TCR sequencing of epitope-specific T cells from SARS-CoV-2 convalescent individuals

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BACKGROUND

T cells have been shown to play a role in the immune response to SARS-CoV-2. Identification of T cell epitopes and a characterization of the size and diversity of the T cell repertoire can provide important insights into how T cells impact antiviral immunity. In addition, these sorts of analyses can help to better understand the factors that drive T cell recognition of individual epitopes. Here, we identified T cell epitopes within the Spike (S), Nucleocapsid (N) and Membrane (M) proteins from SARS-CoV-2 convalescent individuals and performed TCR sequencing on epitope-specific T cells.

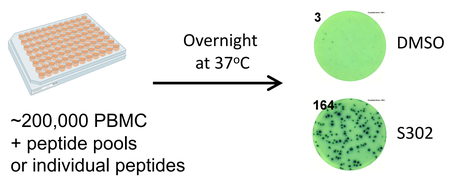
METHODS

VRC 200 Cohort

- Convalescent individuals (n=19)
- Mild or moderate disease
- Between 28-236 days post-symptom onset

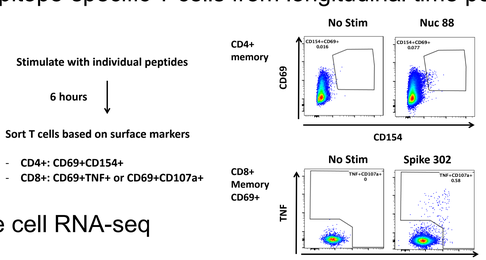
Experimental Outline

1. Determine T cell epitopes by IFNγ ELISpot



2. Identify core epitopes by ICS with truncated peptides

3. Sort epitope-specific T cells from longitudinal time points



4. Single cell RNA-seq

5. Epitope-specific TCR analysis

RESULTS

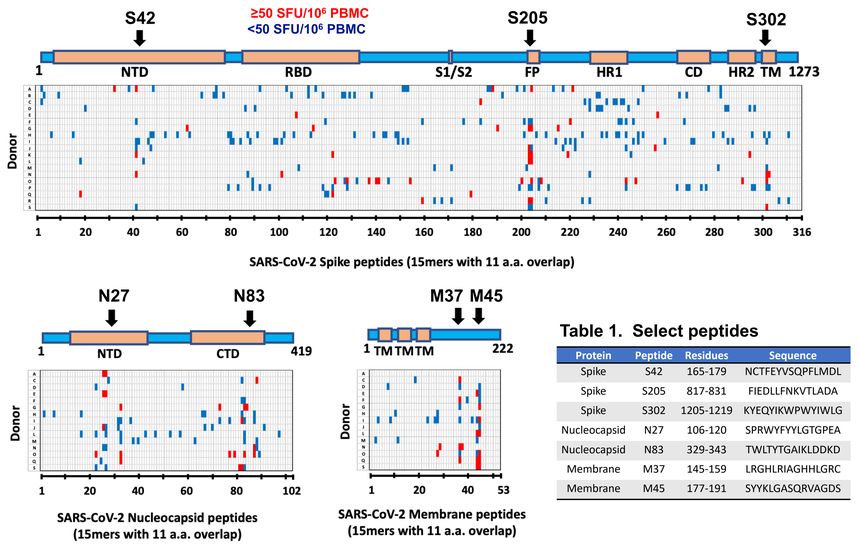


Table 1. Select peptides

Protein	Peptide	Residues	Sequence
Spike	S42	165-179	NCTFEYVSQPFLMDL
Spike	S205	817-831	FIEDLLFNKVTLADA
Spike	S302	1205-1219	KYEQYIKWPWYIWLG
Nucleocapsid	N27	106-120	SPRWYFYYLGTGPEA
Nucleocapsid	N83	329-343	TWLTYTGAIKLDDKD
Membrane	M37	145-159	LRGHLRIAGHHLGRC
Membrane	M45	177-191	SYYKLGASQRVAGDS

FIGURE 1: T cell epitopes identified within the Spike, Nucleocapsid and Membrane proteins. T cell epitopes were determined by IFNγ ELISpot using a matrix approach, where cells were first incubated with peptide pools and positive responses were then confirmed with individual peptides. Positive responses are indicated with a shaded box, with each individual donor represented by a row and individual peptides represented by columns. Responses with ≥50 SFU/10^6 PBMC are shown in red and responses with <50 SFU/10^6 PBMC are shown in blue. Arrows indicate where select dominant T cell epitopes are located within each protein. Select peptides are shown in Table 1.

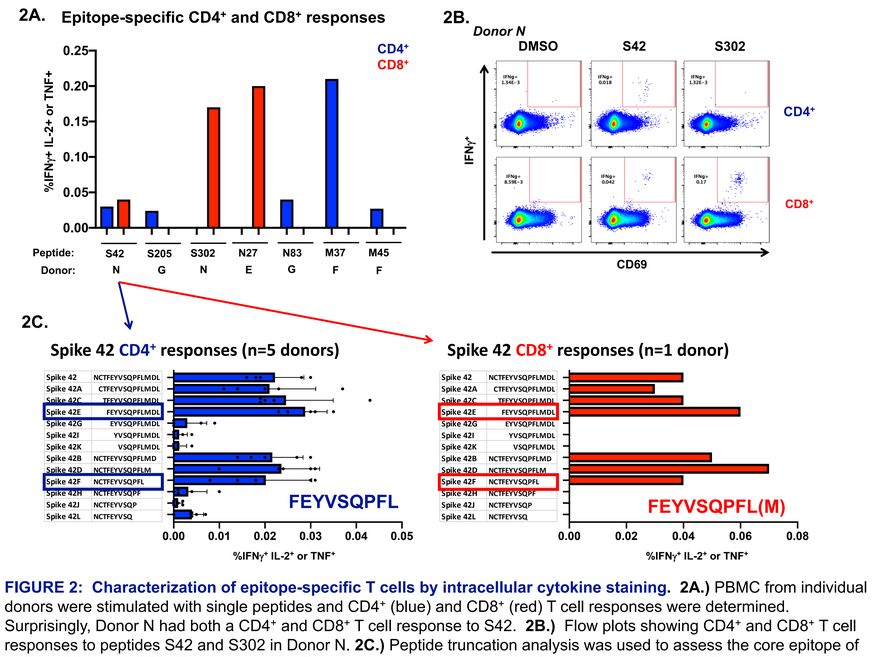


FIGURE 2: Characterization of epitope-specific T cells by intracellular cytokine staining. 2A.) PBMC from individual donors were stimulated with single peptides and CD4+ (blue) and CD8+ (red) T cell responses were determined. Surprisingly, Donor N had both a CD4+ and CD8+ T cell response to S42. **2B.)** Flow plots showing CD4+ and CD8+ T cell responses to peptides S42 and S302 in Donor N. **2C.)** Peptide truncation analysis was used to assess the core epitope of S42 for CD4+ and CD8+ T cells. Of of the 5 donors tested, all donors had CD4+ responses with only Donor N having both a CD4+ and CD8+ response. The core epitope for S42 was determined to be FEYVSQPFL for both CD4+ and CD8+ T cells. Further studies are underway to examine the potential impact of flanking residues on CD4+ versus CD8+ responses.

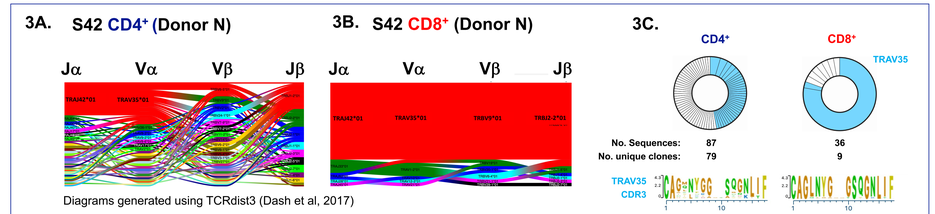
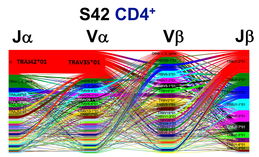
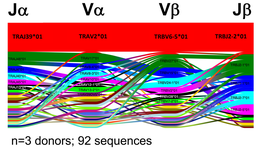


FIGURE 3. Comparison of TCR sequences for CD4+ and CD8+ S42-specific T cells in Donor N. 3A & 3B.) Diagrams show the TCR gene usage of S42-specific T cells for CD4+ and CD8+ responses. CD4+ responses heavily utilize the TRAV35 and TRAJ42 genes, with more diverse gene usage for TCRb. S42-specific CD8+ T cells were more clonal compared to the CD4+ T cells and TRAV35 and TRAJ42 were heavily utilized for TCRa, where TRBV9 and TRBJ2-2 were utilized for TCRb. **3C.)** Pie charts represent the total number of sequences analyzed with each slice representing a unique clone (paired TCRa and TCRb sequences). TRAV35-expressing clones are highlighted in blue with sequence logos of the CDR3 regions shown underneath.



Select S42-specific TCRs

Donor	# Cells	TRAJ	TRAV	CDR3α	TRBV	TRBJ	CDR3β
F	2/80	42	35	CAGQNYGGSQGNLIF	12-3	1-2	CASSPRTGSLYGYTF
N	3/87	42	35	CAGMNYGGSQGNLIF	6-5	2-1	CASRNGLYAFF
J	2/53	42	35	CAGQNYGGSQGNLIF	6-5	2-2	CASSYSRSGTTPNTGELFF
S	2/88	42	35	CAGQNYGGSQGNLIF	7-2	2-2	CASSPGRAGANTGELFF
S	1/88	42	35	CAGMNYGGSQGNLIF	9	1-2	CASSLTGGSGYTF
N	4/87	42	35	CAGMNYGGSQGNLIF	24-1	1-2	CATSPRGAQGYTF



Select N27-specific TCRs

Donor	# Cells	TRAJ	TRAV	CDR3α	TRBV	TRBJ	CDR3β
E	25/61	39	2	CAVEGPNAGNMLTF	6-5	2-2	CASSYRPYTGELFF
E	6/61	48	17	CATERDFGNEKLTF	15	1-1	CATSSAGGEVAFF
A	4/21	48	17	CATESNFGNEKLTF	15	2-5	CATSIIAGETQYF

FIGURE 4: TCR gene usage of S42- and N27-specific T cells across multiple donors. TCR gene usage for S42-specific CD4+ T cells **(4A)** and N27-secific CD8+ T cells **(4B).** Gene usage diagrams are shown on the left and select unique T cell clones are shown in the tables to the right. Similar gene usage and CDR3 sequences can be identified across multiple donors for each epitope.

SUMMARY

- We identified multiple dominant T cell epitopes in SARS-CoV-2 convalescent individuals
- Peptide S42 elicited CD4+ T cell responses in multiple donors and both a CD4+ and CD8+ response in Donor N
- Peptide truncation analysis indicates that CD4+ and CD8+ cells may share the same core epitope for S42
- The S42-specific CD4+ response is made up of a diverse TCR repertoire with biased gene usage of TRAV35 and TRAJ42 within the TCRα chain.
- The S42-specific CD8+ repertoire from Donor N is was made up of mostly clonally expanded cells that also share the TRAV35 and TRAJ42 gene signature
- Similar TCR gene usage patterns and CDR3 sequences can be obversed across multiple donors with T cells that recognize the same epitope

CONCLUSION

In SARS-CoV-2 convalescent individuals, epitope-specific CD4+ and CD8+ T cells can differ in their clonal diversity and related TCRs can be identified across multiple donors. Ongoing longitudinal analysis will provide a better understanding of different epitope-specific TCR repertoires and T cell transcriptional profiles, and how they evolve after infection.