SARS-CoV-2-Specific T Cells Correlate with Airway Virus Control During Early Acute Infection

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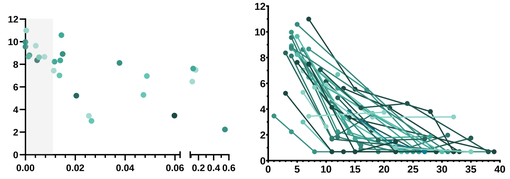
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Introduction

Responses directed against the viral Nucleocapsid (**N**)[1] and Replication-Transcription Complex (**RTC**)[2] of SARS-CoV-2 have been associated with aborted infection prior to seroconversion.

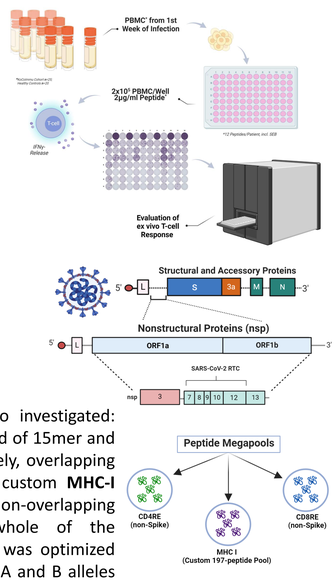


Figures from Esert et al.

In this study, we examine the breadth and specificity of T cell responses to the whole viral proteome in **n=25** study participants, previously naïve to SARS-CoV-2, during days 01-07 after symptom onset. All participants were recruited between May and December 2020. A separate cohort of **n=20** Healthy Controls **(HC)** was likewise tested.

Methods

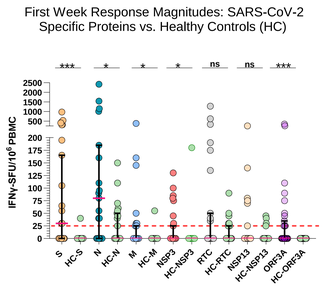
Peripheral Blood Mononuclear Cells (**PBMC**) from **Days 01-35** were thawed and analyzed via **ELISPOT**; each 96-well plate held 6 samples. Cells were stimulated with Spike (**S**), Nucleocapsid(**N**), Membrane (**M**), Non-structural Protein 3 (**nsp3**), Replication-Transcription Complex (**RTC**: nsp7, 8-10, 12), Helicase (**nsp13**), and Open Reading Frame 3a (**ORF3a**).Recognition cutoff was 25 Spot Forming Units (**SFU**) per 10^6 PBMC.

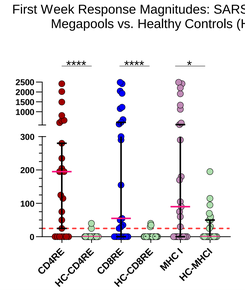


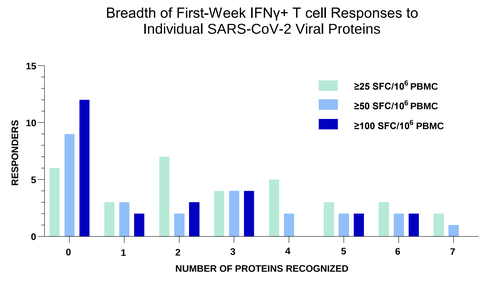
Three Megapools were also investigated: **CD4RE** and **CD8RE**, comprised of 15mer and 9-14mer peptides, respectively, overlapping by 10 amino acids, and a custom **MHC-I** restricted pool of 8-14mer non-overlapping peptides spanning the whole of the proteome. This MHC-I pool was optimized to the 5 most frequent HLA-A and B alleles in the cohort.

Results

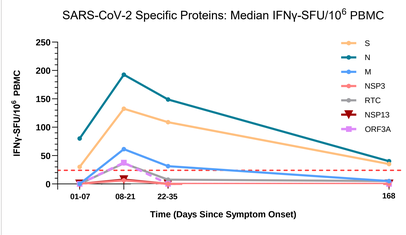
Breadth of T cell responses targeting SARS-CoV-2 individual proteins during **Days 01-07 after symptom onset.** The **Nucleocapsid-specific T cell response was** by far the most immunodominant; Megapool responses were dominated by **IFNγ+ CD4+** T cell activity.

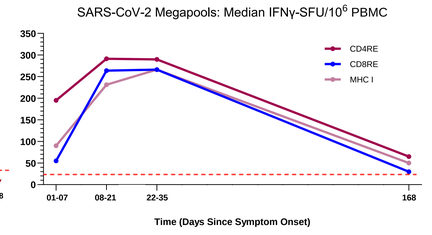




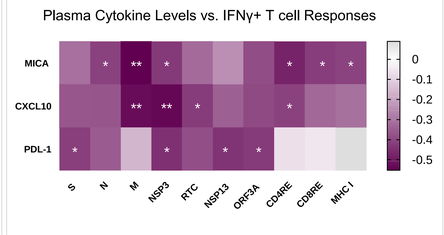


Response kinetics from Days 01-07 through the peak response period between Days 08-21, up to Day 168.

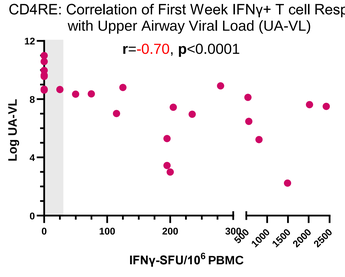


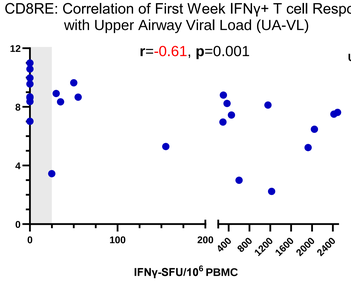


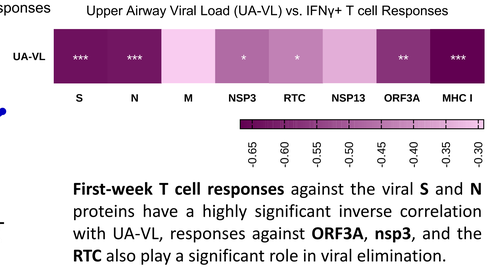
Markers of systemic inflammation correlate inversely with IFNγ+ T cell responses during Days 01-07.



CD4+ T cell activity has a highly significant inverse correlation with Upper Airway Viral Load during Days 01-07 after symptom onset.

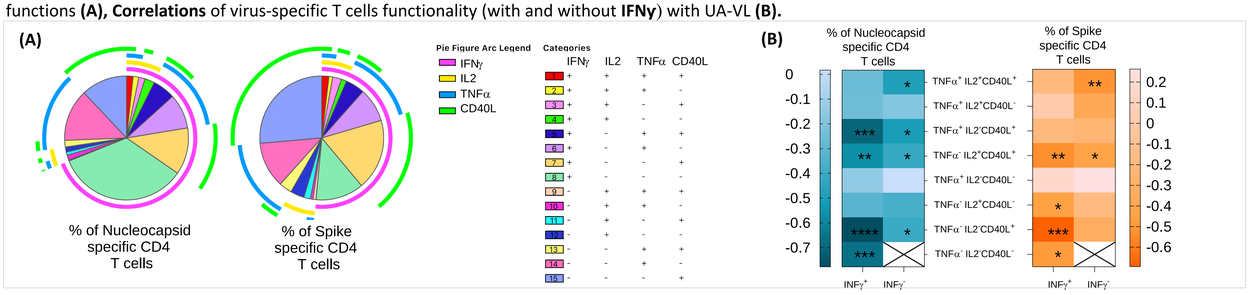






First-week T cell responses against the viral **S** and **N** proteins have a highly significant inverse correlation with UA-VL, responses against **ORF3A**, **nsp3**, and the **RTC** also play a significant role in viral elimination.

Characterization of First-Week SARS-CoV-2-Specific T Cell Functionality in relation to upper airway viral load: Detailed First-Week analysis of specific CD4+ cell functions **(A), Correlations** of virus-specific T cells functionality (with and without **IFNγ**) with UA-VL **(B).**



Conclusions

SARS-CoV-2-specific IFNγ+ T cell responses correlate with early virus control before seroconversion:

- N-specific T cell responses dominate the early SARS-CoV-2-specific T cell response, while other viral antigens are also frequently recognized before seroconversion.
- T cell responses against the viral S, N, NSP3, RTC, and ORF3A have a significant inverse correlation with Upper Airway Viral Load (UA-VL) during acute infection.
- CD4+ and CD8+ T cell responses against the non-Spike viral Megapools correlate inversely with markers of systemic inflammation during acute infection, as do those against the viral M and NSP3 proteins.
- Frequency of CD40L+ NC-specific CD4+ T cells inversely correlates strongly with UA-VL independent of IFNγ co-expression.

Outlook

Early HLA Class 1 T cell epitopes will be mapped for frequent alleles (HLA-A 01:01, 02:01, 03:01, 11:01, 24:02 and HLA-B 07:02, 08:01, 15:01, 35:01, 44:02) and their recognition compared to epitope variants from Common Cold Coronaviruses.

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[1]Eser, T.M., Baranov, O., Huth, M. *et al.* Nucleocapsid-specific T cell responses associate with control of SARS-CoV-2 in the upper airways before seroconversion. *Nat Commun* **14**, 2952 (2023)

[2]Swadling, L., Diniz, M.O., Schmidt, N.M. *et al.* Pre-existing polymerase-specific T cells expand in abortive seronegative SARS-CoV-2. *Nature* **601**, 110–117 (2022)

* p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001 (Mann-Whitney)

----- Symbols indicate cutoff threshold of 25SFU per 10^6 PBMC