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Differential T-Cell Reactivity to Endemic Coronaviruses and SARS-CoV-2 in Community and Health Care Workers

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Herein we measured CD4$^+$ T-cell responses against common cold coronaviruses (CCC) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in high-risk health care workers (HCW) and community controls. We observed higher levels of CCC-reactive T cells in SARS-CoV-2–seronegative HCW compared to community donors, consistent with potential higher occupational exposure of HCW to CCC. We further show that SARS-CoV-2 T-cell reactivity of seronegative HCW was higher than community controls and correlation between CCC and SARS-CoV-2 responses is consistent with cross-reactivity and not associated with recent in vivo activation. Surprisingly, CCC T-cell reactivity was decreased in SARS-CoV-2–infected HCW, suggesting that exposure to SARS-CoV-2 might interfere with CCC responses, either directly or indirectly. This result was unexpected, but consistently detected in independent cohorts derived from Miami and San Diego.

Keywords. coronaviruses; SARS-CoV-2; health care workers; COVID-19; T cells.

Health care workers (HCW) that provide frontline care during the global pandemic of coronavirus disease 2019 (COVID-19) are at increased risk of infection due to frequent close and prolonged exposure to patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. SARS-CoV-2 infection rates among HCW are still largely undetermined and highly variable depending on the geographical and temporal distribution among other factors [2–5] but higher prevalence has been documented during periods of upsurge [6, 7]. However, only a minority have developed mild to severe disease manifestations and the majority have remained seronegative for SARS-CoV-2 antibodies despite having close contact with SARS-CoV-2–infected patients [2–4, 8] (Vallejo A et al 2000 unpublished).

Robust T-cell immunity has been consistently reported in multiple studies in asymptomatic, acute, and convalescent COVID-19 individuals (Vallejo A et al 2000 unpublished), [9–12]. Furthermore, we and others have previously reported significant preexisting immune memory responses to SARS-CoV-2 sequences in unexposed subjects [9, 11–14]. Here, we aimed to characterize preexisting SARS-CoV-2 T-cell responses in this HCW cohort.

Due to close contact with patients, HCW are particularly prone to exposure to respiratory pathogens such as human coronaviruses (HCoVs) and particularly to endemic common cold corona virus (CCC) [15–18]. Human CCC are seasonal endemic circulating viruses that cause only mild upper and lower respiratory infections. They are globally distributed with higher incidences in winter months. Little is known about their pattern of infection, transmission rates, or duration of immunity [19–21]; however, detailed analysis of CCC reactivity from healthy donors and COVID-19 patients have been reported in recent studies [22–24]. As expected, on the basis of their common phylogeny, CCC share varying degrees of sequence homology with SARS-CoV-2 and we and others have shown that cross-reactive CD4$^+$ T-cell memory responses against SARS-CoV-2 can be detected in unexposed donors [13, 22, 24–26], although preexisting reactivity cannot solely be explained by prior exposure to CCC [27].

However, it is still unclear how preexisting immunity impacts disease severity or clinical outcome after SARS-CoV-2 exposure [28, 29] and if this could translate into a protective effect. While some studies suggest this could be the case [23, 30–32], and exposure to CCC concomitantly results in a faster response of preexisting memory cells to control SARS-CoV-2 infection, it cannot be excluded that CCC cross-reactivity could contribute

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to drive COVID-19 immunopathogenesis [33]. Thus, it is important to study differences in CCC reactivity and preexisting immunity in different cohorts, particularly HCW.

METHODS

Peripheral Blood Mononuclear Cells and Serum Isolation and Handling

For the Miami cohorts, peripheral venous blood was collected in EDTA vacutainer tubes and peripheral blood mononuclear cells (PBMCs) were isolated by density gradient isolation using Ficoll-Paque (Lymphoprep; Nycomed Pharma) as previously described [34], and stored in liquid nitrogen until use. Serum was collected and stored at −80°C. For the San Diego cohorts, whole blood was collected in heparin-coated blood bags (healthy unexposed donors) or in acid citrate dextrose tubes (COVID-19 donors) and PBMCs isolated as above. All samples were obtained after written informed consent from the participants in an anonymous fashion and with protocols approved by the respective institutional review boards.

OC43, NL63, HKU1, 229E, and SARS-CoV-2 Enzyme-Linked Immunosorbent Assay

The CCC (OC43 spike, 229E spike, NL63 spike, or HKU1 spike) enzyme-linked immunosorbent assays (ELISAs) were performed as previously described [35] and the endpoint titers determined. The SARS-CoV-2 ELISAs for all cohorts, with the exception of the Miami cohort designated shelter in place (SIP), were performed as previously described in detail [35] following a 2-step ELISA protocol and results interpreted in accordance with the manufacturer's cutoff calculations. Limits of detection were set at 1:80 and 1:50 for CCC and SARS-CoV-2 ELISAs, respectively. All data were plotted as 1:25. For the SIP cohort, an N-antigen ELISA assay for immunoglobulin G (IgG) and immunoglobulin M (IgM) that was purely qualitative was performed. All donors had undetectable levels of antibodies.

Epitope Predictions and Peptide Selection

To investigate CCC $CD4^+$ T-cell responses, we performed prediction of peptides for HLA class II spanning the entire sequence of the 4 CCC strains utilizing the Immune Epitope Database and Analysis Resource (IEDB) [36]. After selection of promiscuous binders, epitopes composed of 15-mer were generated and further divided into 2 different peptide pools (MP) to encompass epitopes sharing 60% or less homology with SARS-CoV-2 sequences or more than 67% homology (Supplementary Table 1). Responses were measured against the 2 different MPs separately and summed together for graphic display. The CMV MP is a pool of previously reported class I and class II epitopes [37]. To study T-cell responses against SARS-CoV-2, we used the entire SARS-CoV-2 genome (GenBank: MN908947) and we generated MPs of 15-mer peptides overlapping by 10 spanning the entire protein sequence (6–253 peptides per pool) or alternatively an MP for the remainder genome consisting of dominant HLA class II predicted $CD4^+$ T-cell epitopes, as previously described [36, 38]. Supplementary Table 1 lists the number of peptides pooled for each of the viral proteins. Alternatively, HLA class I $CD8^+$ T-cell epitopes prediction was performed as previously reported, using NetMHC pan EL 4.0 algorithm [39] (Supplementary Table 1). All peptides were synthesized as crude material (A&A).

Activation-Induced Markers Assay and Memory Phenotype

Cryopreserved cells were thawed, washed, and stimulated for flow cytometry determinations using activation-induced cell marker (AIM) assays as previously described [34, 40]. Antibodies used in the AIM assay as well as the gating strategy used to define AIM reactive cells and memory subpopulations are listed in Supplementary Table 2 and Supplementary Figure 1. All samples were acquired on a ZE5 Cell Analyzer (Bio-rad Laboratories) and analyzed with FlowJo software (Tree Star).

Statistical Analysis

Data and statistical analyses were done in FlowJo 10 and GraphPad Prism 8.4, unless otherwise stated. Nonparametric Mann-Whitney or Kruskal-Wallis test were applied for unpaired 2-group or 3-group comparisons, respectively. Correlation analysis were performed using nonparametric Spearman test. Details pertaining to significance are also noted in the respective figure legends and $P < .05$ was defined as statistically significant. Additional data analysis details are described in the respective figure legends.

RESULTS

Characteristics of the Donor Cohorts Investigated

Five different cohorts of subjects were enrolled in the study (Table 1). Three cohorts were recruited in the Miami metropolitan area and 2 cohorts were recruited in the San Diego metropolitan area. Two cohorts from Miami encompassed high-risk HCW, further classified as seronegative health care workers (NHCW) or antibody or polymerase chain reaction (PCR) positive health care workers (PHCW). Effort was placed by the study team to balance these cohorts in gender, age, and medical specialty. A third Miami cohort, designated SIP, of community volunteers who were all seronegative and with no exposure to known infected persons was included as a control group. The 2 additional cohorts were asymptomatic unexposed and seronegative donors from San Diego (NSD), and COVID-19–seropositive subjects also from the San Diego region (COVID-19SD) (see Supplementary data for more details on the selection process of all the cohorts). All subjects were assigned to positive or negative SARS-CoV-2 categories on the basis of PCR and/or serological tests.

Serological Analysis of the Different Donor Cohorts

Serum samples for all 5 donor cohorts were tested for SARS-CoV-2 using ELISA (see "Methods" for detail). The results are

Table 1. Description of Donor Cohort Characteristics and Demographics

Cohort	NHCW[a]	PHCW[b]	SIP[c]	NSD[d]	COVID-19 SD[e]
Geographical location	Miami	Miami	Miami	San Diego	San Diego
Number of donors	32	26	33	15	10
Sex, M/F	17, 15	13, 13	16, 17	7, 8	3, 7
Mean age, y	41	38	41	41	32
Sample collection date	Apr–Jun 2020	Aug 2020	Jun 2020	Mar–Jun 2020	Apr–Jul 2020
SARS-CoV-2 status	Ab –	Ab + or PCR +	Ab –	Ab –	Ab +
SARS-CoV-2 PCR, n (%)					
Positive	0 (0)	22 (84.6)	0 (0)	0 (0)	7 (70)
Unknown	…	4 (15.4)	…	…	3 (30)
Antibody response, n (%)					
Positive	0 (0)	23 (88.5)	0 (0)	0 (0)	10 (100)
Negative	32 (100)	3 (11.5)	33 (100)	15 (100)	0 (0)
Interval exposure to sample collection, d					
Range	…	20–145	…	…	43–140
Median	…	44	…	…	92
Symptoms, n (%)					
Asymptomatic	32 (100)	7 (26.9)	33 (100)	15 (100)	0 (0)
Mild	…	19 (73)	…	…	8 (80)
Moderate	…	0 (0)	…	…	1 (10)
Severe	…	0 (0)	…	…	1 (10)
Medical specialty, n (%)					
Otolaryngology	17 (53.1)	6 (23.1)	…	…	…
Anesthesiology	5 (15.6)	3 (11.5)	…	…	…
Emergency medicine	5 (15.6)	5 (19.2)	…	…	…
Ophthalmology	2 (6.3)	2 (7.7)	…	…	…
Other, internal medicine, surgery, etc.	3 (9.4)	10 (38.5)	…	…	…

Abbreviations: Ab, antibody; COVID-19 SD, coronavirus disease 2019 seropositive San Diego; NHCW, negative health care workers; NSD, negative San Diego; PCR, polymerase chain reaction; PHCW, positive health care workers; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SIP, shelter in place.

[a]Seronegative high-risk health care workers from Miami.

[b]Antibody/PCR positive high-risk health care workers from Miami.

[c]Seronegative community donors with no patient exposure from Miami.

[d]Seronegative unexposed donors from San Diego.

[e]Seropositive donors from San Diego.

shown in Figure 1A. Significant SARS-CoV-2 titers were detected in almost all cases of individuals in the HCW cohort with COVID-19 disease from Miami (23/26). Conversely, the seronegative cohorts from Miami (NHCW) had undetectable titers or below the limit of detection. Likewise, all COVID-19SD had significant SARS-CoV-2 titers, while none of the NSD donors was seropositive for SARS-CoV-2 spike antibodies.

In parallel, seropositivity for the spike proteins of the 4 endemic CCCs (229E, NL63, HKU1, and OC43), was also determined in the 3 donor cohorts from Miami (Figure 1B). All donors had detectable titers and variable reactivity for each of the CCC strains, consistent with the majority of the general population having detectable responses for the CCCs [19, 20]. In conclusion, these data define the serological status of the donor cohorts for which the T-cell reactivity was investigated.

CD4$^+$ T-Cell Reactivity Against CCC Is Higher in NHCW Compared to SIP and PHCW

To test the various Miami cohorts for CD4$^+$ T-cell reactivity, we performed AIM assays [34, 40], previously utilized to characterize viral responses including SARS-CoV-2 CD4$^+$ T-cell responses [11, 12, 14], using sets of predicted dominant class II-restricted T-cell peptides for each of the 4 CCCs (Supplementary Table 1). This epitope prediction strategy was previously applied in multiple studies [34, 36, 40] and was envisioned to capture the top 50% of the predicted response.

The CD4$^+$ T-cell reactivity to the 229E, NL63, HKU1, and OC43 viruses was higher in the NHCW cohort as compared to the SIP cohort (Figure 2A and 2B show absolute magnitude and stimulation index plots). This difference was most pronounced for NL63 and least pronounced for HKU1 (P values ranged from .03 to .0005 by the Kruskal-Wallis test).

By contrast, NHCW CD4$^+$ T-cell reactivity was significantly higher compared to PHCW against 229E, NL63, and OC43 (P values ranging from .004 to .002). For HKU1 there was a trend toward higher responses (P = .12). No difference was noted with a control MP composed of epitopes derived from the unrelated ubiquitous cytomegalovirus (CMV) pathogen [37]. Representative flow cytometry plots with CCC-specific and CMV CD4$^+$ T-cell responses are shown in Figure 2C.

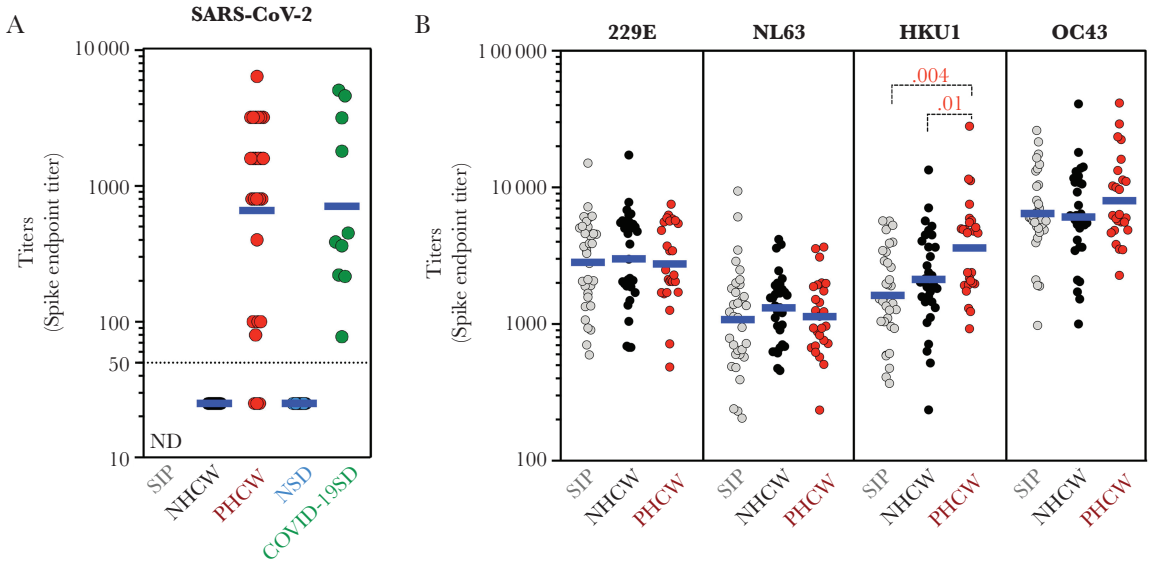

Figure 1. SARS-CoV-2 and CCC serological reactivity for the donor cohort. *A*, Serum ELISA titers to SARS-CoV-2 spike receptor-binding domain protein. *B*, Serum ELISA titers to CCC (HCoV-229E, HCoV-NL63, HCoV-HKU1, and HCoV-OC43) spike protein. Nonparametric Kruskal-Wallis multiple comparison test was applied for each individual CCC strain. Geometric mean titers are indicated by blue lines and *P* values are shown for the statistically significant comparisons. SIP n = 33, NHCW n = 31, PHCW n = 26, NSD n = 15, COVID-19SD n = 10. Dotted line indicates limit of detection (1:50). Abbreviations: CCC, common cold coronavirus; COVID-19SD, coronavirus disease 2019 seropositive San Diego; ELISA, enzyme-linked immunosorbent assays; HCoV, human coronavirus; ND, not determined; NHCW, seronegative health care workers; NSD, seronegative San Diego; PHCW, antibody- or polymerase chain reaction-positive health care workers; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SIP, shelter in place community volunteers.

The SARS-CoV-2–infected donors analyzed were associated with either mild or asymptomatic disease (Table 1). We have analyzed responses to CCC in the cohort of PHCW, segregating asymptomatic individuals (n = 7) versus individuals with mild disease (n = 19) and no differences were observed (data not shown).

CD4$^+$ T-Cell Reactivity Against CCC Is Higher in Unexposed Compared to COVID-19 Donors in an Independent Cohort

To validate these results further, we assessed CCC responses in 2 additional cohorts recruited in the San Diego region, selected on the basis of being asymptomatic and seronegative (NSD) or symptomatic and seropositive (COVID-19SD) for SARS-CoV-2 infection (Table 1). Both cohorts were recruited between March and July of 2020, similar to the Miami cohort.

The 229E, NL63, HKU1, and OC43 epitope pools displayed higher CD4$^+$ T-cell reactivity in the unexposed donors, as compared to the COVID-19–diagnosed donors (Figure 3A and B). No differences between groups were observed in the responses against the CMV control MP. These results indicate that healthy unexposed donors demonstrate higher CD4$^+$ T-cell reactivity against CCC than COVID-19 donors.

CD4$^+$ T-Cell Reactivity to SARS-CoV-2 Spike and CD4R MPs

Next, we tested the various cohorts from the Miami area for SARS-CoV-2 CD4$^+$ T-cell reactivity, using the AIM assay as before and previously described MPs, one encompassing overlapping peptides spanning the entire sequence of the SARS-CoV-2 spike protein (S), and one encompassing predicted CD4$^+$ T-cell epitopes from the remainder of the genome (CD4R) [11, 36] (Supplementary Table 1). The results are shown in Figure 4, which depict CD4$^+$ T-cell responses in the various cohorts plotted as background subtracted data or as stimulation index. A representative flow cytometry AIM$^+$ gating is shown in Supplementary Figure 2.

CD4$^+$ T-cell responses from PHCW cohort were highest, in accordance with their recent exposure to SARS-CoV-2, followed by responses measured in the NHCW and then the SIP cohort. More specifically, the total CD4$^+$ T-cell reactivity of the PHCW cohort to the SARS-CoV-2 pools was significantly higher than both NHCW ($P = .03$ and $P = .003$ by the Kruskal-Wallis test for absolute and stimulation index readouts, respectively) and SIP ($P = .002$ and $P < .0001$ by the Kruskal-Wallis test for absolute and stimulation index readouts, respectively). Of further interest, the total CD4$^+$ T-cell reactivity of NHCW was also higher than that observed in the SIP cohort ($P = .04$ for both absolute and stimulation index readouts). No difference was noted in the case of the CMV MP. As shown in Supplementary Figure 3, the differences noted above are further confirmed by assessing the total reactivity obtained by summing the responses to the various individual SARS-CoV-2 antigen pools as previously reported [11]. Analysis of the expression of the CCR7 and CD45RA memory markers confirmed that the CD4$^+$

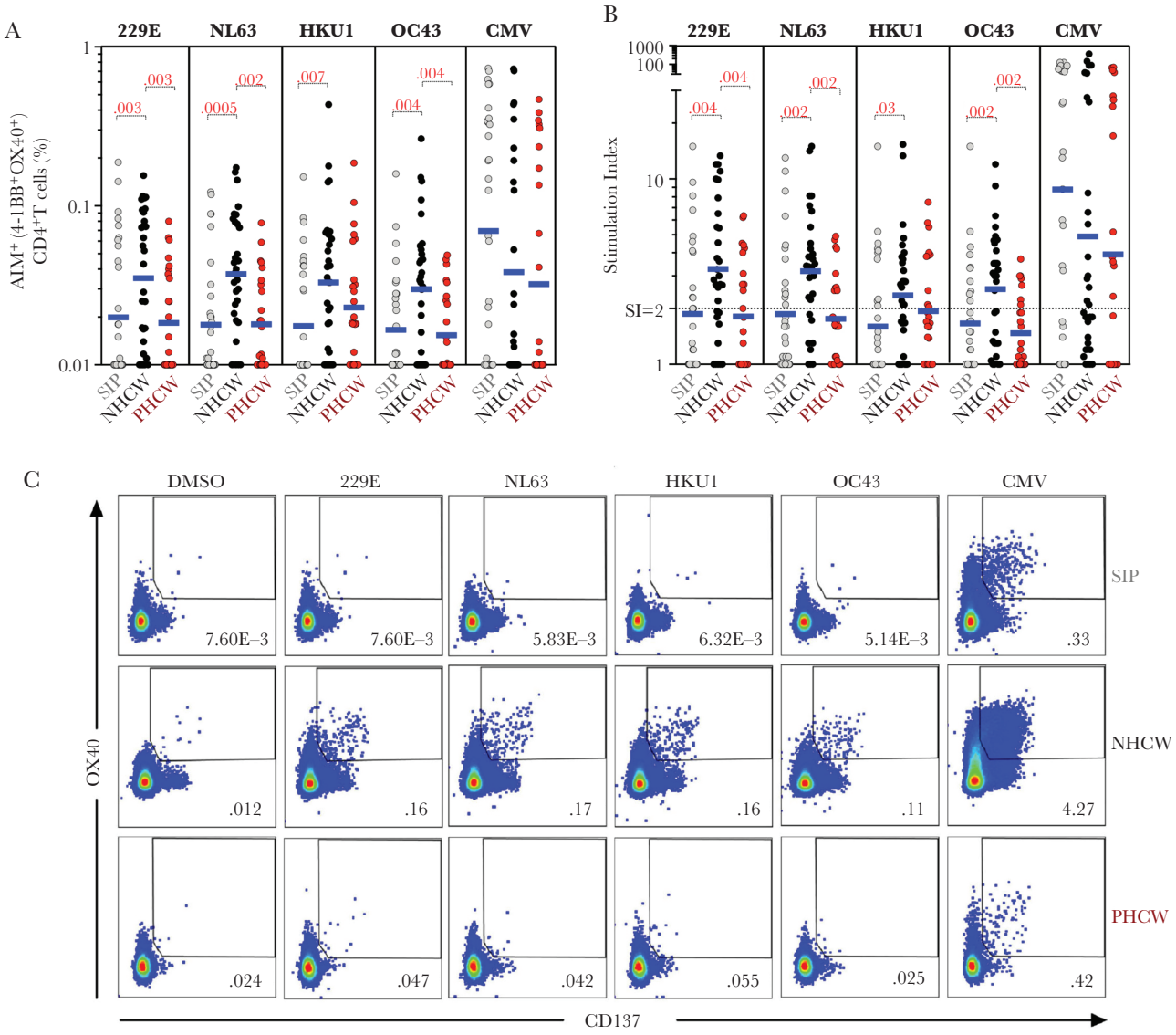

Figure 2. CD4+ T-cell immune responses to CCC epitopes from Miami were higher in NHCW. CCC-specific CD4+ T cells (HCoV-229E, HCoV-NL63, HCoV-HKU1, and HCoV-OC43) and ubiquitous control CMV-specific CD4+ T cells were measured as percentage of AIM+ (OX40+CD137+) CD4+ T cells after stimulation of peripheral blood mononuclear cells with CCC and CMV peptide pools. *A*, Data background subtracted or (*B*) SI against DMSO negative control are shown with geometric mean for the 3 different groups. Nonparametric Kruskal-Wallis multiple comparison test was applied for each individual CCC strain and CMV. *P* values are shown for the statistically significant comparisons. SIP n = 33, NHCW n = 31, PHCW n = 26. *C*, Representative FACS plots, gated on total CD4+ T cells for the 4 CCCs in addition to the DMSO and CMV controls across all the cohorts. Cell frequency for AIM+ cells in each condition is indicated. Abbreviations: AIM, activation-induced marker; CCC, common cold coronavirus; CMV, cytomegalovirus; FACS, fluorescence-activated cell sorting; HCoV, human coronavirus; NHCW, seronegative health care workers; PHCW, antibody- or polymerase chain reaction-positive health care workers; SI, stimulation index; SIP, shelter in place community volunteers.

T-cell reactivity in all 3 cohorts was mediated by memory T-cell subsets (Supplementary Figure 4).

SARS-CoV-2 Reactivity in NHCW Is Not Likely Due to Resolved SARS-CoV-2 Infections in the Absence of Seroconversion

We also analyzed the AIM+ CD4+ T cells for expression of the HLA-DR/CD38 markers, which have been found to be increased in donors from mild to acute SARS-CoV-2 infection, and therefore to be associated with recent in vivo activation [10, 41]. The data shown in Figure 5 demonstrate that the CD4+ T-cell reactivity to SARS-CoV-2 peptides is associated with an increased fraction of recently activated T cells in the case of the PHCW cohort, as compared to the NHCW or SIP cohorts. No difference was detected in the case of the epitope pool derived from the control ubiquitous antigen CMV. In conclusion, the analysis of HLA DR/CD38 markers results are most consistent with recent SARS-CoV-2 infection of the PHCW cohort but not of the NHCW or SIP cohorts.

Having measured CCC-specific responses we further examined responses on a donor-by-donor basis, and asked

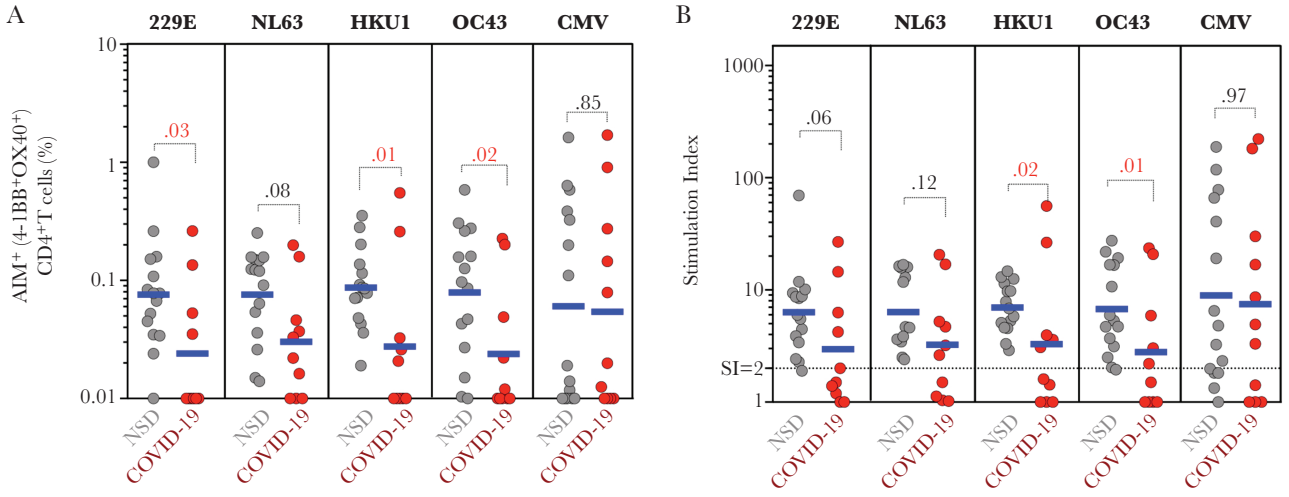

Figure 3. Reactivity of CD4$^+$ T cells against CCC epitopes in an independent cohort from San Diego. CCC-specific CD4$^+$ T cells (HCoV-229E, HCoV-NL63, HCoV-HKU1, and HCoV-OC43) and ubiquitous control CMV-specific CD4$^+$ T cells were measured as percentage of AIM$^+$ (OX40$^+$CD137$^+$) CD4$^+$ T cells after stimulation of peripheral blood mononuclear cells with CCC and CMV peptide pools. *A*, Data background subtracted or (*B*) SI against DMSO negative control are shown with geometric mean for the 2 different groups. Samples were from unexposed seronegative donors (NSD, n = 15) and recovered COVID-19 patients (COVID-19, n = 10). Statistical comparisons across cohorts were performed with the Mann-Whitney test. *P* values are shown with $P < .05$ defined as statistically significant. Abbreviations: AIM, activation-induced marker; CCC, common cold coronavirus; CMV, cytomegalovirus; HCoV, human coronavirus; SI, stimulation index.

whether donors with high CCC CD4$^+$ T-cell reactivity also have high SARS-CoV-2 CD4$^+$ T-cell reactivity. A strong correlation was detected between total CD4$^+$ T-cell responses to CCC and SARS-CoV-2 (Supplementary Figure 5) in all the cohorts and for all CCC strains (significant *P* values ranged from .015 to <.0001 and correlation rank from 0.47 to 0.78), while no correlation was observed between SARS-CoV-2 and CMV responses.

CD8$^+$ T-Cell Reactivity to SARS-CoV-2 Epitopes

Finally, we measured CD8$^+$ T-cell reactivity to SARS-CoV-2 epitopes (Supplementary Table 1) in the various cohorts as previously described [11, 12], utilizing a pool of overlapping peptides spanning the S antigen and 2 MPs containing SARS-CoV-2 predicted HLA binders for the 12 most common HLA A and B alleles (CD8A and CD8B MPs) (Supplementary Table 1). Figure 6 shows CD8$^+$ T-cell responses plotted as background

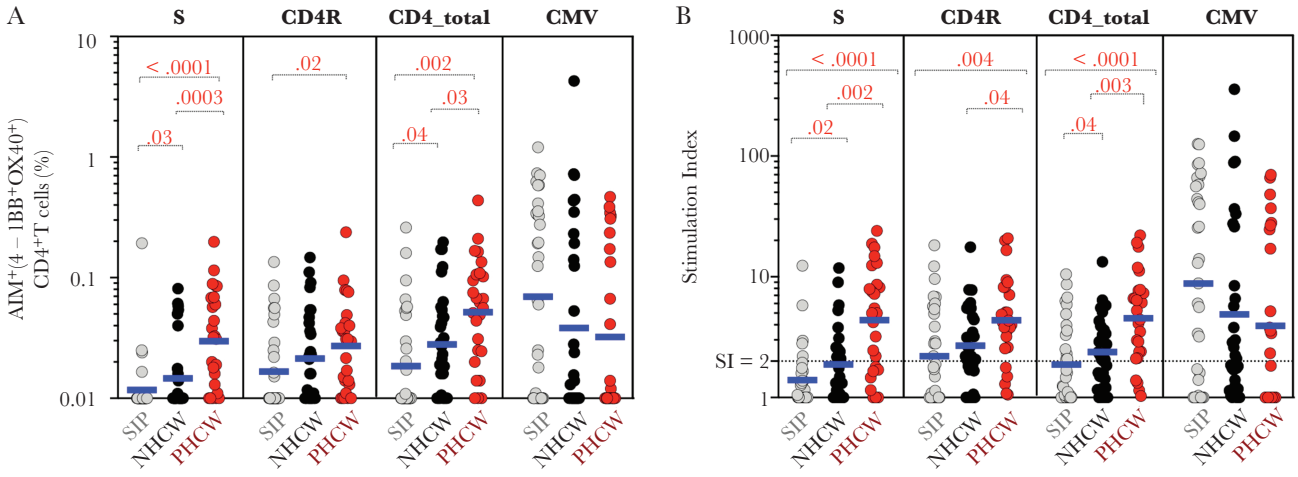

Figure 4. CD4$^+$ T-cell response to SARS-CoV-2 epitopes were highest in PHCW and lowest in SIP. SARS-CoV-2–specific CD4$^+$ T cells were measured as percentage of AIM$^+$ (OX40$^+$CD137$^+$) CD4$^+$ T cells after stimulation of peripheral blood mononuclear cells with peptide pools encompassing spike (S) or representing all the proteome without S (CD4R). Graphs show data for specific responses against S, CD4R, or the combination of both (CD4 total) and against CMV as a control, and plotted as (*A*) background subtracted or (*B*) as SI against DMSO negative control. Geometric mean for the 3 different groups is shown. Nonparametric Kruskal-Wallis multiple comparison test was applied. *P* values are shown for the statistically significant comparisons. SIP n = 33, NHCW n = 31, PHCW n = 26. Abbreviations: AIM, activation-induced marker; NHCW, seronegative health care workers; PHCW, antibody- or polymerase chain reaction-positive health care workers; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SI, stimulation index; SIP, shelter in place community volunteers.

subtracted data, or plotted as stimulation index, against the S pool, the 2 different CD8A and CD8B epitope summed together, and the control CMV pool. A representative flow cytometry AIM$^+$ gating is shown in Supplementary Figure 6.

In the case of the S pool, the CD8$^+$ T-cell response to SARS-CoV-2 spike protein was highest in PHCW (and similar between SIP and NHCW). More specifically, the total CD8$^+$ T-cell reactivity of the PHCW cohort to the SARS-CoV-2 pools was significantly higher than both NHCW (P = .001 and P < .0001 by the Kruskal-Wallis test for both absolute and stimulation index readouts) and SIP (P = .0003 and P = .0003 for both absolute and stimulation index readouts), as expected on the basis of the SARS-CoV-2 infection. The reactivity of the SIP and NHCW was not significantly different.

Similarly, with the CD8A plus B pools, the total CD8$^+$ T-cell reactivity of the PHCW cohort to the SARS-CoV-2 pools was higher than both NHCW (P = .004 and P = .0003 for both absolute and stimulation index readouts) and SIP (P < .0001 and 0.0003 for both absolute and stimulation index readouts). CD8$^+$ T-cell reactivity in all 3 cohorts was mediated by memory T-cell subsets and associated with recently activated HLA-DR$^+$CD38$^+$ cells in the PHCW cohort (Supplementary Figure 7). Overall, these data suggest that the higher reactivity observed in NHCW as compared to SIP is largely confined to CD4$^+$ T-cell responses and only marginally seen in the case of CD8$^+$ T-cell responses, further suggesting that it is not resulting from infected individuals rapidly becoming seronegative.

DISCUSSION

Here we present evidence for differential reactivity to endemic CCC and SARS-CoV-2 epitopes. Although previous reports studied responses to CCC or SARS-CoV-2 in either unexposed or COVID-19 survivors [22–24], this is the first study, to the best of our knowledge, investigating T-cell and antibody responses measured simultaneously for both CCC and SARS-CoV-2 and presenting evidence for differential T-cell reactivity among high-risk HCW and community workers. In particular, we show that a cohort of HCW with presumed exposure to respiratory viruses is associated with higher levels of CCC-reactive T cells as compared to a community SIP cohort, with presumed lower CCC exposure. Interestingly, similar CCC antibody levels were observed across all cohorts.

We hypothesized that this elevated level of CD4$^+$ T-cell reactivity was associated with higher reactivity against SARS-CoV-2 sequences, and indeed we show significantly higher levels of reactivity to SARS-CoV-2 sequences in the NHCW

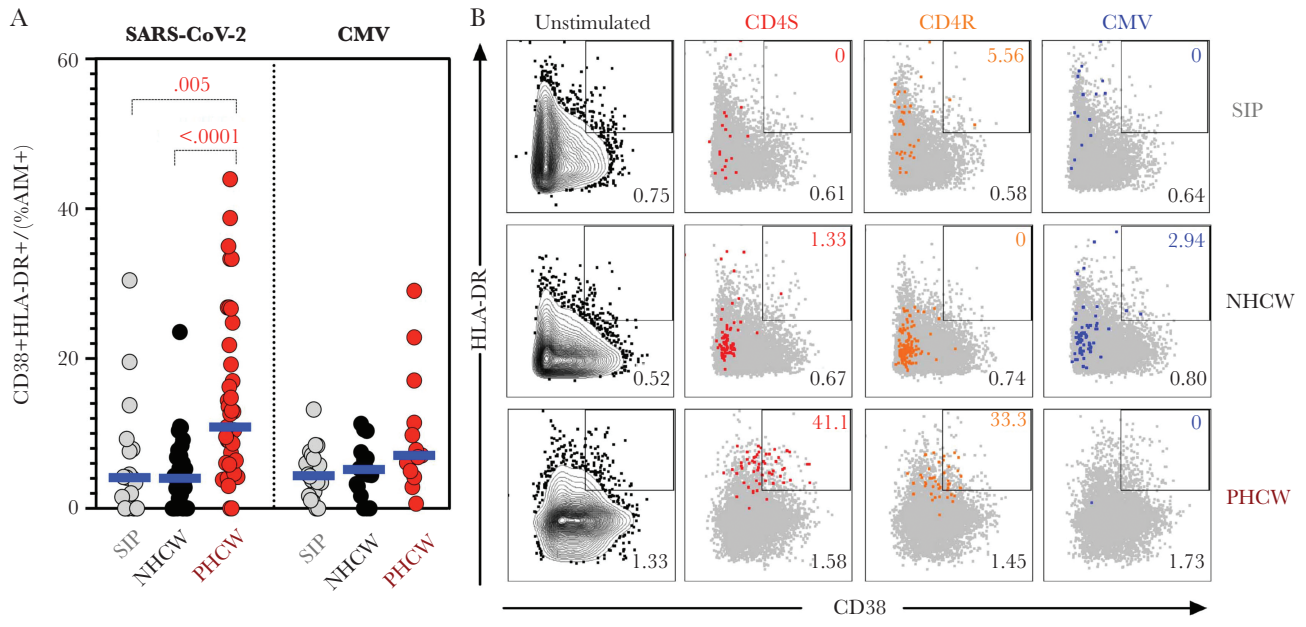

Figure 5. Highest PHCW reactivity in CD4$^+$ T-cell responses was associated with recent infection. *A*, Recently activated SARS-CoV-2–specific CD4$^+$ T cells were measured as percentage of CD38$^+$/HLA-DR$^+$ cells in AIM$^+$ (OX40$^+$CD137$^+$) CD4$^+$ T cells after stimulation of peripheral blood mononuclear cells with peptide pools encompassing a spike only (S) MP and MP representing all the proteome without spike (CD4R). Graphs show data for specific responses against SARS-CoV-2 (both S and CD4R) and the ubiquitous pathogen CMV responses with SI > 2. Each dot represents the response of an individual subject to an individual pool. Geometric mean for the 3 different groups is shown. Nonparametric Kruskal-Wallis multiple comparison test was applied. *P* values are shown for the statistically significant comparisons. SIP n = 20, NHCW n = 33, PHCW n = 39. *B*, Representative FACS plots of HLA-DR/CD38$^+$ cells in AIM$^+$ (OX40$^+$CD137$^+$) CD4$^+$ T cells (colored) overlapped with total HLA-DR$^+$/CD38$^+$ expression (gray) for all the cohorts in the different unstimulated or stimulated conditions. Cell frequency of HLA-DR$^+$/CD38$^+$ in AIM$^+$ cells or total CD4$^+$ T cells is indicated on the top and bottom right corner, respectively. Abbreviations: AIM, activation-induced marker; CMV, cytomegalovirus; FACS, fluorescence-activated cell sorting; MP, 15-mer peptide pool; NHCW, seronegative health care workers; PHCW, antibody- or polymerase chain reaction-positive health care workers; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SIP, shelter in place community volunteers.

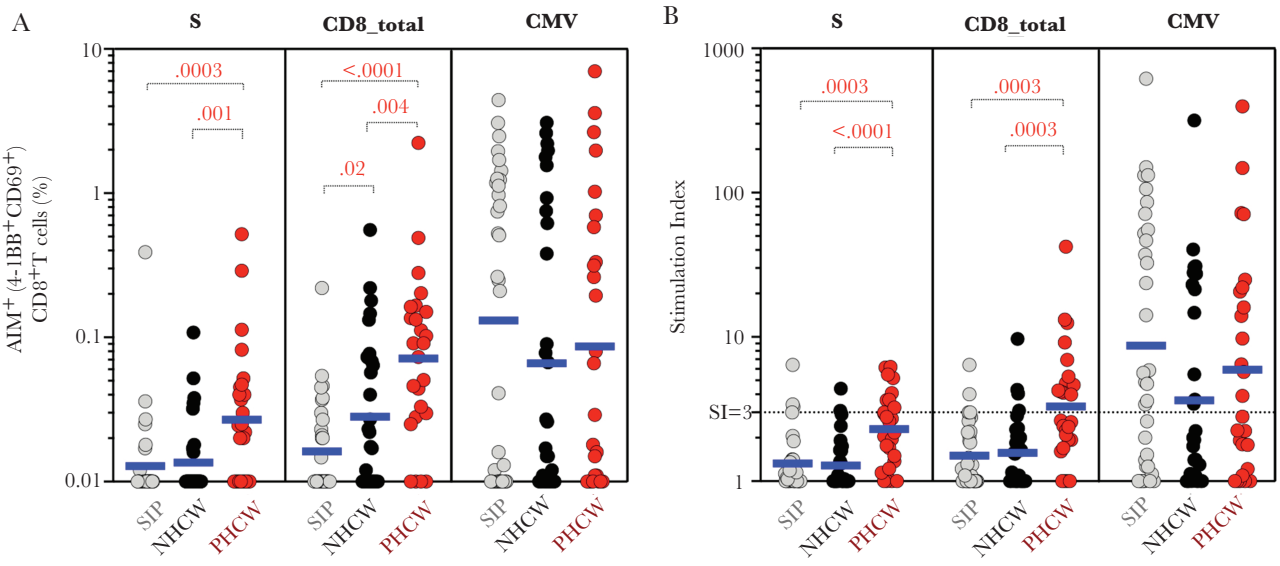

Figure 6. CD8+ T-cell response to SARS-CoV-2 epitopes were highest in PHCW and lowest in SIP. SARS-CoV-2–specific CD8+ T cells were measured as percentage of AIM+ (CD69+CD137+) CD8+ T cells after stimulation of peripheral blood mononuclear cells with spike only (S) MP or class I MPs (CD8A, CD8B). Graphs show data for specific responses against S, the combination of both CD8 MPs (CD8 total), and against CMV as a control, and plotted as (*A*) background subtracted or (*B*) as SI against DMSO negative control. Geometric mean for the 3 different groups is shown. Nonparametric Kruskal-Wallis multiple comparison test was applied. *P* values are shown for the statistically significant comparisons. SIP n = 33, NHCW n = 31, PHCW n = 26. Abbreviations: AIM, activation-induced marker; CMV, cytomegalovirus; MP, 15-mer peptide pool; NHCW, seronegative health care workers; PHCW, antibody or polymerase chain reaction-positive health care workers; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SI, stimulation index; SIP, shelter in place community volunteers.

cohort. There is a correlation between CD4+ T-cell responses to SARS-CoV-2 and CCC. While this correlation is not unexpected in SARS-CoV-2–negative individuals [14, 26, 28], it was not expected in the COVID-19 survivors. It is possible that this finding is reflective of the fact that while most of the response in the COVID-19 survivors, in whom much of the T-cell response is expected to be SARS-CoV-2 specific, also the CCC–cross-reactive component is expanded, thus maintaining a positive correlation. Further studies are required to clarify the evolution of repertoires in exposed and unexposed individuals.

We also analyzed SARS-CoV-2 CD8+ T-cell responses and observed a trend towards higher levels of SARS-CoV-2 cross-reactive CD8+ T cells in the NHCW compared to SIP controls. Both COVID-19 survivors and uninfected controls have SARS-CoV-2–specific T-cell responses that are statistically different but not distinguishable on an individual basis (ie, there is extensive overlap between populations). While it is possible that some of the NHCW may have been infected in the absence of seroconversion or have been associated with transient seroconversion, we believe this is unlikely and/or infrequent. Furthermore, our analysis found expression of cell markers associated with recent in vivo activation [10, 41] exclusively elevated in PHCW for both CD4+ and CD8+ T-cell responses against SARS-CoV-2. As such, the patterns of reactivity detected in the NHCW are likely representative of a sampling of uninfected Miami HCW.

Samples from SARS-CoV-2–infected subjects were associated with lower levels of CCC reactivity as compared to nonexposed donors. This result was unexpected but consistently detected in independent cohorts derived from Miami and San Diego. Several possibilities exist regarding the potential mechanisms underlying this effect. It is possible that SARS-CoV-2 infection may result in a generalized inhibition of CD4+ T-cell responses to other CCCs but not unrelated viruses such as CMV. Impaired responses particularly associated with type I interferon activity in COVID-19 patients were also described in a recent report [42], suggesting that SARS-CoV-2 might interfere with innate immunity. SARS-CoV-2 infection may also result in expansion of SARS-CoV-2–specific, non-CCC reactive T cells, competing with the preexisting CCC specificities [22, 43]. Preexisting CCC reactivity and different preexposure history can also influence disease severity and infection [28]. Indeed, the repertoire of cross-reactive T cells in HCW might have a protective effect against SARS-CoV-2 infection, as suggested in other studies [23, 30, 31]. Based on our current understanding of viral dynamics, it appears unlikely that CD4+ T cells might be able to prevent disease, but it is possible that their presence may lead to rapid termination of infection and only transient seropositivity ([26, 44] and see above). It is also possible that CD8+ T cells might mediate or contribute to rapid termination of infection as described for SARS-CoV [45, 46] and other viral infection diseases [47, 48].

A limitation of the present study is that all the recruited SARS-CoV-2–infected donors were associated with mild or asymptomatic disease, and the small sample size of the study

does not allow us to address whether levels of preexisting cross-reactive CCC T-cell responses might influence disease severity [28, 30]. Also, it would be expected that HCW would wear personal protective equipment during the pandemic period, so it seems unlikely that they would be exposed to CCC to a great extent, at least in the workplace. It is therefore possible that, despite our effort to balance HCW and SIP control cohort, other demographic differences could explain the results. Larger sample sizes will be required to analyze this issue in this type of cross-sectional design, but it is likely that a prospective longitudinal design might be necessary to firmly address this point based on evaluation of CCC reactivity in preinfection samples and its correlation with disease severity after SARS-CoV-2 infection. Also, collection of larger numbers of cells per sample would allow performance of a more granular analysis of the CCC-specific responses and address if T-cell response to CCC in HCW is multispecific or if it becomes more focused to only few epitopes after SARS-CoV-2 infection. Functional characterization or assessment of T-cell phenotypes was also not performed. It would certainly be of interest to elucidate cytokine responses or other functional assays in ensuing studies involving HCW. This study has focused on dissecting the CCC $CD4^+$ T cells responses by design, as scarce ex vivo cross-reactivity has been previously observed for the $CD8^+$ T-cell counterpart [11]. Nevertheless, we cannot exclude an involvement of $CD8^+$ T cells and future studies should be focused to specifically address this point. An additional limitation of this study is the unknown history of previous CCC exposure. Therefore, the results may not be necessarily generalizable to other situations with different patterns of prior exposure.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential conflicts of interest. A. S. is a consultant for Gritstone, Flow Pharma, Merck, Epitogenesis, Gilead, and Avalia. S. C. is a consultant for Avalia. La Jolla Institute for Immunology has filed for patent protection for various aspects of T-cell epitope and vaccine design work. Icahn School of Medicine at Mount Sinai has licensed serological assays to commercial entities, and has filed for patent protection for serological assays with D. S., F. A., and F. K. listed as inventors, and Newcastle disease virus-based SARS-CoV-2 vaccines with F. K. named as inventor. All other authors declare no potential conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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