Circulating CD4 T cells elicited by endemic coronaviruses display vast disparities in abundance and functional potential linked to both antigen specificity and age

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Abstract

Repeated infections with endemic human coronaviruses are thought to reflect lack of long-lasting protective immunity. Here, we evaluate circulating human CD4 T cells collected prior to 2020 for reactivity towards hCoV spike proteins, probing for the ability to produce IFN-γ, IL-2 or granzyme B. We find robust reactivity to spike-derived epitopes, comparable to influenza, but highly variable abundance and functional potential across subjects, depending on age and viral antigen specificity. To explore the potential of these memory cells to be recruited in SARS-CoV-2 infection, we examined the same subjects for cross-reactive recognition of epitopes from SARS-CoV-2 nucleocapsid, membrane/envelope, and spike. The functional potential of these cross-reactive CD4 T cells was highly variable, with nucleocapsid-specific CD4 T cells, but not spike-reactive cells showing exceptionally high levels of granzyme production upon stimulation. These results are considered in light of recruitment of hCoV-reactive cells into responses of humans to SARS-CoV infections or vaccinations.

Key Words: CD4 T cells, coronavirus, SARS-CoV-2, cell-mediated immunity

Summary

Human CD4 T cell reactivity to endemic human coronavirus and SARS-CoV-2-derived peptides were assessed by quantifying IFN-γ, IL-2 and granzyme-B production directly ex vivo, revealing striking variability in abundance and functionality that may impact responsiveness to SARS-CoV-2 infections and vaccination.

Introduction

Human endemic coronaviruses (hCoV) are the source of seasonal infections (reviewed in [1-5]). Although only recently identified, it is speculated that hCoV have been circulating in humans for centuries ([2, 6]). The endemic hCoV, HCoV-229E and HCoV-NL63, belong to the alphacoronavirus group 1 genera and HCoV-OC43 and HCoV-HKU1 belong to the group 2 betacoronavirus genera. Though human subjects often present with similar symptoms, these viruses possess distinctive molecular features and modes of infection. The endemic hCoV share a common genomic organization, with viral replicase genes at the 5'end, followed by structural proteins spike (S), envelope (E), membrane (M) and nucleocapsid (N). The spike facilitates attachment to the target cell through its receptor binding domain (RBD), which plays a major role in viral tropism and pathogenesis. HCoV-229E and NL63 are the most phylogenetically related of the hCoV, but interestingly, the target receptors they use are different, with 229E using the human aminopeptidase N (hAPN) and NL63 employing the Angiotensin Converting Enzyme (ACE2). HCoV-OC43 and HKU1 use O-acetylated sialic acid-linked glycoproteins as their surface receptor. Additionally, the betacoronaviruses also encode hemagglutinin esterase (HE), which serves as a receptor-destroying enzyme, dissociating HCoV-OC43 and HKU1 S1 domain-encoded receptor from its target [7]. Together, these receptor specificities represent a diverse array that may impact the fate of the viruses and host responses after infection.

Endemic hCoV co-circulate annually worldwide [8] and surveillance of adults in US metropolitan areas revealed a seroprevalence rate of approximately 90% against HCoV-OC43, NL63 and 229E and 59% against HCoV-HKU1 [9]. Shared sequence conservation across these strains with the recently emerged SARS-CoV-2 have raised the possibility that previously established immunity against hCoV may provide cross-protective immunity against SARS-CoV-2 that emerged in 2019, causing a world-

wide pandemic. Additionally, SARS-CoV-2 and seasonal hCoV-specific CD4 T cells have recently been

identified in apparently unexposed, healthy donors [10-13], consistent with shared epitopes.

Although immunity to hCoV is apparently not sufficiently protective to prevent infections with hCoV,

memory CD4 T cell subsets established by infection with these endemic strains may influence the

quality and kinetics of responses to SARS-CoV-2 infection and vaccination.

Because of potential impact on responses to related highly pathogenic human CoV, it important to

understand how repeated infections with hCoV impact the abundance, specificity and functionality

of the human CD4 T cell repertoire. In this study, we sought to examine three questions related to

this issue. First, we asked whether circulating CD4 T cells collected prior to 2020 exhibit evidence of

immunity to endemic hCoV, as evidenced by recognition of hCoV-peptide epitopes. Second, we

asked if qualitative features of human CD4 T cells reactive with hCoV were similar across diverse

subjects, an issue probed by quantifying several distinct mediators produced by CD4 T cells specific

for hCoV-derived peptide epitopes. Finally, we directly examined whether human CD4 T cells

collected prior to 2020 exhibited cross reactivity with epitopes from SARS-CoV-2, including spike

nucleocapsid, membrane and envelope proteins. We speculated that recruitment of memory cells

established by intermittent contact with seasonally circulating hCoV might account for some of the

variable outcomes of infection with this SARS-CoV-2.

Materials and Methods:

Synthetic peptides: 17mer peptides overlapping by 13 amino acids encompassing the entire

translated sequences of spike from hCoV OC43, 229E and NL63, and SARS-CoV-2 spike, nucleocapsid,

membrane and envelope proteins, as well as peptide sets from Influenza HA-B/Florida/04/2006 and

Sin Nombre Virus, used as positive and negative controls, respectively. Peptides were obtained from

5

the NIH Biodefense and Emerging Infections Research Repository, NIAID, NIH. For spike proteins, peptides were divided into pools representing the S1 and S2 segments, with each peptide at a final assay concentration of 1 μM.

Isolation of PBMCs from human blood: Following approval from DMID and the University of Rochester Research Subjects Review Boards (protocols 07-009 and 14-0064), blood was obtained from 31 healthy subjects in the Rochester, NY area throughout years 2012-2018 that had provided informed consent. Isolated PBMCs were frozen in fetal calf serum containing 10% DMSO. After thawing and overnight rest in culture, cells were washed and depleted of CD8 and CD56 cells using MACS microbeads per manufacturer instructions (Miltenyi Biotec).

EliSpot Assay: EliSpot assays were performed as previously described [14, 15]. Briefly, CD8- and CD56-depleted PBMCs were cultured with peptides on plates coated with antibodies to human IFNγ or IL-2 for 36 hr at 37°C, 5% CO_2 or with anti-human granzyme B (GzmB) for 60 hrs at 37°C, 5% CO_2. After incubation, and washing, plates were incubated with biotinylated detection antibodies as described [16]. Quantification of mediator-secreting cells was performed with an Immunospot reader series 5.2, using Immunospot software, v5.1. Data are presented as the frequency of mediator-producing cells per million CD8- and CD56-depleted PBMCs. Spot counts three-fold over background were considered positive responses.

Results

Reactivity to endemic seasonal hCoV. Because spike proteins are known to be targets of T cell recognition for SARS and SARS-CoV-2 [10, 11, 17-21], we quantified memory to circulating seasonal hCoV using overlapping peptide libraries corresponding to the entire translated sequences of spike proteins from circulating hCoV. Reactivity to influenza HA-B was used as a positive control, as our

previous studies indicated robust reactivity in most human subjects [14]. EliSpot assays, enriched for CD4 T cells and antigen presenting cells (APC), were used to quantify reactivity, based on the sensitivity and objectivity of these assays [22]. Spike-derived peptides were distributed into S1 and S2 pools to separately query CD4 T cell reactivity to these segments. The symbols and demographics of the subjects are shown in **Supplemental Table I** and responses tabulated in **Supplemental Table II**.

Several interesting observations were made from our initial studies, shown in **Figure 1**, where average values are indicated by grey transparent bars and the reactivity of different subjects is shown by unique symbols. First, reactivity of CD4 T cells specific for endemic spike proteins is readily detectable in many subjects. When response is calculated as the percent of subjects demonstrating at least 3-fold over background for spike, from each of the seasonal CoV spike proteins, at least 90% of subjects demonstrated reactivity. However, a striking range in response magnitude was detected among these subjects, with responding cells differing by as much as 1000-fold. Although some of the variability in CD4 T cell levels can be attributed to HLA class II diversity, selecting for different epitopes in each viral pathogen, the spike proteomes tested (> 195 peptides) coupled with the overall diversity of HLA class II molecules expressed in humans, it is expected that hCoV spike proteins will offer many potential epitopes to CD4 T cells. Next, because each pool of peptides has a different number of peptides, to directly compare the relative reactivity to each source of antigen, responses were normalized to the total number of peptides, shown in **Supplemental Figure 1**. Collectively, these data demonstrate that CD4 T cell reactivity to the alpha CoV (NL63 and 229E) to be more abundant in most subjects, relative to the beta CoV tested (OC43). Statistical treatment of these is shown in **Supplemental Table III**. Similar conclusions were made when complete pools of spike peptides were tested recently for IFN-γ in SARS-CoV-2 serologically negative subjects recently collected [23].

Subject-dependent functional phenotype of CD4 T cells reactive to hCoV spike proteins.

Two additional mediators, IL-2 and granzyme B, were chosen to quantify the hCoV-specific CD4 T cell repertoire (shown in **Figure 1, Panels B and C**) and to explore the functional heterogeneity among responding CD4 T cells. Both mediators have important activities in CD4 T cell function. IL-2 fosters CD8 T cell expansion and memory formation [24-26], and influences the germinal center reaction [27, 28], while granzyme can provide direct cytotoxic function [29, 30]. EliSpot assays, in contrast to intracellular assays that measure stored as well as induced cytotoxic mediators, are of particular advantage for granzyme B detection.

These assays revealed that IL-2 was produced by many hCov-reactive CD4 T cells, although lower in abundance than IFN-γ, indicating potential multifunctionality of many circulating hCoV-reactive CD4 T cells. As in the IFN-γ-specific responses, there was considerable variation in the magnitude of response, with some individuals showing only minimal CD4 T cell responses, while others showed robust CD4 T cell immunity. Interestingly, granzyme secretion was also readily detectable by spike-reactive CD4 T cells, indicating that seasonal hCoV elicits CD4 T cells that have cytotoxic potential. This reactivity was particularly enriched for the S2 segment of NL63 (**Figure 1C**). The abundance of CD4 T cells producing different mediators, relative to the others, was quite disparate among the different subjects. Figure 2 shows analyses of this variability, normalizing for the absolute abundance of CD4 T cells producing each mediator by each subject's CD4 T cells and indicated that granzyme production was the most variable, and particularly deficient in the CD4 T cells specific for OC43 and 229E. These results indicate that individuals that possess robust reactivity to hCoV by production of

one mediator may be deficient in others. Therefore, recruitment of the CD4 T cells into the response to a future infection could have diverse phenotypic consequences in different subjects.

We were intrigued by the differences between reactivity to the S1 and S2 segments of spike proteins. This enrichment for S2 reactive-CD4 T cells relative to S1 was most striking for the alpha-hCoV (NL63 and 229E) (p=0.00001.17, and 0.000001.95, respectively), whereas the beta-hCoV OC43 has a more equivalent distribution of epitopes between S1 and S2 (p=0.19, ns). To examine whether selective accumulation of CD4 T cells specific for the S2 domains might be attributable to cross boosting between strains, we examined sequence relatedness in the spike across the two strains in each hCoV lineage. **Figure 3A** shows sequence alignments for the alpha CoV (NL63 and 229E), with S1 indicated in the top panel and S2 in the bottom. Sequence identity is in black and divergence in gold. It is clear from inspection of these alignments, as well as the calculations of sequence identity, indicated in the legend, that the S2 segments have many options for cross-reactive recognition by CD4 T cells between the alpha-hCoV. This could allow accumulation of S2-specific CD4 T cells during intermittent infections with either of these hCoV over a lifetime. In terms of absolute sequence identity, 229E and NL63 have 31% sequence identity in S1 and 75% in S2. In contrast, the circulating beta-hCoVs (OC43 and HKU1) have more equivalent distribution of shared reactivity toward S1 and S2 (55% vs. 73%, respectively), when these sequences are aligned (**Figure 3B**), suggesting a more balanced boosting of CD4 T cells during sequential cross-strain infections.

CD4 T cell cross reactivity to SARS-CoV-2 derived epitopes. After observing the robust reactivity to endemic hCoV in subjects' CD4 T cells collected prior to 2020, we evaluated if this apparent memory to hCoV was associated with CD4 T cell cross reactivity to SARS-CoV-2-derived epitopes. Evidence of cross-reactivity in unexposed individuals have been examined by other groups, using such methods

9

as *in vitro* expansion of subjects collected prior to 2020 [13], and upregulation of activation markers on serologically negative subjects collected in parallel with COVID subjects [11] or in earlier years [10].

In our studies, direct *ex vivo* cytokine production was used to quantify SARS-Cov-2 reactive cells in the previously examined cohort. CD4 T cells were analyzed for reactivity with SARS-CoV-2 spike (S1/S2), as well as nucleocapsid, membrane and envelope. The latter two peptides pools were combined into one pool because they are both quite small proteins (**Supplemental Table IV**). These SARS-CoV-2 proteins have been shown to be the target of T cell recognition in SARS and SARS-CoV-2 [18, 31]. The experiments, probing cross-reactive immunity to SARS-CoV-2 are shown in **Figure 4 and Supplemental Figure 2.** There was dramatically uneven and statistically significant recognition between SARS-CoV-2 S1 and S2 segments (p<0.001). When quantified by IFN-γ, SARS-CoV-2 nucleocapsid and spike-S2 exhibited the most apparent cross-reactive recognition, followed by M/E. S1-derived epitopes elicited little reactivity, undetectable in most subjects. Interestingly, although SARS-CoV-2 spike S2 elicited similar numbers of nucleocapsid-reactive cells when IFN-γ production was tested, these cells produced undetectable levels of granzyme, in contrast to cells specific for N and M/E. These results indicate a functional divergence in the potential cross-reactivity that might occur upon infection of these types of subjects with SARS-CoV-2.

To better understand the basis for cross-reactive recognition between seasonal hCoV and SARS-CoV-2 derived epitopes, sequence alignments with the hCoV and SARS-CoV-2 were made (**Figure 5**), with amino acid identity in black and divergence in gold. Figure 5 shows that there are intermittent segments of these identical amino acids across all of the seasonal hCoV and SARS-CoV-2 in S2, N, M, but these are rare in S1 and E. A closer inspection of these alignments shows that SARS-CoV-2, as a

10

member of the beta-hCoV, has more regions of identity with OC43 and HKU1 proteins, particularly in the N and S2 regions, suggesting that infections with these hCoV most likely lead to cross-reactive recognition of SARS-CoV-2 spike. Preferential recognition of S2 has also been noted in healthy adults when reactivity has been quantified by flow cytometry, assaying for up-regulation of surface markers upon antigenic stimulation [11], rather than cytokine production as we have here.

Finally, we examined whether in healthy adult cohort, any age-dependence of circulating CD4 T cell memory to endemic hCoV spike protein-derived epitopes was detectable. The number of each subject's CD4 T cell cytokine-producing cells was plotted vs. age at sampling. Strikingly, with increasing age, the number of responding cells producing IFN-γ decreased, with a strong statistical relationship for each of the spike derived pools of peptides (**Figure 6A-C**), across the whole age range, from subjects in their 20s to those over 65. Particularly notable was the comparative analyses of influenza HA-B response patterns among the same subjects, where no age-dependent patterns were observed (**Figure 6D**). The same negative trend in response magnitude with age was seen in responses measured with IL-2 producing cells and granzyme-producing cells but did not reach statistical significance (not shown).

Discussion

In the experiments presented here, we have probed the reactivity of circulating human CD4 T cells to seasonally circulating, endemic hCoV among a cohort of adults sampled prior to 2020. Our studies revealed readily detectable but highly variable hCoV-specific CD4 T cells in both their magnitude and functional potential. Enriched reactivity for S2 relative to S1 segments coincided with the sequence relatedness of the spike proteins of different hCoV. These findings support the view that infections

with hCoV strains cross-boost CD4 T cells established by the alternate strains. It should also be noted that several recent studies have characterized drift in hCoV, in particular locations [32, 33], and genetic variation tends to be localized to regions associated with binding to host cells, which may be enriched by positive selection, that are localized to the S1 domain. This drift might also contribute to enriched reactivity to relatively conserved S2 segments. Additionally, our studies revealed that the CD4 T cell reactivity in samples collected prior to 2020 displayed apparent cross-reactivity to SARS-CoV-2, consistent with amino acid identity between hCoV and corresponding regions of SARS-CoV-2. This is particularly notable in the spike protein, where almost all reactivity is contained in S2, which has much greater sequence relatedness to SARS-CoV-2 than S1. This conclusion is consistent with the suggestions of other studies finding cross-reactivity [11, 13, 23], although a recent study has challenged this view [34]. To further support direct cross reactivity, we have separately quantified reactivity to some of the more conserved, and relatively unique segments of SARS, relative to the analogous segments of nucleocapsid and find highly enriched reactivity to the most conserved segments (Supplemental Figure 3). Approaches involving extended *in vitro* culture, not performed here, have recently been used to support cross-reactive recognition of SARS-CoV-2 specific CD4 T cells in SARS-unexposed humans [11, 13, 23].

One question raised by our studies is the source of variability in CD4 T cell response magnitude toward seasonal hCoV. At least three possibilities can account for these findings. The first is that HLA variability determines the abundance of presented CD4 T cell epitopes available to initiate responses during hCoV infection. A highly complex array of HLA class II molecules, coupled with the size of the spike proteome, would seemingly offer many peptide:class II complexes for recruitment of CD4 T cells. Therefore, we think this first possibility is unlikely. The second possibility is that infection and immune responses with different hCoV is followed by a rapid decay of circulating CD4 T cell memory. This would lead to variable "snapshots" of recent infections in each subject. Sampling of respiratory

infections suggests human experience multiple infections over the course of lifetimes (reviewed in [4, 5]), a conclusion predicted by early serological studies [35]. Repeated infections with hCoV over sequential seasons has also been noted [36], suggesting protective immunity is short-lived. The final possibility to explain the range in abundance of CD4 T cell memory is that CD4 T cells can accumulate over time with repeated confrontations with hCoV, but that the persistence is highly variable across the population.

The striking age-related hierarchy of responses may reflect age-dependent boosting or persistence over time. There is little evidence that frequencies of hCoV infections vary significantly with age in adults [37-39], and thus it seems unlikely that lowered infection rates account for the diminished memory CD4 T cells with increasing age. The decline in memory CD4 T cells may reflect less robust boosting upon infection with increasing age or more acute decay of boosted cells with increasing age. In previous studies probing the memory repertoire for many influenza antigens in a similarly aged cohort, as well as the HA-B specific responses tracked in parallel here, we have found no apparent impact of age on circulating memory. Similar patterns were observed with influenza NA-reactive CD4 T cells that we analyzed in a separate cohort (data not shown). Like hCoV, influenza infections occur periodically in adults, supporting the possibility that factors unique to hCoV infections may be involved in the age disparities identified here. The age-dependent susceptibility to the eldest among us to severe influenza virus infection [40], despite the apparent steady levels of circulating CD4 T cells with age may be related to rare subsets of cells in peripheral blood that can participate in protection being recruited into the response to infection and in general to the overall complexity of human CD4 T cell functionality [41]. Boosting of CD4 T cells by yearly vaccination, which is at the moment is restricted mainly to influenza virus may also contribute to age disparities in circulating CD4 T cells and the CD4 T cells boosted by these protein-based vaccines delivered intramuscularly may not contribute substantially to protection from infection.

13

The variability in functional potential associated with epitope specificity among the CD4 T cells specific for hCoV was also striking, particularly in granzyme production. Granzyme secretion was enriched in SARS-CoV-2 nucleocapsid- and M/E-reactive CD4 T cells, but was undetectable in CD4 T cells specific for the entire SARS-CoV-2 spike protein. Granzyme production was readily apparent in the alpha-hCoV S2 segments but overall less so in beta-CoV-reactive cells. We do not yet understand the factors that lead to these differences in granzyme production among endemic and SARS-CoV-2 cross-reactive cells but it could relate to repeated boosting, and perhaps association of nucleocapsid with viral RNA during priming. Also important in hCoV CD4 T cell functionality is the possible impact of co-infections with other respiratory pathogens. Multiple viruses, including RSV, adenovirus and influenza have been detected in single isolates of human respiratory samples (reviewed in [4, 37] and [42, 43]) and such co-infections could alter the microenvironment of T cell priming and thus the phenotype of the elicited cells.

Independently of mechanisms responsible for differences in functional potential of the CD4 T cells, these patterns of memory at the time of SARS-CoV-2 infection could impact host responses to infection in either a positive or negative way. For example, if SARS cross-reactive, granzyme-positive cells are recruited into the lung, they could eliminate SARS-infected cells and serve a protective function. Alternatively, if recruited into the lymph node where protective initiate, they could potentially eliminate APC, thus being associated with negative outcomes. Disparities in CD4 T cell phenotype are particularly important when one considers the impact of memory on SARS-CoV infections or vaccination. The earliest responders will be memory CD4 T cells, rather than naive CD4 T cells specific for unique SARS-CoV-2, because of their greater abundance and sensitivity to antigen (reviewed in [44]). Cytokines and chemokines produced by SARS-CoV-2 cross-reactive CD4 T cells would likely impact the ultimate phenotype of responding CD4 T cells. The decreasing abundance of

circulating memory to the endemic hCoV, coupled with the apparent greater susceptibility of the elderly to SARS-CoV-2 that has been observed in many studies (reviewed in [45]) may relate to disparities in recruitment or the potential for protective CD4 T memory at different ages. Future detailed analyses of the memory CD4 T cells within the human host will likely reveal the impact of pre-existing memory on the responses to SARS-CoV-2 infection and vaccination.

Figure Legends

Figure 1. Seasonal human coronavirus-reactive CD4 T cells, secreting IFN-γ, interleukin 2 (IL-2) and granzyme B (GrzB), range in response magnitude and functional potential. Enzyme-linked immunospot (EliSpot) analyses were performed following re-stimulation with peptide pools spanning the S1 and S2 regions of betacoronavirus OC43, and alphacoronaviruses 229E and NL63. A pool of peptides spanning the entire coding sequence of influenza HA-B was used as a positive control. CD4 T cell mediators, measured by IFN-γ (A), IL-2 (B) and granzyme B (C) production, are shown as the spot count per million CD8- and CD56- depleted PBMC with background subtracted. Donors are indicated by unique symbols (Supplemental Table I), with the mean value illustrated by a grey shaded bar. Statistical treatment of the data shown in panel A is shown in Supplemental Table III.

Figure 2. Distinct functional potential of hCoV-reactive CD4 T cells across subjects and hCoV protein specificity. Using the previously quantified cell frequencies as measured by EliSpot analysis, the ratio of IFN-γ producing CD4 cells to IL-2 producing CD4 cells (IFN-γ /IL-2, top); ratio of GrzB producing CD4 cells to IL-2 producing CD4 cells (GrzB/IL-2, middle) and ratio of GrzB producing CD4 cells to IFN-γ−producing CD4 cells (GrzB/IFN-γ, bottom) was calculated. Each subject is identified by their unique symbol and the mean values is illustrated by a grey shaded bar.

Figure 3. Comparison of the spike protein sequences across the endemic human coronaviruses reveals possible source of CD4 T cell cross boosting. The amino acid sequence alignments of alphacoronaviruses, 229E and NL63 (3A), and betacoronaviruses, OC43 and HKU1 (3B) were performed, with sequences divided into the S1 and S2 regions of the spike protein. Gold bars represent segments of sequence variation and black bars indicate stretches of sequence identity. For the alphacoronaviruses (3A), the sequence identity was 31% for S1, 75% for S2 and 54% for the total protein. The sequence identity for the betacoronaviruses (3B) was 55% for S1, 73% for S2 and 63% for the whole protein. Sequence files were downloaded from PubMed. The accession numbers are shown in Supplemental Table V. The conservation profiles were constructed using CLC Sequence Viewer 7 software.

Figure 4. SARS-CoV-2 reactive CD4 T cells are readily detectable in unexposed, healthy adults but differ in functional potential. CD4 T cells were evaluated for potential cross reactivity to SARS-CoV-2 proteins spike (S1 and S2), nucleocapsid (N), and Membrane and Envelope (M/E) using EliSpot analyses. Responses are shown as the spot count per million CD8- and CD56- depleted PBMC with background subtracted for each mediator (IFNγ, A; IL-2, B; and granzyme B, C). Responses are represented as the frequency per number of peptides present in the pool. In each panel, donors are indicated by their unique symbols, with the mean value illustrated by a grey shaded bar. Differences in IFNγ reactivity between SARS S1 and S2 were assessed using two sample Wilcoxon tests, p<0.001.

Figure 5. Comparison of the sequence relatedness across the structural proteins endemic hCoV and SARS-CoV-2 reveals multiple regions of preserved identity and cross-reactive

potential. Amino acid sequence alignments for the nucleocapsid, membrane/envelope (indicated by M and E, respectively) and spike proteins of 229E, NL63, OC43 and HKU1, and SARS-CoV-2 were performed. Yellow bars represent segments of sequence variation and black segments indicate stretches of sequence identity. Sequence files were downloaded from PubMed (accession numbers in Supplemental Table IV) and the conservation profiles were constructed using CLC Sequence Viewer 7 software.

Figure 6. Age-related decline in hCoV CD4 T cell memory with increasing age. The number of IFN-γ producing CD4 T cells reactive to S1 (top) and S2 (bottom) for each of the endemic sCoV, OC43 (A), 229E (B), and NL63 (C), as well as HA-B (panel D) are shown as a function of age at the time of blood draw for each subject. The best-fit line is indicated and the r and p values shown below each panel. The Spearman correlation coefficients were calculated in R with the significance of the correlations adjusted for multiple testing by controlling for the false discovery rate.

Footnotes:

3) The research in this manuscript has not been presented at any meetings.

4) No author has changed affiliation since completion of this study.

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Figure 1.

Figure 2.
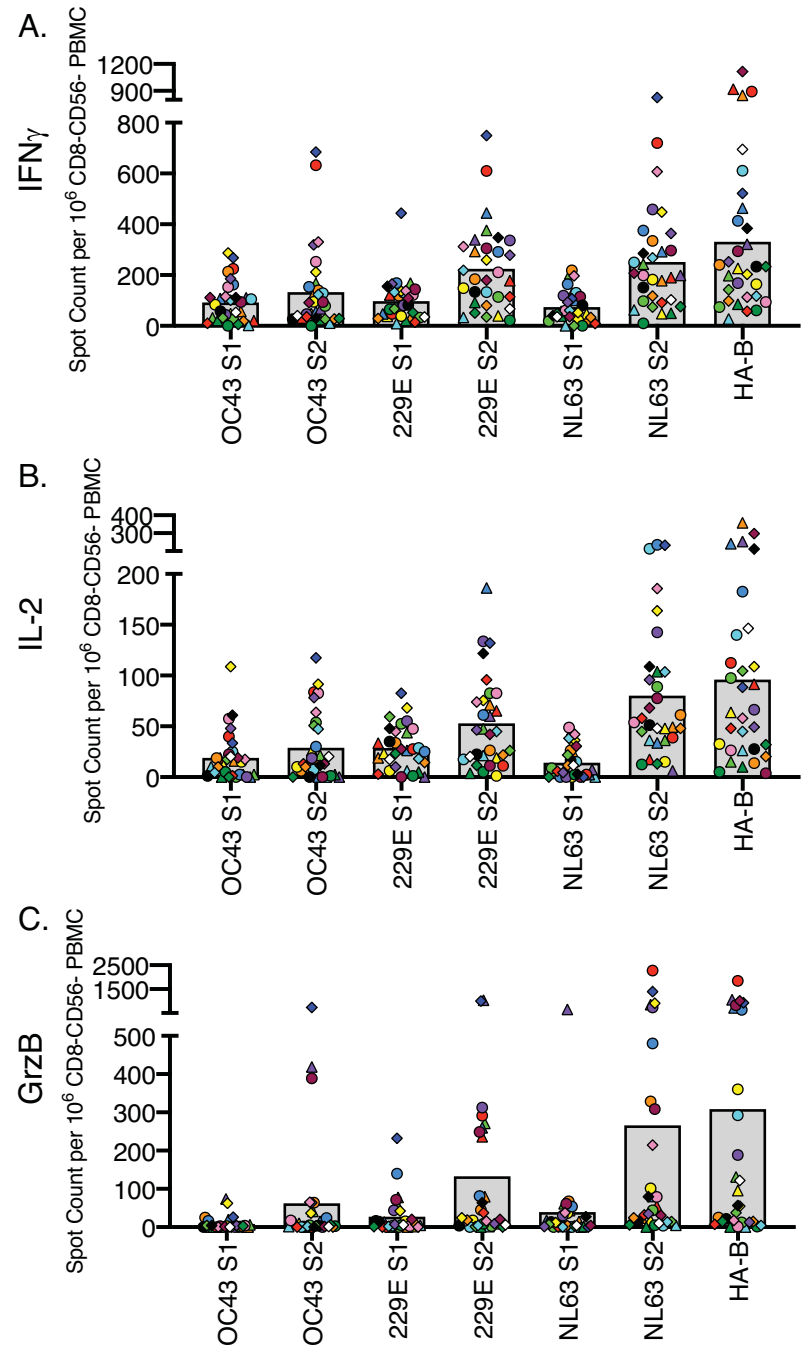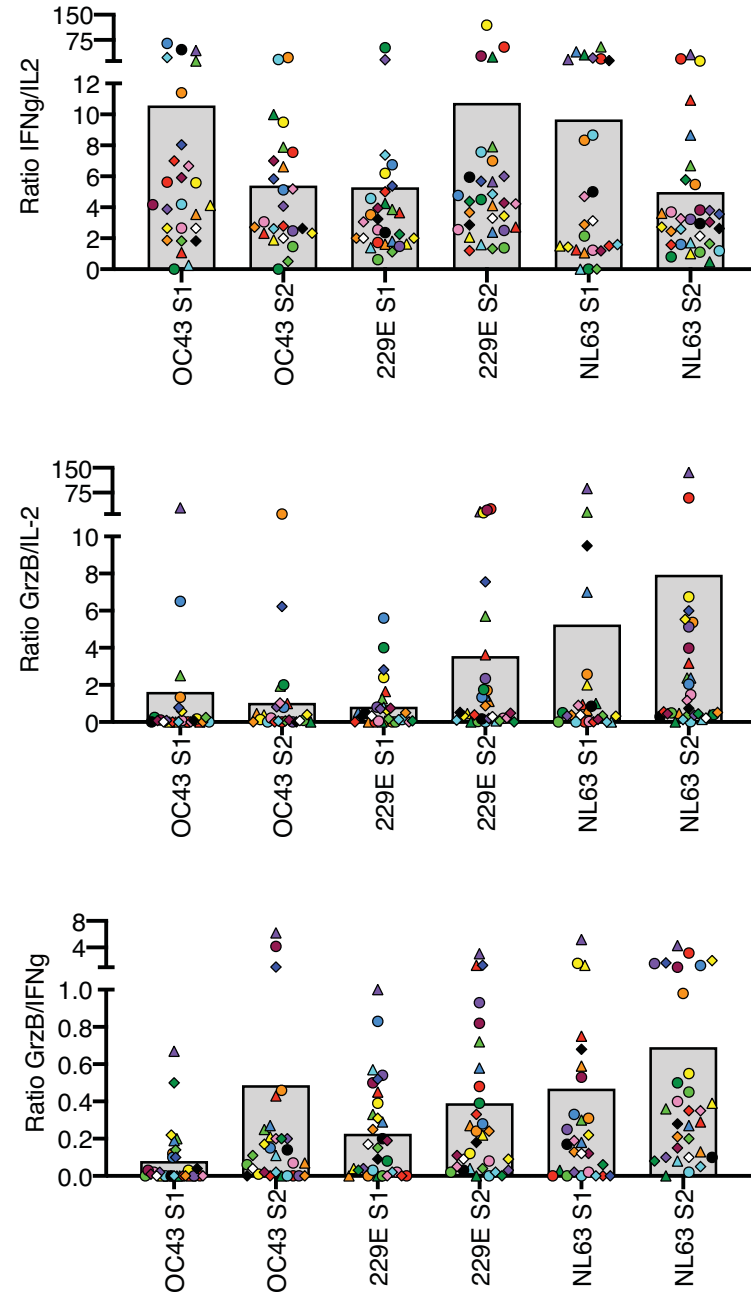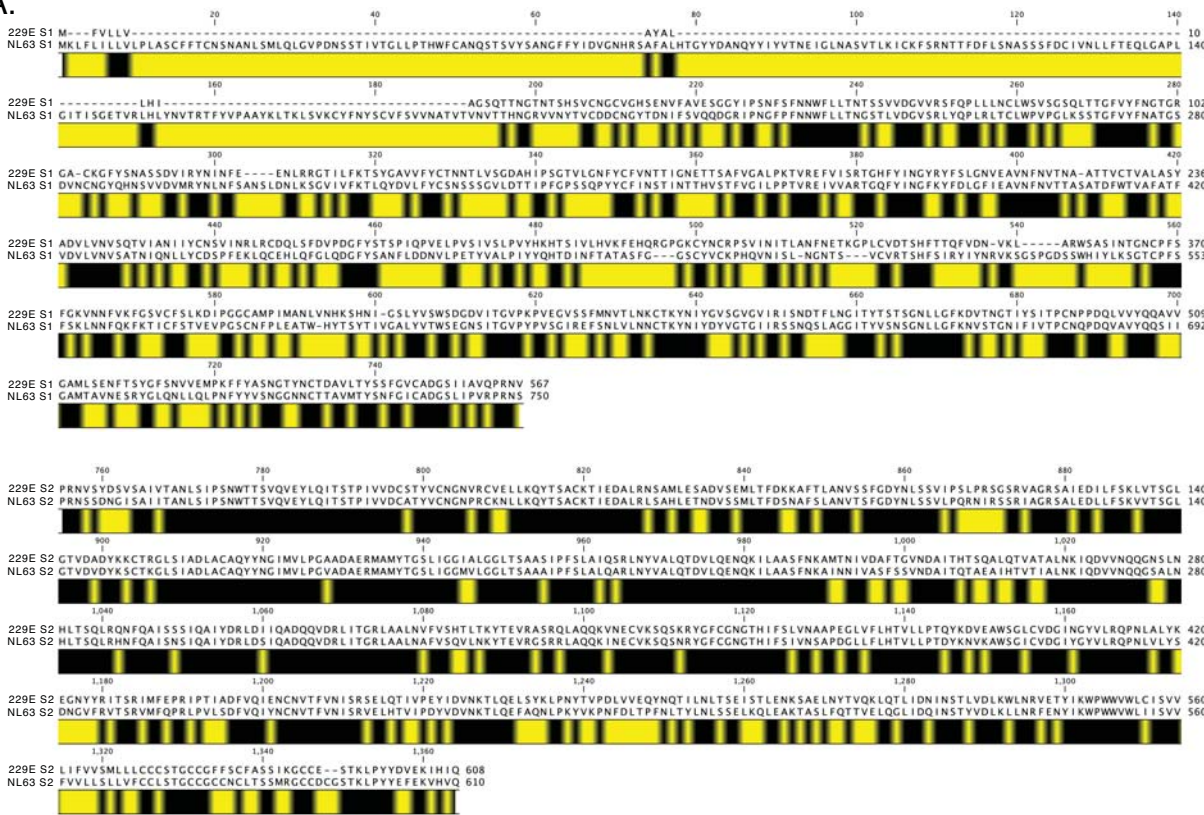
Figure 3.

Figure 4

Figure 5.

A. OC43 — S1: r= -0.4569 p=0.017097*

S2: r= -0.4217 p=0.024649*

B. 229E — S1: r= -0.5272 p=0.016154*

S2: r= -0.4124 p=0.024649*

C. NL63 — S1: r= -0.4749 p=0.016199*

S2: r= -0.4869 p=0.016199*

D. HA-B — r= -0.06521 p=0.7275

Figure 6.