Age-Associated Heterogeneity of Ty21a-induced T Cell Responses to HLA-E Restricted *Salmonella* Typhi Antigen Presentation

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Salmonella Typhi

- S. Typhi is a gram negative human-restricted facultative intracellular pathogen
- Transmitted via fecal-oral route and invades through the gut epithelia
- Can disseminate into lymphatics and bloodstream by targeting DC and MΦ leading to systemic, sometimes chronic, infection

Nature Immunology 3, 1026 - 1032 (2002)
Ty21a Vaccine

- Licensed Vi-neg oral live-attenuated vaccine derived from the wt Ty2 strain of S. Typhi

- Responses generated
  - Antibodies and memory B-cells to S. Typhi-specific O-LPS and H-flagella. However, these responses do not appear to correlate with protection in human wild-type challenge studies
  - CD4+ and CD8+ Cell-mediated immune (T-CMI) responses
    - $T_{c1}/T_{c17}$ cytokine effectors
    - Contact-dependent cytotoxicity (CTL)
    - Helper T cell responses (e.g., Th1, Th17)

Some of these T-CMI responses have been associated with protection in a human wild-type challenge study
## Ty21a Efficacy in Children - Field Studies

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Placebo</th>
<th>Vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-9 yr</td>
<td>7193</td>
<td>7034</td>
</tr>
<tr>
<td># of children</td>
<td>25</td>
<td>10</td>
</tr>
<tr>
<td>Efficacy</td>
<td>-</td>
<td>59% (16-80%)</td>
</tr>
<tr>
<td>10-14 yr</td>
<td>9710</td>
<td>9992</td>
</tr>
<tr>
<td># of children</td>
<td>32</td>
<td>11</td>
</tr>
<tr>
<td>Efficacy</td>
<td>-</td>
<td>67% (35-83%)</td>
</tr>
<tr>
<td>≥ 15 yr</td>
<td>5001</td>
<td>5142</td>
</tr>
<tr>
<td># of children</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>Efficacy</td>
<td>-</td>
<td>85% (42-96%)</td>
</tr>
</tbody>
</table>

Ty21a vaccine recipients (enteric-coated capsules)

Adapted from: Levine et al., The Lancet 329: 1049-1052, 1987

- There is a poor understanding of human pediatric CMI immune responses
- Typically, younger children are less likely to develop robust, long-lasting CMI than adults and older children

Ty21a efficacy was previously observed to exhibit a trend to be lower at a younger age; however, these data showed large overlapping confidence intervals.
Human Leukocyte Antigen – E (HLA-E)

• Non-classical MHC class Ib

• HLA-E presents a conserved set of peptides
  • HLA-A2 leader peptide (inhibitory)
  • Bacterial chaperonins (stimulatory)
  • Heat-shock proteins (stimulatory)

• HLA-E restricted CD8+ T cell responses are present for up to 2 years following Ty21a vaccination in adult volunteers and are also induced upon wt S. Typhi challenge

• Differences in effector cytokine responses to autologous vs. HLA-E restricted S. Typhi-infected cells in Ty21a vaccinated adults


Adapted from: Rodgers et al. Nat Rev Immunol 5: 459-71, 2005
HLA-E Restriction Experimental Protocol

Target Cells
721.221.AEH

S. Typhi Infection

γ-irradiation

Infected Co-culture

PBMC

γ-irradiation

Target Cells
721.221.AEH

Uninfected Co-culture (Control)

Mass Cytometry
(38 parameters/cell)

14-18 hr incubation
Younger children are less likely to have activated CD8+ T cells at baseline than older children or adults.

Rudolph, Sztein et al. Frontiers Immunology 2019
No significant differences were observed among age groups in the percentages of participants who exhibited increased activation to HLA-E-restricted S. Typhi-specific CD8+ T cell stimulation following Ty21a vaccination.

Rudolph, Sztein et al. Frontiers Immunology 2019
No significant age-associated differences were observed in CD8+ T<sub>EM</sub> effector responses following vaccination.

Rudolph, Sztein et al. Frontiers Immunology 2019
Significant age-associated differences in CD8+ T_{EMRA} effector mono and trifunctional responses following Ty21a vaccination.

Rudolph, Sztein et al. Frontiers Immunology 2019
• **Non-linear** variation of principal component analysis

• Groups all variables of interest into a multiple one-dimensional representations containing every cell event

• Combines one-dimensional representations to generate the most descriptive two-dimensional plot

• Clustering algorithms divide the resulting plot into groups based on each cells’ similarity to its neighbors

• Unbiased means of identifying key population features that would be impossible to discern using conventional gating methods
HLA-E Restricted CD8+ T_{EM} tSNE Data

Pre-Vaccination

6-15 yrs

n = 10

Adults (16-65 yrs)

n = 21

Post-Vaccination

n = 10

n = 21

Rudolph, Sztein et al. Frontiers Immunology 2019
HLA-E Restricted CD8+ T_{EM} tSNE Data

Pre-Vaccination Abundance

Post-Vaccination Abundance

Average Cells per Cluster (%)

Cluster (#)

Yrs 6-15 (n=10)

Yrs 16-65 (n=21)

Rudolph, Sztein et al. Frontiers Immunology 2019
Clusters made up of higher percentages of adult HLA-E-restricted *S. Typhi*-responsive CD8+ T_{EM} cells are generally more multifunctional and have more diverse homing potential than those dominated by pediatric cells.

Rudolph, Sztein et al. Front Immun. 2019
Ty21a vaccination only significantly changed percentages of adult HLA-E-restricted S. Typhi-responsive cells within select tSNE clusters

Rudolph, Sztein et al. Frontiers Immunology 2019
Clusters that increase in circulation following Ty21a vaccination are made up of cells that are more diverse in effector function and homing potential than those that decrease following vaccination.
**CD8+ HLA-E Restricted Responses: Summary**

### T Cell Subtype

<table>
<thead>
<tr>
<th>Children</th>
<th>Adults*</th>
</tr>
</thead>
<tbody>
<tr>
<td>↓ Baseline activation (CD69+)</td>
<td>↑ Baseline activation (CD69+)</td>
</tr>
<tr>
<td>Heterogeneous $T_{EM}$ post-Ty21a</td>
<td>Heterogeneous $T_{EM}$ post-Ty21a</td>
</tr>
<tr>
<td>↓ $T_{EMRA}$ MIP1β, TNFα</td>
<td>↑ $T_{EMRA}$ MIP1β, TNFα</td>
</tr>
<tr>
<td>Fewer cells in $T_{EM}$ and $T_{EMRA}$ tSNE clusters dominated by gut/inflammation homing MF populations</td>
<td>More cells in $T_{EM}$ and $T_{EMRA}$ tSNE clusters dominated by gut/inflammation homing MF populations</td>
</tr>
</tbody>
</table>

* 16-17 year old T cell activation and functions are more adult-like

Rudolph, Sztein et al. Frontiers Immunology 2019
CD8+ HLA-E Restricted Responses to Ty21a Immunization in Children: Overall Conclusions

- These data demonstrate the presence of major differences in the “fine granularity” of HLA-E-restricted CD8+ responses between children and adults before and after Ty21a immunization.

- These include an increased multifunctionality of the responses in adults and marked differences in the cytokines being produced and the homing potential of the responding cells.

- These data emphasize the importance of in-depth studies of the CMI immune response in children during the development of future generations of typhoid vaccines, as well as other vaccines targeted for children.
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