Identification of *In Vivo*-Induced Protein Antigens of *Salmonella enterica* serovar Typhi

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Introduction: Salmonellae of mice and humans

- *S. enterica* is comprised of over 2000 serovars.
  - Non-human pathogens
  - Broad-host-range pathogens causing gastroenteritis in humans (e.g. serovar Typhimurium)
  - Human specific pathogens (e.g. serovar Typhi)
- Serovar Typhi causes serious systemic illness--typhoid fever; a chronic infection of macrophages.
  - Fever is the dominant characteristic, often no diarrhea.
  - 20 million cases per year. Increasing antibiotic resistance.
- Most of our understanding of typhoid fever is based on the mouse model (serovar Typhimurium).
- Significant genetic and clinical differences between Typhi and Typhimurium (10-15% of ORFs).
Introduction: IVIAT

• To study serovar Typhi infection directly in the human host we applied IVIAT – *In Vivo* Induced *Antigen* *Technology*

• IVIAT is a screening method to identify immunogenic proteins expressed by microbial pathogens during infection that are *NOT* expressed during *in vitro* growth.

• We applied IVIAT to *S. enterica* serovar Typhi with a goal of identifying proteins involved in pathogenesis, as well as proteins with potential utility in the diagnosis, treatment and prevention of typhoid fever.
IVIAT Method Overview

**DNA Expression Library**
- Genomic DNA
- Ligate
- Inducible expression vector
- Expression library in *E. coli*
- Replicate library on medium with IPTG
- Purify plasmid
- Sequence Insert
- Identify and characterize IVIAT antigen

**Sera Preparation**
- Pooled patient sera
- Microorganism grown *in vitro*
- Extensive adsorption process
- Probe expression library with adsorbed sera

**IVIAT Method Overview**

- DNA Expression Library
- Sera Preparation
150,000 clone library, derived from serovar Typhi CT18 genomic DNA and large plasmid DNA, screened with pooled adsorbed convalescent serum from 8 bacteremic patients. Reactive clones were then screened in triplicate. 58 robustly positive clones identified. These clones contain 47 expressible genes on 34 unique inserts. Tertiary screening was performed by cloning selected full length ORFs into the expression vector and comparing to a no insert control. A total of 35 IVIAT antigens were identified in this screen.
IVIAT Antigens

- A total of 35 IVIAT antigens identified (<1% of the genome)
- 10 identified in multiple inserts
- 5 plasmid-encoded antigens
  - 4 from MDR plasmid, 1 from cryptic plasmid
- 3 genes encoded on CT18 chromosome had no homolog in serovar Typhimurium
- Functional classification assigned to 25 proteins
## Functional Classification of 35 IVIAT Antigens

<table>
<thead>
<tr>
<th>Function</th>
<th>Number of IVIAT Antigens</th>
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<td>Pilus structure and biogenesis</td>
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<tr>
<td>Antimicrobial resistance</td>
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<td>Other pathogenesis</td>
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<td>Heavy metal transport</td>
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<td>Other extracellular transport and binding</td>
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<td>Metabolic and regulatory function</td>
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Quaternary Screen of IVIAT Antigens: Cross-reactivity with Control Sera

Of the 35 IVIAT antigens identified, 4 exhibited no cross-reactivity with sera from population in which serovar Typhi is not endemic, indicating these antigens are specific to infection with serovar Typhi.
Evaluation of IVIAT Antigens (Cont.)

- The four serovar Typhi-specific IVIAT antigens included:
  - **PagC**: A well-studied virulence factor conserved across *S. enterica* serovars and required for survival in macrophage phagosomes
  - **TcfB**: The major structural subunit of a fimbrial operon termed Typhi colonization factor, encoded on SPI 6, 37% homology to CooA (ETEC), but no homolog in Typhimurium
  - **STY0860**: Conserved across serovars
  - **STY3683**: No known homologs in Salmonellae
## Evaluation of IVIAT Antigens: TcfB

### Table 1

Distribution of known fimbrial operons among all (sub)species of *Salmonella* and a number of serotypes of *Salmonella enterica* subspecies I, as determined by Southern hybridization.

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- The genes encoding TCF are not found in serovar Typhimurium and are restricted to relatively few serovars compared to other Typhi fimbrial operons.

Evaluation of IVIAT Antigens: TcfB

Anti-TcfB IgG responses after clinical infection with V. cholerae or serovar Typhi
Evaluation of IVIAT Antigens: PagC

- **pagC** is a conserved virulence factor required for survival in the macrophage but its exact function is unknown.
- **pagC** is not expressed during *in vitro* growth, but is highly expressed in macrophages in serovar Typhimurium.
- PagC is not a known immunogen.
- To further characterize immune responses to PagC we compared acute and convalescent IgG responses by Western blot analysis in individual patients with serovar Typhi bacteremia from Dhaka, Bangladesh.
Evaluation of IVIAT Antigens: PagC

Responses to PagC in paired serum samples from patients infected with serovar Typhi and V. cholerae
Conclusions

• We identified 35 potential *in vivo*-expressed antigens using IVIAT; four of these had no discernable cross-reactivity.

• The validity of IVIAT is supported by the identification of PagC, a known *in vivo*-expressed protein, as immunogenic in 11/14 patients with serovar Typhi bacteremia.

• Our data provide evidence that TcfB is expressed and immunogenic in human infection, and support the hypothesis that TCF may play a role in the host specificity of serovar Typhi.

• The potential role of the IVIAT antigens as diagnostic, therapeutic or vaccine targets warrants further study.
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