Immune response and correlates of protection against *Shigella*

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Shigellosis

- Common all over the world and hyperendemic in developing countries.

- *Shigella spp.* was one of the four major pathogens significantly associated with moderate-to-severe diarrhea in children aged <60 months in the recent GEMS study.

- Children with the disease have an increased risk for persistent diarrhoea, nutritional faltering, and death.

Genus *Shigella*

- *S. sonnei* is the leading *Shigella* species in industrialized countries

- *S. flexneri* (mostly serotypes 2a and 6) prevails in developing countries

- *S. boydii* and *S. dysenteriae* are responsible for around 10-15% of cases of shigellosis

- *S. sonnei* emerges globally with improvement in sanitation and socio-economic level of countries, regions and populations (Ex. Vietnam, China, Bangladesh)

Shigellosis in Israel

- Highly endemic

- Mean incidence rate of 80-100 culture-proven cases per 100,000 per year

- About 10-20 times higher than the incidence rate in the US

- Children aged 1-4 and soldiers serving under field conditions – at highest risk

Cohen D et al. 2001; 2014
Natural *Shigella* Infection

- Induces around 70% serotype specific protection
- Length of protection not clear (~2 years)
- Solid protection is probably attained after consecutive exposures to *Shigella* antigens
- Potential correlates of protection, important for vaccine development and evaluation, are incompletely defined.

Criteria for potential correlates of protection against *Shigella*

- Significantly elicited by *Shigella* natural infection.

- Associated with a reduced risk of disease under natural conditions of exposure or in human challenge studies.

- Associated with protection induced by a candidate vaccine in efficacy studies.

- Have functional capabilities.
Components of the immune response to Shigella LPS following natural infection

- Serum antibodies (IgG, IgA, IgM)
- Secretory antibodies (sIgA)
- Urinary antibodies (sIgA)
- Antibody Secreting Cells
- B memory cells
- T cell response (cytokines)
Soldiers in field units, high incidence of shigellosis in 1980s and 1990s; S. sonnei and S. flexneri equally distributed together responsible for 90% of the cases of disease,

Serum IgG anti-LPS antibodies

* Case-control studies (outbreaks).
* Prospective studies.

Serum anti-*Shigella* LPS antibodies
   Non-IgM fraction detected by passive HA after treatment of sera with 2-ME or
   IgG fraction detected by ELISA.

Strongly associated with protection against disease caused by the homologous strain of *Shigella*.

Pre-existing anti-LPS antibodies & S. sonnei Shigellosis.

- OR=4.2, p<.0001
- OR=1.2, NS

Shigella Conjugate Vaccines

Detoxified O-specific polysaccharide covalently bound to a protein:

- *S.flexneri* 2a – rEPA.
- *S.sonnei* – rEPA.

With the capability to elicit high serum LPS antibodies when injected IM

Antibody response to *S. sonnei* LPS after immunization with the *S.sonnei conjugate*

**IgA** **IgG** **IgM**

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**Time after vaccination**

- 2 Wks
- 6 Wks
- 6 Mths
- 1 Yr
- 2 Yrs
- 4 Yrs
- 5 Yrs

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Cohen & al., Infect. Imm. 1996
GMT of IgG antibodies to Shigella LPS before and after natural infection (n=37) or vaccination (n=23) with S. sonnei conjugate

Conjugate vaccine  Natural infection

- Fold increase = 12.7
- Fold increase = 3.4

p value = 0.8445
p value = 0.0016
**Antibody-Secreting Cell Response (ASC) – IgA (Shigella sonnei & flexneri Conjugate Vaccines)**

<table>
<thead>
<tr>
<th></th>
<th>No./Total (Percent) with Significant ASC Response*</th>
<th>Arithmetic Mean of Positive Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ss-LPS</td>
<td>Sf-LPS</td>
</tr>
<tr>
<td><strong>S. Sonnei vaccinees</strong></td>
<td>18/23 (78%)</td>
<td>0/8 (0%)</td>
</tr>
<tr>
<td><strong>S. Flexneri vaccinees</strong></td>
<td>0/6 (0%)</td>
<td>13/19 (68%)</td>
</tr>
</tbody>
</table>

* ASC result >=18 spots/Mcells: based on the mean (3.33) + 2SD (2x6.97) found in non-vaccinees
Double-blind vaccine-controlled randomised efficacy trial of an investigational *Shigella sonnei* conjugate vaccine in young adults

Dani Cohen, Shai Ashkenazi, Manfred S Green, Michael Gdalevich, Guy Robin, Raphael Slepon, Miri Yavzori, Nadav Orr, Colin Block, Isaac Ashkenazi, Joshua Shemer, David N Taylor, Thomas L Hale, Jerald C Sadoff, Danka Pavliakova, Rachel Schneerson, John B Robbins

74% protective efficacy in young adults

Lancet 1997; 349:155-159
GMT of IgG antibodies to S. sonnei LPS among recipients of S. sonnei-rEPA in group D*

* An outbreak of S. sonnei shigellosis occurred immediately after vaccination
Age-related efficacy of *Shigella* O-specific polysaccharide conjugates in 1–4-year-old Israeli children

Justen H. Passwell\(^a,1\), Shai Ashkenzi\(^b\), Yonit Banet-Levi\(^a\), Reut Ramon-Saraf\(^a\), Nahid Farzam\(^a\), Liat Lerner-Geva\(^c\), Hadas Even-Nir\(^d\), Baruch Yerushalmi\(^d\), Chiayung Chu\(^e\), Joseph Shiloach\(^f\), John B. Robbins\(^e\), Rachel Schneerson\(^e,\,*\), The Israeli Shigella Study Group\(^2\)

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\(^f\) National Institute of Diabetes, Digestive Diseases and Kidney, NIH, Bethesda, MD 20892, USA
Efficacy of 2 doses of Shigella sonnei conjugate vaccine among Israeli children by age

<table>
<thead>
<tr>
<th>Age</th>
<th>Vaccine administered</th>
<th>Efficacy</th>
<th>(95% CI)</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>S. sonnei</td>
<td>S. flexneri 2a</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>Cases</td>
<td>N</td>
<td>Cases</td>
</tr>
<tr>
<td>a. Shigella sonnei</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–2 years</td>
<td>516</td>
<td>18</td>
<td>476</td>
<td>16</td>
</tr>
<tr>
<td>&gt;2–3 years</td>
<td>497</td>
<td>8</td>
<td>481</td>
<td>12</td>
</tr>
<tr>
<td>&gt;3–4 years</td>
<td>371</td>
<td>3</td>
<td>358</td>
<td>10</td>
</tr>
<tr>
<td>All ages</td>
<td>1384</td>
<td>29</td>
<td>1315</td>
<td>38</td>
</tr>
<tr>
<td>b. Shigella flexneri 2a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–2 years</td>
<td>516</td>
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<td>8</td>
<td>1315</td>
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</table>

Passwell JH et al. 2010
Age-related IgG anti-LPS levels 2-3 weeks after second vaccine dose of Shigella conjugates

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Age (years)</th>
<th>N</th>
<th>S. sonnei Ag</th>
<th>S. flexneri 2a</th>
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</thead>
<tbody>
<tr>
<td>S. sonnei</td>
<td>1-2</td>
<td>38</td>
<td>1.4</td>
<td>3.43</td>
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<tr>
<td></td>
<td>&gt;2-3</td>
<td>44</td>
<td>3.71</td>
<td>7.53</td>
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<tr>
<td></td>
<td>&gt;3-4</td>
<td>29</td>
<td>6.38</td>
<td>9.51</td>
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<tr>
<td>S. flexneri 2a</td>
<td>1-2</td>
<td>43</td>
<td>0.25</td>
<td>18.98</td>
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<tr>
<td></td>
<td>&gt;2-3</td>
<td>53</td>
<td>0.42</td>
<td>26.96</td>
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<tr>
<td></td>
<td>&gt;3-4</td>
<td>30</td>
<td>0.76</td>
<td>43.86</td>
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</table>

Passwell JH et al. 2010
Shigella antigen-specific B memory cells are associated with decreased disease severity in subjects challenged with wild-type Shigella flexneri 2a

Rezwanul Wahid a, Jakub K. Simon c, Wendy L. Picking d, Karen L. Kotloff a, Myron M. Levine a, Marcelo B. Sztein a, b, *

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Shigella-specific IgA B memory cells and serum IgG LPS antibodies might play a protective role in humans
Conclusions

• Serum IgG antibodies to *Shigella* LPS emerge as a correlate of protection with mechanistic capabilities.

• We continue to evaluate the possible added value of other immune parameters following exposure to natural infection and candidate vaccines.

• Highly immunogenic vaccines are needed to immunize better than natural infection especially in pediatric populations.
S. flexneri 2a –PS tetanus toxoid synthetic glycoconjugate made at Institut Pasteur (projected phase 1 in adults in Israel, 2016)
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