Vaccine development against *Neisseria meningitidis*

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**Summary**

Meningococcal disease is communicable by close contact or droplet aerosols. Striking features are high case fatality rates and peak incidences of invasive disease in infants, toddlers and adolescents. Vaccine development is hampered by bacterial immune evasion strategies including molecular mimicry. As for *Haemophilus influenzae* and *Streptococcus pneumoniae*, no vaccine has therefore been developed that targets all serogroups of *Neisseria meningitidis*. Polysaccharide vaccines available both in protein conjugated and non-conjugated form, have been introduced against capsular serogroups A, C, W-135 and Y, but are ineffective against serogroup B meningococci, which cause a significant burden of disease in many parts of the world. Detoxified outer membrane vesicles are used since decades to elicit protection against epidemic serogroup B disease. Genome mining and biochemical approaches have provided astounding progress recently in the identification of immunogenic, yet reasonably conserved outer membrane proteins. As subcapsular proteins nevertheless are unlikely to immunize against all serogroup B variants, thorough investigation by surrogate assays and molecular epidemiology approaches are needed prior to introduction and post-licensure of protein vaccines. Research currently addresses the analysis of life vaccines, meningococcus B polysaccharide modifications and mimotopes, as well as the use of *N. lactamica* outer membrane vesicles.

**Introduction**

*Neisseria meningitidis*, the meningococcus, is a Gram-negative bacterium belonging to the β-proteobacteria. The species’ natural habitat is the human nasopharynx. Animal or environmental habitats are unknown. Asymptomatic colonization of the retropharyngeal wall and the tonsils is observed at high frequency in the second decade of life with a maximum in young adulthood (Claus et al., 2005; Caugant et al., 2007). Carriage frequencies have been scarcely studied in elder individuals. In one Norwegian study, carriage rates of male, but not of female subjects in the third and fourth decade of life were comparable to the high rates in adolescents (Kristiansen et al., 1988). Transmission is dependent on close contact between individuals or exposure to droplet aerosols.

*Neisseria meningitidis* is closely related to the pathogenic species *N. gonorrhoeae*, a sexually transmitted organism, and to the commensal *N. lactamica*, which colonizes the same niche as meningococci and exchanges DNA therewith (Linz et al., 2000). *Neisseria lactamica* may provide protection against invasive meningococcal disease (IMD) (Coen et al., 2000).

Genome sequences have been published for several strains of *N. meningitidis* (Parkhill et al., 2000; Tettelin et al., 2000; Bentley et al., 2007; Peng et al., 2008; Schoen et al., 2008). In total, the NCBI genome resource lists 38 neisserial genome projects, which are either in progress or already completed. Genome sequences provide an invaluable repository for phylogenetic analyses, studies on the evolution of virulence, and of course for mining for vaccine targets.

The major pathogenicity factor of meningococci is the polysaccharide capsule. The ecological role of capsule expression is unclear, as unencapsulated strains thrive well in the nasopharynx. Furthermore, IMD is an accident during the bacterium’s life cycle and may be considered as an evolutionary dead-end implying costs only acceptable to very fit lineages of the species (Buckee et al., 2008). The capsule possibly provides protection against desiccation during aerosol transmission. Furthermore, one might suggest that it protects the bacteria during colonization of inflamed mucosal tissue. However, the identification of
apathogenic capsule null locus (cnl) meningococci proofs that meningococci might well proliferate in the population without capsule expression (Claus et al., 2002).

There are 12 biochemically distinct capsular polysaccharides. Serogroups B, C, W-135 and Y, which are frequently observed in IMD, contain N-acetyl-neuraminic acid (Neu5Ac, sialic acid) (Bhattacharjee et al., 1975; 1976). Serogroup A, a major cause of epidemics in Africa, expresses a capsule of \((\rightarrow 6)\alpha-D-ManpNAC(1\rightarrow OPO_3\rightarrow)\) (Bundle et al., 1974). The \(\alpha(2\rightarrow 8)\) linked sialic acid homopolymer of serogroup B is identical to a modification of the mammalian neural cell adhesion molecule (NCAM) (Toikka et al., 1998), which explains why the serogroup B polysaccharide is poorly immunogenic. The serogroup B polysaccharide is structurally related to the serogroup C polysaccharide, an \(\alpha(2\rightarrow 9)\) linked sialic acid homopolymer (Bhattacharjee et al., 1975). This polysaccharide and those found in serogroup A, W-135 and Y meningococci are highly immunogenic. W-135 and Y meningococci express heteropolymeric polysaccharides composed of disaccharide units of sialic acid with either galactose or glucose respectively. The capsule polymerases of serogroups W-135 and Y are more than 99% identical (Claus et al., 1997) with a single amino acid determining substrate specificity (Claus et al., 2009). Serogroup A, C, W-135 and Y polysaccharides can be modified by O-acetylation (Jennings et al., 1977; Michon et al., 2000; Claus et al., 2004; Gudlavalleti et al., 2004). O-acetylated polysaccharide formulations of serogroup C were shown to elicit slightly lower antibody responses than de-O-acetylated ones, but this may also be an effect of the carrier protein of the polysaccharide, the conjugation chemistry and the length of the oligosaccharide (Richmond et al., 2001). O-acetylation is mandatory for immunogenicity of serogroup A polysaccharide (Berry et al., 2002). Serogroup X meningococci have recently emerged in Africa (Djibo et al., 2003), but do not yet play a global role.

Serogroup distribution varies on a global scale (Stephens, 2007), which makes it necessary to adapt vaccine strategies to regional needs. Whereas in Europe serogroups B and C dominate, serogroup Y plays an additional role in Northern America. Devastating epidemics due to serogroup A meningococci are observed in the African Meningitis Belt. Figure 1 demonstrates the serogroup distribution in Germany as an example. Of note, in contrast to several other European countries, meningococcus C (MenC) conjugate vaccination has been implemented in Germany quite late in 2006 and without a catch-up campaign including adolescents. Therefore, serogroup C still plays a significant role.

Incidences of meningococcal disease, i.e. sepsis and meningitis, in Europe and Northern America are low with values undulating around 1 per 100 000 inhabitants per year. Peak incidences are seen in infants, toddlers and adolescents, explaining the need for childhood vaccination programs. Figure 2 exemplifies age-specific incidences using Germany as an example. The figure highlights differences between serogroup B and C. The low incidences of meningococcal disease provide a challenge for vaccine evaluation, because vaccine efficacy cannot be established in clinical trials and surrogates of protection need to be relied on before licensure.

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Meningococci exchange DNA by genetic transformation and homologous recombination. Recombination and selection shape clonal complexes, which are groups of related genotypes (Achtman and Wagner, 2008). Successful clonal complexes have been shown to be distributed internationally and to persist for decades (Caugant et al., 2004). Members of a clonal complex tend to share alleles of immunogenic outer membrane proteins (Urwin et al., 2008). Nevertheless, immune selection drives forward an extensive microevolution of surface antigens (Thompson et al., 2003; Russell et al., 2004; Brehony et al., 2009), which must be considered a major obstacle for the development of protein based vaccines, as vaccines need to cover a major share of variants and immune-escape needs to be monitored.

Surveillance of meningococcal disease in many countries relies on statutory notification and a complementary active or passive laboratory surveillance program. Representative strain collections assembled by reference laboratories are a major resource for evaluation of protein based vaccines, because they can be used to study antigenic variability as well as susceptibility of strains towards bactericidal antibodies elicited by vaccines.

**General problems faced in vaccine development**

A universal meningococcal vaccine is lacking (Table 1). Due to molecular mimicry, serogroup B capsular polysaccharide is poorly immunogenic, and manufacturers have been deterred from polysaccharide vaccine development by theoretical considerations of autoimmunity and potential fetal damage. A recent population based study from Denmark, however, failed to exhibit evidence for autoimmunity elicited by natural meningococcus serogroup B (MenB) infection (Howitz et al., 2007).

Vaccine development against MenB is hampered by variability of surface antigens. Between 2002 and 2005 in Germany alone 33 and 69 variants of the PorA variable regions (VR) 1 and VR2, respectively, were observed (Elias et al., 2006). The meningococcal population is versatile and dynamic. There are emerging clones and lineages, and even neglected serogroups such as X may rise as a new problem as observed in Africa (Boisier et al., 2007).

Specific aspects need to be addressed during meningococcal vaccine development. Licensure of modern meningococcal vaccines is mostly based on safety data, serological correlates of protection, and – for MenB vaccines – strain coverage. Efficacy studies are mostly conducted after vaccine introduction due to the low incidence of disease. Strain coverage is assessed by the analysis of protein expression in a representative strain panel and by determination of the allelic diversity (Bambini et al., 2009; Lucidarme et al., 2009; Murphy et al., 2009). Serum bactericidal assays are a major effort for MenB vaccines, as in contrast to polysaccharide vaccines, several strains have to be tested. If proteins are used, which are not expressed under routine culture conditions, assay formats have to be adapted, which complicates assay standardization.

Furthermore, one has to consider that asymptomatic carriage is a double edged sword in light of vaccine development. The meningococcal serogroup C conjugate (MCC) vaccine campaign in the UK resulted in a reduction of carriage of a limited subset of strains (Maiden et al., 2008), which probably will not affect natural boosting massively. Broadly active meningococcal vaccines, however, might also reduce carriage of apathogenic meningococci, such as cni meningococci (Claus et al., 2002), and of *N. lactamica* due to cross reactive antigens (Gold et al., 1978; Gorrige et al., 2009). Since it is likely that apathogenic strains contribute to natural immunity against disease, an ideal vaccine would rather not touch these variants or species. On the other hand, the vaccine should strongly impact carriage of pathogenic variants to provide for herd immunity as evidenced for polysaccharide conjugate vaccines (Trotter et al., 2008).

**Meningococcal polysaccharide conjugate vaccine development**

Several MCC vaccines have been licensed, which differ with regard to O-acetylation of the polysaccharide, protein conjugate, conjugation chemistry and adjuvant. The vaccines are considered as safe (Pollabauer et al., 2005). The UK in 1999 introduced MCC vaccines, which was highly successful and as an added value provided striking scientific knowledge. The efficacy of the vaccines was about 90%. However, there was an age-dependent decline of efficacy over time following vaccination (Snape et al., 2005; 2006; Borrow and Miller, 2006). This finding led to the recommendation of a booster vaccination in the infant immunization schedule (Trotter et al., 2004). Maintenance of protective titres seems to be essential as the time required for an effective booster response elicited by acquisition of a MenC strain probably exceeds the incubation period (Auckland et al., 2006). The success of the vaccination campaign was in large parts due to the fact that herd immunity was elicited (Ramsay et al., 2003; Trotter et al., 2006). Herd immunity depended on mucosal immunity towards MenC carriage in the UK, which was considerably reduced (Maiden and Stuart, 2002; Maiden et al., 2008). Vaccination of adolescents is most effective in this regard, as carriage rates of meningococci increase in the second decade of life (Claus et al., 2005). One might have speculated that rates of serogroup switching (genetic alteration of a clone) or replacement (increased occurrence of a variant not expressing the vaccine antigen), which have been shown for meningococci on
Table 1. Summary of vaccine concepts discussed.

| Vaccine composition | Indication | Status | Advantage | Disadvantage |
|---------------------|------------|--------|-----------|--------------|
| **Polysaccharide vaccines** |           |        |           |              |
| Plain polysaccharide | Epidemic control | On the market | Low cost | Uneffective in small children |
|                      | Travel medicine | | | |
|                      | Lab workers | | | |
| Protein conjugated polysaccharide | Routine toddler/infant/adolescent vaccination | MenC: on the market | Elicit herd immunity (proven for MenC) | Cost (for most preparations with the exception of the meningococcal A conjugate (PsA-TT) vaccine). |
|                      | | | | |
|                      | Epidemic control | MenACWY: on the market/close to be marketed/in clinical trials | Efficacy in infants and toddlers | Waning immunity in young vaccinees |
|                      | Travel medicine | Lab workers | Memory response | |
| **OMV approaches** | Tailor made (OMVs of epidemic clones) | Epidemic control | Programs have been introduced on several occasions | Effective control of MenB outbreaks and epidemics |
| | | | | Several doses required Immunogenicity in small children may be unsatisfying. |
| | | | | Lack of cross-reactivity |
| | | | | Time consuming pre-clinical and clinical trials. |
| | | | | Poor antibody persistence. |
| **Multivalent PorA vaccines** | Broad protection against meningococci | In clinical trials | Theoretically covers most strains | Some PorA variants are poorly immunogenic |
| **OMV from GMO expressing one or more recombinant minor antigens, in some cases in a PorA negative background** | Broad protection against meningococci | Pre-clinical | May confer protection against a large panel of strains | Pre-clinical and clinical assessment of protection may be a difficult issue with regard to in vivo expression of antigens |
| | | | May avoid dominant effect of PorA | |
| **OMV from N. lactamica** | Broad protection against meningococci | In clinical trials | May confer protection against diverse meningococcal lineages, however, probably by mechanisms independent of bactericidal antibodies. Avoids dominant effect of PorA. | N. lactamica carriage in early childhood, which is likely to confer protection against meningococci, might be affected. |
| **Subunit vaccines** | Broad protection against MenB | In clinical trials | Combination of several targets ensures targeting of many lineages Self-adjuvating effects of OMVs | Complex design |
| Genome derived recombinant multicomponent vaccine | | | | |
| Factor H binding protein presented in two allelic variants as lipoprotein | Broad protection against MenB | In clinical trials | Two antigenic variants for broad coverage Application of lipoprotein with self adjuvating effect | Depends on expression of factor H binding protein and the presence of cross-reactive alleles |
| | | | | |
| Meningococcal secretome | Broad protection against meningococci | Animal models | Many components may ensure broad coverage | Secretome yet not completely deciphered |
| **MenB capsule derived approaches** | Broad protection against MenB | In clinical trials | Independent of antigenic variability | Theoretically possible induction of autoantibodies |
| N-propionylated polysaccharide | | | | Poor induction of bactericidal antibodies in human volunteers |
Table 1. cont.

| Vaccine composition | Indication | Status     | Advantage                                      | Disadvantage                                      |
|---------------------|------------|------------|------------------------------------------------|---------------------------------------------------|
| de-N-acetylated polysaccharide | Broad protection against MenB | Animal models | Independent of antigenic variability | Theoretically possible induction of autoantibodies? |
| Mimotope            | Broad protection against MenB | Animal models | Independent of antigenic variability | Theoretically possible induction of autoantibodies? |
| Live vaccine carriers |           |            |                                                |                                                   |
| S. gordoniae expressing NadA and NhhA/Hsf | Broad protection against meningococci | Animal models | Use of attenuated or commensal organisms | Regulatory issues: Release of GMOs |
| Attenuated unencapsulated N. meningitidis strains (ΔsiaD,ΔrfaF or ΔsiaD,ΔmetH) | Broad protection against meningococci | Animal models | Protection of mice against heterologous strains | Regulatory issues: release of GMOs |

OMV, outer membrane vesicle; GMO, genetically modified organism.

several occasions (Vogel et al., 2000), will increase under selective pressure induced by vaccination campaigns (Maiden and Spratt, 1999). However, the UK disease surveillance did not evidence for increased serogroup switching and replacement (Balmer et al., 2002; Maiden et al., 2008). The occurrence of serogroup switch variants was reported for Spain (Cano et al., 2004). It is unclear whether this observation was due to a different vaccine introduction strategy.

The success of MCC stimulated the introduction and development of novel MenACWY conjugate vaccines (Keyserling et al., 2005; Snape et al., 2008; Ostergaard et al., 2009) and also of combinations of conjugated meningococcal polysaccharide with other components, such as the H. influenzae type b polysaccharide (Borrow et al., 2010). The serological data published until now for tetavalent polysaccharide vaccines are promising; instruments to control MenACWY disease seem to be available now. However, data are needed on the induction of herd immunity. Of interest is further the development of a serogroup A conjugate vaccine by the Meningitis Vaccine Project (MVP) and collaborating agencies (Kshirsagar et al., 2007; LaForce et al., 2007), which hopefully will provide a solution to the devastating serogroup A epidemics in the African meningitis belt.

**Subcapsular vaccine targets**

There is a variety of highly immunogenic structures in the outer membrane, which serve as possible vaccine components. A catalogue has been published recently (Feavers and Pizza, 2009) that categorizes possible candidates as major outer membrane proteins, iron uptake proteins, adhesins, other virulence factors, antigens with unknown function or those involved in membrane architecture, and enzymes. Major outer membrane proteins such as porins are expressed at high amounts (Frasch and Gotschlich, 1974). Other proteins are repressed under in vitro culture conditions, but can be observed, e.g. after iron depletion (Gritanti et al., 2003). Outer membrane proteins frequently are hard to express or purify in native conformation, so that alternative strategies have to be employed such as the production of outer membrane vesicles (OMVs) (Bjune et al., 1991; Sierra et al., 1991; de Moraes et al., 1992). A variety of lipidated proteins have been described as possible meningococcal vaccine antigens (Fletcher et al., 2004; Delgado et al., 2007; Hsu et al., 2008), which are attractive due to their self-adjuvanting activity.

Besides the induction of bactericidal antibodies, which activate serum complement resulting in bacterial cell death, antibodies against some targets block their function. Some monoclonal antibodies against the meningococcal factor H binding protein (FHBP) bind in close proximity to the factor H binding site and block factor H binding (Beernink and Granoff, 2009). Although the structure of FHBP has been resolved (Cantini et al., 2009; Mascioni et al., 2009; Schneider et al., 2009), precise binding sites of antibodies elicited in vaccinees have not been reported to our knowledge.

Proteins involved in iron uptake have been investigated intensively for their vaccine potential. The transferrin binding protein TbpA and even more so the TbpB are immunogenic and protect mice from meningococcal challenge, e.g. when delivered as recombinant antigen (West et al., 2001). Antigenic variability has to be considered for
A highly significant fraction of meningococcal strains are harbored by subunit vaccines containing recombinant meningococcal proteins now play a prominent role in the field of investigational vaccines for meningococci. The groundbreaking approach of ‘reverse vaccinology’ demonstrates how genome research results in potential products (Rinaudo et al., 2009), and along the way enhances the understanding of meningococcal pathogenicity (Pizza et al., 2000; Comanducci et al., 2002; du-Bobie et al., 2004). Predicted surface exposed proteins were tested for immunogenicity. Consecutively, it was investigated whether the proteins elicited bactericidal antibodies. Finally, a broadly reactive subunit vaccine was designed of recombinant proteins partly presented as fusion proteins (Giuliani et al., 2006). One of the proteins is FHB, previously referred to as GNA1870 (Madico et al., 2006), a regulator of the complement cascade. Recruitment of factor H on the bacterial surface blocks consecutive complement activation. Wyeth, also identified FHB as a vaccine target using biochemical approaches (Fletcher et al., 2004; Pillai et al., 2005; McNeil et al., 2009). The protein was designated LP2086 and is included in two antigenic lipoprotein variants in the investigational vaccine.

Alternative concepts

The MenB polysaccharide, a poor antigen eliciting low affinity antibodies, has been modified by N-propionylation to reduce cross-reactivity towards human glycosylated proteins and augment immunogenicity (Jennings et al., 1987; Ashton et al., 1989; Fusco et al., 1997). Unfortunately, N-propionylated polysaccharides elicited antibodies cross-reactive with human α-2,8-linked polysialic acid, the glycosylation of the neural cell adhesion molecule NCAM (Granoff et al., 1998). Furthermore, a human trial with an N-propionylated MenB capsular polysaccharide conjugated to tetanus toxoid was dissatisfying, because the vaccine did not elicit functional antibodies (Bruge et al., 2004). Another MenB polysaccharide modification currently tested in animal models is de-N-acetyl MenB polysaccharide (Moe et al., 2009). Removing N-acetyl groups at the non-reducing end of the polysaccharide obviously stimulates T cell help and supports the induction of IgG in mice. The search for MenB polysaccharide modifications further stimulated the investigation of polysaccharide mimotopes, e.g. by screening phage display libraries with group B specific monoclonal antibodies.
lacking affinity to human polysialic acid (Shin et al., 2001; Park et al., 2004). Vaccination of mice with mimotopes resulted in bactericidal antibodies (Lo Passo et al., 2007). Another concept pursued is the vaccination of animals with secreted proteins of meningococci obtained from cell- and vesicle-free supernatants (Li et al., 2009). The secretome of meningococci has been reviewed recently (van Ulsen and Tommassen, 2006). Li and colleagues (2009) suggest that secreted proteins partly stick to the outer membrane, thereby serving as targets for bactericidal antibodies.

Of interest is the investigation in animal models of attenuated meningococcal strains as live vaccines such as unencapsulated strains with the genotype $\Delta siaD\Delta rfaF$ or $\Delta siaD\Delta metH$ (Li et al., 2004). Furthermore, Ciabattini and colleagues (2008) reported Streptococcus gordonii strains expressing the adhesin NadA (Comanducci et al., 2002) and the putative serum resistance modulator NhhA (Sjolinder et al., 2008), which induced the mucosal secretion of specific IgA in mice (Ciabattini et al., 2008). NadA is probably not the best choice for broad protection against meningococci, as it is not expressed in a variety of IMD-associated strains (Comanducci et al., 2004; Elias and Vogel, 2007; Lucidarme et al., 2009). Nevertheless, vaccination with live bacteria is an interesting issue to pursue. We suggest to consider also $cni$ meningococci, which are constitutively unencapsulated and widely present among healthy carriers (Claus et al., 2002; 2005). IMD caused by $cni$ meningococci is extremely rare. We reported one case in a severely immunocompromised patient (Vogel et al., 2004), who was the only $cni$/IMD case among more than 3400 cases investigated by the reference laboratory between 2002 and 2009. There are two further case reports of invasive disease due to $cni$ meningococci with one fatal case from Canada and three cases from Burkina Faso (Hoang et al., 2005; Findlow et al., 2007). There is evidence that $cni$ meningococci represent ancestors of meningococci (Schoen et al., 2008). Uptake of the capsule locus is possible in the laboratory (own unpublished observation), but unlikely to occur in nature based on genetic analysis of carrier isolates and theoretical consideration taking into account the size of the DNA fragment harbouring the capsule locus. Data are needed for the persistence of carriage of capsule null locus meningococci and the induction of bactericidal antibodies during carriage. Furthermore, the effect of live immunization practices on the population structure of the bacteria and natural boosting would need to be addressed.

Conclusions

The next years will see the introduction of conjugated meningococcus A vaccines in Africa, of new conjugated tetravalent vaccines, and hopefully also of the first broadly cross-reactive MenB vaccines. A variety of MenB protein vaccine candidates with possibly broad cross-reactivity also among other serogroups are under investigation, with the ones using the FHBP being advanced farthest. It is difficult to provide an estimate of the potential coverage of FHBP vaccines due to geographic effects, the unknown impact of carriage, and the unknown velocity and effectiveness of immune escape once effective herd immunity has been established. Many questions will only be answered after licensure, and one may assume that the

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**Fig. 3.** Development of meningococcal vaccines and depiction of the interaction between research institutions, industry and the public health sector.
first generation of universal MenB protein vaccines will be a starting point of continuous development. For sure, MenB vaccines might affect the population structure of the bacteria and consequently the development of natural immunity. Therefore, carriage studies are necessary, as well as an effective post-licensure surveillance of disease. The integration of various players during MenB vaccine development and introduction is summarized in Fig. 3.

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References

Achtman, M., and Wagner, M. (2008) Microbial diversity and the genetic nature of microbial species. Nat Rev Microbiol 6: 431–440.

van Alphen, L., and van den Dobbelsteen, G. (2008) Meningococcal B vaccine development and evaluation of efficacy. Hum Vaccin 4: 158–161.

Ashton, F.E., Ryan, J.A., Michon, F., and Jennings, H.J. (1989) Protective efficacy of mouse serum to the N-propionyl derivative of meningococcal group B polysaccharide. Microb Pathog 6: 455–458.

Auckland, C., Gray, S., Borrow, R., Andrews, N., Goldblatt, D., Ramsay, M., and Miller, E. (2006) Clinical and immunologic risk factors for meningococcal C conjugate vaccine failure in the United Kingdom. J Infect Dis 194: 1745–1752.

Balmer, P., Borrow, R., and Miller, E. (2002) Impact of meningococcal C conjugate vaccine in the UK. J Med Microbiol 51: 717–722.

Bambini, S., Muzzi, A., Olcen, P., Rappuoli, R., Pizza, M., and Comanducci, M. (2009) Distribution and genetic variability of three vaccine components in a panel of strains representative of the diversity of serogroup B meningococcus. Vaccine 27: 2794–2803.

Beernink, P.T., and Granoff, D.M. (2009) The modular architecture of meningococcal factor H-binding protein. Microbiology 155: 2873–2883.

Bentley, S.D., Vernikos, G.S., Snyder, L.A., Chucher, C., Arrowsmith, C., Chillingworth, T., et al. (2007) Meningococcal Genetic Variation Mechanisms Viewed through Comparative Analysis of Serogroup C Strain FAM18. PLoS Genet 3: e23.

Berry, D.S., Lynn, F., Lee, C.H., Frasch, C.E., and Bash, M.C. (2002) Effect of O acetylation of Neisseria meningitidis serogroup A capsular polysaccharide on development of functional immune responses. Infect Immun 70: 3707–3713.

Bhattacharjee, A.K., Jennings, H.J., Kenny, C.P., Martin, A., and Smith, I.C.P. (1975) Structural determination of the sialic acid polysaccharide antigens of Neisseria meningitidis serogroups B and C with carbon 13 nuclear magnetic resonance. J Biol Chem 250: 1926–1932.

Bhattacharjee, A.K., Jennings, H.J., Kenny, C.P., Martin, A., and Smith, I.C. (1976) Structural determination of the polysaccharide antigens of Neisseria meningitidis serogroups Y, W-135, and BO1. Can J Biochem 54: 1–8.

Bjune, G., Hoily, E.A., Gronnesby, J.K., Arnesen, O., Fredriksen, J.H., Halstensen, A., et al. (1991) Effect of outer membrane vesicle vaccine against group B meningococcal disease in Norway. Lancet 338: 1093–1096.

du-Bobie, J., Lupetti, P., Brunelli, B., Granoff, D., Norais, N., Ferrari, G., et al. (2004) GNA33 of Neisseria meningitidis is a lipoprotein required for cell separation, membrane architecture, and virulence. Infect Immun 72: 1914–1919.

Boisier, P., Nicolas, P., Djibo, S., Taha, M.K., Jeanne, I., Mainassara, H.B., et al. (2007) Meningococcal meningitis: unprecedented incidence of serogroup X-related cases in 2006 in Niger. Clin Infect Dis 44: 657–663.

Borrow, R., and Miller, E. (2006) Long-term protection in children with meningococcal C conjugate vaccination: lessons learned. Expert Rev Vaccines 5: 851–857.

Borrow, R., Andrews, N., Findlow, H., Waigt, P., Southern, J., Crowley-Luke, A., et al. (2010) Kinetics of antibody persistence following administration of a combination meningococcal serogroup C and haemophilus influenzae type b conjugate vaccine in healthy infants in the United Kingdom primed with a monovalent meningococcal serogroup C vaccine. Clin Vaccine Immunol 17: 154–159.

Brehony, C., Wilson, D.J., and Maiden, M.C. (2009) Variation of the factor H-binding protein of Neisseria meningitidis. Microbiology 155: 4155–4169.

Brute, J., Bouveret-Le, C.N., Danve, B., Rougon, G., and Schulz, D. (2004) Clinical evaluation of a group B meningococcal N-propionylated polysaccharide conjugate vaccine in adult, male volunteers. Vaccine 22: 1087–1096.

Buckee, C.O., Jolley, K.A., Recker, M., Penman, B., Kriz, P., Gupta, S., and Maiden, M.C. (2008) Role of selection in the emergence of lineages and the evolution of virulence in Neisseria meningitidis. Proc Natl Acad Sci USA 105: 15082–15087.

Bundle, D.R., Smith, I.C., and Jennings, H.J. (1974) Determination of the structure and conformation of bacterial polysaccharides by carbon 13 nuclear magnetic resonance. Studies on the group-specific antigens of Neisseria meningitidis serogroups A and X. J Biol Chem 249: 2275–2281.

Cano, R., Larrauri, A., Mateo, S., Alcala, B., Salcedo, C., and Vazquez, J.A. (2004) Impact of the meningococcal C conjugate vaccine in Spain: an epidemiological and microbiological decision. Euro Surveill 9: 11–15.

Cantini, F., Veggi, D., Dragonetti, S., Savino, S., Scarselli, M., Romagnoli, G., et al. (2009) Solution structure of the factor H-binding protein, a survival factor and protective antigen of Neisseria meningitidis. J Biol Chem 284: 9022–9026.

Caugant, D.A., Froholm, L.O., Bovre, K., Holten, E., Frasch, C.E., Mocca, L.F., et al. (1986) Intercontinental spread of a genetically distinctive complex of clones of Neisseria
meningitidis causing epidemic disease. Proc Natl Acad Sci USA 83: 4927–4931.

Caught, D.A., Tzanakaki, G., and Kriz, P. (2007) Lessons from meningococcal carriage studies. FEMS Microbiol Rev 31: 52–63.

Ciabattini, A., Giomarelli, B., Parigi, R., Chiavollini, D., Pettoni, E., Arico, B., et al. (2008) Intranasal immunization of mice with recombinant Streptococcus gordonii expressing NadA of Neisseria meningitidis induces systemic bactericidal antibodies and local IgA. Vaccine 26: 4244–4250.

Claus, H., Vogel, U., Mühlenhoff, M., Gerardy-Schahn, R., and Frosch, M. (1997) Molecular divergence of the sia locus in different serogroups of Neisseria meningitidis expressing polysialic acid capsules. Mol Gen Genet 257: 28–34.

Claus, H., Maiden, M.C., Maag, R., Frosch, M., and Vogel, U. (2002) Many carried meningococci lack the genes required for capsule synthesis and transport. Microbiology 148: 1813–1819.

Claus, H., Borrow, R., Achtmann, M., Morelli, G., Kanteberg, C., Longworth, E., et al. (2004) Genetics of capsule O-acetylation in serogroup C, W-135 and Y meningococci. Mol Microbiol 51: 227–239.

Claus, H., Maiden, M.C., Wilson, D.J., McCarthy, N.D., Jolley, K.A., Urwin, R., et al. (2005) Genetic analysis of meningococci carried by children and young adults. J Infect Dis 191: 1263–1271.

Claus, H., Stummeley, K., Batzilla, J., Mühlenhoff, M., and Vogel, U. (2009) Amino acid 310 determines the donor substrate specificity of serogroup W-135 and Y capsule polymerases of Neisseria meningitidis. Mol Microbiol 71: 960–971.

Coen, P.G., Cartwright, K., and Stuart, J. (2000) Mathematical modelling of infection and disease due to Neisseria meningitidis and Neisseria lactamica. Int J Epidem 29: 180–188.

Comanducci, M., Bambini, S., Brunelli, B., du-Bobie, J., Arico, B., and Capecchi, B., et al. (2002) NadA, a novel vaccine candidate of Neisseria meningitidis. J Exp Med 195: 1445–1454.

Comanducci, M., Bambini, S., Caugant, D.A., Mora, M., Brunelli, B., Capecchi, B., et al. (2004) NadA diversity and carriage in Neisseria meningitidis. Infect Immun 72: 4217–4223.

Delgado, M., Yero, D., Niebla, O., Gonzalez, S., Climent, Y., Perez, Y., et al. (2007) Lipoprotein NMB0928 from Neisseria meningitidis serogroup B as a novel vaccine candidate. Vaccine 25: 8420–8431.

Djibo, S., Nicolas, P., Alonso, J.M., Djibo, A., Courlet, D., Riou, J.Y., and Chippaux, J.P. (2003) Outbreaks of serogroup X meningococcal meningitis in Niger 1995–2000. Trop Med Int Health 8: 1118–1123.

Elias, J., and Vogel, U. (2007) IS1301 fingerprint analysis of Neisseria meningitidis strains belonging to the ET-15 clone. J Clin Microbiol 45: 159–167.

Elias, J., Harmens, D., Claus, H., Hellenbrand, W., Frosch, M., and Vogel, U. (2006) Spatiotemporal analysis of invasive meningococcal disease, Germany. Emerg Infect Dis 12: 1689–1695.

Feavers, I.M., Heath, A.B., Bygraves, J.A., and Maiden, M.C. (1992) Role of horizontal genetic exchange in the antigenic variation of the class 1 outer membrane protein of Neisseria meningitidis. Mol Microbiol 6: 489–495.

Feavers, I.M., and Pizza, M. (2009) Meningococcal protein antigens and vaccines. Vaccine 27 (Suppl 2): B42–B50.

Findlow, H., Vogel, U., Mueller, J.E., Curry, A., Njannop-Lafourcade, B.M., Claus, H., et al. (2007) Three cases of invasive meningococcal disease caused by a capsule null locus strain circulating among healthy carriers in Burkina Faso. J Infect Dis 195: 1071–1077.

Finney, M., Vaughan, T., Taylor, S., Hudson, M.J., Pratt, C., Wheeler, J.X., et al. (2008) Characterization of the key antigenic components and pre-clinical immune responses to a meningococcal disease vaccine based on Neisseria lactamica outer membrane vesicles, Hum Vaccin 4: 23–30.

Fletcher, L.D., Bernfield, L., Barniak, V., Farley, J.E., Howell, A., Knauf, M., et al. (2004) Vaccine potential of the Neisseria meningitidis 2086 lipoprotein. Infect Immun 72: 2088–2100.

Frasch, C.E., and Gotschlich, E.C. (1974) An outer membrane protein of Neisseria meningitidis group B responsible for serotype specificity. J Exp Med 140: 87–104.

Fusco, P.C., Michon, F.-R., Tai, J.Y., and Blake, M.S. (1997) Preclinical evaluation of a novel group B meningococcal conjugate vaccine that elicits bactericidal activity in both mice and nonhuman primates. J Infect Dis 175: 364–372.

Gigliani, M.M., du-Bobie, J., Comanducci, M., Arico, B., Savino, S., Santini, L., et al. (2006) A universal vaccine for serogroup B meningococcus. Proc Natl Acad Sci USA 103: 10834–10839.

Gold, R., Goldschneider, I., Lepow, M.L., Draper, T.F., and Randolph, M. (1978) Carriage of Neisseria meningitidis and Neisseria lactamica in infants and children. J Infect Dis 137: 112–121.

Gorringe, A.R., Taylor, S., Brookes, C., Matheson, M., Finney, M., Kerr, M., et al. (2009) Phase I safety and immunogenicity study of a candidate meningococcal disease vaccine based on Neisseria lactamica outer membrane vesicles. Clin Vaccine Immunol 16: 1113–1120.

Granoff, D.M., Bartoloni, A., Ricci, S., Gallo, E., Rosa, D., Ravenscroft, N., et al. (1998) Bactericidal monoclonal antibodies that define unique meningococcal B polysaccharide epitopes that do not cross-react with human polysaccharide. J Immunol 160: 5026–5036.

Grifantini, R., Sebastian, S., Frigimelica, E., Draghi, M., Bartolini, E., Muzzi, A., et al. (2003) Identification of iron-activated and -repressed Fur-dependent genes by transcripome analysis of Neisseria meningitidis group B. Proc Natl Acad Sci USA 100: 9542–9547.

Gudlaivaletti, S.K., Datta, A.K., Tzeng, Y.L., Noble, C., Carlson, R.W., and Stephens, D.S. (2004) The Neisseria meningitidis serogroup A capsular polysaccharide O-3 and O-4 acetyltransferase. J Biol Chem 279: 42765–42773.

Harrison, O.B., Maiden, M.C., and Rohkbi, B. (2008) Distribution of transferrin binding protein B gene (tpdB) variants among Neisseria species. BMC Microbiol 8: 66.

Hoang, L.M., Thomas, E., Tyler, S., Pollard, A.J., Stephens, G., Gustafson, L., et al. (2005) Rapid and fatal meningococcal disease due to a strain of Neisseria meningitidis containing the capsule null locus. Clin Infect Dis 40: e38–e42.

Holst, J., Feiring, B., Naess, L.M., Norheim, G., Kristiansen, P., Holby, E.A., et al. (2005) The concept of ‘tailor-made’,
protein-based, outer membrane vesicle vaccines against meningococcal disease. *Vaccine* 23: 2202–2205.

Hou, V.C., Koeberling, O., Welsch, J.A., and Granoff, D.M. (2005) Protective antibody responses elicited by a meningococcal outer membrane vesicle vaccine with overexpressed genome-derived neisserial antigen 1870. *J Infect Dis* 192: 580–590.

Howitz, M., Krause, T.G., Simonen, J.B., Hoffmann, S., Frisch, M., Nielsen, N.M., et al. (2007) Lack of association between group B meningococcal disease and autoimmune disease. *Clin Infect Dis* 45: 1327–1334.

Hsu, C.A., Lin, W.R., Li, J.C., Liu, Y.L., Tseng, Y.T., Chang, C.M., et al. (2008) Immunoproteomic identification of the hypothetical protein NMB1468 as a novel lipoprotein ubiquit inous in *Neisseria meningitidis* with vaccine potential. *Proteomics* 8: 2115–2125.

Jennings, H.J., Bhattacharjee, A.K., Bundle, D.R., Kenny, C.P., Martin, A., and Smith, I.C. (1977) Structures of the capsular polysaccharides of *Neisseria meningitidis* as determined by 13C-nuclear magnetic resonance spectroscopy. *J Infect Dis* 136(Suppl.): S78–S83.

Kelly, C., Arnold, R., Galloway, Y., and O’Hallahan, J. (2007) A prospective study of the effectivity of the New Zealand meningococcal B vaccine. *Am J Epidemiol* 166: 817–823.

Keyserling, H., Papa, T., Koranyi, K., Ryall, R., Bassily, E., Bybel, M.J., et al. (2005) Safety, immunogenicity, and immune memory of a novel meningococcal (groups A, C, Y, and W-135) polysaccharide diphtheria toxoid conjugate vaccine (MCV-4) in healthy adolescents. *Arch Pediatr Adolesc Med* 159: 907–913.

Kristiansen, B.E., Lind, K.W., Mevold, K., Sorensen, B., Froholm, L.O., Bryn, K., et al. (1988) Meningococcal pheno typic and genotypic characteristics and human antibody levels. *J Clin Microbiol* 26: 1988–1992.

Kshirsagar, N., Mur, N., Thatte, U., Gogtay, N., Viviani, S., Preziosi, M.P., et al. (2007) Safety, immunogenicity, and antibody persistence of a novel meningococcal group A conjugate vaccine in healthy Indian adults. Vaccine 25 (Suppl. 1): A101–A107.

LaForce, F.M., Konde, K., Viviani, S., and Preziosi, M.P. (2007) The meningitis vaccine project. *Vaccine* 25 (Suppl. 1): A97–100.

van der Ley, P., Steeghs, L., Hamstra, H.J., ten Hove, J., Zomer, B., and van Alphen, L. (2001) Modification of lipid A biosynthesis in *Neisseria meningitidis* lpxL mutants: influence on lipopolysaccharide structure, toxicity, and adjuvant activity. * Infect Microb* 69: 5981–5990.

Li, Y., Sun, Y.H., Ison, C., Levine, M.M., and Tang, C.M. (2004) Vaccination with attenuated *Neisseria meningitidis* strains protects against challenge with live Meningococci. * Infect Immun* 72: 345–351.

Li, Y., Wooldridge, K.G., Javed, M.A., Tang, C.M., and Ala’Aldeen, D.A. (2009) Secreted proteins of *Neisseria meningitidis* protect mice against infection. *Vaccine* 27: 2320–2325.

Linz, B., Schenker, M., Zhu, P., and Achtman, M. (2000) Frequent interspecific genetic exchange between commensal neisseriae and *Neisseria meningitidis*. *Mol Microbiol* 36: 1049–1058.

Lo Passo, C., Romeo, A., Perrone, I., Donato, P., Midiri, A., Mancuso, G., et al. (2007) Peptide mimics of the group B meningococcal capsule induce bactericidal and protective antibodies after immunization. *J Immunol* 178: 4417–4423.

Lucidarme, J., Comanducci, M., Findlow, J., Gray, S.J., Kaczmierski, E.B., Guiver, M., et al. (2009) Characterization of fHbp, nhba (gna2132), nadA, porA, sequence type (ST), and genomic presence of IS1301 in group B meningococcal ST269 clonal complex isolates from England and Wales. *J Clin Microbiol* 47: 3577–3585.

McNeil, L.K., Murphy, E., Zhao, X.J., Guttmann, S., Harris, S., Scott, A., et al. (2009) Detection of LP2086 on the cell surface of *Neisseria meningitidis* and its accessibility in the presence of serogroup B capsular polysaccharide. *Vaccine* 27: 3417–3421.

Madian, M.C., and Spratt, B.G. (1999) Meningococcal conjugate vaccines: new opportunities and new challenges. *Lancet* 354: 615–616.

Madden, M.C., and Stuart, J.M. (2002) Carriage of serogroup C meningococci 1 year after meningococcal C conjugate polysaccharide vaccination. *Lancet* 359: 1829–1831.

Maiden, M.C., Ibarz-Pavon, A.B., Urwin, R., Gray, S.J., Andrews, N.J., Clarke, S.C., et al. (2008) Impact of meningococcal serogroup C conjugate vaccines on carriage and herd immunity. *J Infect Dis* 197: 737–743.

Mascioni, A., Bentley, B.E., Camarda, R., Dilts, D.A., Fink, P., Gasusirova, V., et al. (2009) Structural Basis for the Immunogenic Properties of the Meningococcal Vaccine Candidate LP2086. *J Biol Chem* 284: 8738–8746.

Michon, F., Huang, C.H., Farley, E.K., Hronowski, L., Di, J., and Fusco, P.C. (2000) Structure activity studies on group C meningococcal polysaccharide-protein conjugate vaccines: effect of O-acetylation on the nature of the protective epitope. *Dev Biol (Basel)* 103: 151–160.

Moe, G.R., Bhandari, T.S., and Flinker, B.A. (2009) Vaccines containing de-N-acetyl sialic acid elicited antibodies protective against neisseria meningitidis groups B and C. *J Immunol* 182: 6610–6617.

de Moraes, J.C., Perkins, B.A., Camargo, M.C., Hidalgo, N.T., Barbosa, H.A., Sacchi, C.T., et al. (1992) Protective efficacy of a serogroup B meningococcal vaccine in Sao Paulo, Brazil. *Lancet* 340: 1074–1078.

Murphy, E., Andrew, L., Lee, K.L., Dills, D.A., Nunez, L., Fink, P.S., et al. (2009) Sequence diversity of the factor H binding protein vaccine candidate in epidemiologically relevant strains of serogroup B *Neisseria meningitidis*. *J Infect Dis* 200: 279–289.

Oliver, K.J., Reddin, K.M., Bracegirdle, P., Hudson, M.J., Borrow, R., Feavers, I.M., et al. (2002) *Neisseria lactamica* protects against experimental meningococcal infection. *Infect Immun* 70: 3621–3626.

Oster, P., Lennon, D., O’Hallahan, J., Mulholland, K., Reid, S., and Martin, D. (2005) MenZB: a safe and highly effective meningococcal conjugate vaccine for serogroup B meningococcal disease. *Lancet* 365: 215–222.

Van der Lelie, P., Steeghs, L., Hamstra, H.J., ten Hove, J., Zomer, B., and van Alphen, L. (2001) Modification of lipid A biosynthesis in *Neisseria meningitidis* lpxL mutants: influence on lipopolysaccharide structure, toxicity, and adjuvant activity. *Infect Microb* 69: 5981–5990.

Li, Y., Sun, Y.H., Ison, C., Levine, M.M., and Tang, C.M. (2004) Vaccination with attenuated *Neisseria meningitidis* strains protects against challenge with live Meningococci. *Infect Immun* 72: 345–351.

Li, Y., Wooldridge, K.G., Javed, M.A., Tang, C.M., and Ala’Aldeen, D.A. (2009) Secreted proteins of *Neisseria meningitidis* protect mice against infection. *Vaccine* 27: 2320–2325.
immunogenic tailor-made vaccine against the New Zealand Neisseria meningitidis serogroup B disease epidemic strain. Vaccine 23: 2191–2196.

Ostergaard, L., Lebaq, E., Poolman, J., Maechler, G., and Boutiau, D. (2009) Immunogenicity, reactivity and persistence of meningococcal A, C, W-135 and Y-tetanus toxoid candidate conjugates (MenACWY-TT) vaccine formulations in adolescents aged 15–25 years. Vaccine 27: 161–168.

Park, I., Choi, I.H., Kim, S.J., and Shin, J.S. (2004) Peptide mimotopes of Neisseria meningitidis group B capsular polysaccharide. Yonsei Med J 45: 755–758.

Parkhill, J., Achtman, M., James, K.D., Bentley, S.D., Pizza, M., Scarlato, V., Masignani, V., Giuliani, M.-M., Arico, B., Comanducci, M., et al. (2005) Identification of vaccine candidates against serogroup B meningococcus by whole-genome sequencing. Science 237: 1816–1820.

Pollabauer, E.M., Petermann, R., and Ehrlich, H.J. (2005) Group C meningococcal polysaccharide-tetanus toxoid conjugate vaccine: a meta-analysis of immunogenicity, safety and posology. Hum Vaccin 1: 131–139.

Ramsay, M.E., Andrews, N.J., Trotter, C.L., Kaczmarski, E.B., and Miller, E. (2003) Herd immunity from meningococcal serogroup C conjugate vaccine vaccination in England: database analysis. BMJ 326: 365–366.

Richmond, P., Borrow, R., Goldblatt, D., Findlow, J., Martin, S., Morris, R., et al. (2001) Ability of 3 different meningococcal C conjugate vaccines to induce immunologic memory after a single dose in UK toddlers. J Infect Dis 183: 160–163.

Rinaudo, C.D., Telford, J.L., Rappuoli, R., and Selib, K.L. (2009) Vaccinology in the genome era. J Clin Invest 119: 78–87.

Rouaud, P., Perrocheau, A., Tah, M.K., Sesboue, C., Forgues, A.M., Parent du Châtelet, I., and Levy-Bruhl, D. (2006) Prolonged outbreak of B meningococcal disease in the Seine-Maritime department, France, January 2003 to June 2005. Euro Surveill 11: 178–181.

Russell, J.E., Jolley, K.A., Feavers, I.M., Maiden, M.C., and Suer, J. (2004) PorA variable regions of Neisseria meningitidis. Emerg Infect Dis 10: 674–678.

Schneider, M.C., Prosser, B.E., Caesar, J.J., Kugelberg, E., Li, S., Zhang, C., et al. (2009) Neisseria meningitidis recruits factor H using protein mimicry of host carbohydrates. Nature 458: 890–893.

Schoen, C., Blom, J., Claus, H., Schramm-Gluck, A., Brandt, P., Muller, T., et al. (2008) Whole-genome comparison of disease and carriage strains provides insights into virulence evolution in Neisseria meningitidis. Proc Natl Acad Sci USA 105: 3473–3478.

Shin, J.S., Lin, J.S., Anderson, P.W., Insel, R.A., and Nahm, M.H. (2001) Monoclonal antibodies specific for Neisseria meningitidis group B polysaccharide and their peptide mimotopes. Infect Immun 69: 3335–3342.

Sierra, G.V., Campa, H.C., Varcael, N.M., Garcia, I.L., Izquierdo, P.L., Sotolongo, P.F., et al. (1991) Vaccine against group B Neisseria meningitidis: protection trial and mass vaccination results in Cuba. NIPH Ann 14: 195–207.

Sjolinder, H., Eriksson, J., Maudsdotter, L., Aro, H., and Jonsson, A.B. (2008) Meningococcal outer membrane protein NhhA is essential for colonization and disease by preventing phagocytosis and complement attack. Infect Immun 76: 5412–5420.

Snape, M.D., Kelly, D.F., Salt, P., Green, S., Snowden, C., Diggle, L., et al. (2006) Serogroup C meningococcal glycoconjugate vaccine in adolescents: persistence of bactericidal antibodies and kinetics of the immune response to a booster vaccine more than 3 years after immunization. Clin Infect Dis 43: 1387–1394.

Snape, M.D., Perrett, K.P., Ford, K.J., John, T.M., Pace, D., Yu, L.M., et al. (2008) Immunogenicity of a tetravalent meningococcal glycoconjugate vaccine in infants: a randomized controlled trial. JAMA 299: 173–184.

Stephens, D.S. (2007) Conquering the meningococci. FEMS Microbiol Rev 31: 3–14.

Stoddard, M.B., Pinto, V., Keiser, P.B., and Zollinger, W. (2010) Evaluation of a whole-blood cytokine release assay for use in measuring endotoxin activity of group B Neisseria meningitidis vaccines made from lipid A acylation mutants. Clin Vaccine Immunol 17: 98–107.

Tettelin, H., Saunders, N.J., Heidelberg, J., Jeffries, A.C., Nelson, K.E., Eisen, J.A., et al. (2000) Complete genome sequence of Neisseria meningitidis serogroup B strain MC58. Science 287: 1809–1815.

Thompson, E.A., Feavers, I.M., and Maiden, M.C. (2003) Antigenic diversity of meningococcal enterobactin receptor FetA, a vaccine component. Microbiology 149: 1849–1858.

Tolkia, J., Aalto, J., Hayrinen, J., Pellinieni, L.J., and Finne, J. (1998) The polysialic acid units of the neural cell adhesion molecule N-CAM form filament bundle networks. J Biol Chem 273: 28557–28559.

Trotter, C.L., Edmunds, W.J., Ramsay, M.E., and Miller, E. (2002) Whole-blood cytokine release assay for use in measuring endotoxin activity of group B Neisseria meningitidis vaccines made from lipid A acylation mutants. Clin Vaccine Immunol 17: 98–107.

van Ulsen, P., and Tommassen, J. (2006) Protein secretion and secreted proteins in pathogenic Neisseria meningitidis and Streptococcus pneumoniae. Vaccine 24: 6940–6944.
Urwin, R., Russell, J.E., Thompson, E.A., Holmes, E.C., Feavers, I.M., and Maiden, M.C. (2004) Distribution of surface protein variants among hyperinvasive meningococci: implications for vaccine design. *Infect Immun* **72**: 5955–5962.

Vogel, U., Claus, H., and Frosch, M. (2000) Rapid serogroup switching in *Neisseria meningitidis*. *N Engl J Med* **342**: 219–220.

Vogel, U., Claus, H., von Muller, L., Bunjes, D., Elias, J., and Frosch, M. (2004) Bacteremia in an immunocompromised patient caused by a commensal *Neisseria meningitidis* strain harboring the capsule null locus (cnl). *J Clin Microbiol* **42**: 2898–2901.

West, D., Reddin, K., Matheson, M., Heath, R., Funnell, S., Hudson, M., *et al.* (2001) Recombinant *Neisseria meningitidis* transferrin binding protein A protects against experimental meningococcal infection. *Infect Immun* **69**: 1561–1567.

Weynants, V.E., Feron, C.M., Goraj, K.K., Bos, M.P., Denoel, P.A., Verlant, V.G., *et al.* (2007) Additive and synergistic bactericidal activity of antibodies directed against minor outer membrane proteins of *Neisseria meningitidis*. *Infect Immun* **75**: 5434–5442.

Weynants, V., Denoel, P., Devos, N., Janssens, D., Feron, C., Goraj, K., *et al.* (2009) Genetically modified L3,7 and L2 lipooligosaccharides from *Neisseria meningitidis* serogroup B confer a broad cross-bactericidal response. *Infect Immun* **77**: 2084–2093.