Shigella Pathogenesis and Vaccine Development

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Causes of Travelers’ Diarrhea

Etiology:

Bacteria: Responsible for 50-75% of the cases of travelers’ diarrhea in travelers and military personnel.

- Enterotoxigenic *E. coli* (ETEC) (5-40%)
- *Shigella* spp. (2-10%)
- *Campylobacter jejuni* (3-45%)
- *Salmonella* spp. (non-typhoid)
- *Plesiomonas*
- *Aeromonas* spp.
- *Vibrio cholerae* and non-cholera spp.

Viruses

Parasites

Shigella Disease Burden and Epidemiology

Genus: *Shigella*
Species or Serogroup: *flexneri, sonnei, dysenteriae, boydi*

Risk of shigellosis per 100,000 travelers’ to different regions of the world

Shigellosis

Food and water borne disease transmitted by the fecal oral route

Fecal-oral transmission

As little as 100-1000 bacteria can reproducibly cause disease!

Baron S. Medical Microbiology, 4th Edition, The University of Texas Medical Branch at Galveston, 1996.
Immune Response and Vaccine Approaches to Infection with Shigella

Innate immune response:
The initial inflammatory response induced by the release of IL-1β, IL-6, TNF-α and IFN-γ from infiltrating PMNs, necrotic macrophages and other lymphocytes.

Cellular immune response:
Although IFN-γ and IL-10 are specifically elevated in patients 21 days after experimental infection T cell responses still remain ill defined.

Humoral immune response:
Most evidence points towards serotype-specific immunity. Natural infections induce serum (IgA and IgG) and mucosal (sIgA) immune responses to O-antigen polysaccharide (LPS) as well as TTSS-associated invasion proteins (IPAs).
Immune Response and Vaccine Approaches to Infection with *Shigella*

**Subcellular vaccines:**

- **Invaplex:**
  
  Combination of invasion proteins IpaB and IpaC & LPS.
  
  Phase I clinical trial for safety and immunogenicity (WRAIR)

- **LPS conjugate vaccines:**
  
  Phase III trials (SHIGELLVAC)
  
  Preclinical (EndoBiologics International corp. & WRAIR)

**Non-living or inactivated whole-cell vaccines:**

* S. sonnei vaccine recently been published

**Live attenuated vaccines strains:**

Deletion of virulence genes and metabolic genes

- Center for Vaccine Development (CVD)
- WRAIR (SC602, WRSS1, WRSd1)
Recent Live Attenuated *Shigella* Vaccines Tested in Human Clinical Trials

**CVD**

Aromatic and enterotoxin attenuation

- **CVD1204** (ΔguaBA)
- **CVD1208** (ΔguaBA, ΔshET2-1, ΔshET1)

**WRAIR**

virG-based attenuation

- **SC602** (ΔvirG, Δiuc)
- **WRSS1** (ΔvirG)
- **WRSd1** (ΔvirG, ΔstxA, Δfnr)
Symptoms of Shigellosis Associated with Live Vaccines

Macrophages, DC, T and B cells

Enterocyte

Toxins

2457T

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Symptoms of Shigellosis Associated with Live Vaccines

Significant protection from experimental challenge!

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Symptoms of Shigellosis Associated with Live Vaccines

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Symptoms of Shigellosis Associated with Live Vaccines

<table>
<thead>
<tr>
<th>Strain</th>
<th>Fever</th>
<th>Diarrhea</th>
<th>Dysentery</th>
</tr>
</thead>
<tbody>
<tr>
<td>2457T</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SC602</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CVD1204</td>
<td></td>
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<tr>
<td>CVD1208</td>
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<table>
<thead>
<tr>
<th>% with symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
</tr>
</tbody>
</table>

Toxins

Macrophages, DC, T and B cells
Symptoms of Shigellosis Associated with Live Vaccines

Toxins

Macrophages, DC, T and B cells

2457T
SC602
CVD1204
CVD1208
53G

% with symptoms

Fever
Diarrhea
Dysentery

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Symptoms of Shigellosis Associated with Live Vaccines

Toxins

Macrophages, DC, T and B cells

enterocyte

2457T
SC602
CVD1204
CVD1208
53G
WRSS1

% with symptoms

Fever
Diarrhea
Dysentery
Symptoms of Shigellosis Associated with Live Vaccines

Conclusions:

virG based vaccines given at low oral doses are safe immunogenic and effective against dysentery

Enterotoxins present in Shigella contribute to reactogenicity associated with live vaccines
Second Generation *Shigella* vaccines at WRAIR

Principle attenuating lesion is $\Delta virG$

- Diarrhea:
  - Enterotoxin ($shet1$)
  - Enterotoxin ($shet2-1$)
  - Enterotoxin ($shet2-2$)
  - Autotransporter ($sigA$)

- Fever:
  - LPS or lipid A ($msbB$)

= Regulatory concerns
Construction of Second Generation Live *Shigella* Vaccine Strains Using Linear (PCR) DNA-Mutagenesis

Genomic DNA - Linear DNA preparation

Transformation

Transformation + FLP out

Generation of vaccine strains with up to 4 gene deletions
Construction of Second Generation *Shigella* vaccines at WRAIR

- 2457T (wild-type)
- WRSf2G(Δ*virG*)
- WRSf2G10(Δ*virG*, Δ*shET2*-1)
- WRSf2G11(Δ*virG*, Δ*sheET2*-1, Δ*shET1*)
2nd Generation Preclinical Bridging Study

<table>
<thead>
<tr>
<th>Group (# Guinea Pigs)</th>
<th>Vaccine Strain</th>
<th>Dose</th>
<th>Route</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (13)</td>
<td>Naïve</td>
<td>NA</td>
<td>IN</td>
</tr>
<tr>
<td>2 (8)</td>
<td>SC602</td>
<td>$5 \times 10^7$</td>
<td>IN</td>
</tr>
<tr>
<td>3 (8)</td>
<td>SC602</td>
<td>$5 \times 10^8$</td>
<td>IN</td>
</tr>
<tr>
<td>4 (8)</td>
<td>WRSf2G11</td>
<td>$5 \times 10^7$</td>
<td>IN</td>
</tr>
<tr>
<td>5 (8)</td>
<td>WRSf2G11</td>
<td>$5 \times 10^8$</td>
<td>IN</td>
</tr>
</tbody>
</table>

Schedule:

- **D0**: Immunize Serum mucosal wash
- **D14**: Immunize Serum, mucosal wash
- **D28**: Serum, mucosal wash
- **D49**: Challenge

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Summary of 2nd Generation Bridging Studies

Results from Guinea Pig Immunogenicity and Challenge Studies:

• Serum immune responses to LPS and IPAs are comparable in both strains.

• Mucosal responses to LPS are comparable in both strains.

• Protection from wild-type challenge is comparable to SC602 in two separate preclinical guinea pig studies.
Second Generation *Shigella* vaccines at WRAIR

Principle attenuating lesion is \(\Delta{\text{virG}}\)

- **Diarrhea**
  - Shet1
  - Shet2-1
  - Shet2-2
  - sigA

- **Fever (inflammation)**
  - LPS or lipid A (msbB1)
  - LPS or lipid A (msbB2)
Lipid A as a Virulence Factor and Mediator of Inflammation and Tissue Destruction in Shigellosis

- *Shigella* has two copies of the *msbB* gene found on both the chromosome and invasion plasmid (EHEC).

- Both copies are needed for full acylation of lipid A.

- Reduced levels of TNF-α produced in human monocytes.

- Reduced acylation led to lower TNF-α production, fluid acc., and tissue destruction.

- Study didn’t address issues related to live vaccine strain development.

Structure of the lipid A molecule in *Shigella flexneri*

Hauteville et al. J. Immunology, 2002
Strain Construction and Summary of *In Vitro* Phenotypic Characterization for *Shigella msbB* mutants

**2457T (Wild-type)**

- **2457TΔmsbB1**
- **2457TΔmsbB2**

- **2457TΔmsbB1,ΔmsbB2**

**Poster presentation for *in vitro* phenotypes:**

- Reduced capacity for intracellular invasion and replication.
- Filamentous growth during intracellular replication.
- Inability to form plaques in BHK cell monolayers.
- Filamentous growth during log phase at 30 and 37 °C.
- Sensitivity to growth on MacConkey Agar.
Virulence and Colonization for *Shigella msbB* Mutants

<table>
<thead>
<tr>
<th>Group (# Mice)</th>
<th>Shigella Strain</th>
<th>Dose</th>
<th>Route</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (8)</td>
<td>Naïve</td>
<td>PBS</td>
<td>IN</td>
</tr>
<tr>
<td>2 (8)</td>
<td>2457T</td>
<td>$5 \times 10^7$</td>
<td>IN</td>
</tr>
<tr>
<td>3 (8)</td>
<td>2457T</td>
<td>$1 \times 10^8$</td>
<td>IN</td>
</tr>
<tr>
<td>4 (8)</td>
<td>2457T&lt;sub&gt;∆msbB1&lt;/sub&gt;</td>
<td>$5 \times 10^7$</td>
<td>IN</td>
</tr>
<tr>
<td>5 (8)</td>
<td>2457T&lt;sub&gt;∆msbB1&lt;/sub&gt;</td>
<td>$1 \times 10^8$</td>
<td>IN</td>
</tr>
<tr>
<td>6 (8)</td>
<td>2457T&lt;sub&gt;∆msbB2&lt;/sub&gt;</td>
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<td>IN</td>
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<tr>
<td>7 (8)</td>
<td>2457T&lt;sub&gt;∆msbB2&lt;/sub&gt;</td>
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<td>IN</td>
</tr>
<tr>
<td>8 (8)</td>
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<td>$5 \times 10^7$</td>
<td>IN</td>
</tr>
<tr>
<td>9 (8)</td>
<td>2457T&lt;sub&gt;∆msbB1,∆msbB2&lt;/sub&gt;</td>
<td>$1 \times 10^8$</td>
<td>IN</td>
</tr>
</tbody>
</table>

Schedule:

- **D0**
  - Intrapulmonary inoculation
  - LD<sub>50</sub>

- 24H
  - LD<sub>50</sub>

- 48H
  - LD<sub>50</sub>

- 72H
  - LD<sub>50</sub>
  - Lung Colonization
  - Cytokine Production
Summary of Virulence and Colonization for *Shigella msbB* Mutants

**DATA Summary:**

- *Shigella msbB* mutants are significantly attenuated in the mouse lung model for virulence.

- Colonization of *Shigella msbB* mutants in mouse lungs is comparable to wild-type.

- Significantly reduced levels of some proinflammatory cytokines can be detected after 72 hours post infection.

**On-going Studies:**

Evaluate the **immunogenicity** and **protective capacity** of each strain using the mouse pulmonary model of infection.

- Measure mucosal, serum and proliferative responses following two sublethal doses.

- Measure protection from a wild-type challenge.
Construction of second generation vaccine strains for *S. sonnei*, *S. flexneri*, *S. dysenteriae* with precise deletions in VirG and enterotoxins is complete.

- WRSf2G12(\(\Delta\)virG, \(\Delta\)shet1, \(\Delta\)shet2-1, \(\Delta\)shet2-2)
- WRSS3 (\(\Delta\)virG, \(\Delta\)shet2-1, \(\Delta\)shet2-2)
- WRSd1 (\(\Delta\)virG, \(\Delta\)stxAB, \(\Delta\)shet2-1, \(\Delta\)shet2-2)

Continue preclinical testing in guinea pigs

Evaluation of additional virulence genes (enterotoxins, *msbB*) is ongoing.

Finish construction of vaccine strains with deletions in additional virulence genes is complete for *S sonnei* and *S. flexneri* and *S. dysenteriae* await preclinical testing in guinea pigs and monkeys.
Conclusions and Acknowledgements

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