INTRODUCTION
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection [coronavirus disease 2019 (COVID-19)] presents with a myriad of organ involvement, variable duration of symptoms, and diverse clinical presentations that range from asymptotic infection to death (1–6). The underlying determinants for variable responses to COVID-19 and other infections remain incompletely understood. Age, sex, comorbidities, and host genetics have emerged as key factors that increase the risk for severe COVID-19 (7–12). Studies in mice have additionally demonstrated a key role of environment on pathogen resistance, with an accumulation of memory T cells that modulate host protection in mice raised in a free-living environment (13, 14). What remains less clear is how the environment shapes human immune responses to SARS-CoV-2 and other pathogens.

Prior studies have identified T cells that recognize SARS-CoV-2 in unexposed individuals, including cells that expressed an antigen-experienced phenotype (15–22). The identification of SARS-CoV-2–specific memory cells with presumed functional superiority against infection has led to excitement over the source of antigens that drive these types of responses (23). A widely held view is that preexisting memory to SARS-CoV-2 reflects past exposures to common circulating coronaviruses (CCCoV) (24). Consistent with this, short-term lines expanded by SARS-CoV-2 peptides identified T cells that responded to analogous sequences from CCCoV (16, 25). However, other studies found weak T cell cross-reactivity between SARS-CoV-2 and CCCoV (22, 26). The existence of other potential sources was suggested by a sequence conservation analysis, which did not find a higher proportion of matches between SARS-CoV-2 and other sequenced members of Coronaviridae family than expected by chance (27). Furthermore, preexisting memory is not unique to SARS-CoV-2. We and others have identified preexisting memory T cells that recognized other pathogens, including yellow fever virus (YFV), that do not circulate in the United States and lack a common close relative to simulate prior exposure (28–31). The acquisition of a memory phenotype in the absence of similar pathogens suggests other avenues for acquiring an antigen-experienced state at baseline.

Here, we performed an in-depth analysis of SARS-CoV-2–specific T cells in unexposed individuals and investigated alternative sources of antigens that could have driven this preexisting T cell differentiation. Rare SARS-CoV-2–specific CD4+ T cells were identified directly ex vivo using class II peptide–major histocompatibility complex (pMHC) tetramer enrichment. Blood samples collected before year 2020 were used to ensure the absence of prior SARS-CoV-2 exposure. The activation of naïve T cells in an anatomical niche imprints selective trafficking properties (32). For example, lamina propria–derived dendritic cells (DCs) in the mesenteric lymph nodes efficiently induce gut-tropic receptors CCR9 and α4β7 (33–36). Conversely, cutaneous DCs imprint CCR10 expression to enable T cells primed in the skin to traffic back to the cutaneous tissue (37). Hypothesizing that the imprints of initial antigen engagement are retained in preexisting T cells, we used trafficking molecules to gain insights into where prior antigen engagement might have occurred. We showed that preexisting SARS-CoV-2–specific memory CD4+ T cells expressed diverse phenotypic markers.
of various CD4+ T cell lineages, displayed gut and skin tropism, and cross-reacted with commensal bacteria. Cross-reactivity to bacterial antigens was identified for multiple SARS-CoV-2 epitopes and occurred at a similar overall frequency as cross-reactive responses to homologous sequences from CCoV for a spike-specific population. These findings highlight the breadth of SARS-CoV-2–specific T cells and implicate noninfectious microbial stimuli as a major factor that guides preexisting immune responses to SARS-CoV-2.

RESULTS

Preexisting SARS-CoV-2–specific T cells in unexposed adults

We analyzed peripheral blood mononuclear cells (PBMCs) from twelve unexposed human leukocyte antigen (HLA)–DRA/B1*0401 (DR4) healthy donors (HDs) using prepandemic blood samples collected before 2020 (table S1). SARS-CoV-2–specific CD4+ T cells were identified using a direct ex vivo approach with pMHC tetramers. We recombinantly expressed HLA-DR4 monomers. A set of 12 peptides that stimulated T cells in patients with COVID-19 based on prior studies and/or predicted to bind HLA-DR4 were selected for generation of tetramers (table S2). We coupled tetramer staining with magnetic column–based enrichment to enable enumeration of rare tetramer-labeled T cells in the unprimed repertoire. The baseline differentiation states of tetramer-labeled T cells were delineated by anti-CD45RO and CCR7 antibody staining. In total, we analyzed 117 SARS-CoV-2–specific CD4+ populations in 12 healthy unexposed adults. SARS-CoV-2 precursors varied by epitope specificity and showed heterogeneity between individuals (Fig. 1, A and B, and fig. S1, A and B). On average, the frequency of spike-specific T cells was lower compared with open reading frame 8 (ORF8)– and ORF9–specific T cells.

Fig. 1. Identification of SARS-CoV-2–specific T cells in unexposed individuals. (A) Direct ex vivo staining, before and after magnetic enrichment, of representative SARS-CoV-2 tetramer+ cells using cryopreserved cells obtained before year 2020. Plots represent one of two independent experiments. (B) The frequency of SARS-CoV-2–specific CD4+ T cells across 12 unexposed HDs by direct ex vivo tetramer staining. Each symbol represents data from a SARS-CoV-2–specific population repeated 2.04 times (±0.07). (C) Plot summarizes the frequencies of T cells that recognized different regions of SARS-CoV-2. (D) Frequency of T cells that recognized epitopes from SARS-CoV-2, YFV, or influenza virus. Plot combines 12 SARS-CoV-2–specific populations and HA306 tetramer+ cells from 12 donors. YFV–specific T cells before (pre) or after (post) vaccination include 11 YFV–specific populations from HD1 and HD2. (E) Plots show CD45RO and CCR7 staining to divide cells into naive or memory subsets. Plots represent one of two independent experiments. (F) The abundance of preexisting memory T cells as a percentage of each tetramer+ population shown in (B). (G) The averaged differentiation phenotype of all SARS-CoV-2 tetramer+ cells examined: naive (CD45RO−CCR7+), CM (CD45RO+CCR7+), EM (CD45RO+CCR7−), and TEMRA (CD45RO−CCR7−). (H) The proportion of naive cells as a percentage of tetramer+ cells shown in (D). (I) Correlation between the abundance of memory precursors and donor age. Distinct tetramer+ populations from the same donor were combined and represented as an average (n = 12). (J) ICOS staining on a representative tetramer+ population and tetramer− CD4+ T cells. Each symbol represents data repeated 1.59 times (±0.17). Line connects averaged data from the same donor (n = 5). (B) to (D), (F), and (H) used Welch’s ANOVA. P values for pairwise comparisons were computed using Dunnett’s T3 procedure. For (I), Spearman correlation was computed. Line represents least square regression line. For (K), a paired t test was performed. Means ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001.
that recognized epitopes outside of the spike region (Fig. 1C). Next, we compared SARS-CoV-2–specific T cells with other antigen-naïve or experienced populations (table S3). Two of the study volunteers had previously participated in a YFV vaccine study and had YFV–specific CD4+ T cells analyzed before and after vaccination (30). Because most people likely have been exposed to influenza virus by vaccination and/or infection, we also stained for T cells that recognized hemagglutinin antigen (HA) of influenza virus as another antigen–experienced population. Consistent with an antigen–inexperienced state, the overall frequency of SARS-CoV-2–specific T cells was similar to that of YFV–specific T cells in unexposed individuals and significantly lower than HA–specific T cells or YFV–specific T cells after vaccination (Fig. 1D and fig. S2).

To examine the differentiation state of SARS-CoV-2–specific preimmune T cells, we broadly divided tetramer-labeled cells by CD45RO and CCR7 staining. This revealed heterogeneous memory phenotypes that differed by epitope specificity and varied across donors (Fig. 1, E and F, and fig. S1C). Collectively, only 35.2% of SARS-CoV-2–specific precursors were naïve. The remaining cells expressed a memory phenotype, which included 39.6% central memory cells (CM), 20.7% effector memory cells (EM), and 4.7% terminal effector cells (TEMRA) (Fig. 1G). Although naïve SARS-CoV-2–specific T cells composed a minor subset, they were still substantially more abundant than the naïve component of post-exposure T cells that recognized HA or YFV (Fig. 1H). SARS-CoV-2–specific T cells contained a slightly smaller naïve fraction compared with YFV–specific T cells in unexposed individuals (Fig. 1H). Previous exposures to endemic coronaviruses may contribute to this difference, although the abundance of preexisting memory cells did not correlate with the conservation of SARS-CoV-2 epitopes with CCCoV sequences (fig. S1D and table S4). Beyond CCCoV, past studies show a relationship between the proportion of memory cells within SARS-CoV-2–specific T cells and the overall immunological experience of the donor (22). Consistent with this, the abundance of preexisting SARS-CoV-2–specific memory T cells positively correlated with donor age (Fig. 1I) and showed a weak association with total memory cell frequency in our dataset (fig. S1E). To investigate whether antigen stimulation was occurring on an ongoing basis, we used ICOS as an activation marker to analyze T cell activation state (30, 38). This showed higher ICOS expression on SARS-CoV-2–specific T cells compared with tetramer-negative CD4+ cells, suggesting that some preexisting cells perceived stimulation signals at baseline (Fig. 1, J to K). Collectively, direct ex vivo tetramer staining provided a quantification of SARS-CoV-2–specific precursors and identified preexisting memory T cells in unexposed individuals. Baseline up-regulation of activation markers further suggested recognition of antigens beyond conserved epitopes from other coronaviruses.

**Preexisting SARS-CoV-2–specific T cells expressed gut-homing markers**

The intestinal tract is home to trillions of microbial organisms (39). The composition of the microbiome is heavily dependent on the living environment and has a critical impact on human health.
In addition, previous studies demonstrate that homeostatic interactions with microbes in the gut are sufficient to drive human memory T cell differentiation (41). We therefore hypothesized that the gut microbiome could be a source of ongoing antigenic stimulation that shapes the preexisting SARS-CoV-2–specific T cell repertoire. Flexible engagement of T cell receptors (TCRs) may then allow some commensal-activated T cells to be detected as memory cells to a new pathogen. To begin to test this idea, we used gut-homing receptors, integrin β7 and CCR9, to infer priming by and/or the potential to engage intestinal antigens (35, 42, 43). Tetramer staining for SARS-CoV-2–specific T cells was combined with antibody staining for trafficking receptors. HA-specific T cells were identified for comparison. This revealed a higher proportion of SARS-CoV-2–specific integrin β7⁺ or CCR9⁺ cells compared with the background level on tetramer-negative memory CD4⁺ T cells (Fig. 2, A to C). SARS-CoV-2–specific T cells coexpressed CCR9 and integrin β7 (Fig. 2D), which showed a variable pattern but, as a group, was more abundant within SARS-CoV-2 tetramer⁺ populations compared with influenza-specific T cells or tetramer-negative memory cells (Fig. 2, E to G). Together, these data support the possibility that chance encounters with antigens in the intestinal environment could promote differentiation of T cells that recognize an unrelated pathogen.

**Preexisting SARS-CoV-2 tetramer⁺ cells expressed a variety of trafficking receptors and differentiation phenotypes**

To further investigate the differentiation state and phenotypic diversity of SARS-CoV-2–specific T cells at baseline, we designed a 25 fluorochrome spectral cytometry panel that focused on trafficking and chemokine receptor expression. We pooled 12 SARS-CoV-2 tetramers on the same fluorochrome to maximize the capture efficiency of SARS-CoV-2–specific T cells from a limited amount of prepandemic blood sample from six HDs. Staining with a few select tetramers was performed separately on cells from three of these individuals with more stored samples. Tetramers loaded with HA306 peptide were included for comparison. In total, 932 SARS-CoV-2 tetramer–labeled T cells were identified by manual gating and combined for analyses using the Spectre pipeline (44). SARS-CoV-2–specific T cells expressed CD45RO, integrin β7, and CCR9 as expected (Fig. 3A and fig. S3). A subset of tetramer⁺ cells also expressed typical markers of follicular helper (T FH ) T cells (CXCR5).

### Fig. 3. SARS-CoV-2–specific CD4⁺ T cells in unexposed donors are phenotypically heterogeneous.

(A and B) UMAPs display the staining intensity of the indicated markers (A) and phenograph-defined clusters (B). Data combined 932 SARS-CoV-2–specific T cells from six healthy individuals that were identified using S166, S236, S936, and ORF8 43 tetramers or a pool of 12 SARS-CoV-2 peptide–loaded tetramers. (C) Heatmap shows the median staining signal of individual markers for clusters shown in (B). (D) Bar graph shows the relative cluster abundance within tetramer⁺ cells from each donor. (E) Individually labeled tetramer⁺ cells of the indicated specificity are projected onto an UMAP that includes all SARS-CoV-2–specific T cells. (F) Plots summarize the abundance of indicated subsets within SARS CoV-2 (CoV2) and HA306 tetramer–labeled CD4⁺ T cells. Each symbol represents averaged data from one donor based on two independent experiments (n = 6). Paired t tests were performed. Means ± SEM. *P < 0.05 and ***P < 0.001.
or T helper 1 cells (Th1; CXCR3), suggesting polarization of some preexisting memory cells into defined T helper subsets (Fig. 3A). Cutaneous lymphocyte-associated antigen and CCR10 guide T cell trafficking to the skin (45–47). We observed high expression of these markers in some SARS-CoV-2–specific T cells (Fig. 3A), raising the possibility that relevant prior antigen experiences could extend beyond the intestinal compartment to involve other barrier tissues. Phenotypic relationship between cells was analyzed using Phenograph and visualized on uniform manifold approximation and projection (UMAP) (48, 49). Phenograph clustered tetramer+ cells into six subpopulations (Fig. 3B), which were shown in a heatmap by median intensity of individual markers (Fig. 3C). SARS-CoV-2 tetramer+ cells were broadly divided into naïve or memory subsets by CD45RO and CCR7 staining (naïve, clusters 1 and 2; memory, clusters 3 to 6). Memory phenotype clusters were further separated by gut and skin trafficking receptors, which localized to distinct parts of the UMAP and coextensive with chemokine receptors in distinct patterns (Fig. 3, A to C). Subdividing tetramer+ cells by donor of origin showed variable distribution of different clusters within cells from different individuals (Fig. 3D).

To investigate how different SARS-CoV-2–specific populations contributed to phenotypic heterogeneity, we examined T cells labeled individually with S166, S236, S936, or ORF8 43 tetramers. This showed that T cells stained by a particular tetramer can have a range of phenotypes and localized to distinct regions of UMAP (Fig. 3E and fig. S4A). Between T cells stained by different tetramers, we observed both shared and unique features. For example, S166, S936, and ORF8 43 tetramer+ cells expressed overlapping phenotypes and were dominated by cells in clusters 2, 3, and 4 (fig. S4B). By contrast, S236-labeled T cells differed from other SARS-CoV-2–specific
populations and primarily mapped to cluster 6 on the UMAP (Fig. 3E and fig. S4, A and B). Variations between precursor populations could have reflected differences in prior antigen experiences. However, as a group, there may be similarities that distinguish them from classic memory cells generated in the context of infection and/or vaccination. To test this, we compared SARS-CoV-2-tetramer+ and HA306-tetramer+ T cells from the same individuals (Fig. 3F and fig. S4C). Memory tetramer+ cells were subdivided by CD25 and CD127 expression into cells with (CD25hiCD127lo) or without (CD25lo) regulatory T cell (Treg) features. CD25lo cells were further gated by CXCR5 and CXCR3 staining to mark Th1 and Th17-associated phenotypic subsets (fig. S4C). We did not focus on Treg due to low frequency of CD25hiCD127lo cells in both HA- and SARS-CoV-2 tetramer–labeled populations. For CXCR3 and CXCR5, SARS-CoV-2–specific T cells contained a lower proportion of CXCR3+CXCR5+ and CXCR3+CXCR5− cells, but the percentage of CXCR3+CXCR5− subset was not significantly different between SARS-CoV-2 precursors and HA-specific T cells (Fig. 3F). Collectively, these data revealed distinct features of the preexisting memory pool. SARS-CoV-2–specific T cells expressed receptors that enable access across barrier tissues and included small subsets of Th1- and Th17-polarized cells in unexposed individuals.

**SARS-CoV-2–specific T cells responded to predicted microbial peptides**

Whereas T cells recognize antigens in a highly specific manner, TCRs can also flexibly dock onto pMHC complexes (50, 51). This feature of the TCR that allows a single TCR to bind multiple distinct pMHCs is referred to as cross-reactivity. Here, we investigated whether SARS-CoV-2–specific T cells could cross-react with commensal bacteria–derived antigens using single-cell–derived T cell clones. We initially focused on T cells that recognized the spike amino acid sequence 936 to 952 (S936) as a population that showed robust integrin β7 and CCR9 expression. We sorted single S936 tetramer+ cells individually into 96-well plates using prepandemic cells from HD1 and HD10 (table S5). Expanded T cells were restained by tetramers and tested for response to S936 peptide. In total, we generated 17 clones and confirmed the correct specificity for 16 clones by tetramer staining and/or peptide response (fig. S5). Six candidate peptides from commensal bacteria identified on the basis of similarity to the predicted S936 core sequence were used to investigate the ability of S936–specific T cells to respond to microbe-derived sequences (table S3). We tested twelve S936 clones that grew well in culture for tumor necrosis factor–α (TNF-α) production after a 5-hour stimulation with peptide-treated monocyte-derived DCs. This revealed high levels of responses to nonspike sequences. S936 clones were most responsive to P3, P5, and P6 and had cross-reactivity to at least one microbial peptide per clone (Fig. 4, A, C, and D). A subset of these interactions probably had high avidity and could also be detected by tetramer staining (Fig. 4B). We identified several S936 clones that also bound P3- and P5–loaded tetramers and showed a positive correlation between tetramer staining and the robustness of cytokine response (Fig. 4, E and F). To further quantify the responsiveness to cross-reactive peptides, we stimulated three clones with different levels of sensitivity to S936 with decreasing concentration of cognate and cross-reactive P5 peptides. Functional avidity was not significantly different between S936 and P5 sequences (Fig. 4G and fig. S6). We further evaluated the high avidity 1C5 clone using additional bacterial sequences and found largely comparable sensitivity to cognate and cross-reactive sequences (Fig. 4H and fig. S6). Collectively, these data demonstrated that SARS-CoV-2–specific T cells can recognize and respond to nonviral sequences. Cross-reactivity involved a substantial number of virus-specific precursors and included cells with high avidity to SARS-CoV-2.

**SARS-CoV-2–specific T cells responded to fecal and bacterial lysates**

Next, we examined cross-reactivity to naturally occurring microbial components. S936 T cell clones were stimulated with DCs incubated with fecal lysates from seven healthy individuals. This showed a significant response to four fecal lysates (F04, F06, F22, and F26) compared with phosphate-buffered saline (PBS) (Fig. 5, A and B). Pretreating lysate-loaded DCs with HLA II blocking antibodies inhibited TNF-α production, indicating that cytokine response to these fecal lysates was MHC dependent (Fig. 5C and fig. S7). Although these data provided evidence that a SARS-CoV-2–specific T cell clone could recognize naturally processed microbial epitopes, the relevant sources of antigens were challenging to identify due to the complexity of microbes in the stools. To analyze more defined bacterial antigens, we cultured commensal bacteria and generated lysates for stimulation. We broadened our analyses to include bacteria from different barrier tissues—using *Porphyromonas gingivalis* from
the oral cavity; *Prevotella copri*, *Bacteroides ovatus*, and *Akkermansia muciniphila* from the gut; and *Staphylococcus epidermidis* from the skin. To determine whether SARS-CoV-2–specific T cells besides those recognizing S936 could respond to microbial antigens, we generated additional T cell clones for other SARS-CoV-2 epitopes and confirmed the specificity of tetramer-stained cells as before. Single S462, ORF8 43, or ORF9 NF127 tetramer+ cells were sorted and expanded in culture. Antigen specificity was validated for 94% of the clones by staining with the same tetramer and/or response to the cognate peptide (nine of nine S462 clones, two of two ORF8 clones, and four of five ORF9 clones; fig. S8). In total, 25 SARS-CoV-2–specific T cell clones were stimulated with five cultured bacterial lysates. This identified two responsive clones from different donors that recognized distinct SARS-CoV-2 epitopes (fig. 6). The S936-specific 1C5 clone from HD10 responded robustly to *S. epidermidis* and moderately to *P. copri* (fig. 6C). The 2B8 clone from HD5 recognized ORF9 NF127 and showed preferential cross-reactivity to *B. ovatus* (fig. 6D). These data provided evidence that SARS-CoV-2–specific T cells from unexposed individuals could cross-react with naturally processed antigens from commensal bacteria on the skin and in the gastrointestinal tract.

**Ex vivo detection of cross-reactive T cells**

CCCoV is a known source of cross-reactive antigen that contributes to preexisting responses to SARS-CoV-2 (16, 22, 24, 25, 52). Here, we compared cross-reactivities to bacterial peptides with responses to CCCoV to gain insights into the relative contribution of different types of exposures. S936 T cell clones were stimulated with homologous sequences from HKU1, OC43, NL63, and 229E. Unexpectedly, DCs treated with CCCoV peptides activated fewer T cell clones compared with bacterial sequences (fig. 7, A and C). None of the S936 T cell clones analyzed showed a positive staining for the CCCoV tetrathers (fig. 7, B and D). To eliminate the possibility that the lack of CCCoV response was due to poor growth of this type of cross-reactive cells in culture, we performed direct ex vivo tetramer staining on cells that were used to generate the clones from HD1. We produced tetramers for two bacterial and two CCCoV peptides (fig. 7E). The beta coronavirus sequences from HKU1 and OC43 were used because they were most similar to S936. Bacterial peptides P3 and P5 were selected because they stimulated greater numbers of T cell clones. In agreement with T cell clones generated from the same donor, we found S936 tetramer+ T cells that bound P3 and P5 tetramers but not HKU1 or OC43 tetramers by direct ex vivo staining (fig. 7F and fig. S8A). To test whether the lack of CCCoV cross-reactivity was donor specific, we expanded our analyses to include cells from HD2, HD3, HD4, and HD5. Tetramer staining identified S936*CCCoV* double tetramer–positive cells in these donors (fig. 7G and fig. S9A). The type and degree of cross-reactivity varied between individuals but showed a similar overall frequency between bacterial and CCCoV sequences (fig. 7, G to I). As a percentage of HKU1, OC43, P3, or P5 tetramer+ populations, S936 cross-reactivity was not significantly different between CCCoV and bacterial

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**Fig. 6. SARS-CoV-2–specific T cells respond to lysates from gut and skin bacteria.** (A) Cytokine response after stimulation by PBS or the indicated bacterial lysates. Plots represent one of four (S926 1C5) or three (ORF9 2B8) independent experiments. (B) Heatmap shows T cell response to PBS or the indicated lysates as a percentage of TNF-α response to the cognate peptide, repeated two to four times. SARS-CoV-2 specificity is indicated above the heatmap and separated by color. (C) S936 1C5

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Fig. 7. T cell cross-reactivity to CCCoV. (A and B) The percentage of cells within a clone that responded to homologous sequences from CCCoV by TNF-α production (A) or tetramer staining (B). Each symbol represents measurements from one clone (n = 12), repeated two or three times (A) or one or two times (B). (C and D) Plots summarize the frequency of response to a CCCoV peptide (HKU1, OC43, NL63, or 229E) or a bacterial peptide (P1 to P6) by TNF-α response (C) or tetramer staining (D). (E) Sequence alignment of S936 from SARS-CoV-2 with bacterial sequences P3, P5, and homologous CCCoV sequences from HKU1 and OC43. (F) Identification of CD4+ T cells from HD1 that recognized S936, HKU1, OC43, P3, or P5 peptides by direct ex vivo tetramer staining. Plots represent one of two independent experiments. (G) Bar graphs show the frequency of T cells that cross-reacted with HKU1 or OC43 sequences as a percentage of total S936 tetramer+ cells. (H) Cross-reactivity to P3 and P5 as a percentage of total S936 tetramer+ cells. Each bar represents data from one individual. (I) The frequency of S936-specific T cells that cross-reacted with bacterial sequences (P3 or P5) or CCCoV peptide (HKU1 or OC43). (J) S936+HKU1+, S936+OC43+, S936+P3+, or S936+P5+ cells as a percentage of total HKU1, OC43, P3, or P5 tetramer+ T cells. For (G) to (J), data were generated from one or two independent experiments depending on cell availability. For (A) and (B), Friedman test with Dunn’s multiple comparisons test was used. For (C) and (D), Mann-Whitney test was used. For (I), Welch’s t test was used. For (J), Kruskal-Wallis test and Dunn’s multiple comparisons test were used. Means ± SEM. *P < 0.05, **P < 0.001, and ****P < 0.0001.

peptide-specific T cells (Fig. 7J) and fig. S9B). These data provided further evidence in support of cross-reactivity between SARS-CoV-2 and bacterial antigens. Interactions with commensal bacteria could be a major source of antigen experience that complements CCCoV in the development of preexisting memory to SARS-CoV-2.

**DISCUSSION**

T cells that recognize SARS-CoV-2 are found in unexposed individuals (15–22), but the composition of these cells remains poorly defined. Here, we examined the preexisting state of 117 SARS-CoV-2-specific CD4+ T cell populations in healthy adults. A tetramer-based enrichment approach was used to analyze rare antigen-specific T cells directly ex vivo. This approach enabled quantitative analyses of T cell frequency and cellular states with minimal perturbation. The specificity of tetramer staining was confirmed for over 90% of labeled cells using single-cell-derived clones generated from individually sorted tetramer+ cells. The experiments were performed using blood collected before the year 2020 to ensure the absence of prior SARS-CoV-2 exposure. Our analyses of SARS-CoV-2–specific T cells showed variation in precursor frequencies across donors and between T cells that recognized the same antigen. Among SARS-CoV-2 epitopes surveyed, T cell frequency was highest for a peptide from ORF8, an immune modulatory protein that shared little sequence homology with CCCoV (53). Irrespective of the degree of similarity to endemic coronaviruses, we were able to identify memory phenotype T cells that recognized SARS-CoV-2 epitopes. Overall, more than 60% of SARS-CoV-2–specific T cells examined acquired a memory phenotype in unexposed individuals.

Although prior exposures to endemic coronaviruses influence the preexisting repertoire of SARS-CoV-2–specific T cells, the high proportion of preexisting memory T cells remains incompletely explained. Because a TCR can interact with pMHC ligand with considerable flexibility, we sought to investigate cross-reactivity to other environmental antigens beyond related viruses. The intestinal tract is home to trillions of microbial organisms that influence health and disease (13, 39, 40, 54). Similarly, the skin houses a highly diverse microbial community with essential immune functions (55).

Using trafficking receptor expression to infer tissue tropism, we performed phenotypic analyses of ex vivo isolated SARS-CoV-2–specific
CD4+ T cells and identified skin and gut-tropic populations. Whether SARS-CoV-2–specific T cells could cross-react with microbial antigens in these tissue compartments was evaluated using T cell clones and by tetramer staining. These studies revealed specific T cell recognition and response to microbial peptides, stool lysates, and defined bacteria from skin and gastrointestinal tract.

Intriguingly, a substantial portion of SARS-CoV-2–specific CD4+ precursor cells could recognize microbial antigens. A subset of these cross-reactive interactions had high functional avidity and bound bacterial epitopes with sufficient strength to be detected by tetramers. Cross-reactivity to microbial peptides was heterogeneous but was also readily detectable as a group and showed a similar overall frequency as homologous CCCoV sequences in our experimental system. Because our data were generated from limited sampling of SARS-CoV-2–specific populations and small numbers of T cell clones, they provided a selective view of the preexisting repertoire that likely contains other T cells with different cross-reactive potentials. Another limitation is that we do not know whether SARS-CoV-2–specific T cells had in fact encountered a particular bacterial antigen and the timing or the order of these exposures. Relevant antigens could also come from fungal and viral components of the microbial environment that were not analyzed in this study. Broader studies that link microbiome composition with antibody and T cell responses in people of different age groups living in distinct environments would further inform the development of human preexisting repertoire to novel pathogens.

How preexisting states influence host protection remains incompletely understood. The presence of a preexisting pool of T cells that can recognize SARS-CoV-2 has generated a great deal of excitement for the possibility that they might provide superior responses to infections (15–22). The frequency of preexisting SARS-CoV-2–specific T cells is associated with beneficial features such as milder disease and abortive infection (52, 56, 57). However, SARS-CoV-2 T cells from unexposed individuals required more peptides to respond in culture, a feature that was shared with T cells from patients with severe COVID-19 infection (22). By analyzing the phenotype of tetramer-labeled T cells, we showed that the preexisting memory pool contained a diverse population of SARS-CoV-2–specific T cells with distinct differentiation features and trafficking potentials. The heterogeneity within the preexisting repertoire is consistent with priming by various cross-reactive sources and may contribute to the diversity of responses after exposure. We speculate that the expression of trafficking receptors may enable accelerated migration of preconditioned memory T cells into tissues to enhance local defense in the event of infection. Preexisting Tfh cells might orchestrate B cells to further jump-start responses to a pathogen. Alternatively, hyperinflammatory cells that infiltrate tissues may contribute to acute and/or persistent damage. Extension of T cell cross-reactivity to self-antigens may perpetuate immune processes and result in maladaptive host responses after viral clearance. Cross-reactive T cells activated by COVID-19 infection may in turn target the microbiome and further modify the relationship between microbes and the immune system. Future studies will be needed to investigate how preexisting populations respond to perturbations and converge to modulate the overall quality of immune response to pathogens.

In summary, our analyses of SARS-CoV-2 precursor repertoire showed that preexisting SARS-CoV-2–specific memory T cells express diverse phenotypes and display broad tissue tropism. By examining the cross-reactivities of individual T cells, our data further revealed a wide range of responses to commensal bacteria. These findings highlight the breadth of T cell recognition and suggest that lifelong exposures to a diverse microbial environment could have profound impacts on the composition of the immune repertoire. Preexisting immune memory is typically studied in the context of exposure to related pathogens. Our data provide the basis for considering a broader range of antigen experiences in the education of the baseline immune responses. Interindividual differences in the abundance and differentiation states of preexisting cells resulting from different life experiences may contribute to the heterogeneity of human responses to SARS-CoV-2 and potentially other infections.

**MATERIALS AND METHODS**

**Study design**

The goal of the study was to define the preexisting state of SARS-CoV-2–specific T cells in unexposed individuals. Cryopreserved cells were stored from past collection of de-identified donors from the Stanford Blood Bank or from prior studies at the University of Pennsylvania (30). Subject characteristics are shown in table S1. Stool samples were baseline samples from a controlled feeding study in healthy adult volunteers (aged 18 to 60 years) (58). All samples were de-identified and obtained with institutional review board regulatory approval from the University of Pennsylvania.

**Direct ex vivo T cell analyses and cell sorting**

HIS-tagged HLA-DR protein monomers with tethered thrombin cleavable CLIP peptide were produced by Hi5 insect cells and extracted from culture supernatant using Ni–nitrilotriacetic acid (Ni-NTA) agarose (Qiagen). HLA-DR monomers were biotinylated overnight at 4°C using BirA (Avidity), analyzed by streptavidin gel-shift assay to confirm biotinylation, and purified by size exclusion chromatography using a Superdex 200 size exclusion column (AKTA, GE Healthcare). Peptide exchange and tetramerization were performed using standard protocols as previously described (28, 59). Briefly, HLA-DR proteins were incubated with thrombin (Millipore) at room temperature for 3 to 4 hours and exchanged with peptides of interest in 50-fold excess at 37°C for 16 hours. Peptide-loaded HLA-DR monomers were incubated with fluorochrome-conjugated streptavidin at 4 to 5:1 ratio for 2 min at room temperature, followed by a 15-min incubation with an equal volume of biotin–agarose slurry (Millipore). Tetramers were buffered-exchanged into PBS, concentrated using Amicon ULTRA 0.5 ml 100 kDa (Millipore), and kept at 4°C for no more than 2 weeks before use. For tetramer staining, 30 to 90 million CD3- or CD4-enriched T cells were stained at room temperature for 1 hour using 5 µg of each tetramer in 50 to 100 µl of reaction. Tetramer-tagged cells were enriched by adding anti-fluorochrome and anti-HIS MicroBeads (Miltenyi Biotec) and passing the mixture through LS columns (Miltenyi Biotec). Column-bound cells were washed and eluted according to the manufacturer’s protocol. For antibody staining, eluted cells were stained with viability dye (Thermo Fisher Scientific) and anti-CD19 and anti-CD11b (BioLegend) to exclude dead and non–T cells. Other antibodies used are listed in table S6. Surface staining was carried out in 50 to 100 µl of fluorescence-activated cell sorting (FACS) buffer [PBS plus 2% fetal calf serum (FCS), 2.5 mM EDTA, and 0.025% sodium azide] for 30 min at 4°C. Samples were acquired by flow cytometry using LSRII (BD) or sorted on FACS Aria (BD). Frequency calculation was obtained by mixing one-tenth of pre- and postenrichment
samples with 200,000 fluorescent beads (Spherotech) for normalization (28). Nonzero populations were included in the analyses performed using FlowJo (BD). Spectral flow cytometric analyses were performed with the following modifications: For pooled tetramer staining, 2 μg of tetrarmers loaded with each of the 12 SARS-CoV-2 peptides used in this study was tagged to the same fluorochrome and combined in the staining reaction. Tetramer-enriched cells were stained with live/dead dyes, exclusion markers, and a panel of additional surface antibodies (table S6) for 1 hour at 4°C, followed by fixation with 2% paraformaldehyde. Samples were acquired on spectral flow Cytek Aurora (ARC 1207i).

**Microbial culturing and preparation of lysates**

*P. copri* German Collection of Microorganisms and Cell Cultures (DSMZ) 18205, *S. epidermidis* American Type Culture Collection (ATCC) 14990, *A. muciniphila* ATCC BAA-835, *P. gingivalis* ATCC 33278, and *B. ovatus* ATCC 8483 were obtained from DSMZ (Braunschweig, Germany) or ATCC (Manassas, VA) and grown in Schaedler broth, nutrient broth, brain heart infusion broth, supplemented tryptic soy broth, or modified chopped meat medium, respectively. *P. copri*, *A. muciniphila*, and *B. ovatus* were grown in an anaerobic glove box containing about 2.5% CO₂, 2.5% H₂, and 95% N₂. All strains were grown at 37°C until the mid–late-log phase growth. Two hundred fifty milliliters of the culture were spun down at 3000g for 10 min, the supernatant was removed, and the pellet was resuspended in 4 ml of PBS and sonicated using a probe sonicator (30 s, followed by 1 min of rest, repeated three times) for the preparation of bacterial lysates. The bacterial lysates were clarified by centrifugation at 10,000 rpm for 1 min at 4°C. The clarified bacterial lysates were then treated with Polymyxin B (Sigma-Aldrich) to remove endotoxin content, followed by estimation of total protein content in the lysates through the bicinchoninic acid (BCA) protein assay method (Thermo Fisher Scientific). Fecal lysates were generated by diluting 100 mg of fecal sample in 1 ml of PBS and sonicated three times for 1 min at 4°C with 30 s of rest in between. The fecal lysates were clarified by centrifugation at 15,000 rpm for 5 min before use.

**Generation of T cell clones**

**Generation of T cell clones**

Cells were stained with tetrarmers and enriched by magnetic isolation as described above. Tetramer-positive cells were sorted as single cells into 96-well plates using the purity mode on FACSAria II (BD). Each well contained 10⁵ irradiated PBMCs and the 10⁴ JY cell line (an Epstein–Barr virus–immortalized B cell lymphoblastoid line; Thermo Fisher Scientific), phytohemagglutinin (PHA; 1:100, Thermo Fisher Scientific), interleukin-7 (IL-7; 25 ng/ml; PeproTech), and IL-15 (25 ng/ml; PeproTech). IL-2 (50 IU/ml; PeproTech) was added on day 5 and replenished every 3 to 5 days. Cells were re-supplied with fresh medium with IL-2 (50 IU/ml), PHA (1:100), and 10⁵ irradiated PBMCs every 2 weeks.

**Generation of DCs**

Monocytes from PBMCs of HD1 and HD2 were isolated using RosetteSep Human Monocyte Enrichment Cocktail (StemCell). Five million cryopreserved monocytes were thawed in 6.4 ml of DCs media (RPMI 1640 plus glutamine, 10% FCS, penicillin-streptomycin, and Hepes) containing granulocyte-macrophage colony-stimulating factor (GM-CSF; 100 ng/ml) and IL-4 (500 U/ml). On the third day, 3.2 ml of fresh media with GM-CSF (100 ng/ml), IL-4 (500 U/ml), and 0.05 mM 2-mercaptoethanol was added to the ongoing culture. For maturation of DCs with bacterial lysates or fecal lysates, we harvested the immature DCs on day 4. A total of 25,000 DCs were plated in a U-bottom 96-well plate, incubated with polymyxin bead–treated bacterial lysates (50 μg/ml) or fecal lysates (50 μg/ml) for 36 hours, followed by the addition of lipopolysaccharide (LPS; 100 ng/ml) on day 6. The bacterial lysate– or fecal lysate–treated DCs were cocultured with T cell clones on day 7. For peptide stimulation, we harvested immature DCs on day 5. A total of 0.1 million DCs were plated in a flat bottom 96-well plate and matured with LPS (100 ng/ml) and incubated with peptide (20 μg/ml) for 16 to 24 hours before coculturing with T cells.

**Stimulation of T cell clones**

T cell clones were rested overnight in fresh media without IL-2 and added to wells containing matured DCs. Cells were incubated for 5 hours in the presence of monensin (2 μM, Sigma-Aldrich) and brefeldin A (5 μg/ml, Sigma-Aldrich). For MHC blocking experiments, anti–MHC class II antibodies L243 (0.37 μg/ml, BioLegend) and TU39 (0.01 μg/ml, BioLegend) were added to T cells before combining with DCs. Intracellular cytokine staining with anti–TNF-α and anti–interferon-γ (BioLegend) was performed after stimulation using the BD Cytofix/Cytoperm Fixation/Permeabilization Kit according to the manufacturer’s protocol (BD).

**Bacterial and CCCoV sequence analyses**

**Bacterial peptides**

To identify potential cross-reactivity to microbial peptides, we predicted the DR4 binding register in S936 using NetMHCIIP 2.3. We then performed Protein BLAST analyses against the nonredundant protein sequence database using the S936 core sequence (LSSTASALG), excluding SARS-CoV-2– and coronavirus–related sequences (taxid: 2901879, 2697049, 694009, 2697049, 1508227, 694002, 694003, and 1928434). We curated six sequences from commensal bacteria allowing for one–amino acid mismatch and extended these nine–amino acid oligomer peptides by two amino acids on both ends based on the bacterial protein sequence.

**Coronavirus sequences**

All spike and nonspike sequences of SARS-CoV-2 and common coronaviruses were derived from the National Center for Biotechnology Information (NCBI) database (SARS-CoV-2 spike,YP_009724390.1; SARS-CoV-2 ORF8, YP_009724396.1; SARS-CoV-2 NP, QSM17284.1; HKU1 spike, ABD96198.1; HKU1 ORF8, AZS52623.1; HKU1 NP, YP_173242.1; 229E spike, AW652679.1; 229E NP, P15130.2; NL63 spike, APF29063.1; NL63 NP, Q6Q1R8.1; OC43 spike, QEG03803.1; OC43 NP, P33469.1). Pairwise alignment using Clustal Omega was performed to identify aligned regions. Similarity between the aligned sequences was calculated by Sequence Identity and Similarity using BLOSUM62 matrix. A score of 0 was used if no aligned region was found. Missing sequences were excluded from the analyses.

**High-dimensional phenotypic analyses**

Spectral flowometric data were analyzed by manual gating to select SARS-CoV-2 tetramer–labeled cells from each sample. Tetramer⁺ cells stained using pooled or individual tetrarmers were exported from FlowJo. A total of 932 tetramer⁺ cells were read into R by flowCore and combined into one single dataset for subsequent data processing and high-dimensional analyses using the Spectre package in R (42). Staining intensities were converted using Arcsinh transformation with a cofactor of 2000. Batch alignment was performed by first coarse aligning the batches with quantile conversions of
marker intensities calculated with a reference sample included in all the batches and then applying this conversion to all samples via the CytoNorm algorithm. Clustering was performed using Phenograph with nearest neighbors set to 60 (k = 60) (44). UMAP was used for dimensional reduction and visualization (43). Color scale was modified to use the same color for 0 and values under 0 after Arcsinh transformation.

Statistical methods
Data transformation was performed using the logarithmic function or inverse hyperbolic sine transformation $\sinh^{-1}(X) = \ln(X + \sqrt{1 + (X^2)})$ when data contained zero values. Assessment of normality was performed using D’Agostino-Pearson test. Spearman correlation was used if either of the two variables was nonnormal. Otherwise, Pearson correlation was used to measure the degree of association. The best-fitting line was calculated using least squares linear regression. Statistical comparisons of two means were performed using two-tailed Student’s t test or paired t test using a P value of < 0.05 as the significance level. P values were adjusted for multiple comparisons. Multiple comparisons procedures were performed when the Welch’s one-way analysis of variance (ANOVA; Dunnett’s T3). Friedman’s repeated-measures one-way ANOVA (Dunn), or two-way ANOVA (Tukey) was significant. Statistical analyses were performed using GraphPad Prism. Lines and bars represent mean, and variability is represented by SEM. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, and ns (not significant).

SUPPLEMENTARY MATERIALS

REFERENCES AND NOTES
1. L. Pan, M. Mu, P. Yang, Y. Sun, R. Wang, J. Yan, P. Li, B. Hu, J. Wang, C. Hu, Y. Jin, N. Xiu, R. Peng, Y. Du, T. Li, G. Xu, Q. Hu, L. Tu, Clinical characteristics of COVID-19 patients with digestive symptoms in Hubei, China: A descriptive, cross-sectional, multicenter study. J. Autoimmun. 115, 766–773 (2020).
2. Y. R. Nobel, M. Phipps, J. Zucker, B. Lebwohl, T. C. Wang, M. E. Sobieszczyk, D. E. Freedberg, Gastrointestinal symptoms and coronavirus disease 2019: A case-control study from the United States. Gastroenterology 159, 373–375.e2 (2020).
3. A. M. Baig, E. C. Sanders, Potential neuroinvasive pathways of SARS-CoV-2: Deciphering the spectrum of neurological deficit seen in coronavirus disease-2019 (COVID-19). J. Med. Virol. 92, 1845–1857 (2020).
4. R. Ramlah, Acute pancreatitis disease in the patient with COVID-19 infection: A systematic review. Annals of hepatobiliary-pancreatic surgery 25, 5372 (2021).
5. D. P. Oren, E. J. Topol, Prevalence of asymptomatic SARS-CoV-2 infection. Ann. Intern. Med. 173, 362–367 (2020).
6. J. F. Ludwigsson, Systematic review of COVID-19 in children shows milder cases and a better prognosis than adults. Acta Paediatr. 109, 1088–1095 (2020).
7. J. G. Ray, M. J. Schull, M. J. Vermeulen, A. L. Park, Association between ABO and Rh blood groups and SARS-CoV-2 infection or severe COVID-19 illness: A population-based cohort study. Ann. Intern. Med. 174, 308–315 (2021).
8. P. Bastard, L. B. Rosen, Q. Zhang, E. Michailidis, H. H. Hoffmann, Y. Zhang, K. Dhompon, O. Philippot, J. Rosain, V. Béziat, J. Manney, E. Shaw, L. Galamjans, P. Peterson, L. Lorenz, L. Bézin, S. Trouillet-Assant, K. Dobbs, A. A. de Jesus, A. Bollet, A. Kallaste, E. Catherinot, Y. Tandjaoui-Lambiotte, J. Le Pen, S. Hodeib, C. Korol, J. Rosain, K. Bilguvar, J. Ye, A. Bollet, B. Bigio, R. Yang, A. A. Arias, L. Bizien, S. Trouillet-Assant, K. Dobbs, A. A. de Jesus, A. Belot, A. Kallaste, E. Catherinot, A. Iwasaki, Sex differences in immune responses that underlie COVID-19 disease outcomes. Nature 588, 315–320 (2020).
9. C. Wu, X. Chen, Y. Cai, J. Xia, X. Zhou, S. Xu, H. Huang, L. Zhang, X. Zhou, C. Du, Y. Zhang, J. Song, S. Wang, Y. Zhao, Z. Yang, J. Xu, X. Zhou, D. Chen, W. Xiong, L. Xu, F. Zhou, J. Jiang, C. Bai, J. Zheng, Y. Song, Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pandemic in Wuhan, China. JAMA Intern Med 180, 934–943 (2020).
10. T. Takahashi, M. K. Ellingson, P. Wong, B. Israelow, C. Lucas, J. Klein, J. Silva, T. Mao, J. E. Oh, M. Tokuyama, P. L. Wu, A. Venkataraman, A. Park, F. Liu, A. Meir, J. Sun, E. Y. Wang, A. Casanova-Massana, A. A. Wyllie, C. B. Vogels, R. Earnest, S. Lapidus, I. M. Ott, A. J. Moore; Yale IMPACT Research Team; Albert Shaw, A. Shaw, J. F. Bournier, C. D. Oidio, S. Farhadian, C. dela Cruz, N. D. Graubaugh, W. L. Schultz, A. M. Ring, A. K. G. B. Omer, A. Iwasaki, Sex differences in immune responses that underlie COVID-19 disease outcomes. Nature 588, 315–320 (2020).
11. C. Wu, X. Chen, Y. Cai, J. Xia, X. Zhou, S. Xu, H. Huang, L. Zhang, X. Zhou, C. Du, Y. Zhang, J. Song, S. Wang, Y. Zhao, Z. Yang, J. Xu, X. Zhou, D. Chen, W. Xiong, L. Xu, F. Zhou, J. Jiang, C. Bai, J. Zheng, Y. Song, Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pandemic in Wuhan, China. JAMA Intern Med 180, 934–943 (2020).
12. W.-J. Guan, W.-H. Liang, Y. Zhao, H.-R. Liang, Z.-S. Chen, Y.-M. Li, X.-Q. Li, R.-C. Chen, C.-L. Tang, Wang, C.-Q. Ou, Li, P.-Y. Chen, Sang, W. Wang, F.-J. Li, C.-C. Li, L.-M. Ou, B. Cheng, S. Xiong, Z.-Y. Ni, X. Jiang, Y. Hu, L. Liu, Shang, C.-L. Lei, X.-Y. Peng, L. Wei, Y. Liu, Y.-H. Hu, P. Peng, J.-M. Wang, J.-Y. Liu, Z. Chen, G. Li, Z.-J. Zheng, S.-Q. Qiu, J. Luo, C.-J. Ye, S.-Y. Zhu, L.-H. Cheng, F. Ye, S.-Y. Li, J.-P. Zheng, N.-F. Zhang, N.-S. Zheng, J.-X. He; Chinese Medical Treatment Team of Patients with COVID-19; China, Comorbidity and its impact on 1590 patients with COVID-19 in China: A meta-analysis. Eur. Respir. J. 55, 2000547 (2020).
13. L. K. Beura, S. E. Hamilton, K. Bi, J. M. Schenkel, O. A. Odumade, K. A. Casey, E. A. Thompson, K. A. Frazer, P. C. Rosato, A. Filali-Mouhim, R. P. Sekaly, M. K. Jenkins, V. Vezy, W. N. Haining, S. C. Jameson, D. Masopust, Normalizing the environment recapitulates adult human immune traits in laboratory mice. Nature 532, 512–516 (2020).
14. S. P. Rosbash, G. B. Vassallo, D. Angeletti, D. S. Hutchinson, A. P. Morgan, K. Takeda, H. D. Hickman, A. J. McCulloch, J. H. Badger, N. J. Ajami, G. Trinchieri, P. Fard-Mendel de Villena, J. W. Yewdell, B. Rehemtulla, Wild mouse gut microbiota promotes host fitness and improves disease resistance. Cell 171, 1015–1028.e13 (2018).
15. A. Grifoni, D. Weiskopf, S. I. Ramirez, J. Mateus, J. Man, D. C. Rodbercher, S. A. Rawlings, A. Sutherland, L. Premkumar, R. S. Jadi, D. Marrama, A. M. de Silva, A. Fraizer, A. F. Carlin, J. A. Greenbaum, B. Peters, F. Kramer, D. M. Smith, S. Croty, A. Sette, Targets of T cell responses to SARS-CoV-2 in humans with COVID-19 disease and unexposed individuals. Cell 181, 1489–1501.e15 (2020).
16. J. Mateus, A. Grifoni, A. Tarke, J. Sidney, S. I. Ramirez, J. M. Dan, Z. C. Burger, S. A. Rawlings, D. Smith, E. Phillips, S. Mallal, M. Lammers, P. Rubiro, L. Quimabao, A. Sutherland, E. D. Yu, R. da Silva Antunes, J. Greenbaum, A. Fraizer, A. J. Markmann, L. Premkumar, A. de Silva, B. Peters, S. Croty, A. Sette, Weiskopf, Selective and cross-reactive SARS-CoV-2 T cell epitopes in unexposed humans. Science 370, 89–94 (2020).
17. N. E. Tert, A. T. Tan, K. Runseagenar, C. Y. T. Tham, M. Hafezi, A. Chia, H. M. Y. Chng, M. Lin, N. Tan, M. Linsner, W. N. Chia, M. I. Chen, L. F. Wang, E. E. Ooi, S. Kaimuddin, P. A. Tambayeb, J. G. Low, Y. J. Tan, J. Barrientos, SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls. Nature 584, 457–462 (2020).
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