Dissecting HLA-E-restricted T-cell responses against Mtb as future targets for vaccination

Global Forum on TB Vaccines

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HLA-E in innate and adaptive immunity

**HLA-E**
- Non-classical HLA-I antigen presentation molecule with only two functional variants (HLA-E*0101 and *0103)
- 1 amino acid difference between alleles located outside peptide binding groove
- Dimorphism makes HLA-E an interesting vaccine candidate

**HLA-E and ligands**
- HLA-E interacts with NKG2A/C + CD94 co-receptor complex expressed on NK-cells
- HLA-E can present peptides from bacterial and viral origin, as well as tumour derived peptides, to TCRs

Ottenhoff and Joosten, Science 2019
HLA-E & Mtb infections

- HLA-E present in Mtb-containing phagosomes
- HLA-E is not downregulated during HIV infections
- HLA-E Mtb T-cells identified in TB, latent TB and HIV-co-infected individuals
- HLA-E Mtb T-cells have both regulatory as well as cytolytic phenotype, and can inhibit intracellular mycobacterial growth
Identification of HLA-E Mtb binding peptides

1. Test peptide binding
2. Generate binding motif
3. Peptide prediction
4. Peptide selection
5. Test T-cell recognition

Table of Mtb peptides:
- TVCVIWC1H 0.07414313828
- YFSPVLDEG 0.173300151338
- MYPVSILD 0.660690583462
- NEEAAEWDRE 0.148922248602
- QMAVFIHNF 0.105999140042
- EKIINALV 0.228524424669
...
P2 and P9 are critical anchor residues for HLA-E binding and show a preference for hydrophobic residues. P7 containing a Pro is also important for binding to HLA-E*01:03.
## Novel predicted Mtb-derived HLA-E binding peptides have a higher affinity

Improved peptide affinity to four different MHC-E molecules after 3 rounds of prediction and testing

Stronger predictive power with new training model

<table>
<thead>
<tr>
<th>Prediction 1</th>
<th>Prediction 2</th>
<th>Prediction 3</th>
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<tbody>
<tr>
<td>% binders</td>
<td>HLA-E*01:03</td>
<td>HLA-E*01:01</td>
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<td>1.50</td>
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<td>1.35</td>
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<td>2.65</td>
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<td>0.75</td>
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**Figure:**

- **HLA-E*01:03**
- **HLA-E*01:01**

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**Graph:**

- **HLA-E*01:03**
- **HLA-E*01:01**
T-cell recognition of HLA-E binding peptides

HLA-E restricted CD8+ T-cells specific to newly identified peptides can be detected in TST positive humans
Understanding HLA-E T-cell interactions for optimal vaccine design

TCR repertoire analysis of HLA-E TM sorted T-cells directly *ex vivo* using two Mtb-derived peptides predicted in 2010 (Joosten et al. 2010)

*Image of a diagram showing the process of TCR repertoire analysis.*

*SOP with courtesy of the Doherty Laboratory, Department of Microbiology and Immunology, University of Melbourne*
HLA-E restricted T-cells have a highly diverse TCR repertoire

Diversity in both TRAV and TRBV sequences → polyclonal TCR repertoire

D1 – p55⁺
0101-0103

D16 – p34⁺
0101

Clustering of TCR sequences based on TCR sequence similarity, enriched motifs in the CDR3 regions and clonal expansion bias (GLIPH2 cluster algorithm (Glanville et al. 2017))
Different GLIPH2 clusters have been identified for p55 & p34 specific TCRs

**p34 and p55 TCR-enriched GLIPH clusters (≥75% vs. CD8 bulk)**

- **p55 (% of total p55 TCR sequences)**
  - 9
  - 11
  - 10
  - 8
  - 7
- **p34 (% of total p34 TCR sequences)**
  - 6
  - 5
  - 4
  - 3
  - 2

**Legend**
- **% of p55 specificity**
  - 0 - 100
- **% of p34 specificity**
  - 20 - 80
- **# of TCR sequences**
  - 7
  - 5
  - 3
Mtb specific TCRs derived from sequencing are functional

*Proof of principle:* Anti-CD3CD28 bead activation of transduced TCR sequences
Take home messages

• HLA-E is an interesting molecule to target donor-unrestricted immunity against (Mtb) infection in a vaccination setting

• A prediction method based on improved peptide binding motifs can lead to the identification of novel and high affinity HLA-E-presented Mtb-derived peptides

• TCRs of HLA-E Mtb restricted CD3^+CD8^+ T-cells are highly diverse but can be discriminated from the total pool of CD8^+ TCRs

• Understanding the fundamentals of TCR recognition of HLA-E/peptide complexes is important to optimize vaccine design