

Bacterial Polysaccharides

Animal Pathogens

- *Salmonella enteritidis*
- *E. coli*
- *Neisseria meningitidis*
- *Haemophilus influenzae*
- *Proteus mirabilis*
- *Proteus vulgaris*
- *Mycobacteria*
- (many others)

Soil Bacteria

- *Rhizobiaceae*
- *Pseudomonas solanacearum*
- *Erwinia amylovora*
- (many others)

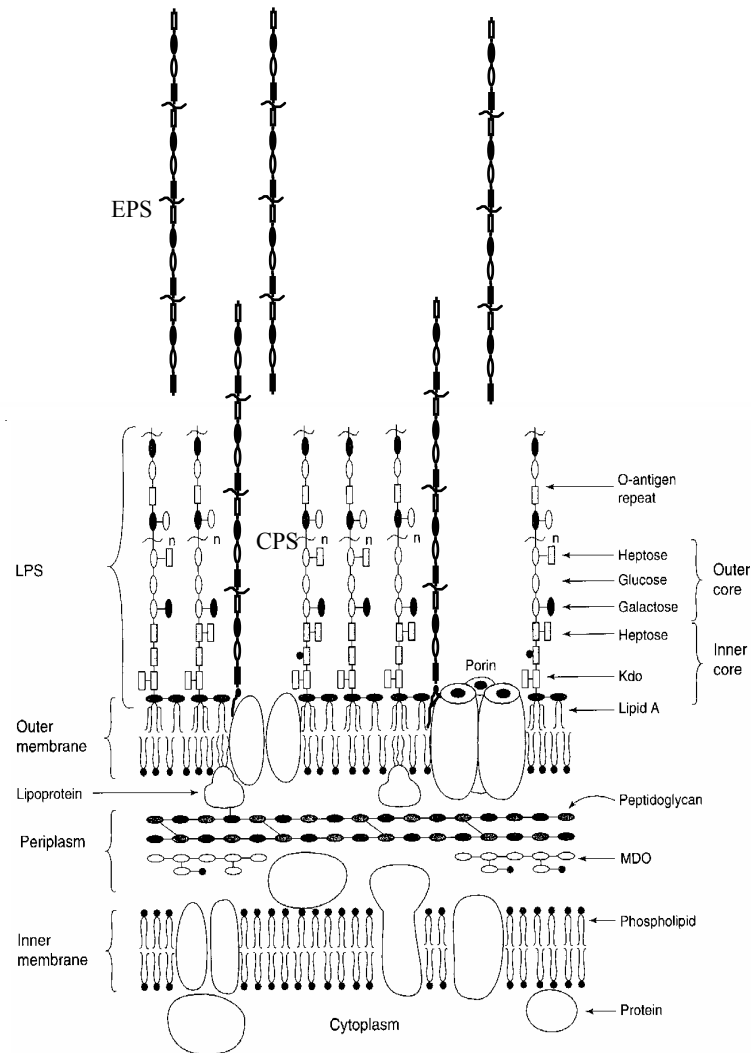
Polysaccharides

- **Extracellular polysaccharides (EPSs)**
 - Excreted heteropolysaccharides
 - Most often acidic
 - Frequently form solutions of high viscosity
 - Gram-positive and Gram-negative products
- **Capsular Polysaccharides (CPSs)**
 - Cell-associated heteropolysaccharides
 - Most often acidic, but some are neutral
 - Solutions can be highly viscous
 - Gram-positive and Gram-negative products
- **Lipopolysaccharides (LPSs)**
 - Component of the outer membrane
 - Amphiphilic
 - Gram-negative bacterial component
- **Teichoic acid**
 - Gram-Positive
- **Mycobacterium cell wall components**
 - Mycolyl arabinogalactan
 - Lipooligosaccharides (LOSs)
 - Glycopeptidolipids (GPLs)
 - Phenolic glycolipids (PGLs)

Polysaccharide Functions

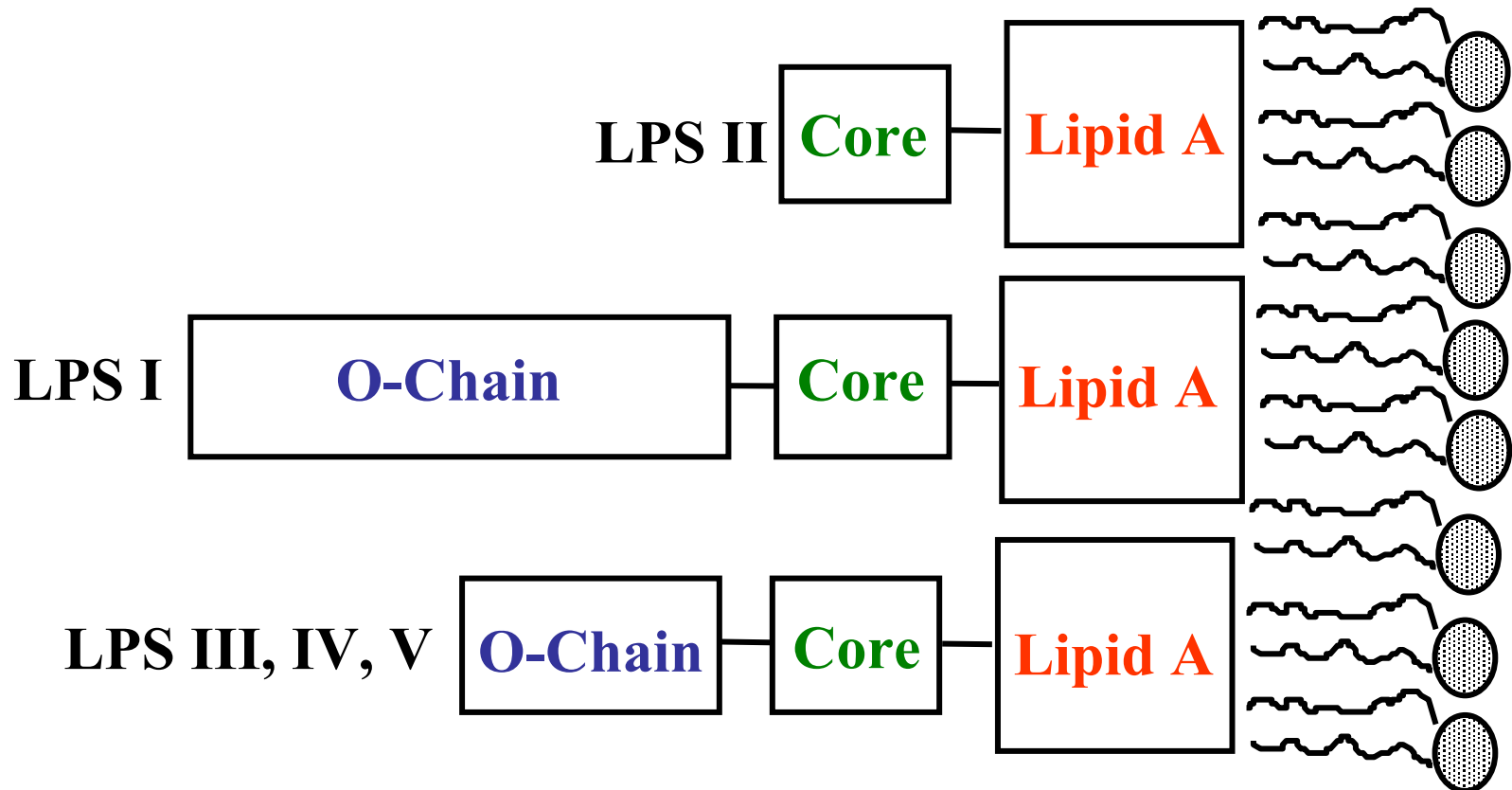
- Prevention of desiccation
 - Survival
- Adherence
 - Colonization of oral surfaces
 - Colonization of catheters
 - Bacteria-plant interactions
- Resistance to non-specific host immunity
 - Complement-mediated phagocytosis
 - Complement-mediated killing
- Resistance to specific host immunity
 - Poor antibody response
- Cell-cell recognition signaling
 - Bacteria-plant interactions

Gram-Negative Bacterial Cell Wall



Lipopolysaccharides: Structure, Synthesis, Function

READ: Raetz, and Whitfield. 2002. Lipopolysaccharide Endotoxins. *Ann. Rev. Biochem.* 71:635-700.



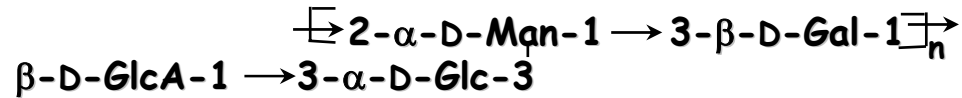
Schematic Diagram of a Lipopolysaccharide

Examples of Capsular Polysaccharides from Enteric Bacteria

Group I

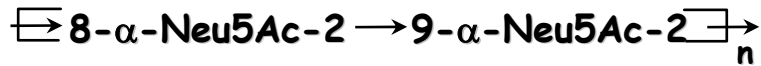


E. coli K40 CPS

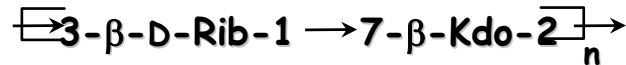


E. coli K30 CPS

Group II



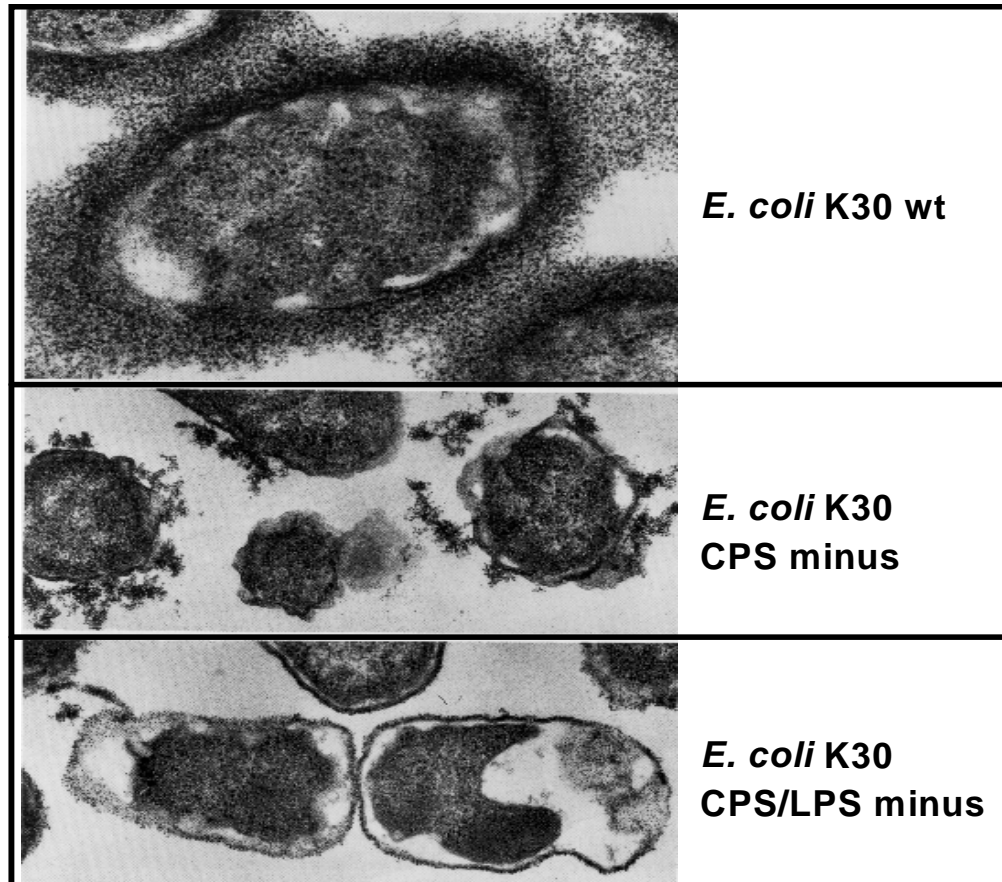
E. coli K92 CPS



E. coli K23 CPS

(Whitfield and Valvano. 1993. Biosynthesis and expression of cell-surface polysaccharides in gram-negative bacteria. *Adv. Microbial. Physiol.* 35:135-246).

E. coli Capsular Polysaccharide

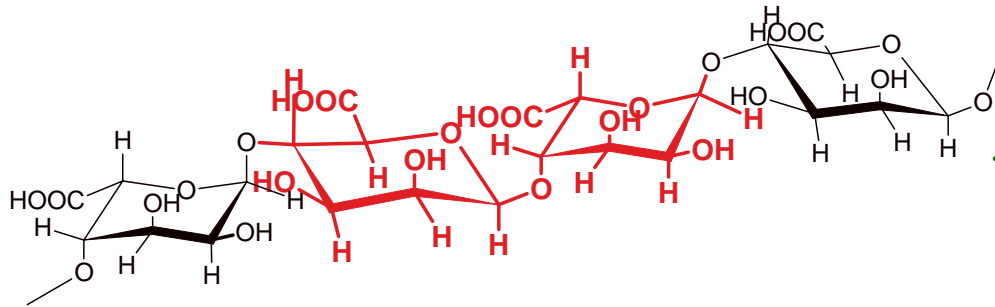


From "Current Topics in Microbiology and Immunology", 1990, Ed. by K. Jann and B. Jann

Virulence Functions of Bacterial Capsular and Lipopolysaccharides

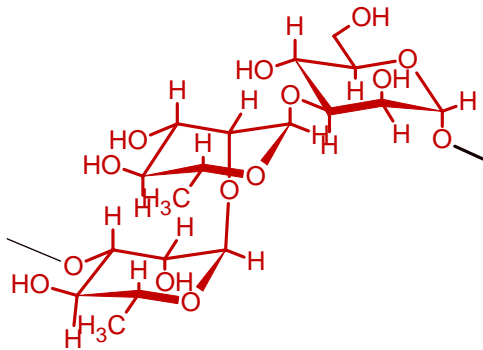
Pseudomonas aeruginosa

(Rocchetta, Burrows, and Lam. 1999. Genetics of O-antigen biosynthesis in *Pseudomonas aeruginosa*. *Microbiol. and Molecular Biol. Rev.* 63:523-553. READ pages 523-528)



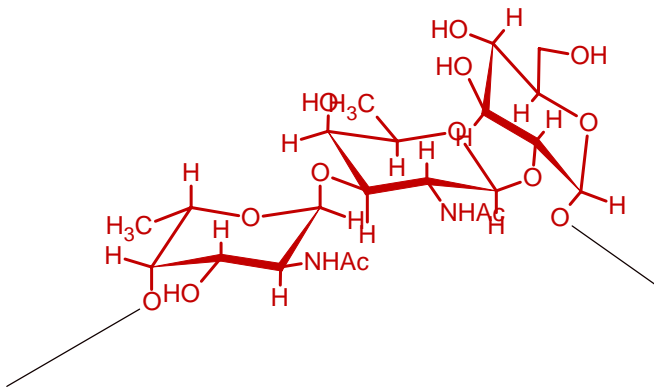
Alginate

-4- β -D-ManA-1-4- α -L-GulA-1-



A-Band LPS O-Polysaccharide

-3- α -D-Rha-1-2- α -D-Rha-1-3- α -D-Rha-1-



O11 B-Band LPS O-Polysaccharide

-3- α -L-FucNAc-1-3- β -D-FucNAc-1-2- β -D-Glc-1-

Contributions of *Pseudomonas aeruginosa* Polysaccharides to Pathogenicity

Mutant strains which can not produce an LPS O-chain polysaccharide are much less infective, and much more sensitive to serum killing than are wild-type strains that have polysaccharide.

O-chain defective mutants are bound more efficiently by a receptor on the surface of epithelial cells, presumably, resulting in greater ingestion and destruction of *P. aeruginosa* by these cells.

B-band polysaccharide is both immunogenic and able to confer serum resistance, while A-band polysaccharide is relatively non-immunogenic and does not confer serum resistance.

Bacteria isolated from lungs of CF patients produce EPS (alginic acid), and LPS with A-band but not B-band polysaccharide.

Alginic acid is correlated with microcolony growth within the lungs which takes place on biofilm consisting of alginic acid.

(Rocchetta, Burrows and Lam. 1999. Genetics and O-antigen biosynthesis in *Pseudomonas aeruginosa*. *Microbiol. and Mol. Biol. Rev.* 63:523-553.)

Pathogenicity of *Neisseria meningitidis*

350,000 deaths/year world wide.

- Fatality rate in children is 20 fold greater than in adults
- In epidemics, there is a shift to older children and adults (15 to 20 year-olds).

The organism is common in the mucous membranes.

Pathogenicity

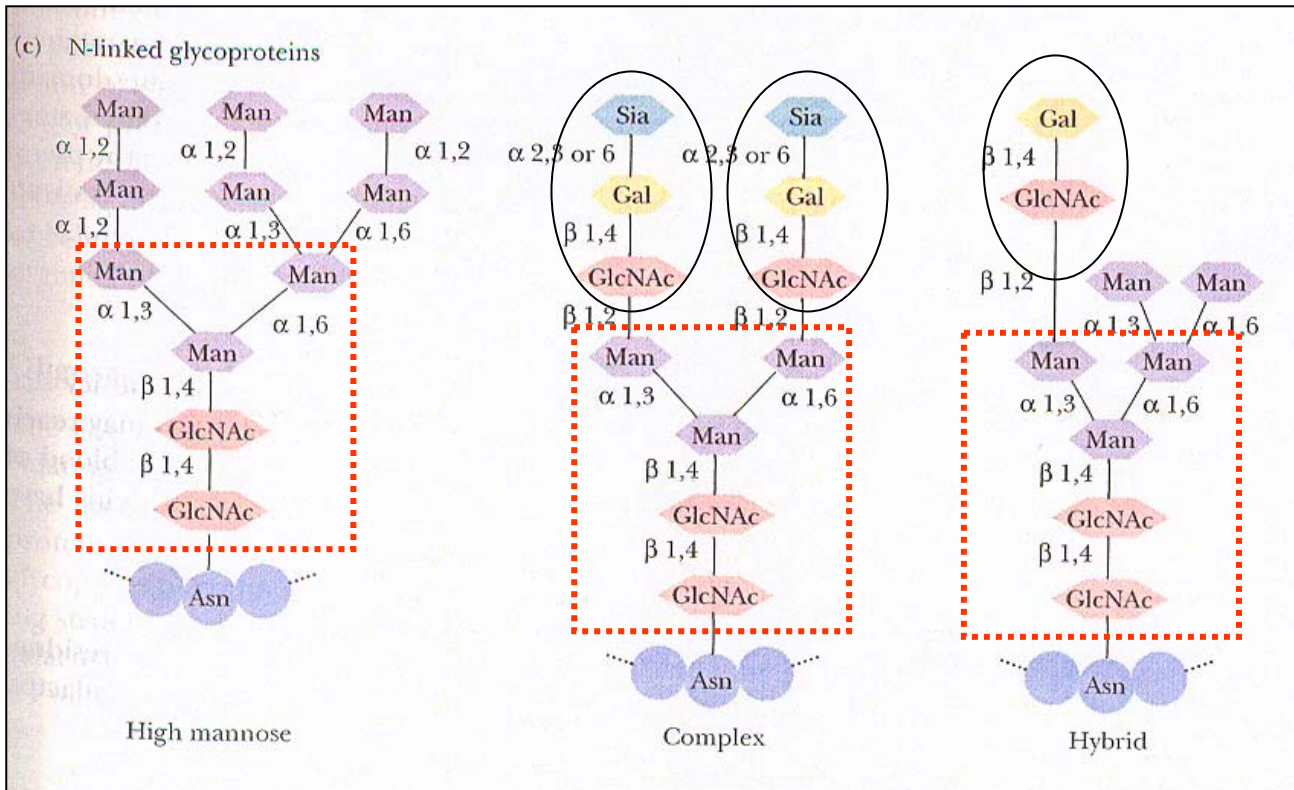
Attachment

- Piliated acapsular cells containing LPS with terminal β -Gal-1-4-Glc- moiety that is not sialylated.
- An outer membrane protein, Opc;, mediates attachment.
- Enzymes involved in sialylation of LOS and in production of sialic acid-containing CPS are down-regulated (e.g. Lst, or *lgt* cluster).
- Attachment is followed by cell invasion, multiplication and entry into the blood stream.

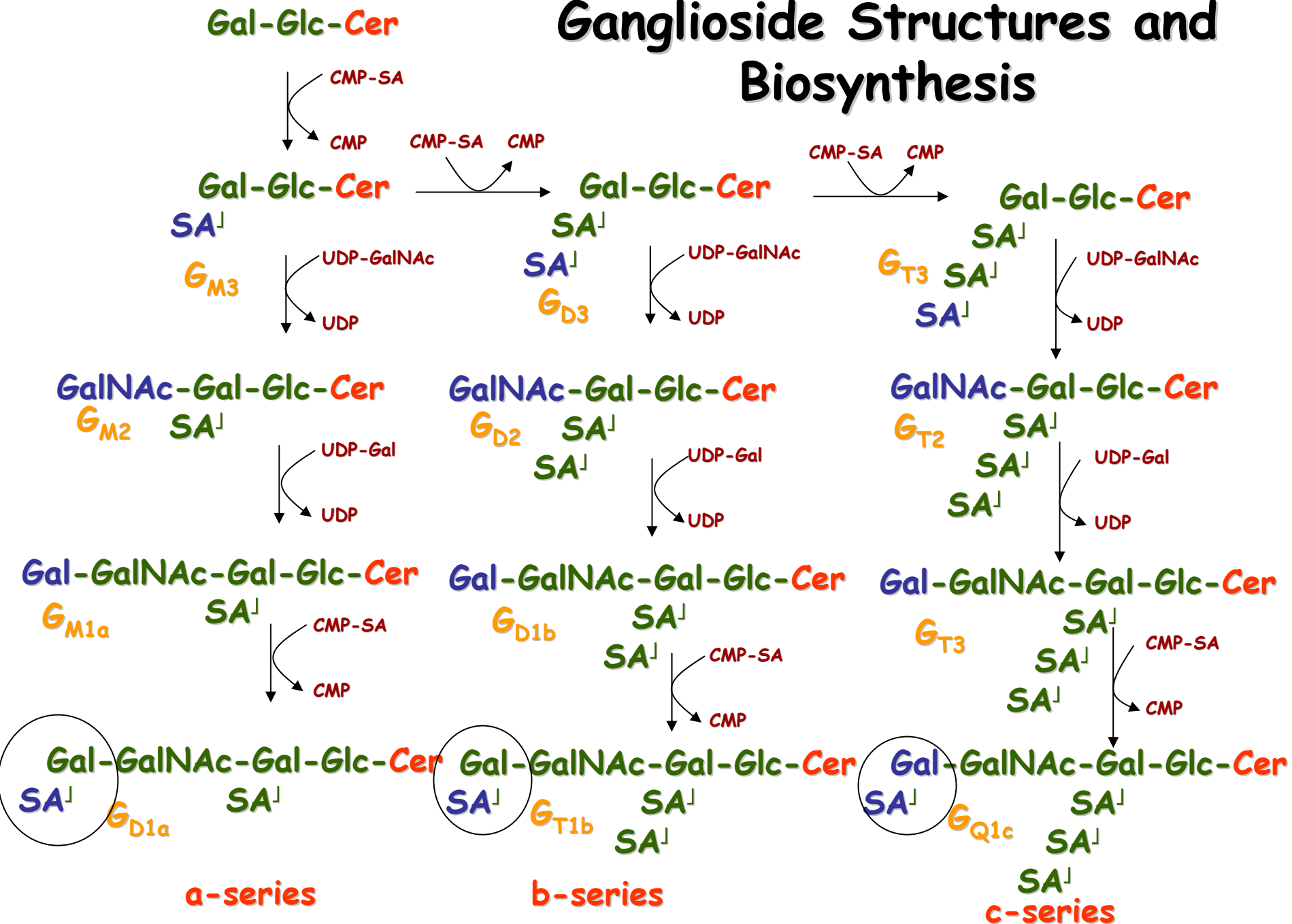
Invasive Cells

- Once in the blood stream, sialylated LPS and CPS are produced.
- CPS inhibits immune response.
- LPS oligosaccharides mimic those of host glycosphingolipids.
- Sialylated LPS structures contribute to serum resistance.
- Release of LPS into the blood stream leads to endotoxemia.

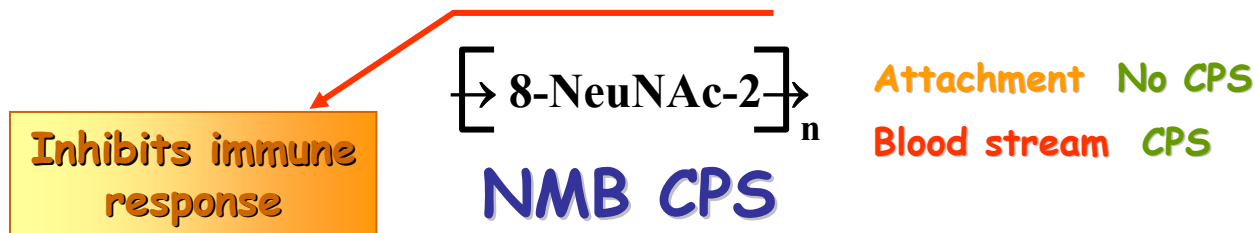
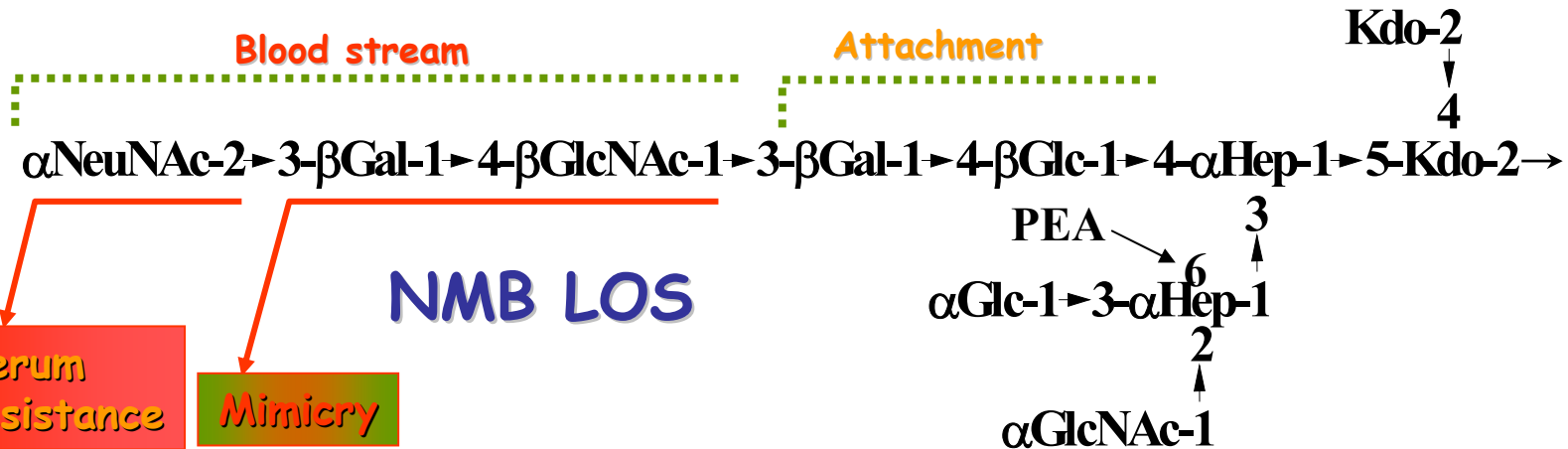
N-Linked Glycoproteins



Ganglioside Structures and Biosynthesis



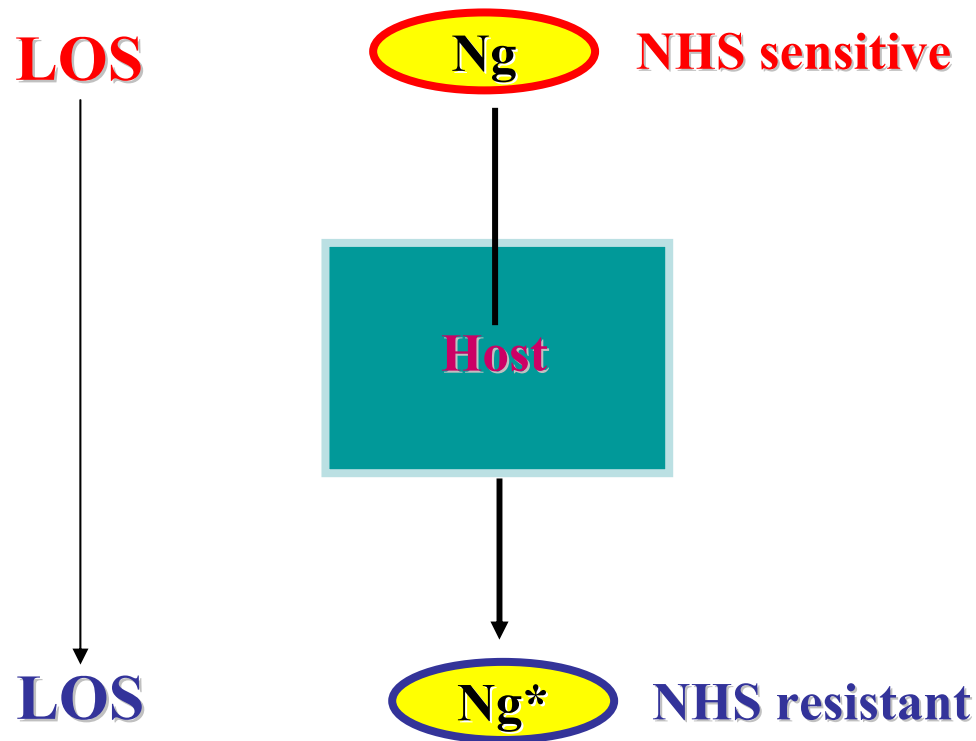
Neisseria meningitidis NMB



(Kahler and Stephens. 1998. Genetic basis for biosynthesis, structure, and function of meningococcal lipooligosaccharide (Endotoxin). *Crit. Rev. Microbiol.* 24:281-334.)

Phase Variable Changes in Genes *lgtA* and *lgtC* within the *lgtABCDE* Operon of *Neisseria gonorrhoeae* Can Modulate Gonococcal Susceptibility to Normal Human Serum

William Shafer, Emory University
Anup Datta, Kumar Kolli, Russell Carlson, CCRC, UGA



Regulation of Glycosyl Transferases by Slip-Strand Mismatching Events

Glycosyl Transferase

12

NNNNNGGGGGGGGGGGGGNNNNNN

Phase-on

11

NNNNNGGGGGGGGGGGGGNNNNNN

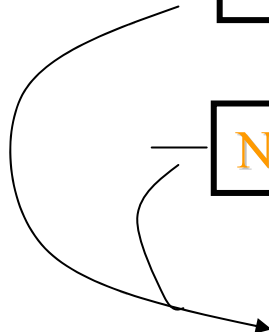
Phase-off

13

NNNNNGGGGGGGGGGGGGGNNNNNN

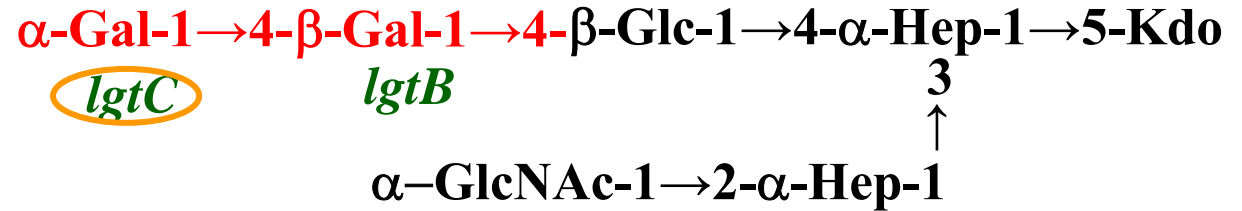
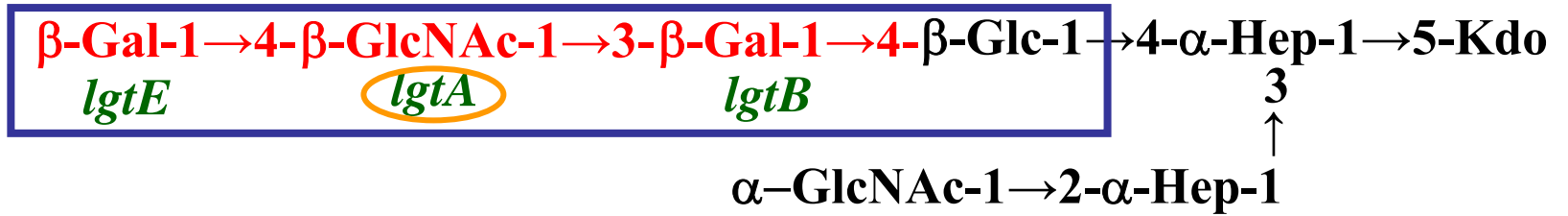
Phase-off

Truncated proteins

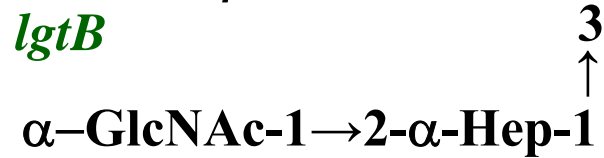
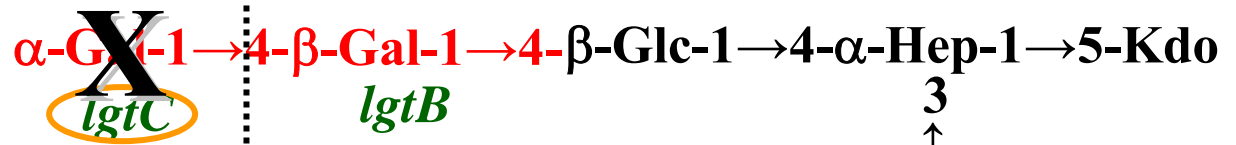
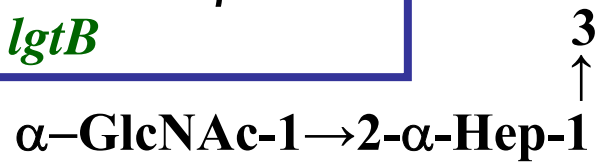


Neisseria gonorrhoeae LOS Oligosaccharides

α -chain



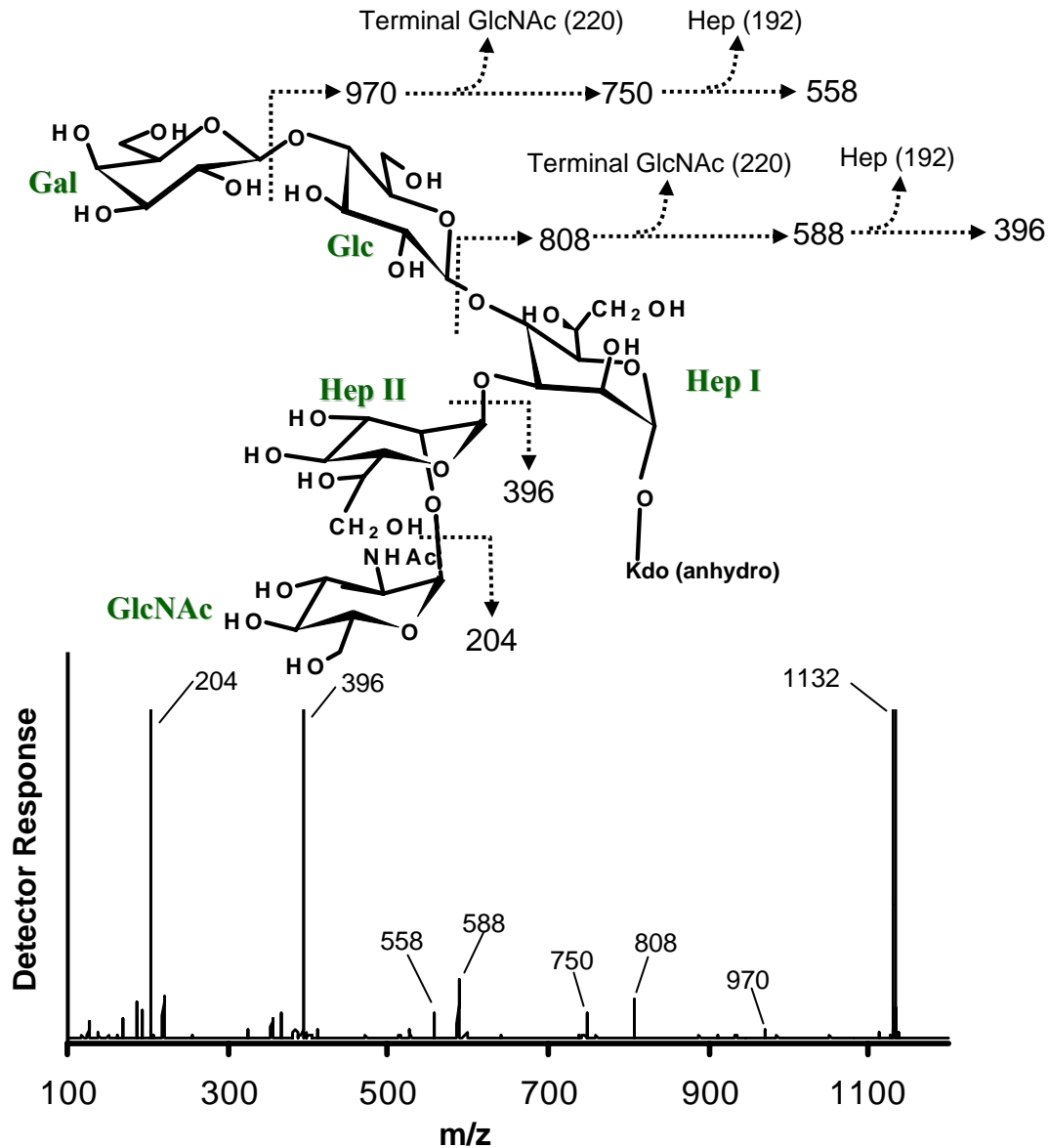
α-chain



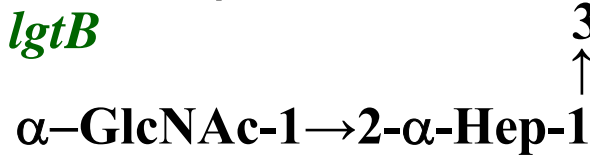
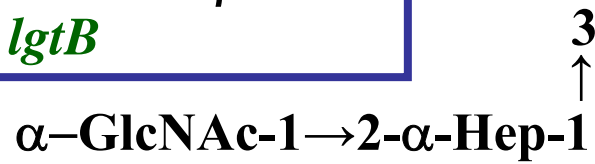
FA19 & SS2B (Serum resistant, 97% survival)

- NNNNGGGGGGGGGGGGGGNNNNN- *lgtA* on, 346 aa
- NNNNGGGGGGGGGGGGGGNNNNN- *lgtA* off, 105 aa
- NNNNGGGGGGGGGGGGGGGGNNNNN- *lgtC* on, 305 aa
- NNNNGGGGGGGGGGGGGGGGGNNNNN- *lgtC* off, 57 aa

MS/MS Analysis of Oligosaccharides from FA19 and SS2B



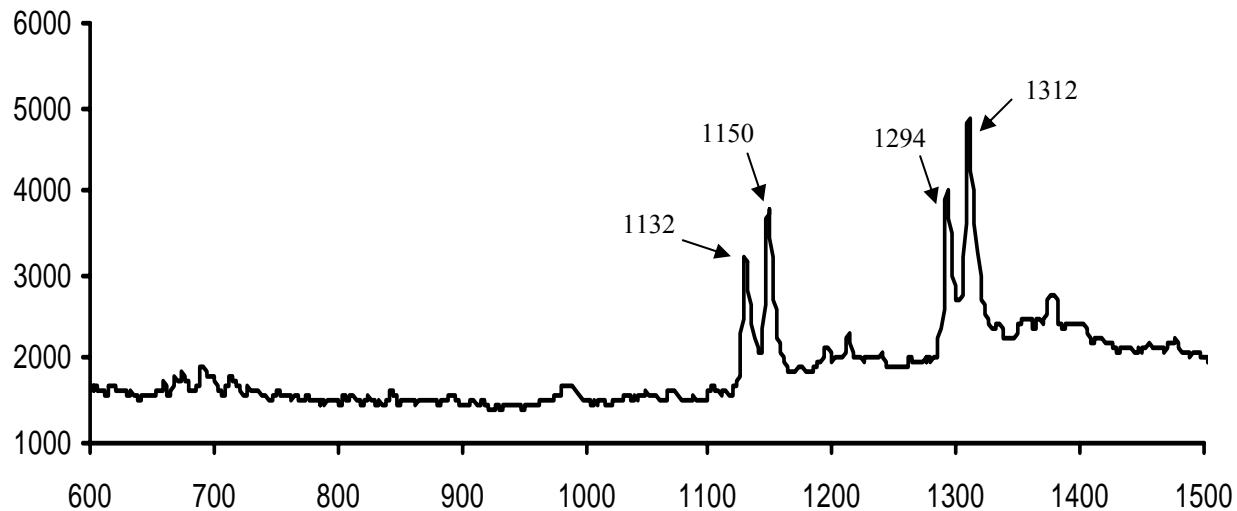
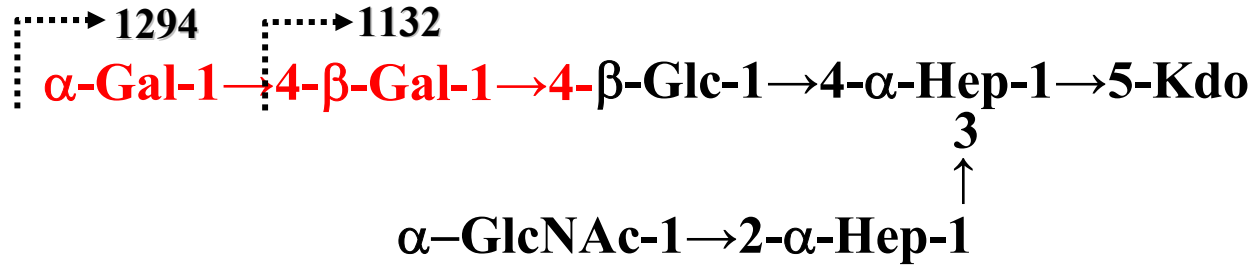
α-chain



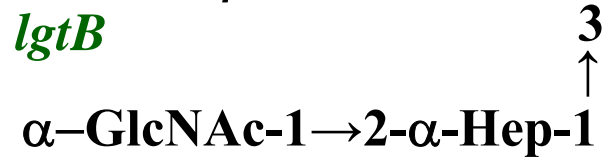
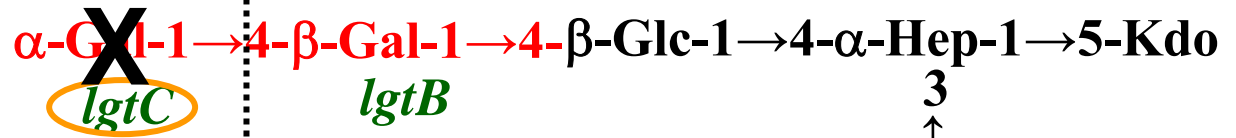
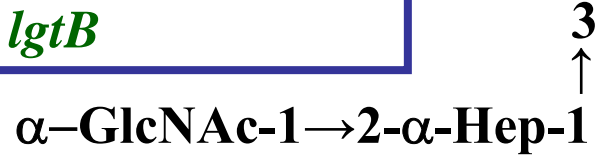
SS9 (partially serum sensitive,
 37% survival)

- NNNNGGGGGGGGGGGGNNNNN- *lgtA* on, 346 aa
- NNNNGGGGGGGGGGGGNNNNN- *lgtA* off, 105 aa
- NNNNGGGGGGGGGGGGGGGGNNNNN- *lgtC* on, 305 aa
- NNNNGGGGGGGGGGGGGGGGGNNNNN- *lgtC* off, 57 aa

Analysis of Oligosaccharides from SS9



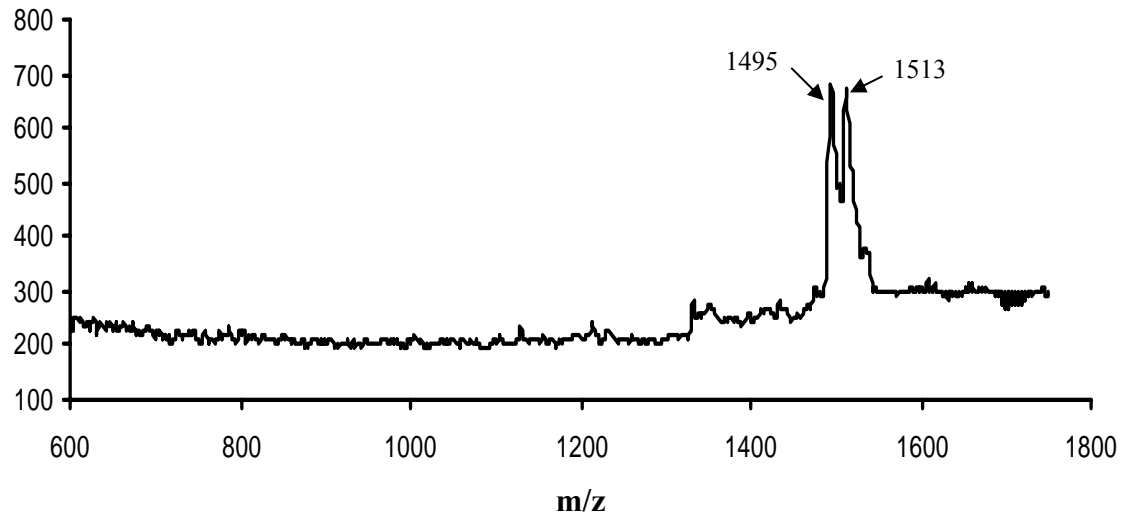
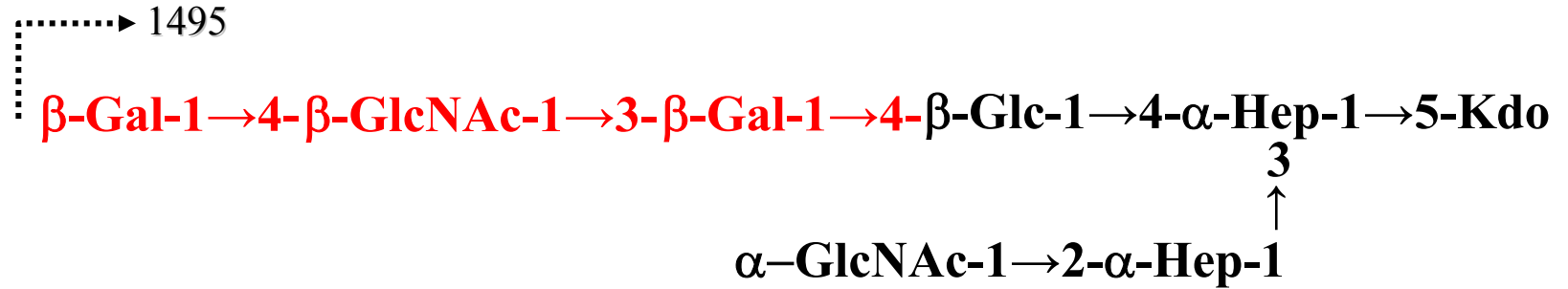
α-chain



SS2 (serum sensitive, 0.03% survival)

- **-NNNNGGGGGGGGGGGGNNNNN-** *lgtA* on, 346 aa
- **-NNNNGGGGGGGGGGGGNNNNN-** *lgtA* off, 105 aa
- **-NNNNGGGGGGGGGGGGNNNNN-** *lgtC* on, 305 aa
- **-NNNNGGGGGGGGGGGGGNNNNN-** *lgtC* off, 57 aa

Analysis of Oligosaccharides from SS2



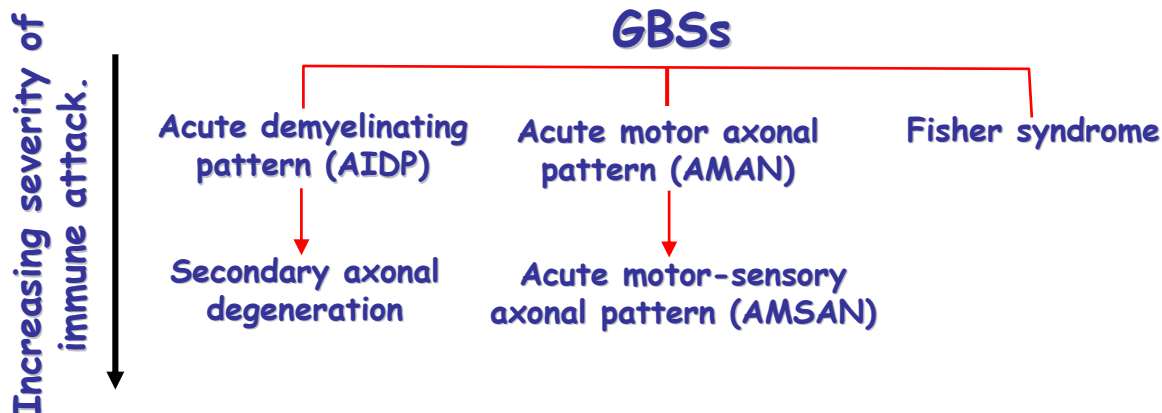
Campylobacter jejuni Lipopolysaccharides And Guillain-Barre Syndrome

(Nachamkin, et al. 1998. *Campylobacter* Species and Guillain-Barre Syndrome.
Clinical Microbiol. Rev. 11:555-567 ON WEB SITE)

What is Guillain-Barre Syndrome?

An autoimmune disorder of the peripheral nervous system. Weakness of limbs, respiratory muscles, reflexes. 15 to 20% of patients are left with severe neurological defects.

C. jejuni-associated GBS results in more severe disease, a greater requirement for ventilation assistance, more severe neurological disorders, and more deaths,



Gangliosides & *C. jejuni* LPS

Gangliosides	<i>C. jejuni</i> LPS
<p style="text-align: center;">GalNAc-Gal-Glc-Cer SA[↓] G_{M2}</p>	<p style="text-align: center;">PEA₇ GalNAc-Gal-Gal-Hep-Hep-Kdo-Lipid-A SA[↓] Gal[↓] Glc[↓] Glc[↓] Cj O:1, O:23/36</p>
<p style="text-align: center;">Gal-Glc-Cer SA[↓] G_{M3}</p>	<p style="text-align: center;">PEA₇ Gal-Gal-Hep-Hep-Kdo-Lipid-A SA[↓] Gal[↓] Glc[↓] Glc[↓] Cj O:2</p>
<p style="text-align: center;">Gal-GalNAc-Gal-Glc-Cer SA[↓] SA[↓] G_{D1a}</p>	<p style="text-align: center;">PEA₇ Gal-GalNAc-Gal-Hep-Hep-Kdo-Lipid-A SA[↓] SA[↓] Glc[↓] Cj O:4, O:19</p>
<p style="text-align: center;">Gal-Glc-Cer SA[↓] SA[↓] G_{D3}</p>	<p style="text-align: center;">P₇ Gal-GalNAc-Gal-Hep-Hep-Kdo-Lipid-A SA[↓] SA[↓] Glc[↓] Glc[↓] Cj O:1</p>

Carbohydrate Based Vaccines

READ (on the WEB SITE):

Lindberg, A.A. 1999. Glycoprotein vaccines. *Vaccine*:S28-S36

Weintraub, A. 2003. Immunology of bacterial polysaccharide antigens. *Carbohydr. Res.* 338:2539-2547.

Why Carbohydrate Vaccines?

Historical Factors (by the 1940s):

1. Polysaccharides (PS), on killed cells or purified, produced protective immune responses.
2. Infants and young children did not produce antibodies (either from the PS, the killed cells, or from actual disease).
3. Specific PS molecules characteristic of the particular strain (or type) produced the protective response.
4. Vaccination with PS reduced the need to vaccinate with the organism itself.
5. When the PS was coupled to a protein, it produced a much higher titer (in rabbits).

Why the Late Development of Carbohydrate Vaccines?

1. The introduction of antibiotics.
2. By 1970s it was recognized that antibiotics would not be the ultimate solution.
3. Numerous failures due to the rise of resistant forms of the disease organisms.
4. Advances in understanding of the immune system.
5. Structural determination of numerous carbohydrate structures leads way to development of defined glycoconjugates.

Haemophilus influenzae type b (Hib)

Diseases: meningitis, epiglottitis, septicemia, facial cellulitis, pneumonia, arthritis, and others.

Prevaccine era:

1. 30 to 60 cases per 100,000 in children under 5 years of age in Europe and 50 to 100 cases/100,000 in the U.S.
2. Native Americans and Australian aborigines had up to 300 cases per 100,000.
3. Case study of 100,000 children: >18 months were protected; 12 to 18 months some protection; 3 to 12 months had no protection.

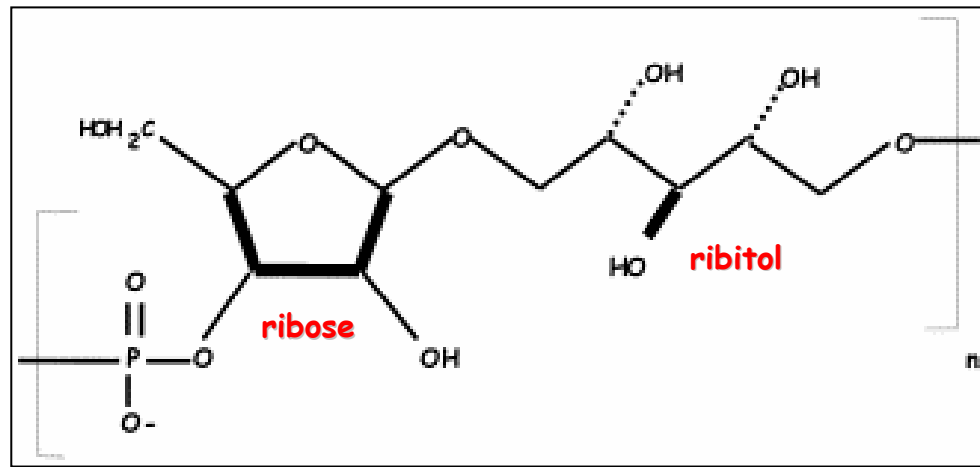
Hib Carbohydrate Vaccine

1. *H. influenzae* strains are mostly non-encapsulated and are, therefore, “non-typeable” (NTHi).
2. A number do have CPS and there are six different CPS types.
3. One CPS type, type “b”, is the most common and most virulent.

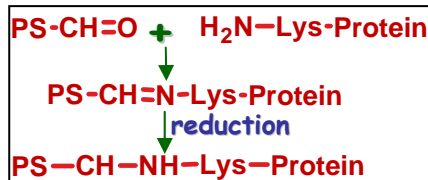
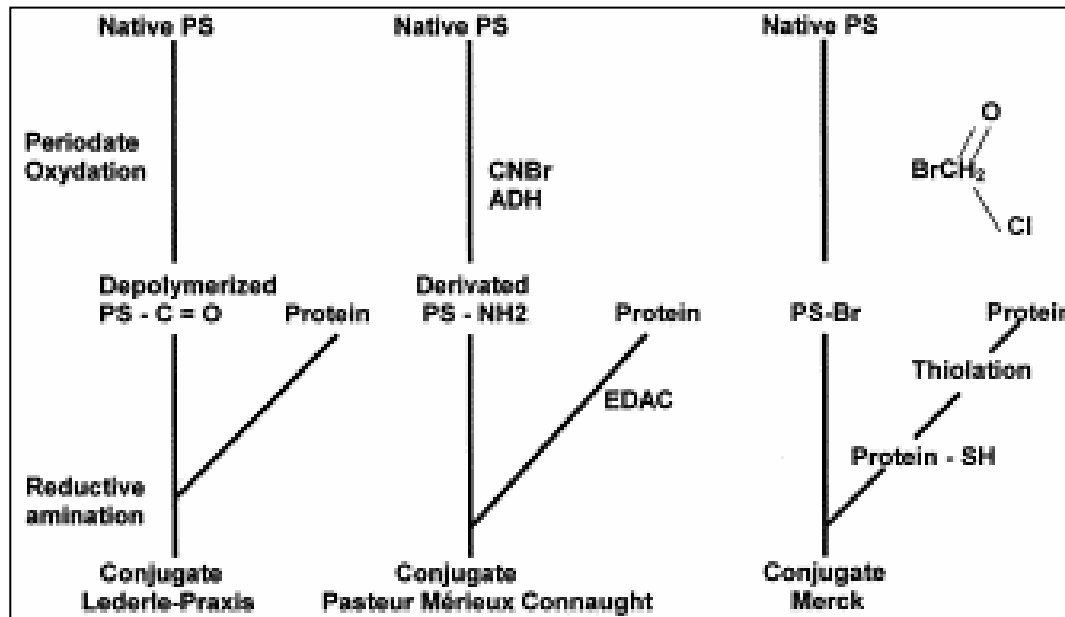
Hib Carbohydrate Vaccine

The lack of protection in children under 12 months (i.e. no Hib antibodies) together with the role of T and B cells in immune memory, suggested the need for the development of Hib CPS-protein conjugate vaccines.

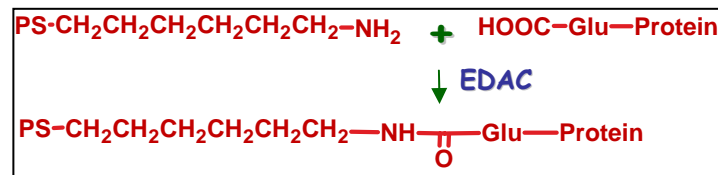
Hi type b (Hib)
capsular
polysaccharide,
polyribitol/ribose
phosphate (PRP).



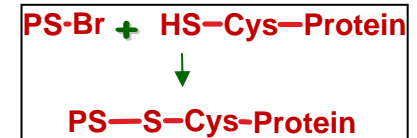
Hib Carbohydrate-Conjugate Vaccine



HbOC (Hib Titer)
Lederle-Praxis



PRP-D or T (Act Hib)
Pasteur-Merieux



PRP-OMP (PedVax Hib)
Merck, Sharpe & Dohme

Streptococcus pneumoniae

Diseases: otitis media, bronchopneumonia, meningitis.

In the U.S. each year: 3000 cases of meningitis, 50,000 bacteremia, 500,000 pneumonia, 7,000,000 otitis media; 40,000 deaths. World wide deaths per year: more than 4,000,000, most under age of 5.

S. pneumoniae Capsular Polysaccharides

1. 90 different CPS serogroups (different CPS structures).
2. 7 CPS types cover 85% of all infections.
3. 23 CPS types cover up to 90% of all infections.
4. CPS vaccines offer >90% protection, if over 2 years of age.
5. CPS-protein conjugate vaccines are under development.

S. pneumoniae Capsular Polysaccharide-Conjugate Vaccine

Multivalent vaccines needed to cover majority of infections.

A 23 valent conjugate vaccine resulted in an 89.1% efficacy against invasive disease in children under 5 years of age.

“In infants and vulnerable children throughout the world, PNCRM7 vaccine has the potential to reduce the mortality and morbidity rates associated with *S. pneumoniae* infections. In disabling infections but its impact in developing countries will be more pronounced with the potential to greatly reduce mortality.”

(Darkes and Plosker. 2002. *Paediatr. Drugs* 4:609-630)

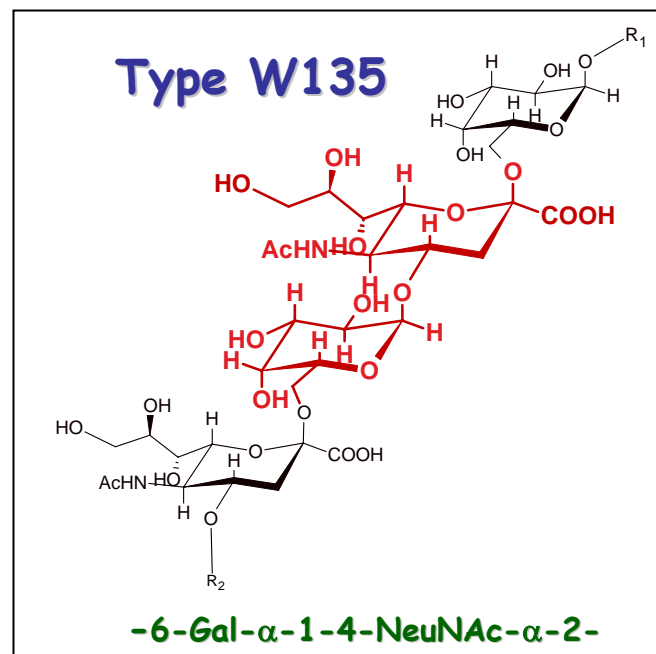
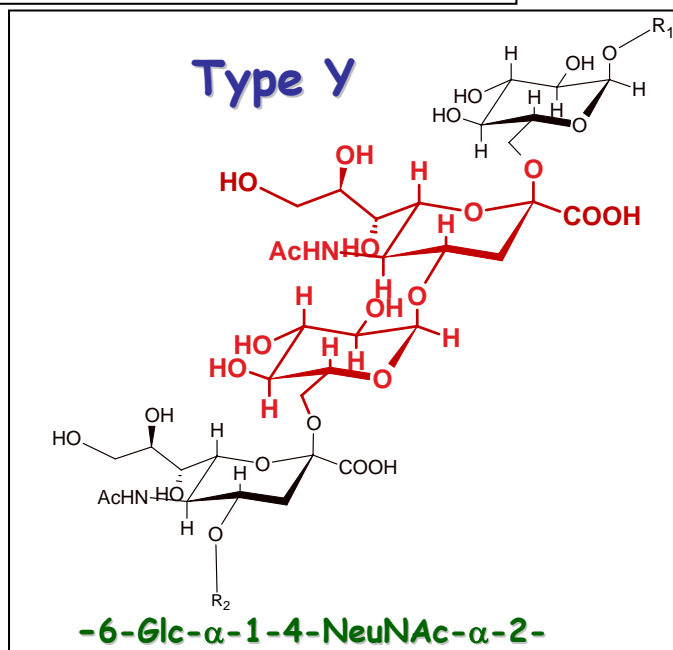
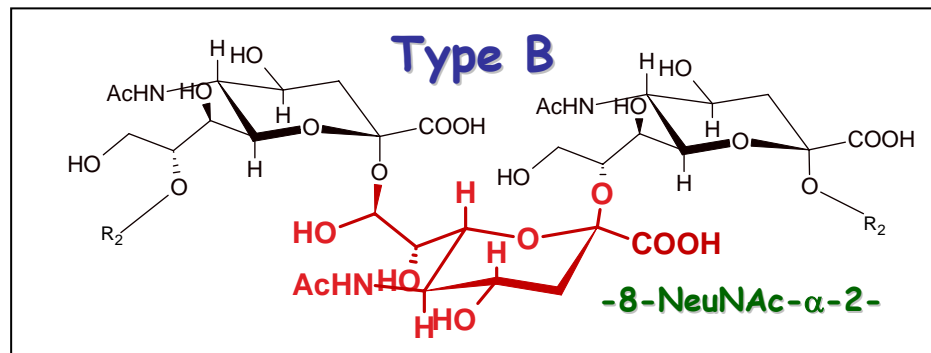
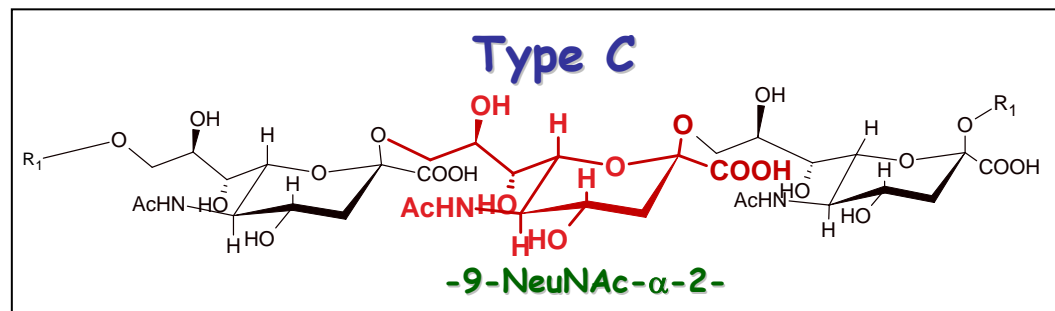
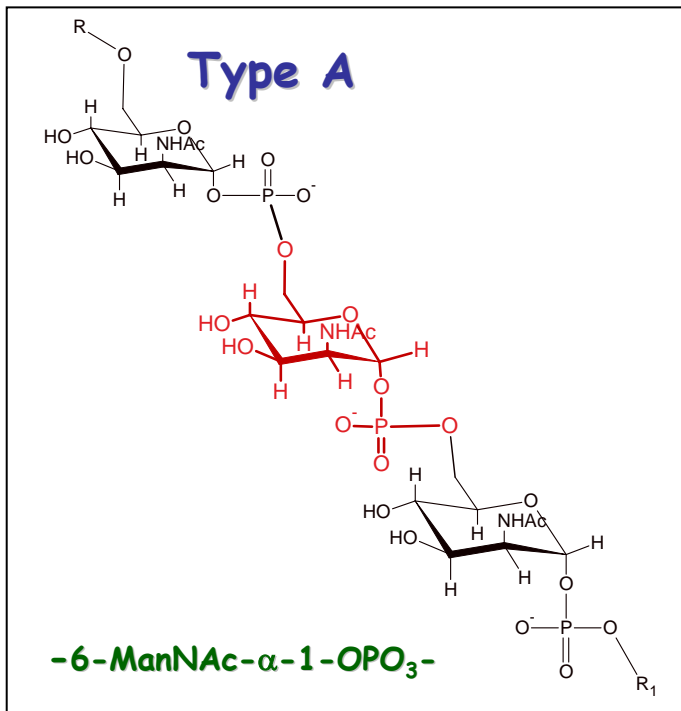
Neisseria meningitidis

Infections are more rare than other infections we have discussed but it causes meningitis with a high mortality rate.

33% of infections occur from 0 to 4 years old, 33% from 5 to 9 years of age, and 33% at age 20 and above.

There are 12 serogroups based on their differing CPS structures; A, B, C, 29E, H, I, K, L, W135, X, Y, and Z. Almost all infections are caused by types A, B, C, W135, and Y. A=sub-Saharan Africa; B=Europe and Latin America; C=North America; W135=Saudi Arabia (Hajj outbreak)

There are currently CPS-based vaccines against A, C, W135, and Y. They are not conjugate vaccines (except for C) and, therefore, do not work in young children.



Neisseria meningitidis Capsular Polysaccharide-Conjugate Vaccine

Meningitec®: Wyeth

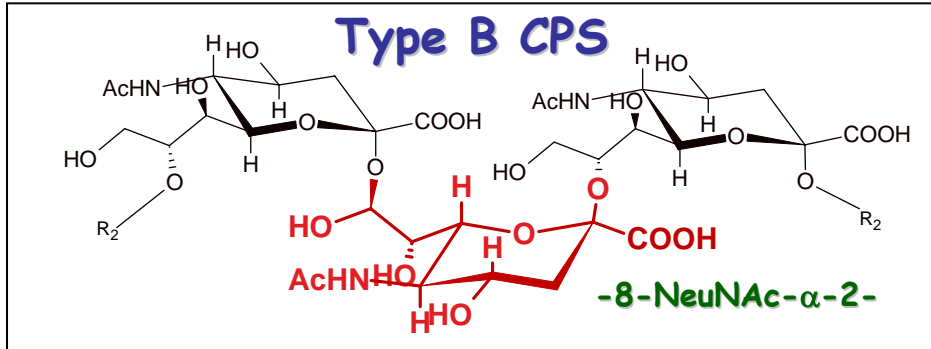
Menjugate®: Chiron

NeisVac-C®: Baxter

Type C conjugate Vaccines.

Protein-CPS conjugate vaccines are currently under preparation for W135, A, and Y. The goal will be to make a tetravalent vaccine.

What about *Neisseria meningitidis* Type B?



Not suitable for a vaccine as it mimics host structures and may be (a.) non-immunogenic, or (b.) induce and autoimmune reaction.

Type B LOS: There are seven different immunotypes (structures), L1-L7.

