Lecture outline & learning objectives

Outline

- Introduction to *N. meningitidis* and meningococcal disease (MD)
- Main meningococcal virulence attributes (Part I)
- Major advances in meningococcal vaccine development (Part II)

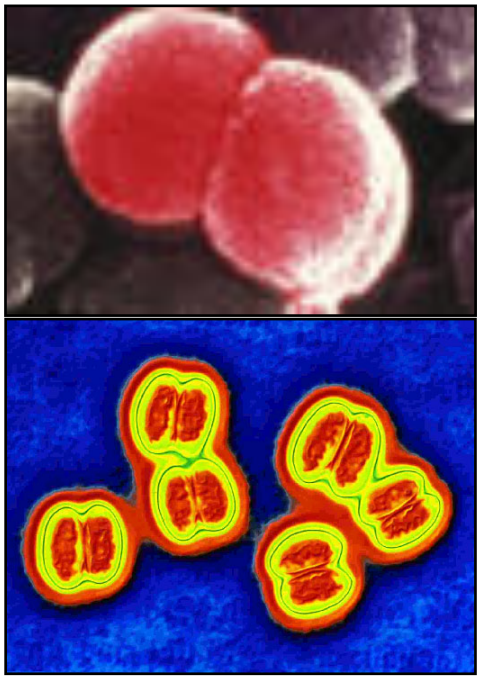
You should be able to

- Describe the outcomes of *N. meningitidis* infection
- Give examples of major meningococcal virulence factors and define how they contribute to the disease process
- Explain the mechanisms of variation of surface molecules and describe how this contributes to virulence
- Comment on the need for meningococcal vaccines
- Describe the various strategies used to design meningococcal vaccines

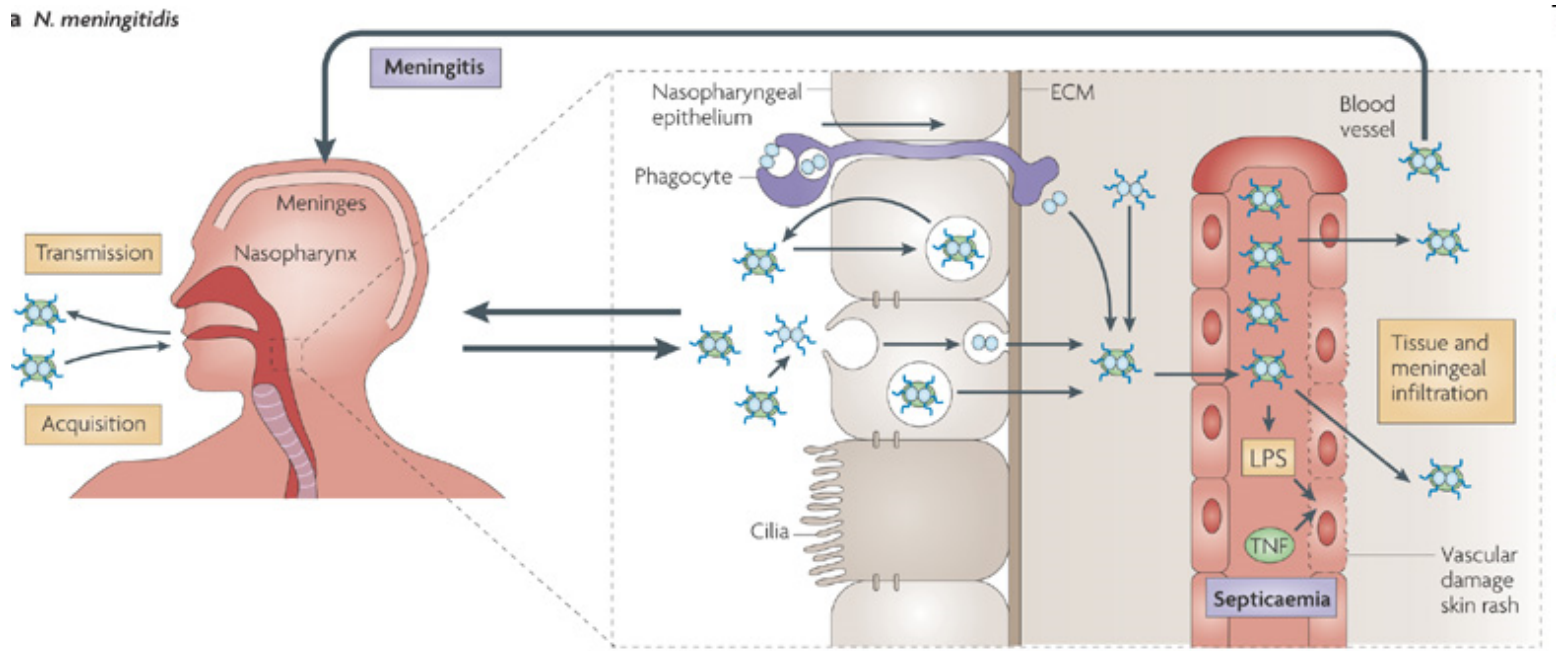
Some facts about *N. meningitidis*

- first report of meningococcal disease in Geneva by Vieusseux as *"fièvre cérébrale maligne non contagieuse"* in 1805

- Gram negative diplococcus
- Obligate human parasite, *i.e.* only reservoir is the human host
- Extracellular pathogen causing meningitis and septicaemia
- Common classification into serogroups based on capsular polysaccharide
- Serogroups A, B, C, W135, Y & X most commonly associated with disease



Outcomes of infection with *N. meningitidis*



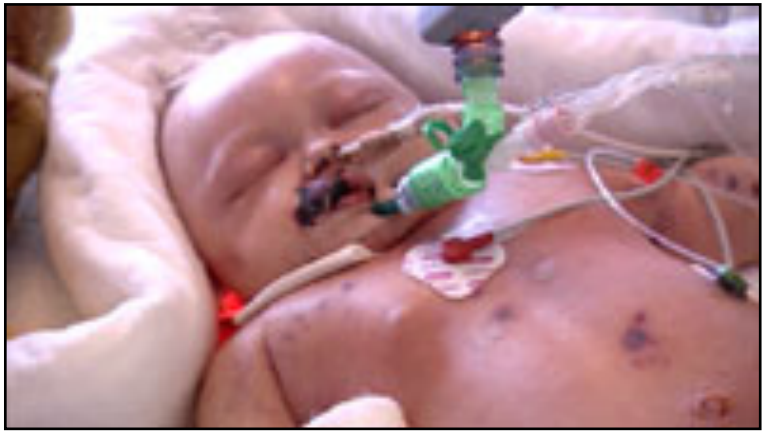
Nasopharyngeal colonisation

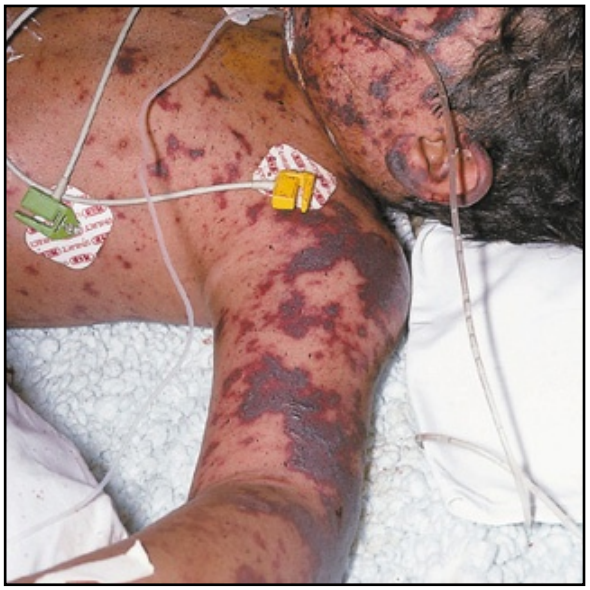
- asymptomatic process
- carriage rates 10-40% of population
- colonisation varies with age
- immunising process

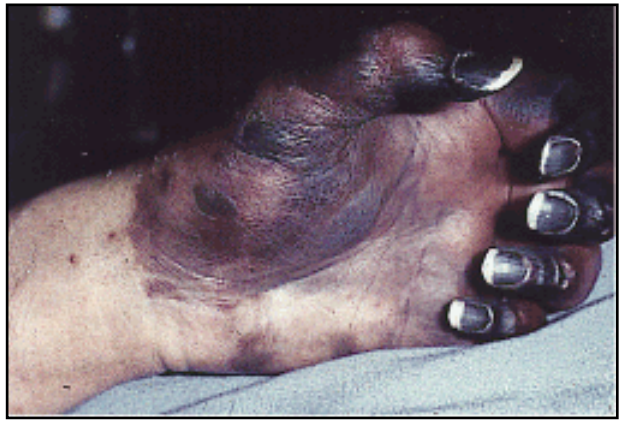
Invasive MD

- bacteria present in systemic circulation
- presents as septicaemia +/- meningitis
- approx. 1/100,000 case per year (UK)
- sporadic cases, outbreaks or epidemics

Meningococcal disease

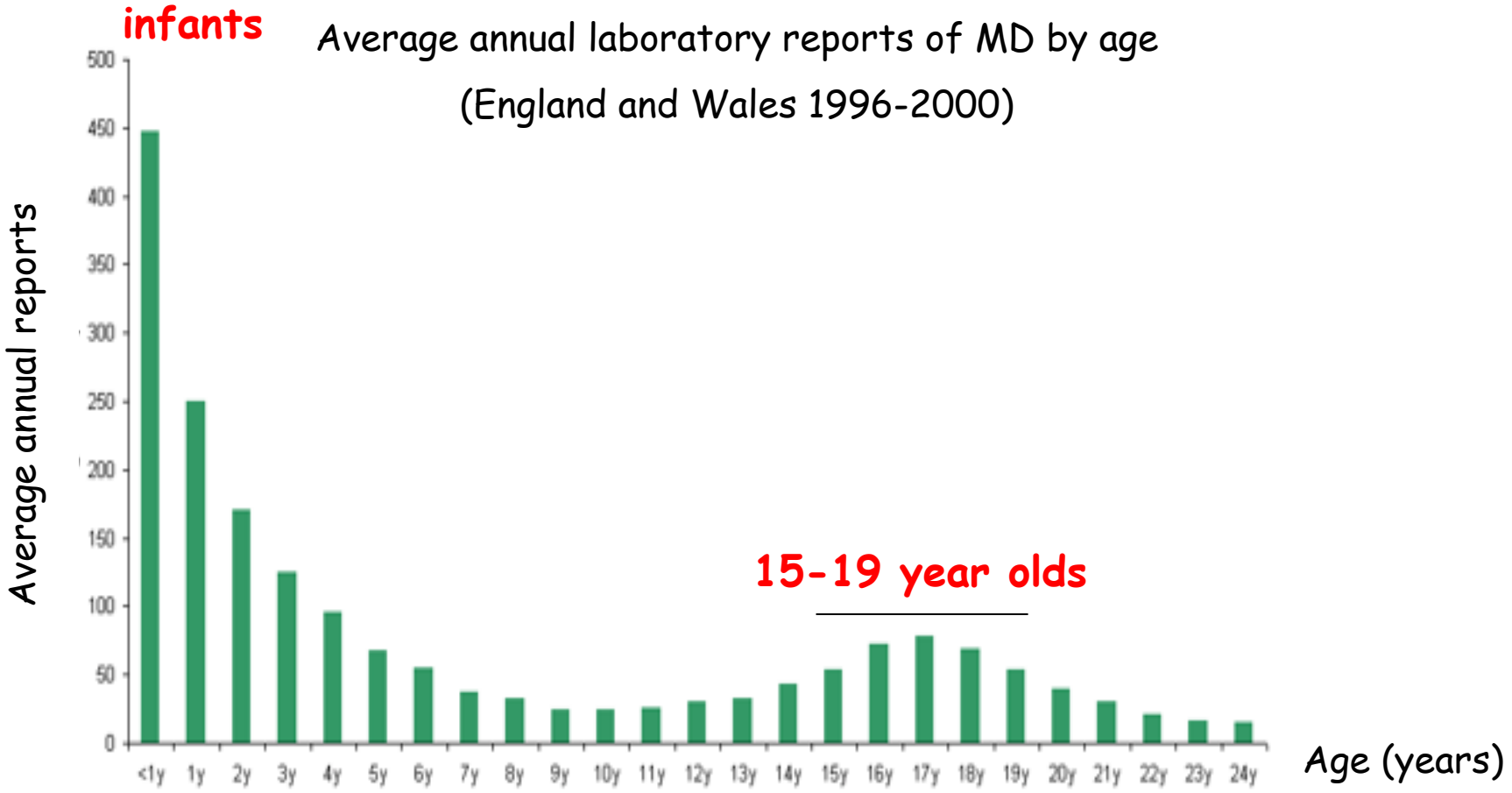




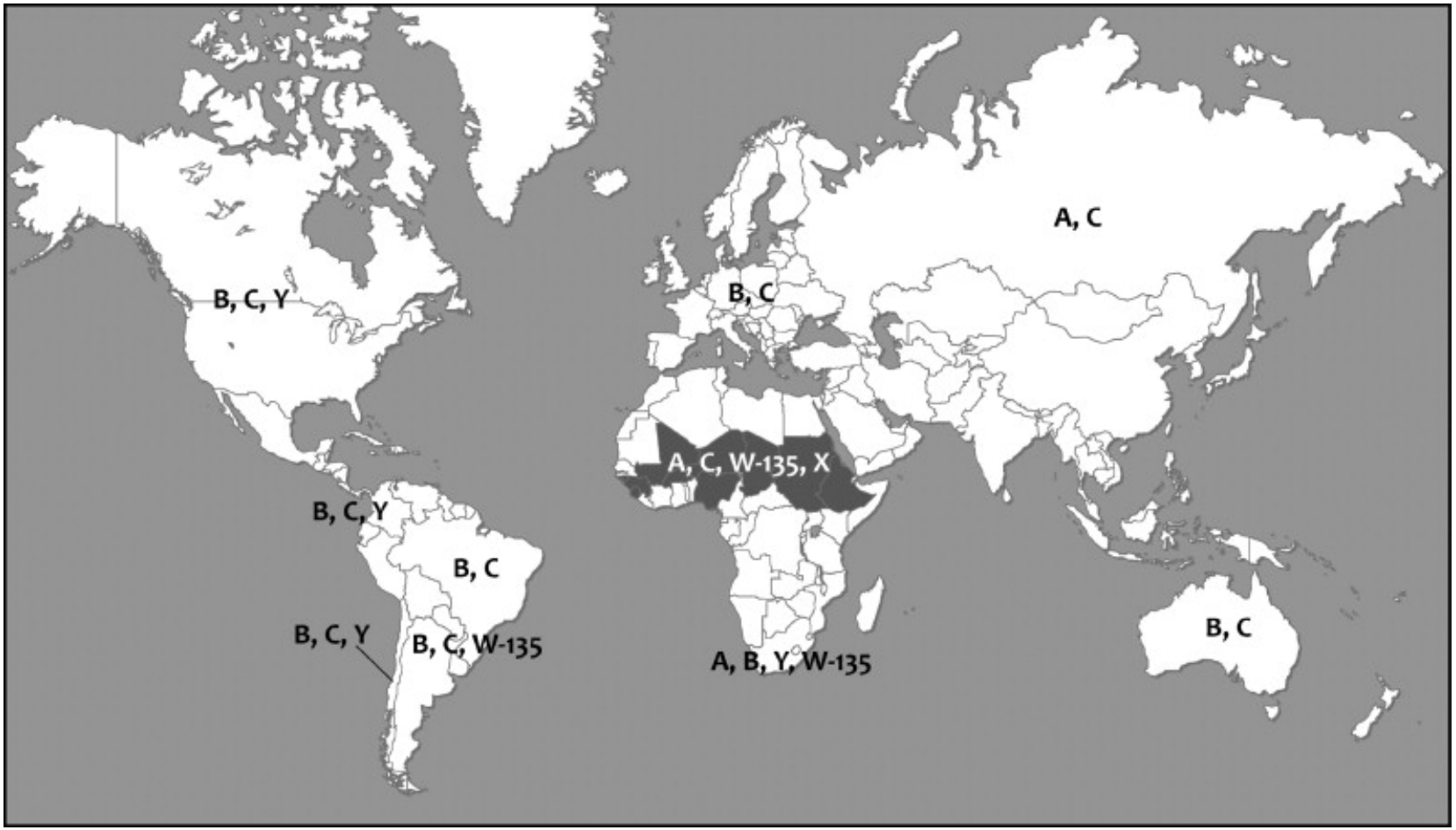


MD affects mainly infants and young adults

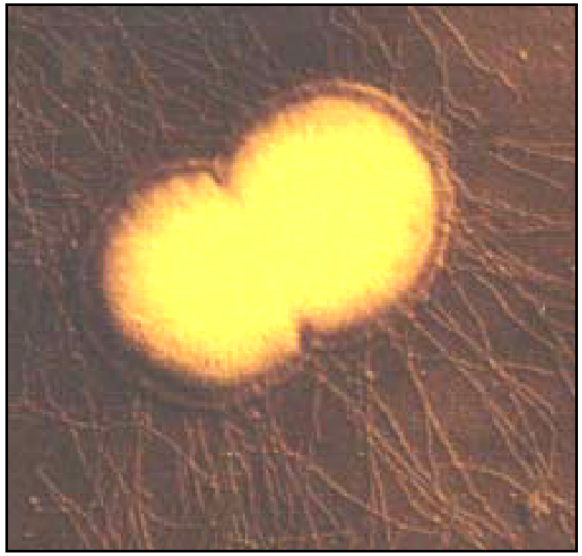
- Commonest infectious cause of death in children and young people
- Number one infectious killer of children aged 1-5 years



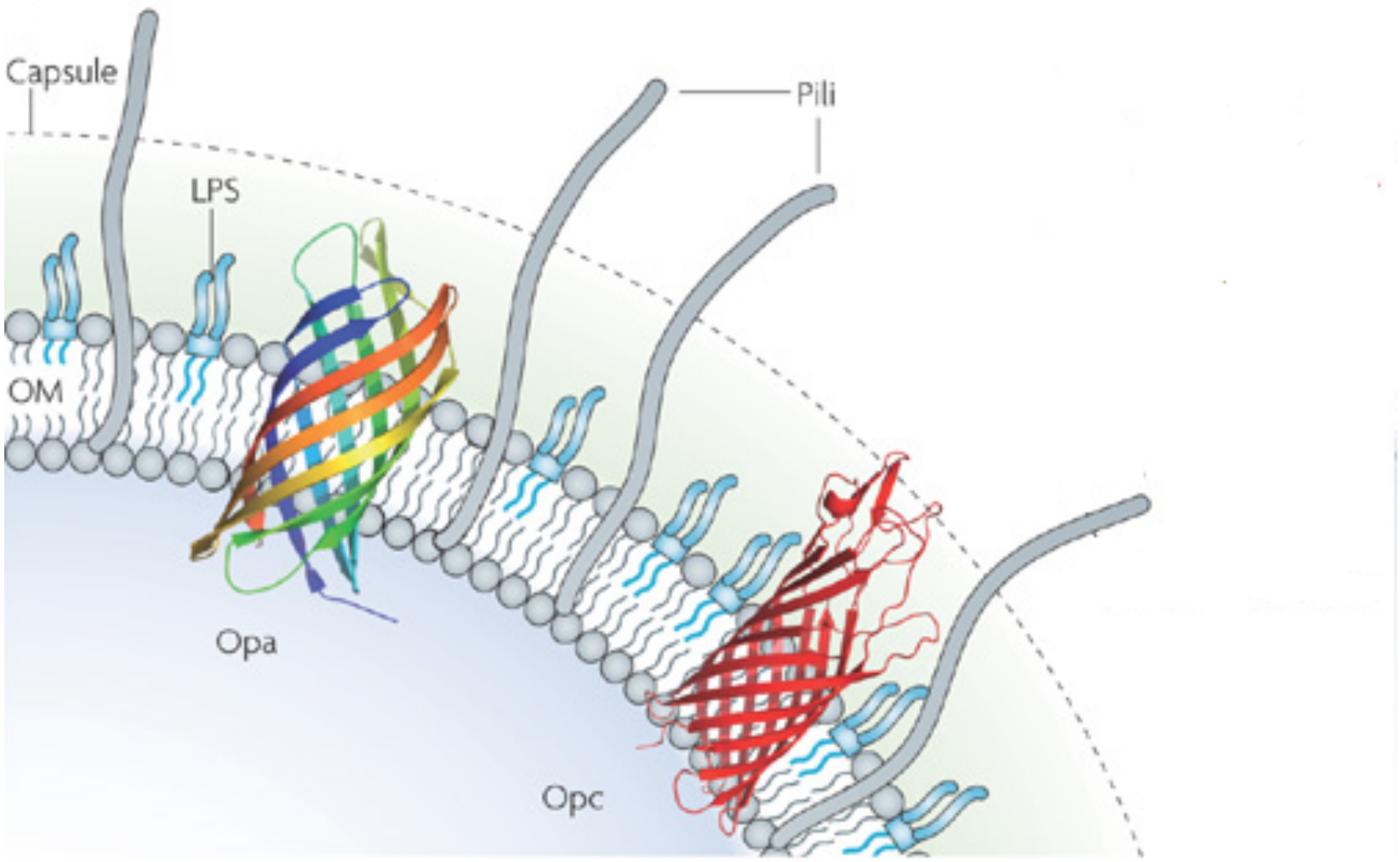
MD is a global problem



Pathogenesis of *Neisseria meningitidis*
Part I



Virulence attributes of *N. meningitidis*

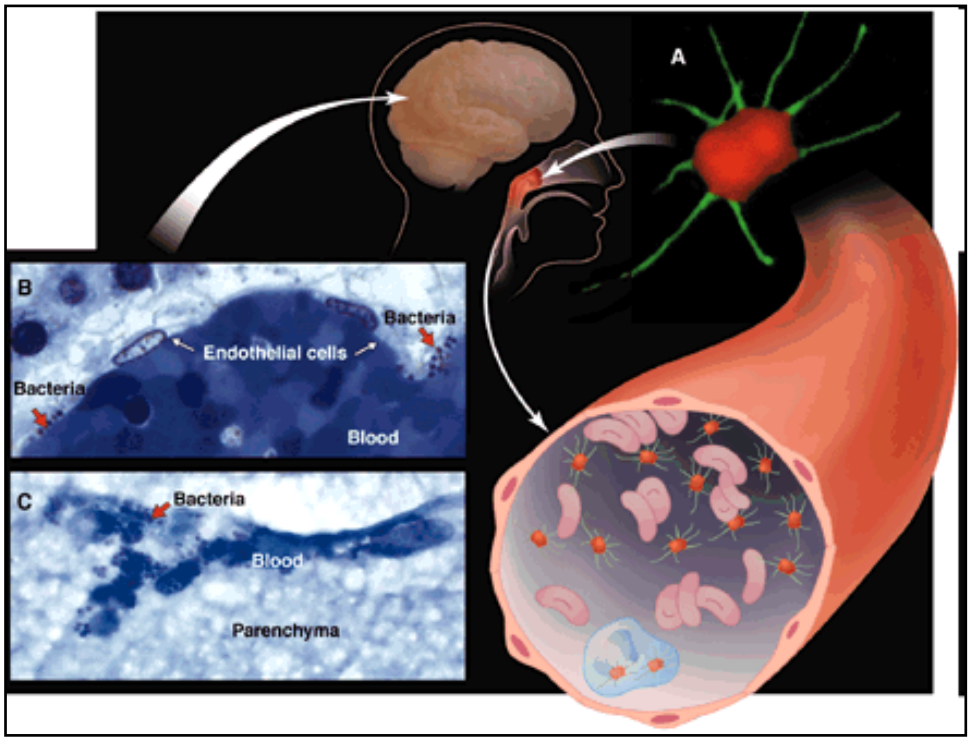


Virji. 2009. Nature Reviews Microbiology **7**: 274-286

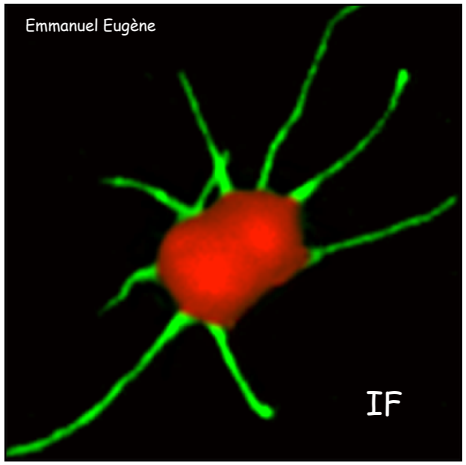
What makes *N. meningitidis* a dreadful pathogen?

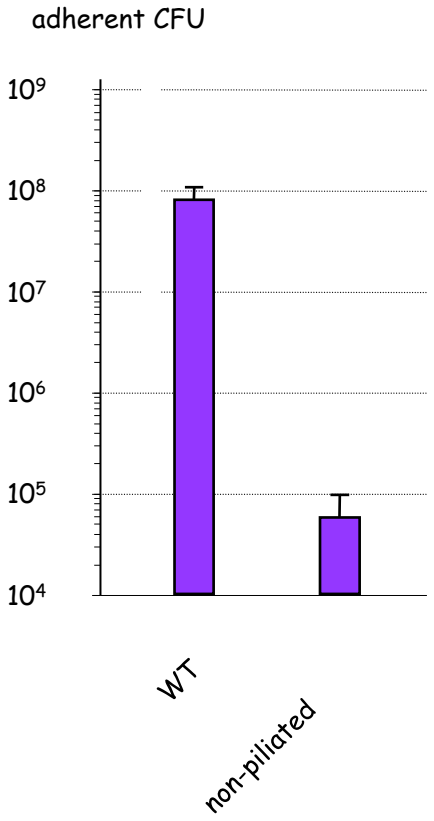
- Adhere to and cross several host barriers (**Tropism**)
- Evade and/or resist immune system (**Evasion**)
- Induce strong inflammatory response (**Damage**)

Tropism

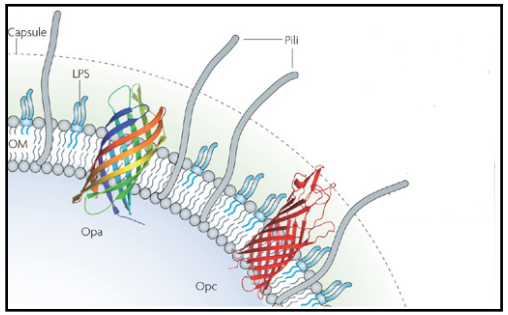

A
B
Bacteria
Endothelial cells
Bacteria
Blood
C
Bacteria
Blood
Parenchyma

1. Type IV pili (Tfp)





2. Opacity proteins



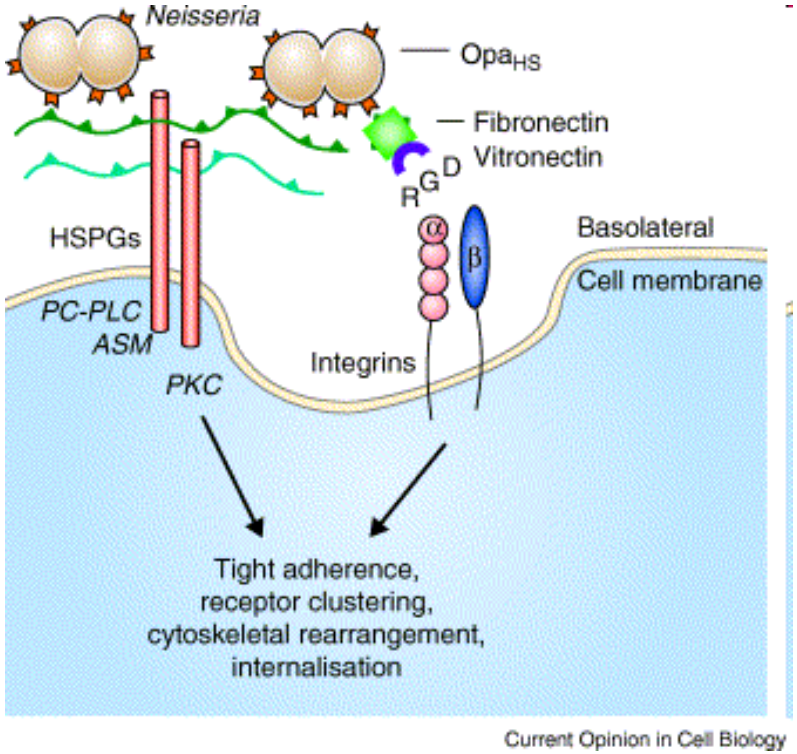
Opa proteins

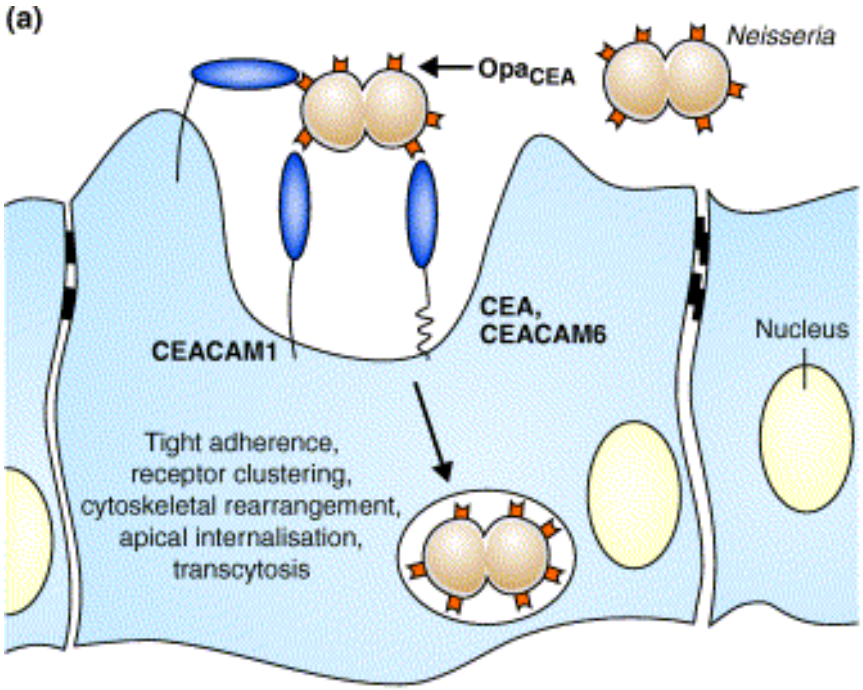
- 24-35 kDa outer membrane proteins
- 8-stranded ß-barrels with 4 exposed surface loops
- *N. meningitidis* has 3-4 copies of *opa* genes

Opc

- 24-35 kDa integral outer membrane protein
- 10 stranded ß-barrel with 5 exposed surface loops
- encoded by a single gene, *opcA*

Opacity proteins mediate adhesion



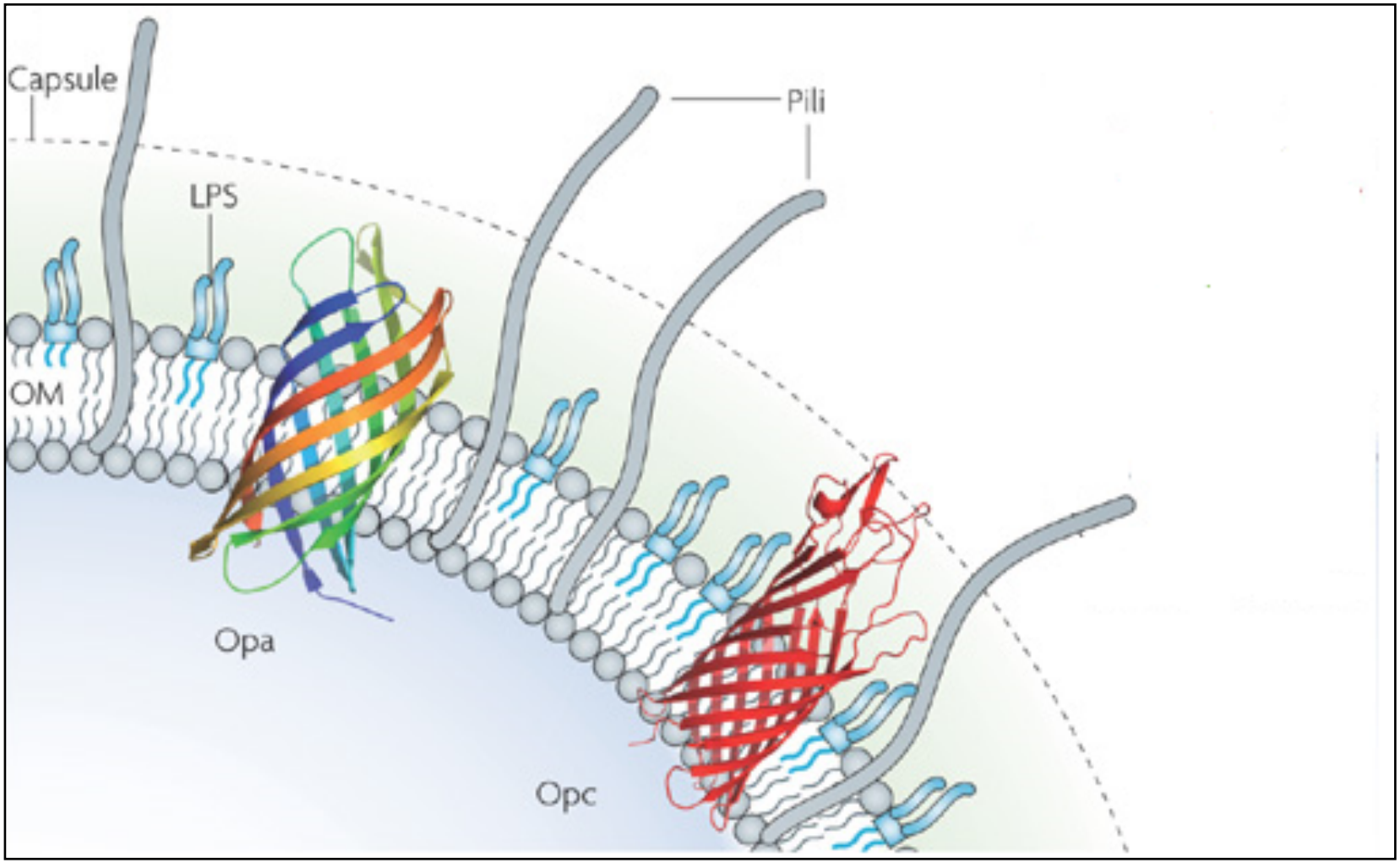


- binds to HSPG (heparan sulphate proteoglycan) and sialic acid
- direct or indirect interactions
- can lead to internalisation

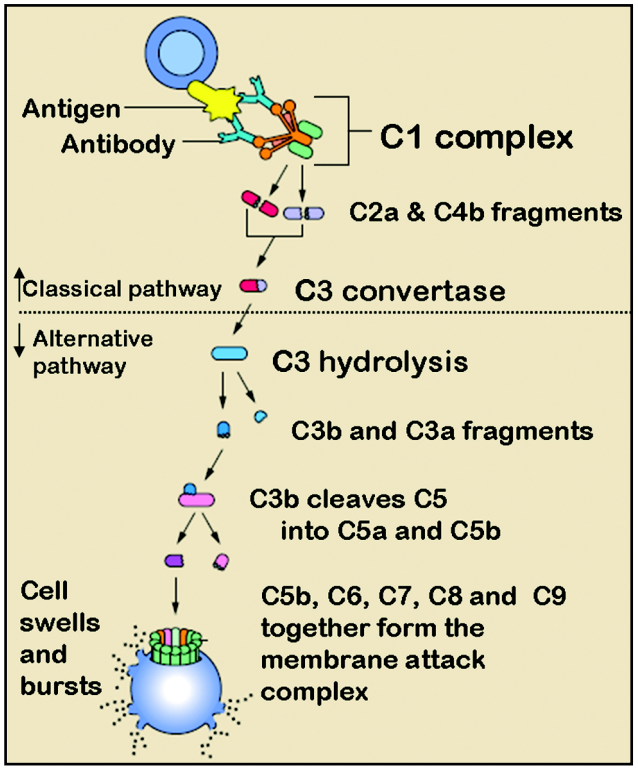
- bind to CEACAMs (carcino embryonic antigen receptors)
- leads to uptake and transcytosis

Hauck et al. 2003. Curr Opin Microbiol. **6**:43-9

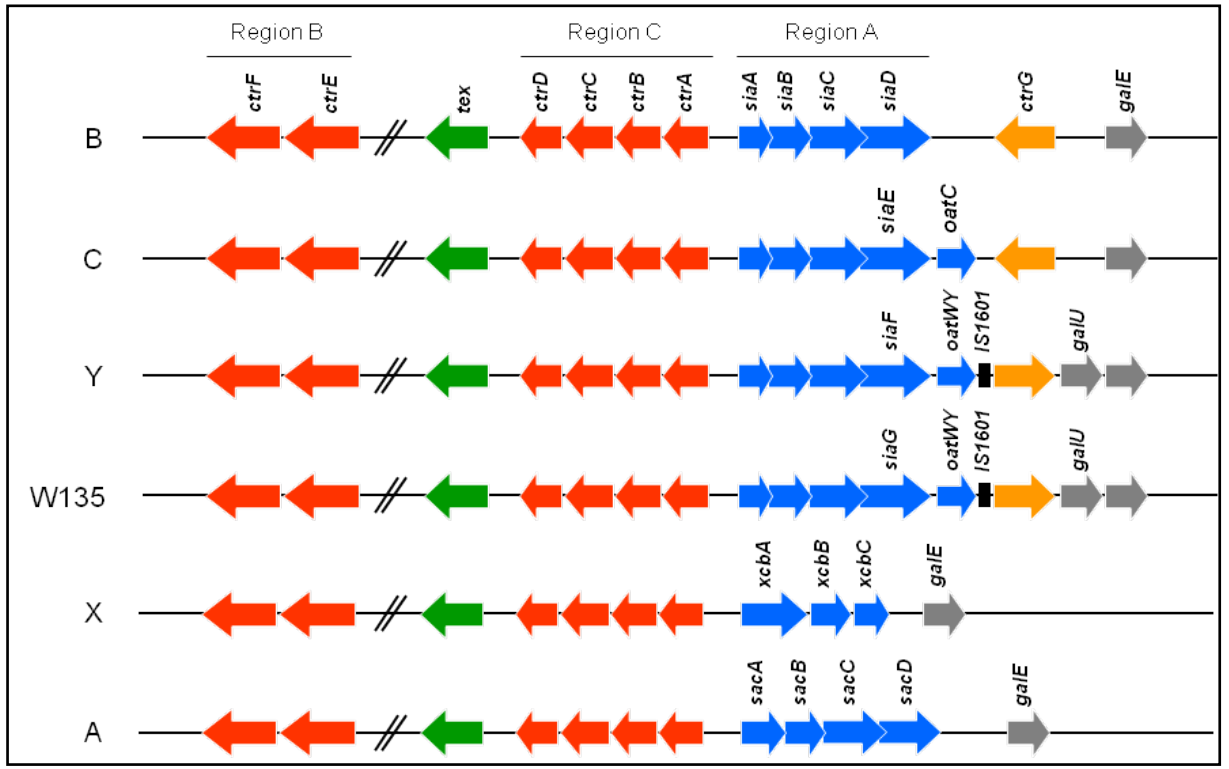
Capsule inhibits Opa-mediated adhesion



Immune evasion



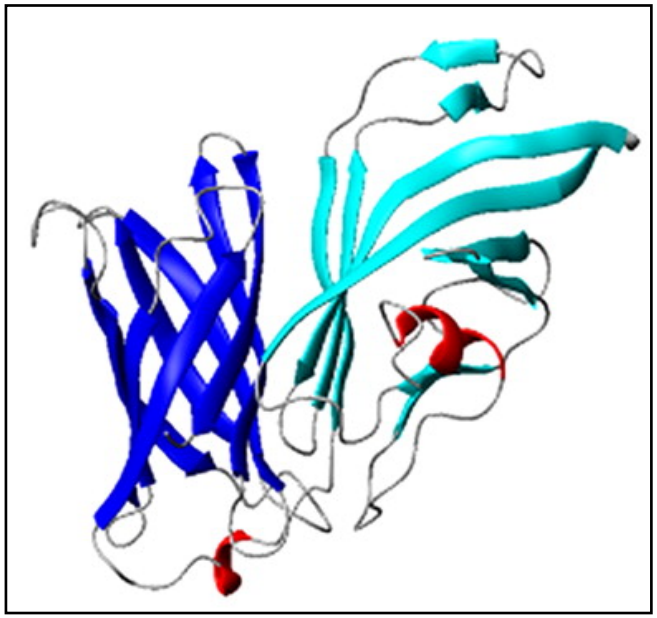
3. Capsule



- island of horizontal gene transfer, absent in the closely related *N. gonorrhoeae*
- all invasive strains have capsule (only 50% of carriage isolates are encapsulated)

Hobb *et al.* 2010. Microbes and Infection **12**:476-487; Tzeng *et al.* 2003. I & I **71**:6712-6720; Wang *et al.* 2010. I & I. **78**:3883-3888

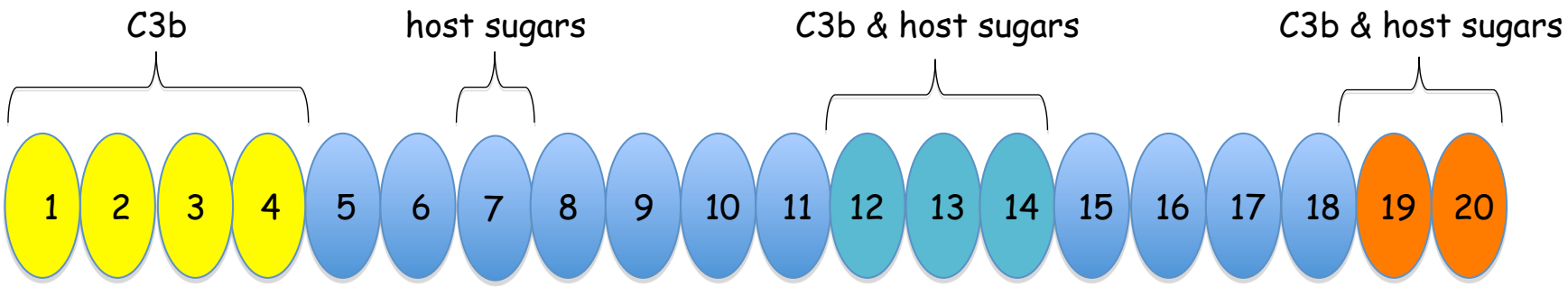
4. FHbp



- surface lipoprotein 26.9 kDa
- folds into 2 ß-barrels
- expressed in most isolates but at different levels
- several variants exist

Cantini *et al.* 2006. J Biol Chem **281**:7220-7

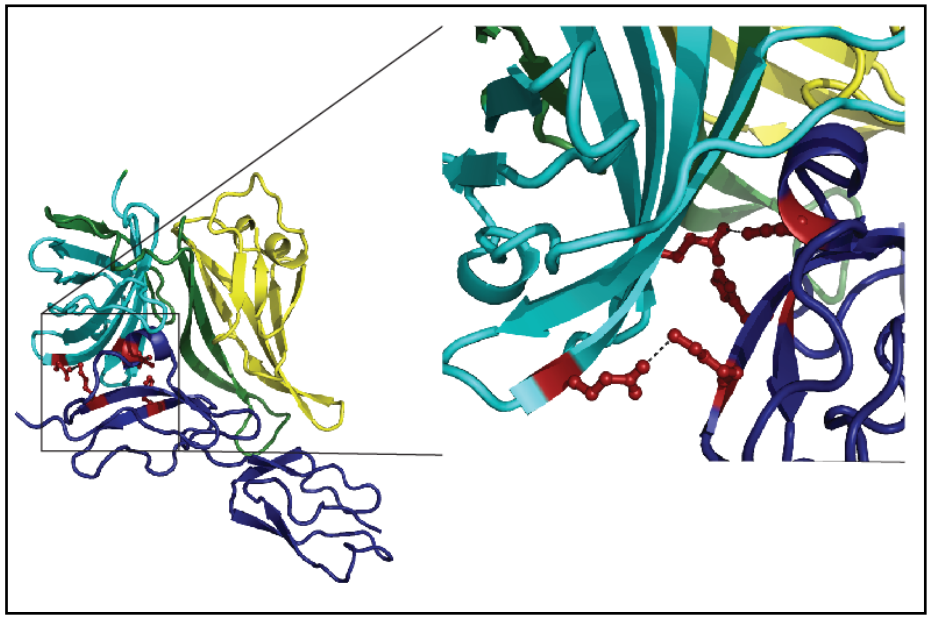
Factor H



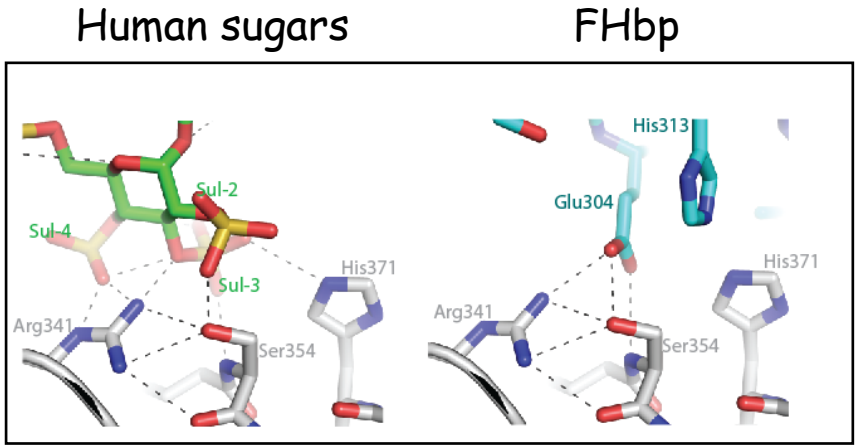
- large (115 kDa) soluble glycopoprotein of human plasma
- member of the regulators of complement activation family
- composed of 20 SCR (short consensus repeats of 60 aa)
- protects host cells and tissues from damage by complement activation

Rodríguez de Córdoba *et al.* 2004. *Mol Immunol* **41**:355-67

FHbp binds human factor H using mimicry of host sugars



Factor H SCR 6/7 + FHbp
(two Glu residues important on FHbp)



Factor H

Schneider *et al.* 2009. Nature. **458**:890-893

5. Modification of surface structures

Antigenic variation

•change in DNA sequence which alters protein sequence

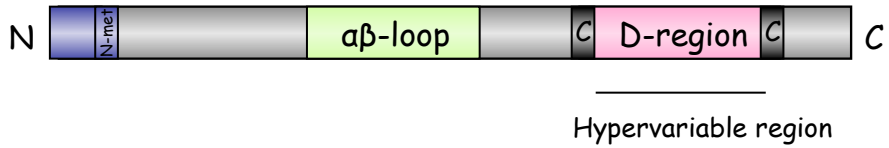
Phase variation

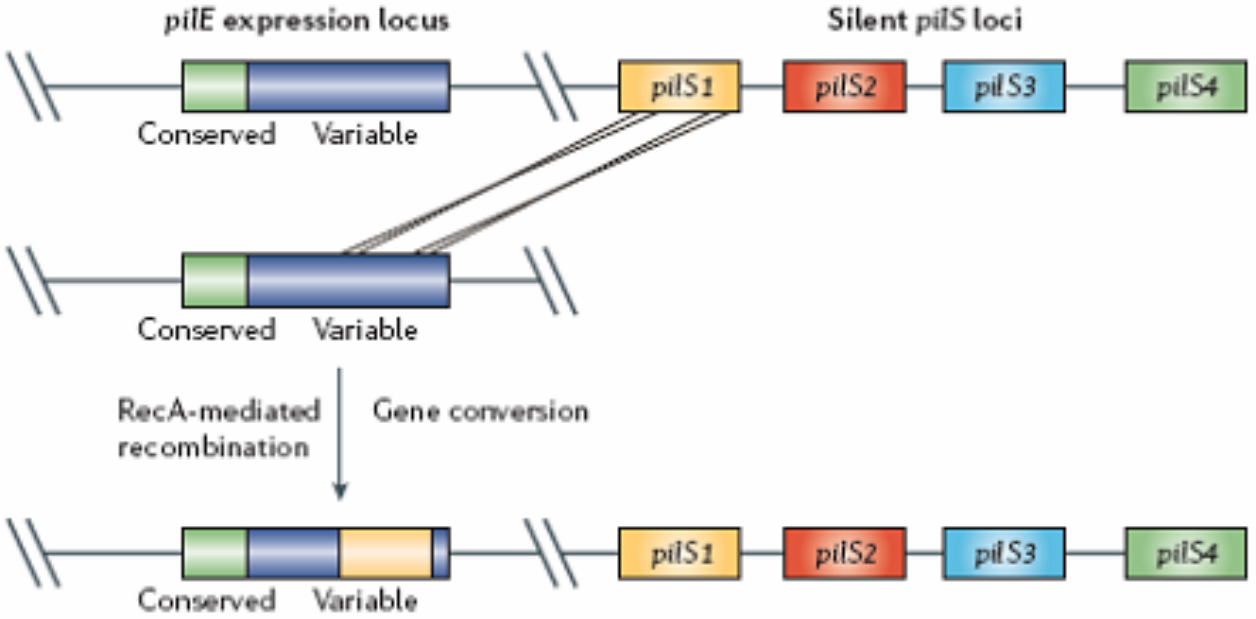
• reversible change in DNA sequence which alters gene expression

• occurs primarily through slipped strand mispairing (SSM)

• induced by repetitive sequences within genes or their promoters

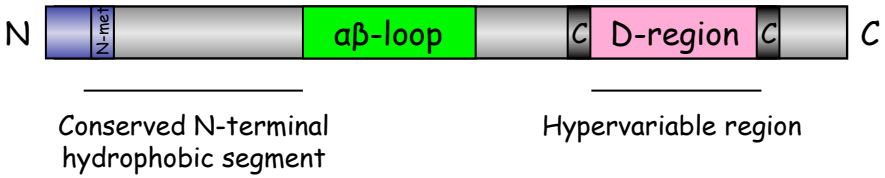
Pilin antigenic variation arises via gene conversion

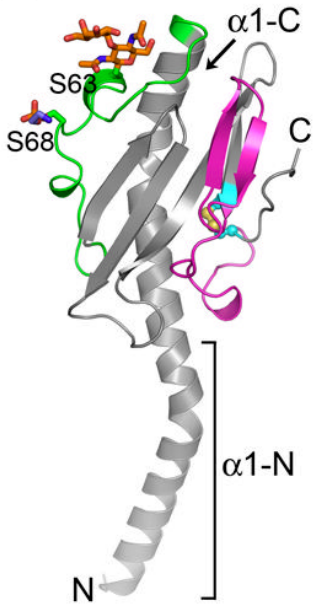


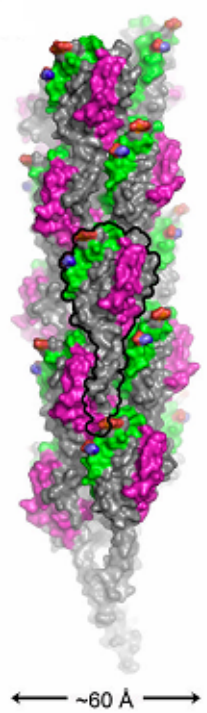


Davidsen *et al.* 2006. *Nat Rev Microbiol* **4**:11–22

Variable pilin regions are exposed on the surface of Tfp







Craig *et al.* 2008. Curr Opin Struct Biol **18**:267-77

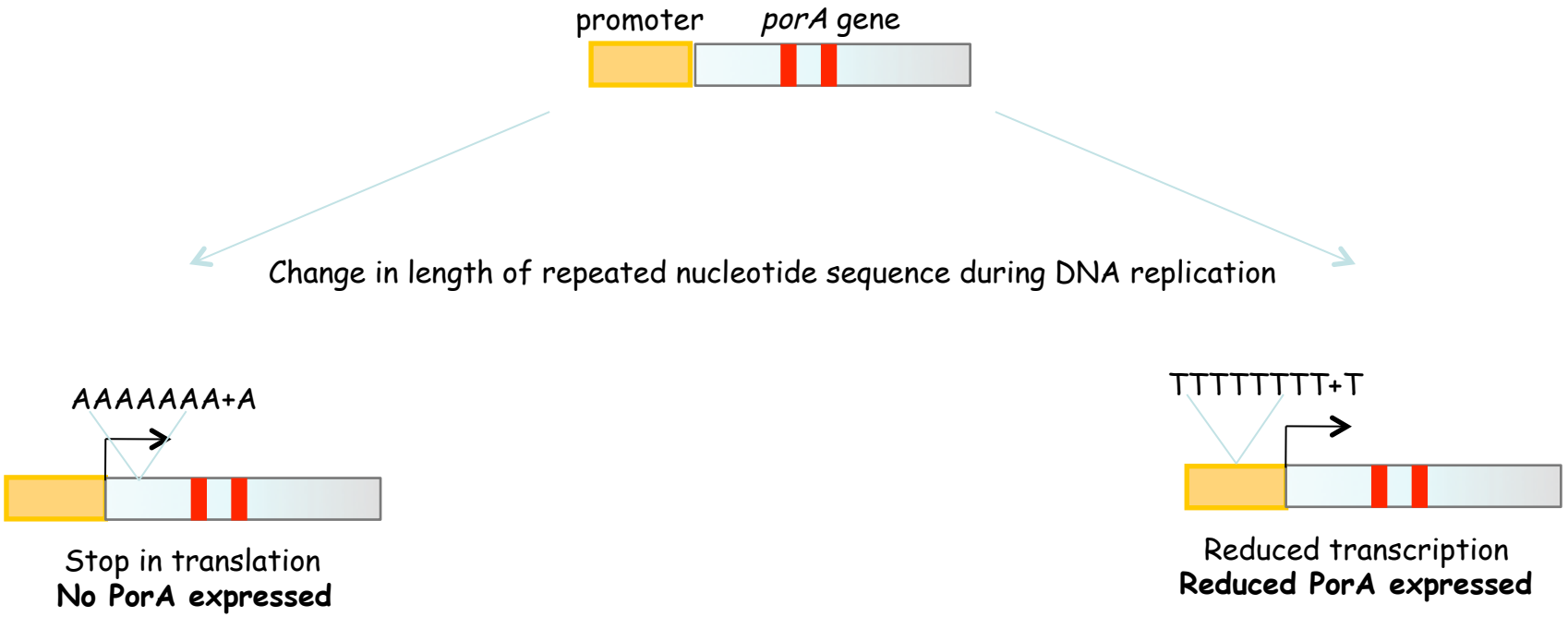
Phase-variable genes associated with meningococcal virulence

- 60 phase variable genes
- can generate 2^{60} different variants

Phenotype	Gene	Variation*	Mechanism‡
Type IV pili	*pilE, pilS*	P+A	Rec
Type IV pilus adhesin	*pilC*	P	SSM
Opacity protein	*opa*	P+A	SSM
Outer-membrane protein	*opc*	P	SSM
Outer-membrane protein	*porA*	P	SSM
Adhesin	*nadA*	P	SSM
Lipo-oligosaccharide modification	*lgtA,C,D,G*	P	SSM
Haemoglobin receptor	*hpuAB, hmbR*	P	SSM
Capsule	*siaD*	P	SSM

Davidsen *et al.* 2006. *Nat Rev Microbiol* **4**:11–22

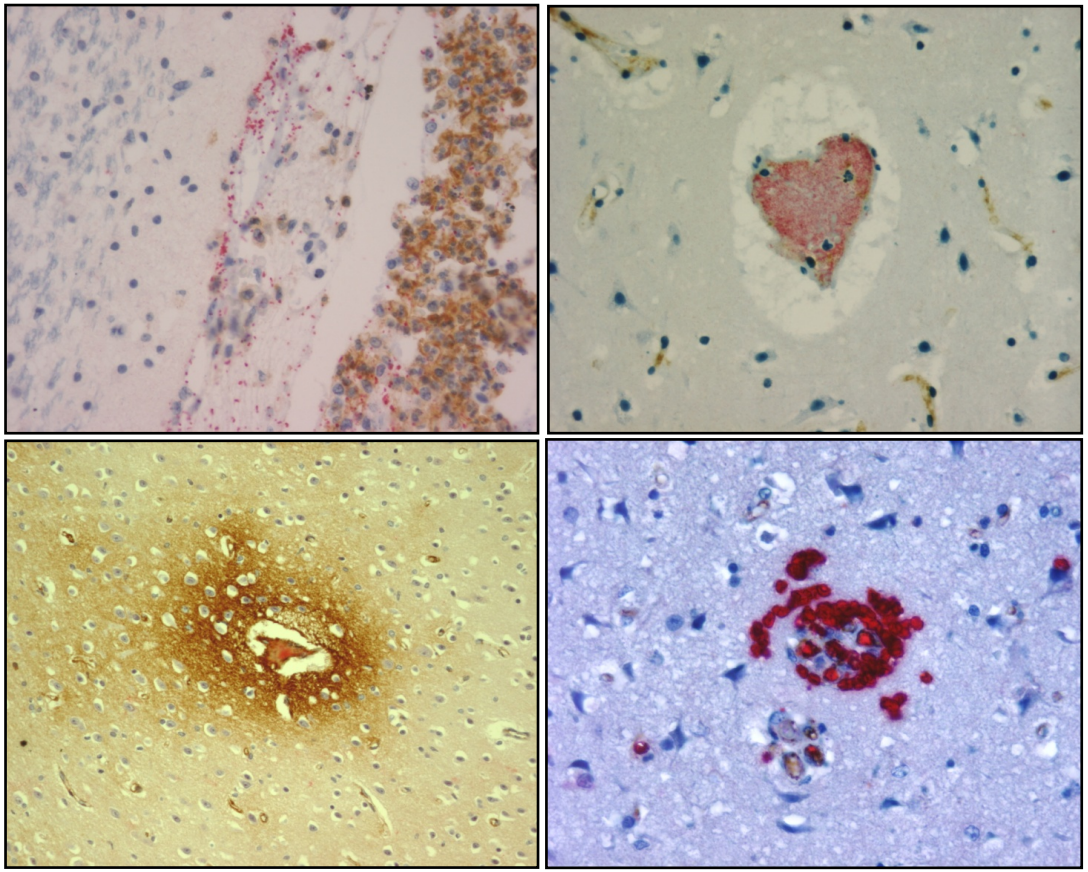
Mechanisms of phase variation



Damage

Infiltration of leukocytes

N.meningitidis in blood vessels

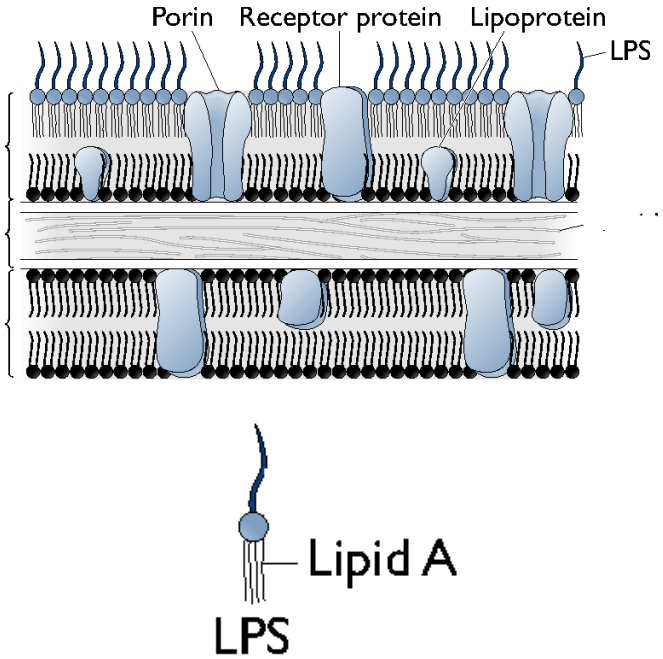


Fibrinogen leakage from vessel

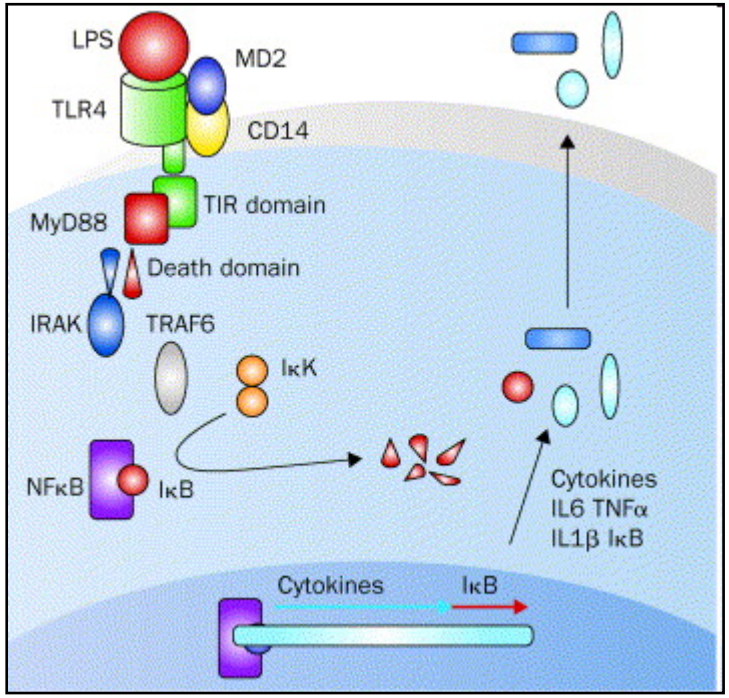
RBC leakage from blood vessel

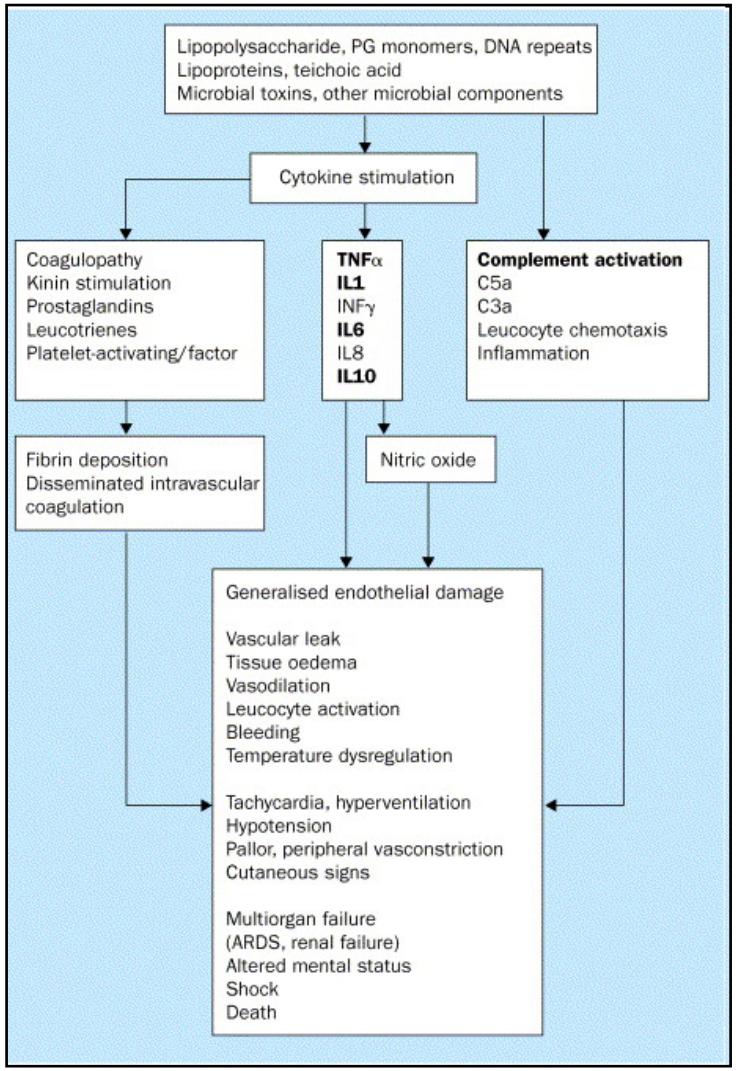
6. Lipopolysaccharide (LPS)

- LPS molecules make up the outer leaflet of the outer membrane in Gram negative bacteria
- composed of lipid A moiety and sugar chain
- certain immunotypes modified by sialic acid addition



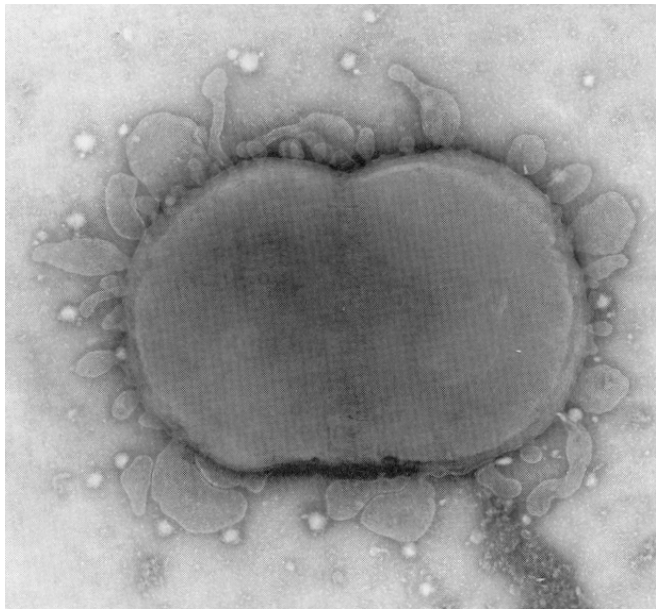
LPS induces inflammatory responses





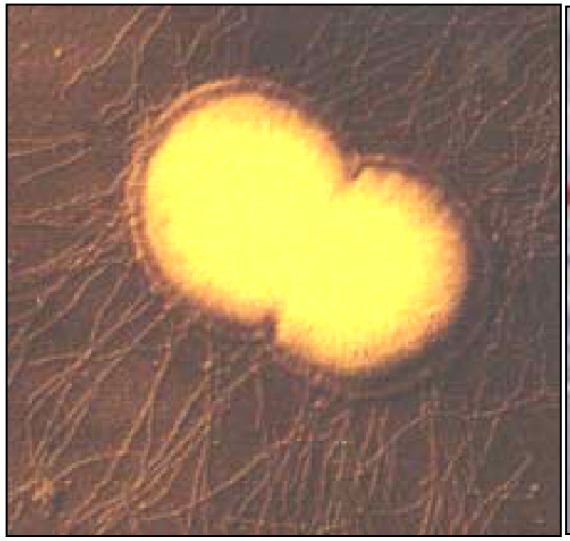
Emonts *et al.* 2003. Lancet **3**:565-577

Meningococci release huge amounts of LPS in the bloodstream



- Among the highest levels of bacteraemia
- *N. meningitidis* releases blebs of outer membrane
- Circulating levels of LPS correlate with infection outcome (15/15 patients with >700 ng/l developed septic shock)

Meningococcal vaccines
Part II



The need for meningococcal vaccines

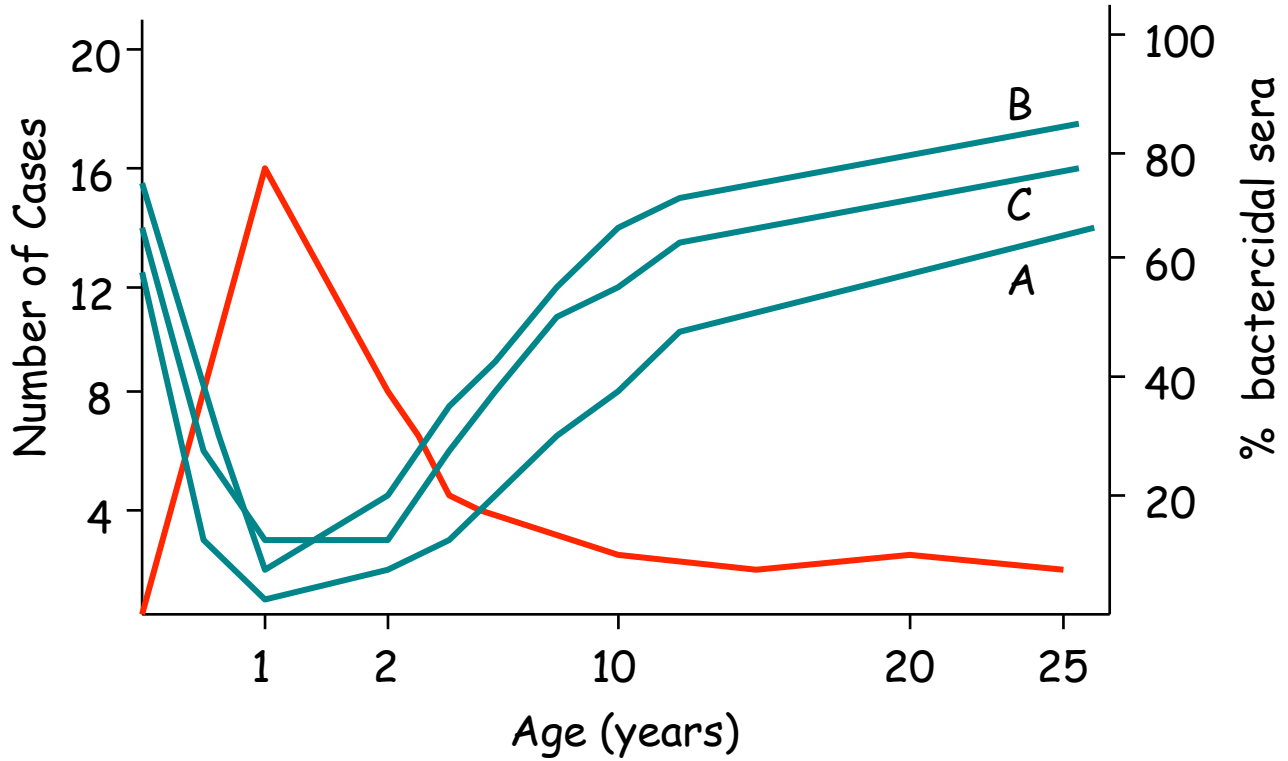
- non-specific early symptoms (fever, headache and vomiting)
- rapid (fulminant) progression to disease (hours)
- fatality rates 2-10%
- sequelae: deafness, limb amputation, brain damage

5 major advances in meningococcal vaccine development

1. Recognition of serological correlate of protection
2. Development of polysaccharide vaccines
3. Polysaccharide-conjugate vaccines
4. Outer membrane vesicle vaccines
5. Reverse Vaccinology

1. Recognition of serological correlate of protection

Goldschneider *et al.* 1969. Human immunity to the meningococcus. I. The role of humoral antibodies. J Exp Med **129**:1307-1326



2. Polysaccharide vaccines

- First developed in 1960s
- Contain capsular polysaccharides (CPS) as target antigen. Easy to produce
- Quadrivalent vaccine against serogroups A, C, W135 and Y
- T-cell independent, short-term protection, no immunological memory
- Response is age-dependent and is lowest <2 years of age when needed most

3. Conjugate-polysaccharide vaccines

- Use of a technology mastered in production of Hib conjugate vaccine that has almost eradicated invasive Hib disease
- CPS attached to a carrier protein
    - tetanus toxoid
    - CRM_{197} (mutant diphtheria toxin)
- T-cell dependent response, immunological memory and prolonged protection
- Good response in infants

MenC vaccines in the UK

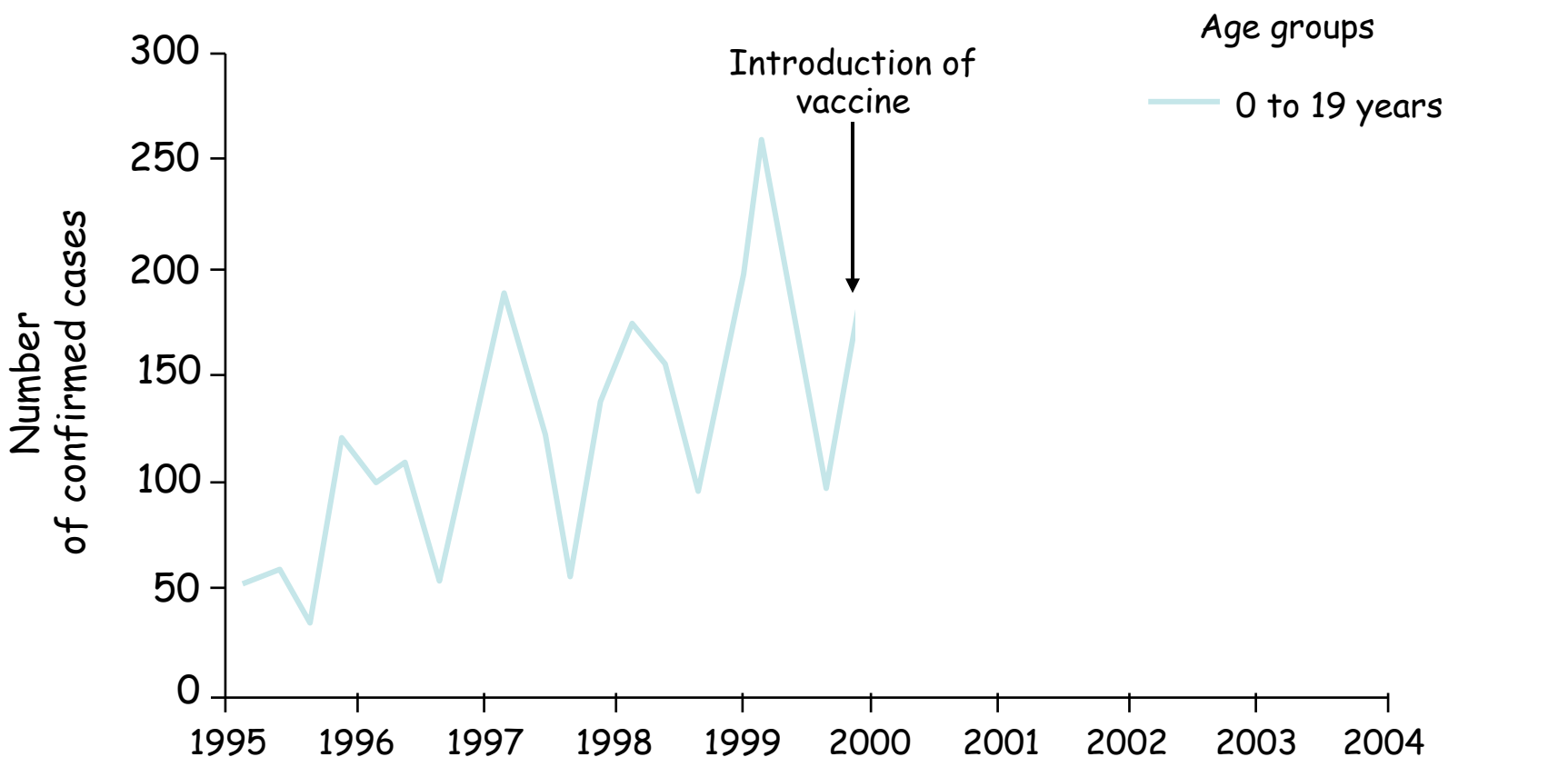
- In 1999, the UK introduced meningococcal serogroup C conjugate (MCC) vaccines into the primary immunisation schedule at 2, 3 and 4 months of age
- A catch up to 18 years of age was also performed
- Three MCC vaccines were licensed

Vaccine	Manufacturer	Carrier protein
NeisVac-C	Baxter	Tetanus toxoid
Meningitec	Wyeth (Pfizer)	CRM_{197}
Menjugate	Novartis	CRM_{197}

From Jamie Findlow (Health Protection Agency Meningococcal Reference Unit)

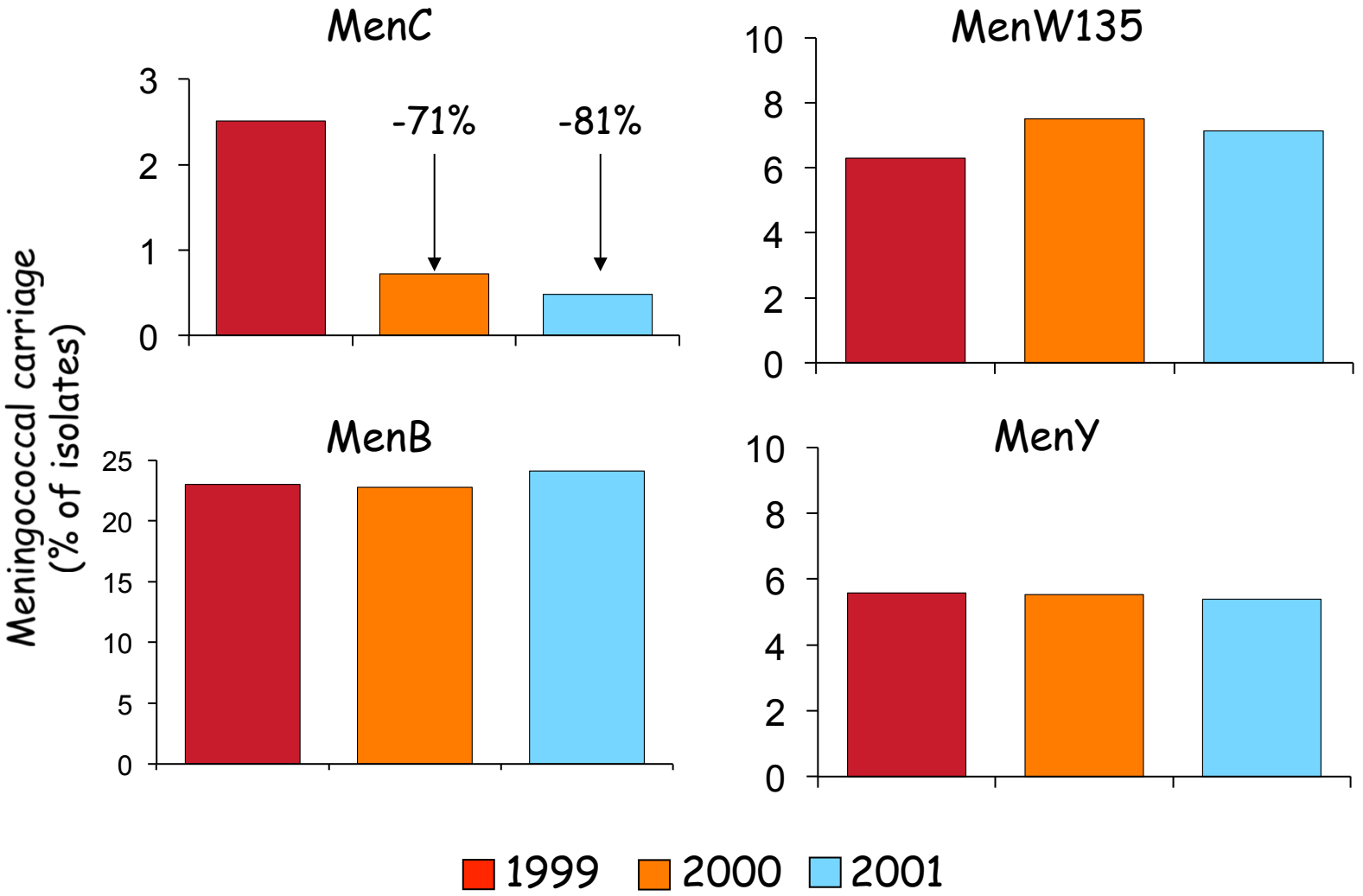
Success of MenC vaccination in UK

Confirmed cases of MenC disease pre- and post-introduction of MCC vaccines



From Jamie Findlow (Health Protection Agency Meningococcal Reference Unit)

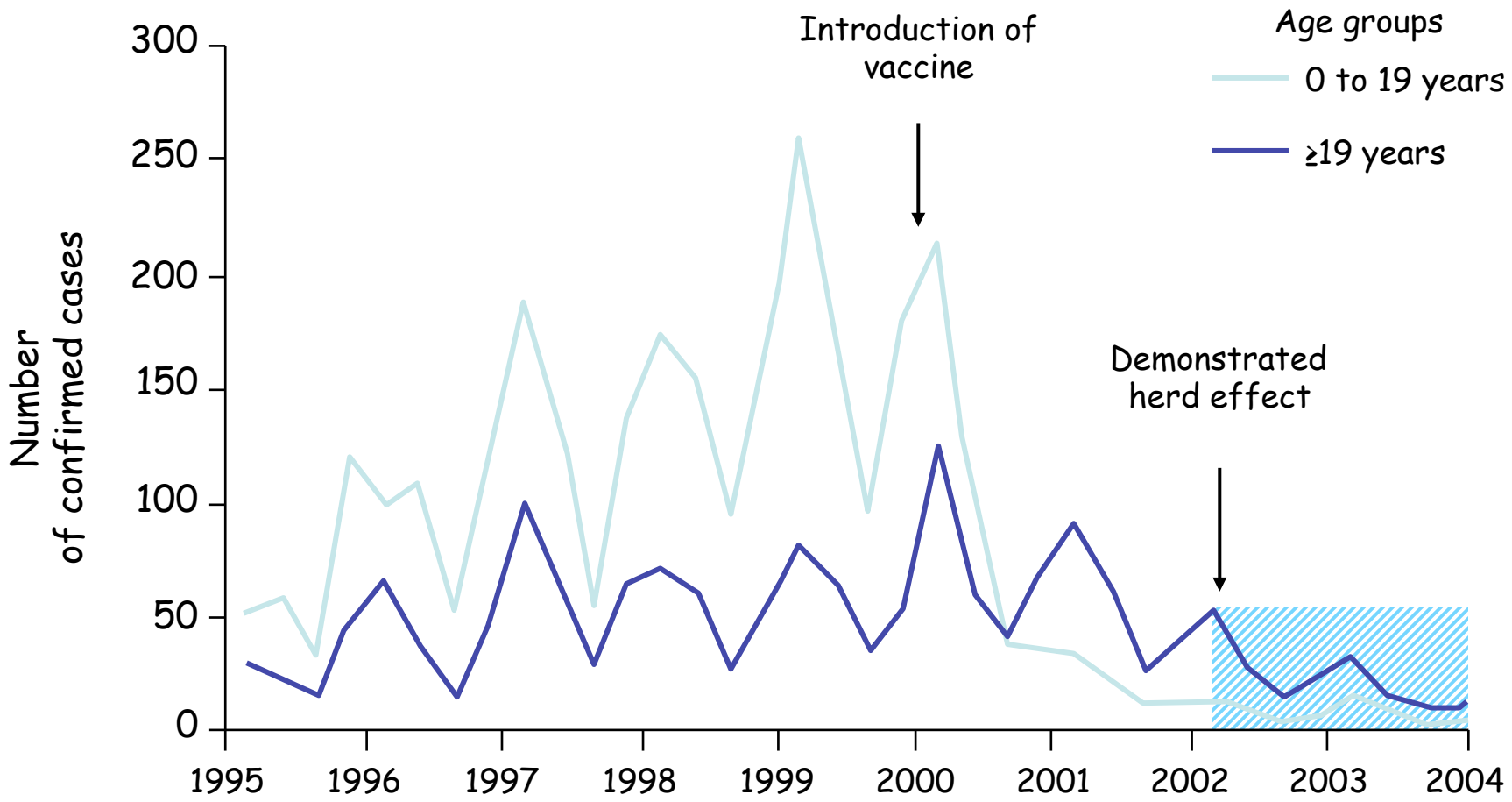
Reduction in MenC carriage following introduction of MCC vaccines



From Jamie Findlow (Health Protection Agency Meningococcal Reference Unit)

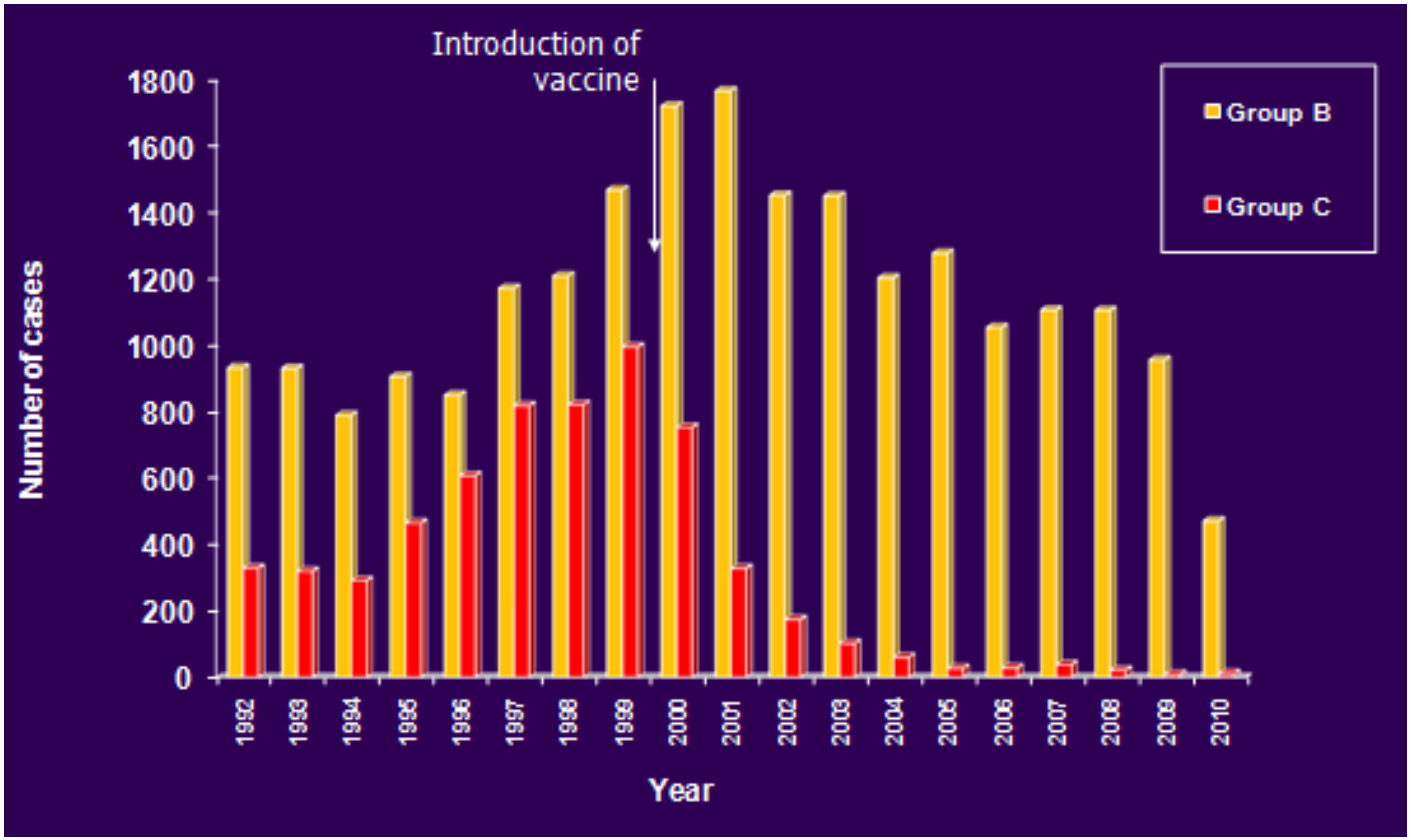
MenC vaccination and herd immunity

Confirmed cases of MenC disease pre- and post-introduction of MCC vaccines



From Jamie Findlow (Health Protection Agency Meningococcal Reference Unit)

Serogroup B meningococcal disease remains a problem



Annual cases of laboratory confirmed meningococcal disease in England & Wales 1992 to 2010 (up to 7/8/2010)

From Jamie Findlow (Health Protection Agency Meningococcal Reference Unit)

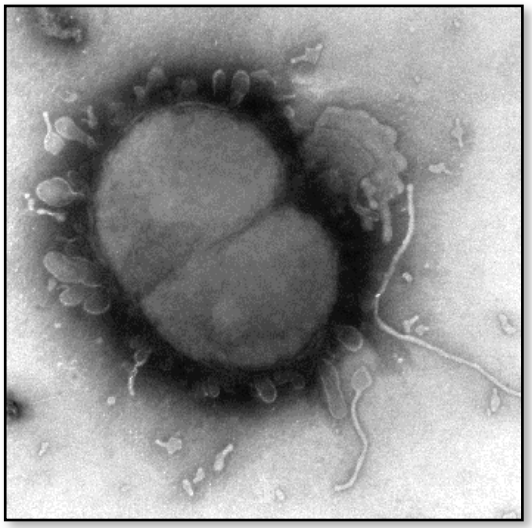
The problem with serogroup B capsule

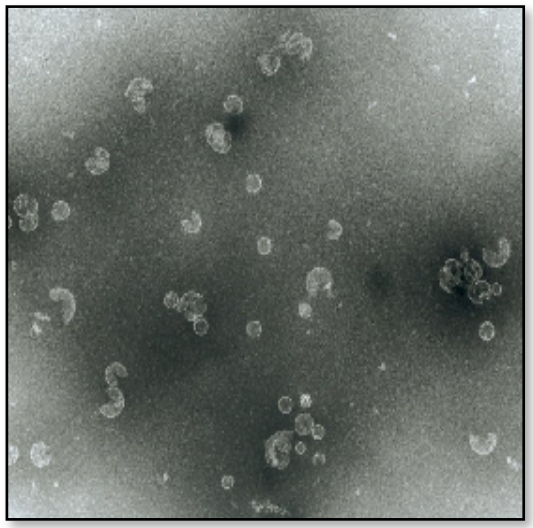
B	(α2–8)- *N*-acetylneuraminic acid	• Europe • Asia • North America • South America • Australia • New Zealand	Homopolymer of sialic acid; mimics structures present on neuronal cell adhesion molecules; poor immunogen
C	(α2–9)- *N*-acetylneuraminic acid	• Europe • Asia • North America • South America • Australia • Africa	Homopolymer of sialic acid; immunogenic

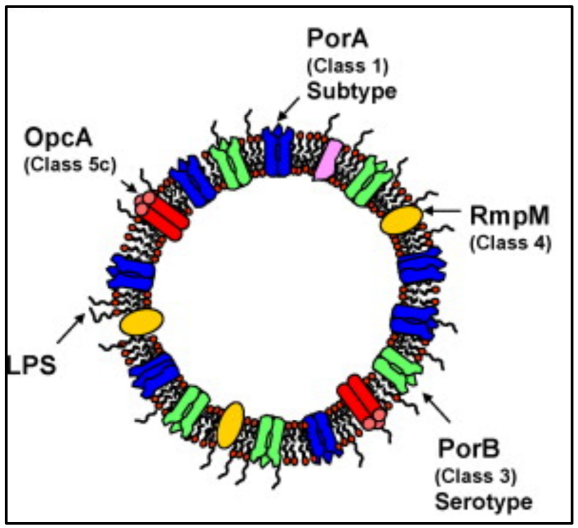
- little or no immune response
- shares moieties with host cells NCAM (neural cell adhesion molecule)

4. OMV vaccines

- OMVs are released spontaneously by meningococci *in vivo* /culture
- contain outer membrane proteins, lipids and LPS
- vaccines are produced by detergent extraction
- used in Cuba, Brazil, Chile and New Zealand



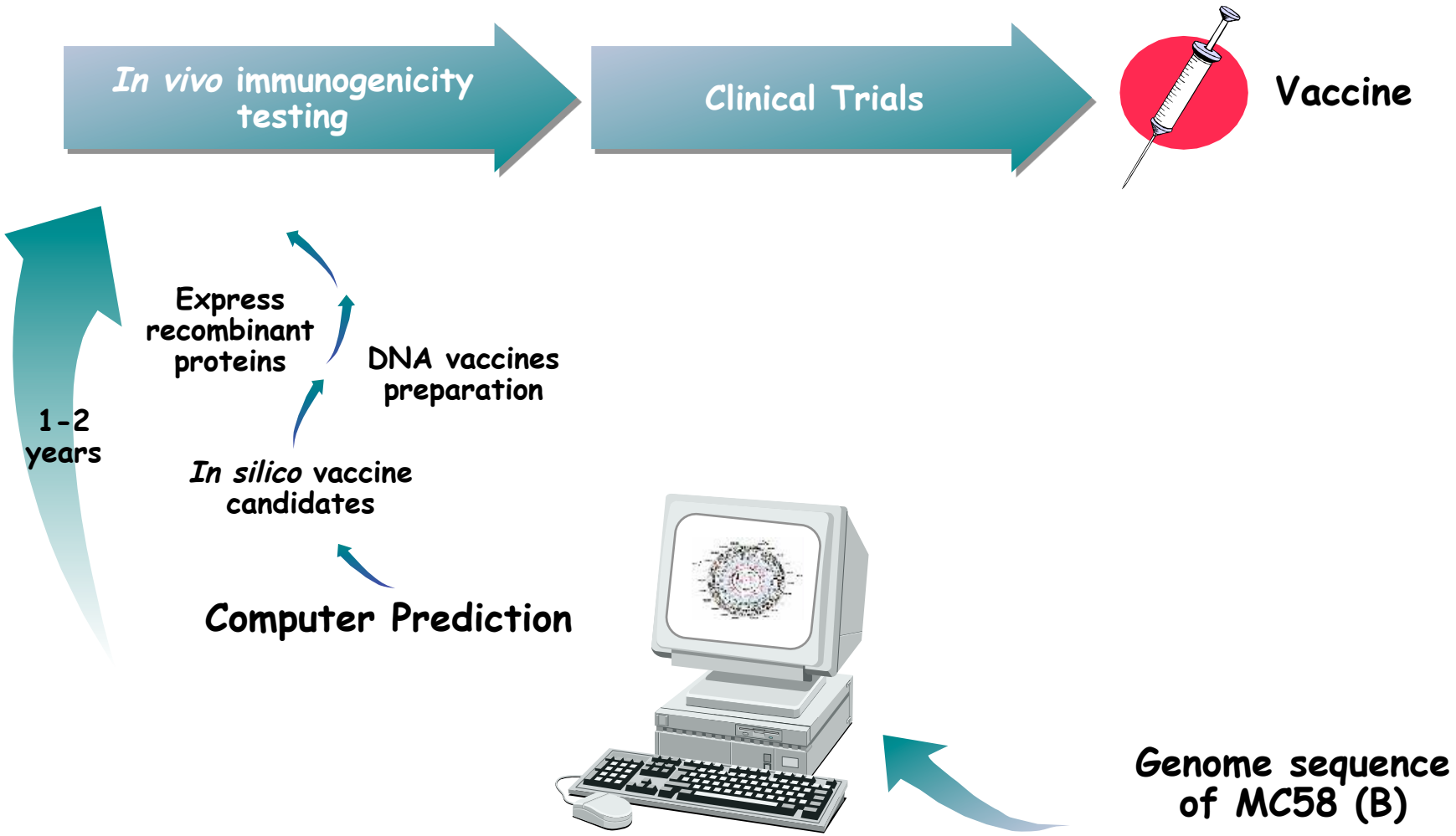




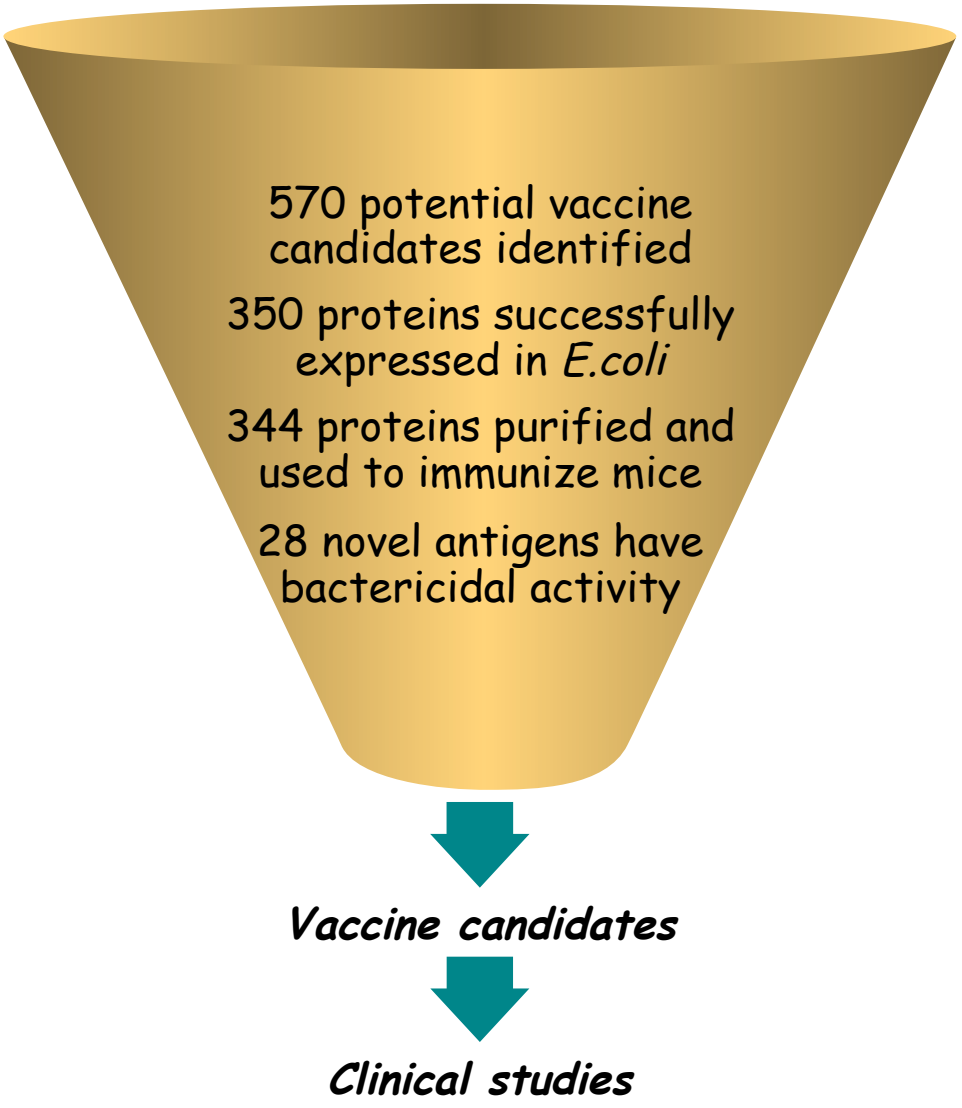
OMV vaccines examples: New Zealand

- since 1991 NZ experienced an epidemic of serogroup B disease with more than 10/100,000 cases per year
- almost 90% of MD were due to one serogroup B strain (B:4:P1.7b, 4)
- an OMV vaccine, MeNZB™, has been developed by Novartis using the epidemic strain
- between 2004 and 2006 MeNZB™ vaccination was offered to anyone under the age of 20. Routine immunisation for babies and preschoolers continued until June 2008
- the number of people developing serogroup B MD decreased from over 300 cases in 2001 to less than 30 cases in 2010

5. Reverse vaccinology (Novartis)



Reverse vaccinology: numbers and facts



- identified more candidates in 18 months than in previous 40 years
- 5 most immunogenic proteins selected for inclusion in a vaccine

NOVARTIS MenB Vaccine

PNAS

A universal vaccine for serogroup B meningococcus

Marzia M. Giuliani*, Jeannette Adu-Bobie*, Maurizio Comanducci*, Beatrice Aricò*, Silvana Savino*, Laura Santini*, Brunella Brunelli*, Stefania Bambini*, Alessia Biolchi*, Barbara Capecchi*, Elena Cartocci*, Laura Ciucchi*, Federica Di Marcello*, Francesca Ferlicca*, Barbara Galli*, Enrico Luzzi*, Vega Masignani*, Davide Serruto*, Daniele Veggi*, Mario Contorni*, Maurizio Morandi*, Alessandro Bartalesi*, Vanda Cinotti*, Donatella Mannucci*, Francesca Titta*, Elisa Ovidi†, Jo Anne Welsch‡, Dan Granoff‡, Rino Rappuoli*§, and Mariagrazia Pizza*

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This contribution is part of the special series of Inaugural Articles by members of the National Academy of Sciences elected on May 3, 2005.

Contributed by Rino Rappuoli, May 12, 2006

PNAS 2006 **103**:10834

5 Antigens discovered by reverse vaccinology

GNA1030 - unknown function

Nhba – heparin-binding lipoprotein

FHbp – factor H binding protein

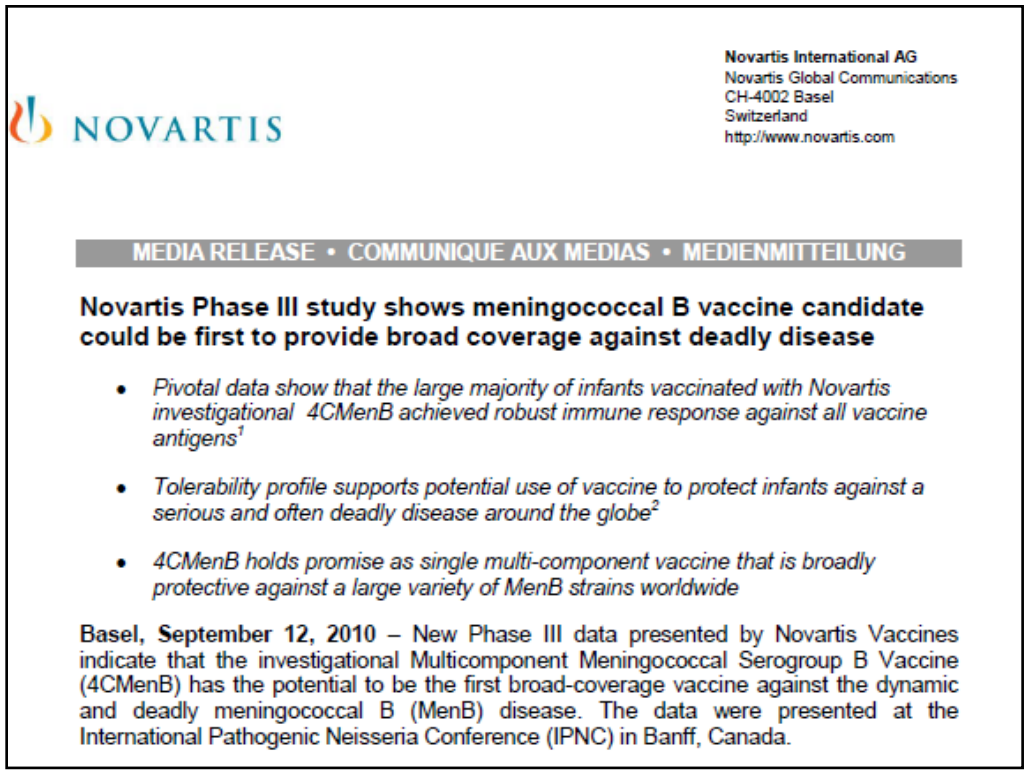
GNA2091 - unknown function

NadA – protein involved in adhesion and invasion into epithelial cells

4CMenB = Nhba-GNA1030+GNA2091-FHbp+NadA+OMV MeNZB™

4CMenB Vaccine Progress

- generated bactericidal antibodies (mice) against 78% of 85 serogroup B strains
- coverage increased to >90% using different adjuvants
- elicits serum bactericidal antibody in adults and infants



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Novartis Phase III study shows meningococcal B vaccine candidate could be first to provide broad coverage against deadly disease

- *Pivotal data show that the large majority of infants vaccinated with Novartis investigational 4CMenB achieved robust immune response against all vaccine antigens*[1]
- *Tolerability profile supports potential use of vaccine to protect infants against a serious and often deadly disease around the globe*[2]
- *4CMenB holds promise as single multi-component vaccine that is broadly protective against a large variety of MenB strains worldwide*

Basel, September 12, 2010 – New Phase III data presented by Novartis Vaccines indicate that the investigational Multicomponent Meningococcal Serogroup B Vaccine (4CMenB) has the potential to be the first broad-coverage vaccine against the dynamic and deadly meningococcal B (MenB) disease. The data were presented at the International Pathogenic Neisseria Conference (IPNC) in Banff, Canada.

Summary

N. meningitidis biology:

- is mainly a commensal of the human nasopharynx that can cause invasive disease with often devastating consequences
- efficiently adhere and crosses several cellular barriers (Tfp, Opa)
- resists complement-mediated lysis (capsule, FHbp)
- evades the immune system by very efficiently varying its surface structures (antigenic and phase variation)
- provokes major damage (LPS)

N. meningitidis vaccines:

- serum bactericidal activity correlates with protection
- capsular polysaccharides have been used as antigens for vaccines against serogroups A, C, W135, & Y, but not serogroup B
- OMVs have been used to prevent serogroup B disease
- subcapsular antigens are providing promising results in clinical trials

Suggested reading

M. Virji. 2009. Pathogenic neisseriae: surface modulation, pathogenesis and infection control. Nature Reviews Microbiology **7**:274-286

Lo *et al*. 2009. Mechanisms of avoidance of host immunity by *Neisseria meningitidis* and its effect on vaccine development. Lancet Infectious Diseases **9**:418-427

Hill *et al*. 2010. Cellular and molecular biology of *Neisseria meningitidis* colonization and invasive disease. Clinical Science (London) **118**:547-564

Tan *et al*. 2010. Advances in the development of vaccines against *Neisseria meningitidis*. New England Journal of Medicine **362**:1511-1520

Serruto *et al*. 2009. Genome-based approaches to develop vaccines against bacterial pathogens. Vaccine **27**:3245-3250

Holst *et al*. 2009. Properties and clinical performance of vaccines containing outer membrane vesicles from *Neisseria meningitidis*. Vaccine. **27(Suppl 2)**:3-12

Meningitis Research Foundation (http://www.meningitis.org)