# CROSS-RECOGNITION OF A CONSERVED CD4+ T-CELL EPITOPE PRESENT IN DIVERSE CORONAVIRUSES

318

**Bezawit A. Woldemeskel**[1], Arbor G. Dykema[2,3], Caroline C. Garliss[1], Saphira Cherfils[4], Kellie N. Smith[2,3], Joel N. Blankson[1]

[1]Department of Medicine, Johns Hopkins Medicine, Baltimore, MD, USA, [2]Bloomberg-Kimmel Institute for Cancer Immunotherapy, Johns Hopkins Medicine, Baltimore, MD, USA ,
[3]Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University, Baltimore, Maryland, USA, [4]Hunter College, CUNY, New York. New York. USA

## BACKGROUND

Recent studies have shown that vaccinated individuals harbor cross-reactive T cell responses that can cross-recognize SARS-CoV-2 and endemic human common cold coronaviruses (HCoVs). However, it is still unknown whether CD4+ T cells from vaccinated individuals recognize peptides from bat coronaviruses that may have the potential of causing future pandemics.

In this study, we identified a SARS-CoV-2 spike protein epitope ($S_{815-827}$) that is conserved in coronaviruses from different genera and subgenera including SARS-CoV, MERS-CoV, multiple bat coronaviruses and a feline coronavirus. We hypothesized that $S_{815-827}$ is recognized by vaccinated individuals, and that $S_{815-827}$-reactive T cells can cross-recognize homologues bat coronaviruses.

## METHODS

Study participants (age range 21-60) received either the Pfizer-BioNTech (n=33) or Moderna (n=5) COVID-19 vaccines. Blood was drawn and processed between June - Aug 2021. Peptides used in the study are listed in Figure 1A, and were synthesized by Genscript.

To evaluate CD4+ T cell responses, we isolated CD8 depleted PBMCs from COVID-19 vaccinated individuals and performed IFN-γ ELISpot assay with relevant peptides.

To assess T cell cross-reactivity, we generated $S_{815-827}$ reactive T cell lines and re-stimulated them with homologous coronavirus peptides to assess cytokine production with intracellular cytokine staining and flow cytometry. Furthermore, we performed the Vira-FEST assay (which utilizes antigen specific T cell expansion for 10 days, followed by TCR Vβ CDR3 sequencing of CD4+ T cells) to identify cross-reactive CD4+ T cell clones.

Statistical comparisons were done using Mann-Whitney test, Wilcoxon matched-pairs signed rank test or Friedman test with Dunn's multiple comparison as appropriate.

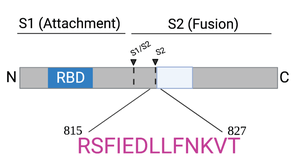



Illustration of SARS-CoV-2 spike protein, with location of the epitope $S_{815-827}$ within the fusion peptide domain indicated.

**Some COVID-19 vaccine recipients have CD4+ T cells that can cross-recognize the conserved SARS-CoV-2 epitope $S_{815-827}$ and homologous peptides from diverse coronaviruses including bats not known to infect humans.**

## RESULTS

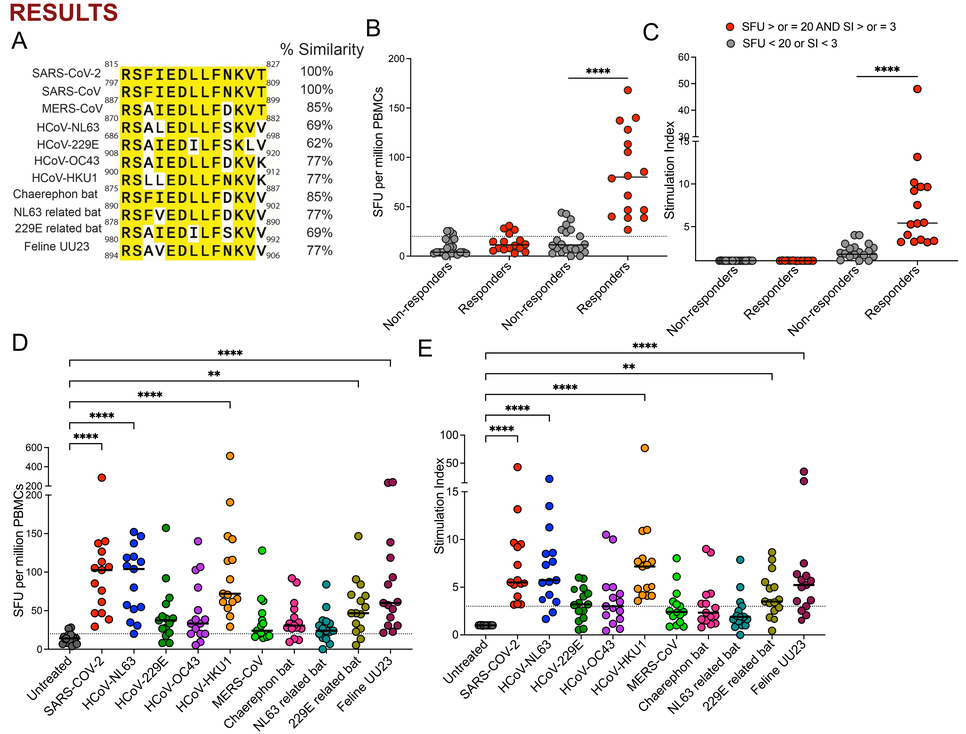



**Figure 1: Some COVID-19 mRNA vaccinated individuals have CD4+ T cells that recognize the conserved SARS-CoV-2 epitope $S_{815-827}$ and homologous peptides from diverse coronaviruses.** (A) Peptide sequence alignment for coronaviruses used in this study are shown. (B and C) IFN-γ ELISpot assay was done with S815-827 or untreated control (n=38 volunteers). Spot forming units (B) and stimulation index (C) were plotted. 16/38 donors (42%) recognized $S_{815-827}$ (termed responders and shown in red). Responders were determined based on the cut-off SFU ≥ 20 and SI ≥ 3. (D and E) $S_{815-827}$ responders (n=15 volunteers) were further assessed for CD4+ T cell responses to homologous coronavirus peptides with IFN-γ ELISpot. Homologous peptides from HCoV-NL63, HCoV-HKU1, 229E-related bat, and Feline coronavirus were recognized robustly by responders.

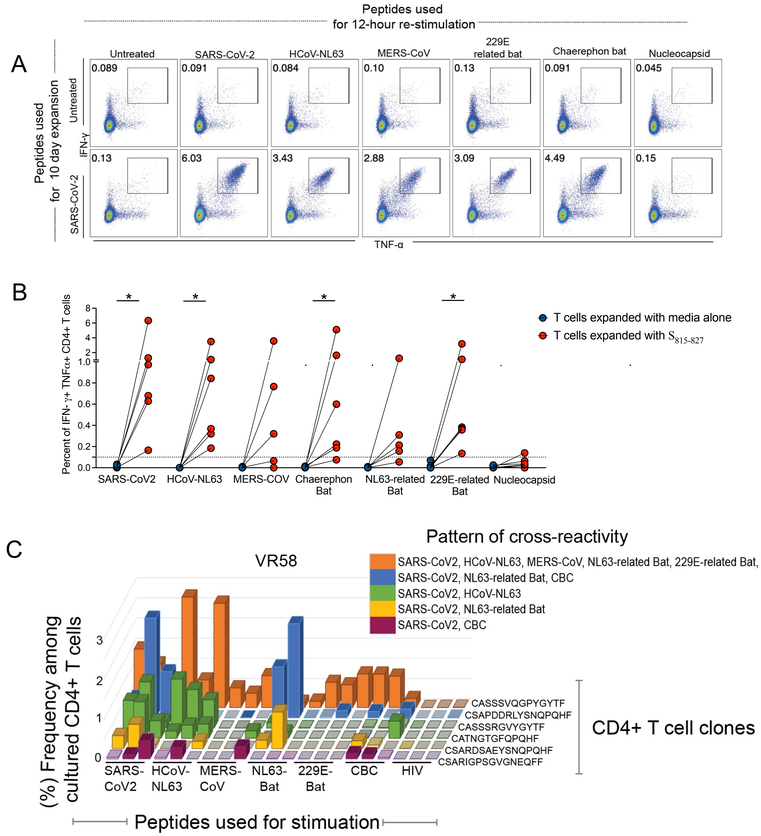



**Figure 2: $S_{815-827}$-Specific CD4+ T cells cross-recognize homologous peptides from diverse coronaviruses.** (A, B) $S_{815-827}$-Specific CD4+ T cell lines respond to restimulation with homologous coronavirus peptides. (A) Representative flow plots shown, with peptides used for expansion indicated on the left, peptides used for re-stimulation indicated at the top and double positive population gated. (B) Responses for all donors tested (n=6) are shown. (C) The ViraFEST assay, which uses antigen specific expansion followed by TCR Vβ CDR3 sequencing, identified multiple CD4+ T cell clonotypes that cross-recognize both $S_{815-827}$ and homologous peptides from diverse coronaviruses in a representative donor shown. Assay was done in triplicate. CBC = Chaerephon bat coronavirus, HIV = HIV-1 Nef

## CONCLUSIONS

- 42% (16/38) of COVID-19 mRNA vaccinated participants in our cohort recognize the conserved SARS-CoV-2 epitope $S_{815-827}$
- Most participants who recognize $S_{815-827}$ also recognized homologous peptides from diverse coronaviruses including bats not known to infect humans
- Responses are a result of cross-reactive CD4+ T cells that can cross-recognize SARS-CoV-2 and homologous coronavirus peptides
- **Our results will inform pan-coronavirus vaccine strategies that hope to elicit cross-protective immunity against diverse coronaviruses**

**Author Contact Information:** bwoldem1@jhmi.edu