immunodominant nucleocapsid (N) and spike (S) proteins using commercial ELISA. As previously observed in other studies (Ripperger et al., 2020), N-specific IgG antibodies showed a rapid decrease in both cohorts (**Figure 1A**). This decrease was particularly pronounced in M-CoV patients, among which 9 out of 18 had titers below the threshold of positivity at 6 months (**Figure 1A, Table S1**). In contrast, S-specific IgG antibodies appeared quite stable with time and only two M-CoV patients showed anti-S antibodies titers below detection threshold at the time of our last sampling (**Figures 1B-C**). Levels of S-specific IgG antibodies at 3 and 6 months, however, were significantly higher in patients who had developed a more severe form of the disease (**Figures 1B-C**). Elevated titers of anti-S IgG strongly correlated with enhanced neutralization potential of patients' sera in vitro (**Figure 1D, Figures S1A-B**), confirming the spike trimeric glycoprotein as a primary target of the protective immune response in patients.

To further track the SARS-CoV-2-specific memory B cell response, we next implemented two complementary in-depth approaches on four S-CoV patients' samples. First, we generated His-tagged trimeric SARS-CoV-2 spike ectodomain and used successive staining with His-tagged spike protein and two fluorescently labeled anti-His antibodies to stain and perform high purity single-cell sorting of SARS-CoV-2 spike-specific B cells (**Figure 1E**). Single B cells were further cultured in an optimized in vitro culture assay (McCarthy et al., 2018) (**Figure 1F**). After 21 days of culture, RNA was extracted from single-cell cultures to determine their immunoglobulin heavy chain (IgH) sequence. Culture supernatants with IgG concentration over 1 μ g/ml were additionally tested by ELISA to

validate the cell specificity towards SARS-CoV-2 spike protein (over 95 % purity in this assay (**Figure S1C**)), investigate its recognition of the SARS-CoV-2 spike receptor binding domain (RBD) and its neutralization potential, and analyze potential cross-reactivity to spike proteins from seasonal coronaviruses (HCoV-HKU1/HCoV-OC43). In parallel, to get a broader view of the overall B cell response towards SARS-CoV-2 at both early (M0) and late time points (M6), we sorted CD19⁺IgD⁻ B cells from the same patients (**Figure S1D-H**) and performed scRNA-seq using the 10X Genomics technology. This allowed us to couple both 5' single-cell RNA sequencing, V(D)J profiling and Feature Barcoding of 20 known B cell surface markers, and we developed as well anti-His barcoded antibodies to reveal His-tagged SARS-CoV-2 spike-specific B cells.

Spike-responding B cells harbor phenotypically and transcriptionally an activated phenotype and a time-dependent maturation toward the memory B cell lineage.

Unsupervised clustering analysis of our scRNA-seq dataset revealed that CD19⁺IgD⁻ B cells could be divided in 5 major clusters according to their gene expression profile (**Figure 2A-B, S2A-B and Table S2**), a clustering which correlated nicely with the expression of barcoded surface markers included in our dataset (**Figure 2C-D, Figure S2C**). Two of these clusters corresponded to antibody-secreting cells (ASCs), with both a short-lived plasmablast cluster (PB), enriched for expression of cell proliferation-related genes, and non-dividing plasma cells (PC). Both PB/PC populations were strongly reduced by 6 months, confirming the resolution with time of the primary extrafollicular response in these severe COVID-19 patients (**Figure 2B, Table S2**). The remaining B cells were separated in 3 populations: a mixture of naïve/transitional B cells, a resting memory B cell population (MBCs) and a CD95⁺ activated cluster. This activated cluster could be further subdivided in 3 distinct populations: CD21^{low}CD27⁺CD38⁺CD71⁺ activated B cells (ABC), CD21^{low}CD27^{low}CD38⁻CD71^{low}CD11c⁺FcRL5⁺ cells, likely corresponding to atypical memory and/or double-negative 2 population (DN2) (Sanz et al., 2019) and CD21⁺CD27^{int/+}CD38⁻CD71^{low}CD95⁺ cells corresponding to a cluster with intermediate characteristics between ABC and memory B cells (**Figures 2C-D and S2B-C**). The contraction of the primary response, clearly observed for ASCs, could also be seen, albeit less pronounced, for ABCs, with a parallel increase in resting MBCs. All other clusters remained stable (**Fig. 2B**).

To gain insight into the specificity of this B cell response and the clonal relationship between the different B cell clusters, we first used the initial burst of ASCs seen at M0 as a proxy for the overall SARS-CoV-2-specific B cell response. We thus focused on the top 10%

ASC clones which represents 40-55% of the PB/PC analyzed (**Table S2**). Analysis of this top 10% ASC clones revealed a strong link between the ABC cluster and the ASC population (**Figures 2E and S2F, Table S2**). The bimodal distribution of somatic hypermutation (SHM) seen in ASCs further suggests the existence of a strong extra-follicular reaction recruiting newly engaged naïve and memory B cells, which fuels the primary response towards SARS-CoV-2 (**Figure 2G and 2 H**). In line with the resolution of the immune response, most of these clones were no longer present in ASCs six months after SARS-CoV-2 infection, with only few cells, clonally-related to the original ASC burst, still detectable in residual PC, while they were increased in the resting MBC compartment by that time (**Figures 2E and 2F, S2D and S2E**). Parallel analysis of barcoded-spike reads in our dataset allowed us to identify clear populations of spike-specific ABCs and ASCs at M0 (**Fig. 2E, S2E and Table S2**). By 6 months, spike-barcoded specific cells mainly localized in the resting MBC cluster (**Fig. 2E**). We performed a second analysis of spike-specific cells in our dataset, identified either as barcoded-spike cells or as cells clonally related to cells identified as spike-specific by single cell culture, to increase their representation (**Fig. S2 G-F and description in Methods**). We found limited clonal overlap of spike-specific memory B cells at M6 with the initial ASC response, suggesting that two distinct, albeit synchronous responses, take place in Covid-19 patients (**Fig. S2F-H and Table S2**).

Spike-specific memory B cells mature from ABCs and accumulate up to 6 months post-infection in COVID-19 patients.

To further analyze the dynamics of the overall and SARS-CoV-2 S-specific B cell response at the level of our entire cohort, we next performed multi-parametric FACS analysis using a flow panel which included 7 surface markers identified in our scRNA-seq dataset as major markers of activated B cells and ASC subsets involved in the initial response (CD19, CD21, CD27, CD38, CD71, CD11c, IgD, as well as His-tagged trimeric S protein). Unsupervised analysis of CD19⁺IgD⁻ switched B cell populations (**Figures S3A-C**), concatenated from 83 samples collected at M0, M3 and M6 from patients in our cohort, confirmed our initial observation that spike-specific B cells were enriched in a cluster of CD27⁺CD38^{int/+}CD71⁺ ABCs and in ASCs at M0 (**Figures 3 A-C, Table S3**). The enrichment in ASCs was most prominent in severe COVID-19 patients and is in line with the strong ASC burst seen in these patients (**Figures S3 A-C**). Spike-specific ASCs were marginally detectable at 6 months, at which time point spike-specific B cells mostly resided in the CD21⁺CD27⁺CD38⁻CD71^{int/-} resting memory B cell compartment in both severe and mild

COVID-19 convalescent patients. Of note, some spike-specific cells were still found in the CD71⁺ ABC cluster at M6 in both groups of patients but were much less frequent in the DN2/atypical compartment (**Figure 3C**).

Based on these results, and using a more traditional gating strategy, we next focused our analysis on the switched CD27⁺IgD⁻CD38^{int/-} memory B cell compartment, which includes both ABCs and resting memory B cells (**Figure S1C**). The first overall analysis confirmed a contraction of the ASC compartment, with a stable size of both IgD⁻CD27⁺ B cells and activated B cells with time (**Figure S3 D-F**). In contrast to the rapid disappearance of spike-specific ASCs, both the percentage and absolute number of spike-specific CD27⁺IgD⁻ B cells appeared stable up to 6 months in the vast majority of patients in our cohort, and even continuously increased up to that time point in a subset of convalescent S-CoV patients (**Figures 3D-E and S3G and S3H**). Of note, and as previously mentioned, the double-negative population contained few spike-specific cells (**Figures S3I and S3J**). Convalescent S-CoV patients showed significantly higher frequencies of spike-specific MBCs at the 6-month time point of our study. Most M-CoV patients, however, still harbored a sizeable population of spike-specific memory B cells at 6 months post-infection (mean 0.94% ±0.17 of MBCs) and only one (M-CoV24) out of the 16 M-CoV patients analyzed at 6 months in our study showed a frequency of spike-specific switched CD27⁺ memory B cells below that of pre-pandemic healthy donors. Even more striking was that both M-CoV patients whose serum levels of S-specific IgG had dropped below detectable levels by 6 months (correlating with an absence of in vitro neutralizing potential) still harbored a clear population of spike-specific MBCs at that time point (0.41 and 0.60% of MBCs, respectively, both above the threshold of 0.20% (> 2 SD of the mean value observed in healthy donors)). Altogether, these results demonstrate the induction of a robust and stable spike-specific MBC population in both mild and severe COVID-19 patients. Aside from severity, none of the clinical parameters or treatment that we could monitor, notably corticosteroids, appeared to have any clear influence on the long-term establishment of these spike-specific MBCs (**Figures S3M-Q**).

To ensure that such stable levels of spike-specific CD27⁺ switched-memory B cells did not simply reflect a sustained immune response in these convalescent COVID-19 patients, we further used CD19 and CD71 to separate ABCs (CD19^{high}CD71⁺) and resting MBCs (CD19⁺CD71^{low}) among spike-specific cells over time, as previously described (Ellebedy et al., 2016). Confirming our initial observation in the four S-CoV patients included in the scRNAseq dataset (**Figure 2E**) and the clear enrichment of spike-specific B cells in cluster 3

of our unsupervised FACS analysis (**Figures 3A-C**), the majority of spike-specific memory B cells displayed a CD19^{high}CD71⁺ ABC phenotype in the days following infection (**Figure 3F**). In both S-CoV and M-CoV patients, the proportion of spike-specific ABCs steadily decreased over time, along with an increase of spike-specific classical, resting MBCs (**Figures 3G and 3H**). Interestingly, ABCs were still detectable in both cohorts at 6 months (approximately 20% in both cohorts), suggesting continued antigen-driven activation, as has been described after Ebola infection (Davis et al., 2019a). In S-CoV patients, a small fraction of these early responding MBCs expressed the transcription factor T-bet, but such expression appeared only transient (**Figure S3K and 3L**). More interestingly, we noted a significantly higher frequency of ABCs among spike-specific MBCs in S-CoV as compared to M-CoV patients, as well as a large inter-patient variability for this parameter in both groups (**Figure 3G**). This heterogeneity could not be simply explained by inter-patient variations in the time of initial sampling. To evaluate whether early B cell activation may impact later formation of memory B cells, we plotted the frequency of CD27⁺CD71⁺ ABC cells at M0 vs. the frequency of spike-specific memory B cells at M6. Interestingly, there was a positive, albeit modest, correlation between both values, indicating that such early B cell activation does not prevent the development of B cell memory against SARS-CoV-2 (**Figure 3I**).

Acquisition of somatic mutations in RBD-specific Ig VH genes of MBCs.

In order to better understand the origin, fine specificity and developmental pathways involved in the generation of memory B cells against SARS-CoV-2, we next performed single cell sorting and culture of spike-specific memory B cells at 3 and 6 months post-infection in all 4 donors previously analyzed in the context of our scRNA-seq experiment (**Figure 1E**). A total of 2412 cells at M3 and 2423 cells M6 from the 4 donors were sorted (**Table S2**), and 488 and 1027 supernatants (respectively at M3 and M6) post single-cell culture were tested for specificity against the SARS-CoV-2 spike protein as well as spike proteins from two other related seasonal Betacoronavirus (HCoV-HKU1 and HCoV-OC43) (Huang et al., 2020), and against the RBD domain of the SARS-CoV-2 spike protein which is the main target of neutralizing antibodies in COVID-19 patients (**Figures 4A, Table S4**). We found that a sizeable proportion of SARS-CoV-2 spike-specific memory B cells also recognized the spike protein of H-CoV-HKU1 and H-CoV-OC43, with many of these cells recognizing both, as previously reported (Shrock et al., 2020, Wec et al., 2020). The proportion of HCoV-HKU1 and HCoV-OC43 specific MBCs clones, however, readily decreased between M3 and M6 ($12.4\% \pm 4.9$ to $4.8\% \pm 1.7$ respectively), suggesting that these specificities, while actively

participating in the early response, are not preferentially recruited in the later memory B cell compartment. In contrast, the proportion of SARS-CoV-2 RBD-specific MBCs clones clearly increased between the M3 and M6 time points in all donors ($11.1\% \pm 2.1$ and $26.3\% \pm 5.1$ respectively) (**Figures 4A and 4B, Figure S4A**). SARS-CoV-2 RBD-specific clones were mostly sorted from resting CD71⁻CD27⁺ switched memory B cells at these late time points (**Figures S4B and S4C**) and were detectable within the same proportion in M-CoV and S-Cov patients at 6 months (**Figure 4B**). Finally, only a minority of SARS-CoV-2 RBD-specific clones cross-reacted with HCoV-HKU1 and HCoV-OC43 spike protein, as anticipated by the low similarity of the RBD domain between these different viruses (**Figures 4A**). Overall, these results suggested an early and rapid recruitment of cross-reactive memory B cells through the extra-follicular pathway, in parallel with the establishment of a new immune response against epitopes unique to SARS-CoV-2, with a delayed and progressive output of memory B cells.

To evaluate whether such memory B cell output corresponds to an ongoing GC response, we next evaluated the occurrence and evolution of somatic mutations in spike-specific MBCs. Heavy chain V_H sequencing from SARS-CoV-2 spike- or RBD-specific wells (**Table S2**) in our in vitro culture assay, at both M3 and M6 (**Figure S4D**), allowed us to identify clonal relationships with sequences obtained in our scRNA-seq dataset at both M0 and M6. Mutations in V_H sequences of SARS-CoV-2 S-specific clones at M0 displayed a bimodal distribution with both near germline and highly mutated sequences. In line with an initial recruitment of cross-reactive memory B cells into the anti-SARS-CoV-2 early humoral response, HCoV-HKU1 and HCoV-OC43 specific clones contained sequences that were already highly mutated at M0 (**Figure 4C**). In contrast, SARS-CoV-2 RBD-specific clones displayed low mutation numbers in V_H sequences, as previously described in numerous studies during the early stages of this pandemic (Brouwer et al., 2020; Kreer et al., 2020; Liu et al., 2020; Robbiani et al., 2020; Seydoux et al., 2020; Shi et al., 2020; Wec et al., 2020; Zost et al., 2020). Both early SARS-CoV-2 spike- and RBD-specific Ig V_H sequences showed high level of convergence between donors in our dataset, but also with published SARS-CoV-2 spike- and RBD-specific antibody sequences (43/874 sequences in clonal relationship with cells from our dataset) (**Figure S4E**). Although near germline sequences were initially present in spike-specific clones, and even predominated in those with SARS-CoV-2 RBD specificity in both our M0 dataset and in the literature, longitudinal tracking of clonal relationships at 3 and 6 months revealed a progressive acquisition of mutations in V_H sequences over time

(**Figures 4D-E and S4G-H**). Most importantly, over 85% of SARS-CoV-2 RBD-specific culture supernatants at M6 showed intermediate to strong neutralizing potential against SARS-CoV-2 *in vitro* in S-CoV patients and over 50% in M-CoV patients (**Figure 4F, S4I, Table S4**). Progressive accumulation of mutations in V_H sequences was not associated with a loss of neutralization potential (**Figure 4G, Table S2**). In contrast, a strong neutralizing capacity was identified along lineage trees including previously described near germline anti-SARS-CoV-2 RBD antibodies and the mutated versions that we identified at M6 in this work. (**Figures 4H and S4J**). Dynamics of such clones and emergence of highly mutated neutralizing sequences, along with the robustness of the memory response observed in S-CoV and M-CoV patients, demonstrate that MBCs bearing unique specificities and neutralizing potential against SARS-CoV-2 can expand and mature over time through an ongoing germinal center response.

Discussion

The long-term stability of humoral immune memory following viral infection is provided by separate pools of protective long-lived plasma cells (LLPCs) and memory B cells that can last for a lifetime (Amanna et al., 2007; Crotty et al., 2003; Mamani-Matsuda et al., 2008). Upon re-infection, long-lived memory B cells generate a new wave of short-lived plasma cells and LLPC, while preserving the pool of memory B cells. In this study, we focused on SARS-CoV-2 infected patients with mild or severe disease and investigated the establishment of humoral memory and how this memory relates to the primary B cell response that emerges at the early stage of infection.

Experimental infections of volunteers with Betacoronaviruses responsible for common colds and longitudinal serological studies have suggested the absence of durable immunity against these seasonal infections (Sariol and Perlman, 2020). Furthermore, early reports have shown potentially defective GC formation in severe COVID-19 patients in intensive care unit (Kaneko et al., 2020) and predominantly near-germline anti-SARS-CoV-2 neutralizing antibodies (Ju et al., 2020; Nielsen et al., 2020; Robbiani et al., 2020). Such observations suggested a strong bias of the anti-SARS-CoV-2 immune response towards the extrafollicular pathway in these patients, which may preclude the formation of long-term memory.

Our study demonstrates, in contrast, a remarkable stability of the overall spike-specific memory B cell population up to 6 months after infection, and even its expansion in severe

patients, extending recent observations on memory persistence in COVID-19 (Gaebler et al., 2020, Rodda et al., 2020). This is also consistent with recent results showing in a large cohort of patients that SARS-Cov-2 antigen-specific memory B -cells persist up to 8 months after infection (Dan et al., 2021).

Tracking of spike-specific B cells provided a more complex picture than previously anticipated, characterized by two synchronous responses with distinct dynamics throughout the extra-follicular reaction. Indeed, near germline B cell clones recognizing the SARS-CoV-2 spike protein were mobilized, but also pre-existing highly mutated MBCs specific for the spike protein of other seasonal Betacoronaviruses.

The longitudinal VDJ sequencing of spike-specific MBCs revealed that neutralizing SARS-CoV-2 RBD-specific clones, including convergent antibody rearrangements across donors, accumulated with time and acquired somatic mutations in their variable region genes, the hallmark of a GC-dependent immune response. Such GC imprinting is of major importance because it usually predicts the generation of long-lived memory B cells (Mesin et al., 2016). Along this line, a recent report observed the presence of viral proteins in the intestine of patients several months after the initial diagnostic, which could sustain such B cell response (Gaebler et al., 2020).

Cross-reactivity against the spike domain of Betacoronaviruses has been already documented in serological studies showing reactivity to conserved regions of the spike protein (Aydillo et al., 2020; Wang et al., 2020), by the presence of pre-existing antibodies recognizing conserved epitopes in the S2 protein of SARS-CoV-2 in uninfected individuals (Ng et al., 2020) and by cross-reactivity of sorted spike-specific B cells with seasonal coronavirus (Song et al., 2020; Wec et al., 2020). Such cross-reactivity also exists for T-cells (Braun et al., 2020; Grifoni et al., 2020; Le Bert et al., 2020), but its protective potential remains debated (Ng et al., 2020). The proportion of cross-reactive clones, however, decreased from 3 to 6 months, suggesting that, after an initial activation, these cross-reactive clones were not positively selected in the memory pool.

As observed previously by others for antibody titers (Ripperger et al., 2020), the overall strength and stability of the memory B cell response was positively correlated with the initial severity of COVID-19-associated pathologies in convalescent patients. Interestingly, the magnitude of the initial SARS-Cov-2-specific ABC response was not predictive of an impaired B cell memory, displaying in contrast a positive, albeit modest, correlation with the frequency of spike-specific memory B cells at 6 months. Activated B cells in COVID-19 patients have been previously reported with various phenotypes (Juno et al., 2020; Mathew et

al., 2020; Oliviero et al., 2020; Woodruff et al., 2020), including a double-negative CD11c-positive population (DN2). One study has also suggested the presence of SARS-CoV-2 RBD-specific DN2 cells as a correlate of critical illness (Kaneko et al., 2020). We found here, that most spike-responding B cells harbored an activated phenotype that notably differed from DN2 but was similar to the one described after Ebola or Influenza virus infection (Ellebedy et al., 2016). These SARS-CoV-2 spike-specific ABCs were in clonal relationship with expanded cells from the initial burst of antibody-secreting cells, but also with resting memory B cells that persisted at 6 months. Given the limited clonal overlap between the early ASC burst and the later mutated memory B cell response, it is tempting to suggest that a distinct part of ABCs is dedicated to fueling the GC response in these patients, while another part contribute to the ASC burst. Of note, the ABC population remains detectable up to 6 months post-infection in convalescent patients, with some, albeit few spike-specific cells, suggesting, as described for Ebola (Davis et al., 2019), an antigen-driven activation that persisted for months after infection.

Collectively, taking into account this report and previous studies, the COVID-19 infection seems in most non-critical cases to induce both an immediate, possibly protective antibody response, together with the ongoing maturation of memory B cells, which should give rise to neutralizing antibody-secreting cells upon reinfection. While this overall picture is positive, notably in the context of vaccine campaigns to come, the long-term duration of the protective memory B cell response remains an open question.

Limitations of Study

The relatively small size of the cohort does not allow us to identify patients' characteristics that would predict the maintenance of the immune memory. Furthermore, patients with severe and mild COVID-19 were not matched for age and comorbidities, thus differences between these two groups must be interpreted with caution. Finally, this study did not investigate whether memory B cells are generated after asymptomatic COVID-19. Thus, larger cohorts of individuals with a wider spectrum of disease severity would be mandatory in the future to provide a comprehensive picture of B cell memory against SARS-CoV-2.

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Figure 1. Longitudinal characterization of the humoral response against SARS-Cov-2 in severe and mild COVID-19 patients.

(A-B) Anti-SARS-Cov-2 nucleocapsid (N) (A) and spike (S) (B) serum IgG levels were measured by ELISA in 21 severe COVID-19 (S-CoV) and 18 mild COVID-19 (M-CoV) at M0 (white), M3 (light orange) and M6 (dark orange). The dashed line indicates the positivity threshold provided by the manufacturer. (C) Evolution of anti-SARS-Cov-2 S serum IgG levels over time post symptom onset in S-CoV (black dots, dark blue line) and M-CoV (white dots, light blue line) patients. Continuous lines indicate linear regression, colored area between dashed-lines indicates error bands ($R^2=0.049$ for S-CoV, ns and 0.0061 for M-CoV, ns, Pearson correlation). (D) Correlation between sera anti-SARS-Cov-2 S IgG levels and in vitro neutralization potential (% neutralization achieved at a 1/90 dilution) at M6 (n=10 S-CoV and 11 M-CoV patients). The line represents a simple linear regression (R^2 and p-value with Pearson correlation are shown) (E) Representative FACS plot of His-tagged SARS-Cov-2 S staining in gated live $CD19^+CD38^{int/-}CD27^+IgD^-$ B cells at M0 in two representative S-CoV (upper plot) and M-CoV patients (lower plot). (F) Overall study design. Anova and two-tailed Mann-Whitney tests (A-B). Linear regression with Pearson correlation analysis (D). **** $P < 0.0001$, ** $P < 0.01$, * $P < 0.05$.

See also Figure S1 and Table S1.

Figure 2. Characterization of the B cell response against SARS-CoV-2 in acute (M0) and convalescent (M6) severe COVID-19 patients.

(A) UMAP and clustering of 41083 B cells analyzed by scRNA-seq from four S-CoV patients at M0 (left panel) and M6 (right panel) (see Table S2). Upper square in both panels shows the results of increased clustering resolution for the “Activated” cell cluster. (B) Relative cluster distribution at M0 and M6 for all sorted cells (Top panel) and cells falling in the “Activated” cluster at initial clustering resolution. Bar indicates mean with SEM. (C) Feature Plots showing scaled normalized counts for CD27 and CD38 barcoded antibodies as well as S score and G2/M signature scores in all cells. (D) Feature plots showing scaled normalized counts for CD21, CD71, CD38, CD11c, CD95, CD27 and CD307e (FcRL5) barcoded antibodies in cells from the “Activated” cluster. (E) UMAP of all cells at M0 or M6, with cells belonging to one of the top 10% antibody secreting cell (ASC) clones highlighted (light blue). Cells stained by the barcoded-anti-His/His-tagged SARS-Cov-2-S protein combination (barcoded-SARS-Cov-2-S stained cells) are also highlighted (red when members of one of the top 10%

ASC clones at M0 ("shared"), dark blue otherwise). **(F)** Circus plots showing clonal relationships between cells from different UMAP clusters and time points. Blue lines indicate clonal relationships with one of the top 10% ASC clones at M0 and grey line all other clonal relationships. **(G)** Histograms showing the number of mutations in VH genes for cells belonging to one of the top 10% ASC clones at M0 according to their barcoded-SARS-Cov-2-S staining (top = unstained; bottom = stained). **(H)** Graph showing the frequencies of cells at the M0 time-point harboring 0-1 (white), 2-9 (grey) or ≥ 10 (black) mutations in their VH genes for all sorted cells according to their cluster of origin (left) as well as for cells stained by the barcoded-SARS-Cov-2 S protein and/or members of the top 10% ASC clones at M0, as defined in panel (E). RM two-way ANOVA and Sidak's multiple comparison tests (B). **** $P < 0.0001$, *** $P < 0.001$, * $P < 0.05$.

See also Figure S2 and Table S2.

Figure 3. Phenotypic evolution of the SARS-Cov-2 spike-specific B cell response up to six months post infection in mild and severe COVID-19 patients.

(A) UMAP projections of concatenated multi-parametric FACS analysis of CD19⁺IgD⁻ cells from all S-CoV (n=15) and M-CoV (n=16) patients analyzed over time in our cohort (Table S1). His-tagged labeled SARS-Cov-2 spike-specific cells are overlaid as red dots. **(B)** Unsupervised clustering (FlowSOM) was performed on the concatenated FACS dataset based on IgD, CD71, CD27, CD38, CD11c, CD19, CD21 fluorescence intensity. 95% density contours for each identified cluster is overlaid in the UMAP projection. **(C)** Cluster distribution for all analyzed SARS-Cov-2 spike-specific cells in identified clusters, at indicated time point. Bars indicate mean with SEM. **(D)** Proportion of SARS-Cov-2 spike-specific CD19⁺IgD⁻CD27⁺CD38⁻ MBCs at M0, M3 and M6 in S-CoV and M-CoV patients, as compared to 6 pre-pandemic healthy donor controls (HD). Each dot represents one patient, bars indicate mean with SEM. Dashed line indicates the mean + 2 SD of the detected frequency of SARS-Cov-2 spike-specific MBCs in HD. **(E)** Proportion of spike-specific MBCs over time post symptom onset in S-CoV (black dots, dark blue line) and M-CoV (white dots, light blue line) patients. Lines indicates linear regression ($R^2=0.051$, P -value=0.02 for S-CoV, ns, and $R^2=0.018$ for M-CoV, ns. Pearson correlation). Colored area between dashed-lines indicates error bands. Red dots and lines represent data obtained for the four S-CoV patients analyzed as part of our scRNA-seq dataset. **(F)** Representative dot plot showing CD71 and CD19 expression in IgD⁻CD19⁺CD38⁻ B cell at indicated time points from

two representative S-CoV and M-CoV patients. SARS-Cov-2 spike-specific cells are overlaid as red dots. Gating strategy for ABCs and classical MBCs according to CD71 expression is further displayed. **(G)** Proportion of SARS-Cov-2 spike-specific cells displaying an ABC (CD19⁺CD27⁺IgD⁻CD71⁺) (left) or MBC (CD19⁺CD27⁺IgD⁻CD71^{low/int}) (right) phenotype at indicated time points. Bars indicate mean with SEM **(H)** Proportion of activated MBCs over time post symptom onset in S-CoV (black dots, dark blue line) and M-CoV (white dots, light blue line) patients. Lines represents the linear regression. Colored area between dashed lines indicates error bands. R² and *P*-value with Pearson correlation. **(I)** Correlation between the frequency of SARS-Cov-2 spike-specific MBCs at M6 (expressed as % of CD27⁺IgD⁻ B cells) and of ABCs at M0 in all M-CoV and S-CoV patients. RM two-way ANOVA and Sidak's multiple comparison tests (C). ANOVA and two-tailed Mann-Whitney tests (D and G). Linear regression with Pearson correlation analysis (I). *****P* < 0.0001, ****P* < 0.01, ***P* < 0.01, **P* < 0.05

See also Figure S3 and Table S3.

Figure 4. Maturation of the SARS-Cov-2 spike-specific repertoire up to six months post infection in mild and severe COVID-19 patients.

(A) Left: Pie charts showing the average percentage of RBD⁺ and cross-reactive specificities (RBD⁺OC43⁺, RBD⁺HKU1⁺OC43⁺, RBD⁺HKU1⁺, OC43⁺, HKU1⁺, OC43⁺HKU1⁺) among single-cell cultured spike-specific B cells as determined by ELISA. Three and four S-CoV patients, all previously included in our scRNA-seq dataset, were analyzed at M3 and M6, respectively. Right: Bar plot showing the proportion of RBD specificity and cross-reactive specificities among spike-specific cells for each patient. **(B)** Average percentage of RBD-specific cells among SARS-Cov-2 spike-specific sorted cells from indicated S-CoV and M-CoV patient at M3 and M6. **(C)** Violin plot showing the number of mutations in the Ig VH segment of cells in the original M0 10x scRNAseq VDJ dataset found to be in clonal relationship with M3 or M6 SARS-Cov-2 spike-specific sorted cells showing specificity against RBD (RBD⁺ clones), seasonal Beta-coronavirus spike proteins (HKU1-CoV or OC43-CoV) or against SARS-Cov-2 spike (Spike⁺ clones). **(D-E)** Histograms showing the relative distribution of mutation numbers in the Ig VH segment from all SARS-Cov-2 spike- **(D)** or RBD- **(E)** specific clone members (10x scRNA-seq dataset and single-cell heavy chain sequencing data) at M0, M3 and M6 as well as from sequences from the literature (Brouwer et al., 2020; Kreer et al., 2020; Liu et al., 2020; Robbiani et al., 2020; Shi et al., 2020; Wec et

al., 2020; Zost et al., 2020), mainly determined between M0 and M3). **(F)** Plot showing the percentage of neutralization, normalized to the IgG concentration for single cell culture supernatants from identified SARS-Cov-2 RBD-specific B cells at M6. Two dilutions (1/2 and 1/20) were assayed for each supernatant tested. Dashed lines indicate 80% and 50% neutralization. **(G)** Violin plot representing the number of mutations in the Ig VH sequence in strong neutralizing antibodies at M6 (neutralization >80% at 1/2 dilution) vs. the number of mutations in the Ig VH sequence of all cells from anti-RBD clones at M6. **(H)** Evolutionary tree of an RBD-specific and neutralizing clone, built on sequences from 10X scRNA-seq, cell culture and literature. Each circle represents a unique sequence from that clone. Circle color indicates time-point of origin and the number inside indicates the calculated number of mutations from an inferred unmutated common ancestor ("germline"). Grey indicates a theoretically inferred common precursor. * indicate that the antibody associated with that sequence has been validated as neutralizing in vitro. CDR3 from all sequences in the tree are represented as a frequency plot logo (Top left) as well as below the tree, where each amino-acid in red indicates a change compared to the top listed CDR3. ANOVA and two-tailed Mann-Whitney tests (B-C). *** $P < 0.01$, * $P < 0.05$.

See also Figure S4 and Table S4.

Figure S1. Quantification and functional assessment of the anti-SARS-Cov-2 humoral immune response in COVID-19 convalescent patients (Related to Figure 1).

(A) Percentage of in vitro neutralization shown by individual sera from S-CoV (n=10) and M-CoV (n=12) at M6 at increasing dilutions. **(B)** Representative wells for the neutralization assay. Blue spots represent SARS-Cov-2 positive cells. **(C)** Representative results of an anti-SARS-Cov-2 S IgG ELISA on supernatants from sorted SARS-Cov-2 S-specific MBCs (dark dots) and non spike-specific MBCs (white dots), as validation of FACS SARS-Cov-2 S-staining. Lines indicate median value. Dashed line indicates the positivity threshold ($\geq 3 \times$ blank). **(D-H)** Flow cytometric gating strategies for the analysis and sorting of major B cell populations from PBMCs of convalescent COVID-19 patients. **(D)** Gating strategy to analyze SARS-Cov-2 S-specific B cell population. Lymphocytes were first gated based on morphology, before exclusion of doublets, dead cells and CD3/CD14 cells. CD19⁺ cells were next gated before exclusion of CD38^{hi} plasma cells. CD38^{int/-} cells were then divided in four quadrants using CD27 and IgD. Upper left quadrant defines memory B cells (MBCs), lower

left quadrant double-negative (DN), upper right quadrant CD27⁺IgD⁺ cells (MZB) and lower right quadrant naive B cells (excluding CD38^{hi} transitional). SARS-Cov-2 S-specific B cells were then analyzed within the B cell population of interest using a double-staining strategy with anti-His antibodies of a His-tagged SARS-Cov-2 S protein. **(E)** Gating strategy to separate activated and classical switched B cells using CD71, within the IgD⁻CD27⁺ gate **(F)** Gating strategy to analyze CD27^{hi}CD38^{hi} plasma cells in CD3⁻CD14⁻ live cells. **(G)** Gating strategy for sorting of CD19⁺IgD⁻ cells from PBMCs for 10X-Chromium single cell experiment. Lymphocytes were gated before exclusion of doublets and of CD14/CD15/CD56/IgD⁺ cells, before sorting of CD19⁺ cells. **(H)** Gating strategy for single-cell sorting of SARS-Cov-2 S-specific B cells for single cell culture at M3 and M6. Lymphocytes were gated before double exclusion of doublets, dead cells and CD3/CD14 cells. CD19⁺ cells were then gated before exclusion of CD38^{hi} plasma cells. CD38^{int/-} cells were further divided into four quadrants using CD27 and IgD. Double stained SARS-Cov-2 S specific cells were sorted from all three non-naïve quadrants (not IgD⁺CD27⁻).

Figure S2. Gene expression markers and cluster assignments for the main single cell populations identified in acute and convalescent severe Covid-19 patients (Related to Figure 2).

(A-B) Dot plots showing expression of selected genes in cells from the main clusters **(A)** or in cells from the “Activated” cluster **(B)**. Size of dots represents the percentage of cells in the cluster in which transcripts for that gene are detected. Dot color represents the average expression level (scaled normalized counts) of that gene in the population. **(C)** Feature plots showing scaled normalized counts for CD21, CD71, CD11c, CD95 barcoded antibodies in all sorted CD19⁺IgD⁻. **(D-E)** Relative cluster distribution at M0 and M6 for cells belonging to one of the top 10% ASC clones **(D)** or stained by the barcoded-anti-His/His-tagged SARS-Cov-2-S protein combination (barcoded-SARS-Cov-2-S) **(E)**. Top panels represent cluster distribution for all cells, middle panels represent cluster distribution for non-ASC cells and bottom panels represent cluster distribution for cells belonging to the “Activated” cluster. Bars indicate mean with SEM. **(F)** UMAP of all cells at M0 or M6, with cells belonging to one of the top 10% ASCs clones highlighted (light blue). Cells belonging to Spike-specific clones, as defined in the Methods section, are also highlighted (red when members of one of the top 10% ASC clones at M0, dark blue otherwise). **(G)** Relative cluster distribution at M0 and M6 for cells belonging to Spike-specific clones. Top panel represent cluster distribution for all cells, middle panel represent cluster distribution for non-ASC cells and bottom panels

represent cluster distribution for cells belonging to the “Activated” cluster. Bars indicate mean with SEM. **(H)** Circus plot showing clonal relationships between cells from different UMAP clusters and time points. Blue lines indicate clonal relationships with one of the Spike-specific clones and grey line all other clonal relationships. RM two-way ANOVA and Sidak’s multiple comparison tests (D-E, G). **** $P < 0.0001$, *** $P < 0.01$, ** $P < 0.01$, * $P < 0.05$.

Figure S3. Phenotypic characterization of total and SARS-Cov-2 spike-specific B cell populations in convalescent COVID-19 patients (Related to Figure 3).

(A) Cluster distribution of all analyzed IgD⁻CD19⁺ B cell across FlowSOM_identified clusters from S-CoV (n=15) and M-CoV (n=16) donors (Table S1) at all three time points (M0, M3 and M6). **(B)** UMAP of concatenated down-sampled cells from all 83 samples analyzed colored according to the scaled fluorescence intensity for IgD, CD71, CD27, CD38, CD11c, CD19, CD21 expression. **(C)** Heat map representing the mean fluorescent intensity for IgD, CD71, CD27, CD38, CD11c, CD19, CD21 in each identified FlowSOM cluster. **(D-F)** Absolute number of antibody secreting cells (PC+PB) (D), IgD⁻CD27⁺ MBCs (E), and ABCs (F) at indicated time points in S-CoV and M-CoV patients. Bars indicate the mean with SEM. Dashed line indicates the threshold based on healthy donors (mean + 2 SD) **(G-H)** Percentage (G) and absolute numbers (H) of SARS-Cov-2 spike-specific CD27⁺IgD⁻ MBCs at indicated time points in S-CoV and M-CoV patients. **(I-J)** Percentage (I) and absolute number (J) of SARS-Cov-2 spike-specific cells among CD27⁺IgD⁻ DN cells. (G and I) Each dot represents a patient and lines connect the different time points for each patient. Dashed line indicates the threshold based on healthy donors (mean + 2 SD). (H and J) Bars indicate the mean with SEM. **(K)** Representative dot plot for T-bet staining in CD19⁺CD38⁻ B cells from COVID-19 patients. **(L)** Percentage of intra-cellular T-Bet staining in SARS-Cov-2 spike-specific CD19⁺IgD⁻CD27⁺CD38⁻ cells from S-CoV (n=2) and M-CoV (n=3) patients at indicated time points. Bar indicate the mean with SEM. **(M-O)** Correlation between the number of SARS-Cov-2 spike-specific CD27⁺IgD⁻ MBCs at M6 and age at diagnosis (M), maximum oxygen flow during hospitalization (liters/minutes, only in S-CoV) (N) and maximum CRP during hospitalization (mg/L, available only in S-CoV) (O) using linear regression. **(P)** Number of spike-specific CD27⁺IgD⁻ MBCs at M6 according to the initial disease severity (S-CoV and M-CoV) and the sex of the patient (Women, W, blue and Men, M, green). Bars indicate the mean with SEM. **(Q)** Plot showing the number of spike-specific CD27⁺IgD⁻ cells in the 21 S-CoV patients analyzed at M6 colored according to the treatment received during initial hospitalization. Standard of care included oxygen, low dose anticoagulant, antibiotic if needed

and symptomatic treatments. RM two-way ANOVA and Sidak's multiple comparison tests (A). ANOVA and two-tailed Mann-Whitney tests (D-F, H, J, L). Linear regression with Pearson correlation analysis (M-0). **** $P < 0.0001$, ** $P < 0.01$, * $P < 0.05$.

Figure S4. Cross-reactivity, convergence and accumulation of somatic mutations in anti-SARS-Cov-2 S-specific memory B cells from convalescent COVID-19 patients (Related to Figure 4).

(A) Heat map showing the RBD, OC43 and HKU1 ELISA blank ratio for all tested SARS-Cov-2-specific single cell culture supernatant at M3 and M6. Each line represents one tested supernatant. Light red lines indicate that no value is available for that cell (B) FACS plots representing index sorting data of spike-specific cells according to their specificity for SARS-Cov-2 spike or RBD. (C) Repartition of the SARS-Cov-2 spike- and RBD-specific cells at M3 and M6 between the MBCs, DN or IgD⁺CD27⁺ compartments. (D) Pie chart representing clone size in all the sequences generated via single cell culture for each of the 4 patients previously included in our scRNAseq analysis. Total number of sequenced cells is indicated in the middle of the pie. (E) Circus plot showing SARS-Cov-2 spike- (grey) or RBD- (light blue) specific clones shared between patients in our dataset or with sequences from the literature. (F) Histogram showing log-normalized counts for both barcoded his-tagged SARS-Cov-2 S proteins in all cells from the M6 time point of our 10X scRNA-seq dataset (white) and cells in clonal relationship with sequence from single cell culture (dark grey) (G-H) Histograms showing the distribution of mutations in Ig VH for SARS-Cov-2 spike- (G) and RBD- (H) specific clones at M0, M3 and M6 according to the assay of origin. (I) Plot showing the raw percentage of neutralization for single cell culture supernatants from identified SARS-Cov-2 RBD-specific B cells at M6. Two dilutions (1/2 and 1/20) were assayed for each supernatant tested. Dashed lines indicate 80% and 50% neutralization (see Figure 4F). (J) Evolutionary tree of a convergent RBD-specific and neutralizing clone, built on sequences from 10X scRNA-seq and cell culture from two patients and from the literature. Each circle represents a unique sequence from that clone. Circle color indicates time-point of origin and the number inside indicates the calculated number of mutations from an inferred unmutated common ancestor (germline). Grey indicates a theoretically inferred common progenitor. CDR3 from all sequences in the tree are represented as a frequency plot logo (top

left) as well as below the tree, where each amino-acid in red indicates a change compared to the first listed CDR3 sequence.

STARMethods

Key resources table

Reagent or resource	Source	Identifier
Antibody		
CD3	Biologend	UCHT1 ; Cat#300425 ; RRID : AB_830754
CD14	BD Bioscience	M φP9 ; Cat#561709; RRID : AB_1645464
CD19	BD Bioscience	HIB19 ; Cat# 562321 ; RRID : AB_11154408
CD38	BD Bioscience	HIT2 ; Cat# 551400 ; RRID AB_394184
CD27	Biologend	M-T271 ; Cat# 356417 ; RRID: AB_2562598
CD11c	BD Bioscience	S-HCL-3 ; Cat#744436 ; RRID : AB_2742232
IgD	Life technologies	Polyclonal ; Cat# H15501 ; RRID : AB_2536563
His Tag	Biologend	J095G46 ; Cat# 362605 ; RRID:AB_2563634
His Tag	Biologend	J095G46 ; Cat# 362603 ; RRID:AB_2715818
CD71	Biologend	CY1G4 ; Cat# 334111 ; RRID:AB_2563118
CD21	BD Bioscience	B-ly4; Cat#563163 ; RRID:AB_2741028
CD14	BD Bioscience	M5E2 ; Cat#555398 ; RRID:AB_396590
CD15	BD Bioscience	HI98 ; Cat#562371 ; RRID:AB_395802
CD56	BD Bioscience	B159 ; Cat# 556647 ; RRID:AB_395906
IgD	BD Bioscience	IA6-2 ; Cat# 555779 ; RRID:AB_396114
CD3	BD Bioscience	UCHT1 ; Cat#561027RRID:AB_10561682
T-Bet	Biologend	4B10 ; Cat#644823; RRID:AB_2561760
CD38	Biologend	HIT2 ; Cat#303509 ; RRID:AB_314361
CD38 (Totalseq-C)	Biologend	HB-7; Cat# 356637 ; RRID:AB_2820007
CD138 (Totalseq-C)	Biologend	MI15; Cat#356539 ;RRID:AB_2810567
CD27 (Totalseq-C)	Biologend	O323; Cat#302853 ;RRID:AB_2800747
CD11c (Totalseq-C)	Biologend	S-HCL-3; Cat#371521 ;RRID:AB_2801018
CD21 (Totalseq-C)	Biologend	Bu32; Cat#354923 ;RRID:AB_2800953
CD71 (Totalseq-C)	Biologend	CY1G4; Cat#334125 ; RRID:AB_2800885
CXCR4 (Totalseq-C)	Biologend	12G5; Cat#306533 ; RRID:AB_2800791
CXCR3 (Totalseq-C)	Biologend	G025H7; Cat#353747 ;

		RRID:AB_2800949
CD69 (Totalseq-C)	Biolegend	FN50; Cat#310951 ; RRID:AB_2800810
HLA-DR (Totalseq-C)	Biolegend	L243; Cat#307663 ; RRID:AB_2800795
CD307d (FCRL4; Totalseq-C)	Biolegend	413D12; Cat#340213 ; RRID:AB_2832666
CD307e (FCRL5; Totalseq-C)	Biolegend	509f6; Cat#340309 ; RRID:AB_2819969
CD95 (Fas; Totalseq-C)	Biolegend	DX2; Cat#305651 ; RRID:AB_2800787
Integrin B7	Biolegend	FIB504; Cat#321229 ;RRID:AB_2810481
CD49d (Integrin alpha 4; Totalseq-C)	Biolegend	9F10; Cat#304345 ; RRID:AB_2814137
CD103 (Totalseq-C)	Biolegend	Ber-ACT8; Cat#350233 ;AB_2800933
LAG-3 (Totalseq-C)	Biolegend	11C3C65; Cat#369335 ; RRID:AB_2814327
TIM3 (Totalseq-C)	Biolegend	F38-2E2; Cat#345049 ; RRID:AB_2800925
CD305 (LAIR1; Totalseq-C)	Biolegend	NKTA255; Cat#342807 ; RRID:AB_2832668
NKG2D (Totalseq-C)	Biolegend	1D11; Cat#320837 ; RRID:AB_2800844
CD73 (Totalseq-C)	Biolegend	AD2; Cat#344031 ; RRID:AB_2800916
His Tag (Totalseq-C)	Biolegend and custom conjugation	J095G46 ; Cat#362601 ; RRID : AB_2721736
His Tag (Totalseq-C)	Biolegend and custom conjugation	J095G46 ; Cat#362601 ; RRID : AB_2721736
N Sars-CoV-2 (Rabbit polyclonal)	Institut Pasteur, N. Escriou	N/A
S Sars-CoV-2 (Human monoclonal)	Institut Pasteur, H. Mouquet	N/A
Biological samples		
Cryopreserved PBMCs from S-Cov and M-Cov patients	Henri Mondor Hospital, Assistance Publique des Hôpitaux de Paris	N/A
Cryopreserved PBMCs from pre-pandemic subjects	French Blood Agency (EFS)	N/A
SARS- CoV-2 virus (BetaCoV/France/IDF0372/2020)	Institut Pasteur, CNR Respiratory Viruses (S.Van der Werf)	N/A
Chemical, peptides, and recombinant proteins		
Sars-CoV-2 Spike	Institut Pasteur, Virologie Structurale (F. Rey)	N/A
Sars-CoV-2 RBD	Institut Pasteur, Virologie Structurale (F. Rey)	N/A
HKU1 Spike	Institut Pasteur, Virologie Structurale (F. Rey)	N/A
OC43 Spike	Institut Pasteur, Virologie Structurale (F. Rey)	N/A
Live dead aqua	Life technologies	Cat#L34957

Recombinant human IL-2	PeptoTech	Cat#200-02
Recombinant human IL-4	PeptoTech	Cat#200-04
Recombinant human IL-21	PeptoTech	Cat#210-21
Recombinant human BAFF	PeptoTech	Cat#310-13
Oligonucleotide Conjugation Kit	Abcam	Cat#ab218260
Softwares and algorithms		
Kaluza v2.1	Beckman Coulter	https://www.beckman.fr
Flowjo v10.7.1	FlowJo, LLC	https://www.flowjo.com
GraphPad Prism v8	GraphPad	https://www.graphpad.com
Codon Code Aligner v9	Codon Code Corporation	https://www.codoncode.com/
Python v3.7.6	Python Software Foundation	https://www.python.org/
R v4.0.2	R Foundation	https://www.r-project.org
RStudio v1.3.1056	RStudio	https://rstudio.com
IgBLASTn v1.16.0	NCBI	https://www.ncbi.nlm.nih.gov/igblast/
Docker desktop v2.5.0.0	Docker, Inc	https://www.docker.com/products/docker-desktop

RESOURCE AVAILABILITY

Lead Contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Matthieu Mahévas (matthieu.mahevas@aphp.fr).

Materials Availability

No unique materials were generated for this study.

Data and Code Availability

All single cell RNA sequencing data associated with this study have been deposited to ArrayExpress and are available under the following accession number: E-MTAB-9995. Single cell culture VDJ sequencing data reported in **Figure 4** are directly included in this study as part of **Table S4**.

Additional Supplemental items are available from Mendeley Data at <http://dx.doi.org/10.17632/76j5vnb6h.1>.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Study participants

In total 39 patients with COVID-19 were recruited. We included 21 COVID-19 patients requiring oxygen (S-CoV) and 18 healthcare workers, with a mild COVID-19 disease (M-

CoV). SARS-Cov-2 infection was defined as confirmed reverse transcriptase polymerase chain reaction (RT-PCR) on nasal swab or clinical presentation associated with typical aspect on CT-scan and/or serological evidence. Samples were collected in mean 18.8 (\pm SD: 8.8 days) days after disease onset in S-CoV, and in mean 35.5 days (\pm SD: 12.8 days) for M-CoV. Samples were additionally collected 3 months (mean \pm SD: 89.8 \pm 15.8 days for S-CoV and 94.2 \pm 8.2 for M-CoV), and 6 months (mean \pm SD: 170.5 \pm 12.9 days for S-CoV and 184.6 \pm 10.2 for M-CoV) after onset. Clinical and biological characteristics of these patients are summarized in Table S1. B cells from four S-CoV patients were further analyzed by scRNAseq, these four patients being selected for the absence of specific treatment (corticosteroids, IL6/IL6R inhibitors) that could interfere with the B-cell response. Patients were recruited at Henri Mondor University Hospital (AP-HP), between March and May 2020. Samples from healthy donors were obtained at EFS Henri-Mondor and frozen before 2019. MEMO-COV-2 study (NCT04402892) was approved by the ethical committee Ile-de-France VI (Number: 40-20 HPS), and was performed in accordance with the French law. Written informed consent was obtained from all participants.

METHOD DETAILS

SARS-Cov-2 viral detection by RT-PCR

Nasopharyngeal (NP) swabs in transport media and plasma samples were held at 4 °C (or -80°C if > 12h) prior to testing on the Alinity M SARS CoV-2 Amp kit and/or Cepheid Xpert Xpress SARS-Cov-2 kit. The GeneXpert ®Dx System (Cepheid) and Alinity M (Abbott) performed automated specimen processing and real-time RT-PCR analysis. The Cepheid Xpert Xpress SARS-Cov-2 kit detects N and E genes of the SARS CoV-2 genome; Alinity M SARS CoV-2 Amp kit detects RdRp and N genes.

Anti-S and anti-N commercial assays

Serological assays were performed for IgG anti-N, IgG anti-S and total antibodies (ab) anti-S detection. Serum samples were processed for anti-Nucleoprotein (N) detection on Abbott SARS-Cov-2 IgG chemiluminescent microparticle immunoassay following the manufacturer's instructions. Serum samples were further analyzed using the VITROS Immunodiagnostic Products, for IgG anti-spike (S) SARS-Cov-2 detection and for total Ab anti-spike (S) detection (Ortho Clinical Diagnostics). All assays were performed by trained

laboratory technicians according to the respective manufacturer's standard procedures. Qualitative results and index values reported by the instruments were used in analysis.

Recombinant protein purification

Construct design

The human coronavirus Spike (S) proteins were expressed as ectodomains that were stabilized to preserve their trimeric prefusion conformation. The SARS-Cov-2 spike protein (residues 1-1208) was stabilized by introducing six proline substitutions (F817P, A892P, A899P, A942P, K986P, V987P), a GSAS substitution at the furin cleavage site (residues 682–685) and a C-terminal Foldon trimerization motif (Hsieh et al., 2020), followed by Hisx8 and Strep tags. This construct was cloned with its endogenous signal peptide in pcDNA3.1(+). The OC43 S ectodomain (residues 15-1263) was cloned in the pCAGGS vector with a CD5 N-terminal signal peptide and a C-terminal GCN4 trimerization motif, a thrombin cleavage site and a single Strep-tag. Additionally, it contains mutations to abolish the furin cleavage (754-RRSRG-758 to 754-GGSGG-758) at the S1-S2 junction (Tortorici et al., 2019). The HKU1-CoV S protein (residues 14-1276) was stabilized by a double-proline mutation (N1067P, L1068P), the substitution of the RRSRG motif by GGSGG (residues 754-758) to avoid a potential S1/S2 furin cleavage, and a C-terminal Foldon motif. This construct was cloned in pcDNA3.1(+) with an IgK signal peptide and a thrombin cleavage site at the C-terminus that was followed by a Hisx8 tag.

The SARS-CoV-2 Receptor Binding Domain (RBD) was cloned in pcDNA3.1(+) encompassing residues 331-528 from the Spike ectodomain, and it was flanked by an N-terminal IgK signal peptide and a C-terminal Thrombin cleavage site followed by a Hisx8-tag.

Protein expression and purification

The plasmids coding for the recombinant proteins were transiently transfected in Expi293F™ cells (Thermo Fischer) using FectoPRO® DNA transfection reagent (Polyplus), according to the manufacturer's instructions. The cells were incubated at 37 °C for 5 days and then the culture was centrifuged and the supernatant was concentrated. The proteins were purified from the supernatant by affinity and size-exclusion chromatography (SEC). The first purification step was performed using StrepTactin columns (IBA) (SARS-Cov-2 S, OC43 S) or His-Trap™ Excel columns (GE Healthcare) (HKU1 S, SARS-Cov-2 RBD). The different S ectodomains were further purified using a Superose6 10/300 column (GE Healthcare) equilibrated in PBS, while a Superdex200 10/300 column was used for SARS-Cov-2 RBD.

Flow cytometry and cell sorting

PBMCs were isolated from venous blood samples via standard density gradient centrifugation and used after cryopreservation at -150°C . Cells were thawed using RPMI-1640 (Gibco) 10% FBS, washed twice and incubated with 10 μg of the SARS-CoV-2 His-tagged spike protein in 100 μL of PBS (Gibco) 2% FBS during 20 min on ice. Cells were washed and resuspended in the same conditions, then the fluorochrome-conjugated antibody cocktail including the 2 anti-His antibodies was added at pre-titrated concentrations for 20 min at 4°C and viable cells were identified using a LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (Thermo Fisher Scientific) incubated with conjugated antibodies (see **Table S4**). If a permeabilization was needed (T-Bet staining), cells were washed and resuspended in 250 μL of Fix/perm (eBioscience) for 25 min, then washed with appropriate buffer before adding the conjugated antibody on the cell pellet for a further 25 min at 4°C . Samples were acquired using a LSR Fortessa SORP (BD Biosciences). For cell sorting, cells were stained using the same protocol and then sorted in 96 plates using an ultra-purity mode on a MA900 cell sorter (SONY), or Aria III (BD Biosciences). Data were analyzed with FlowJo or Kaluza softwares. Detailed gating strategies for individual markers are depicted in Figure S1. Control samples included PMBC collected from healthy donors before SARS-Cov-2 pandemic and incubation of S-CoV and M-CoV samples with anti-His antibodies but no His-tagged spike protein to determine the labelling background.

For UMAP generation and visualization (**Figure 3A-C**), data from all 83 samples from patients with complete panel acquisition at M0, M3 and M6 (**Table S1**) in our dataset were individually down-sampled to 3000 cells each using the Downsample (v3.3) plugin in FlowJO. All samples were subsequently concatenated and FlowJO UMAP (v3.1) plugin was used to calculate the UMAP coordinates for the resulting 249,000 cells (with 30 neighbors, metric = euclidian and minimum distance = 0.5 as default parameters) (**Figure 3A**). The FlowSOM plugin was used in parallel on the same downsampled dataset to create a self-organizing map (using $n = 9$ clusters as default parameter) (**Figure 3B**). Upsampling was then performed via the FlowSOM plugin by applying this self-organizing map to the initial FCS files from all 83 samples to calculate both total and spike-specific memory B cell repartition in identified clusters on all collected cells for each donor (**Figure 3C**, **Figure S3A** and **Table S3**). Both UMAP and FlowSOM plugin were run taking into account fluorescent intensities from the following parameters: FSC-A, SSC-A, CD19, CD21, CD11c, CD71, CD38, CD27 and IgD. Contour plots (equal probability contouring, with levels set to 5% of gated

populations) for each identified cluster were further overlaid on UMAP projection in FlowJO. For visualization purposes, only the outermost density representing 95% of the total gated cells was kept for the final figure (**Figure 3B**), all other levels were removed in Adobe Illustrator.

Single-cell culture

Single cell culture was performed as previously described (Crickx et al., 2019): single B cells were sorted in 96-well plates containing MS40 cells expressing CD40L (kind gift from G. Kelsoe). Cells were co-cultured at 37°C with 5% CO₂ during 21 or 25 days in RPMI-1640 (Invitrogen) supplemented with 10% HyClone FBS (Thermo Scientific), 55 µM 2-mercaptoethanol, 10 mM HEPES, 1 mM sodium pyruvate, 100 units/mL penicillin, 100 µg/mL streptomycin, and MEM non-essential amino acids (all Invitrogen), with the addition of recombinant human BAFF (10 ng/ml), IL2 (50 ng/ml), IL4 (10 ng/ml), and IL21 (10 ng/ml; all Peprotech). Part of the supernatant was carefully removed at days 4, 8, 12, 15 and 18 and the same amount of fresh medium with cytokines was added to the cultures. After 21 days of single cell culture, supernatants were harvested and stored at -20°C. Cell pellets were placed on ice and gently washed with PBS (Gibco) before being resuspended in 50 µL of RLT buffer (Qiagen) supplemented with 10% 2-mercaptoethanol and subsequently stored at -80°C until further processing.

ELISA

IgG and SARS-Cov-2, HKU1-CoV and OC43-CoV spike-specific IgG from culture supernatants were detected by home-made ELISA. Briefly, 96 well ELISA plates (Thermo Fisher) were coated with either goat anti-human Ig (10 µg/ml, Invitrogen) or recombinant SARS-Cov-2, HKU1 or OC43 spike or SARS-Cov-2 RBD protein (2.5 µg/ml each) in sodium carbonate during 1h at 37°C. After plate blocking, cell culture supernatants were added for 1hr, then ELISA were developed using HRP-goat anti-human IgG (1 µg/ml, Immunotech) and TMB substrate (Eurobio). OD450 and OD620 were measured and Ab-reactivity was calculated after subtraction of blank wells. Supernatants whose ratio of OD450-OD620 over control well (consisting of supernatant from wells that contained single-cell sorted spike-negative memory B cells from the same single cell culture assay) was over 3 were considered as positive for RBD ELISA. A cut-off at a ratio of 5 was chosen for HKU1 and OC43 spike ELISA. PBS was used to define background OD450-OD620.

Single-cell IgH sequencing

Clones whose culture had proven successful (IgG concentration $\geq 1 \mu\text{g/mL}$ at day 21-25) were selected and extracted using the NucleoSpin96 RNA extraction kit (Macherey-Nagel) according to the manufacturer's instruction. A reverse transcription step was then performed using the SuperScript IV enzyme (ThermoFisher) in a 14 μl final volume (42°C 10 min, 25°C 10 min, 50°C 60 min, 94°C 5 min) with 4 μl of RNA and random hexamers (GE Healthcare). A PCR was further performed based on the protocol established by Tiller et al (Tiller et al., 2008). Briefly, 3.5 μl of cDNA was used as template and amplified in a total volume of 40 μl with a mix of forward L-VH primers (Table S4) and reverse C γ primer and using the HotStar® Taq DNA polymerase (Qiagen) and 50 cycles of PCR (94°C 30 s, 58°C 30 s, 72°C 60 s). PCR products were sequenced with the reverse primer CHG-D1 and read on ABI PRISM 3130XL genetic analyzer (Applied Biosystems). Sequence quality was verified with the CodonCode Aligner software (CodonCode Corporation) and data were analyzed with the IMGT/HighV-QUEST web portal (from The International Immunogenetics Information System) or in parallel with the VDJ sequences generated as part of our scRNA-seq dataset (see below).

Oligonucleotide conjugation of anti-His antibody

Unconjugated anti-His antibodies were purchased from Biolegend and custom oligonucleotides were ordered from Integrated DNA Technologies, following the 10Xgenomics protocol available at <https://support.10xgenomics.com/single-cell-gene-expression/overview/doc/demonstrated-protocol-cell-surface-protein-labeling-for-single-cell-rna-sequencing-protocols> and the barcode whitelist. Sequences of the 2 HPLC purified barcoded oligonucleotides were: /5AmMC12/CGGAGATGTGTATAAAGAGACAGNNNNNNNNNNNTGCATAGCCTGTGG ANNNNNNNNNCCCATATAAGAAA and /5AmMC12/CGGAGATGTGTATAAAGAGACAGNNNNNNNNNNNCTCTCCAATGTACTC NNNNNNNNNCCCATATAAGAAA. Oligonucleotide antibody conjugation was performed using the Oligonucleotide Conjugation Kit from Abcam, according to the manufacturer instructions using an oligonucleotide/antibody ratio of 5:1.

Single-cell RNA-seq library preparation and sequencing

Peripheral (CD3⁻CD14⁻CD15⁻CD56⁻CD19⁺IgD⁻) B cells were FACS-sorted (MA900, Sony) in PBS/0.08% FCS from 4 patients (S-CoV) at baseline (M0) and 6 months (M6). 5×10^4 to

10x10⁵ cells were obtained for each subset. The scRNA-seq libraries were generated using Chromium Next GEM Single Cell V(D)J Reagent Kit v.1.1 with Feature Barcoding (10x Genomics) according to the manufacturer's protocol. Gene expression (mRNA), ADT and VDJ BCR libraries were constructed. Briefly, cells were counted and up to 20 000 cells were loaded in the 10x Chromium Controller to generate single-cell gel-beads in emulsion. After reverse transcription, gel-beads in emulsion were disrupted. Barcoded complementary DNA was isolated and amplified by PCR. Following fragmentation, end repair and A-tailing, sample indexes were added during index PCR. The purified libraries were sequenced on a Novaseq S2 flowcell (Illumina) with 26 cycles of read 1, 8 cycles of i7 index and 91 cycles of read 2, targeting a median depth of 50000 reads per cell for gene expression and 5000 reads per cell for each other two libraries (BCR VDJ and ADT Feature barcoding).

Single-cell gene expression analysis

Paired-end FASTQ reads for all three libraries were demultiplexed and aligned against the GRCh38 human reference genome (GENCODE v32/Ensembl 98; July 2020) using 10x Genomics' Cell Ranger v4.0.0 pipeline. Outputs of Cell Ranger were directly loaded into Seurat v3.2.2 (Stuart et al., 2019) for further QC steps and analysis. Following manual inspection of cell quality, only genes detected in at least 10 cells and cells with more than 500 unique genes detected and less than 25% of UMI counts mapped to mitochondrial genes were kept. Reads mapping to the immunoglobulin genes locus were further stored in a separate assay at this step to avoid unwanted clustering based solely on differential isotype expression and cells with exactly one heavy chain sequence were retained for final analysis. UMI counts were then log-normalized. The top 2,000 highly variable genes were identified using the FindVariableFeatures() function in Seurat and the default vst method. Normalized counts were then scaled and centered using the ScaleData() function, removing unwanted variation related to the percentage of mitochondrial UMI counts at that step. After principal component analysis, potential donor and sort-specific batch effects were removed using the Harmony algorithm (Korsunsky et al., 2019). The first 30 corrected PCA dimensions were then used to construct a knn graph (k=20 neighbors) and perform graph-based clustering (Louvain) with a resolution parameter of 0.2 as well as compute the UMAP coordinates for each cell. Further separation of the "Activated" cell cluster was performed using a resolution parameter of 0.4. Two small clusters with T (0.2%) and monocyte (0.1%) signatures were removed from further analysis as clear doublets, as well as a separate cluster of poor quality cells (2.1% of all cells), grouped solely based on high percentages of mitochondrial UMI count. G2M and S cell cycle

signatures were calculated using the CellCycleScoring() function and the associated gene lists in Seurat (G2M scoring: HMGB2, CDK1, NUSAP1, UBE2C, BIRC5, TPX2, TOP2A, NDC80, CKS2, NUF2, CKS1B, MKI67, TMPO, CENPF, TACC3, FAM64A, SMC4, CCNB2, CKAP2L, CKAP2, AURKB, BUB1, KIF11, ANP32E, TUBB4B, GTSE1, KIF20B, HJURP, CDCA3, HN1, CDC20, TTK, CDC25C, KIF2C, RANGAP1, NCAPD2, DLGAP5, CDCA2, CDCA8, ECT2, KIF23, HMMR, AURKA, PSRC1, ANLN, LBR, CKAP5, CENPE, CTCF, NEK2, G2E3, GAS2L3, CBX5, CENPA; S scoring: MCM5, PCNA, TYMS, FEN1, MCM2, MCM4, RRM1, UNG, GINS2, MCM6, CDCA7, DTL, PRIM1, UHRF1, MLF1IP, HELLS, RFC2, RPA2, NASP, RAD51AP1, GMNN, WDR76, SLBP, CCNE2, UBR7, POLD3, MSH2, ATAD2, RAD51, RRM2, CDC45, CDC6, EXO1, TIPIN, DSCC1, BLM, CASP8AP2, USP1, CLSPN, POLA1, CHAF1B, BRIP1, E2F8).

Computational analyses of VDJ sequences:

Processed FASTA sequences from cultured single-cell heavy chain sequencing, 10x single-cell RNA sequencing and 874 published SARS-CoV-2 RBD and/or S-specific antibodies (Brouwer et al., 2020; Kreer et al., 2020; Liu et al., 2020; Robbiani et al., 2020; Seydoux et al., 2020; Shi et al., 2020; Wec et al., 2020; Zost et al., 2020) were annotated using Igbblast v1.16.0 against the human IMGT reference database. Non-productively rearranged sequences were removed at that step as well as sequences from cells that did not pass the initial QC cut-offs from our scRNA-seq analysis. Cases of 10x barcodes with two or more consensus heavy chain sequences for which more than ten UMI were detected were generally flagged as potential doublets for removal from our scRNA-seq analysis. Similarly, cases where no clear heavy chains could be attributed (none above 10 UMIs) were also flagged for removal. Two exceptions were made: 1/ in cases of identical CDR3s but differing isotypes (c_call), in which case the isotype switched sequence was kept and UMI counts from both contigs were aggregated; and 2/ in cases when one the heavy chains was clearly over represented at the UMI level and the second most represented sequences did not exceed ten UMIs, in which case the most represented sequence was kept.

Clonal cluster assignment (DefineClones.py) and germline reconstruction (CreateGermlines.py) was performed using the Immcantation/Change-O toolkit (Gupta et al., 2015) on all heavy chain V sequences. Sequences that had the same V-gene, same J-gene, including ambiguous assignments, and same CDR3 length with maximal length normalized nucleotide hamming distance of 0.15 were considered as potentially belonging to the same clonal group. Mutation frequencies in V genes were then calculated using the

calcObservedMutations() function from Immcantation/SHazaM v1.0.2 R package. For the analysis of the initial ASC response in our 10x dataset (**Figure 2E/F**), clonal assignments were further corrected using available light chain information (light_cluster.py script from Immcantation). Further clonal analyses were implemented in R.

Based on light chain-corrected clonal affectation, clones were ranked based on the total number of ASC (PC + PB) from the M0 time point of our analysis found in each clone for each individual donor. Clones with equal number of ASC were further ranked based on their total number of Activated and MBC cells from the M0 time point of our analysis. The top ten percent highest ranked clones were further labeled as Top 10% ASC clones (**Figure 2E-F and Figure S2D-H**). Based on heavy-chain only clonal affectation, clones were defined as SARS-Cov-2 S specific (Spike-specific clones) if they contained 1 or more validated single-cell culture sequence or if more than ten percent of the cells from that clone were positively stained by our barcoded His-tagged S protein in our scRNAseq dataset. All Spike-specific clones described in **Figure 4** and **Figure S2F-G** were manually curated based on available light chain information (10x and published antibodies only) and CDR3 sequences. Cut-off for positive staining with barcoded His-tagged S protein in our scRNAseq dataset were set at log normalized count values of 1.2 for both barcodes. Cells in clonal relationship with single-cell culture sequences at the M6 time point served as reference (**Figure S4F**). Clones were defined as SARS-Cov-2 RBD or HKU1/OC43 spike-specific (**Figure 4C-E-G**) if they contained 1 or more validated single-cell culture sequence with a positive ELISA against one of these proteins. Graphics were obtained using the ggplot2 v3.3.2 and circlize v0.4.10 packages. Phylogenetic trees were generated using the Immcantation/IgPhyML toolkit (Immcantation/suite v4.0.0 docker image) and further visualized in R using the Alakazam v1.0.2 and igraph v1.2.6 packages.

Virus neutralization assay

Virus neutralization was evaluated by a focus reduction neutralization test (FRNT). Vero E6 cells were seeded at 2×10^4 cells/well in a 96-well plate 24h before the assay. Two hundred focus-forming units (ffu) of Sars-CoV-2 virus (BetaCoV/France/IDF0372/2020 strain, a kind gift from the National Reference Centre for Respiratory Viruses at Institut Pasteur, Paris, originally supplied through the European Virus Archive goes Global platform) were pre-incubated with serial dilutions of heat-inactivated sera or supernatant from B-cell clones for 1h at 37°C before infection of cells. After 2h infection, the virus/antibody mix was removed and foci were left to develop in presence of 1.5% methylcellulose for 2 days. Cells were then

fixed with 4% formaldehyde and foci were revealed using either a rabbit anti-SARS-CoV N or a human anti-SARS-CoV-2 S (H2-162) antibody and matching secondary HRP-conjugated secondary antibodies. Foci were visualized by diaminobenzidine (DAB) staining and counted using an Immunospot S6 Analyser (Cellular Technology Limited CTL). Purified IgG from healthy donors were used as negative controls (TEM10 at 100 µg/ml; TEM11 at 90 µg/ml; TEM13 at 130 µg/ml) and purified human monoclonal C3-235 as positive control (IC₅₀ 0.3 nM). Percentage of virus neutralization was calculated as $(100 - (\#foci\ Ab / \#foci\ control) * 100)$. The total amount of IgG present in the supernatants from B cells varied between S-CoV (12 µg/ml ± 7.6) and M-CoV patients (48 µg/ml ± 26). Neutralization values were normalized to the total IgG concentration present in the supernatant. As the neutralization activity may not linearly correlate with the amount of IgG, such normalization may lead to an underestimate of the neutralizing potency of B cell supernatants from M-CoV patients. Variation of the assay in weak neutralizers (i.e. < 50 %) was due to intrinsic variation in counting.

Quantification and Statistical Analysis

RM two-way ANOVA, Sidak's multiple comparison tests, Kruskal-Wallis test and Mann-Whitney test were used to compare continuous variables as appropriate (indicated in Figures). A *P*-value ≤ 0.05 was considered statistically significant. Statistical analyses involved use of GraphPad Prism 8.0 (La Jolla, CA, USA).

Additional resources

ClinicalTrials.gov Identifier: MEMO-CoV2, NCT04402892.

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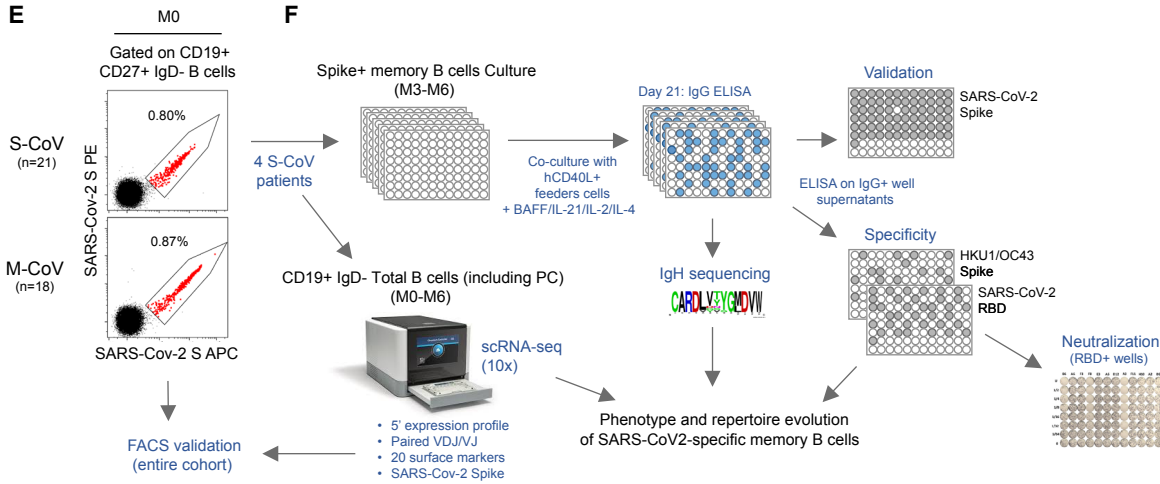
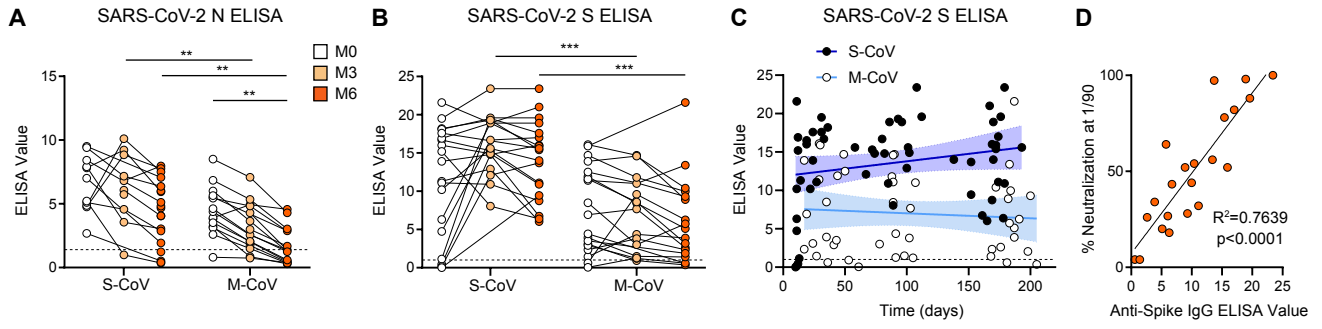
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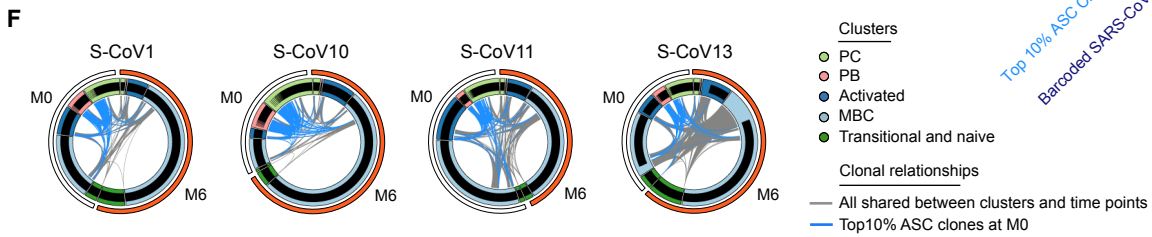
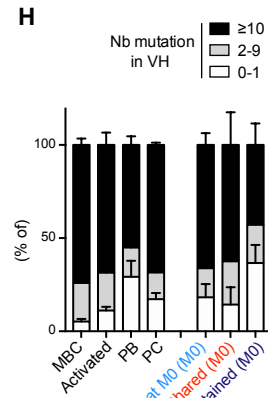
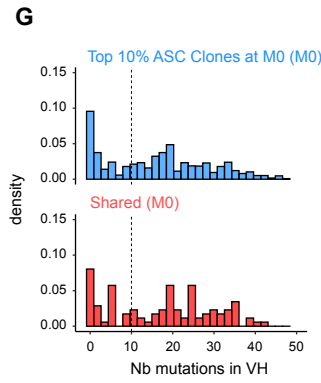
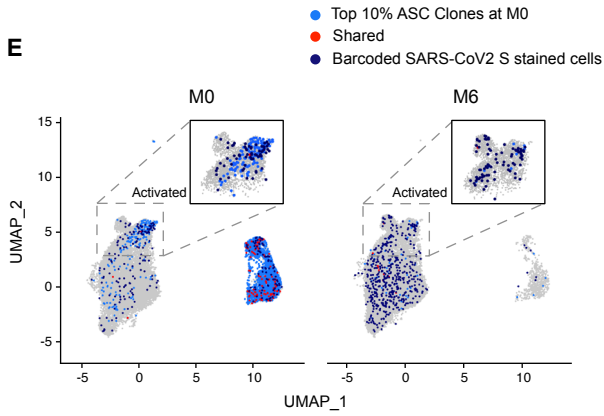
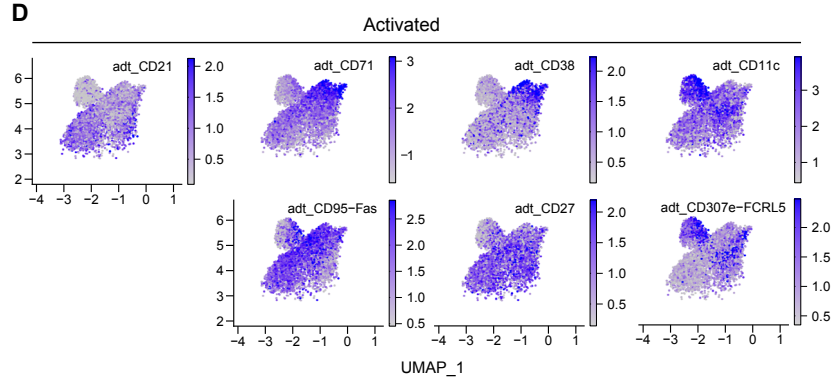
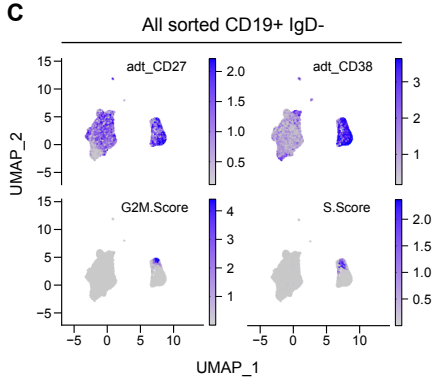
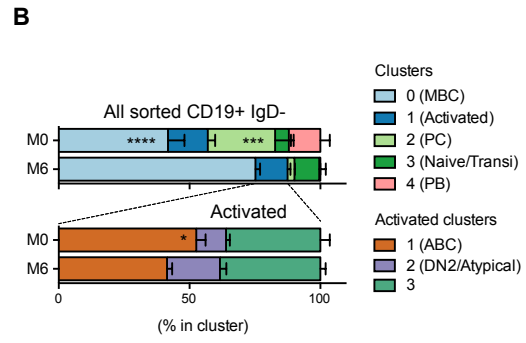
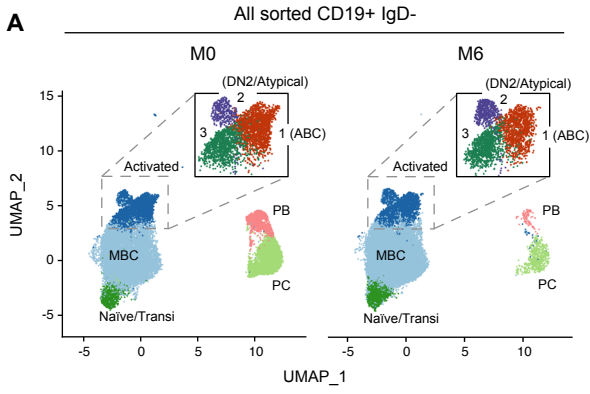
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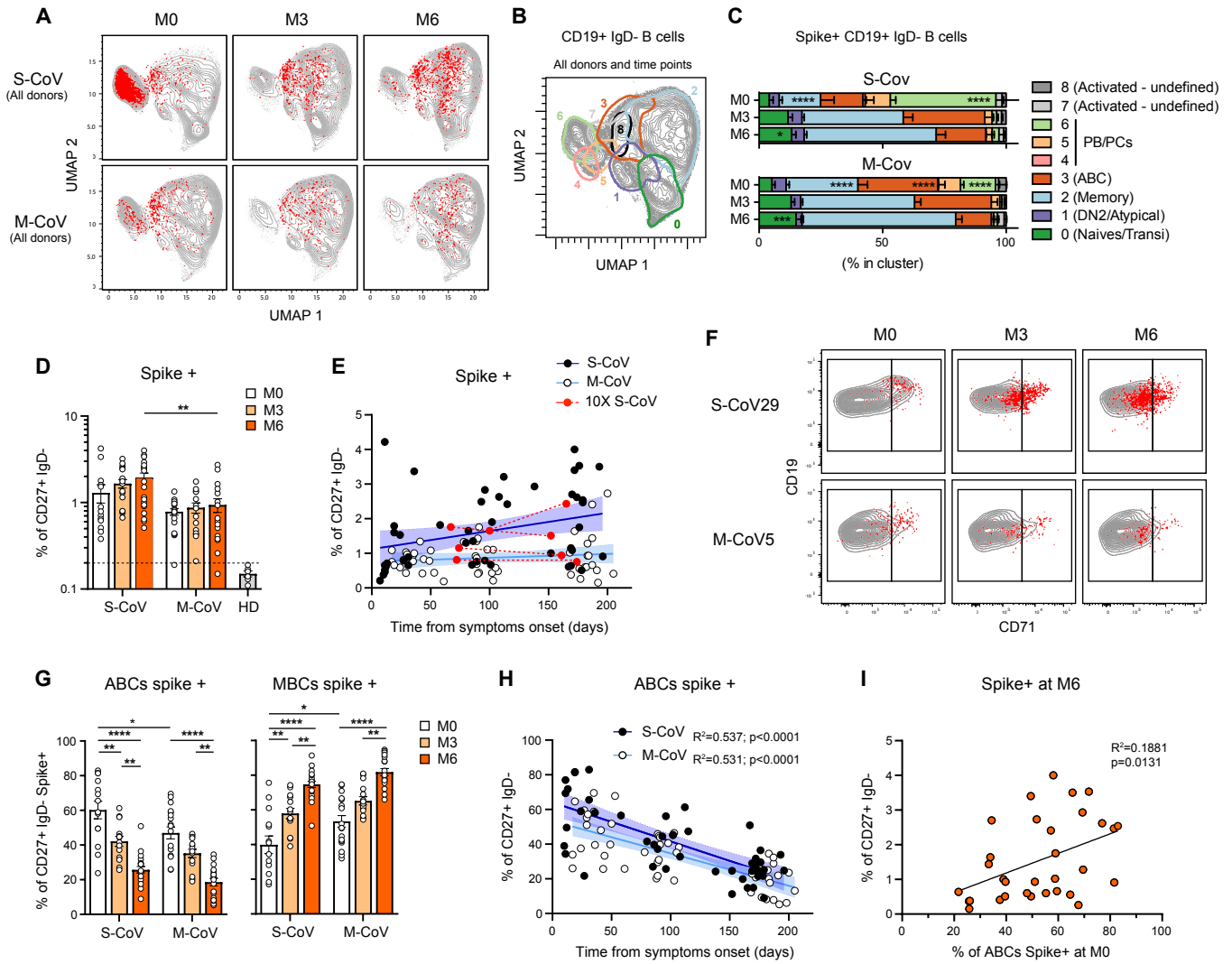
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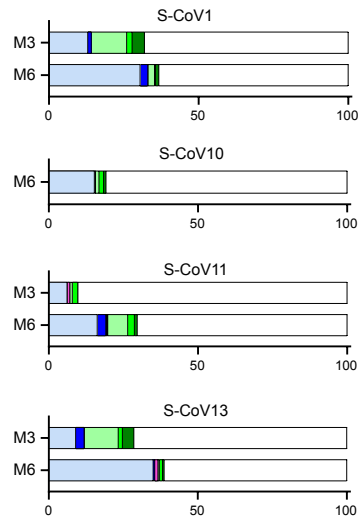
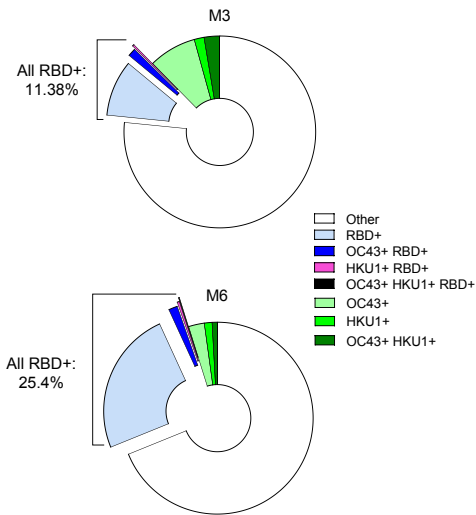
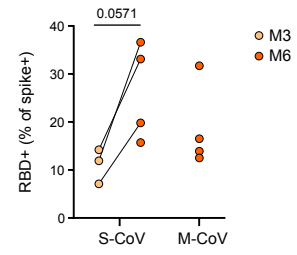
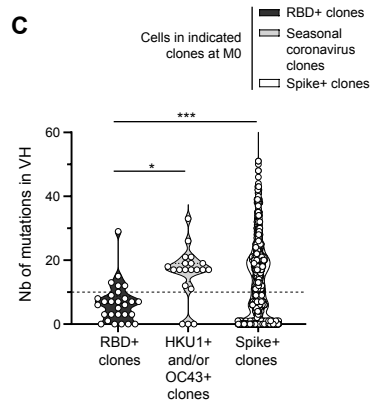
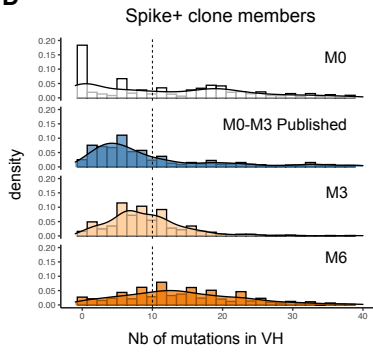
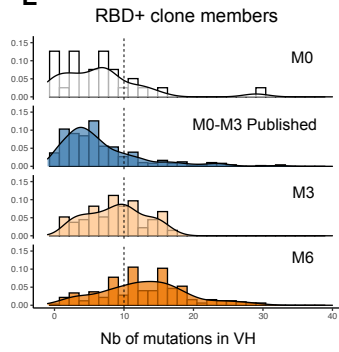
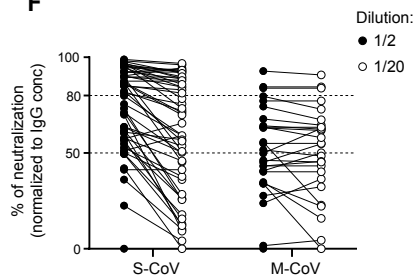
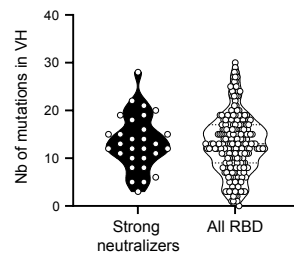
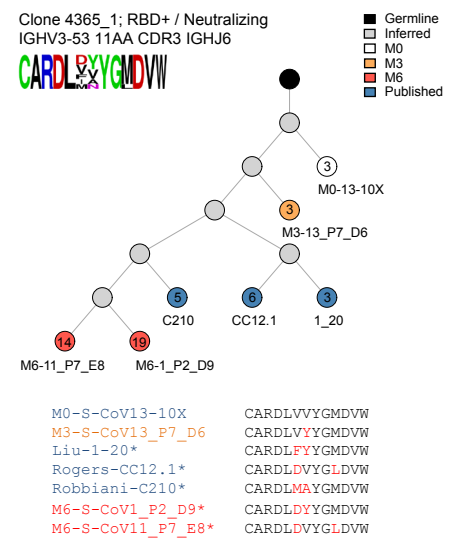
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A**B****C****D****E****F****G****H**

**early
anti-spike response**

**memory
anti-spike response
(6 months)**

