Vaccine against shigellosis:

dream or reality?

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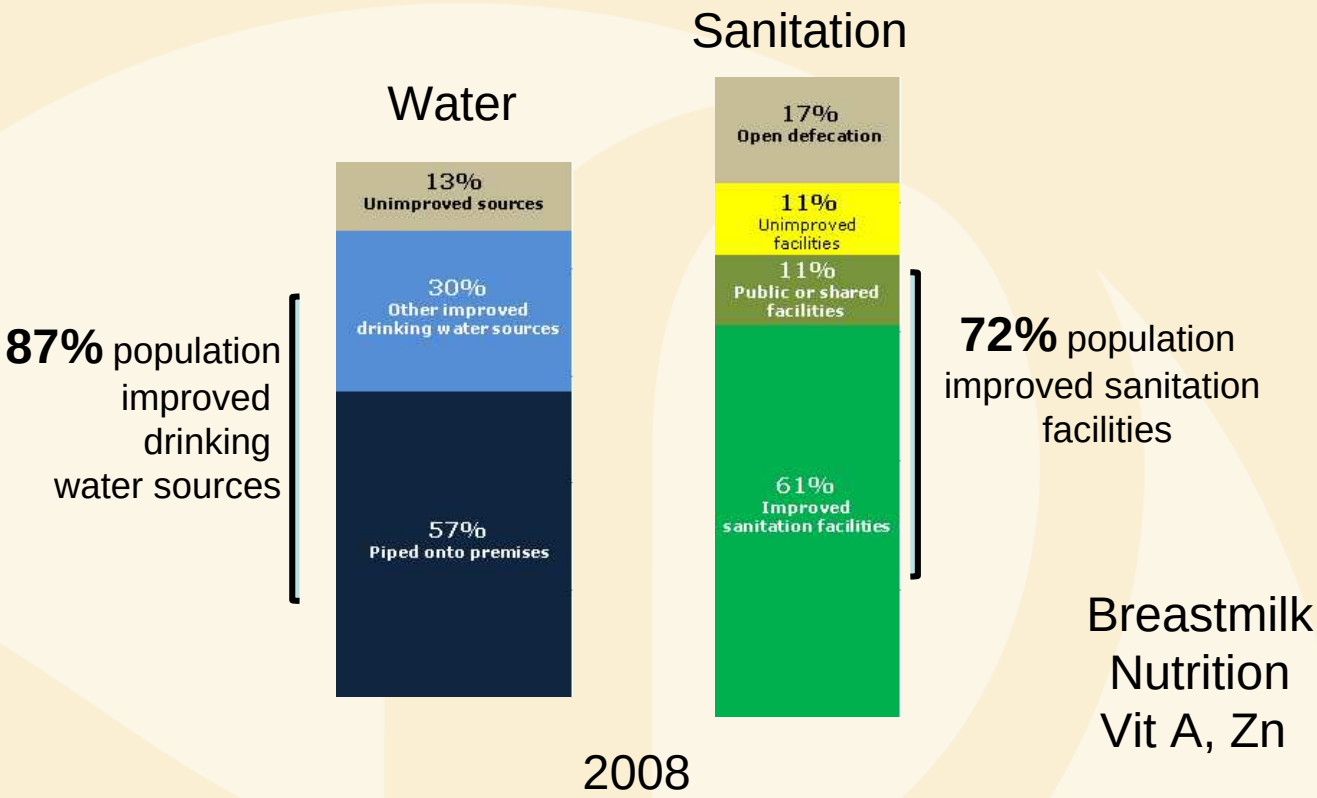
Group Leader « Adaptive immunity and vaccine development »

Molecular Microbial Pathogenesis Unit

INSERM U786

Headed by Professor Philippe Sansonetti

Significant efforts to prevent diarrhoeal diseases

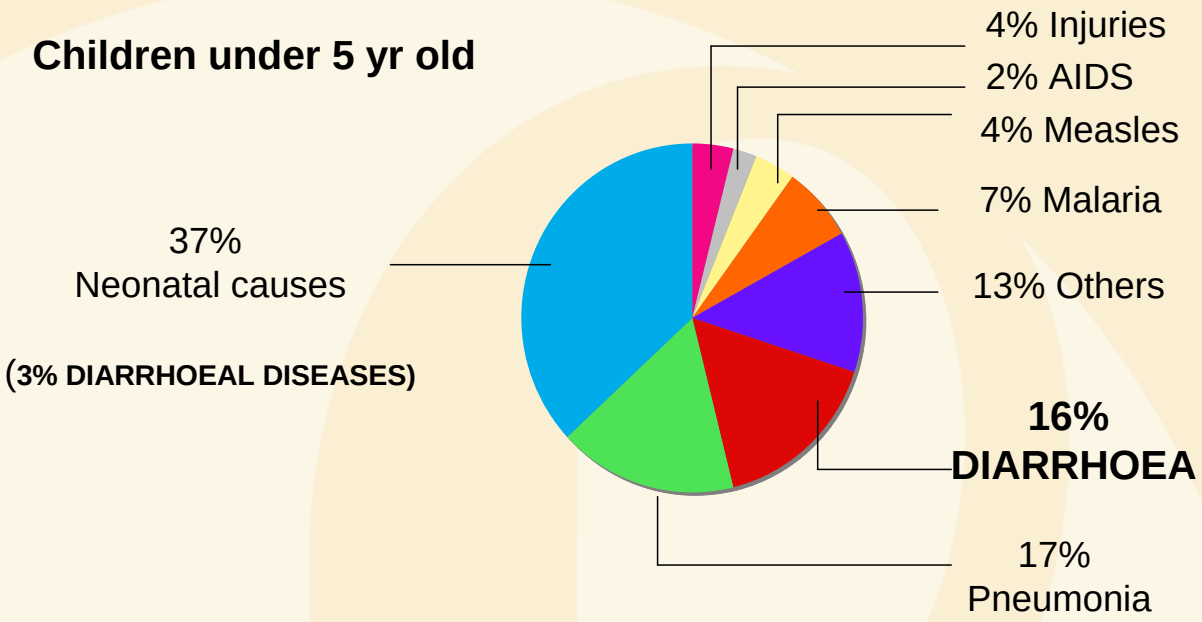


87% population improved drinking water sources

72% population improved sanitation facilities

Breastmilk
Nutrition
Vit A, Zn

Diarrhoea: second leading cause of child deaths worldwide



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

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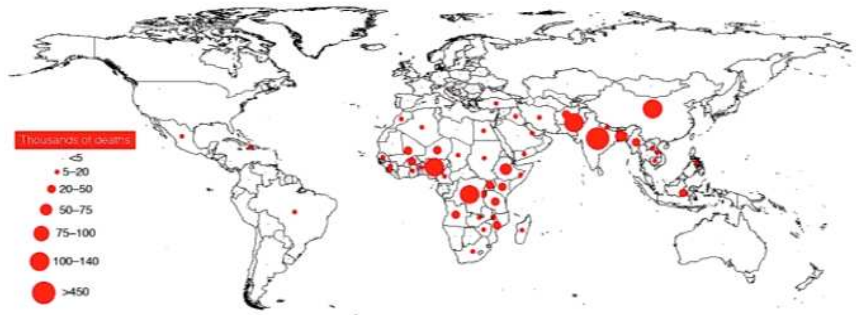
Incidence of diarrhoeal diseases



Thousands of deaths

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

From Keusch, G.T., et al. 2006.

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

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Morbidity linked diarrhoeal diseases

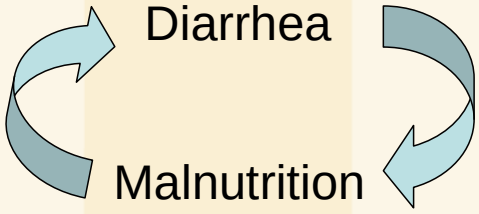
3-4 diarrhoea episodes per child per year
(active surveillance between 1992-2000)

Lasting disability effects

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

DALYs : HIGH

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



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

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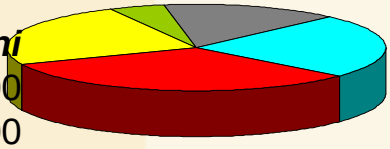
Diarrhea: vaccine-preventable diseases

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, 1999

Kosek M. et al. 2003, Bull. WHO

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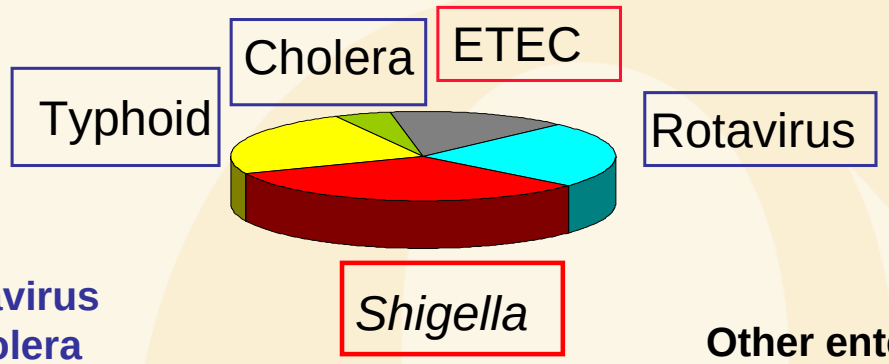
Vaccine availability



Rotavirus
Cholera
Typhoid

Licensed vaccines some under-utilized

Shigella
ETEC

No licensed vaccine yet

Other enteric pathogens to be considered
Non typhoid *Salmonellae*
Enteric parasites
Human Caliciviruses

WHO Diarrhoeal and Enteric Vaccine Advisory Committee, Geneva October 2008

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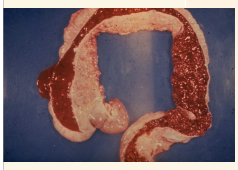
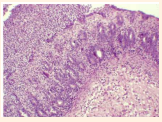
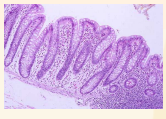
Shigella: the causal agent of shigellosis or bacillary dysentery

*Gram negative enteroinvasive bacterium

*Rectocolitis

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

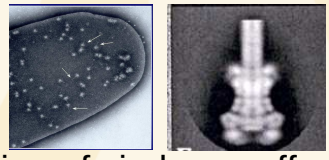


Acute inflammation
Neutrophil infiltration
Massive tissue destruction

Invasive and pro-inflammatory phenotype

Type III secretion system



Injection of virulence effectors to subvert host cell functions

Prokovskii Vi et al. 1973; Korshunov MF, 1974;Levine M. et al. 1973; Racz P. et al. 1973; Mathan M. and Mathan V. , 1986; Raqib R. et al., 1995,1997, 2000, 2002,2003; Parsot C., 2005; Ogawa M. et al.., 2008

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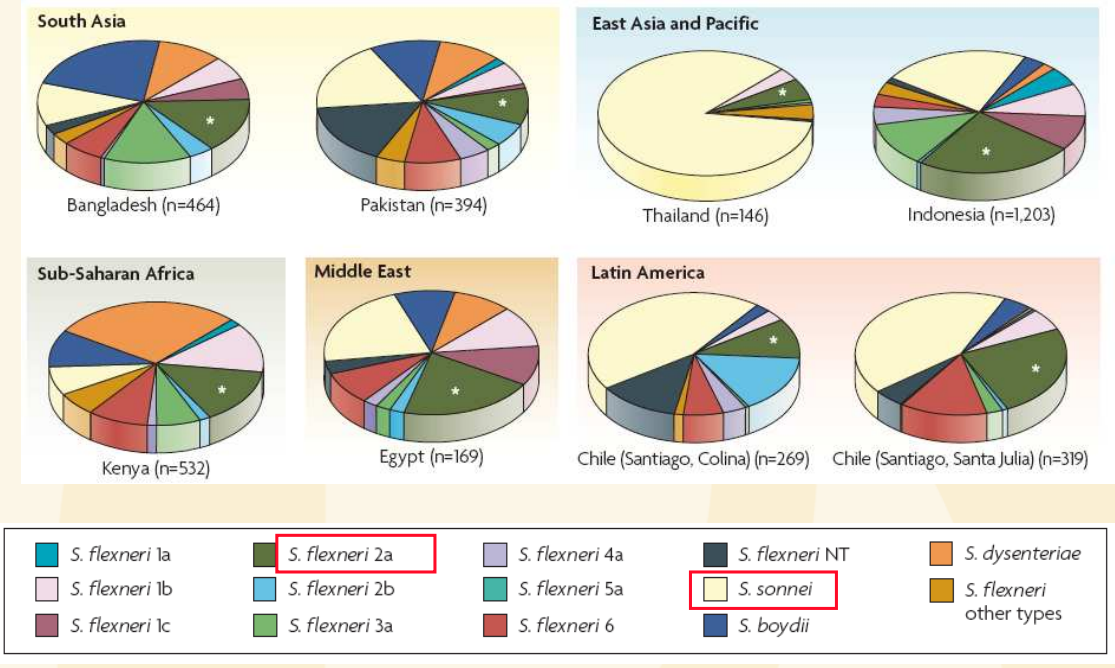
Shigella: issue of serotype diversity

S. flexneri
S. sonnei
S. boydii

S. dysenteriae



From Levine M. et al., 2007

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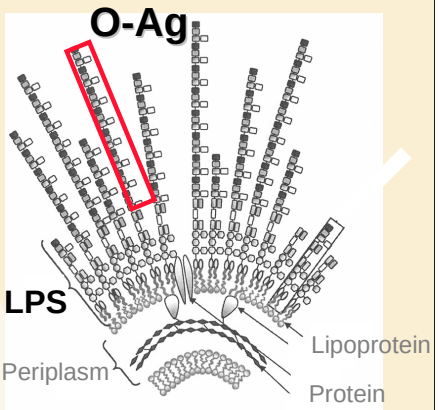
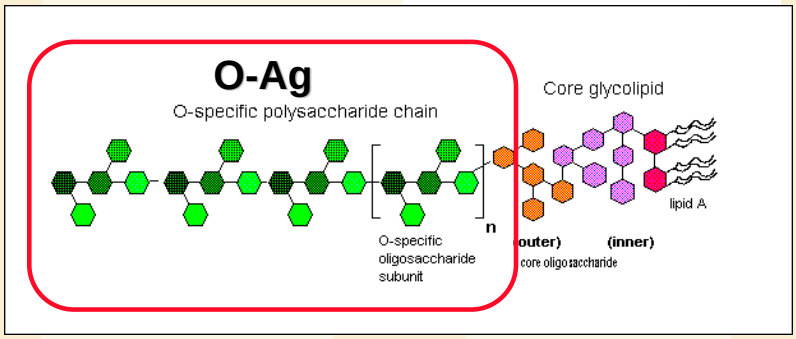
Protective immunity to shigellosis

Protection serotype-specific mediated by anti-LPS Abs



O-Ag polysaccharide
=
the major protective Ag



Repeating unit

Reviews: Phalipon and Sansonetti, 2003, 2007; Levine M. 2007

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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

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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

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The two main vaccine strategies

Subunit vaccines parenterally administered

*Induction of anti-LPS serum IgG
*Several strategies

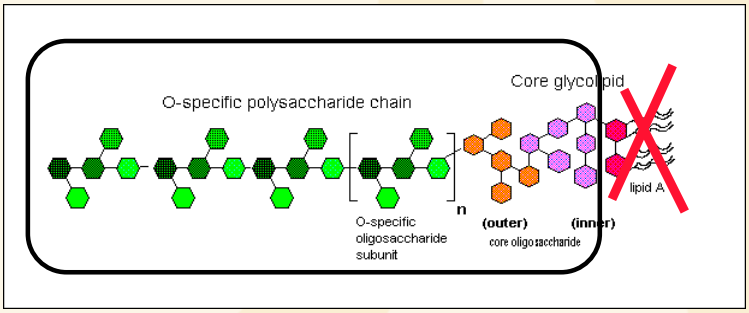
Conjugate vaccines/ J. Robbins

Capsular polysaccharides coupled to a carrier protein

Efficient pneumo, meningitis, Hib vaccines in young children

Gram negative bacteria

Detoxified LPS



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

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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 ot 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

Taylor DN et al. 1993; Cohen D et al. 1996, 1997; Robin G et al. 1999; Passwell JH et al. 2001, 2003;

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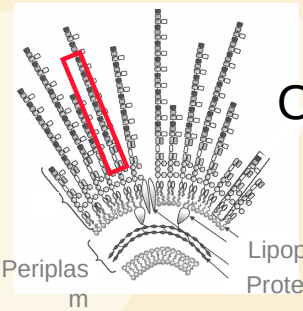
Glycoconjugate vaccines based on surrogates of LPS O-Ag

LPS

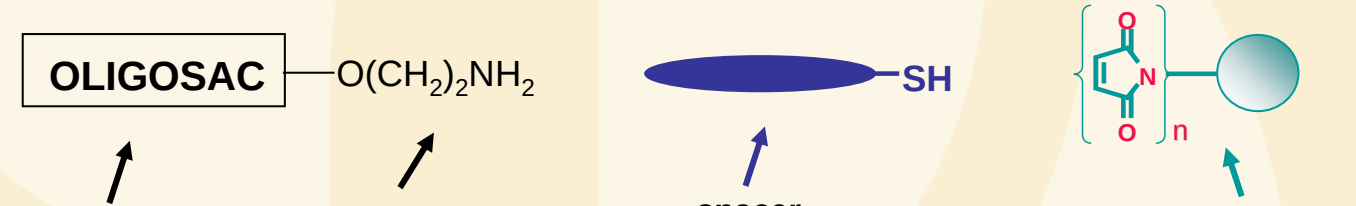


O-Ag

Laurence Mulard IP

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 TT

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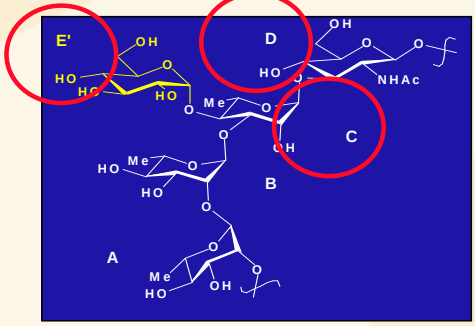
Characterization of the serotype 2a specific determinants

1- Synthetic oligosaccharides



Tri-	Tetra-	Penta- and longer oligosaccharides
D''AB	ECDA'	B(E)CDA'
BCD	AB(E)C	D''AB(E)C
CDA'	B(E)CD	AB(E)CD
ABC		ECDA'B'
ECD		
B(E)C		B(E)CDA'B'(E')C'
		D''AB(E)CDA'B'(E')C'
		$[AB(E)CD]_2$ $[AB(E)CD]_3$

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

L.Mulard et al. 2000, 2004; Costachel et al. 2000; Ségat et al. 2002; Bélot et al. 2002, 2004; Phalipon et al. 2006

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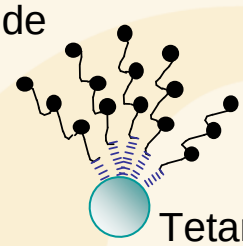
Obtention of different chemically defined semi-synthetic glycoconjugates

Synthetic oligosaccharide



Tetanus toxoid (TT)

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

Phalipon et al. 2006

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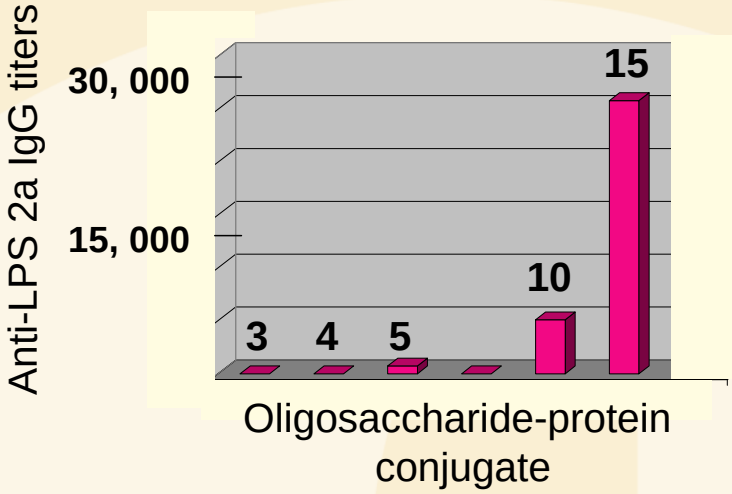
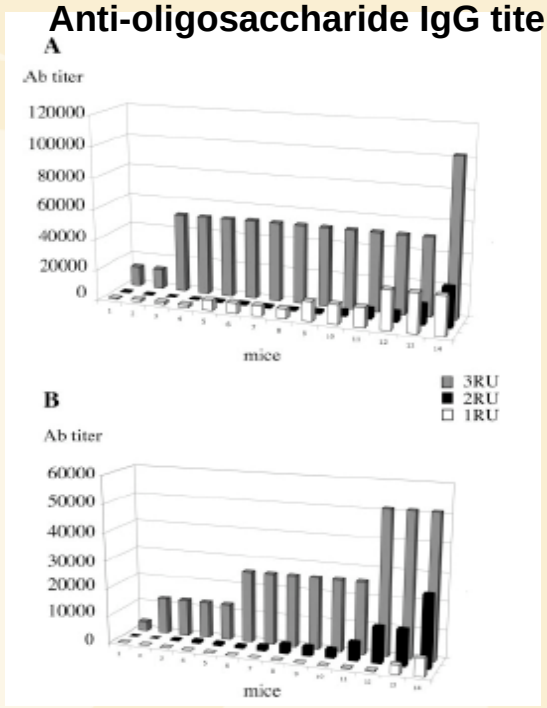
The pentadecasaccharide-conjugate induces the highest anti-SF2a IgG titer



Immunization protocol:
10 µg/oligosaccharide **without adjuvant**
3 immunizations at 3 week-interval
+ one boost one month later

Anti-oligosaccharide IgG titer



Anti-SF2a LPS IgG titer

Phalipon et al. 2006

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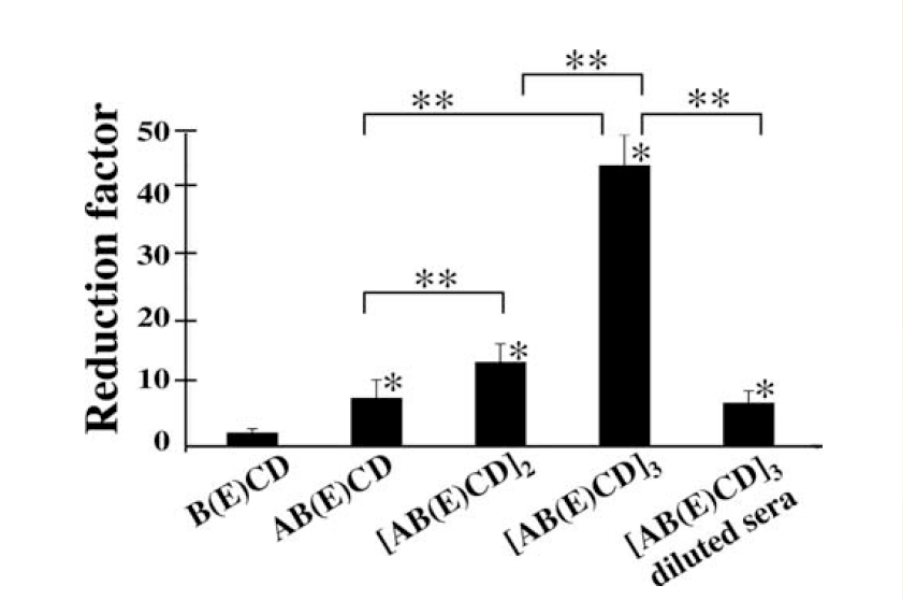
The pentadecasaccharide-conjugate induces protective anti- SF2a LPS Abs

Reduction factor: control group receiving pre-immune serum



Reduction of bacterial load upon passive immunization in mice

[AB(E)CD] 3 = 3RUs = Pentadecasaccharide= functional mimic of LPS O-Ag

Phalipon et al. 2009

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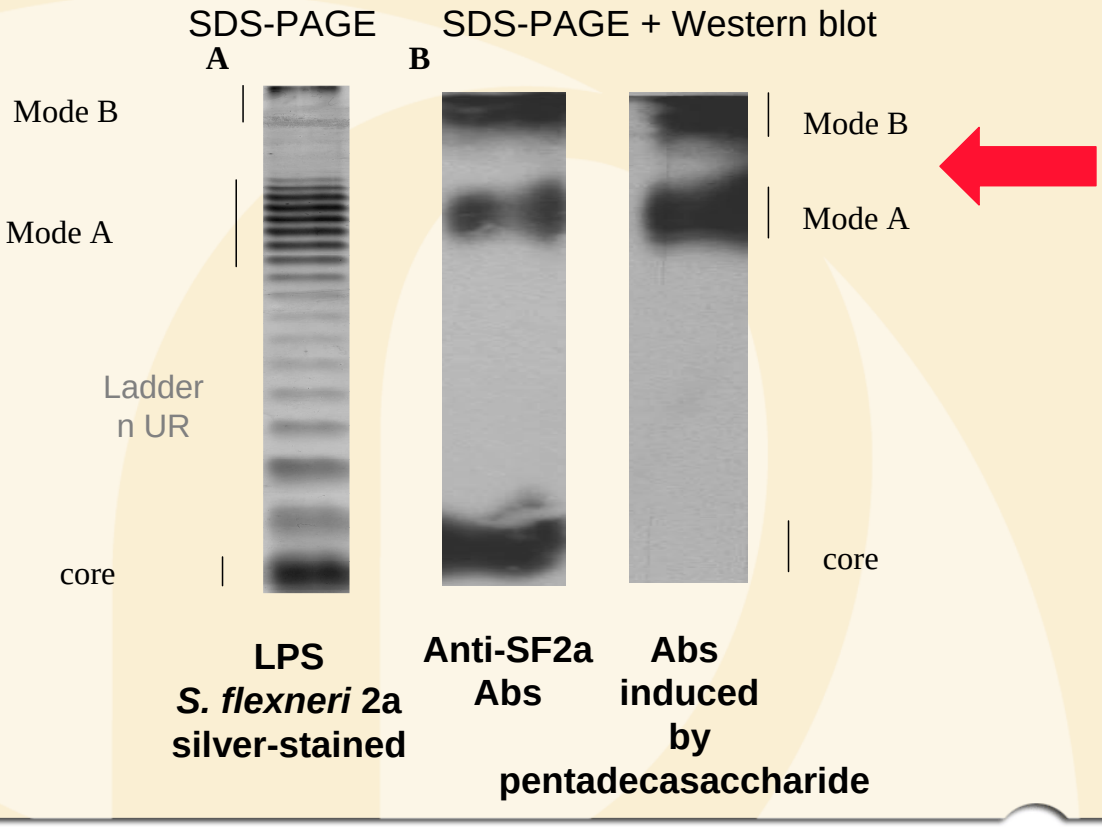
Recognition of SF2a LPS by Abs induced by the pentadecasaccharide-conjugate



Phalipon et al. 2009

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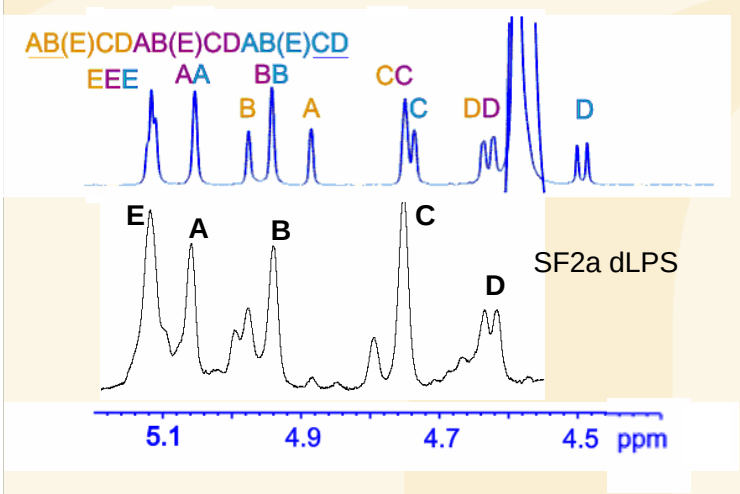
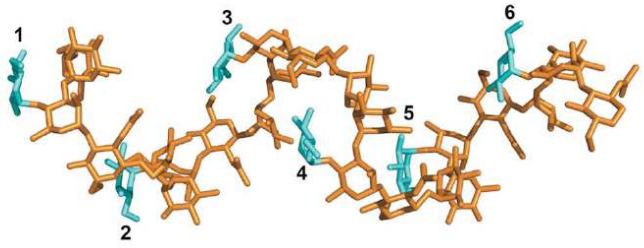
The pentadecasaccharide is a structural mimic of the SF2a O-Ag

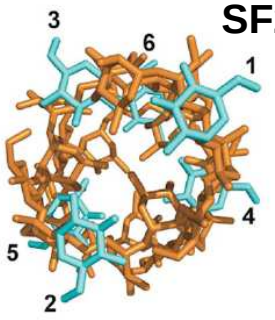


^{1}H NMR anomeric region
(600 MHz, D_2O, 50°C)

X Ray/Molecular modeling



SF2a O-Ag



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

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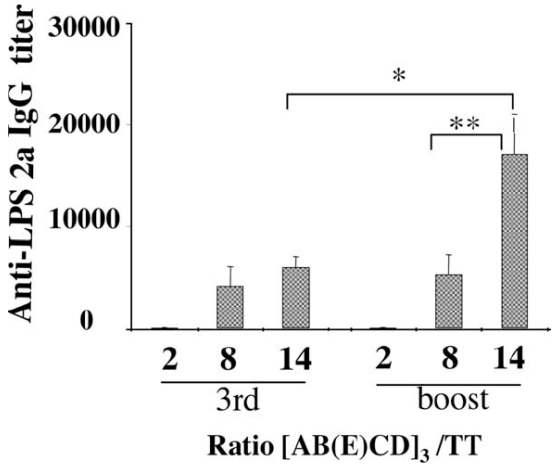
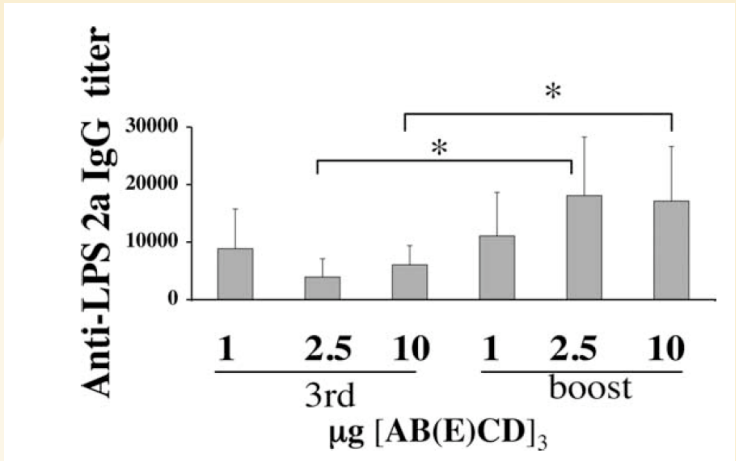
Optimal Ab response induced by the pentadecasaccharide-conjugate

Ratio oligosaccharide/protein



Higher ratio: no significant increase in Ab response

Optimal immunizing dose: 1µg



Classical glycoconjugates in humans: 2.5 µg

Phalipon et al. 2009.

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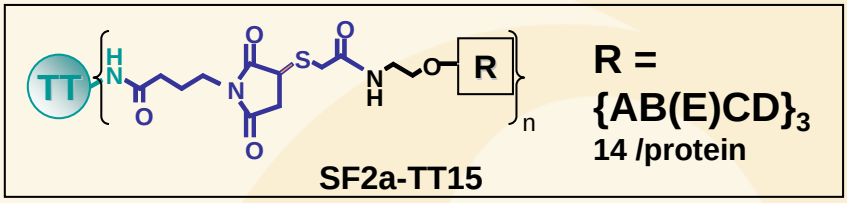
First synthetic oligosaccharide-protein conjugate against SF2a



SF2a-TT15

R = {AB(E)CD}$_3$
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

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STOPENTERICS Consortium

EU Call 2010

15 Equipes
12 million euros
4yr project

Institut Pasteur	France
Université de Bergen	Norvège
Imperial College	Angleterre
Nederlands Vaccine Institut	Hollande
Université Libre de Bruxelles	Belgique
Université de Rome La Sapienza	Italie
Université de Goteborg	Suède
Genome Research Limited	Angleterre
Health Sciences eTraining Foundation	Suisse
ICDDR,B Dhaka	Bangladesh
Université de Tel Aviv	Israel
Université Ghana	Ghana
Sanofi Pasteur	France
Novartis Institute for Global Health	Italy
Centre Pasteur-Cochin de Vaccinologie	France

IP Coordonateur: Philippe Sansonetti

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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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Tel Aviv University

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K. Wright

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sanofi pasteur
The vaccines business of sanofi-aventis Group

gift of TT