Vaccine against shigellosis: dream or reality?

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Eau et maladies infectieuses/ Enjeux du 21ème siècle CEMI 15 Paris Mai 2010

Significant efforts to prevent diarrhoeal diseases

Water

87% population improved drinking water sources

Sanitation

72% population improved sanitation facilities

Breastmilk
Nutrition
Vit A, Zn

2008

WHO-UNICEF/ CHILDINFO www.childinfo.org
Diarrhoea: second leading cause of child deaths worldwide

Children under 5 yr old

- 37% Neonatal causes
- 4% Injuries
- 2% AIDS
- 4% Measles
- 7% Malaria
- 13% Others
- 16% DIARRHOEA
- 17% Pneumonia

(3% DIARRHOEAL DISEASES)

Source: WHO Global burden of diseases/ update 2008
WHO/UNICEF www.childinfo.org

Incidence of diarrhoeal diseases

Thousands of deaths

Worldwide distribution of deaths caused by diarrhea in children under 5 years in 2000.


Significant decrease of mortality rate:
- 13.6 deaths per 1,000 children per year (1954-1979)
- 4.9 deaths per 1,000 children per year (1992-2000)

From Von Seidlein L. et al. 2006
Kotloff K et al. Bull. WHO, 1999
Kosek M. et al. 2003, Bull. WHO
Morbidity linked diarrhoeal diseases

3-4 diarrhoea episodes per child per year
(active surveillance between 1992-2000)

Lasting disability effects

Early childhood diarrhea cuts 8 cm growth, 10 IQ pts and 12m schooling
(favela children, Brazil)

DALYs: HIGH
Disability adjusted life years [Yrs of life lost + Yrs lost to disability]

Diarrhea

Malnutrition

Petri et al. JCI, 2008; Guerrant et al. Nutr Rev. 2008;
Checkley et al. Int’l J Epi, 2008; Copeland et al. JWH, 2009

Diarrhea: vaccine-preventable diseases

2.1 million annual deaths
1.5 million <5yr

**Vibrio cholerae**
Cases/year: 5,000,000
Deaths/year: 120,000

**ETEC**
Cases/year: 650,000,000
Deaths/year: 380,000

**Salmonella typhi**
Cases/year: 17,000,000
Deaths/year: 600,000

**Rotavirus**
Cases/year: 130,000,000
Deaths/year: 650,000

**Shigella**
Cases/year: 163,000,000
Deaths/year: 700,000

The « big five »

From Von Seidlein L. et al. 2006 Kotloff K et al. Bull. WHO
Kosek M. et al. 2003, Bull. WHO

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**Shigella**: the causal agent of shigellosis or bacillary dysentery

*Gram negative enteroinvasive bacterium

*Rectocolitis

*Dysentery with fever, intestinal cramps, and mucoid bloody stools

Acute inflammation
Neutrophil infiltration
Massive tissue destruction

Type III secretion system
Invasive and pro-inflammatory phenotype

Injection of virulence effectors to subvert host cell functions

Shigella: issue of serotype diversity

S. flexneri
S. sonnei
S. boydii

S. dysenteriae

From Levine M. et al., 2007

Protective immunity to shigellosis

Protection serotype-specific mediated by anti-LPS Abs

O-Ag polysaccharide = the major protective Ag

Justification of vaccine-based prevention

*1-Multiresistance to “first-line” antibiotics
sulfonamides/trimethoprim, tetracyclin, ampicillin, chloramphenicol, nalidixic acid.

*2- Poor benefit of oral rehydration therapy

*3- Acute complications (often cause of death).
- Bacteriemia / Septicemia (50% Shigella, malnourished children).
- Hypoglykemia
- Toxic megacolon: perforation, peritonitis, septis chock
- Hemolytic uremic syndrome (HUS).

Targeted population:
* Toddlers
* Travelers

The two main vaccine strategies

Live, rationally attenuated, orally administered, vaccine strains

* Induction of local anti-LPS S-IgA
  and serum IgG
* Several kinds of attenuation

- One oral dose of live, specifically attenuated vaccine strains:
  proof of concept in western volunteers (safe and protective)
- Disappointing results on the field: safe but non immunogenic

Phalipon and Sansonetti 2003; Nataro 2004; Vankatesen and Ranallo 2006;
Girard et al. 2006; Levine et al. 2007; Phalipon et al. 2008
**The two main vaccine strategies**

**Subunit vaccines parenterally administered**

- Induction of anti-LPS serum IgG
- Several strategies

Conjugate vaccines/ J. Robbins

**Capsular polysaccharides coupled to a carrier protein**

*Efficient pneumo, meningitis, Hib vaccines in young children*

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**Gram negative bacteria**

**Detoxified LPS**

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**Shigella glycoconjugate vaccine candidates**

*S. sonnei / S. flexneri 2a (SF2a) detoxified LPS-protein conjugates**

- Protection induced in adults
  - Phase III randomized, controlled, double blind efficacy trial *S. sonnei* conjugate single dose in Israeli soldiers:
    - 74% protection related to the level of conjugate-induced anti-LPS IgG

- Safe and immunogenic in 1-4 yr-old children
  - Phase II *S. sonnei* and SF2a/ 2 doses spaced 6wks apart/ Israeli 1-4 year-old
  - High Ab titers 2yrs after vaccination

- Protection in 1-4 yr-old children unpublished data
- Lower level of protection in < 1 yr-old children unpublished data

**Main limitation: detoxification step**

- Loss of immunogenicity
- Lack of reproducibility

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Vankatesen and Ranallo 2006; Girard et al. 2006; Levine et al. 2007; Phalipon et al. 2008; Kaminski and Oaks, 2009

To identify synthetic oligosaccharides mimicking the protective serotype-specific determinants as surrogates to induce protective anti-LPS antibodies.

A modular approach: 3 levels of flexibility

serotype-specific haptens
selectivity

spacer (chemistry, length, valence)

activated carrier

Characterization of the serotype 2a specific determinants

1- Synthetic oligosaccharides

2- Recognition by the protective mAbs (Inhibition ELISA- IC50 measurement)

Results

*ECD: minimal sequence required for recognition

*Additional flanking residues leading to B(E)CD and B(E)CDA or AB(E)CD: optimal recognition

*Elongating the sequence significantly improves the recognition

Obtention of different chemically defined semi-synthetic glycoconjugates

Trisaccharide: ECD-TT
Tetrasaccharide: B(E)CD-TT
Pentasaccharide (1 UR): AB(E)CD-TT
Decasaccharide (2UR): AB(E)CDAB(E)CD-TT
Pentadecasaccharide (3UR): AB(E)CDAB(E)CDAB(E )CD-TT

Synthetic oligosaccharide

Tetanus toxoid (TT)

The pentadecasaccharide-conjugate induces the highest anti-SF2a IgG titer

Immunization protocol:
10 µg/oligosaccharide without adjuvant
3 immunizations at 3 week-interval + one boost one month later
The pentadecasaccharide-conjugate induces protective anti-SF2a LPS Abs

Reduction factor: control group receiving pre-immune serum

Reduction of bacterial load upon passive immunization in mice

$$[\text{AB(E)CD}]_3 = 3\text{RUs} = \text{Pentadecasaccharide} = \text{functional mimic of LPS O-Ag}$$

Phalipon et al. 2009

Recognition of SF2a LPS by Abs induced by the pentadecasaccharide-conjugate

SDS-PAGE

Mode A

Mode B

Ladder

n UR

core

LPS

S. flexneri 2a silver-stained

Anti-SF2a Abs

Abs induced by pentadecasaccharide

SDS-PAGE + Western blot

Mode B

Mode A

core

Phalipon et al. 2009
The pentadecasaccharide is a structural mimic of the SF2a O-Ag

\[ \text{SF2a } \text{dLPS} \]

\[ \text{SF2a } \text{O-Ag} \]

\[ \text{\textsuperscript{1}H NMR anomeric region} \]
\[ (600 \text{ MHz, } \text{D}_2\text{O, }50^\circ\text{C}) \]

Right-handed helix
pitch ~23 Å, diameter ~15 Å
Close to three RUs per turn

Vulliez-Le Normand B. et al. PNAS, 2008
Theillet F et al. JMB 2009

Optimal Ab response induced by the pentadecasaccharide-conjugate

Ratio oligosaccharide/protein

Optimal immunizing dose: 1 \( \mu \)g

Classical glycoconjugates in humans: 2.5 \( \mu \)g

First synthetic oligosaccharide-protein conjugate against SF2a

\[
\text{SF2a-TT15} \quad R = \{AB(E)CD\}_3 \\
\text{14/protein}
\]

*Characterization of the products: requirements for regulatory agencies
*Feasibility of the synthesis at an industrial scale and at an acceptable cost

Proof of concept in humans? Phase I clinical trial

### STOPENTERICS Consortium

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15 Equipes
12 million euros
4yr project

IP Coordonateur: Philippe Sansonetti
STOPENTERICS Consortium:

Objectives

1/ Proof of concept for new vaccine candidates
   Phase 1 clinical trials with *Shigella* vaccine candidates ready to be evaluated in humans
   2 subunit vaccines to be tested

2/ To identify new protein antigens that induce protection across a variety of *Shigella* serotypes
   (and across serotypes and CFs in ETEC)

Conclusion/Perspectives
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(P. Sansonetti)

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The Vaccine Business of Sanofi-Aventis Group