Supplemental information

Longitudinal analysis shows durable and broad immune memory after SARS-CoV-2 infection with persisting antibody responses and memory B and T cells

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Table S1. Cohort Demographics and Baseline Characteristics (Related to STAR Methods Subject Details).

| Characteristic                                      | All (N=254) |
|-----------------------------------------------------|-------------|
| Age, median (range)— years                          | 48.5 (18-82) |
| Female sex at birth— no. (%)                         | 141 (55.6)  |
| Race or ethnic group— no. (%)                        |             |
| White                                              | 226 (89.0)  |
| Hispanic or Latino                                  | 21 (8.3)    |
| Black or African American                           | 15 (5.9)    |
| Asian                                              | 11 (4.3)    |
| Other\(^a\)                                         | 7 (2.8)     |
| Median time from symptom onset to enrollment (range)— days | 53.5 (1-203) |
| Comorbid conditions— no. (%)                        |             |
| Hypertension                                        | 46 (18.1)   |
| Obesity                                            | 41 (16.1)   |
| Chronic lung disease                                | 23 (9.3)    |
| HIV-1 and/or autoimmune disease                     | 19 (7.7)    |
| Type 2 diabetes mellitus                            | 18 (7.3)    |
| Heart disease                                       | 15 (6.0)    |
| Cancer                                              | 10 (3.9)    |
| Symptoms with initial illness— no. (%)              |             |
| Myalgia, fatigue                                    | 231 (90.9)  |
| Headache                                            | 168 (66.1)  |
| Fever                                               | 167 (65.7)  |
| Cough                                               | 161 (63.4)  |
| Loss of smell                                       | 146 (57.5)  |
| Loss of taste                                       | 143 (56.3)  |
| Shortness of breath                                 | 108 (42.5)  |
| Diarrhea                                            | 102 (40.2)  |
| Sputum production                                   | 43 (16.9)   |
| None                                                | 9 (3.5)     |
| Disease severity (WHO Score)—no. (%)                |             |
| Mild (1-2)                                          | 180\(^b\)  (70.9) |
| Moderate (3-4)                                      | 62 (24.4)   |
| Severe (5-10)                                       | 12 (4.7)    |
| Maximum number of visits—total                      |             |
| 1                                                   | 9           |
| 2                                                   | 103         |
| 3                                                   | 62          |
| 4                                                   | 51          |
| 5-7                                                 | 29          |

\(^a\)Individuals identifying as Other included: American Indian or Alaska Native; White (n=1); Asian, Black or African American (n=1); Asian; White (n=3); Native Hawaiian or other Pacific Islander; White (n=2); \(^b\)6 participants had a positive Abbott SARS-CoV-2 Abbott SARS-CoV-2 IgG assay test but did not have a positive nasal SARS-CoV-2 PCR test.
**Figure S1. Modeling of antibody titer decline.** Decline of IgG antibody titers was analyzed by an exponential decay model (red) and a power law model (green) for antibodies reactive to SARS-CoV-2 antigens (A) and SARS-CoV-1 spike (B). The half-lives estimated by the exponential and power law models (C). The half-lives estimated by the power law were calculated at day 120 after symptom onset. The fold difference in IgG antibody titers to endemic coronaviruses between COVID-19 patients and pre-pandemic controls plotted over days since symptom onset (D). Related to Figure 1 and 2.
Figure S2. Longitudinal SARS-CoV-2 nucleocapsid binding antibody responses. IgG (A), IgA (B), and IgM (C) antibodies reactive to SARS-CoV-2 nucleocapsid were measured by an electrochemiluminescent multiplex immunoassay in triplicate and reported as arbitrary units per ml (AU/ml) as normalized by a standard curve. Longitudinal antibody titers of COVID-19 patients (in blue, n=222 COVID-19+ for IgG; n=190 COVID-19+ for IgA and for IgM) are plotted over days since symptom onset, whereas longitudinal pre-pandemic donor samples (in red, n=51 for IgG, IgA and IgM) were collected in the course of a non-SARS-CoV-2 vaccine study before 2019 and plotted over days since immunization. IgG decay curves and half-lives estimated by an exponential decay model are shown in black, whereas the decay curves and half-lives at day 120 post symptom onset estimated by a power law model are shown in green. Related to Figure 1.
Figure S3. SARS-CoV-2 uninfected controls have few if any memory B and T cells recognizing SARS-CoV-2 antigens. Spike+ (A) and RBD+ (B) IgG+, IgA+ and IgM+ memory B cells in SARS-CoV-2 negative subjects are shown from PBMC collected before 2019 (n=29; tested in singlet). Line is at the median. Low frequencies of T cells recognizing SARS-COV-2 antigens are shown from donor samples not infected with SARS-CoV-2 (n=51). Background-subtracted CD4+ T cells expressing IFN-γ, IL-2 and/or CD40L (C), and IFN-γ+ CD8+ T cells (D) in response to stimulation with the SARS-CoV-2 antigens (on the x-axis) are shown. Positive T cell stimulations (as determined by MIMOSA) are indicated by a solid black circle, whereas samples that are negative are indicated by gray open triangles and the percent of positive responders are shown above the T cell graphs. Related to Figure 4, 5 and 6.
Figure S4. Representative individual-level estimates of SARS-CoV-2 B and T cell responses from 30 days post-symptom onset. Post-day 30 S+ IgG+ B cell responses (log scale) for individuals with data at 3 or more time points (A) and 1-2 time points (B) with fitted curves from a linear mixed effects model with random effects for the intercept and slope. Post-day 30 CD4+ T cell responses to SARS CoV-2 (log scale) for individuals with data at 3 or more time points (C) and 1-2 timepoints (D), with fitted curves from a nonlinear mixed effects model with random effects for the intercept and slope. The CD4+ T cell analyses only included individuals with a positive response to at least one SARS-CoV-2 antigen at one or more time points, where positive responses were determined by MIMOSA. Related to Figures 4 and 5.
Figure S5. CD4+ T cell responses among SARS-CoV-2 convalescent subjects to individual SARS-CoV-2 peptide pools. (A) Representative SARS-CoV-2 specific CD4+ T cell responses to multiple SARS-CoV-2 antigens by intracellular cytokine staining (ICS) assay in PBMCs from a SARS-CoV-2 patient. Background-subtracted frequencies of IFN-γ+, IL-2+ and/or CD40L+ CD4+ T cells responding to: (B) S1, (C) S2, (D) envelope and membrane (EM), (E) N, (F) ORF3a and 6, (G) ORF7a, 7, and 8 (n=114; tested in single replicates). Positive responses as determined by MIMOSA are indicated by a solid circle and negative responses are indicated by open triangles. The bold black line represents the median fitted curve from a nonlinear mixed effects model of post-day 30 responses with random effects for the intercept and slope. The mixed effects models only include individuals with a positive response to the antigen(s) under consideration at one or more time points. Related to Figure 5.
Figure S6. CD8 T+ cell responses among COVID-19 patients to individual SARS-CoV-2 peptide pools. (A) Representative SARS-CoV-2-specific CD8+ T cell responses to multiple SARS-CoV-2 antigens by intracellular cytokine staining (ICS) assay in PBMCs from a SARS-CoV-2 patient. Background-subtracted frequencies of IFN-γ+ CD8+ T cells responding to: (B) S1, (C) S2, (D) envelope and membrane (EM), (E) N, (F) ORF3a and 6, (G) ORF7a, 7, and 8 (n=114; tested in single replicates). Positive responses as determined by MIMOSA are indicated by a solid circle, and negative responses are indicated by open triangles. The bold black line represents the median fitted curve from a nonlinear mixed effects model of post-day 30 responses with random effects for the intercept and slope. The mixed effects models only included individuals with a positive response to the antigen(s) under consideration at one or more time points. Related to Figure 6.