Discovery of immunodominant T cell targets in COVID-19 patients and design of novel T cell-based vaccines

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The delta variant of SARS-CoV-2 is now widespread

• The delta variant is now responsible for more than 58% of new infections in the United States

• Six New York Yankee players just tested positive for COVID-19
  • Five were fully vaccinated
The SARS outbreak of 2002/2003 suggests that CD8+ T cells may be important for establishing long-term immunity

- Long-term follow up studies of SARS patients (2, 6, 11, and 17 years later) showed that convalescent patients rapidly lost their anti-viral antibodies and memory B cells but retained their memory T cells\(^1\)\(^-\)\(^4\).

- Animal studies showed that vaccination with a single immunodominant CD8+ T cell epitope conferred complete protection from lethal exposure to SARS-CoV\(^5\)\(^,\)\(^6\).

References

Studies of COVID-19 patients also suggest that a T cell-eliciting vaccine may be necessary for long-term immunity

- Neutralizing antibodies against the spike protein rapidly wane following infection with SARS-CoV-2\(^1\).

- Germinal centers are largely absent in patients with acute COVID-19, impairing the formation of memory B cells and long-lived plasma cells\(^2\).

- SARS-CoV-2-specific memory T cells are found in most convalescent individuals, including asymptomatic cases and those with undetectable antibody responses\(^3\).

References

Unbiased genome-wide screen enables identification of the targets of CD8+ memory T cells in COVID-19 patients

1. Co-culture

2. Enrich (Annexin V MACS)

3. Purify (FACS)

4. Sequence (NGS)

CD8+ memory T cells + Genome-wide library of target cells

- Class I peptide
- GzB-activated scramblase
- 61-aa protein fragment
- Proteasome
- GzB-activated fluorescence

Pre-apoptotic cells

Purified target cells
Unbiased genome-wide screen enables identification of the targets of CD8⁺ memory T cells in COVID-19 patients

SARS-CoV-2 (104 sequenced isolates), SARS-CoV, HKU1, OC43, 229E, NL63

**ORF1**
- Each clone expresses a 61-aa protein fragment (tile)
- Each tile is represented 10 times (DNA bar-coded)
- Library contains 43,420 total clones

**ORF2**

20-aa steps
TScan screen identified eight dominant targets in an HLA A*02:01 patient.
TScan screens of nine A*02:01 patients show that their T cells are largely recognizing the same epitopes.
The precise T cell epitopes were identified and found to be immunodominant (shared across patients)

- Also validated by CD137 expression and tetramer staining

Top three epitopes are broadly shared among patients

Nine patients from screen

27 patients including independent test-set
Immunodominant epitopes were observed in five additional common HLA types

- HLA-A*01:01
- HLA-A*03:01
- HLA-A*11:01
- HLA-A*24:02
- HLA-B*07:02
TScan discovered a total of 29 immunodominant epitopes

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**Table 1. Shared CD8+ T Cell Epitopes Identified in COVID-19 Convalescent Patients**

<table>
<thead>
<tr>
<th>Allele</th>
<th>Peptide Name</th>
<th>Full Peptide</th>
<th>Parent Protein</th>
<th>Start</th>
<th>End</th>
<th>Affinity* (nM)</th>
<th>% of Patients (Screen)</th>
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<tbody>
<tr>
<td>A’02:01</td>
<td>KLW</td>
<td>KLWAOCVGQL</td>
<td>ORF1ab</td>
<td>3,886</td>
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<td>17.7</td>
<td>88.9</td>
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<td>A’02:01</td>
<td>YLQ</td>
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<td>44.4</td>
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<td>FTS</td>
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<td>ATSRTLSSY</td>
<td>M</td>
<td>171</td>
<td>179</td>
<td>16.7</td>
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<td>CTDONALAY</td>
<td>ORF1ab</td>
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<td>4,091</td>
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<td>A’11:01</td>
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<td>6.3</td>
<td>100</td>
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<td>ORF1ab</td>
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<tr>
<td>A’11:01</td>
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<td>ATSRGLSSY</td>
<td>M</td>
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<td>SPR</td>
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<td>105</td>
<td>113</td>
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<td>80</td>
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<td>IPRRINVATL</td>
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<td>5,916</td>
<td>5,924</td>
<td>5.1</td>
<td>20</td>
</tr>
</tbody>
</table>

*Affinity (equilibrium dissociation constant) predicted by using NetMHC4.0.
Of the 29 immunodominant epitopes in SARS-CoV-2, only 3 are found in the Spike protein

- ~90% of immunodominant epitopes are located outside the Spike protein
- No mutations with frequency >1% are observed in 27 of the 29 epitopes (>10,000 sequenced isolates)
- None of the mutations in the UK, South African, Brazilian, or Delta variants occur in these epitopes
Trend observed between anti-viral T cells and disease severity

Virus-specific T cells negatively correlate with disease severity

T cell contraction is not driving the correlation

Kruskal-Wallis, $p = 0.040$

$p = 0.041$

$p = 0.91$

$p = 0.23$

$r = -0.59$

$p = 0.0012$
T cells don’t cross-react with other coronaviruses

SARS-CoV-2

SARS-CoV

‘Common Cold’ Coronaviruses

Coronaviruses
>400 TCRs for SARS-CoV-2 were discovered, explaining immunodominance and enabling T cell-based therapeutics

See: “An Allogeneic TCR-T Cell Therapy for COVID-19” – Poseida Therapeutics
Assay developed by QIAGEN to detect prior exposure to SARS-CoV-2 based on anti-viral T cells

1. Add mixture of immunodominant peptides from SARS-CoV-2

2. Incubate

3. Spin

4. IFN-γ ELISA

- Analogous to QuantiFERON-TB Gold Plus test for TB
- IFNγ assay is compatible with processing tens of thousands of samples in high-throughput.

- Immunodominant peptides provide specificity, as they are unique to SARS-CoV-2 and not endemic coronaviruses.
Several polyepitope vaccine candidates were designed based on the discovered immunodominant sequences

- Different lengths of native adjacent sequence
- Optimized proteasomal cleavage motif
- Optimized to minimize generation of junctional neoepitopes
- Top 3-4 epitopes per allele (19 total)
- All immunodominant epitopes (27 total)
Next-generation vaccine constructs were designed with and without the Spike protein

Polyepitope vaccine alone

Two versions: 19 epitopes and 27 epitopes.

S protein plus polyepitope vaccine

Stabilizing proline mutations

S protein plus immunodominant regions

Stabilizing proline mutations

- These constructs can be delivered using a variety of technologies, including mRNA/LNP
Human cells efficiently process and present epitopes from the polyepitope vaccine candidates, but not from full-length Spike

- HEK 293 cells engineered to express A*02:01 were transduced with lentiviral vectors delivering each vaccine candidate
- Memory CD8+ T cells from two A*02:01-positive COVID-19 patients were co-cultured with the transduced HEK cells and secreted IFN-γ was measured after 18 hours

Data from 7 additional patients further support these conclusions.
Data available in *Immunity* publication

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**Article**

**Unbiased Screens Show CD8⁺ T Cells of COVID-19 Patients Recognize Shared Epitopes in SARS-CoV-2 that Largely Reside outside the Spike Protein**

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