Establishment of an intragastric challenge model for wild-type Shigella dysenteriae-1

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AFRIMS Mission

Medical research, disease surveillance, and development & evaluation of medical products for tropical infectious diseases
Shigellosis

- Shigellosis is endemic in developing countries
- Shigella dysenteriae-1 (Shiga bacillus), is the most virulent of the four serogroups of Shigella
- Shigella dysenteriae-1 is the cause of epidemic dysentery
- Shigella dysenteriae-1 cases in developing country/refugee populations are often fatal
- Due to the presence of the potent Shiga toxin, S. dysenteriae-1 infection complications include hemolytic-uremic syndrome, seizures, sensis, rectal prolapse, and toxic megacolon
S. dysenteriae-1 Vaccine

• An effective S. dysenteriae-1 vaccine could:
  – Prevent outbreaks in refugee and disaster settings from contaminated food and water
  – Preclude the need for new antimicrobials to treat multi-drug resistant species of S. dysenteriae-1

• WRSd1 is an oral live attenuated vaccine
  – Derived from S. dysenteriae-1 parent strain 1617
  – Genes necessary for inter- and intracellular spread virG(icsA) and for Shiga toxin (stx) are deleted
  – Has significant side effects in early human trials making further development unlikely
Animal Models

• Animal model required for the required pre-clinical evaluation of the vaccine candidates
• Rhesus monkeys and other primates are naturally susceptible to intestinal infections with Shigella
• Shigellosis in non-human primates closely mimics the disease and immune response seen in humans
• Availability of multiple biological samples (blood, colonic mucosal tissue, colonic lavage, and stool) helpful
Objectives

• Determine optimal dose of S. dysenteriae-1 1617 strain required to establish dysentery in the nonhuman primates (rhesus monkey)
• Document reactogenicity and immune responses elicited by the optimal dose of S. dysenteriae-1 1617 strain
• Demonstrate that previous infection with 1617 strain can protect monkeys against subsequent challenge with the same organism
Study Design

- 20 monkeys (4 groups of 5 each) assigned to one of four groups.
- Optimal challenge dose will reproducibly produce dysentery in 3-4 monkeys of 5
- Escalating doses - $2 \times 10^8$ cfu (group-1 monkeys), $2 \times 10^9$ cfu (group-2 monkeys), $2 \times 10^{10}$ cfu (group-3 monkeys) with repeat at optimal dose
• Optimal dose monkeys are re-challenged with the same dose after one month to evaluate any protectivity elicited by the first challenge.
• At the same time group-4 monkeys are challenged with the same dose preparation.
• Group-4 monkeys will be re-challenged after one month of challenge (similar to optimal dose monkeys).
• Total of 10 monkeys are challenged with the optimal dose and re-challenged after one month to evaluate reproducibility & protection.
## Preliminary Results

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (cfu)</th>
<th>Significant Clinical Signs (5 monkeys in each group)</th>
<th>Change in CBC</th>
<th>Histopathology with Acute inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$2 \times 10^8$</td>
<td>None with dysentery 4 with transient anorexia</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>$2 \times 10^9$</td>
<td>2 with dysentery 4 with transient anorexia 4 with reduced activity</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>$2 \times 10^{10}$</td>
<td>2 with dysentery 4 with transient anorexia 3 with reduced activity</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>$2 \times 10^{10}$ (re-challenge)</td>
<td><strong>2 monkeys with soft stool probably will get dysentery</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>$2 \times 10^{10}$</td>
<td><strong>1 monkey died 4 monkeys developed dysentery</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Dysentery = Loose or watery stool with blood and mucus*  
CBC = complete blood cell count; Change in CBC = Neutrophilia with left shift and toxic change  
** result until day-3 post challenge
Colonic biopsy with normal features

(before challenge)

(3 days post challenge)

**Acute neutrophilic colitis** (moderate) with crypt abscess (increased numbers of neutrophils, decreased numbers of goblet cells and an increase in mitotic figures)
One monkey of group-4 (challenged with $2 \times 10^{10}$ cfu) died in the evening of challenge day.

Only blood & mucus as feces
Inflamed intestine with bloody fluid in ileum (possibly effect of Shiga toxin)
Plasma Antibody Responses

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (cfu)</th>
<th>IgA antibody titers</th>
<th>IgG antibody titers</th>
<th>IgM antibody titers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LPS Invaplex</td>
<td>LPS Invaplex</td>
<td>LPS Invaplex</td>
</tr>
<tr>
<td>1</td>
<td>$2 \times 10^8$</td>
<td>5 4</td>
<td>8 67</td>
<td>8 5</td>
</tr>
<tr>
<td>2</td>
<td>$2 \times 10^9$</td>
<td>7 6</td>
<td>90 192</td>
<td>14 24</td>
</tr>
<tr>
<td>3</td>
<td>$2 \times 10^{10}$</td>
<td>5 9</td>
<td>43 368</td>
<td>4 8</td>
</tr>
</tbody>
</table>

Fold increase (average of 5 monkeys) of antibody titers against *Shigella* antigens in plasma samples from convalescent phase compared to baseline plasma samples.
## Fecal Cytokine Levels

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Study Day</th>
<th>Group-1</th>
<th>Group-2</th>
<th>Group-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1 beta</td>
<td>Day-0</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>Day-2</td>
<td>19 (43)</td>
<td>111 (165)</td>
<td>3 (8)</td>
</tr>
<tr>
<td></td>
<td>Day-5</td>
<td>129 (259)</td>
<td>195 (303)</td>
<td>48 (82)</td>
</tr>
<tr>
<td></td>
<td>Day-7</td>
<td>111 (194)</td>
<td>85 (88)</td>
<td>64 (84)</td>
</tr>
<tr>
<td>IL-6</td>
<td>Day-0</td>
<td>2 (2)</td>
<td>2 (1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>Day-2</td>
<td>2 (1)</td>
<td>72 (9157)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>Day-5</td>
<td>29 (59)</td>
<td>162 (257)</td>
<td>32 (67)</td>
</tr>
<tr>
<td></td>
<td>Day-7</td>
<td>5 (11)</td>
<td>27 (60)</td>
<td>227 (436)</td>
</tr>
<tr>
<td>IL-8</td>
<td>Day-0</td>
<td>1 (1)</td>
<td>4 (9)</td>
<td>7 (7)</td>
</tr>
<tr>
<td></td>
<td>Day-2</td>
<td>13 (29)</td>
<td>164 (260)</td>
<td>11 (19)</td>
</tr>
<tr>
<td></td>
<td>Day-5</td>
<td>449 (936)</td>
<td>1455 (1842)</td>
<td>440 (910)</td>
</tr>
<tr>
<td></td>
<td>Day-7</td>
<td>186 (378)</td>
<td>132 (226)</td>
<td>787 (1445)</td>
</tr>
</tbody>
</table>
Fecal IgA Responses

Fecal IgA titers against *S. dysenteriae* 1 LPS
Pending Results

- Analysis of antibody secreting cells by ELISPOT assay
- Analysis of expression of different cytokines in gut tissue samples at m-RNA levels by Real-Time PCR assay
- Immunohistochemical staining of frozen gut tissue sections for: i) identification of recruitment of different immune cells in gut tissue samples and ii) infiltration of other inflammatory mediators (myeloperoxidase and lactoferrin)
Future Plans

• Complete lab assays on samples from current studies
• Evaluate candidate S. dysenteriae-1 vaccines as available
• Compare candidate S. dysenteriae-1 vaccines to existing vaccine WRSd1
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