Typhoid fever in Bangladesh: from infection to protection

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Outline of this talk

• Understanding the pathogens using high throughput techniques
• Immune responses in natural infections
• Diagnosis of typhoid and paratyphoid fever
• Prevention and vaccines
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Understanding the pathogens using high throughput techniques

• Diagnosis of typhoid and paratyphoid fever
• Prevention and vaccines
High throughput microarray techniques to provide insight into the bacterial adaptation and modifications that may need to survive within infected humans and for identifying novel antigens and virulence factors.

Do bacteria produce novel factors during \textit{in vivo} growth?

Can high throughput DNA microarray and proteomics give better insight into the mechanism?
Detection/analysis of captured products by
Selective Capture of Transcribed Sequences (SCOTS)

- Capture transcribed sequence and amplification
- Microarray
- Real-time PCR

Composite array of an in vivo specimen

Expression of mRNAs for 2,046 *S. Typhi* genes (44% of the *S. Typhi* genome) in human blood - **25 genes in vivo only**

1,798 *S. Paratyphi A* mRNAs expressed in the blood of infected humans (43.9% of the ORFeome) - **41 genes in vivo only**

- *In vivo* expression of *Salmonella enterica* serotype Typhi genes in the blood of patients with typhoid fever in Bangladesh
- Analysis of *Salmonella enterica* serotype Paratyphi A gene expression in the blood of bacteremic patients in Bangladesh

Using a high throughput immunoscreening technique, *in vivo*-induced antigen technology (IVIAT), have identified subsets of immunogenic bacterial proteins expressed in infected humans-absorbed sera from patients used to screen genome library of *S*.Typhi/*S*.Paratyphi

- 35 proteins in *S*.Typhi and 20 proteins in *S*. Paratyphi A infections

Immunoproteomic analysis and Mass Spectrometry- 57 proteins of which HlyE is important
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Recent infections can be detected in secretions from circulating lymphocytes

Activated mucosal lymphocytes migrate from intestinal tissue and circulate within peripheral blood before rehoming to mucosal tissues.

This migration peaks around 5-7 days after intestinal infection.

The immune response can be measured from peripheral blood mononuclear cells (PBMC) using lymphocyte secretions.
Membrane protein specific-IgA responses in lymphocyte secretions

- S. Typhi bacteremic young children had similar MP-IgA responses as older kids and adults at early stage of the disease (day 3-7 of onset of fever).
- This response thus can be used as a marker of active infection even in young children (~6-9 months of age).
- Lower S. Typhi specific MP-IgA in young children in plasma.

Poster P20 Farhana Khanam

Khanam et al. 2013, 2015
Antigens detected by high throughput techniques are capable of stimulating Interferon-γ responses to S. Typhi infection.
Immune response to HlyE antigen

Tested using lymphocyte secretions from patients
Outline of this talk

• Understanding the disease using high throughput techniques
• Immune responses in natural infections

Diagnosis of typhoid and paratyphoid fever

• Prevention and vaccines
**TPTest**, a diagnostic method for early diagnosis of enteric fever

**Our existing method:**

1. **Density gradient centrifugation for separation of peripheral blood lymphocytes**
2. **37°C incubator with a constant 5% CO₂ supply**
3. **ELISA reader**
The method is useful for diagnosis of patients with enteric fever caused by both S. Typhi and S. Paratyphi fever.

**TPTest (Typhoid and Paratyphoid Test)**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. of individuals</th>
<th>TPTest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Patients with <em>S. Typhi</em> bacteremia</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>Patients with <em>S. Paratyphi A</em> bacteremia</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Clinically suspected enteric fever but blood culture negative</td>
<td>204</td>
<td>44</td>
</tr>
</tbody>
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The sensitivity and specificity of the TPTest is **100% and 78-97%** respectively.

Specificity based on the definition of the true negative using blood culture for comparison.

Specimens from patients with other febrile illnesses also tested.

Test compared with other available diagnostic kits - Tubex, Typhidot.

Latent class modelling - 96% specific and sensitive (Jason Andrews, Stanford University)
The TPT test is being used in a nationwide disease surveillance as well as in the Clinical Diagnostic Services at the icddr,b for outpatients.
We have evaluated a simplified cell separation procedure i.e. cell separation by RBC lysis- Heparinized blood treated with NH4Cl

Incubation of the cell culture in incubator at 37°C without the supply of 5% CO₂

We are currently working on developing a diagnostic test to make the TPTest more applicable for field settings- ELISA, dot blot, Immunochromatography (ICT)

Farhana et al. 2013 ongoing
Specimens from healthy controls and patients with enteric fever and other febrile illness tested

<table>
<thead>
<tr>
<th>Healthy Control</th>
<th>Bacillus</th>
<th>Micrococcus</th>
<th>Strep</th>
<th>V.chol</th>
<th>TB</th>
<th>Kala-azar</th>
<th>Dengue</th>
<th>S.Typhi Positive</th>
</tr>
</thead>
</table>

Results of Strip Test

Collaboration Incepta and icddr,b

POSTER P17- Islam et al
Immunoproteomic analysis of lymphocyte secretions of S. Typhi infected patients

- Salmonella protein arrays –
  » ~ 2000 proteins including membrane proteins and others predicted by software to be potentially immunogenic

- Arrays probed- **49 antigens detected**
  - Typhoid Positive samples, n=10
  - Healthy controls, n=5
  - Other febrile illness, n=5

**Collaboration P. Felgner** (Univ California)

Charles et al. 2014
Summary of diagnostics

• Using blood specimens, a highly specific and sensitive technique has been optimized for diagnosis of patients with enteric fever

• Simplified lymphocyte extraction and culture followed by Rapid ICT diagnostics developed and being commercialized
Outline of this talk

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• Immune responses in natural infections
• Diagnosis

Prevention and vaccines
Vaccination is an effective public health tool and an effective short term preventive measure

Vi capsular polysaccharide vaccine

Vi polysaccharide given as a single intramuscular dose has been found to be effective in reducing burden of typhoid fever in endemic settings in Pakistan, Nepal, India, China and Vietnam. Protection is better in older compared to younger children. The vaccine is licensed for those > 2 years and above. A booster dose is required every two to three years.
Typhoid Vaccines

Vi conjugate vaccines

**Vi-DT/Vi-TT** conjugate vaccine: Diphtheria toxoid or tetanus toxoid conjugated with Vi polysaccharide

**Vi-rEPA**-conjugated to capsular polysaccharide of *Salmonella typhi*

**Bharat Biotech**- Typbar-TCV- Licensed Typhoid conjugate vaccine

**BioFarma**- Vi-DT

**Bivalent Typhi/Paratyphi vaccines**- Vi-CRM197-Novartis/Biologics E

Live oral attenuated strains

**Ty21a- Vivotif**- Vaccine recommended for those 5 years and above in age
Studies on Ty21A as a vaccine for young children and infants

Vivotif, the oral typhoid vaccine is formulated in capsules and licensed for use in older children and adults

Improving immune responses to oral typhoid vaccine in children 2-5 years of age

Vaccine as a liquid formulation for intake by young children
Immune responses to Ty21A in young children vaccinated with the liquid formulation of Ty21a

**IgA Plasma antibody responses seen in children 2-5 years old:**
Treatment with antiparasitic drugs did not improve responses

↑Responses in ALS specimens

Both mucosal IgA and systemic responses generated

Children, 2 years of age mount T and B cell response

Vaccination induced both antigen specific proliferation and cytokine responses - IFN-γ >IL-13 indicating a TH1 response

Taufiq et al. 2014
Status of Typhoid Vaccine Availability in Bangladesh

• Vaxphoid- Vi-PS vaccine manufactured in Bangladesh

• Development of Typhoid Conjugate Vaccine with the assistance of International Vaccine Institute (IVI), Korea
  Purified Vi polysaccharide conjugated with Diptheria Toxoid (DT)

• This vaccine is awaiting preclinical studies, following which Phase I-III trials will be carried out
Conclusions

• We are taking an all rounded approach to better understand immunological responses to S. Typhi and S. Paratyphi infections; improved diagnostics and studies on vaccines

• Novel genes/antigens and immunological factors determined from high throughput studies will help formulate better diagnostics and vaccines

• Studies and protective interventions using vaccines as they become available are needed in our settings

• Working together in field of enteric fever through the CAT network has helped form a consortium in the field and we look forward to more collaborations
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