Longitudinal analysis of the evolution of cellular immunity to SARS-CoV-2 induced by infection and vaccination

There is emerging evidence that T-cell immunity plays an important role in preventing severe coronavirus disease 2019 (COVID-19) infection and disease and that cellular immune deficiencies render individuals at increased risk of disease progression and COVID-19-related death.[1,2] However, longitudinal studies that comprehensively assess the quantity, quality, diversity, and stability of the T-cell immune response induced by the currently approved vaccines or severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in healthy subjects are lacking. Here we provide such an assessment of COVID-19 T-cell responses in 27 healthy subjects with diverse HLA types (Table 1), five of whom were first infected and then vaccinated and 22 who were vaccinated. In these individuals we serially assessed immunity over a 2-year period in order to provide a detailed characterization (through kinetics of emergence and expansion, magnitude, polyclonality, functional capacity, and longevity) of COVID-19 T-cell responses.

To characterize the tempo of emergence, profile (polyclonality and specificity), and stability of induced cellular immunity over time, we investigated the frequency of interferon gamma (IFNγ)-producing T cells in samples spanning 2 years. First, we performed a single round of *in vitro* stimulation in which peripheral blood mononuclear cells (collected with informed consent under a Baylor College of Medicine institutional review board-approved protocol [H-7634]) were exposed to overlapping peptide libraries (pepmixes) spanning four structural proteins (spike [S], nucleocapsid [N], membrane [M], and envelope [E]), and 14 non-structural proteins (NSP) (AP7a, AP7b, AP8, NSP1, 3, 4, 5, 6, 10, 12, 13, 14, 15, and 16) followed by culture in a G-Rex24 well plate in medium supplemented with the cytokines interleukin-4 and interleukin-7 for 10-13 days. The frequency and specificity of reactive cells were quantified by enzyme-linked immunospot analysis, while polyclonality/T-cell receptor (TCR) diversity was assessed by flow cytometric analysis using the IOTest Beta Mark kit.

Five of the 27 study participants became infected with SARS-CoV-2 (but did not require hospitalization) and were subsequently vaccinated (SARS-CoV-2-infected cohort). At baseline these subjects exhibited minimal anti-SARS-CoV-2 T-cell activity. However, upon infection all mounted potent and robust immune responses to a range of structural and non-structural antigens (Figure 1A). To identify which antigens were immunodominant we examined T-cell reactivity against these antigens individually. All five subjects recognized S, as determined by spot-forming cells (SFC) (median: 3,892; range, 2,917-7,353 SFC/2×10^5 peripheral blood mononuclear cells; peak detection, 3-5 months post-infection), M (median: 1,966; range, 547-11,261), and N (median: 1,994; range, 1,712-6,457), while NSP4 and AP7a reactivity was detected in three and two subjects, respectively. Activity against the other antigens was minimal and varied from subject to subject. The data are summarized in Figure 1B and detailed for each subject and timepoint assessed in *Online Supplementary Table S1*.

We next sought to understand the impact of the spike-targeted vaccines on both spike and non-spike-specific T cells in infected subjects with SARS-CoV-2 memory T-cell responses. To do this we analyzed the frequency of reactive T cells over time. Within 3-6 months of infection, all five subjects had been vaccinated with a primary vaccine series (n=2 Pfizer; n=2 Moderna; n=1 J&J), which resulted in a 1.4-fold increase in spike-responsive T cells (from a peak of 4,909 SFC/2×10^5 peripheral blood mononuclear cells post-infection to a peak of 6,706 SFC/2×10^5 post-vaccination). In contrast, the vaccine had minimal impact on T cells reactive against non-spike SARS-CoV-2 antigens (“bystander” T cells) (Figure 1C). Administration of a booster dose (n=4 Pfizer; n=1 Moderna) resulted in the same pattern of activity with an expansion and subsequent contraction and stabilization of spike-reactive T cells, and minimal impact on bystander T cells. Finally, to assess the stability of the memory T-cell response we examined the frequency of reactive cells in a longitudinal manner. For immunity that was induced by the virus and not boosted thereafter (i.e., bystander cells), T-cell reactivity peaked 3-5 months after the initial infection, then contracted and plateaued approximately 4 months later. Thereafter T-cell levels remained relatively stable for the duration of the study. In contrast, spike-specific T cells induced by the virus were amplified by the primary and booster vaccine series. Hence, proportionally, spike-directed T cells induced by viral infection initially accounted for approximately one third of the total anti-SARS-CoV-2 immune response, but after administration of primary and booster vaccines they accounted for up to 65% of the total anti-SARS-CoV-2 response (Figure 1D).

We next examined T-cell immunity in the 22 infection-naïve individuals whose first immune exposure to SARS-CoV-2 was via vaccination (vaccine-only cohort; n=19 Pfizer; n=3 Moderna). The magnitude, specificity, impact of vaccine (primary and booster), and stability of response

Table 1. Donors' demographics.

Infected donors									
Donor ID	**Age, years**	**Gender**	**Race**	**HLA-A**	**HLA-B**	**HLA-C**	**HLA-DR**	**HLA-DQ**	**Vaccine/Booster**
D#1	58	F	White	02,11	18,44	05,07	04,04	03,03	Pfizer/Pfizer
D#2	34	F	Asian	24,68	15,35	nd	11,15	03,05	Moderna/Moderna
D#3	28	M	Hispanic	02,11	15,40	01,02	08,09	03,04	Moderna/Pfizer
D#4	34	M	Asian	24,24	07,13	04,07	07,12	02,03	J &J/Pfizer
D#5	43	F	African American	02,36	35,58	03,04	11,13	03,06	Pfizer/Pfizer
Vaccinated donors									
Donor ID	**Age, years**	**Gender**	**Race**	**HLA-A**	**HLA-B**	**HLA-C**	**HLA-DR**	**HLA-DQ**	**Vaccine/Booster**
D#6	65	F	White	02,03	13,35	04,04	01,01	01,01	Pfizer/Pfizer
D#7	33	F	White	02,02	44,44	05,16	01,04	05,03	Pfizer/Pfizer
D#8	35	M	White	26,32	38,44	05,12	01,04	03,03	Pfizer/Pfizer
D#9	66	F	White	02,11	07,08	07,07	03,15	02,06	Pfizer/Pfizer
D#10	39	F	Hispanic	02,33	14,15	nd	11, 11	03,03	Pfizer/Pfizer
D#11	50	M	White	30,33	14,41	nd	03,13	02,06	Pfizer/Pfizer
D#12	62	F	Asian	02,02	35,52	nd	15,15	06,06	Pfizer/Pfizer
D#13	48	F	White	25,32	15,53	nd	11,13	03,05	Pfizer/Pfizer
D#14	54	F	White	02,02	27,44	nd	11,16	03,05	Moderna/Moderna
D#15	38	M	Asian	02,24	13,35	03,03	12,15	03,06	Pfizer/Pfizer
D#16	34	F	Asian	02,33	13,51	04,07	04,15	03,06	Pfizer/Pfizer
D#17	46	F	White	01,24	08,18	07,07	01,03	02,05	Pfizer/Pfizer
D#18	26	F	Asian	32,33	15,58	03,07	03,16	02,05	Pfizer/Pfizer
D#19	37	F	Asian	24,31	07,13	03,07	15,15	06,06	Pfizer/Pfizer
D#20	29	F	Asian	24,33	44,54	01,14	04,13	04,06	Pfizer/Pfizer
D#21	53	F	White	03,24	08,35	nd	03,04	02,03	Pfizer/Pfizer
D#22	37	F	Asian	02,33	46,58	03,08	03,09	02,03	Pfizer/Pfizer
D#23	30	M	Hispanic	02,68	15,35	01,04	09,15	03,06	Pfizer/Pfizer
D#24	28	M	White	03,23	07,49	07,07	11,15	03,06	Pfizer/Pfizer
D#25	42	F	White	02,02	07,15	01,07	09,11	03,03	Moderna/Moderna
D#26	32	M	Hispanic	02,24	35,39	04,07	09,11	03,03	Moderna/Moderna
D#27	37	M	Asian	11,24	08,52	02,12	03,03	02,02	Pfizer/Pfizer

F: female; M: male; nd: not done.

over time are summarized in Figure 1E-H while *Online Supplementary Table S1* includes detailed results for each subject and time-point. Prior to vaccine administration these healthy subjects had minimal anti-SARS-CoV-2 T-cell activity. However, within 2 months of primary vaccination all 22 patients mounted a potent and specific response to the spike protein (median: 7,051; range, 721-13,334 SFC/2×10^5 peripheral blood mononuclear cells), with minimal to no evidence of response to any of the other structural/non-structural proteins. After the primary vaccine series, there was a contraction and subsequent stabilization of spike-reactive T cells, which increased with booster vaccination. In the vaccine-only cohort, age had no impact on magnitude or duration of response to vaccine (6 patients >50 years, 16 patients <50 years) (*Online Supplementary Figure S1*).

All participants were monitored for SARS-CoV-2 infection for the duration of the study. Notably, one out of 22 of our initially infection-naïve subjects experienced an infection after administration of the booster dose of vaccine, resulting in the amplification of memory spike-specific T cells as well as the induction of *de novo* T-cell responses against other immunogenic structural and non-structural proteins (*Online Supplementary Figure S2*). Hence, exposure to the virus in this subject induced a broad and polyclonal response against multiple SARS-CoV-2 antigens post-vaccination.

To investigate the magnitude and breadth of T-cell activity induced by the spike vaccine in infection-naïve subjects (n=22) and those with pre-existing immunity prior to vaccination (n=5), we compared spike T-cell responses between the two cohorts. As shown in Figure 2A, the peak magnitude of the anti-spike T-cell immune response was similar in the two cohorts and stabilized at similar levels post-infection/vaccination. Furthermore, when we compared the TCR diversity by isolating spike-directed IFNγ-

producing T cells (IFNγ secretion assay-detection kit, Miltenyi Biotec) and examining the TCRvβ repertoire we saw no difference in the breadth of T-cell activity (Figure 2B). Considering all the data, there was no quantitative or qualitative difference between the spike-directed T-cell immune response induced by vaccine or SARS-CoV-2 virus.

This longitudinal T-cell study revealed that the kinetics of antiviral immunity induced by the anti-SARS-CoV-2 vaccine and the virus itself were similar. As such, the initial challenge induced a robust expansion in antigen-specific T cells, followed by contraction and then stabilization for ≥1 year of follow-up, which is consistent with a typical T-cell response after the effector phase.[3,4] This is in contrast

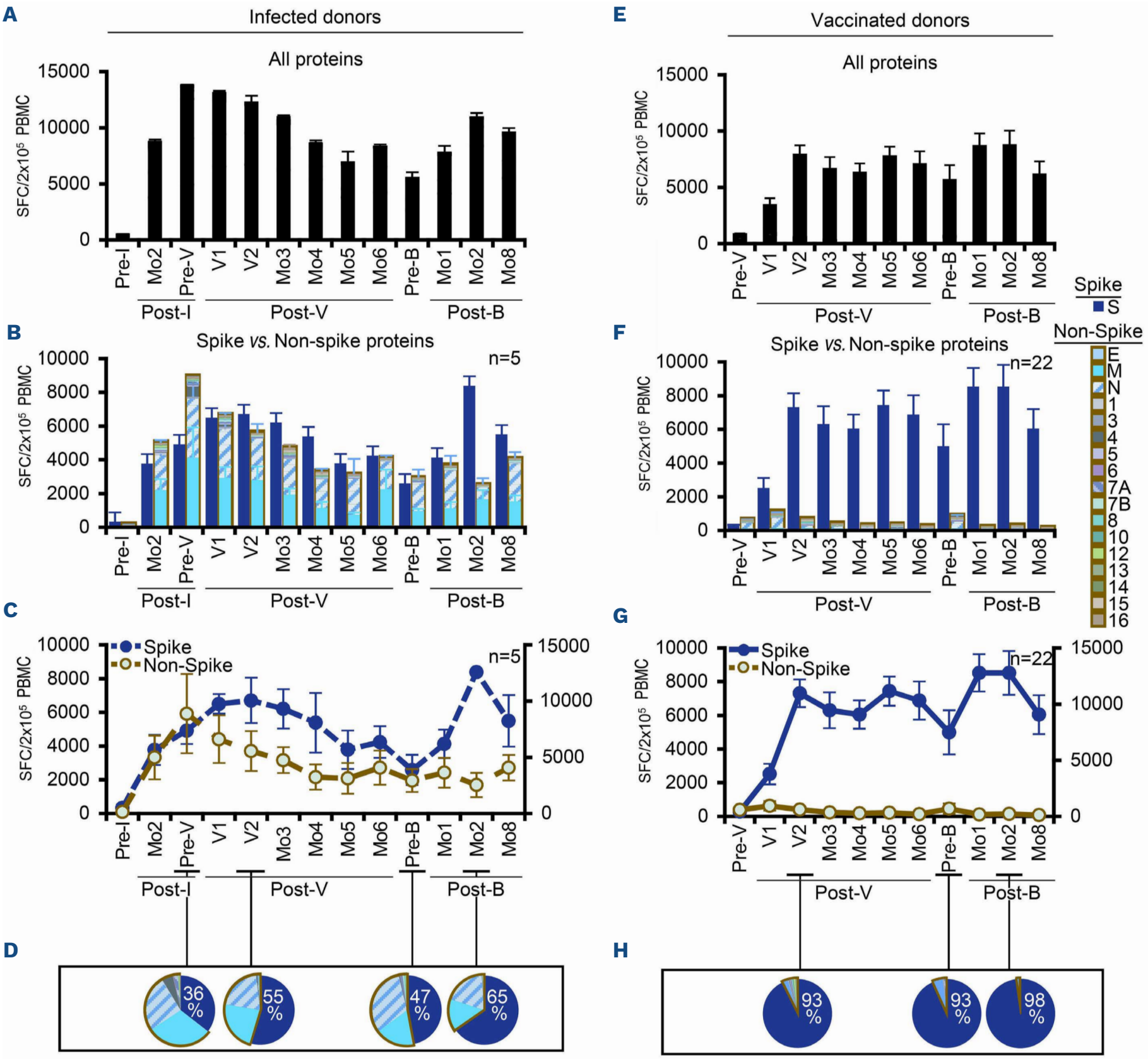

Figure 1. Immunogenicity of SARS-CoV-2-derived antigens and longitudinal assessment of T-cell immunity. Reactivity against 18 SARS-CoV-2-derived antigens pooled (A, E) and individually (B, F) tested in *ex vivo*-expanded SARS-CoV-2-specific T cells in 27 healthy subjects as measured by enzyme-linked immunospot assay. Data are shown as spot-forming cells ± standard error of mean. The frequency of spike- and non-spike-reactive T cells is plotted longitudinally in infected+vaccinated (C) and vaccinated subjects (G). Data are shown as spot-forming cells ± standard error of mean; spike immunity is shown as a blue line and non-spike shown in gold. Proportion of anti-SARS-CoV-2 T cells reactive against spike and non-spike proteins in infected+vaccinated subjects (D) *versus* vaccinated subjects (H). SFC: spot-forming cells; PBMC: peripheral blood mononuclear cells; Pre-I: pre-infection; Mo: month; Post-I: post-infection; Pre-V: pre-vaccination; Post-V: post-vaccination; V1: vaccine dose 1; V2: vaccine dose 2; Pre-B: pre-booster; post-B: post-booster.

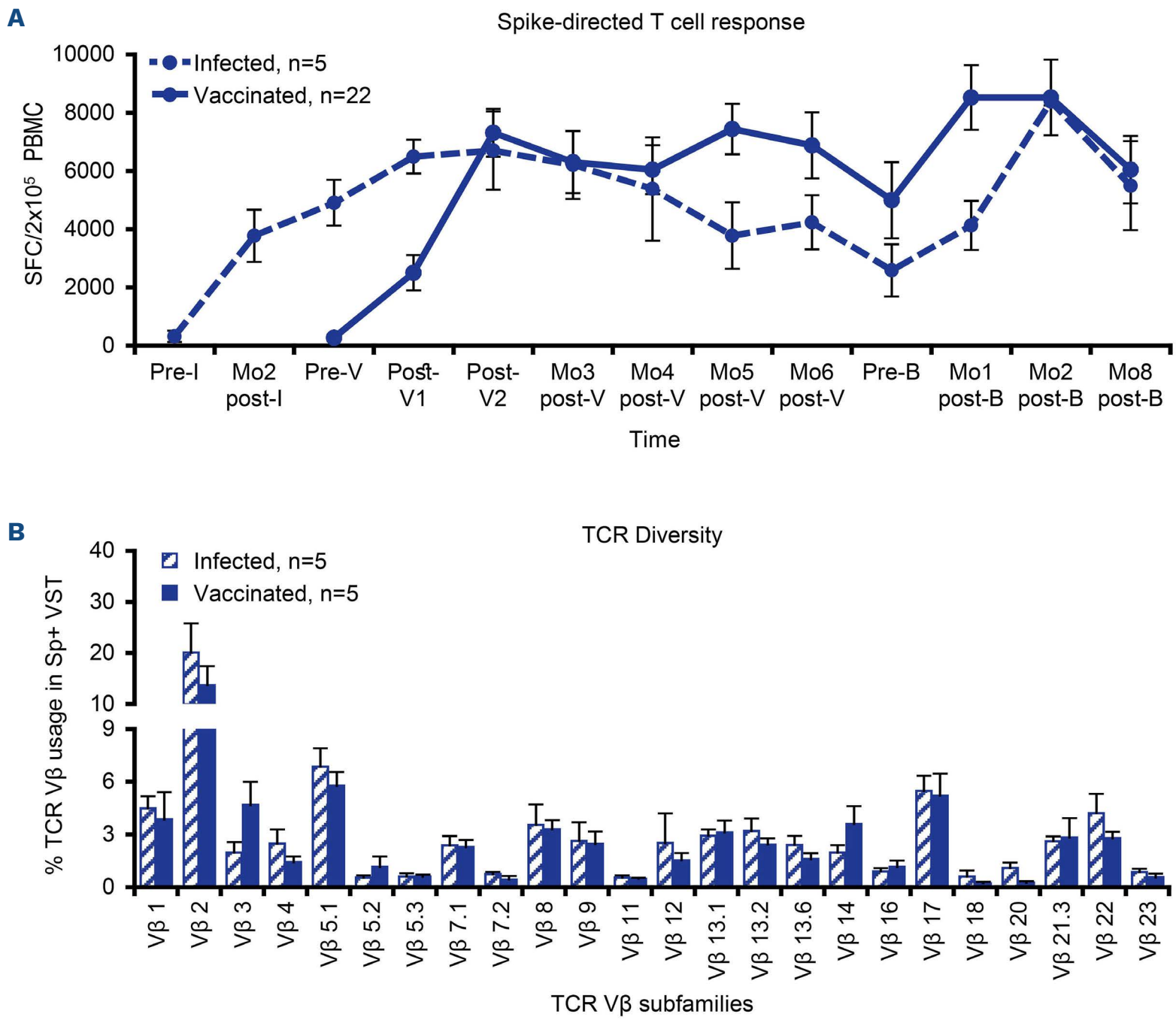

Figure 2. Spike-specific T-cell immunity in infected *versus* vaccinated individuals. (A) Levels of spike-reactive T cells in infected and vaccinated subjects as assessed by enzyme-linked immunospot assay in serial samples. Results are presented as spot-forming cells ± standard error of mean. (B) T-cell receptor vβ repertoire of spike-specific T cells present in infected and vaccinated individuals. SFC: spot-forming cells; PBMC: peripheral blood mononuclear cells; Pre-I: pre-infection; Mo: month; Post-I: post-infection; Pre-V: pre-vaccination; Post-V: post-vaccination; V1: vaccine dose 1; V2: vaccine dose 2; Pre-B: pre-booster; post-B: post-booster; TCR: T-cell receptor; Sp+: spike positive; VST: virus-specific T cells.

to neutralizing antibody levels (induced by either the vaccine or virus) that are associated with protective immunity from re-infection, which decay over time in the majority of individuals.[5] Indeed, in a longitudinal analysis performed by Chen and colleagues[6] in 92 subjects after symptomatic COVID-19, virus-specific IgG levels decayed substantially in the majority of individuals over 100 days. Similarly, Goel and colleagues[7] reported that 61 vaccine recipients had peak antibody levels 1 week after the second vaccine dose and a subsequent decline thereafter with a half-life of ~30 days.

Memory T-cell responses have been shown to be less affected by SARS-CoV-2 viral variants than humoral immunity.[8-10] This is likely due to the diverse repertoire of T cells induced by vaccine/viral challenge, which are polyclonal and recognize multiple epitopes within immunogenic antigens. This vast repertoire of activity enables T cells to react to clinically important viral variants. Given the robust, potent, and stable T-cell activity that is induced upon exposure to the virus and vaccine, as well as the growing evidence of broad T-cell-mediated variant coverage, there are opportunities to exploit this knowledge to guide clinical management. For example, serial monitoring of specific T-cell immunity (in parallel with antibody titers) might serve as a tool to guide the tempo of administration of booster vaccines, particularly in high-risk immune suppressed individuals. Furthermore, a number of groups, including ours, have considered harnessing virus-specific T cells as a COVID-19 therapeutic.[11-14] Indeed, our group prepared and cryopreserved banks of virus-specific T cells, which were generated by stimulating peripheral blood mononuclear cells from convalescent healthy donors with pepmixes (overlapping peptide libraries) spanning structural and non-structural immunodominant antigens (based on the

parental strain sequence). These were administered as a partially HLA-matched product to hospitalized COVID-19 patients and the outcomes are reported in Vasileiou *et al.*[15] We also provided emergency access to a number of investigators including Martits-Chalangari and colleagues,[16] who used these cells to successfully treat recalcitrant COVID-19 (delta strain) in a heart transplant recipient. These proof-of-concept studies provide further evidence of the importance of T cells in mediating protective antiviral effects and suggest the feasibility of adoptive T-cell therapy for the treatment of COVID-19 in high-risk patients.

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SV, MK and YV are consultants to AlloVir. CMR has stock and other ownership interests with Coya, Bluebird Bio, Tessa Therapeutics, Marker Therapeutics, AlloVir, Walking Fish, Allogene Therapeutics, Memgen, Kuur Therapeutics, Bellicum Pharmaceuticals, TScan Therapeutics, and Abintus Bio; has a consulting or advisory role with Abintus Bio, Adaptimmune, Brooklyn Immunotherapeutic, Onk Therapeutics, Tessa Therapeutics, Memgen, Torque, Walking Fish Therapeutics, TScan Therapeutics, Marker Therapeutics, and Turnstone Bio; and receives research funding from Kuur Therapeutics. SG is an employee of AlloVir. AML is a co-founder and equity holder of AlloVir and Marker Therapeutics and a consultant to AlloVir.

Contributions

SV, MK, YV, AW, MN, AGW, MFK and ATC performed research; SV, MK, MN and AGW analyzed data; MK and YV organized the study; CMR and AML supervised the study; SV, SG, CMR and AML wrote the manuscript.

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Data-sharing statement

Datasets are maintained in an electronic database at the Center for Cell and Gene Therapy; data are available from the corresponding author upon reasonable request.

References

1. Fung M, Babik JM. COVID-19 in immunocompromised hosts: what we know so far. Clin Infect Dis. 2021;72(2):340-350.
2. Garcia-Vidal C, Puerta-Alcalde P, Mateu A, et al. Prolonged viral replication in patients with hematologic malignancies hospitalized with COVID-19. Haematologica. 2022;107(7):1731-1735.
3. Dan JM, Mateus J, Kato Y, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. Science. 2021;371(6529):eabf4063.
4. Moss P. The T cell immune response against SARS-CoV-2. Nat Immunol. 2022;23(2):186-193.
5. Qi H, Liu B, Wang X, Zhang L. The humoral response and antibodies against SARS-CoV-2 infection. Nat Immunol. 2022;23(7):1008-1020.
6. Chen Y, Zuiani A, Fischinger S, et al. Quick COVID-19 healers sustain anti-SARS-CoV-2 antibody production. Cell. 2020;183(6):1496-1507.
7. Goel RR, Painter MM, Apostolidis SA, et al. mRNA vaccines induce durable immune memory to SARS-CoV-2 and variants of concern. Science. 2021;374(6572):abm0829.
8. Geers D, Shamier MC, Bogers S, et al. SARS-CoV-2 variants of concern partially escape humoral but not T-cell responses in COVID-19 convalescent donors and vaccinees. Sci Immunol. 2021;6(59):eabj1750.
9. Keeton R, Tincho MB, Ngomti A, et al. T cell responses to SARS-CoV-2 spike cross-recognize Omicron. Nature. 2022;603(7901):488-492.
10. Tarke A, Sidney J, Methot N, et al. Impact of SARS-CoV-2 variants on the total CD4(+) and CD8(+) T cell reactivity in infected or vaccinated individuals. Cell Rep Med. 2021;2(7):100355.
11. Cooper RS, Fraser AR, Smith L, et al. Rapid GMP-compliant expansion of SARS-CoV-2-specific T cells from convalescent donors for use as an allogeneic cell therapy for COVID-19. Front Immunol. 2021;11:598402.
12. Garcia-Garcia I, Guerra-Garcia P, Ferreras C, et al. A phase I/II dose-escalation multi-center study to evaluate the safety of infusion of natural killer cells or memory T cells as adoptive therapy in coronavirus pneumonia and/or lymphopenia: RELEASE

study protocol. Trials. 2021;22(1):674.
13. Keller MD, Harris KM, Jensen-Wachspress MA, et al. SARS-CoV-2-specific T cells are rapidly expanded for therapeutic use and target conserved regions of the membrane protein. Blood. 2020;136(25):2905-2917.
14. Peter L, Wendering DJ, Schlickeiser S, et al. Tacrolimus-resistant SARS-CoV-2-specific T cell products to prevent and treat severe COVID-19 in immunosuppressed patients. Mol Ther Methods Clin Dev. 2022;25:52-73.
15. Vasileiou S, Hill L, Kuvalekar M, et al. Allogeneic, off-the-shelf, SARS-CoV-2-specific T cells (ALVR109) for the treatment of COVID-19 in high-risk patients. Haematologica 2023: doi: 10.3324/haematol.2022.281946 [Epub ahead of print].
16. Martits-Chalangari K, Spak CW, Askar M, et al. ALVR109, an off-the-shelf partially HLA matched SARS-CoV-2-specific T cell therapy, to treat refractory severe COVID-19 pneumonia in a heart transplant patient: case report. Am J Transplant. 2022;22(4):1261-1265.