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SARS-CoV-2 mRNA vaccines induce broad CD4+ T cell responses that recognize SARS-CoV-2 variants and HCoV-NL63

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The authors have declared that no conflict of interest exists

Abstract

Recent studies have shown T cell cross-recognition of SARS-CoV-2 and common cold coronavirus spike proteins. However, the effect of SARS-CoV-2 vaccines on T cell responses to common cold coronaviruses remain unknown. In this study, we analyzed CD4+ T cell responses to spike peptides from SARS-CoV-2 and 3 common cold coronaviruses (HCoV-229E, HCoV-NL63, and HCoV-OC43) before and after study participants received Pfizer-BioNTech (BNT162b2) or Moderna (mRNA-1273) mRNA-based COVID-19 vaccines. Vaccine recipients made broad T cell responses to the SARS-CoV-2 spike protein and we identified 23 distinct targeted peptides in 9 participants including one peptide that was targeted by 6 individuals. Only 4 out of these 23 targeted peptides would potentially be affected by mutations in the UK (B.1.1.7) and South African (B.1.351) variants and CD4+ T cells from vaccine recipients recognized the 2 variant spike proteins as effectively as the spike protein from the ancestral virus. Interestingly, we saw a 3-fold increase in the CD4+ T cell responses to HCoV-NL63 spike peptides post-vaccination. Our results suggest that T cell responses elicited or enhanced by SARS-CoV-2 mRNA vaccines may be able to control SARS-CoV-2 variants and lead to cross-protection from some endemic coronaviruses.

Introduction

T cell cross-recognition of SARS-CoV-2 and common cold coronaviruses (CCCs) has recently been demonstrated (1-10). The Pfizer-BioNTech (BNT162b2) and Moderna (mRNA-1273) mRNA COVID-19 vaccines generate robust T cell responses to spike peptides (11, 12) and we hypothesized that this may also translate to enhanced responses to CCCs. There have been multiple evolving spike protein variants described and recent studies have generally shown some degree of reduction in the ability of mRNA vaccine elicited antibodies to neutralize B.1.351 and/or B.1.1.7 variants (13-22). In this study, we analyzed CD4+ T cell responses to CCCs before and after study participants received mRNA vaccines. We identified peptides targeted by CD4+ T cells and determined whether they would be affected by mutations present in B.1.351 and B.1.1.7 variants. Our data further our understanding of the impact of T cell cross-recognition of coronaviruses.

Results and Discussion

The Pfizer-BioNTech (BNT162b2) and Moderna (mRNA-1273) mRNA vaccines elicit strong T cell responses to SARS-CoV-2 (11, 12). Given that recent studies have demonstrated cross-recognition of common cold coronaviruses (CCCs) and SARS-CoV-2 by T cells (1-10), we asked whether COVID-19 vaccines would enhance T cell responses to the common cold coronaviruses. We performed IFN-y ELISpot assays on peripheral blood mononuclear cells (PBMCs) from individuals before and after vaccination to quantify the frequency of virus-specific T cells. As expected, the vaccines elicited strong T cell responses against SARS-CoV-2 with a median of 222 spot forming units (SFU) per million cells responding to SARS-CoV-2 spike peptide pools post-vaccination, compared to less than 3 SFU per million cells in pre-vaccination samples (figure 1a and 1b). Further, the vaccines elicited a significant increase in the response to HCoV-NL63 with an observed increase in the T cell response from a median of 28 SFU per million T cells pre-vaccination, to a median of 93 SFU per million T cells post-vaccination (Figure 1A and 1B).

We have previously shown that CD4+ T cells were responsible for the majority of T cell responses generated by our peptide pools (5). Consistent with our prior study, we found that CD8+ T cell depletion from PBMCs increased responses to all CCCs and SARS-CoV-2, (figure 1C and 1D) suggesting that most of the T cell responses are due to CD4+ T cells. Specifically, we found that responses to HCoV-NL63 were enhanced from 36 SFU pre-vaccination to 113 SFU post vaccination.

Antigenic imprinting occurs when an initial response to a pathogen shapes the immune response to a subsequent infection by a related pathogen. This concept has been shown to play a role in

CD4+ T cell responses to influenza and other pathogens (23). Given the cross-reactive epitopes present in SARS-CoV-2 and CCC spike proteins (3, 6), we asked whether the ability of the vaccines to induce T cells responses to SARS-CoV-2 was affected by pre-existing T cell responses to CCCs. Interestingly, we found no correlation between T cell responses to CCCs prior to vaccination, and T cell responses to SARS-CoV-2 post vaccination (Figure 1 E-G).

We next assessed whether vaccination enhanced T cell responses to HCoV-NL63 by generating antigen specific T cell lines. We expanded antigen-specific T cells pre- and post-vaccination with peptide pools from HCoV-NL63 or SARS-COV-2 spike peptides for 10-12 days and looked at cytokine production following a 12-hour re-stimulation with the same peptide pools. As expected, vaccination dramatically enhanced T cell responses to SARS-CoV-2 spike peptides with a median of 4.2% of T cells co-expressing INF-y and TNF-α post-vaccination, compared to 0.28% of T cells prior to vaccination (Figure 2A and B). Interestingly, vaccination also dramatically enhanced T-cell responses to HCoV-NL63 spike peptides, with a median of 2.7% of T cells co-expressing IFN-y and TNF-α post-vaccination compared to 0.4% pre-vaccination (Figure 2A and B).

13 out of the 15 vaccine recipients studied in Figure 1 had preexisting T cell responses to HCoV-NL63 and we hypothesized that COVID-19 vaccination enhances responses to this virus due to an expansion of T cells that cross-recognize HCoV-NL63 and SARS-CoV-2 spike peptides. To test this hypothesis, we cultured cells with SARS-CoV2 spike peptide pools for 10 to 12 days, and then re-stimulated with peptide pools from a different virus (for example: cells expanded with SARS-CoV-2 spike peptides were then re-stimulated with SARS-CoV-2, HCoV-NL63, or HCoV-229E spike peptides). As shown in Figure 2C, cells expanded with SARS-CoV-2 spike peptides for 10-12 days

followed by a re-stimulation for 12 hours with SARS-CoV-2 spike peptides had a dramatic increase in IFN-γ and TNFα co-expression. Interestingly, these SARS-CoV-2 expanded T cells also responded to re-stimulation by HCoV-NL63 spike peptides suggesting that vaccine induced SARS-CoV-2 specific T cells also recognize HCoV-NL63 spike peptides in this study participant. Overall, in 9 vaccine recipients who were studied pre- and post-vaccination, the percentage of SARS-CoV-2 spike peptide-specific CD4+ T cells that cross-reacted with HCoV-NL63 spike peptides increased from 0.02% pre-vaccination to 0.28% post vaccination (Figure 2D). Further, following expansion with HCoV-NL63 peptides, the percentage of HCoV-NL63 specific CD4+ T cells that cross-reacted with SARS-CoV-2 spike peptides increased from 0.005% in pre-vaccination to 0.37% post-vaccination (Supplementary figure 2)

Further studies are needed to determine why we saw a significant post-vaccine increase in theCD4+ T cell response to HCoV-NL63 but not to HCoV-229E and HCoV-OC43 spike peptides. The percent sequence identity of the CCC spike proteins to SARS-CoV-2 spike protein has been estimated to be around 30% with the beta coronaviruses (HCoV-OC43 and HCoV-HKU1) having slightly higher identity than the alpha coronaviruses (HCoV-NL63 and HCoV-229E)(4). However, a recent study that analyzed antibodies to all 4 CCCs in plasma from convalescent COVID-19 patients revealed an association between HCoV-NL63 antibody responses and the development of highly neutralizing antibodies to SARS-CoV-2 (24) suggesting that HCoV-NL63 may have more epitopes in common with SARS-CoV-2 than the other CCCs.

We next mapped out individual spike peptides targeted by CD4+ T cells. We performed IFN-γ ELISpot assays with CD8 depleted PBMCs using sequential peptide pools consisting of 10 overlapping peptides. As shown in figure in 3A and 3B, CD4+ T cells recognized broad regions

across SARS-CoV-2 spike in vaccine recipients, with pools containing peptides that covered amino acids 141-220, 351-430, 631-710, and 771-850 generating the most robust CD4+ T cell responses. We then mapped specific peptides targeted by 9 vaccine recipients for whom we had enough cells by repeating the ELISpot with individual peptides from 3 of the 18 pools for each vaccine recipient. The optimal epitope and the predicted binding HLA allele was determined as previously described (5, 25). We identified 23 distinct targeted peptides (Table 1, Supplementary Table 1). One of these peptides (SKRSFIEDLLFNKVTLA, 813-829) was targeted by 6 of the 9 participants. This epitope is present in a motif that is conserved in many coronaviruses (26) and the optimal epitope is predicted to bind to conserved HLA-DP alleles (Table 1).

Several spike variants have been described and studies have shown that they are generally neutralized to a lesser extent by antibodies from mRNA vaccine recipients (13-22). However, it is unclear whether these variants also escape from T cells responses. This is a critical question given the key role cellular immunity plays in controlling viral replication (27). While CD4+ T cell epitopes in convalescent COVID-19 patients have been characterized (3, 6, 28), little is known about epitopes targeted by vaccine recipients. In order to predict whether virus-specific T cells would still recognize B.1.1.7 and B.1351 variants, we determined whether mutations present in these variants were located in any of the targeted peptides we identified. Only 3 mutations (Y144 deletion, D614G, P681H) were present in any of the 23 targeted peptides suggesting that these variants would be effectively recognized by the majority of vaccine-generated CD4+ T cells. We tested this hypothesis by comparing CD4+ T cell recognition of the S1 subunit of spike proteins from the ancestral virus and the B.1.1.7 and B.1351 variants. The responses to the spike S1 subunits were lower than the responses we observed to the spike peptide pools. This could be

partially due to the proteins not being efficiently processed into peptides in the ELISpot assay and the fact that S2 subunit epitopes are not present. We found no significant difference in T cell responses to the S1 subunits from the ancestral virus or B.1.1.7 and B.1351 variants (Figure 3D-E). This finding also held true for the 3 participants who targeted peptides that would be affected by the variant mutations.

Overall, our data suggest that the mRNA vaccines may provide protection against not just SARS-CoV-2, but perhaps also some CCCs as well. Our data also suggest that vaccine elicited CD4+ T cells should effectively recognize some of the common SARS-CoV-2 variants and provide protection from severe disease even if neutralizing antibodies are no longer effective.

Methods

Participants. Blood samples were obtained from 30 healthy health care workers and laboratory donors who had not tested positive for COVID-19. 12 participants were female and 18 were male. 11 participants were between 20 and 29, 7 were between 30 and 39, 7 were between 40 and 49, and 5 were between 50 and 59. All participants in Figure 1 had blood drawn 7 to 14 days after the second shot while all participants used for the variant study had blood drawn at 7 to 11 weeks after the second shot. 28 participants received the Pfizer-BioNTech vaccine and 2 received the Moderna vaccine. For all experiments, PBMCs were collected from whole blood after Ficoll-Paque PLUS gradient centrifugation (GE Healthcare Life Sciences). For some experiments, CD8+ T cells were depleted using Miltenyi Biotec CD8+ T Cell Positive Selection Kits. High-resolution class II typing was performed on PBMCs from 6 HDs at the Johns Hopkins Hospital Immunogenetics Laboratory. The Immune Epitope Database and Analysis Resource (http://www.iedb.org) was used for optimal epitope and HLA-binding predictions using recommended parameters (15).

Peptides and ELISpot assays. Peptides for the spike protein of HCoV-NL63, HCoV-229E, HCoV-OC43, and SARS-Cov-2, as well as the nucleocapsid protein of SARS-CoV-2 were obtained from BEI Resources and were reconstituted with DMSO at a concentration of 10 mg/mL. The HCoV-229E S protein peptide pool has 195 peptides consisting of 17 mer with 11 amino acid overlaps. The HCoV-NL63 S protein peptide pool has 226 peptides made up of 14–17 mer with 11–13 amino acid overlaps. The HCoV-OC43 S protein peptide pool has 226 peptides made up of 17 or 18 mer with 11 amino acid overlaps. The SARS-CoV-2 peptides are 12 mer, 13 mer, or 17 mer,

with 10 amino acid overlaps. The spike protein peptide pool was made up of 181 peptides and the nucleocapsid protein peptide pool was made up of 59 peptides. All the peptides were combined into 1 pool for each viral protein. Pools of 10 peptides were made for the SARS-CoV-2 S protein. Stimulation with anti-CD3 antibody (Mabtech, 1 μg/mL) was used as a positive control for each study participant.

IFN-γ ELISpot assays were performed as previously described (5). Briefly ELISpot Pro and ELISpot Plus kits with pre-coated plates were purchased from Mabtech. The wells were plated with unfractionated PBMCs or CD8+ T cell depleted PBMCs at 250,000 cells/well, and the cells were cultured for 20 hours with HCoV peptides at a concentration of 10 μg/mL. The plates were then processed according to the manufacturer's protocol and read by a blinded independent investigator using an automated reading system. Four replicates per pool were run for the comparison of the different viral proteins. The replicate furthest from the median was not used. If 2 values were equally distant from the median, then the higher value was discarded. Two replicates were run for the SARS-CoV-2 S protein pools that examined the breadth of the T cell responses. For epitope mapping, each individual peptide present in a pool was tested in duplicate wells. A peptide was only considered to be positive if both wells had values that were at least twice the average of the untreated wells and the average stimulation index was above 3 and more than 20 SFU/106 cells were present.

SARS-CoV-2 variant assay. The S1 subunit of spike protein from ancestral SARS-CoV-2 (29) and the B.1.1.7 and B.1351 variants with polyhistidine tags at the C-terminus were purchased from Sino Biological and tested in the ELISpot assay at a concentration of 1ug/ml with a 20 hour incubation period. No significant responses were made to these proteins by T cells from 4

unvaccinated healthy donors with no known exposure to COVID-19. The mutations and deletions present in the variant proteins are shown in Figure 3C.

Expansion culture assay. PBMCs (10^7 cells) were cultured in R10 media with 10 U/mL IL-2 and 5 μg/mL peptides for 10–12 days as previously described (5). The media were not changed during this period. The cells were then washed and replated in fresh R10 with 10 U/mL IL-2 and rested 1 day before they were stimulated again with 5 μg/mL peptide with protein transport inhibitors (GolgiPlug, 1 μg/mL; GolgiStop, 0.7 μg/mL) and antibodies against CD28 and CD49d (all from BD Biosciences). After a 12-hour incubation, the cells were washed and stained with annexin V (BV-421, BD Biosciences, 563973) and antibodies against CD3 (APC-Cy-7, BioLegend, 300426) and CD4 (PerCP-CY-5.5, BioLegend, 300530). The cells were then fixed, permeabilized, and stained intracellularly for TNF-α (PE-Cy-7, BD Biosciences, 557647) and IFN-γ (APC, BD Biosciences, 506510)). Flow cytometry was performed on a BD FACS LSR Fortessa flow cytometer, and data were analyzed using FlowJo, version 10. Data on a minimum of 100,000 events in the lymphocyte gate were collected and analyzed.

Statistics. All statistical analyses were performed using GraphPad Prism 8.4.3 (GraphPad Software). For experiments requiring comparisons between 2 groups, a 2-tailed, paired Student's t test was used to determine significance. A P value of less than 0.05 was considered statistically significant. For experiments requiring multiple comparisons, a 1-way ANOVA with Geisser-Greenhouse correction was used. Dunnett's multiple-comparison test was used to determine difference between groups. A P value of less than 0.05 was considered statistically significant.

Study approval. The study was approved by the IRB of Johns Hopkins University. Written informed consent was obtained from all study participants prior to their inclusion in the study.

Author contributions. BAW performed the experiments and wrote the manuscript. CCG performed experiments. JNB supervised experiments and wrote the manuscript.

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Figure 1

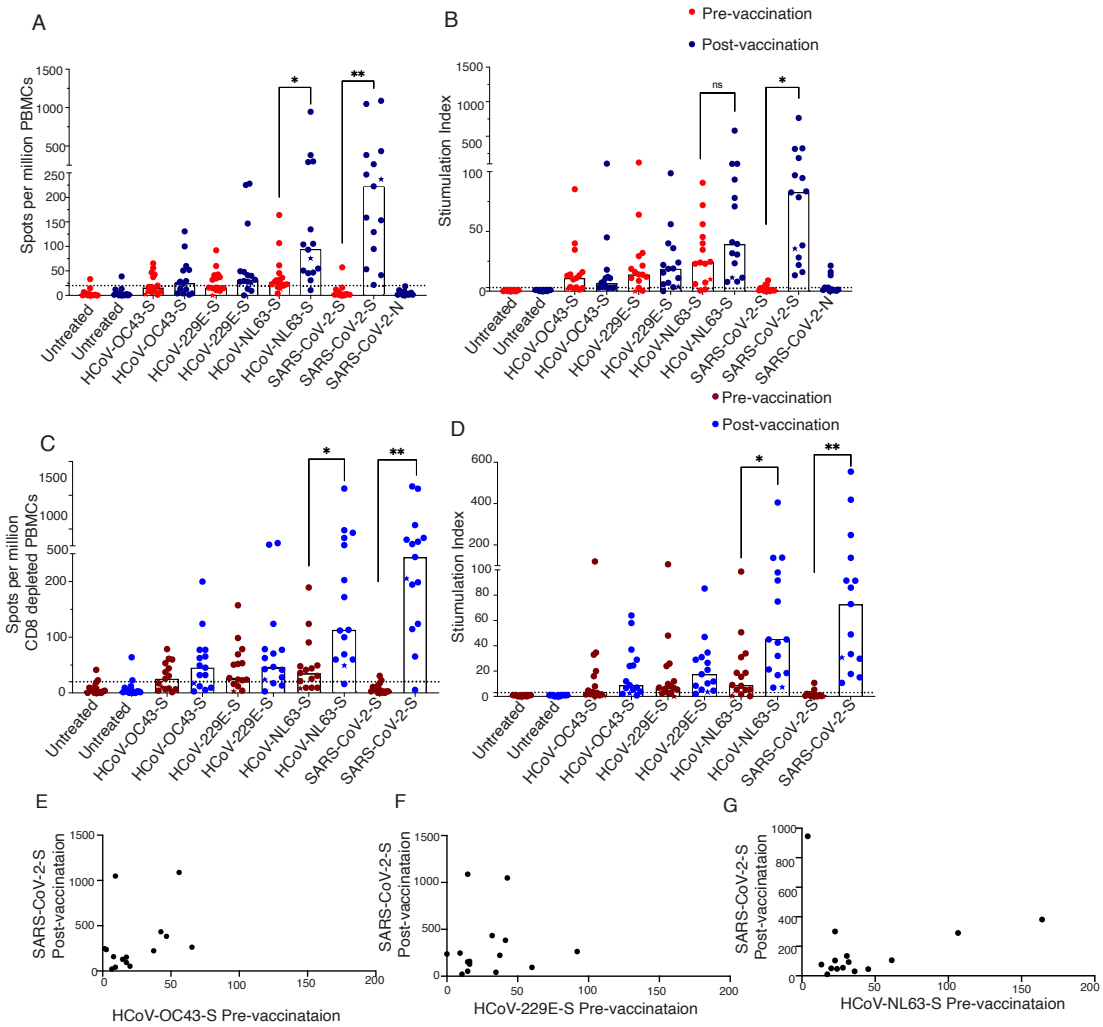

Figure 1: T cell IFN-γ responses to SARS-CoV-2 and CCCs. IFN-γ ELISpot was performed in samples obtained from individuals pre- and post-vaccination. The spot forming units (SFU) and stimulation indices of PBMCs (A, B) and CD8+ T cell depleted PBMCs (C, D) in response to HCoV-NL63, HCoV-229E, HCoV-OC43 and SARS-CoV-2 peptide pools are shown. Each data point represents the mean of 3 replicate values. Horizontal bars represent the median (n=15). The donor who received the Moderna (mRNA-1273) vaccine is indicated with a star symbol. *p = 0.0332 and **P = 0.0021, by 2-tailed, paired Student's t test. (E-G) Correlation between post-vaccination SARS-CoV-2 PBMC ELISpot responses and pre-vaccination responses to HCoV-OC43, HCoV-229E and HCoV-NL63 respectively. Pearson correlation test, r=0.065, 0.36 and 0.12

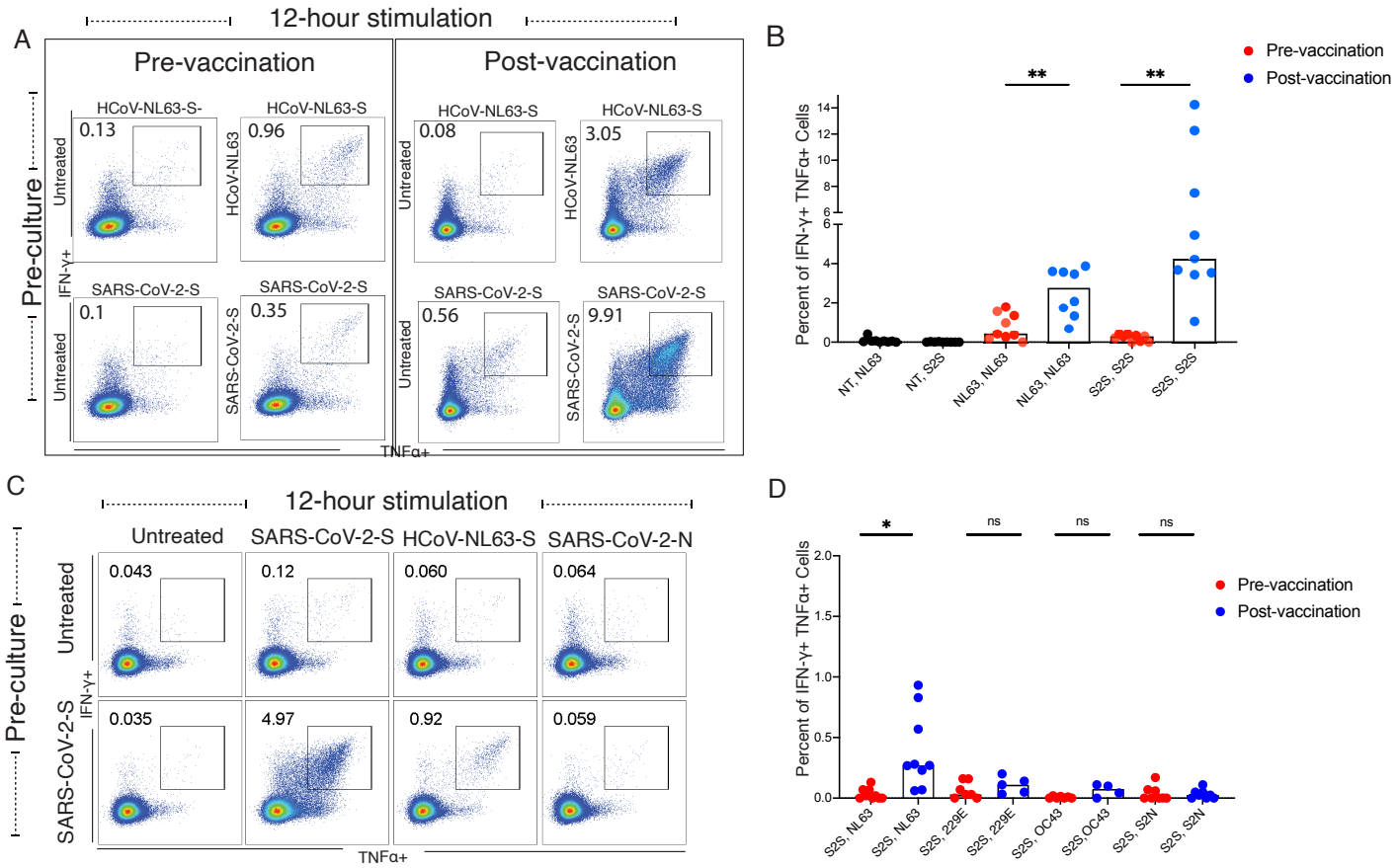

Figure 2: CD4+ T cell responses to SARS-CoV-2 and CCCs. CD4+ T cell responses after cells were cultured for 10-12 days, and then re-stimulated for 12-hours with HCoV-NL63 or SARS-CoV-2 spike peptide pools. TNF-α+ IFN-γ+ CD4+ T cells (shown in gated box) in response CCC or SARS-CoV-2 spike peptides are shown for a representative vaccine recipient (A) and for 9 vaccine recipients (B) pre and post-vaccination. (C-D) CD4+ T cell responses after cells were untreated or cultured for 10- 12 days with HCoV-NL63 or SARS-CoV-2 peptide pools (shown in rows), and then re-stimulated with different peptide pools for 12 hours (shown in columns) to analyze cross-reactive T-cell responses. Responses are shown for a vaccine recipient post-vaccination (C) and for 9 vaccine recipients pre and post-vaccination (D) In panels B and D, the peptide pool used for pre-culturing is shown first, followed by the peptide pool used in the 12-hour stimulation. NT = not treated; NL63 = HCoV-NL63; 229E = HCoV-229E; OC43 = HCoV-OC43; S2N = SARS-CoV-2-N; S2S = SARS-CoV-2-S. *P = 0.0332

Figure 3

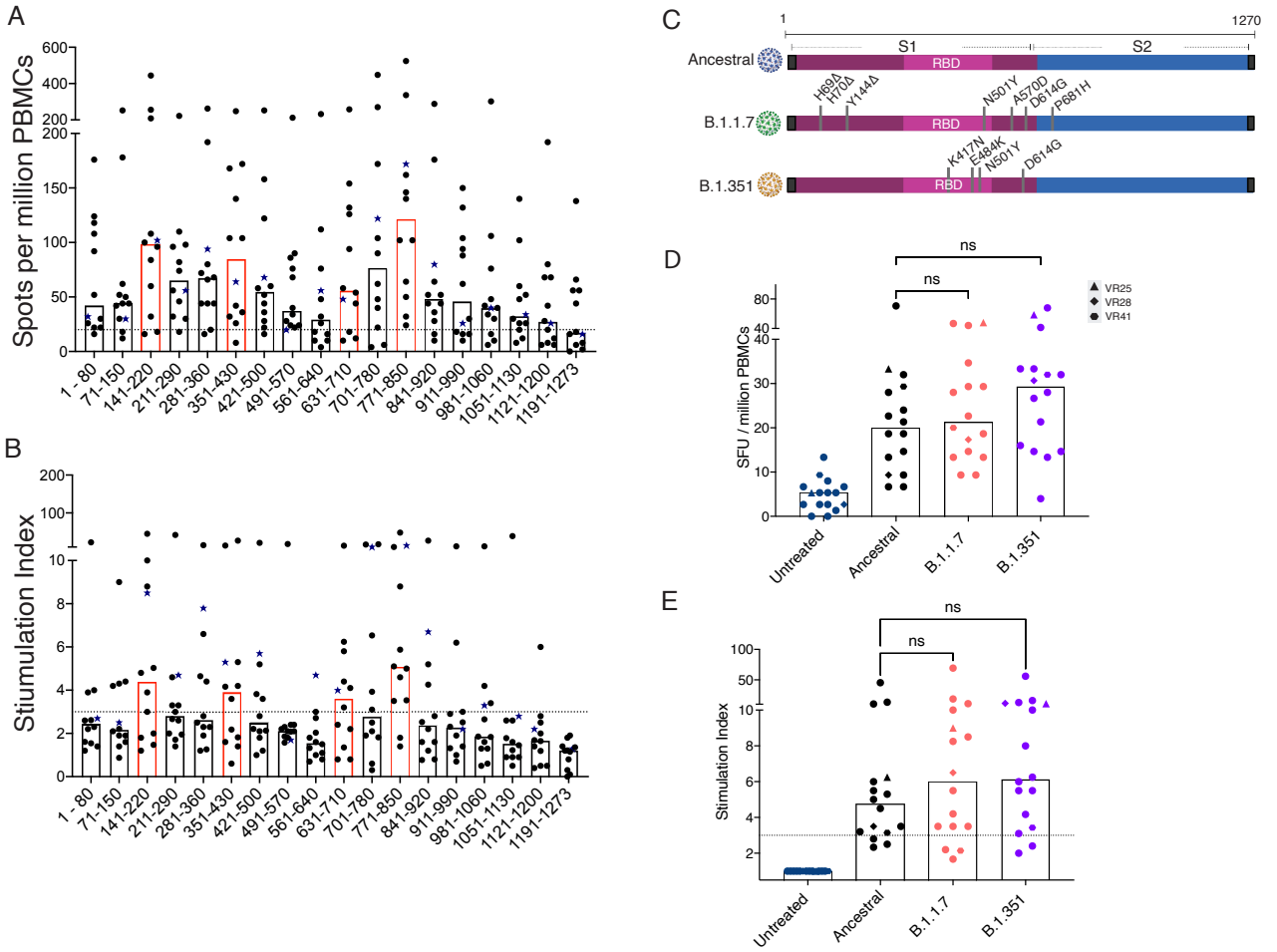

Figure 3: Breadth of T cell responses to SARS-CoV-2 spike protein and responses to spike variants. The numbers of SFU per million CD8+ T cell-depleted PBMCs (A) and stimulation indices (B) generated for pools of 10 peptides are shown for 12 vaccine recipients. The donor who received the Moderna (mRNA-1273) vaccine is indicated with a star symbol. Horizontal bars indicate the median. Pools that elicited the most potent responses are highlighted in red. T cell responses to S1 subunits from ancestral SARS-CoV-2 or B.1.351 and B.1.1.7 variant spike proteins (C) were measured. The numbers of SFU per million CD8+ T cell-depleted PBMCs (D) and stimulation indices (E) generated are shown for 17 vaccine recipients. Horizontal bars represent the median. Statistical comparisons were performed using 1-way ANOVA with Geisser-Greenhouse correction and Dunnett's multiple-comparison test. *P = 0.0332, **P = 0.0021,

Table: Peptides targeted by vaccine recipients' CD4+ T cells

Peptide #	Amino-acid number	Sequence	Responding VR	Predicted restricted HLA allele
2	8-24	LPLVSSQCVNLTTRTQL	VR32	DRB4*01:01
6	36-52	VYYPDKVFRSSVLHSTQ	VR32	DPA1*01:03/DPB1*04:01
13	85-101	PFNDGVYFASTEKSNII	VR28	DRB3*02:02
15	99-115	NIIRGWIFGTTLDSKTQ	VR28	DPA1*01:03/ DPB1*06:01
18	120-136	VNNATNVVIKVCEFQFC	VR28	DRB3*02:02
20	134-150	QFCNDPFLGVYYHKNNK	VR28	DRB1*11:01
21	141-157	LGVYYHKNNKSWMESEF	VR41	DRB3*02:02
24	162-178	SANNCTFEYVSQPFLMD	VR32	DPA1*01:03/DPB1*04:01
28	190-206	REFVFKNIDGYFKIYSK	VR21, VR32	DQA1*01:01/DQB1*05:01 DRB5*01:01
51	351-367	YAWNRKRISNCVADYSV	VR25, VR28	DRB3*02:02, DRB4*01:03
54	372-388	ASFSTFKCYGVSPTKLN	VR20, VR28	HLA-DRB1*15:02 DPA1*01:03/DPB1*06:01
56	386-402	KLNDLCFTNVYADSFVI	VR28	DPA1*02:01/DPB1*14:01
58	400-416	FVIRGDEVRQIAPGQTG	VR28	DQA1*05:05/DQB1*03:01
64	442-458	DSKVGGNYNYLYRLFRK	VR14	DRB1*11:01
88	610-626	VLYQDVNCTEVPVAIHA	VR25	DRB1*13:01
94	652-668	GAEHVNNSYECDIPIGA	VR25	DQA1*02:01/DQB1*02:02
98	680-696	SPRRARSVASQSIIAYT	VR41	DQA1*01:03/DQB1*06:03
103	715-731	PTNFTISVTTEILPVSM	VR32	DRB1*07:01
105	729-745	VSMTKTSVDCTMYICGD	VR20, VR32, VR41	DQA1*02:01/DQB1*02:02 DQA1*02:01/DQB1*03:03
108	750-766	SNLLLQYGSFCTQLNRA	VR21, VR36, VR40	DRB1*15:01
117	813-829	SKRSFIEDLLFNKVTLA	VR14, VR20, VR21, VR25, VR36, VR40	DPA1*01:03/DPB1*04:01 DPA1*02:01/DPB1*01:01 DPA1*02:02/DPB1*02:01
132	918-934	ENQKLIANQFNSAIGKI	VR28	DRB3*02:02
138	960-976	NTLVKQLSSNFGAISSV	VR28	DRB3*02:02

Amino acids in red font are present in B.1.1.7 and/or B.1.351 variants