Identification of immunogenic *Salmonella enterica* serotype Typhi antigens expressed uniquely *in vivo* in chronic biliary carriers of S. Typhi in Kathmandu, Nepal

Enteric Fever

• Enteric fever is caused primarily by *Salmonella enterica* serovar Typhi and Paratyphi A

• They are both human restricted pathogen that causes an acute illness characterized by high fever, malaise, and abdominal pain.

• Endemic throughout the Asian and African continent

• There are 21 million cases per year resulting in 200,000 deaths
Pathogenesis

Typhi carriers

- 1-3% of infected individuals develop chronic infection in the gall bladder which may persist for decades

- May be reservoirs of infection within a community

- May contribute to transmission of infection

- May act as vehicles for introducing S. Typhi or S. Paratyphi A into previously uninfected communities.
Typhi Diagnostics - chronic carriers

- Microbiologic culture
  - stool – intermittent shedding

- Antibody detection of capsular Vi antigen
  - anti-Vi antibody titers a sensitivity 75% and specificity >95%.
  - In Vietnam 3000 potential carries screened.
    - 3% with positive test
    - no S. Typhi could be isolated from fecal samples
Identification of biomarkers for S. Typhi carriage

• We applied an immunoscreening technique, *in vivo*-induced antigen technology (IVIAT) to identify potential biomarkers unique to S. Typhi chronic carriers.

• Hypothesis: S. Typhi surviving in the biliary tract of humans may express a proteomic profile distinct from that expressed in bacteria grown using standard *in vitro* conditions.
Genomic Inducible Expression Library

- *Salmonella enterica* serovar Typhi CT18 (5133713 bp, 4753 genes)
  - Chromosome 4,809,037 bp
  - 2 plasmids
    - pHCM1 218,150 bp
    - pHCM2 106,516 bp
- Library size: 120,000 clones (500-1500 bp fragments) in *E. coli* BL21DE3
Sample Collection

- Individuals undergoing elective cholecystectomy in Nepal were enrolled
  - At the time of cholecystectomy
    - venous blood sample taken and stored
    - bile sample was taken for microbiologic analysis
Sera adsorption

- **Selected sera:**
  - pool of 5 patients with bile cultures positive for S. Typhi
- **Sera adsorbed against the following samples**
  - S. Typhi whole cell
  - S. Typhi lysate
  - *E. coli BL21 DE3* (with empty vector, pet30c) whole cell
  - *E. coli BL21 DE3* (with empty vector, pet30c) lysate
Screening - Immunoblots

2. Incubate colonies on LB/Kan plate + IPTG 37°C for 3 hrs.
3. Transfer colonies to nitrocellulose membrane.
4. Incubate with primary and secondary antibody.
5. Develop Immunoblot.

120,000 clones screened
268 genes
Screening - Immunoblots

268 genes

56 genes

13 genes

S. Typhi Gene

No Insert

Typhi Carrier Sera

S. Typhi Gene

No Insert

Healthy control Sera

S. Typhi Gene

No insert
## Top 13 Hits

<table>
<thead>
<tr>
<th>STY Locus</th>
<th>Gene Name</th>
<th>Sequence</th>
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<tr>
<td>STY1364</td>
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<td>hypothetical periplasmic protein</td>
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<td>xapB</td>
<td>xanthosine permease</td>
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<td>HCM1.137</td>
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<td>STY2386</td>
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<td>putative lipoprotein</td>
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<td>Putative transposase</td>
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<td>STY0712</td>
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<td>haemolysin-related protein</td>
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</table>
YncE (STY1479) response

**A)** YncE-IgG

**B)** YncE-IgA

ELISA Units

- Typhi carrier
- PTA carrier
- GB control
- HB control
- Typhi acute
- Typhi conv

- Typhi carrier
- PTA carrier
- GB control
- HB control
- Typhi acute
- Typhi conv

**p-values:**
- YncE-IgG:
  - p=0.0266
  - p=0.0044
  - p=0.0005
  - p=0.0205
  - p=0.22

- YncE-IgA:
  - p=0.4598
  - p=0.2011
  - p=0.2370
  - p=0.8112
Vi Antigen Response

A) Vi-IgG

B) Vi-IgA

Typhi carrier

PTA carrier

GB control

HB control

Typhi conv

Typhi conv

Typhi conv

Typhi conv
Conclusion

- We have identified a number of immunoreactive antigen in S. Typhi carriers, including YncE.

- Further evaluation of YncE and other antigens could lead to development of an improved diagnostic assay and improved understanding of S. Typhi’s survival within the biliary tract of carriers
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