Immune response and correlates of protection against typhoid

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Correlates of enteric vaccine-induced protection
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Salmonella enterica serovar Typhi

- It is a human restricted **intracellular** Gram-negative bacterium that infects both phagocytic and non-phagocytic cells
- The causative agent of typhoid fever
- >20 million of individuals infected annually
- >200,000 deaths/year
- Appearance of antibiotic resistance in Asia and northeast Africa

http://www.infectionlandscapes.org
Vaccine Development
The never ending search for the optimal balance

Reactogenicity

Immunogenicity

What responses?
Identification of the precise immunological correlates of protection (either mechanistic or non-mechanistic), can:

1. Define the effector immunity to be pursued during vaccine development
2. Help predict long-term protection
3. Accelerate the development of broad spectrum vaccines for enteric fevers (e.g., S. Paratyphi A, S. Paratyphi B)
4. Advance the development of live vector vaccines
Lack of known immunological correlates of protection in typhoid fever

- 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.
Immunological correlates of protection in typhoid fever: Ab summary

It is not known whether Ab to common S. Typhi antigens (e.g., O, H and Vi), particularly 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
Experimental Design: In vitro stimulation with S. Typhi-infected targets

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

**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)
- MAIT
- APC’s, B cells, Others?

**S. Typhi infection**

**γ-irradiation**

**ELISpot (IFN-γ)**

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

14-18 hrs
Key effector CMI to S. Typhi in orally immunized subjects (1)

- **Effector responses to S. Typhi-infected targets:**
  - Cytotoxic T lymphocytes (CTL) activity \(^{51}\text{Cr-release assays; granzyme; CD107 staining by flow cytometry)}\)
  - 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)
  - IFN\(\gamma\), TNF\(\alpha\), IL-10 in the absence of IL-4, IL-5 & IL-6
  - IFN\(\gamma\) produced predominantly by CD4\(^+\) cells

- **Homing to mucosal and non-mucosal tissues:** IFN-\(\gamma\) production by **central** and **effector memory T** subsets that express, or not, the gut homing molecule integrin \(\alpha_4/\beta_7\)

- Presence of **long-term multifunctional** HLA-E-restricted CD8\(^+\) cells co-expressing **IFN-\(\gamma\)**, **TNF-\(\alpha\)** and **CD107**
CMI to S. Typhi-infected autologous cells in Ty21 vaccinees: Multifunctional T cells

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

  ➢ Multifunctional T cells (those producing ≥ 2 cytokines simultaneously, might be critical effectors in protection from infection in animals and humans (e.g., HIV, Mtb, Ebola) and be key determinants in long-term immunity

  ➢ Technological advances and unsupervised flow cytometry analysis packages enable the study of all possible combinations of many cytokines to define multifunctional CD8⁺ T cell subsets
IL-17A production to S. Typhi-infected autologous cells in Ty21 vaccinees

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

- **Findings:**
  - First demonstration of IL-17A production by CD4+ & CD8+ (T_{EM} and T_{EMRA}) subsets elicited by Ty21a immunization
  - Responses were multifunctional and multiphasic (biphasic, triphasic over 1 year). Thus, evaluating a single time point may fail to accurately evaluate responses
Role of $T_{\text{eff}}$, $T_{\text{reg}}$, $pT_{\text{fh}}$, MAIT and $T_{\text{RM}}$ systemically and in the gut mucosa in typhoid fever

Immunity to S. Typhi: What is relevant?

Over the past 2 decades we demonstrated that immunization of volunteers with S. Typhi vaccines elicits complex and heterogeneous CMI responses.

However, a key question remains unanswered: which of these 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
Goals of the wt S. Typhi human challenge model

Determine the dose of S. Typhi required to produce an attack rate of 60-75%

Clinical and laboratory features
- Time course
- Bacteraemia
- Inflammatory response

Development of immunity
- Innate & humoral (e.g., Ab, ASC)
- CMI (T_{eff}, T_{reg}, T_{M}, MAIT)
- APC (MΦ, DC), B memory

Long-term immunity after treatment

Correlates of protection

Variation in genomic response

Diagnostics
- PCR-based
- Mass spectrometry

Human challenge model
Oxford challenge (study 1): Design

- 41 healthy participants received wt S. Typhi
- 2 doses evaluated (20 vol each): low (~$10^3$ cfu) and high (~$10^4$ cfu)
- Typhoid Diagnosis (TD) determined by fever >38°C for ≥12hr or bacteremia
- Outcome: 61% (overall 25/41 developed TD)
- TD developed between 6-14 days after challenge
- Participants received antibiotics at time of diagnosis or at day 14
Oxford human wild-type S. Typhi challenge studies:
Memory & effector T cells
Experimental Design: in vitro stimulation of T effectors with S. Typhi-infected targets

**Target Cells**
- Autologous B-LCL
- AEH-B-LCL (HLA-E-restricted)
- Autologous blasts

**36 subjects**

<table>
<thead>
<tr>
<th>LOW DOSE</th>
<th>HIGH DOSE</th>
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<tr>
<td>TD (n=7)</td>
<td>TD (n=13)</td>
</tr>
<tr>
<td>NoTD (n=9)</td>
<td>NoTD (n=7)</td>
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**Procedures**
- S. Typhi infection
- \(\gamma\)-irradiation
- Flow cytometry
  - 14-color – conventional flow
  - 24-parameter – mass cytometry
- 14-18 hrs
Experimental Design: Flow cytometry gating

- **CD8+ T cells**
- **CD8+ subsets**
  - TCM
  - T N
  - TEM
  - TEMRA

- **Lymphocytes**
- **Singlets**
- **Live T cells**

- **CD3 BV650-A**
  - R4: 23.47%

- **CD8 PerCP-Cy5-5-A**
  - R6: 13.17%
  - R7: 56.02%
  - R8: 15.53%
  - R9: 8.56%

- **CD45RA Qdot 800-A**
- **CD62L APC-EF780-A**

- **YEVID + CD14/19/45 BV570-A**
  - R201: 75.70%

- **CD107a/b FITC-A**
- **CD69 ECD-A**

- **IL-17 BV421-A**
- **IFNg PE-Cy 7-A**
- **TNFa Alexa700-A**
- **IL-2 BV605-A**

- **MIP1b PE-A**
No differences were observed at baseline (pre-challenge) in the absolute numbers of WCC, lymphocytes, CD3, CD4 or CD8 cells between TD and No TD subjects.
S. Typhi-specific CD8+ T<sub>EM</sub> responses: Baseline

EBV, AEH & Blasts combined (low dose)

Higher baseline responses (pre-challenge) are associated with protection.*
Relationship between S. Typhi-specific baseline responses and time to diagnosis

**EBV, AEH & Blasts combined (low dose)**

High pre-challenge S. Typhi-specific baseline responses are associated with delayed time to diagnosis
Multifunctional CD8+ $T_{EM}$ responses: Baseline

MF S. Typhi-specific responses are dominant in NoTD volunteers
Multifunctional CD8+ T\textsubscript{EM} responses: Baseline

EBV, AEH & Blasts combined (low dose)

CD8 T\textsubscript{EM}

% positive cells

CD107  -  +  +  +  -  -  +  +  +
IFN-\gamma  -  -  -  +  +  +  +  +  +
TNF-\alpha  +  -  +  -  +  +  +  +  -
MIP-1b  +  +  +  +  +  +  +  +  -
IL-2  -  -  -  -  -  -  +  -  +

MF S. Typhi-specific responses are dominant in NoTD volunteers
Marked decreases were observed in S. Typhi-specific $T_{EM}$ expressing CD107a and producing cytokines following challenge and before the onset of disease.
Marked decreases were observed in S. Typhi-specific T_{EM} expressing, or not, integrin $\alpha_4\beta_7$ following challenge and before the onset of disease.
This study provides unique insights into the human immune response during the development of typhoid fever

- Uncovered, for the first time, that S. Typhi-specific CD8 T cell baseline responses correlate significantly with clinical outcome after infection. Higher baseline S. Typhi-specific responses are associated with:
  - Protection from typhoid disease
  - Delayed time to diagnosis in subjects who developed TD

- Demonstrated that multifunctional T cells are likely to play an important role in protection from the development of typhoid disease
Salmonella Typhi-specific multifunctional CD8+ T cells play a dominant role in protection from typhoid fever in humans

Stephanie Fresnay¹, Monica A. McArthur¹, Laurence Magder², Thomas C. Darton³, Claire Jones³, Claire S. Waddington³, Christoph J. Blohmke³, Brian Angus⁴, Myron M. Levine¹, Andrew J. Pollard³ and Marcelo B. Sztein¹*
Oxford human wild-type S. Typhi challenge studies:

Regulatory T cells
Regulatory T cells ($T_{reg}$)

- Subset of CD4+ T cells that suppress other immune cells
- Characterized by expression of IL-2R (CD25) and transcription factor FoxP3
- Traffic to sites of specific immune responses
- Regulatory functions
  - CTLA-4 competition for co-stimulatory molecules (CD80 and CD86) on antigen presenting cells (APC)
  - Consumption of IL-2 (IL-2R)
  - Cytokine production (IL-10)
Goals

- Identify the potential role of circulating $T_{reg}$ in a wild-type S. Typhi challenge model in humans.
- Identify the homing potential and activation characteristics of $T_{reg}$ associated with typhoid diagnosis.
Gating Strategy

**Lymphocytes**
- SSC-A vs. FSC-H

**Singlets**
- FSC-A vs. FSC-H

**Live CD3+ T cells**
- Live/dead/CD14/CD19 vs. CD3

**FoxP3+**
- CD4

**T_{reg}**
- 87.58%

**Activation**
- HLA-DR
- LFA-1
- NRP-1
- Tim-3
- PD-1

**Homing**
- $\alpha 4\beta 7$
- CXCR3
- CCR6
No significant difference in mean percentages of total T_{reg} across time segments or between TD and No TD volunteers indicating that changes in total circulating Treg are not associated with TD.
Gut homing potential of circulating $T_{reg}$

- S. Typhi-specific expression of integrin $\alpha 4\beta 7$ is up-regulated on circulating Treg pre-challenge in TD volunteers indicating possible association with disease.
- Down-regulation of S. Typhi-specific integrin $\alpha 4\beta 7$ expression on Treg pos-challenge in TD volunteers indicates potential homing to the gut.
S. Typhi-specific activation of circulating T\textsubscript{reg}

Higher S. Typhi-specific expression of activation molecules (HLA-DR) on T\textsubscript{reg} post-challenge is associated with TD indicating S. Typhi-specific activation of T\textsubscript{reg} may be associated with development of disease.
Kinetics of S. Typhi-specific T_{reg} activation (representative volunteers)

Gated on T_{reg}

TD volunteers have up-regulation of S. Typhi-specific expression of LFA-1 near the time of typhoid diagnosis supporting the association of increased activation of T_{reg} with TD
Depletion of $T_{\text{reg}}$

PBMC

$T_{\text{reg}}$

$T_{\text{EM}}$

Infected target

mock depletion

CD25 depletion

$S.\ Typhi$-infected B-LCL

14-18 hrs

Mass cytometry

14-18 hrs
Depletion of $T_{\text{reg}}$

Gated on CD3+ CD4+

Mock depletion

CD25 depletion

CD25 depletion results in decreased levels of FoxP3+ $T_{\text{reg}}$
Cytokine production by S. Typhi-specific CD8+ T_{EM} is higher in the absence of T_{reg} indicating that T_{reg} may functionally suppress S. Typhi-specific responses
S. Typhi-specific expression of integrin $\alpha4\beta7$ is up-regulated pre-challenge in TD volunteers

Down-regulation of S. Typhi-specific expression of integrin $\alpha4\beta7$ on circulating $T_{\text{reg}}$ occurs post-challenge in TD volunteers

- Higher levels of S. Typhi-specific gut homing potential may result in accumulation of $T_{\text{reg}}$ in the local gut environment resulting in suppression of protective pro-inflammatory responses and TD
T regulatory cells: Conclusions (II)

- S. Typhi-specific expression of activation molecules is increased in TD volunteers
- $T_{\text{reg}}$ are capable of functionally suppressing S. Typhi-specific responses
- $T_{\text{reg}}$ suppress lymphocytes with gut homing potential as well as those that are likely to remain in the periphery
Activation of *Salmonella* Typhi-Specific Regulatory T Cells in Typhoid Disease in a Wild-Type S. Typhi Challenge Model

Monica A. McArthur¹, Stephanie Fresnay¹, Laurence S. Magder², Thomas C. Darton³, Claire Jones³, Claire S. Waddington³, Christoph J. Blohmke³, Gordon Dougan⁴, Brian Angus⁵, Myron M. Levine¹, Andrew J. Pollard³, Marcelo B. Sztein¹*
Balancing the immune response

- Effector T cell responses
- Regulatory T cell responses

Homeostasis (protection?)

- Effector T cell responses
- Regulatory T cell responses

Ineffective protection

Inflammation
Balancing the immune response

**Effector T cell responses**
- CD4+ [Th1, Th2, Th9, Th17, Th1/17, Th22]
- CD8+ [Tc1, Tc2, CTL, conv Fox-P3dim T, others]
- MF, which concomitant cytokines?

**Regulatory T cell responses**
- Treg [FoxP3+ CD25hi CD152+]
- eTreg (CD45RA-), nTreg (CD45RA+), Tr1, Tr3, Th3, Th1/17-10, others
- MF, which concomitant cytokines?

**Humoral immunity**
- APC (Mφ, DC, B) & other cells that bridge adaptive and innate immunity (e.g., MAIT, Tγδ)

Host HLA and other genetic factors, nutrition, microbiota, etc
S. Typhi appears to be such an effective pathogen, at least in part, by being exquisitely stealth

Thus, identifying the effective immunological CoP and their kinetics and homing among a multitude of non-protective or downregulatory immune responses might hold the key for the development of more effective attenuated enteric fever vaccines
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