Advances in meningococcal vaccines

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Practice Points

- Awareness of the early symptoms of bacterial meningitis and septicemia caused by pathogens including the meningococcus can save lives.
- Treatment recommendations for meningitis and septicemia include the use of presumptive therapy with antibiotics in cases where meningococcal disease is strongly suspected.
- As noted by recommending expert groups, presumptive antibiotics should cover all known circulating pathogens that could cause invasive disease and take into account any known antibiotic resistance in circulating strains of these organisms.
- Penicillin is effective in treating invasive meningococcal disease but does not eradicate carriage and may be ineffective against other circulating pathogens.
- Vaccination is the best means of preventing invasive meningococcal disease and should be employed following local guidelines and national immunization schedules.
- Product profiles should be reviewed for all vaccines administered at a single office visit in order to apprise vaccines and their caregivers of expected effects.

SUMMARY During the 20th century, meningococcal vaccine manufacturers took advantage of conjugate technology to provide products that could protect infants, the most vulnerable population, against invasive disease caused by serogroup C. These conjugate vaccines induce anamnestic responses and induce herd protection. Today, quadrivalent conjugate vaccines against serogroups A, C, W-135 and Y are available to protect all age groups. Several combination formulations are available in developed countries and a novel, low-cost serogroup A conjugate has been implemented in three counties in the African meningitis belt. 20th century approaches to serogroup B included two outer membrane vesicle vaccines against specific outbreak strains. The 21st century saw the development of several



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additional promising vaccine candidates based on subcapsular surface proteins. These include a licensed outer membrane vesicle product against the New Zealand outbreak

Background

Invasive meningococcal disease (IMD), which most commonly manifests as meningitis, septicemia or a combination of the two, is an important public health problem worldwide. The meningococcus, which was first identified during the early 19th century, has been a target for vaccine development since the 20th century [1-3]. Initial vaccines used the polysaccharide capsule as the primary antigen to induce a bactericidal antibody responses. Current licensed and investigational vaccines reflect important technological developments and immunological discoveries that will change the future of vaccine research [1,2,4-6]. The last 5 years have seen a number of new advances in the development of vaccines against meningococcal disease and their implementation worldwide. We describe the reasons for continued development of IMD vaccines, and provide a brief review of existing vaccines, recent technological developments and the ongoing public health efforts of governments, physicians agencies and scientists worldwide. We open with a description of meningococcal disease, its treatment and prevention and then review materials considering all types of vaccines against recent developments in treatment to provide a fuller picture of the existing literature and update several recent reviews of conjugate vaccines and vaccines against serogroup B.

Meningococcal disease

Neisseria meningitidis, a diplococcus (Figure 1), is one of the two pathogenic species of the genus: the meningococcus and the gonococcus, both of which are obligate human pathogens that primarily inhabit the mucosa. Additional *Neisseria* species are considered commensal and are not routinely associated with invasive disease. The meningococcus colonizes the nasopharyngeal mucosa and is most typically carried asymptomatically by approximately 10% of the population at any given time. Rates of asymptomatic carriage increase dramatically in certain conditions, such as crowded dormitories or military barracks, the Hajj pilgrimage and some international sporting events [3–11].

IMD generally manifests as sudden illness that occurs in healthy persons and can lead to death or permanent disability in as little as 24 h from the first recognizable symptoms. The disease is most commonly characterized by meningitis or sepsis, although rare presentations like pneumonia or arthritis have been reported. Delays in seeking treatment or obtaining a correct diagnosis can have dire consequences. Approximately 10% of patients in developed nations will die, although this figure varies by country, serogroup and age group. Up to 20% of patients will have permanent sequelae, such as limb amputation, scarring, cognitive impairment or hearing loss [9-14]. The risk of death and disability are strongly increased in patients with sepsis, for which case the fatality rate can exceed 30% [12,13]. Known risk factors for developing IMD include viral respiratory infections, crowded living conditions, travel, pub attendance and cigarette smoking. Of note, IMD often occurs within a week of new exposure or colonization by a novel or virulent strain [1,10-19].

The polysaccharide capsule is the basis for serogroup classification of *N. meningitidis*. Currently, 12 distinct serogroups are accepted, although not all are considered pathogenic [1,3,11]. Epidemiologically, the most important pathogenic serogroups are A, B, C, W-135, X and Y (Table 1). The major reservoir for serogroup A and the only reservoir for serogroup X are in the meningitis belt of sub-Saharan Africa, where annual seasonal epidemics represent the single greatest IMD burden on the planet. Serogroup W-135, which was strongly associated with travel to the Hajj (Mecca, Saudi Arabia) or Umrah (Mecca, Saudi Arabia), is the only other major disease-causing serogroup in this region [14,17,18].

Serogroup epidemiology has been highly mutable over time and space. Serogroup A is an important cause of disease in much of Asia. In Europe, the major cause of IMD is serogroup B, particularly in infants, while in North America disease caused by serogroups B, C, and Y are all considered to be epidemiologically important. The importance of serogroup Y disease is also increasing in some European countries. In the late 20th century, serogroup Y disease, which is associated with a higher case fatality rate compared with serogroup B, emerged in the USA. In Latin American countries, disease caused by serogroup Y is increasingly important as is disease caused by serogroup B. Serogroup C, which was a major cause of infant disease in developed nations a decade ago, has been largely controlled through universal vaccination campaigns [1,10,16–19].

Treatment of meningococcal disease

Treatments for meningococcal disease general include parenteral antibiotics, but case fatality rates and morbidity have remained at consistent levels in many countries, despite advances in prevention and treatment [20-23]. The effectiveness of treatment can be limited by antibiotic resistance, or a failure to detect index cases, particularly in locations where polymerase chain reaction is not used for case confirmation. In some regions, presumptive diagnoses of meningitis are treated similarly, using antibiotics that are known to kill all of the most common bacterial pathogens until a definite diagnosis can be made (Table 2). Knowledge of the most common bacterial pathogens causing meningitis and septicemia is of vital importance in making decisions about antibiotic use for presumptive diagnoses, especially in the context of antibiotic resistance of one or more disease-causing pathogens. As some of these pathogens are not vaccine-preventable, consistent awareness of all possible causes of the symptoms of meningitis and septicemia remains vital [11,20-27].

Strategies for the prevention of IMD

Several major strategies for the prevention of meningococcal disease are commonly employed [1-4,7,9,13,15,19-31]. These generally involve the use of antibiotics to protect at-risk individuals before or after high-risk exposures and vaccination (Table 1). Chemoprophylaxis of close contacts can be undertaken within the first week after an IMD index case. The prevention of IMD can be targeted to specific at-risk individuals or to larger groups. Vaccination policies are generally designed to cover large populations; however, protection of individuals, as with plain polysaccharide vaccines (see below), is sometimes employed as a cost-saving measure. Antibiotic prophylaxis, similarly can be offered to a small group of close contacts of an index case, or can be employed on a larger scale as a measure against outbreaks or epidemic disease. Antibiotics are sometimes used to supplement vaccination, as with specific populations of Hajj pilgrims [1-4,7, 11,13,19-31].

Chemoprophylaxis of close contacts, including vaccinated persons is intended to eradicate carriage and invasive bacteria, as well as to provide



Figure 1. Image of an example of meningococcal serogroup B strain MC58, revealing the surface-exposed Porin A protein.

protection until the body can mount an immune response.

Rifampin, ciprofloxacin, and ceftriaxone are generally recommended for prophylaxis; although pregnant women should avoid ciprofloxacin and rifampin, and other contraindications exist. Azithromycin is a suggested alternative. Agents for IMD treatment and prophylaxis are not always the same. Penicillin, specifically, is not recommended for prophylaxis, given its limited ability to eradicate carriage, although it is effective for treatment. Similarly, tetracycline and erythromycin are not recommended for chemoprophylaxis [13,20-22,25-26]. Little information is available about other antibiotics, including thirdgeneration cephalosporins, as chemoprophylactic agents. Timing is critical, and recommendations suggest administration within 7 days; preventative antibiotics are not recommended once 10 or more days have elapsed from exposure to an index case [20,21].

Vaccination is well established as the best public health measure for the prevention of IMD [1-4,11,19,32,33]. Various vaccination programs have been effective in limiting epidemics and sporadic disease.

Meningococcal vaccines

Sera were suggested to prevent IMD during the early 20th century, and vaccine development began during the latter part of that century (Table 3). The first large-scale field trials of plain polysaccharide vaccines were conducted in military recruits during the 1960s [1,34,35,201]. While some plain polysaccharide vaccines are still in use, conjugation technology, which involves the chemical combination of a capsular

Table 1. Meningococcal serogroups, vaccine types and epidemiology.				
Serogroup	Epidemiology	Regions affected	Existing Vaccine Types ⁺	Existing needs
A	Epidemic/sporadic	African meningitis belt countries	Conjugate: MenA-TT MenACWY-CRM ₁₉₇ MenACWY-DT Polysaccharide: MenAC-PS MenACW-PS MenACWY-PS	Broader dissemination of vaccine in the meningitis belt, increased surveillance and access to medicines and public health services
		Asia		
		Middle East		
В	Epidemic/sporadic	Australia and New Zealand	Serosubtype-specific outer membrane vesicle vaccines	Broad-coverage vaccines for routine use
		Europe		
		Latin America		
		North America		
		South Africa		
С	Epidemic/sporadic	African meningitis belt	Conjugate:	Evaluation of the success of
		Asia	MenC-CRM ₁₉₇	routine vaccine campaigns,
		Australia	Menc-TT MenACWY-CRM	vaccination in areas with
		Europe	MenACW PCNM ₁₉₇ MenACWP-DT Polysaccharide: MenAC-PS MenACW-PS MenACWY-PS	significant disease incidence
		Latin America		
		North America		
W-135	Epidemic/sporadic	Middle East	Conjugate: MenACWY-CRM ₁₉₇ MenACWY-DT Polysaccharide: MenACW-PS MenACWY-PS	Implementation of conjugate vaccination where needed, evaluation of vaccines for meningitis belt
		Argentina		
		South Africa and African meningitis belt		
х	Epidemic	Regions of African Meningitis belt countries and bordering areas	None	Investigation of vaccines for outbreak control
		Middle East		
Y	Sporadic	Chile	Conjugate: MenACWY-CRM ₁₉₇ MenACWY-DT Polysaccharide: MenACWY-PS	Implementation of routine vaccination in populations most affected
		Colombia		
		North America		
		South Africa		
[†] Not all vaccines are Data taken from [1–	available in all regions. 4,7,9,13,15,17–19].			

polysaccharide or oligosaccharide to a carrier protein is now commonly used to increase immunogenicity in infants and other persons who require anamnestic responses for protection from disease (Tables 1 & 4) [1,29,36,37]. Several recent reviews have presented a general overview of ongoing advances in the field of meningococcal vaccines [1,2,5,6,15,19,29,31,38].

Serogroup B polysaccharide is unsuitable for vaccine development because it does not induce immune responses. Consequently, various subcapsular units have been investigated as vaccine antigens (Table 5) [1-6,28,38]. Effective vaccines have employed the outer membrane vesicle (OMV) of specific outbreak strains to successfully limit and prevent hyperendemic and epidemic disease [1,3,4,39]. These vaccines only protect against a specific PorA serosubtype; therefore additional vaccine protein antigens and formulations have been investigated (Table 6) [4-6,40]. A multicomponent vaccine, 4CMenB, which contains proteins initially identified via reverse vaccinology (Figure 2) as well as the wild-type OMV from the New Zealand outbreak strain (Figure 3) is the only such vaccine to have completed Phase III trials and is described below [5,6,41-44].

Plain polysaccharide vaccines to protect against meningococcal disease

Plain polysaccharide vaccines have been employed against several organisms, including the meningococcus. Meningococcal polysaccharide vaccines provide serogroup-specific protection to particular at-risk individuals, such as Hajj pilgrims, persons living in the meningitis belt or military dormitories [7,15,36,37]. Consistent with recommendations by the WHO Strategic Advisory Group of Experts (SAGE) working group, polysaccharide vaccines remain a cost-effective means to obtain short-term protection most usually for individuals who will have limited exposure to high-risk situations or to prevent outbreaks (Tables 1 & 3) [45]. However, some countries will continue to use plain polysaccharide vaccines until affordable conjugate formulations become available [45,46].

Polysaccharide vaccines are well-tolerated in all age groups, yet their immunogenicity profile presents certain obstacles to routine use. The polysaccharide induces a B cell antibody response, which can provide protection to at-risk persons for periods of up to 3 to 5 years (1–3 years in persons aged 2–5 years), depending on the serogroup. However, plain polysaccharide vaccines, with the exception of some vaccines against serogroup A, they have been shown to be ineffective in infants and toddlers. Thus, with the exception of serogroup A vaccines, have not been routinely used in children under 2 years of age [1]. B celldependent responses limit the applicability of such vaccines in the elderly and others, such as those with innate or acquired immune deficiencies, who may be at the highest risk for developing IMD. Furthermore, repeat dosing, which is necessary for those who have sustained exposure to high-risk situations, may result in hyporesponsiveness, possibly because the B cell memory pool may become depleted over time [1,3,11,36,37,46–48]. Protection extends to vaccinated individuals, and reduced transmission is generally not observed, this is likely because the most recent studies show that these vaccines do not have clinically meaningful effects on carriage in many situations and are unlikely to prevent the acquisition of new carriage over time [1,49].

Meningococcal polysaccharide-protein conjugate vaccines

When a capsular polysaccharide is chemically conjugated to a protein carrier, such as tetanus toxoid (TT), diphtheria toxoid (DT), or cross-reacting material 197 (CRM₁₀₇), a T-celldependent response can be elicited, allowing for the induction of immune memory and immunogenicity in infants [4,32,47,49]. While the potential for immune interference, the possibility that a carrier protein or multiple antigens could interfere with immunogenicity of one or more antigen, has been considered, evidence remains equivocal [50,51]. Published data on conjugate vaccines suggest that immune interference may be of greater concern with the toxoid carriers, while bystander interference, or the potential for the vaccine antigens to interfere with one another, may be of greater concern with CRM. These observations remain speculative and further study is necessary to elucidate the potential for immune

Table 2. The most common causes of bacterial meningitis in developed countries.			
Pathogen	Vaccine preventable	Further vaccine development ongoing	
Streptococcus pneumoniae [†]	7-valent (conjugate) 10-valent (conjugate) 13-valent (conjugate) 23-valent (plain polysaccharide)	Yes	
Neisseria meningitidis	Serogroups A, C, W-135, and Y (conjugate) Selected Porin A serosubtypes (OMV)	Yes	
Group B streptococcus ⁺	No	Yes	
Haemophilus influenzae [‡]	Type b (conjugate)	Yes	
Listeria monocytogenes	No	No	
[†] A study of neonatal meningitis in France s pathogens. [†] Also important in neonates in developing pathogens, as does Staphylococcus aureu Data taken from [25–27].	howed that Escherichia coli and group B streptococcus countries; in the developing world, Gram-negative bacil ıs.	were the most important bacterial li appear to be important	

Table 3. Examples of meningococcal vaccines in clinical use or development.			
Туре	Serogroups	Current use	
Plain polysaccharide	A, C, W-135, Y	Individual protection in the context of limited exposure Areas where conjugate vaccines are not available	
Polysaccharide-protein conjugate	A, C, W-135, Y	Universal immunization against serogroups of epidemiologic interest Hajj pilgrimage (if available) At-risk groups	
Wild-type OMV	В	Outbreak and epidemic control against strains with a single Porin A serosubtype	
Recombinant OMV	B, A, W-135	In development	
Recombinant proteins ⁺	В	In development	
Purified proteins ⁺	В	In development	
Combination vaccines	A, B, C, W-135, Y	In development Some vaccines combining serogroup C conjugate with other childhood vaccines are available or in development	
[†] May be formulated with or without additional components.			

OMV: Outer membrane vesicle.

and bystander interference with combination vaccines [50].

The existing clinical trial databases of meningococcal protein conjugate vaccines have been substantively reviewed [10,16,29,37,52]. One review considers the clinical and therapeutic profile of CRM₁₉₇, a naturally nontoxic carrier protein that has been used in several meningococcal conjugate vaccine formulations [53].

Monovalent conjugate vaccines

Monovalent MenC conjugate vaccines first introduced in the UK in 1999 have subsequently shown immunogenicity and safety in all age groups. Routine vaccination programs substantially reduced serogroup C IMD in many countries, including Spain, Italy, Greece, France, Canada, Australia, Brazil and Argentina [19,36,37,46,54,55]. These vaccines should protect against the acquisition of carriage for at least 3 years [45,56,57].

An important observation in the UK was breakthrough IMD in toddlers and young children who had received a MenC vaccine only during infancy, suggesting the need for a booster dose in the second year of life [37,56,57]. A total of 3–6 years after initial vaccination with MenC conjugate vaccines, adolescents in Quebec showed immune memory following a booster of plain polysaccharide or a CRM₁₉₇ conjugate MenC. Protective responses were evident 1 year after the booster doses and were more substantial compared with adolescents who had received only an initial dose of conjugate vaccine [37,54].

Recently, a MenA-TT conjugate vaccine, MenAfriVac, was developed through the

Table 4. Examples of licensed and investigational meningococcal polysaccharide–protein conjugate vaccines.

conjugate vaccines.	
Meningococcal serogroups targeted	Carrier protein(s)
Monovalent vaccines	
с	CRM ₁₉₇ , TT
A	TT
Quadrivalent vaccines	
A, C, W-135, Y	CRM_{197} , DT, TT ⁺
Combination vaccines (additional antigens)	
A, C (DTPw + HBV + Hib)	TT
C, Y (Hib)	TT
C (Hib)	ТТ
C (9-valent pneumococcal) ⁺	CRM ₁₉₇
[†] Investigational products; not yet licensed.	inhtheria totanus whole-cell pertussis vaccines

CRM₁₉₇; Cross-reacting material 197; DT: Diphtheria toxoid; DTPw: Combination diphtheria, tetanus, whole-cell pertussis vaccine; HBV: Hepatitis B vaccine; Hib: Haemophilus influenzae type b; TT: Tetanus toxoid.

Table 5. Examples of approaches for meningococcal serogroup B vaccine development.			
Туре	Major protein antigen(s)	Licensed	
OMV	PorA	In limited areas Control clonal outbreaks and epidemics ⁺	
Recombinant OMVs	PorA fHbp TbpA NhhA NspA OMP 85 NadA OPC Lbp A and B	No	
OMV of Neisseria lactamica	Various	No	
Recombinant or purifi d proteins	fHbp and NadA and NHBA NspA TbpA and TbpB FetA OPA and OPC Lbps App PilQ GNA2091 GNA1030 LctP NM0088 NMB0928 MIP	No	
Lipopolysaccharide/ lipo-oligosaccharides	N/A	No	
⁺ Considered effective against strains with a single PorA serosubtype. N/A: Not applicable; OMV: Outer membrane vesicle; Por: Porin. Data taken from [1,4–6,28,80–82].			

Meningitis Vaccine Project for use in sub-Saharan Africa. In clinical studies and routine use, MenAfriVac was generally well tolerated and provided evidence of seroprotection in infants, adolescents and adults up to 29 years of age [37,58]. The vaccine was introduced in Burkina Faso, Mali and Niger in 2010, and dramatically reduced the incidence of serogroup A IMD. MenAfriVac could prevent more than a million IMD cases over the next 10 years, representing a cost savings of approximately \$350 million dollars [37,59]. Yet, although rates of serogroup A disease were markedly decreased, with four cases (all in unvaccinated persons) observed in Burkina Faso, disease caused by serogroups X and W-135 and also by the pneumococcus was observed. New vaccine development to cover all circulating serogroups in the meningitis belt is ongoing [37,60,61].

Table 6. Vaccines against serogroup B in clinical development.		
Vaccine	Development stage	
4CMenB	Completed Phase III trials	
rMenB + OMV NW rMenB (fHbp, NadA, NHBA) Hexavalent PorA, trivalent LPS OMV	Phase II trials	
nOMV Lpx-overexpressing OpcA Two PorAs, fHbp and NadA Bivalent fHbp (LP2086)	Phase I trials in adults	
LPS: Lipopolysaccharide; OMV: Outer membrane vesicle; Por: Porin. Data taken from [1,4–6,28,40,82].		



Figure 2. Spiral diagram of the reverse vaccinology process from *in silico* analysis through to vaccine development. ORF: Open reading frame.

Quadrivalent conjugate vaccines

Two conjugate vaccines against serogroups A, C, W-135 and Y, Menactra® (MenACWY-D; Sanofi Pasteur Swiftwater, PA, USA) and Menveo® (MenACWY-CRM; Novartis Vaccines and Diagnostics, MA, USA), have been licensed for use following different schedules in various countries and regions including the USA, Canada, South America, Asia and Saudi Arabia. MenACWY-CRM is also licensed in the EU. MenACWY-D is approved for use in individuals aged 9 months to 55 years, and postlicensure data suggest that vaccine effectiveness was between 80% and 85% following the introduction of routine vaccination in adolescents [62]. Similar data are not yet available for MenACWY-CRM, which is licensed for use in persons aged 2 years and older. Of note, the MenACWY-CRM vaccine was the first quadrivalent conjugate vaccine to show robust clinical immunogenicity in infants [62–64]. Clinical data for indications down to 2 months of age are under review. Another quadrivalent conjugate vaccine, MenACWY-T, is currently in clinical trials [37].

The two licensed MenACWY vaccines have shown robust immunogenicity with a generally favorable safety and tolerability profile in a substantial database of infants through adults, with clinical data supporting the use of both vaccines in persons aged 9 months to 55 years of age [10,16,29,63]. Published data also indicates that the CRM conjugate vaccine has clinical immunogenicity in infants from 2 months of age and in persons older than 55 years of age [10,16,51,52]. Another recent review details the chemistry of the CRM-containing vaccine [53]. The safety profiles of both MenACWY-CRM and MenACWY-D have been favorable in children, adolescents and infants with coadministra-

tion of routine vaccines, including multivalent pneumococcal vaccine, measles, mumps, rubella and varicella vaccine, and human papillomavirus vaccine [10,16,29,37,63–68].

One of the potential advantages suggested with the use of conjugate vaccines is that the duration of immune response, as measured by circulating antibody, could be enhanced compared with plain polysaccharide vaccines. Both MenACWY-CRM and MenACWY-D have been evaluated and have been shown to have a protective response with a duration of up to 3 years in adolescents [10,16,29,63]. As previously published, in some studies, statistically significant differences between the two quadrivalent conjugate vaccines, with the CRM conjugate showing greater immunogenicity, have been observed in some parameters and serogroups. The clinical importance, if any, of these differences remains unclear.

Additional persistence data for MenACWY-CRM have also been collected. Data presented at a scientific meeting suggest that MenACWY-CRM provided protective antibody levels for up to 5 years postvaccination in a majority of 11–18-year-old individuals tested, and also primed for anamnestic responses [69]. No similar data for MenACWY-D have been presented or published. Planned studies of MenACWY-CRM up to 7 years postvaccination will provide valuable insight into antibody persistence elicited by conjugate vaccines [70].

Recent data indicate that the MenACWY-T vaccines were immunogenic and well tolerated in two clinical trials: one in children from 12 to 23 months of age and one in children, adolescents and young adults [71-73].

Combination conjugate vaccines

Combination vaccines are commonly used to reduce the number of injections during routine well-infant healthcare visits in developed nations. The most widely used combination vaccines contain diphtheria and TTs in combination with various other antigens or protect against measles, mumps and rubella. Meningococcal combination vaccines are also currently in development or are licensed for use in infants.

A diphtheria, tetanus, acellular pertussis, hepatitis B, *Haemophilus influenzae* type b (Hib); Hib and meningococcal serogroups A and C vaccine is currently in Phase II trials in infants. After dosing at 6, 10, and 14 weeks of age, immune responses were similar to those following the administration of single vaccines for all antigens except the meningococcal serogroups, which were below the accepted levels of protection [74].

A Hib and MenC-TT conjugate vaccine was immunogenic and well tolerated when administered to infants at 2, 3, and 4 months of age, followed by a booster in the second year of life. No diminishment of immune response was evident when the Hib MenC vaccine was coadministered with DTaP-HBV-inactivated poliovirus, PCV7 or MMRV [75,76]. A Hib MenCY-TT vaccine was similarly immunogenic and well tolerated when administered to infants at 2, 4, and 6 months of age. Of interest, while the immune response to Hib was similar compared with a monovalent Hib vaccine, increased levels of bactericidal antibody against the meningococcal serogroups were observed [65,77].

A 9-valent pneumococcal and MenC-CRM₁₉₇ conjugate vaccine elicited a significant immune response to all vaccine antigens when administered to infants in Iceland and France. No plans to register this product exist [78,79].

Serogroup B vaccines

Attempts to formulate broad coverage vaccines against serogroup B have faced major challenges. The most important obstacle to vaccine development has been the serogroup B polysaccharide capsule, which is identical to polysialic acid, a constituent of the fetal neural cell adhesion molecule that can also be found in certain tissues in adults. The body does not recognize the serogroup B polysaccharide as foreign and will not mount an antibody response against it. Consequently, subcapsular antigens have been employed in all further vaccine development efforts against meningococcal serogroup B [1,4,11,37,43,44]. Approaches to vaccine development against serogroup B include the use of purified outer membrane proteins, OMVs, OMV overexpressing antigens, various recombinant proteins, and a macrophage infectivity potentiator



Figure 3. Schematic representation of the 4CMenB antigens. (A) Artist rendering of a meningococcus showing the surface-exposed proteins used in vaccine development. The proteins fHbp, NadA and NHBA (see Table 5) are anchored to the membrane and fully accessible to antibody binding. PorA is embedded within the membrane. Proteins are not to scale. (B) Cartoon depiction of an intact outer membrane vesicle, showing a cross-section of the membrane and of the most abundant outer membrane vesicle proteins embedded in the membrane. (Table 5) [80]. Recent reviews have considered most of these vaccine approaches [4-6,28,38,81,82].

OMV vaccines

Thus far, the only licensed vaccines to protect against meningococcal serogroup B-encapsulated strains have been OMV vaccines against specific outbreak strains [2,37,39,81,82]. Wild-type OMVs can be obtained by detergent extraction or collecting blebs released by the bacteria in culture. Native OMVs are obtained without the use of detergents and need lipooligosaccharide detoxification to reduce reactogenicity. OMVs can also be produced recombinantly for greater control of surface expression and antigenicity of constituent proteins This is of note, because the OMV contains proteins embedded in a membrane, these formulations require the presence of some lipopolysaccharide (LPS), which enables the maintenance of a bilayer conformation. Although membrane-bound LPS is known to be much less toxic than free LPS, additional measures, such as adsorption to aluminum hydroxide, for wild-type OMVs have been necessary to provide an optimal safety and tolerability profile [39].

The three OMV vaccines, VA-MENIN-GOC-BC, MenBVac® (Norwegian Institute for Public Health, Bergen, Norway) and MeNZB® (Novartis Vaccines) were developed to address hyperendemic or prolonged epidemic disease in Cuba, Norway and New Zealand, respectively. Furthermore, MenBVac was used in Normandy to prevent a localized epidemic caused by a prevalent meningococcal serogroup B strain sharing the same PorA serosubtype as the Norway outbreak strain. It continues to be used routinely in this area. Licensed OMV vaccines have also been used to limit disease in Brazil and Chile [1,3,4,9,39]. All three vaccines provided adequate protection from disease to limit or prevent epidemic disease in the regions where vaccine campaigns were implemented. Recent reviews detail the effects and results of these vaccines in clinical use (Box 1) [1,9,28,39].

The public health benefits provided by the use of OMV vaccines have been substantial. Of note, OMV vaccines are understood to protect against all strains of the homologous serosubtype (PorA type). OMV approaches have been suggested for the development of serogroup A and W-135 vaccines for use in the African meningitis belt. Additional uses of OMVs include the development of a novel multicomponent vaccine against serogroup B (4CMenB), which is currently being reviewed for licensure by the European Medicines Agency [5,6].

Identification of novel antigens using genomics

As previously reviewed, [1,2,28,83–85]; although more conventional approaches to antigen identification have yielded some promising subcapsular antigen candidates, a novel approach relying on the examination of whole genome sequences, called 'reverse vaccinology', was developed and first used to address meningococcal serogroup B. This approach has subsequently been refined and used to identify additional possible protein antigens for vaccine development and to identify antigens against other microorganisms [84,86].

The initial examination of the genome of the MC58 strain identified 600 genes encoding for potentially surface-exposed proteins that could represent ideal candidates for a new vaccine against meningococcal serogroup B. These genes were cloned in *Escherichia coli*, and recombinant proteins were purified and used to immunize mice. Then, mouse sera were analyzed for their ability to recognize the proteins on the meningococcal surface and to mediate bacterial killing. About 350 proteins were successfully expressed in *E. coli* and used to immunize mice. Immunological characterization of the immune sera revealed that 92 proteins were

Box 1. Important clinical features of outer membrane vesicle vaccines in clinical use.

- Provides protection against strains expressing the same PorA serosubtype in all age groups
- Age-group differences have been observed, especially against heterologous strains, with diminished effects in infants and young children compared with older persons. However, immunogenicity is observed in children as young as 6 weeks of age
- Immune memory and boosting, while evident, may not be fast enough to counteract proliferation of newly acquired invasive disease
- Reactogenicity profiles are considered moderate. Fever is the most commonly observed reaction in infants. Injection site pain and swelling are commonly observed in adolescents and adults. Headache (adolescents and adults) and irritability (infants) have also been commonly reported

Data taken from [1,4-6,82].

surface-exposed in N. meningitidis and 28 proteins were able to induce bactericidal antibodies that could kill N. meningitidis in a serum bactericidal assay (Figure 2). Antigens were then prioritized on the basis of their ability to induce the killing of multiple, genetically diverse meningococcal strains. Although none of the 28 antigens induced killing of all strains, the most promising vaccine was a combination of three antigens: fHbp, NadA and NHBA. Gene amplification and sequencing on a wider panel of strains indicated that these antigens have some regions of variability and that some antigenic variants were more predominant than others. Therefore the final vaccine combination was defined as the combination of the most represented variants/peptides. Antigen expression, stability and immunogenicity were optimized for fHbp and NHBA by fusion of each with a specific additional accessory antigen.

NadA, NHBA and fHbp have been characterized for their functional, immunological and structural properties. NadA is an outer membrane protein with a trimeric structure, able to mediate adhesion and invasion into epithelial cells. The gene for NadA is present in all strains belonging to three of the four hypervirulent lineages, and is almost absent in carrier strains, defined as strains isolated only from healthy individuals that have not been linked to IMD cases. NadA expression is highly regulated at the gene level, yet antibodies against NadA are present in sera from convalescent individuals suggesting that the antigen is expressed during disease. fHbp is a lipoprotein with a β -barrel structure that has three main variants. It is an important virulence factor due to its ability to bind human factor H, a regulator of the alternative complement cascade; fHbp is also immunogenic during disease. The fHbp gene is present in nearly all strains, although a few strains that do not express fHbp have been identified. Of interest, fHbp is not the only Neisserial protein able to bind factor H, as NspA shares this function. NHBA is a lipoprotein with a β -barrel carboxy-terminal domain. It can bind heparin a property that correlates with increased survival of the unencapsulated bacterium in human serum. Antibodies against NHBA are present in sera from convalescent individuals suggesting that it is expressed and immunogenic during disease [87,88].

Vaccine development against meningococcal

serogroup B, as mentioned above and in recent reviews, has largely focused on the inclusion of multiple antigens and components [3,28]. As discussed below, the combination of fHbp, NadA, and NHBA alone (rMenB) or formulated with the OMV from one of the effective outbreak vaccines discussed above showed clinical immunogenicity and tolerability in early trials in humans [5,6]. These studies and further formulation work resulted in the development of 4CMenB, which is currently the only broadly protective meningococcal serogroup B vaccine to have completed Phase III trials and enter consideration for licensure. Further work to define the genomes of additional serogroup B strains [89], to identify immunogenic proteins, and to present the results of clinical trials of 4CMenB are ongoing [86,90,91]. While a bivalent fHbp vaccine is in development, there are no plans to develop standalone vaccines with NHBA or NadA.

Additional factors in the development of serogroup B vaccines

Although the meningococcus can be divided into serogroups, strains and clonal complexes, these divisions are not mutually inclusive or exclusive. A single serogroup can comprise encapsulated strains from various clonal complexes, and a single clonal complex may include several capsular serogroups as well. Therefore, considerations of meningococcal serogroup B vaccines for general use must include some accounting for strain coverage. This is especially important because it seems unlikely that singlecomponent vaccines will adequately account for phase and sequence variation over time and across geographic regions.

The complications of meningococcal serogroup, strain, subtype, serosubtype and clonal complexes are such that several different nomenclature schemes have been used in the past decade. The most recent nomenclature scheme suggests inclusion of several types of information as follows: serogroup: subserotyping (PorA type): fetA type: multilocus sequence typing (based on seven housekeeping genes) defining clonal complex(es). Of note, multilocus sequence typing is now considered an essential and more refined method of typing as compared with multilocus electrophoretic typing (MLEE) [92]. The current nomenclature scheme adequately accounts for antigens and proteins considered of interest through the development

of the OMV vaccines, but does not account for all antigens currently being examined in vaccine development [93].

Another consideration for the development of vaccines against meningococcal serogroup B is the establishment of an adequate method to assess the expected level of strain coverage a vaccine will afford against circulating strains in a particular country or region [82]. Clinical trials of all types of meningococcal vaccine have long relied on serologic correlates of protection to predict clinical efficacy. The most widely accepted correlate is a titer greater than or equal to four in a serum bactericidal assay using human complement (hSBA) [82,94]. Several approaches to developing adequate representation of strain panels have been proposed [82]; however, given that few of the vaccine antigens being examined in vaccine development are adequately typed, additional methods have been proposed to provide supplemental information for vaccines in clinical trials.

The only published novel method, a meningococcal antigen typing system (MATS) was designed to account for expression and crossreactivity of the primary antigens included in 4CMenB: fHbp, NadA and NHBA, which are evaluated in a specialized ELISA using polyclonal antibodies, in conjunction with the examination of the PorA serosubtype by sequencing. Meningococcal strains that express one or more of the protein antigens above a predefined threshold or have the gene coding for PorA 1.4, which is present on the OMV component of the vaccine, are considered to be covered by 4CMenB. MATS was shown to correlate with hSBA and has subsequently been transferred to several national reference laboratories [93].

Serogroup B vaccines in clinical trials

Several different vaccines against meningococcal serogroup B have been employed in clinical trials (Table 6). For the most part, these products contain one or more OMV components. However, formulations containing only purified proteins have also been investigated. Of the vaccines in clinical trials, a substantial proportion contain OMV, but only two comprised solely of purified proteins (rMenB and bivalent fHbp [LP2086]), have elicited bactericidal activity in the serum bactericidal assay [4–6,28,38]. It has been suggested that vaccine approaches that draw on multiple components would tend to avoid the problem of escape mutants, while the inclusion of multiple variants of a single antigen may mitigate issues with antigen diversity [28].

Hexavalent OMV vaccine

As recently reviewed, a recombinant hexavalent PorA OMV vaccine (HexaMen) developed at the National Institute for Public Health and the Environment (RIVM) in The Netherlands entered several clinical trials [28]. This vaccine was designed to augment the strain coverage expected from the monovalent wild-type OMV vaccines described above. In the hexavalent vaccine, six PorA OMPs are embedded in OMVs (P1.7,16; P1.5-1,2-2; P1.19,15-1; P1.5-2,10; P1.12-1,13 and P1.7-2,4) containing three different types of LPS [3,28,95]. Initial studies in infants, toddlers and school-aged children demonstrated that the vaccine was generally safe and well tolerated, with evidence of robust immunogenicity with multiple doses. These studies did not account for the sequence variability of the VR1 and VR2 regions. To assess additional individual serosubtypes, serum bactericidal assays were performed using samples obtained pre- and post-vaccination from toddlers and children who had received the hexavalent vaccine. Findlow and colleagues found that sequence variation in the VR region had a substantial effect on immunogenicity in the serum bactericidal assay, whereas variation in the V1 region did not. These findings suggest that further study is needed and that accounting for variations in the VR2 region would continue to be important when evaluating strain coverage by OMV vaccines [95]. Furthermore, the immune responses to two of the PorA types was weak in animal models and in clinical studies. A heterologous prime-boost strategy was proposed to address this potential problem [95]. This vaccine has not entered Phase III trials.

Bivalent fHbp vaccine

Preclinical information has been published regarding a bivalent fHbp (LP2086) vaccine [96-98]. The fHbp in this vaccine differs from that in all other fHbp-containing vaccines in clinical trials in that it is lipidated and not joined to an additional fusion protein [38,97,98]. A recent study shows that this vaccine component was recognized by the human immune system [99]. Preclinical publications posit the likelihood of broad strain coverage [100] and show immunogenicity results in animal models [101]. Preliminary data from Phase I studies have been presented at meetings and reviewed [38]. Publication of these promising initial results is forthcoming.

rMenB

Several clinical studies detailing results of clinical trials of a prototype vaccine (rMenB) containing fHbp, NadA and NHBA have been published or presented at scientific meetings [41,43,44,102]. These trials indicated that rMenB was generally safe and well-tolerated in infants, adolescents and adults with repeat dosing. Furthermore, robust immunogenicity was observed for panels of genetically heterologous MenB strains in all age groups. Of note, cross-protection was less commonly observed in studies in infants, in which strain killing in the hSBA was observed primarily for strains expressing a homologous or closely related form of at least one of the vaccine antigens.

A formulation of rMenB with OMV from the Norway outbreak strain showed generally similar results to rMenB, but with added protection against strains carrying homologous PorA and a small increase in reactogenicity [41]. A single study compared the rMenB with NW OMV to 4CMenB, and shows that 4CMenB provided greater cross-coverage when assessed against a panel of 15 heterologous MenB strains [102]. Neither rMenB nor rMenB plus NW OMV have been investigated in Phase III trials [5,6].

4CMenB

Although vaccine development using myriad antigen formulations and combinations is ongoing to address the problem of serogroup B IMD, which is a particular concern for infants in developed countries, only a single vaccine is currently being considered for licensure in Europe, where over 90% of IMD in infants is caused by this serogroup. Two recent reviews have described the vaccine antigens found in 4CMenB and the vaccine itself [5,6]. The reviewers observed that 4CMenB was immunogenic and had a generally acceptable profile in various age groups, including infants. Immune responses have been described as robust, although some concerns about the level of cross-reactivity of the vaccine antigens in infants has been expressed in some contexts, suggesting a need for further study [5,6,103]. Nevertheless, a consistent finding

in all clinical trials published is that 4CMenB consistently elicited robust immune responses against genetically heterologous serogroup B strains [5,6,41–44]. Phase III clinical trials have used a set of 'reference strains' to assess the four individual vaccine antigens [87,104], and results will be bridged to different countries using the MATS methodology. It is hoped that the multicomponent vaccine approach will provide protection against MenB over time and limit possible escape mutants. Further work is needed to establish full information on expected coverage for all geographic regions.

5-year view

Over the next 5 years, it is to be expected that new vaccines will be developed or licensed to address the problem of meningococcal disease. Likely 4CMenB, a multicomponent vaccine that includes fHbp, NadA, NHBA, and OMV from strain NZ 98/254, which caused the clonal outbreak in New Zealand, will be licensed in Europe, Canada, Australia and other countries. Given the current body of information to support the immunogenicity and safety profile of 4CMenB in clinical use, it is anticipated that this new vaccine could effect dramatic reductions in the incidence of meningococcal disease, especially in infants, providing added public health benefits.

In Africa, the recent introduction of a meningococcal serogroup A conjugate vaccine should result in a dramatic decrease in IMD incidence and related mortality. Likely, background rates of IMD caused by additional serogroups or pathogens will be observed in other countries as they were in Burkina Faso. We are confident that efforts to formulate inexpensive vaccines against serogroups A and W-135 will address needs in this region and possibly in low-income countries with populations attending Hajj. In the USA, we anticipate that MenACWY-CRM may be licensed in infants within the next 2-3 years, particularly following the recent expansion of indication for MenACWY-D down to age 9 months. With the recent nadir in the incidence of meningococcal disease in the USA [18], it is unclear how recommendations for immunization of infants or other at-risk groups will develop or change. In Europe, we anticipate licensure of a meningococcal serogroup B vaccine within the next 2-3 years. Given that the vast majority of IMD in this region is caused by



this serogroup, it is likely that the routine use of an appropriate vaccine will result in a reduction of clinical disease.

Additional vaccine development efforts will be ongoing. Recently, a combination vaccine against serogroups A, B, C, W-135 and Y entered clinical trials. This novel vaccine combines polysaccharide from serogroups A, C, W-135 and Y with the vaccine antigens included in 4CMenB. Results of clinical trials should be available over the next 2 years; however, until such data are collected and interpreted it will remain unclear whether this product will be implemented routinely.

Conclusion & future perspective

A burgeoning literature has sprung up around novel protein antigens that can serve as vaccine candidates against meningococcal serogroup B. We anticipate that in the next 5 years much additional information will become available about fHbp, NadA, and NHBA and their function, epidemiology and immunogenicity profiles. Recent work on various recombinant antigens will likely be published over the next few years, as will the results of MATS typing of strain collections by national reference laboratories in the USA, Europe, and other regions. Additional work in molecular epidemiology, typing of existing meningococcal strain collections, and collection of additional pathogenic and carriage isolates is also anticipated. Furthermore, it is likely that additional clinical information will become available about the bivalent fHbp vaccine. Similarly, we anticipate that much additional information will become available describing the effects of the various combinations and recombinant OMV vaccines currently in preclinical and clinical trials. Over the next 5 years, it is to be expected that new vaccines will be developed or licensed to address the problem of meningococcal disease. Likely 4CMenB, a multicomponent vaccine that includes fHbp, NadA, NHBA, and OMV from strain NZ 98/254, which caused the clonal outbreak in New Zealand, will be licensed in Europe, Canada, Australia and other countries. Given the current body of information to support the immunogenicity and safety profile of 4CMenB in clinical use, it is anticipated that this new vaccine could affect dramatic reductions in the incidence of meningococcal disease, especially in infants, providing added public health benefits.

In Africa, the recent introduction of a meningococcal serogroup A conjugate vaccine should result in a dramatic decrease in IMD incidence and related mortality. Likely, background rates of disease caused by additional serogroups or pathogens will be observed in other countries as they were in Burkina Faso. We are confident that efforts to formulate inexpensive vaccines against serogroups A and W-135 will address the needs of this region and possibly in low-income countries with populations attending Hajj. In the USA, we anticipate that MenACWY-CRM will be licensed in infants within the next 5 years, particularly following the recent expansion of indication for MenACWY-D down to the age of 9 months. With the recent nadir in the incidence of meningococcal disease in the USA [18], it is unclear how recommendations for immunization of infants or other at-risk groups will develop or change over the next few years. In Europe, we anticipate licensure of a meningococcal serogroup B vaccine within the next 5 years; given that the vast majority of IMD in this region is caused by this serogroup, it is likely that the routine use of an appropriate vaccine will result in a reduction of clinical disease.

Additional vaccine development efforts will be ongoing. Recently, a combination vaccine against serogroups A, B, C, W-135 and Y entered clinical trials. This novel vaccine combines polysaccharide from serogroups A, C, W-135 and Y with the vaccine antigens included in 4CMenB. Results of clinical trials should be available within the next 2 years; however, until such data are collected and interpreted it will remain unclear whether this product will be implemented routinely.

Although meningococcal disease is a serious public health problem in its own right, the quest to end this dangerous illness has led to the development of reverse vaccinology and the MATS. It is likely that these novel and highly adaptable research methods will be applied to other disease entities to promote public health in the broadest sense.

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