Differentially recognized T cell epitopes in the spectrum of Mtb infection

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The complexity of Mtb infection

IGRA – Interferon Gamma Release Assay

- TB blood test
- Measures cell mediated immune response against Mtb-derived antigens ESAT-6 and CFP10
- Not influenced by BCG vaccination
Characterization of epitope-specific T cells

- Magnitude (higher or lower)
- Specificity (antigens and immunodominance)
- Breadth (diversity of response)
- Functionality (T cell subsets)
21,220 Mtb- and BCG-derived peptide library

- 20,610 Mtb-derived peptides, representing every ORF with 2-10 peptides per ORF
  - Including 1,660 variant sequences present in selected Mtb genomes
- 93 peptides present in BCG (not in Mtb)
- 517 overlapping 15-mers spanning the entire sequence of 12 vaccine candidate and IGRA antigens
A Genome-wide Library (20,610 peptides) defined the targets of CD4 responses in LTBIs

• Identified ~400 epitopes
• 82 antigens were recognized by >10% of subjects and accounted for ~80% of the total response
• Each subject recognizes on average 24 epitopes – heterogeneity of immunity to Mtb

Study subjects

- 31 subjects from Peru (presumed BCG vaccinated at birth)
  - 21 pulmonary ATB 3-4m post diagnosis
  - 10 IGRA- controls
- Age: median 32, range 25-57
- 38% Female, 62% Male

- IFNg Fluorospot
Antigenic islands differ between cohorts

(C) and (D) show the distribution of identified antigens in the H37Rv genome. (C) highlights the percentage of total response across various categories, while (D) illustrates the ORF distribution with stacked bars indicating Healthy IGRA+ and ATB categories.

(E) displays the % of total response for Islands 1, 2, and 3, respectively, with Healthy IGRA+ and ATB categories represented by different colors.
Hierarchy in T cell reactivity against vaccine candidate and IGRA antigens
Can we design peptide pools that can distinguish different Mtb infection states?
An ATB-specific peptide pool

% Responders SFC>50

(A)  

<table>
<thead>
<tr>
<th></th>
<th>ATB at diagnosis (n=9)</th>
<th>ATB mid-treatment (n=12)</th>
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<th>ATB mid-treatment (n=12)</th>
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<tbody>
<tr>
<td>IGRA+</td>
<td>100</td>
<td>35</td>
<td>100</td>
<td>30</td>
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<td>IGRA-</td>
<td>100</td>
<td>81</td>
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(B)  

% Responders SFC>50

(C)  

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Mtbp infection stage-specific T cell responses

- Participants have a large heterogeneity of epitope-specific responses
- The number of frequently recognized antigens are more restricted compared to LTBI
- Active-specific antigens and peptide pool
HLA diversity as a source of heterogeneity

Can we identify HLA alleles associated with active TB?
<table>
<thead>
<tr>
<th>HLA allele</th>
<th>No. subjects with allele</th>
<th>No. subjects lacking allele</th>
<th>Odds ratio</th>
<th>P-value</th>
<th>Corrected p-value</th>
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<tbody>
<tr>
<td></td>
<td>Active TB</td>
<td>QFT+ &amp; QFT-</td>
<td>Active TB</td>
<td>QFT+ &amp; QFT-</td>
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<td>DQA1*03:01</td>
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DQA1*03:01 also identified in a previous study (Sveinbjornsson 2016 Nature Genetics)
HLA association

• 3 HLA alleles were found to be associated with susceptibility to ATB
• Expression of susceptibility alleles causes a decrease in Mtb-specific responses
• Expression of susceptibility alleles leads to decreased expression of APC-related genes.
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