Recent advances on the complex and multifaceted T memory and effector immunity elicited to S. Typhi in humans

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Ab to *S. Typhi* antigens (e.g., Vi, LPS O) are likely to play an important role in defense against typhoid bacilli when they are extracellular.

In contrast, since *S. Typhi* persists intracellularly, thereby avoiding destruction by Ab and C’, CMI is expected to be essential in eliminating *S. Typhi* from the infected cells.

Both adaptive immune mechanisms (CMI & Ab) are expected to provide critical support to innate immunity in the mucosal microenvironment and elsewhere.
**Effector Ab responses to S. Typhi**

1. Serum IgG and IgA Abs to S. Typhi antigens
   - LPS O, flagella, Vi, OMPs, hsp, others? (Elisa)
   - Opsonophagocytosis, ADCC, bactericidal (Functional)

2. IgG and IgA ASC to S. Typhi antigens in circulation
   - LPS O, flagella, Vi, OMPs, others?
   - 7-10 days after immunization (longevity in the gut and other tissues unknown)

3. Anti-S. Typhi specific secretory IgA (SIgA) in intestinal lavage fluids and stools of subjects exposed to S. Typhi

4. B memory cells (e.g., LPS, Vi)
It is not known whether Ab to common S. Typhi antigens, particularly to O, H and Vi, particularalry those with defined functional activities, actually

(1) Mediate protection,

(2) Act in conjunction with other innate and adaptive responses or

(3) Serve as a surrogate for the presence of other more dominant protective immune responses (e.g., CMI) that will eventually lead to the elimination of this intracellular bacteria from the host
Immunological correlates of protection in typhoid fever: Hypothesis

Both CMI and Ab responses play central roles in protection in typhoid fever and are elicited following immunization with attenuated S. Typhi vaccine candidates.
Vaccine Development
The never ending search for the optimal balance

Reactogenicity

Immunogenicity

What responses?
## Attenuated *S. Typhi* vaccine strains

<table>
<thead>
<tr>
<th>Organism</th>
<th>Strain</th>
<th>Mutations</th>
<th>Metabolic Pathways</th>
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<tr>
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<td><em>S. Typhi</em></td>
<td>Ty21a</td>
<td>Chemical mutagenesis</td>
<td>Many</td>
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</table>
Key Effector CMI to *S. Typhi* in orally immunized subjects (1)

- **Effector responses to *S. Typhi*-infected targets:**
  - **Cytotoxic T lymphocytes (CTL) activity** ($^{51}$Cr-release assays; granzyme; CD107 staining by flow cytometry)
  - **IFN$\gamma$ production** (TNF-\(\alpha\), others)
  - **Mediated by both CD8$^+$ (dominant) and CD4$^+$ cells**

- **CD8$^+$ CTL activity restricted by:**
  - Classical class Ia molecules (HLA-A, B, C)
  - Non-classical class-Ib molecules (HLA-E)
Key Effector CMI to S. Typhi in orally immunized subjects (2)

- **Proliferation** and predominant **type-1 cytokine responses** to soluble S. Typhi antigens (e.g., flagella)
  - IFNγ, TNFα, IL-10 in the absence of IL-4, IL-5 & IL-6
  - IFNγ produced predominantly by CD4⁺ cells

- **Homing to mucosal and non-mucosal tissues**: IFN-γ production by **central and effector memory** T subsets that express, or not, the gut homing molecule integrin α₄/β₇

- Presence of **long-term multifunctional** HLA-E-restricted CD8⁺ cells co-expressing IFN-γ, TNF-α and CD107
Detailed CMI studies to S. Typhi-infected autologous cells in Ty21 vaccinees

1. Define the kinetics of CMI to S. Typhi-infected autologous cells elicited by Ty21a in humans

2. Study IL-17A responses elicited in Ty21a vaccinees

- IL-17A is a pro-inflammatory cytokine produced by CD4+ and CD8+ T cells. Recently shown to play a key role in mucosal immunity

- Measure the multi-functionality of T cell responses following Ty21a immunization
Why study multi-functionality of the T cell responses following Ty21a immunization?

- Studies have shown that multifunctional T cells, those producing 2 or more cytokines simultaneously, might be critical effectors in protection from infection in animals and humans (e.g., HIV, Mtb).

- Technological advances and unsupervised flow cytometry analysis packages enable the study of all possible combinations of many cytokines to define multi-functional CD8+ T cell subsets.
Experimental Design: In vitro stimulation with S. Typhi-infected targets

**Target Cells**
Autologous EBV-LCL, 721.221.AEH, blasts

**S. Typhi infection**

**γ-irradiation**

**Effector cells**
*Ex vivo* PBMC collected before and 1, 2, 4, 8, 10, 14, 21, 28, 60, 90, 180, 360 days after Ty21a immunization

- CD8+ T effectors (CTL, cytokines)
- CD4+ T effectors (cytokines)
- Others?

**Flow cytometry**
(Tm subsets, CD107a, IFN-γ, TNF-α, MIP-1β, IL-17A, IL-2, etc)

14-18 hrs
Experimental Design: Flow cytometry gating

### Sequential gating strategy

**Lymphocytes**
- **No Gate**

**Singlets**
- **Gate: R1**
  - R1: 19%
  - R2: 92%

**Live T cells**
- **Gate: R1&R2**
  - R3: 75%

#### Cell phenotypes

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<td>&quot;Dump&quot; channel</td>
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Cytokines/chemokines are secreted primarily from the CD8+ T_{EM} & T_{EMRA} subsets.

Total CD8+

CD8+ T_{CM}

CD8+ T_{N}

CD8+ T_{EM}

CD8+ T_{EMRA}

IL-10 → IL-17A → IL-2 → IFN-γ → TNF-α → MIP-1β
IL-17A is secreted by CD8+ TEM cells in response to Ty21a immunization in humans

Days post immunization
% positive cells

IL-17A
Kinetics of net CD8+ T_{EM} responses to stimulation with S. Typhi-infected autologous cells in Ty21a vaccinees
IL-17A+ multifunctional CD8+ T_{EM} following exposure to S. Typhi-infected autologous cells

% positive cells

Days post immunization

IL-17A+ IFN-γ+ TNF-α+
IL-17A+ IL-2+ IFN-γ+ TNF-α+
IL-17A+ IFN-γ+ TNF-α+ MIP-1β+
IL-17A+ IL-2+ IFN-γ+ TNF-α+ MIP-1β+
Conclusions (I)

- Ty21a-immunized volunteers exhibited CD8+ T_{EM} and T_{EMRA} cell responses following stimulation with S. Typhi-infected autologous EBV-LCL.

- Multifunctional CD8+ T cells are present in response to Ty21a immunization.

- Multifunctional T cells have been reported to correlate with lack of disease progression in HIV, have been identified as a potential correlate of protection for \textit{Leishmania major} immunization in a mouse model, etc.
Conclusions (II)

• This is the first demonstration of IL-17A production in response to Ty21a immunization
  - IL-17A secreting cells have been shown to play a role in mucosal immunity

• IL-17A is secreted from **multifunctional** cells that co-produce IL-2, IFN-γ, TNF-α, and/or MIP-1β

• There is heterogeneity in the kinetics of the immune responses to Ty21a immunization.
  - **Bi-phasic & tri-phasic** responses to *S. Typhi*-infected autologous EBV-LCL are typical. Thus, evaluating a single time point may fail to accurately evaluate responsiveness
**Ag uptake & presentation**

- **S. Typhi**

**Epithelium**

- **M cells**

**Lumen**

**Lamina Propria**

**GALT**

**Peripheral circulation**

**Bacteremia**

**Mesenteric lymph nodes**

**Thoracic duct**

**RES spleen, liver, bone marrow, LN, intracellular replication**

**Biliary tract, other organs**

**T & B activation**

**Cytokines** (e.g., IFN-γ, TNF-α, IL-10, IL-17, IL-4, IL-5, IL-6)

**Chemokines**

**Bact ASC Ab BM**

**Tact**

**Proliferation**

**Th1 cytokine pattern**: IFN-γ, TNF-α, IL-10, IL-17, IL-4, IL-5, IL-6

**CTL** [classical class Ia- and class Ib (HLA-E)-restricted]

**T_M** [**T_EM** **T_EMRA** **T_CM**]  

**T_EM** **T_EMRA** **T_CM** expressing [or not] **integrin α4/β7**

**Common Mucosal Immune System?**
The question remains:

Which of these complex and heterogeneous CMI responses, if any, are associated with protection from typhoid fever?
To answer this question, and to better understand typhoid disease, we recently initiated a collaboration with Dr. Pollard and his team at Oxford who have re-established a human challenge model with wild-type *S. Typhi*
Multiphasic kinetics of CMI responses by CD8+ T_EM to S. Typhi-infected AEH-cells (HLA-E-restriction)

This volunteer (Ox6) did not develop typhoid fever (No TD)

- **CD107a**
- **IFN-γ**
- **TNF-α**
- **IL-2**
- **IL-17**
- **MIP-1β**

Days post-challenge

ABX : antibiotics
Multifunctional CD8+ TEM to S. Typhi-inf B-LCL in wild-type S. Typhi-challenged subjects

Peak d7

No TD:
- Ox4
- Ox6
- Ox7
- Ox8
- Ox12

TD:
- Ox3
- Ox5
- Ox9
- Ox10
- Ox11

% positive cells

CD107a
IFN-γ
TNF-α
IL-2
IL-17A
MIP-1β

Mean
Mass cytometry (CyTOF), a transformational flow cytometry technology to measure human immune responses by simultaneously evaluating >35 parameters/cell
S. Typhi appears to be such an effective pathogen, at least in part, by being exquisitely stealth.

Thus, identifying the effective immunological CoP among a sea of non-protective or downregulatory immunity might hold the key for the development of more effective vaccines.
Identification of the precise immunological correlates of protection (either mechanistic or non-mechanistic), can significantly advance the development of broad spectrum vaccines for enteric fevers (e.g., S. Paratyphi A, S. Paratyphi B)
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