Recent advances in the identification of immunological correlates of protection in a human S. Typhi challenge model

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10th International Conference on Typhoid and Other Invasive Salmonelloses

April 5, 2017 - Kampala, Uganda
Vaccine Development
The never ending search for the optimal balance

Reactogenicity

Immunogenicity

What responses?
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.
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)
  - 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**
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?
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) and other enteric infections (e.g., *Shigella*, ETEC)
To answer this question, and to better understand typhoid disease, we initiated a collaboration with Dr. Pollard and his team at Oxford who have re-established a human challenge model with wild-type S. Typhi (Quailes strain)
Goals of the wt S. Typhi human challenge model

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

Variation in genomic response

Diagnostics
- PCR-based
- Mass spectrometry

Correlates of protection
Oxford human wild-type S. Typhi challenge studies (Quailes strain)

Memory & effector T cells
S. Typhi-responsive CD8+ $T_{EM}$ responses: Baseline

EBV, AEH & Blasts combined (low dose)

Higher baseline responses (pre-challenge) are associated with protection
Marked decreases were observed in S. Typhi-specific $T_{EM}$ expressing CD107a and producing cytokines 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
Oxford human wild-type *S. Typhi* challenge studies (Quailes strain)

Regulatory T cells
**Gut homing of circulating S. Typhi-responsive T\textsubscript{reg}**

- **Gated on T\textsubscript{reg} (CD4\textsuperscript{+} FoxP3\textsuperscript{+} CD25\textsuperscript{hi})**

  ![Graph showing the net percentage of integrin $\alpha4\beta7$ expression over days post-challenge for TD and No TD volunteers.](image)

- **S. Typhi-responsive expression of integrin $\alpha4\beta7$ is up-regulated on circulating Treg pre-challenge in TD volunteers indicating possible association with disease**

- **Down-regulation of S. Typhi-responsive integrin $\alpha4\beta7$ expression on Treg post-challenge in TD volunteers indicates potential homing to the gut**

  * $p \leq 0.05$ (TD vs No TD)
  # $p \leq 0.05$ (time-points)
T regulatory cells: Conclusions

- S. Typhi-specific expression of integrin $\alpha 4\beta 7$ is up-regulated pre-challenge in TD volunteers

- S. Typhi-specific expression of activation molecules is increased in TD volunteers

- $T_{\text{reg}}$ are capable of functionally suppressing S. Typhi-specific responses
Oxford human wild-type S. Typhi challenge studies (Quailes strain)

Antigen Presenting Cells (monocytes, dendritic cells)
Changes in CD38 and CD40 expression was up-regulated in monocytes only in TD subjects.

* p = 0.05; ** p = 0.005
Changes in integrin $\alpha 4\beta 7$ expression

Only monocytes isolated from TD volunteers showed up-regulation of integrin $\alpha 4\beta 7$

** p=0.005
Monocytes binding to S. Typhi

- In TD volunteers a spike in S. Typhi binding was detected during disease days (2 of 4 volunteers)
- In NoTD volunteers a spike in S. Typhi binding was also noted; however, it was present immediately after challenge (D1)
- A MAIT cell paper is In Press (Front. Immunol.)
- Correlation of all data to evaluate whether a single response can be a CoP (best case scenario) or a combination of immune responses is more indicative of short and long-term protection than individual responses
Balancing the immune response

Effector T cell responses

Regulatory T cell responses

Homeostasis (protection?)

Effector T cell responses

Regulatory T cell responses

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-3dim T, others]

**Regulatory T cell responses**

- Treg [FoxP3+, CD25hi CD152+]
- eTreg (CD45RA-), nTreg (CD45RA+), Tr1, Tr3, Th3, Th1/17-10, others

**Homeostasis (protection?)**

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

**Humoral immunity**

- MF, which concomitant cytokines?

**Inflammation**

- Host HLA and other genetic factors, nutrition, microbiota, etc
Overall Conclusion

Identifying **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 new generations of more effective vaccines against infectious diseases.
Acknowledgements

Center Vaccine Development, UMB
Immunology Group
Stephanie Fresnay (Teff/mem)
Monica McArthur (Treg)
Franklin Toapanta (B, APC)
Rosangela Mezghanni (MAIT)
Jay Booth
Rezwan Wahid
Paula Bernal
Mark Rudolph
Cathy Storrer
Regina Harley
Haiyan Chen

Dept. Epidemiology, Biostatistics, UMB
Larry Magder

UK Collaborators
Andrew J Pollard
Claire Jones
Claire Waddington
Thomas Darton
Christoph Blohmke
Gordon Dougan
Jeremy Farrar
Paul Langford
Brian Angus
Derrick Crook
Stephen Lockhart

Clinical studies
Myron Levine

Supported by grants U19 AI082655 (UM-CCHI) & R01 AI-36525 from the NIH and by the Wellcome Trust