Adaptive immune responses to SARS-CoV-2 infection have been extensively characterized in blood; however, most functions of protective immunity must be accomplished in tissues. Here, we report from examination of SARS-CoV-2 seropositive organ donors (ages 10 to 74) that CD4⁺ T, CD8⁺ T, and B cell memory generated in response to infection is present in the bone marrow, spleen, lung, and multiple lymph nodes (LNs) for up to 6 months after infection. Lungs and lung-associated LNs were the most prevalent sites for SARS-CoV-2–specific memory T and B cells with significant correlations between circulating and tissue-resident memory T and B cells in all sites. We further identified SARS-CoV-2–specific germinal centers in the lung-associated LNs up to 6 months after infection. SARS-CoV-2–specific follicular helper T cells were also abundant in lung-associated LNs and lungs. Together, the results indicate local tissue composition of cellular and humoral immune memory against SARS-CoV-2 for site-specific protection against future infectious challenges.
have not been reported. Moreover, the relationship between human B and T cell memory in tissues is largely unexplored.

The use of physiologically healthy tissues from organ donors has enabled study of human immune cells across multiple sites (16, 34, 35). Investigating tissue immunity to SARS-CoV-2 is particularly challenging, as previously infected, but unvaccinated, donors are required. Here, we present an investigation of SARS-CoV-2–specific memory T and B cell populations in lymphoid and mucosal sites of previously infected, seropositive organ donors, which we identified in the bone marrow (BM), spleen, lung, and LNs up to 6 months after infection. Lung and lung-associated LNs were the most prevalent sites for SARS-CoV-2–specific memory T and B cells, with a proportion exhibiting tissue-resident profiles. We also detected virus-specific GC B cells in lung-associated LNs along with Tfh, suggesting ongoing generation of humorally immune. Together, our results reveal local coordination of cellular and humoral memory immune responses for site-specific protective immunity.

RESULTS
Organ donor cohorts for analysis of SARS-CoV-2–specific immune responses in tissues
We have established a human tissue resource for obtaining multiple tissues from organ donors through collaborations with organ procurement organizations (OPOs) (16, 34, 35). Use of organ donor tissue allows for rapid isolation of live immune cells for functional analysis, thus enabling assessment of immune responses in multiple sites within an individual. We identified four organ donors ages 10 to 74 years with previous SARS-CoV-2 infection (Fig. 1A) who died of noninfectious-related causes and were SARS-CoV-2–polymerase chain reaction (PCR) negative at the time of organ procurement. Previous SARS-CoV-2 infection history was based on post-procurement testing for antibodies to N protein (see Materials and Methods) and/or a confirmed history of COVID-19 2 to 6 months previously (table S1). Controls were prepandemic seronegative organ donors procured before November 2019 who also died of noninfectious causes (table S1).

All seropositive donors had detectable serum immunoglobulin G (IgG) to N, S, and RBD, along with SARS-CoV-2–specific neutralizing antibodies, consistent with antibody responses generated from acute infection (36, 37), whereas serum from prepandemic organ donors lacked antibodies to SARS-CoV-2 (Fig. 1, B and C). Using this cohort, we examined SARS-CoV-2–specific T and B cell responses across the blood, BM, spleen, lung, lung-associated LNs, as well as gut-associated LNs of seropositive and seronegative donors.

SARS-CoV-2–specific T cells in lung and lymphoid tissues
SARS-CoV-2–specific T cells in different sites from seropositive and seronegative organ donors were measured based on expression of T cell receptor–dependent activation-induced markers (AIMs) after stimulation with SARS-CoV-2–specific peptide megapools (MPs), which enable simultaneous presentation of a large number of virus-specific epitopes (see fig. S1 and Materials and Methods) (2, 38, 39). Mononuclear cells from each site were stimulated for 24 hours in vitro with four different SARS-CoV-2–specific MPs: MP_S (containing overlapping peptides representing the entirety of S), MP_CD4_R [containing predicted human leukocyte antigen (HLA) class II viral epitopes minus SI], and MP_CD8_A and MP_CD8_B (each containing predicted HLA class I epitopes from all viral proteins) (2). SARS-CoV-2–specific CD4+ T cells were identified on the basis of coexpression of two or more of the following three AIMs [OX40, 4-1BB, and/or CD40 ligand (CD40L)] (Fig. 1D and fig. S2A), whereas virus-reactive CD8+ T cells were identified on the basis of coexpression of CD25 and 4-1BB (Fig. 1E and fig. S2B). Quantification of SARS-CoV-2–specific CD4+ and CD8+ T cell responses was based on dimethyl sulfoxide (DMSO) background–subtracted frequencies of AIM+ CD4+ and CD8+ T cells (Fig. 1, F and H).

For CD4+ T cells, significant responses to S protein were found in all sites examined (blood, BM, spleen, lung, lung-associated LNs, and gut-associated LNs) relative to prepandemic control samples (Fig. 1F, left). For non–S SARS-CoV-2 epitopes, there were significant CD4+ T cell frequencies in the BM, lung-associated LNs, and gut-associated LNs (Fig. 1F, right). Total SARS-CoV-2–specific CD4+ T cell responses largely reflected the pattern observed with S-specific responses (Fig. 1, F and G). SARS-CoV-2–specific CD8+ T cell frequencies were generally lower in magnitude than for CD4+ T cells and more variable between donors (Fig. 1, G to I). Significant SARS-CoV-2–specific CD8+ T cell frequencies above controls were observed for lung- and gut-associated LNs for class I epitopes (Fig. 1, H and I). Comparing across all donors and sites, CD4+ T cells responding to S protein epitopes were the predominant SARS-CoV-2–specific T cells across tissue sites for all individuals (Fig. 1, F and G, and fig. S2C). Although the distribution patterns of SARS-CoV-2–specific T cell responses across tissue sites varied between donors (Fig. 1 and fig. S3), the lung and lung-associated LNs were most consistently the dominant sites for virus-specific CD4+ and CD8+ T cells (fig. S2D). These results indicate that SARS-CoV-2 infection generates virus-specific T cell responses across blood, multiple lymphoid sites, and lungs, with higher frequencies localized in lung tissue and lung-associated LNs.

SARS-CoV-2–specific T cells are maintained in tissues as circulating and resident memory subsets
We analyzed the subset distribution of SARS-CoV-2–specific T cells based on coordinate expression of CD45RA and CCR7, defining Tcm cells (CD45RA-CCR7+), TEM cells (CD45RA-CCR7-), terminally differentiated effector T (TEMRA) cells (CD45RA-CCR7+), and naive or stem-like memory cells (CD45RA-CCR7-) (40, 41). Each tissue had a distinct T cell subset composition that is conserved between individuals as we previously determined (26, 35, 42); T cell phenotypes for each site and for SARS-CoV-2–specific T cells are shown in representative flow cytometry plots (Fig. 2A). The majority of SARS-CoV-2–specific CD4+ T cells were maintained as TEM (≥75%) in the blood and lung and as TEM or TEMRA (≥80%) in lymphoid sites (Fig. 2B). For SARS-CoV-2–specific CD8+ T cells, the majority were maintained as TEM and TEMRA cells (≥50%) for all sites; the proportion of TEMRA was higher than TEM for the BM, spleen, and lung, whereas TEM were more prevalent than TEMRA for LNs (Fig. 2C). Donors D495 and D498 had a particularly high proportion of SARS-CoV-2–specific CD8+ TEMRA cells in the lung, whereas the pediatric donor (HDL113) harbored more SARS-CoV-2–specific CD45RA-CCR7+ CD8+ T cells in the lung and lung-associated LN (Fig. 2C).

We also analyzed coexpression of residency markers CD69 and CD103 by SARS-CoV-2–specific T cells (see fig. S1 for gating), as assessment of CD69 alone as a TEM marker was confounded because of CD69 up-regulation by T cell receptor–stimulated T cells. Virus-specific CD69+CD103+ memory CD4+ and CD8+ T cells (TEMRA) were mostly confined to the lung, whereas lower frequencies of SARS-CoV-2–specific CD8+ TEMRA were also detected in LNs (Fig. 2, D and E).
Fig. 1. SARS-CoV-2–specific CD4+ and CD8+ T cells in blood and tissues of previously infected organ donors. (A) SARS-CoV-2 seropositive donors and tissues used from each donor for this study. (B) Anti–SARS-CoV-2 antibody reactivities for seropositive and seronegative donors. Graphs show ET of IgG specific for SARS-CoV-2 Spike, RBD, and nucleocapsid. (C) SARS-CoV-2 Spike PSV neutralizing titers for seropositive and seronegative donors. Serology statistical analyses were performed using Mann-Whitney U test. *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; ****P ≤ 0.0001. (D) Identification of SARS-CoV-2–specific CD4+ T cells using the AIM assay. Mononuclear cells isolated from blood, BM, spleen, lung, and lung-associated lymph node (LLN) were stimulated with SARS-CoV-2 peptide pools (see Materials and Methods) and responding CD4+ T cells were identified based on induction of OX40, 4-1BB, and CD40L as shown in representative flow cytometry plots from D498 reactive to MP_S. SARS-CoV-2–specific CD4+ T cells were defined based on combined gates CD40L, 4-1BB, or CD40L/OX40 (right) of total CD4+ T cells for each stimulation condition and tissue site (see fig. S1 for gating strategy). (E) AIM assay for detection of SARS-CoV-2–specific CD8+ T cells in blood, BM, lung, LLN, and gut-associated lymph node (GLN) showing induction of 4-1BB and CD25 in representative flow cytometry plots from D495 reactive to MP_S. AIM+ CD8+ T cells were defined based on frequency 4-1BB+CD25+ from total CD8+ T cells for each stimulation condition and tissue site (see fig. S1 for gating strategy). (F) SARS-CoV-2 epitope-specific CD4+ T cells identified following stimulation with MP_S (left) and MP_CD4_R (right) peptide MPs from indicated tissues sites of seropositive and seronegative donors. (G) Total SARS-CoV-2–specific CD4+ T cells in each site from individual donors based on responses to all epitopes. (H) SARS-CoV-2–epitope specific CD8+ T cells identified following stimulation with MP_S (left), MP_CD8_A (middle), and MP_CD8_B (right) peptide MPs from indicated sites of seropositive and seronegative donors. (I) Total SARS-CoV-2–specific CD8+ T cells in each site from individual donors based on compiled responses to all epitopes. Statistical analysis was performed using one-way analysis of variance (ANOVA), corrected for multiple comparisons by false discovery rate (FDR) using two-stage linear step-up procedure of Benjamini, Krieger, and Yekutieli. *q ≤ 0.05, **q ≤ 0.01, ***q ≤ 0.001, and ****q ≤ 0.0001. Datasets were log-transformed before statistical analysis.
Together, these results show that SARS-CoV-2–specific T cells are maintained across diverse tissue sites as memory T cells, with a portion of T cells persisting as tissue-resident populations, particularly within the lung.

**Tissue specificity and heterogeneity of functional responses to SARS-CoV-2**

The functional responses of SARS-CoV-2–responding cells from different sites were assessed by multiplex quantification of 50 immune mediators from culture supernatants of peptide-stimulated mononuclear cells as in Fig. 1. Blood and tissues exhibited distinct functional profiles, and the magnitude of responses varied between donors (Fig. 3A and fig. S4). There was heterogeneity between individuals in the distribution of functional responses across sites. In particular, SARS-CoV-2–specific functional responses were primarily located in the lung for donor D492 and in the lung-associated LN and blood for donor D495, whereas D498 and HDL113 exhibited a broad functional response across multiple sites (Fig. 3A).
Fig. 3. Heterogeneity and tissue specificity of functional responses to SARS-CoV-2 epitopes. (A) Profiles of immune mediators produced for multiple tissue sites within SARS-CoV-2 seropositive donors following stimulation with peptide MPs MP_S (S), MP_CD4_R (R), MP_CD8_A (A), and MP_CD8_B (B), shown as a heatmap. The color intensity of each cell represents DMSO background–subtracted analyte concentration (max absolute scaled per column) within each donor (see Materials and Methods).

(B) Concentration of indicated immune mediators measured within supernatants from in vitro stimulations of blood, BM, spleen, lung, LLN, and GLN mononuclear cells with SARS-CoV-2 MPs for which SARS-CoV-2–specific T cells were identified based on DMSO background–subtracted frequencies of AIM+ CD4+ and CD8+ T cells. Statistical analysis was performed using one-way ANOVA, corrected for multiple comparisons by Tukey's multiple comparisons test. *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, and ****P ≤ 0.0001.

(C) Immune mediator milieu for each site. Heatmap showing log (Mean x + 1) pg/ml levels of immune mediators averaged across donors and stimulation conditions for each tissue site derived from samples for which significant frequencies of SARS-CoV-2–specific T cells were identified above background in Fig. 1. BL, blood; LG, lung.
elderly donors (D492 and D498), lung-associated LN responses were much weaker compared with other tissue sites, even when the frequency AIM C+CD4 + and CD8 + T cells in the lung-associated LN were high relative to other tissue sites (Fig. 3A and fig. S3). Overall, these data show that virus-specific functional responses are differentially maintained across sites and individuals.

Tissue-specific functional profiles were apparent in the profile of soluble mediators produced in response to stimulation with SARS-CoV-2 peptide pools (Fig. 3, B and C). The functional responses in the LN were the most diverse and included type 1 proinflammatory cytokines and cytolytic mediators [interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), granzyme B, perforin, and granulocyte-macrophage colony-stimulating factor (GM-CSF)], type 2 cytokines [interleukin-5 (IL-5), IL-9, and IL-13], and type 3 cytokines [IL-17A, IL-17F, and granulocyte colony-stimulating factor (G-CSF)]—most at increased levels compared with other sites (Fig. 3, B and C). The functional response in the lung was distinct from other sites and included proinflammatory profiles (TNF-α, perforin, granzyme, IL-12, IL-17A, and G-CSF), IL-10 associated with regulation of inflammation during respiratory infections (43), and higher levels of IL-6 and the homeostatic cytokine IL-15 compared with other sites (Fig. 3, B and C). BM responses were mostly TNF-α, perforin, granzyme B, and IL-10 and blood responses were similar to BM (Fig. 3, B and C). Comparing the immune mediator milieu between sites revealed that certain cytokines are produced across sites, whereas others are distinct to specific sites (Fig. 3C). Together, these results indicate that SARS-CoV-2–specific memory T cells in different sites exhibit distinct functional responses to viruses that are likely adapted to the site and contribute to a multifaceted protective response.

**SARS-CoV-2–specific memory B cells and resident phenotypes in tissues**

To characterize the nature of memory B cell responses to SARS-CoV-2 in tissues, we used fluorescently labeled, biotinylated, and multimerized probes of full-length S and RBD proteins to detect antigen-binding B cells among IgM + , IgG + , or IgA + memory B cells (fig. S5A), as previously described (1). SARS-CoV-2–specific memory B cells were detected at frequencies substantially higher in seropositive donors than seronegative donors in all tissue examined, including the lungs, BM, spleen, lung-associated LNs, and gut-associated LNs (Fig. 4A, A and B). IgG + was the dominant isotype of SARS-CoV-2–specific memory B cells in almost all samples, although IgM + and IgA + memory B cells were present (Fig. 4C). For D498, whereas few SARS-CoV-2–specific memory B cells were IgM + in the LNs, >40% were IgM + in the BM and spleen. For D495, whereas almost no IgA + SARS-CoV-2–specific memory B cells were found in the lung, ~25% were IgA + in gut-associated LNs (Fig. 4C). SARS-CoV-2–specific memory B cells were present at significantly higher frequencies in lung and lung-associated LNs than in the spleen or gut-associated LNs (Fig. 4A and D). The highest proportion of SARS-CoV-2–specific IgG + memory B cells was found in the lung and lung-associated LNs (Fig. 4D).

Memory B cells can persist as tissue-resident cells (BRM) in lymphoid or nonlymphoid tissues and are identified by CD69 expression (32, 33, 44). In tissues of seropositive and seronegative donors, we detected significant populations (>50 to 75%) of CD69 + B cells comprising >50% of total B cells in the lungs and LNs, whereas the BM and spleen contained much lower frequencies (0 to 10%) of CD69-expressing B cells (Fig. S5, B and C). Substantial frequencies of SARS-CoV-2–specific memory B cells exhibited CD69 expression indicative of tissue-resident profiles in lungs (50 to 80%) and LNs (20 to 40%) (Fig. 4, E and F). By contrast, negligible frequencies (<3%) of CD69 + SARS-CoV-2 S/RBD–specific memory B cells were detected in the BM of organ donors (Fig. 4, E and F), and in previously obtained peripheral blood samples from convalescent individuals with COVID-19 (fig. S5, D to I), consistent with previous analysis of polyclonal B cells in these sites (33). Together, these results provide direct evidence for human antigen-specific B RM in lungs and LNs that are distinct from corresponding populations in the blood. Thus, SARS-CoV-2 infection leads to the preferential formation and/or retention of antigen-specific B cell memory within lungs and lung-associated LNs, with CD69 + tissue-resident cells representing the majority of the SARS-CoV-2 S–specific memory B cells in lungs.

**SARS-CoV-2–specific GC B cells and T FH cells in LNs**

GCs within lymphoid organs are important microanatomical sites in which activated B cells receive cognate help from T FH to undergo somatic hypermutation to evolve higher affinity antibody recognition of pathogens (45). While previous studies have demonstrated affinity-matured SARS-CoV-2–specific memory B cells in the blood (3), BM plasma cells (46), and circulating virus-specific T FH cells (47), which all indicate GC responses in COVID-19 (48), direct evidence of SARS-CoV-2 antigen-specific GCs (and S–specific GC B cells, in particular) is lacking. We identified GC B cells by assessing coexpression of Bcl6 [a transcription factor required for GC B cell differentiation (49)] and Ki67 (a marker of active cellular proliferation) among total CD19 + B cells (fig. S5A). The frequency of GC B cells (Bcl6 +Ki67 +CD19 + B cells) trended higher in lung-associated LNs of seropositive donors than seronegative donors (q = 0.053; Fig. 5, A and B). SARS-CoV-2–specific GC B cells identified based on binding to S and RBD proteins were identified in lung-associated LNs of three of four seropositive donors and in gut-associated LNs of one donor (Fig. 5, C and D, and fig. S5J). Virus-specific GC B cells were not detected in the BM, spleen, or lung of seropositive donors or in any tissues of seronegative donors (Fig. 5, C and D). These results provide direct evidence that SARS-CoV-2–specific GC responses are induced by SARS-CoV-2 infection and are maintained in lung-associated LNs after resolution of infection. In addition, long-lasting GCs can even be generated in human gut-associated LNs.

T FH cells can be identified by coexpression of CXCR5 and PD-1 (50). T FH–phenotype cells (CXCR5 +PD-1 +CD4 + T cells) were found at low frequencies (5 to 10%) in lymphoid sites (spleen, lung-associated LNs, and gut-associated LNs) and in even lower frequencies in BM and most lungs of seronegative and seropositive donors (fig. S6), demonstrating the relative rarity of this population among total T cells. However, SARS-CoV-2–specific T FH cells (identified by A1Ms as in Fig. 1) were found in multiple sites of seropositive donors comprising 20 to 50% of SARS-CoV-2–reactive CD4 + T cells in LNs and lower but significant frequencies in the lung, spleen, and BM (Fig. 5, E to G). In one seropositive donor, more than 80% of virus-specific CD4 + T cells in the lung were T FH, representing ~4% of non-naïve (NN) SARS-CoV-2–specific CD4 + T cells, consistent with the higher overall frequency of T FH cells in the lungs of that donor (Fig. 5, F and G, and fig. S6). Together, the SARS-CoV-2–specific GC B cell and T FH data indicated robust GC responses to SARS-CoV-2 infection, distributed among lymphoid tissues and sites of viral infection, with some GCs being active for months after infection.
Fig. 4. SARS-CoV-2–specific memory B cells in tissues. (A) Representative flow cytometry plots showing staining patterns of probes for SARS-CoV-2 Spike (top) and RBD (bottom) on memory B cells, defined here as CD19⁺CD20⁺IgD⁻ non-GC B cells (see fig. S5A for gating). Memory B cells in BM, spleen, lung, LLN, and GLN from a SARS-CoV-2 Spike seropositive donor (D498) and memory B cells in lung from a seronegative donor (D340). Percentages are indicated. (B) SARS-CoV-2–specific memory B cells in tissues. Graph shows frequency of memory B cells specific to Spike and/or RBD (S/RBD) in indicated sites expressed as a percentage of CD19⁺CD20⁺ total B cells. (C) Fraction of SARS-CoV-2 S/RBD–specific memory B cells that belong to indicated Ig isotypes. (D) Frequency (percentage) of IgG⁺ memory B cells that are specific to S/RBD. (E) Representative flow cytometry plots showing CD69 expression on memory B cells specific to S/RBD. Percentages are indicated. (F) Frequency (percentage) of SARS-CoV-2 S/RBD–specific memory B cells that are CD69⁺. n = 4 Seropositive donors (n = 4 for lung, LLN, and GLN; n = 3 for spleen; n = 2 for BM). n = 7 Seronegative donors (n = 4 for lung and spleen; n = 5 for LLN, GLN, and BM). Statistical analysis was performed using one-way ANOVA corrected for multiple comparisons by FDR using two-stage linear step-up procedure of Benjamini, Krieger, and Yekutieli. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, and ****p ≤ 0.0001. For (B) and (D), datasets were log-transformed before statistical analysis.
Fig. 5. SARS-CoV-2–specific GC B cells and follicular helper T cells in seropositive donors. (A) Bcl6+Ki67+ GC B cells in different tissues, shown in representative flow cytometry plots from seropositive donor D498 (see fig. S5 for gating). (B) Frequency of Bcl6+Ki67+ GC B cells in tissue sites of seropositive and seronegative donors expressed as a percentage of total CD19+CD20+ total B cells. (C) GC B cells in the LLN for each donor shown in representative flow cytometry plots. GC B cells specific to S/RBD are depicted in red. (D) Frequency of S/RBD–specific GC B cells as a percentage of total B cells. (E) Tfh phenotype and frequency among NN CD4+ T cells per tissue as depicted by the rectangle gate shown in representative flow cytometry plots from seropositive donor D498 (lung, LLN, spleen, and BM) and D495 (GLN); see Materials and Methods for gating strategy. SARS-CoV-2–specific NN CD4+ T cells are highlighted in orange. (F) Frequency of CXCR5+PD-1+ Tfh cells per tissue as percentage of SARS-CoV-2–specific CD4+ T cells. (G) Frequency of SARS-CoV-2–specific Tfh cells per tissue as percentage of total NN CD4+ T cells. Statistical analysis was performed on log-transformed datasets using one-way ANOVA, corrected for multiple comparisons by FDR using two-stage linear step-up procedure of Benjamini, Krieger, and Yekutieli. *q ≤ 0.05, **q ≤ 0.01; ***q ≤ 0.001. S/RBD, SARS-CoV-2 Spike and/or RBD; Tfh, Follicular helper T cell.
**Coordinated adaptive immunity across tissues**

The identification of SARS-CoV-2–specific memory T and B cells in multiple tissues, along with T<sub>FH</sub> and GC B cells in LNs, suggested site-directed coordination of cellular and humoral immunity. To identify potential associations between SARS-CoV-2–specific lymphocyte populations across sites, we performed an exploratory correlation analysis (Fig. 6 and fig. S7). The frequencies of S-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells (but not total SARS-CoV-2–specific T cells; fig. S7A) were positively correlated across tissue sites (P = 0.0116; Fig. 6A). Significant associations also emerged between SARS-CoV-2–specific B and T cells (Fig. 6, B to F, and fig. S7, B to K). SARS-CoV-2–specific CD4<sup>+</sup> T cell frequencies correlated positively with S/RBD-specific memory B cells across all tissues (P = 0.0009), as well as IgG<sup>+</sup> (P = 0.0009) and IgA<sup>+</sup> (P = 0.0203) subpopulations (Fig. 6, B and C, and fig. S7, D and E). For tissue-resident SARS-CoV-2–specific lymphocytes, CD69<sup>+</sup>CD103<sup>+</sup>CD8<sup>+</sup> T<sub>RM</sub> cells correlated with CD69<sup>+</sup> B<sub>RM</sub>—both as frequencies of total lymphocytes (P = 0.0069) and as fractions within their respective antigen-specific populations (P = 0.01) (Fig. 6E and fig. S7). Positive correlations were also observed between CD4<sup>+</sup> T<sub>RM</sub> and B<sub>RM</sub> across all tissues (P = 0.0198; fig. S7). For follicular responses, S/RBD-specific memory B cells correlated with SARS-CoV-2–specific T<sub>FH</sub> cells across tissues (P = 0.0236; Fig. 6F and fig. S7K).

Given that lung and lung-associated LNs contained the highest frequencies of SARS-CoV-2–specific lymphocytes, we performed a targeted correlation matrix analysis to identify potential associations within and between these sites (Fig. 6G). SARS-CoV-2–specific T<sub>FH</sub> cells in the lung-associated LNs were significantly associated with lung memory B cells, whereas SARS-CoV-2–specific CD4<sup>+</sup> T cell frequencies positively correlated with SARS-CoV-2–specific GC B cells lung-associated LNs. Conversely, certain LN and lung populations were inversely correlated. In particular, the amount of S/RBD-specific GC B cells in the lung-associated LNs negatively correlated with S-specific CD4<sup>+</sup> T cells in the lung. Similarly, S/RBD-specific memory B cell frequencies in the lung-associated LNs negatively correlated with SARS-CoV-2–specific and S-specific CD8<sup>+</sup> T cell frequencies in the lung. Similar correlations were observed when including blood and plasma antigen-specific lymphocyte populations in correlation matrix analyses (fig. S8). Together, these results suggest opposing or compensatory effects of humoral and cellular immune responses in lung-associated LNs and lungs.

**DISCUSSION**

Immunological memory is maintained by heterogeneous subsets of virus-specific T and B cells in nonlymphoid tissue sites of infection and multiple lymphoid organs. A comprehensive assessment of memory responses is therefore difficult to accomplish in humans. Here, we reveal the cellular complexity and functional diversity of SARS-CoV-2–specific memory T and B cells in lymphoid and mucosal tissues of previously infected organ donors up to 6 months after infection (see fig. S9 for summary schematic). SARS-CoV-2–specific CD4<sup>+</sup> T, CD8<sup>+</sup> T, and B cells predominantly localized in the lung and lung-associated LNs and were maintained as memory cell populations. Tissue-resident T and B cells, known to participate in protection against secondary viral infections, were found most abundantly in the lung and were correlated across multiple sites. Moreover, SARS-CoV-2–specific GC B cells and T<sub>FH</sub> cells were found in lung-associated LNs, suggesting persisting GC responses months after resolution of infection. Together, these results indicate that the maintenance of SARS-CoV-2–specific immune memory is characterized by localized, ongoing coordination of cellular and humoral immunity within tissues.

SARS-CoV-2–specific memory T and B cells were found throughout the body and localized preferentially to lung and lung-associated LNs, providing direct evidence that those sites are key locations for establishment of immune memory after SARS-CoV-2 infection. Gut-draining LNs in some donors were also significant sites for SARS-CoV-2–specific memory T and B cells (particularly T<sub>RM</sub> and B<sub>RM</sub>), which could be because of the gut being a major site for SARS-CoV-2 replication in some cases (3, 51). The low frequency of SARS-CoV-2–specific memory T or B cells in the spleen further suggests that virus infection is generally limited to mucosal sites of entry. Our results show that a proportion of SARS-CoV-2–specific memory T cells in the lung were T<sub>RM</sub>, consistent with findings in airways of severe COVID-19 (28) and surgical lung samples from previously infected patients (52). Additionally, we show that the majority of the SARS-CoV-2–specific memory B cells were resident. In mouse models of influenza infection, localization and tissue residence of T and B cells to the lung and lung-associated LNs are correlated with optimal protective responses (20, 23, 32). Therefore, tissue-localized and resident memory T and B cells in the lung are likely important for site-specific protection and could be targets for site-specific boosting in vaccination.

SARS-CoV-2–specific memory CD4<sup>+</sup> T cells were identified at significantly higher frequencies than CD8<sup>+</sup> T cells across tissue sites, reflecting previous studies of peripheral blood showing that CD4<sup>+</sup> responses are more robust than CD8<sup>+</sup> responses months after resolution of infection (2, 53). In addition, SARS-CoV-2–specific T cells exhibited tissue-specific functional profiles with cytotoxic proinflammatory, regulatory, and tissue repair functions variably manifested across different sites. In the lung-associated LNs, memory T cells exhibited broad proinflammatory, helper, and regulatory functional profiles. SARS-CoV-2–specific lung T cells produced higher levels of IL-10 compared with other sites consistent with a role for T cell–derived IL-10 in regulating lung inflammation in mice (43). We previously showed in paired airway and blood samples of patients with severe COVID-19 that the cytokine and chemokine profile in airway washes was distinct from that in plasma (28). Here, we further demonstrate that the functional responses of virus-specific T cells are tissue-specific—not only at the site of infection but also across numerous lymphoid tissues. Together, these results suggest that T cells in tissues mediate responses that are functionally adapted to the tissue site, resulting in heterogeneity of immune memory stored throughout the body.

SARS-CoV-2–specific memory B cells were distributed across multiple sites. While frequencies were highest in lung and LNs, there were also significant frequencies in BM. In all sites, virus-specific memory B cells exhibited a predominantly IgG<sup>+</sup> memory phenotype. The finding of S/RBD-specific B<sub>RM</sub> in lung and lymphoid sites was notable, as was the low frequency of IgA<sup>+</sup> SARS-CoV-2–specific memory B cells in mucosal tissue and associate LNs.

Our results directly demonstrate ongoing, persistent GC responses in LNs following resolution of SARS-CoV-2 infection—including at least one example 6 months after infection. Despite a report of potentially impaired GC responses in fatal COVID-19 (54), our data show coordinated T<sub>FH</sub> and GC B cells in the lung-associated LNs to nonfatal SARS-CoV-2 infection. These results provide evidence of
Fig. 6. SARS-CoV-2–specific immune memory relationships across organs. (A) Correlation between SARS-CoV-2 Spike–specific CD4+ T cells as frequency of total CD4+ T cells and Spike–specific CD8+ T cells as frequency of total CD8+ T cells. (B) Correlation between the frequency of SARS-CoV-2–specific CD4+ T cells and SARS-CoV-2 S/RBD–specific memory B cells. (C) Correlation between SARS-CoV-2–specific CD4+ T cells and IgG+ SARS-CoV-2 S/RBD–specific memory B cells. (D) Correlation between SARS-CoV-2–specific CD8+ T cells and SARS-CoV-2 S/RBD–specific memory B cells within lymphoid tissues. (E) Correlation between SARS-CoV-2–specific CD69+CD103+ CD8+ TRM cells and CD69+ CD8+ cells. (F) Correlation between SARS-CoV-2–specific CXCR5+PD-1+ TFH cells as frequency of NN CD4+ T cells and SARS-CoV-2 S/RBD–specific memory B cells as frequency of total memory B cells. (G) Correlogram of SARS-CoV-2–specific lung and LLN lymphocyte populations. Pearson’s R coefficients are shown from blue (−1.0) to red (1.0); R values are indicated by color and circle size. SARS-CoV-2–specific lymphocyte frequencies are depicted as a percentage of the parent population (%) or as counts per million peripheral blood mononuclear cells (/M); SARS-CoV-2–specific TFH cells are a percentage of total NN CD4+ T cells. Statistical analysis was performed on datasets using Pearson correlation. *P < 0.05, **P < 0.01, and ***P < 0.001. GCB, GC B cell; MB, memory B cell; TFH, CD69+ CD103+–resident memory T cell; BRM, CD69+–resident memory B cell; S+, Spike protein–specific; S2+, SARS-CoV-2–specific.
ongoing GC reactions after resolution of infection, consistent with reports of prolonged evolution of humoral responses in peripheral blood up to 6 months after SARS-CoV-2 infection (3, 55). GC B cells were detected in donors spanning a broad age range—from 10 to 74 years, providing compelling evidence that the ability to establish robust GC responses to novel pathogens can be maintained with age.

These results also indicate ongoing interaction and coordination between T and B cells within LNs, which we also found related to memory populations in the lung. Significant correlations were also found between SARS-CoV-2–specific memory B and T cell populations across tissue sites, consistent with correlations between virus-specific T and B cell responses in peripheral blood of previously infected individuals (1, 7). We also identified potential inverse correlations between frequencies of virus-specific CD8+ T cells in the lung and memory B cells in the lung-associated LN, suggesting that lung responses in situ can affect the magnitude or requirement for humoral responses in the associated LN. Together, these findings suggest that dynamic coordination of adaptive immune responses across the body is a feature of antiviral immunity to SARS-CoV-2.

This work has certain limitations. Namely, our study focuses on four seropositive donors across seven decades of life to provide a representative profile of tissue-specific antiviral immune responses. In addition to the challenges of obtaining live cells for immunological studies from organ donors, the findings here also depended on SARS-CoV-2 seropositive donors who had not been vaccinated, thus limiting the size of the donor pool and the timeframe of collection (before December 2020). The consistency in cell type and site-specific trends and correlations across all profiled donors, as well as corroboration of larger scale blood studies, demonstrates how this project provides new insights into tissue-specific immune memory maintenance and persistence of humoral and cellular responses after SARS-CoV-2 infection.

In conclusion, we reveal here that immunological memory from SARS-CoV-2 infection is maintained as heterogeneous subsets across multiple sites, with active and preferential maintenance in lung and associated LNs, as well as site-specific functional adaptations. These findings support the development of site-specific strategies for monitoring immune memory to infections and vaccines and for fortifying immune responses at the infection sites.

**MATERIALS AND METHODS**

**Study design**

The objective of this study was to measure adaptive immune responses to SARS-CoV-2 in blood and tissues of seropositive individuals after resolution of infection. We measured the frequency of SARS-CoV-2–specific CD4+ T, CD8+ T, and B cells in seropositive organ donors compared with prepandemic seronegative donors to understand the maintenance of immunological memory to SARS-CoV-2 as T and B cell subsets across the body, the functional immune response in tissues, and the immune memory relationships across circulating and tissue-resident SARS-CoV-2–specific T and B cell populations.

**Human samples**

Human tissues were obtained from deceased organ donors at the time of organ acquisition for clinical transplantation through an approved protocol and material transfer agreement with LiveOnNY, the OPO for the New York metropolitan area, as previously described (34, 35, 56–60). Human tissues from the pediatric donor (HDL113) were obtained through arrangements with multiple OPOs across the United States through the Human Atlas for Neonatal Development–Immunity program, which is an extension of the coordinating center set up for nPOD (network for Pancreatic Organ Donors with Diabetes) (61). A list of donors from which tissues were used in this study is presented in table S1. Organ donors were tested for SARS-CoV-2 infection (and confirmed as SARS-CoV-2 negative) by PCR testing of nasal swabs, tracheal aspirates, and/or bronchoalveolar lavage. A history of previous COVID-19 (D492 and D498) and positive serology (D495) was provided in the donor summary, and SARS-CoV-2 serology for all donors was measured in the Center for Advanced Laboratory Medicine at Columbia University Irving Medical Center. Tissues from all seropositive donors were obtained before December 2020, and all donors were free of cancer and seronegative for hepatitis B, hepatitis C, and HIV. Because tissues were obtained from brain-dead organ donors, this study does not qualify as “human subjects” research, as confirmed by the Columbia University Institutional Review Board.

**Isolation of single-cell suspensions from tissue samples**

Tissue samples were maintained in cold saline or media and transported to the laboratory within 2 to 4 hours of organ procurement for adult organs and shipped to the laboratory on ice within 24 hours of procurement for pediatric donors. Tissue processing protocols were adapted from protocols previously described (34, 56–60), with some recent optimizations. Briefly, mononuclear cells were isolated from the blood and BM samples by density centrifugation using ficoll-Paque PLUS (GE Healthcare, catalog no. 17-1440-03). Spleen was processed using mechanical dissociation, followed by pushing through 100-μm filters (Thermo Fisher Scientific, catalog no. 50-146-1428), and ficoll-Paque density centrifugation as above. Lung and LN samples were first incubated with collagenase D (1 mg/ml) (Sigma-Aldrich, catalog no. 1108882001) and deoxyribonuclease (0.1 mg/ml) (Thermo Fisher Scientific, catalog no. NC979009) in Iscove’s modified Dulbecco’s medium (Thermo Fisher Scientific, catalog no. 12-140-053) or 0.5 M EDTA (pH 8.0) (Thermo Fisher Scientific, catalog no. 150575-020), filtration, and density centrifugation as above, resulting in high yields of live leukocytes.

**SARS-CoV-2 serology testing**

Blood from deceased organ donors (D495, D498, and HDL113) was collected, and serum was obtained after centrifugation using serum separating clot activator tubes (Thermo Fisher Scientific, catalog no. 22040546). SARS-CoV-2 serology testing for N protein was then performed by the Center for Advanced Laboratory Medicine at Columbia University Irving Medical Center to determine previous exposure to SARS-CoV-2 for inclusion in the study.

SARS-CoV-2 enzyme-linked immunosorbent assay titers were determined as previously described (1). Briefly, Corning 96-well half-area plates (Thermo Fisher Scientific, catalog no. 3690) were coated with SARS-CoV-2 S protein (1 μg/ml), RBD protein, or N protein (Sino Biological, catalog no. 40588-V07E) overnight at 4°C. The next day, plates were blocked with 3% milk (skim milk powder, Thermo Fisher Scientific, catalog no. LP0031) or phosphate-buffered saline (PBS) containing 0.05% Tween 20 (ThermoScientific, catalog no. J06005-AP) for 2 hours at room temperature. Heat-inactivated serum (30 min at 56°C) was then added to the plates and incubated for 1.5 hours at room temperature. Plates were washed five times with 0.05% PBS/Tween.
20. Secondary antibodies were diluted in 1% milk containing 0.05% Tween 20 in PBS. IgG titers were determined using anti-human IgG peroxidase antibody (Hybridoma Reagent Laboratory, catalog no. HP6123-HRP) at 1:1000 dilution. End-point titers were plotted for each sample using background subtracted data. The limit of detection was defined as 1:3 for IgG.

**Pseudovirus neutralization assay**

The pseudovirus (PSV) neutralization assays were performed as previously described (1). Briefly, 2.5 × 10⁴ Vero cells [American Type Culture Collection (ATCC), catalog no. CCL-81] were seeded in clear flat-bottom 96-well plates (Thermo Fisher Scientific, catalog no. 165305) to produce a monolayer at the time of infection. Recombinant SARS-CoV-2 S–D614G–pseudotyped VSV-ΔG-GFP were generated by transfecting human embryonic kidney 293T cells (ATCC, catalog no. CR1-321) with plasmid pCMV3–SARS-CoV-2 S and then infecting with VSV-ΔG-GFP. Pretitrated rVSV–SARS-CoV-2–S–D614G was incubated with serially diluted human heat-inactivated serum at 37°C for 1 to 1.5 hours before addition to confluent Vero cell monolayers. Cells were incubated for 16 hours at 37°C in 5% CO₂, fixed in 4% paraformaldehyde in PBS (pH 7.4) (Santa Cruz, catalog no. sc-281692) with Hoechst (10 μg/ml) (Thermo Fisher Scientific, catalog no. 62249), and imaged using a CellInsight CX5 imager to quantitate the total number of cells and infected green fluorescent protein (GFP)—expressing cells to determine the percentage of infection. Neutralization titers or inhibition dose 50 (ID₅₀) were calculated using the One-Site Fit Log IC₅₀ model in Prism 8.0 (GraphPad). As internal quality control to define the interassay variation, three samples were included across the PSV neutralization assays. Samples that did not reach 50% inhibition at the lowest serum dilution of 1:20 were considered as non-neutralizing.

**In vitro T cell stimulations with SARS-CoV-2 peptide MPs**

Mononuclear cells from the blood, BM, spleen, lung, lung-associated LNs, and gut-associated LNs of SARS-CoV-2 seropositive donors were thawed, and dead cells were removed using the EasySep Dead Cell Removal (annexin V) Kit (STEMCELL Technologies, catalog no. 17899) containing 10% heat-inactivated human AB serum (Gemini, catalog no. 507533010) and penicillin-streptomycin-glutamine (Thermo Fisher Scientific, catalog no. 17899) containing 10% heat-inactivated human AB serum (Gemini, catalog no. 88-8824-00) before staining with antibodies against transcription factors in eBioscience Permeabilization Buffer (Thermo Fisher Scientific, catalog no. L34962) in PBS at 4°C for 30 minutes. Cells were then fixed and permeabilized using eBioscience Intracellular Fixation & Permeabilization Buffer Set (Thermo Fisher Scientific, catalog no. 88-8824-00) before staining with antibodies against transcription factors in eBioscience Intracellular Fixation and Permeabilization Buffer Set (Thermo Fisher Scientific, catalog no. 00-8333-56). Samples were acquired on Cytex Aurora and analyzed using FlowJo V 10.7.1 (BD Biosciences).

For flow cytometry analysis of SARS-CoV-2 antigen-reactive T cells, cells were stained in 96-well U-bottom plates protected from light using fluorochrome-conjugated antibodies (see table S2 for antibodies in the T cell flow cytometry panel). Briefly, cells were washed with fluorescence-activated cell sorting (FACS) buffer (PBS with 2% heat-inactivated fetal bovine serum) and then resuspended with surface staining antibody cocktail for 20 min at room temperature. Surface-stained cells were fixed for 30 min at room temperature in fixation buffer (Tonbo, catalog no. TNB-0607-KIT), washed with permeabilization buffer (Tonbo, catalog no. TNB-0607-KIT), and washed again with FACS buffer. Flow cytometry data were collected using the five-laser Cytex Aurora flow cytometer (Cytex Bio) and analyzed using FlowJo V 10.7.1 and Prism 9.0.1 software.

For flow cytometry analysis of SARS-CoV-2–specific B cells, biotinylated protein antigens were immobilized onto fluorescently labeled streptavidin were used as probes to detect antigen-specific B cells (see table S3 for antibodies used in the B cell flow cytometry panel). Avidiated full-length SARS-CoV-2 S (2P-stabilized, double streptavidin-tagged) and RBD proteins were generated in-house. Biotinylation was performed using biotin protein ligase standard reaction kit (AviDity, catalog no. Bir500A) following the manufacturer’s protocol and dialyzed against PBS. Biotinylated S was mixed with streptavidin BV421 (BioLegend, catalog no. 405225) and streptavidin BV737 (BD Bioscience, catalog no. 612775) at 20:1 ratio (−61 molar ratio). Biotinylated RBD was mixed with streptavidin phycoerythrin (PE)–Cy7 (BioLegend, catalog no. 405206) and streptavidin BUV661 (BD Bioscience, catalog no. 612979) at 2:2:1 ratio (−4:1 molar ratio). Streptavidin PE–Cy5.5 (Thermo Fisher Scientific, catalog no. SA1018) was used as a decoy probe for nonspecific streptavidin-binding B cells. The probes were then mixed in Brilliant Stain Buffer (BD Bioscience, catalog no. 566349) containing 5 μM free d-biotin (AviDity, catalog no. Bir500A). Cells (×10⁷) were prepared in U-bottom 96-well plates and stained with 50 μl of antigen cocktail containing 400 ng of S (200 ng per probe), 100-ng of RBD (50 ng per probe), and 20 ng of streptavidin PE–Cy5.5 at 4°C for 1 hour followed by staining for surface markers in Brilliant Stain Buffer at 4°C for 30 min. Dead cells were stained using the LIVE/DEAD Fixable Blue Stain Kit (Thermo Fisher Scientific, catalog no. L34962) in PBS at 4°C for 30 min. Cells were then fixed and permeabilized using eBioscience Intracellular Fixation and Permeabilization Buffer Set (Thermo Fisher Scientific, catalog no. 88-8824-00) before staining with antibodies against transcription factors in eBioscience Permeabilization Buffer (Thermo Fisher Scientific, catalog no. 00-8333-56). Samples were acquired on Cytex Aurora and analyzed using FlowJo V10.7.1 (BD Biosciences). In each
experiment, peripheral blood mononuclear cells from a known convalescent individual with COVID-19 and an unexposed individual were included to ensure consistent sensitivity and specificity of the assay.

**Multiplex detection of cytokines**
Cryopreserved supernatant from in vitro T cell stimulation experiments was sent to Eve Technologies Corp. (Calgary, Alberta) for quantification of 50 total human cytokines, chemokines, and growth factors. Luminex xMAP technology was used for multiplexed quantification of two human cytokines in one array (perforin and granulocyte colony stimulating factor, G-CSF), GM-CSF, IFN-α, IL-1α, IL-1β, IL-1a, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-17A, IL-17E/IL-25, IL-17F, IL-18, IL-22, IL-27, IP-10, MCP-1 (monocyte chemoattractant protein 1), MCP-3, M-CSF, MDC (macrophage-derived chemokine) (CCL22), MIG (monokine induced by gamma interferon), MIP-1α, MIP-1β, PDGF-AA (platelet-derived growth factor AA), PDGF-AB/BB, RANTES, TNF-α (transforming growth factor-α), TNF-β, VEGF-A (vascular endothelial growth factor A). The multiplexing analysis was performed using the Luminex 200 system with assay kits sourced by Millipore MILLIPLEX (MilliporeSigma, Burlington, Massachusetts, USA) according to the manufacturer’s protocol.

Observed concentrations were calculated with the standard curve based on the fluorescence intensity of the bead population for a specific analyte. For analysis and visualization of cytokine/chemokine production by antigen-responding cells in multiple tissues sites within each individual donor, observed concentrations for each analyte were first subtracted from DMSO-negative control and then scaled across samples for each individual donor on a maximum absolute scale, with values ranging from −1 to 1 across all analytes using the MaxAbsScaler.preprocessing function of the Python scikit-learn library (36, 63). Heatmap visualizations were generated using the Python data visualization library seaborn (64). For analysis comparing the production of analytes across donors and tissue sites either absolute observed concentrations were used or observed concentrations for each analyte were normalized to DMSO-negative control.

**Correlogram plot and visualization**
Correlograms were analyzed and plotted using the Pearson product moment correlation coefficient (r) between all parameter pairs from blood, lung, and lung-associated LN lymphocyte populations (see data file 2 for raw data, R and P values). Correlograms were created with the corplot package (v0.88) (65) running under R (v4.0.2) in RStudio (1.4.1103). Visual clustering of parameters was performed using the “hclust” option of correMatOrder. Two-sided P values were calculated using corr.test (stats v4.0.2) and graphed (corplot v0.88) based on *P ≤ 0.05, **P ≤ 0.01, and ***P ≤ 0.001.

**Statistical analysis**
Descriptive statistics of compiled flow cytometry data and statistical testing were performed using Prism (GraphPad). Graphs were generated using Prism (GraphPad), Python matplotlib and seaborn libraries (65, 67), and RStudio corplot package (66). Differences in means between two sample groups were compared using nonparametric test of null hypothesis Mann-Whitney U test. Pearson correlations were used to evaluate immune memory relationships. Multiple group comparisons were done using one-way analysis of variance (ANOVA), corrected for multiple comparisons by false discovery rate using two-stage linear step-up procedure of Benjamini, Krieger, and Yekutieli when comparing seropositive and seronegative donors. For comparing immune mediator profiles across tissue sites, statistical analyses were performed via one-way ANOVA corrected for multiple comparisons by Tukey’s multiple comparison test. 

**REFERENCES AND NOTES**
1. J. M. Dan, J. Mateus, Y. Kato, K. M. Hastie, E. D. Yu, C. E. Faliti, A. Grifoni, S. I. Ramirez, S. Haupt, A. Frazier, C. Nakao, V. Rayaprulu, S. A. Rawlings, B. Peters, F. Krammer, V. Simon, E. O. Saphire, D. Smith, W. Deischof, A. Sette, S. Crotty, Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. Science 371, eabf4063 (2021).
2. A. Grifoni, D. Weiskopf, S. I. Ramirez, J. Mateus, J. M. Dan, C. R. Moderbacher, A. S. Rawlings, A. Sutherland, L. Prekmurkar, R. S. Jadi, D. Marrama, A. M. de Silva, A. Frazier, A. F. Carl, J. A. Greenbaum, B. Peters, F. Krammer, D. M. Smith, S. Crotty, A. Sette, Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. Cell 181, 1489–1501.e15 (2020).
3. C. Gaebler, Z. Wang, J. C. C. Lorenzi, F. Muecksch, S. Finkin, M. Tokuyama, A. Cho, M. Jankovic, D. Schafer-Babajev, T. Y. Oliveira, M. Cipolla, C. Viant, C. O. Barnes, Y. Bram, G. Breton, T. Hagglof, F. Mendoza, A. Hurley, M. Turroja, K. Gordon, K. G. Millard, V. Ramos, F. Schmidt, Y. Weisblum, D. Jha, M. Tankeliehe, G. Martinez-Delgado, J. Yee, R. Patel, J. Dizon, C. Unson-O’Brien, I. Shimeliovich, D. F. Robbiana, Z. Zhao, A. Gazuymun, R. E. Schwartz, T. Hatziioannou, P. J. Bjorkman, S. Mehandru, P. D. Bieniasz, M. Caskey, M. C. Nussenzweig, Evolution of antibody immunity to SARS-CoV-2. Nature 591, 639–644 (2021).
4. Z. Wang, F. Muecksch, D. Schafer-Babajev, S. Finkin, C. Viant, C. Gaebler, H. H. Hoffmann, C. O. Barnes, M. Cipolla, V. Ramos, T. Y. Oliveira, A. Cho, F. Schmidt, J. D. Isaeva, S. F. Bednarz, L. Aguado, T. Erfur, M. Daga, M. Turroja, K. G. Millard, M. Jankovic, A. Gazuymun, Z. Zhao, C. M. Rice, P. D. Bieniasz, M. Caskey, T. Hatziioannou, M. C. Nussenzweig, Naturally enhanced neutralizing breadth against SARS-CoV-2 one year after infection. Nature 595, 426–431 (2021).
5. L. B. Rodda, J. Netland, L. Shehata, C. B. Pruner, P. A. Moravwski, C. D. Thousenel, K. K. Takehara, J. Eggengerben, E. A. Hemann, H. R. Waterman, M. L. Fahning, Y. Chen, M. Hale, J. Rathe, C. Stokes, S. Wrenn, B. Fiala, L. Carter, J. A. Hamburger, P. King, M. Gale Jr., D. J. Campbell, D. J. Rawlings, M. Pepper, Functional SARS-CoV-2-specific immune memory persists after mild COVID-19. Cell 184, 169–183.e17 (2021).
6. T. Blrich, A. Neile, J. S. Heitmann, Y. Maringer, M. Roerden, J. Bauer, J. Riehl, M. Wacker, A. Peter, S. Horber, D. Rachalski, M. Marklin, S. Stevanovic, H. G. Rammsenee, H. R. Salih, J. S. Winz, T cell and antibody kinetics delineate SARS-CoV-2 peptides mediating long-term immune responses in COVID-19 convalescent individuals. Sci. Transl. Med. 13, eab7517 (2021).
7. K. W. Cohen, S. L. Lindnerman, Z. Moodie, J. Czartowski, L. Lai, G. Mantus, C. Norwood, L. E. Nyhoff, V. V. Edara, K. Floyd, S. C. De Rosa, H. Ahmed, R. Whaley, S. N. Patel, B. Pigrmoore, M. P. Lemoso, C. W. Davis, S. Furth, J. B. O’Keefe, M. P. Gharpure, S. Gunsetty, K. Stephens, R. Antia, V. I. Zarnitsyna, D. S. Stephens, S. Eseputaganti, N. Roupheal, E. J. Anderson, A. K. Mehta, J. Wrammert, M. S. Suthar, R. Ahmed, M. J. McLellan, Longitudinal analysis shows durable and broad immune memory after SARS-CoV-2 infection with persisting antibody responses and memory B cells. Cell Rep. Med. 2, 100354 (2021).
8. J. Zuo, A. C. Dowell, H. Pearce, K. Verma, H. M. Long, J. Begum, F. Aiano, Z. Amin-Chowdhury, K. Hoschel, T. Brooks, S. Taylor, J. Hevison, B. Hallis, L. Stapley, R. Borrow, E. Linley, S. Ahmad, B. Parker, A. Horsley, G. Amirthalingam, K. Brown, M. E. Ramsay, S. Ladhani, P. Moss, Robust SARS-CoV-2-specific T cell immunity is maintained at 6 months following primary infection. Nat. Immunol. 22, 620–626 (2021).
9. D. S. Khoury, D. Cromer, A. Reynolds, T. E. Schub, A. K. Wheatley, J. A. Juno, K. Subbarao, S. J. Kent, J. A. Triccas, S. Wrenn, B. Peters, R. Borrow, S. Peters, M. Pepper, Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. Nat. Med. 27, 1205–1211 (2021).
13. R. E. Chen, X. Zhang, J. B. Case, E. S. Winkler, Y. Liu, L. A. VanBlargan, J. Liu, J. M. Errico, L. J. Abu-Raddad, H. Chemaitelly, A. A. Butt; National Study Group for COVID-19

16. D. L. Farber, Tissues, not blood, are where immune cells function.

21. T. Wu, Y. Hu, Y. T. Lee, K. R. Bouchard, A. Benechet, K. Khanna, L. S. Cauley, Lung-resident memory CD4 T cells mediate optimal protection to respiratory virus infection.

22. J. Zhao, A. K. Mangalam, R. Channappanavar, C. Fett, D. K. Meyerholz, S. Agnihothram, J. G. de Jesus, P. S. Andrade, T. M. Coletti, G. M. Ferreira, C. A. M. Silva, E. R. Manuli, F. C. S. Sales, I. Hawryluk, J. T. McCrone, R. J. G. Hulswit, L. A. M. Franco, M. S. Ramundo, Reveals a site of vulnerability for SARS-CoV-2.

Poon et al., Sci. Immunol. 6, eabl9105 (2021)
60. P. Dogra, C. Rancan, W. Ma, M. Toth, T. Senda, D. J. Carpenter, M. Kubota, R. Matsumoto, P. Thapa, P. A. Szabo, M. M. Li Poon, J. Li, J. Arakawa-Hoyt, Y. Shen, L. Fong, L. L. Lanier, D. L. Farber, T. M. Caradonna, J. Chevalier, F. Chowdhury, T. J. Diefenbach, K. Einkauf, J. Fallon, J. Feldman, K. K. Finn, P. Garcia-Broncano, C. A. Hartana, B. M. Hauser, C. Jiang, P. Kaplanow, M. Karpel, E. C. Koscher, X. Lian, H. Liu, J. Liu, N. L. Ly, A. R. Michell, M. Perrot, E. Duchesnay, Scikit-learn: Machine learning in Python. J. Open Source Soft. 6, 90–95 (2021).

61. A. Pugliese, M. Yang, I. Kusmarteva, T. Heiple, F. Vendrame, C. Wasserfall, P. Rowe, J. M. Moraski, S. Ball, L. Jebsen, D. A. Schatz, R. Gianani, G. W. Burke, C. Nieras, T. Staeva, J. S. Kaddis, M. Campbell-Thompson, M. A. Atkinson, The Juvenile Diabetes Research Foundation Network for Pancreatic Organ Donors with Diabetes (nPOD): Goals, operational model and emerging findings. Pediatr. Diabetes 15, 1–9 (2014).

62. D. Weskopf, K. S. Schmitz, M. P. Raadse, A. Grifoni, N. M. A. Obka, H. Endeman, J. P. van den Akker, R. Molenkamp, M. P. G. Koopmans, E. C. M. van Gorp, B. L. Haagmans, R. L. de Swart, A. Sette, R. D. de Vries, Phenotype and kinetics of SARS-CoV-2-specific T cells in COVID-19 patients with acute respiratory distress syndrome. Sci. Immunol. 5, eabz2071 (2020).

63. S. Reiss, A. E. Baxter, K. M. Cirilli, J. M. Dan, A. Morou, A. Daigneault, N. Brassard, G. Silvestri, J. P. Routy, C. Havenar-Daughton, S. Crotty, D. E. Kaufmann, Comparative analysis of activation induced marker (AIM) assays for sensitive identification of antigen-specific CD4 T cells. PLOS ONE 12, e0186998 (2017).

64. F. Pedregosa, G. Varoquaux, A. Gramfort, V. Michel, B. Thirion, O. Grisel, M. Blondel, P. Prettenhofer, R. Weiss, V. Dubouj, J. Vanderplas, A. Passos, D. Kourmep, M. Brucher, M. Porro, E. Duchesnay, Scikit-learn: Machine learning in Python. JMLR 12, 2825–2830 (2011).

65. W. L. Waskorn, seaborin: Statistical data visualization. J. Open Source Soft. 6, 3021 (2021).

66. T. Wei, V. Simko, R package "corplot": Visualization of a correlation matrix (Version 0.89) (2021).

67. J. D. Hunter, Matplotlib: A 2D graphics environment. Comput. Sci. Eng. 9, 90–95 (2007).

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