# Serogroup B meningococcal vaccines—an unfinished story

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**Most invasive meningococcal disease in developed countries is caused by *Neisseria meningitidis* with a serogroup B capsule. However, despite availability of vaccines for other serogroups since the 1960s, no serogroup B vaccine exists. In this Review we look at the development of serogroup B vaccines over the past 40 years. Outer membrane vesicle vaccines have been successfully used to control geographically isolated epidemics, but most have not been highly immunogenic in young children or provided broad cross-protection from infections with other strains. Vaccines based on subcapsular antigens have recently produced promising results in early clinical trials, and the disease burden might be substantially reduced over the next few years.**

## Introduction

The first description of a disease with the characteristics of infection with *Neisseria meningitidis* was made by Gaspard Vieusseux[1] in Geneva, Switzerland, during a meningitis epidemic in 1805. Invasive meningococcal disease is now endemic worldwide and most cases are caused by five of the 13 meningococcal serogroups (A, B, C, Y, and W135). Serogroup B disease accounts for many cases in Europe, the Americas, and Australasia, where the incidence of invasive meningococcal disease ranges from less than one case per 100 000 per year to six cases per 100 000 per year,[2,3] peaking in children between ages 6 months and 2 years.

Meningococci can be characterised by serological methods, on the basis of surface structures of the organism (figure 1),[4] or by multilocus sequence typing (MLST)[5] during surveillance and vaccine development. The latter uses DNA sequence data to identify variation in housekeeping genes, and assigns genetically related isolates into sequence types, and related sequence types into clonal complexes. Most serogroup B disease since the 1960s has been caused by isolates from sequence type 32 and sequence type 41/44 clonal complexes.[6]

Introduction of a serogroup C conjugate vaccine (MenC) into several countries since 1999 reduced the incidence of serogroup C disease ten-fold,[7] and an ACYW135 tetravalent conjugate vaccine has recently been introduced into the USA for adolescents (aged 11–18 years),[8] although data are not yet available on its effectiveness. Because available vaccines can control A, C, Y, and W135 disease, serogroup B is the main cause of invasive meningococcal disease in most temperate countries, and accounts for 85–90% of cases in the UK.[7,9–12] Until a safe and effective serogroup B vaccine is developed, invasive meningococcal disease will continue to cause substantial morbidity and mortality in children worldwide.

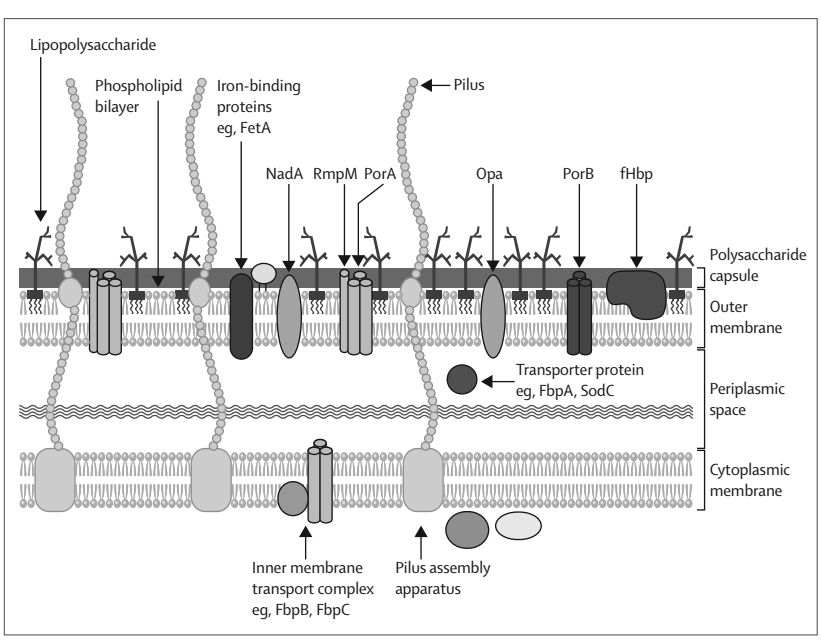


***Figure 1:*** **Surface structures of *Neisseria meningitidis***
Serological classification of *N meningitidis* is based on capsule (serogroup), the outer membrane proteins PorB (serotype) and PorA (serosubtype), and lipopolysaccharide (immunotype). Other major outer membrane components include the new vaccine candidates fHbp and NadA.

## Immunity and surrogate markers of protection

Serogroup B disease has a low incidence in countries where it is endemic, so the large sample size needed for vaccine efficacy studies prohibits trials that use disease as an outcome. Surrogate markers of protection are therefore needed that can be measured in participants of the trials. The importance of so-called bactericidal substances in human blood in protecting against invasive meningococcal disease, and higher concentrations of such factors in adults compared with children, was first proposed in the early 1900s.[13,14] In 1969, Irving Goldschneider and colleagues[15] provided evidence that circulating antibody was the crucial substance, showing an inverse correlation between the incidence of disease and the prevalence of complement-dependent serum bactericidal antibody (figure 2). This finding led to accepted use of the serum bactericidal antibody assay as a surrogate marker of protection.

In the modern serum bactericidal antibody assay, *N meningitidis* target strains are killed in the presence of meningococcal-specific antibody (from postvaccination serum) and exogenous complement.[16] For MenC, a serum bactericidal antibody titre of 1/8 or greater (with rabbit complement) correlated strongly with postlicensure vaccine effectiveness.[16] For serogroup B disease the data are less secure, but the proportions of vaccine recipients

with four-fold or greater rises in serum bactericidal antibody after vaccination or serum bactericidal antibody titres of 1/4 or greater, using human complement, have been correlated with clinical efficacy in trials of outer membrane vesicle vaccines.[17–19] These cutoffs are therefore thought to be the protective threshold in assessment of experimental vaccines, and are required to support vaccine licensure.

Several limitations of the serum bactericidal antibody assay exist. There is substantial interlaboratory variation, particularly when measuring serum bactericidal antibody titre of 1/4 or greater and no consensus about representative target strains. Recent data suggest that rabbit complement factor H (fH) does not bind to *N meningitidis*, unlike human fH. Binding of fH to the bacterial surface downregulates complement activation, leading to higher titres of serum bactericidal antibody and overestimation of vaccine efficacy with rabbit complement.[20] These data suggest that either human complement or exogenous human fH should be used in these assays, but there is difficulty in getting human complement sources for all test strains.

Evidence is increasing that alternative mechanisms are important in establishing protection against invasive meningococcal disease. First, the relation between incidence of disease and prevalence of serum bactericidal antibody described by Goldschneider and colleagues[15] was not seen in more recent studies in the UK and Canada,[21,22] where a decline in disease incidence throughout childhood was not associated with a change in the prevalence of serum bactericidal antibody. In the UK study, the second peak of disease in teenagers coincided with a paradoxical increase in the proportion with a serum bactericidal antibody titre of 1/4 or greater, and adults had a low risk of disease despite a much lower prevalence of serum bactericidal antibody activity. Second, in a large study in Iceland, titres of serum bactericidal antibody gave an underestimate of vaccine efficacy.[23] Third, disease in individuals with complement deficiency has a different age distribution, less severe clinical features, and involves unusual serogroups.[24] Indeed, serogroup B disease has only occasionally been described in series of complement-deficient individuals with invasive meningococcal disease.

Alternative surrogate markers of protection include the opsonophagocytic assay[25] and antibody avidity,[26] but there are no data linking these with vaccine efficacy or even population protection. Protection in the absence of serum bactericidal antibodies is probably conferred by opsonophagoytosis.[27] This protection is seen in complement factor C6-deficient rats, which permit opsonisation but not bacteriolysis. Both serum bactericidal antibody and opsonophagocytic activity have been elicited in animals and human beings after immunisation with serogroup B protein and lipopolysaccharide antigens. However, few data suggest that opsonophagocytic activity in the absence of serum bactericidal antibody can prevent or improve outcome from invasive meningococcal disease. The importance of antibody avidity and the ability of vaccines to stimulate avidity maturation have been shown for MenC, and vaccines against *Streptococcus pneumoniae* and *Haemophilus influenzae* type b.[27] Serum bactericidal antibody titre and IgG concentration after vaccination with an outer membrane vesicle vaccine correlated poorly, possibly because only high avidity antibodies were bactericidal,[28] and further investigation for serogroup B vaccines is warranted.

The serum bactericidal antibody assay continues to be widely used, but whether mechanisms of protection for new vaccine candidates will be the same as for polysaccharide and outer membrane vesicle vaccines is unknown. New vaccines might elicit different mechanisms of killing, and other immune responses might be important. However, serum bactericidal

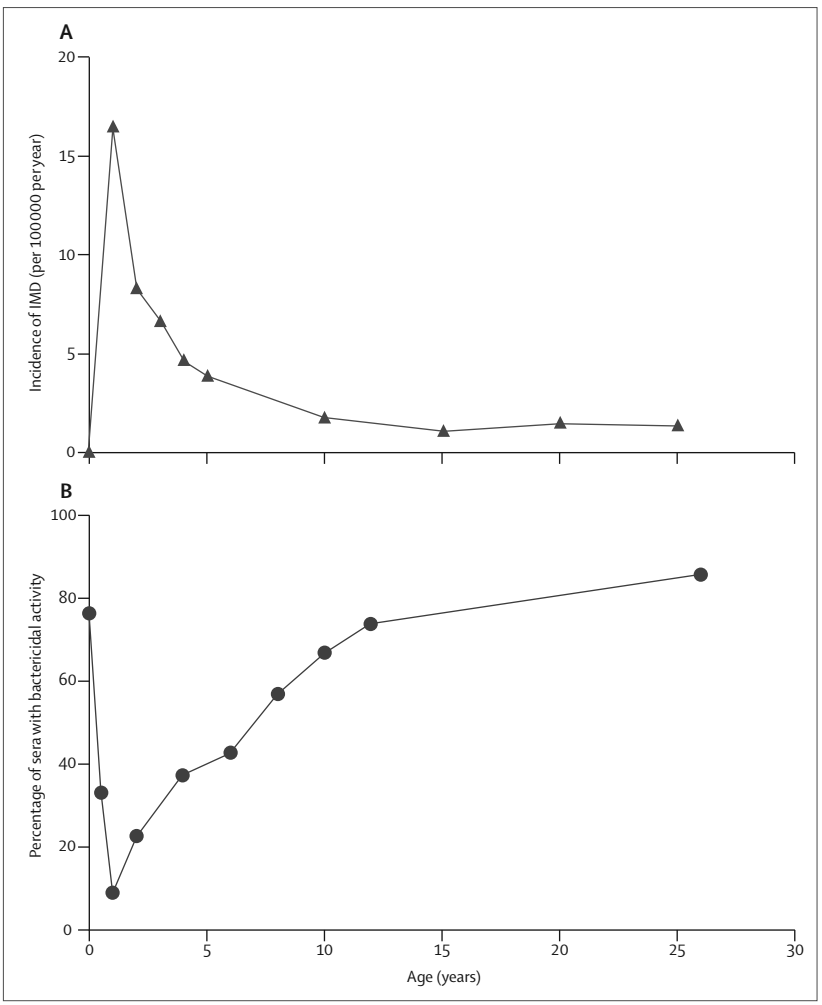


***Figure 2:*** **Relation of incidence of disease and serum bactericidal antibody activity of serogroup B *N meningitidis* with age**
Incidence of disease (A) is greatest and bactericidal activity (B) lowest among children aged 6 months to 2 years. A similar relation was described for serogroups A, B, and C. IMD=invasive meningococcal disease. Adapted from Goldschneider and colleagues.[15]

antibody remains the only surrogate of protection for which there are any supportive data from human beings, although vaccine effectiveness will only be accurately evaluated during postmarketing surveillance.

## Capsular polysaccharide vaccines

The serogroup B capsule is the most obvious candidate for a universal vaccine, but this has not elicited serum bactericidal antibody in humans beings[29] or animals[30] (table 1). Some studies of complexes of the B polysaccharide and outer membrane proteins showed antibody responses to polysaccharide and protein components,[52,53] but this has not been confirmed by other studies.[54] When polysaccharide-specific antibodies are present, they are mostly of the IgM class, not bactericidal, and therefore might not be protective. The most likely explanation for

| | Vaccine | | Number of doses | Number of people | Age group | Proportion with serum bactericidal antibody titre against vaccine strain | | Vaccine efficacy (95% CI) |
|---|---|---|---|---|---|---|---|---|
| | Strain | Components | | | | 1/4 or greater | Four-fold increase or more | |
| **Capsular polysaccharide vaccines** | | | | | | | | |
| USA (1972)[29] | NA | B polysaccharide | 1 | 113 | Adults | 0% | 0 | .. |
| France (2004)[31] | NA | N-propionylated B polysaccharide | 1 | 17 | Adult men | 0% | 0 | .. |
| **Outer membrane vesicle vaccines** | | | | | | | | |
| South Africa (1983)[32] | M986* | Outer membrane protein and B polysaccharide | 1 | 4400 | 4 months to 5 years | 41% (titre 1:8 or greater) | .. | .. |
| Cuba (1991)[18] | Cu385/83† | Outer membrane vesicle, C polysaccharide, and 65–95 kDa envelope proteins | 2 | 106 252 | 10–14 years | .. | 85% | 83% (42–95) |
| Norway (1991)[17,33] | 44/76‡ | Outer membrane vesicle | 2 | 171 800 | 14–16 years | 97% | 80% | 57% (27–87), 87% in first 10 months |
| Norway (1991)[17] | 44/76‡ | Outer membrane vesicle | 2 and 1 | 311 | 13–14 years | 96% | 96% | .. |
| Norway (1991)[34] | 44/76‡ | Outer membrane vesicle | 3 and 1 | 374 | 12–17 years | 93% | 90% | .. |
| Sao Paulo, Brazil (1992)[35] | Cu385/83† | Outer membrane vesicle, C polysaccharide, and 65–95 kDa envelope proteins | 2 | About 2·4 million in all three groups | 4–6 years | 52% | 52% | 74% (16–92) |
| Sao Paulo, Brazil (1992)[35] | Cu385/83† | Outer membrane vesicle, C polysaccharide, and 65–95 kDa envelope proteins | 2 | | 24–47 months | 43% | 45% | 47% (–72 to 84) |
| Sao Paulo, Brazil (1992)[35] | Cu385/83† | Outer membrane vesicle, C polysaccharide, and 65–95 kDa envelope proteins | 2 | | 3–23 months | 13% | 22% | –37% (–100 to 73) |
| Rio de Janeiro Brazil (1995)[36] | Cu385/83† | Outer membrane vesicle, C polysaccharide, and 65–95 kDa envelope proteins | 2 | About 1·6 million in all four groups | 6 months to 9 years | .. | .. | 58% (31–74) |
| Rio de Janeiro, Brazil (1995)[36] | Cu385/83† | Outer membrane vesicle, C polysaccharide, and 65–95 kDa envelope proteins | 2 | | 4–9 years | .. | .. | 70% (38–85) |
| Rio de Janeiro, Brazil (1995)[36] | Cu385/83† | Outer membrane vesicle, C polysaccharide, and 65–95 kDa envelope proteins | 2 | | 24–47 months | .. | .. | 42% (–47 to 77) |
| Rio de Janeiro, Brazil (1995)[36] | Cu385/83† | Outer membrane vesicle, C polysaccharide, and 65–95 kDa envelope proteins | 2 | | 6–23 months | .. | .. | 23% (–119 to 73) |
| Chile (1995)[37] | 8529§ | Outer membrane protein and C polysaccharide | 2 | 40 811 in all three groups | 1–21 years | .. | .. | 51% (–11 to 80) |
| Chile (1995)[37] | 8529§ | Outer membrane protein and C polysaccharide | 2 | | 5–21 years | .. | 35–65% | 69% (14–91) |
| Chile (1995)[37] | 8529§ | Outer membrane protein and C polysaccharide | 2 | | 1–4 years | .. | 12% | –23% (less than –100 to 73) |
| New Zealand (2005)[38] | NZ98/254¶ | Outer membrane vesicle | 3 | 75 | Adults | .. | 96% | .. |
| New Zealand (2005)[38] | NZ98/254¶ | Outer membrane vesicle | 3 | 608 | 8–12 years | .. | 76% | .. |
| New Zealand (2005)[38,39] | NZ98/254¶ | Outer membrane vesicle | 3 or 4 | 325 | 16–24 months | 75% (titre 1:8 or greater) | 75% (100% after fourth dose) | .. |
| New Zealand (2005)[38,39] | NZ98/254¶ | Outer membrane vesicle | 3 | 294 | 6–8 months | 74% (titre 1:8 or greater) | 74% | .. |
| New Zealand (2005)[39] | NZ98/254¶ | Outer membrane vesicle | 3 or 4 | 375 | 6–8 weeks | 54% (titre 1:8 or greater) | 53% (69% after fourth dose) | .. |
| Netherlands (2000)[40] | H44/76‡ | Hexavalent PorA outer membrane vesicle‖ | 3 | 172 | 2–3 years | 28–98% | .. | .. |

(Continues on next page)

| | Vaccine | | Number of doses | Number of people | Age group | Proportion with serum bactericidal antibody titre against vaccine strain | | Vaccine efficacy (95% CI) |
|---|---|---|---|---|---|---|---|---|
| | Strain | Components | | | | 1/4 or greater | Four-fold increase or more | |
| (Continued from previous page) | | | | | | | | |
| Netherlands (2000)[40] | H44/76‡ | Hexavalent PorA outer membrane vesicle‖ | 3 | 165 | 7–8 years | 16–100% | .. | .. |
| UK (1999)[41] | H44/76‡ | Hexavalent PorA outer membrane vesicle‖ | 3 and 1 | 111 | 8–12 weeks | 98–100% | 19–81% (three doses), 78–95 (fourth dose) | .. |
| Norway (2007)[42] | 44/76† + NZ98/254¶ | Bivalent outer membrane vesicle | 3 and 1 | 91 | Adults | 68–87% | .. | .. |
| Spain and Belgium (2007)[43] | Cu385/83† + NZ228/985¶ | Bivalent outer membrane vesicle | 3 | 478 | 12–18 years | .. | 42–76% | .. |
| **Other vaccines** | | | | | | | | |
| UK (2008)[44] | NZ98/254¶ (outer membrane vesicle) | 5CVMB: NadA, fHbp, NHBA, and outer membrane vesicle | 3 | 147 | 2 months | 63–100% | .. | .. |
| UK (2008)[45] | NZ98/254¶ (outer membrane vesicle) | 5CVMB: NadA, fHbp, NHBA, and outer membrane vesicle | 3 | 60 | 6–8 months | 96–100% | .. | .. |
| Australia (2008)[46] | NA | fHbp A and B | 3 | 103 | Adults | .. | 22–100% | .. |
| Canada (2007)[47] | NA | NspA | 3 | 122 | Adults | 0 | 0 | .. |
| UK (2009)[48] | *Neisseria lactamica* | Outer membrane vesicle | 3 | 97 | Adults | 54–96% | 8–31% | .. |
| Norway (1998)[49] | 44/76‡ | Intranasal outer membrane vesicle | 4 and 1 | 23 | Adults | .. | 45–72% | .. |
| USA (1999)[50] | 9162** | Intranasal outer membrane vesicle | 3 | 32 | Adults | .. | 75% | .. |
| USA (2002)[51] | 9162** | Intranasal outer membrane vesicle | 3 or 4 | 44 | Adults | .. | 43% | .. |

*M986=B:2a:P1.2. †Cu385/83=B:4:P1.19,15:L3,7,9. ‡44/76 or H44/76=B:15:P1.7,16:L3,7,9. §8529=B:15:P1.3:L3,7. ¶NZ98/254 and NZ228/98=B:4:P1.7-2,4. ||Contains P1.7,16; P1.5-1,2-2; P1.19,15-1; P1.5-2,10; P1.12-1,13; P1.7-2,4. **9162=-:15:P1.3:L3,7,9.

***Table 1:*** **Clinical trials of serogroup B meningococcal vaccines**

this lack of immunogenicity is structural homology between the B polysaccharide and human tissue, leading to immunological tolerance. The key component of the serogroup B capsule is an α2–8-linked sialic acid homopolymer, which contains epitopes that are cross-reactive with the polysialylated form of the neural cell adhesion molecule.[55] Any anticapsular antibodies elicited could crossreact with host antigens and contribute to autoimmune disease.

A modified polysaccharide in which the N-acetyl groups were replaced with N-propionyl groups, which were then conjugated with tetanus toxoid, elicited bactericidal antibodies in mice[56] but not in humans beings (table 1).[31] Although there was no evidence that this vaccine induced autoantibodies, worries remain about this possibility. Failure to derive a capsule-based vaccine and worries about autoimmunity have shifted focus to subcapsular antigens.

## Outer membrane vesicle vaccines

Bactericidal antibodies directed against subcapsular antigens develop after disease and carriage, suggesting that outer membrane proteins are candidates for vaccine development.[57,58] The first outer membrane protein vaccines in the 1970s were immunogenic in animals but did not elicit serum bactericidal antibody in human beings,[59,60] leading to the development of soluble protein vaccines in the form of outer membrane vesicles. In these preparations, some of the lipopolysaccharide is removed from outer membrane fragments by detergent, after which the detergent is removed and proteins in the vesicles solubilised. The vesicles are similar to outer membrane blebs naturally released by *N meningitidis* during culture and infection and contain lipopolysaccharide, outer membrane proteins, periplasmic proteins, and phospholipid (figure 3).[61] Studies of early outer membrane vesicle vaccines during the late 1970s and early 1980s showed a four-fold or greater rise in serum bactericidal antibody in 70–85% of adults after a single dose, but children younger than 6 years had lower responses.[32,52,62,63] Immunogenicities were enhanced with the addition of capsular polysacharide[63,64] or aluminium hydroxide,[54] and the latter was included as an adjuvant in most subsequent outer membrane vesicle vaccines. A number of serogroup B outer membrane vesicle vaccines have since undergone efficacy trials (table 1).

A Cuban outer membrane vesicle vaccine, which had an efficacy of 83%, was the first serogroup B vaccine to be highly efficacious.[18] After a mass vaccination campaign, the incidence of invasive meningococcal disease in Cuba fell from a peak of 14·4 per 100 000 people per year in 1983 to 0·8 per 100 000 people per year in 1993–94, although it was already in decline before the introduction of the vaccine.[65] Two subsequent case–control studies of this vaccine in Brazil[35,36] were the first to estimate its efficacy in young children. The efficacy was 70–74% in children 4–9 years of age, but the vaccine was ineffective in younger children. Similar results were reported for an outer membrane protein vaccine in Chile.[37] These studies also suggested that responses to outer membrane vesicle

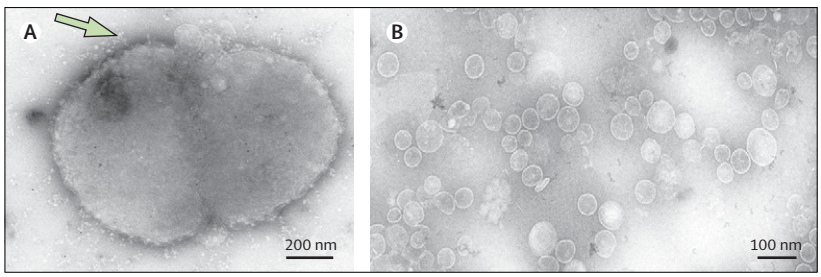


***Figure 3:*** **Electron micrographs of *Neisseria meningitidis* (A) and outer membrane vesicles (B)**
*N meningitidis* shown as a diplococcus (A); arrow denotes naturally occurring blebs of the outer membrane. Provided courtesy of David Ferguson and Gunnstein Norheim, University of Oxford, Oxford, UK.

vaccines were largely restricted to the vaccine strain, although older individuals seemed to have some crossprotective responses.

In Norway, an outer membrane vesicle vaccine based on an epidemic strain had an efficacy of 57% after two doses, which was deemed insufficient to justify a public vaccination campaign.[33] There was substantial reduction of immunity over time, with an efficacy of 87% in the first 10 months after vaccination, compared with only 30% after 21–29 months.[17] Subsequent studies showed boosting of serum bactericidal antibody titres with additional doses, which also resulted in serum bactericidal antibody activity against strains of a different serosubtype, suggesting this strategy could induce broader protection.[17,34] The Norwegian vaccine is being used to aid control of a serogroup B epidemic in Normandy, France, which started in 2003 and has had a high case-fatality rate (19%).[66] A vaccination programme started in 2006 and data on its effect are awaited.

For more on **PorA variants** see http://www.neisseria.org

The second serogroup B meningococcal vaccine to be licensed after the Cuban vaccine was used in New Zealand. From 1991, New Zealand experienced an epidemic of invasive meningococcal disease, reaching a peak in 2001,[67] predominantly caused by one serogroup B strain. After three doses of an outer membrane vesicle vaccine based on this strain, an increase in serum bactericidal antibody titre of four-fold or greater was seen in 53% of young infants (aged 6–8 weeks),[38] 74–76% of older children (aged 6 months to 12 years), and 96% of adults.[39] A four-fold or greater increase and a postvaccination titre of 1/8 or greater (rather than 1/4 or greater) was needed for patients to be designated as seroresponders in these studies, which could result in these data underestimating population immunity. Again there was substantial waning of immunity over time with only 3–34% of children aged between 6 weeks and 24 months sustaining a response 4–16 months after the third dose. The proportion of seroresponders increased to 100% of toddlers (aged 16–24 months) and 69% of young infants after a fourth dose, which was given to a subset of participants.[38]

The vaccine was rolled-out nationally in 2004, contributing to a decrease in number of cases, although the disease burden was already waning. The effectiveness of this vaccine, which depends on a number of variables in addition to vaccine efficacy at an individual level, has been estimated by two methods. A theoretical statistical model that included confounding variables such as age, ethnicity, socioeconomic status, and geographical region derived a vaccine effectiveness of 73% overall over 2 years.[68] This number has been disputed because of lack of inclusion of overcrowding and other factors, and the failure to account for different disease confirmation rates.[69] This model showed no benefit of the vaccine in infants. A cohort analysis estimated that the effectiveness of the vaccine was 80% in children aged between 6 months and 5 years, and 85% in those aged 6 months to 3 years.[70] There was no evidence, however, of effectiveness in any age group 13–24 months after the third dose. This study did not consider the youngest infants, who have the highest rates of disease in New Zealand.

The vaccination programme was stopped in 2008 by the New Zealand Ministry of Health.[71] The rationale for stopping the programme is not clear given the lack of long-term protection induced by this vaccine and the persistence of disease rates above pre-epidemic levels. Ongoing surveillance will establish the longer term effect of the vaccine campaign and its cessation, and provide important information for strategies to control future outbreaks.

The serum bactericidal antibody response induced by outer membrane vesicle vaccines is largely specific to the serosubtype, being predominantly directed against PorA.[72–74] There are over 600 PorA variants, although only a few have been associated with most isolates that cause disease.[75] To increase vaccine coverage, a hexavalent PorA outer membrane vesicle vaccine was developed in the Netherlands, consisting of two outer membrane vesicles each expressing three different PorA proteins (table 1). In phase 2 trials in children aged between 8 weeks and 8 years in the Netherlands and the UK,[40,41] the proportion with significant serum bactericidal antibody responses was 16–100%, dependent on the PorA variant, with the most common serosubtype inducing the lowest response. By 32–42 months of age, serum bactericidal antibody titres of immunised infants had returned to titres found after the first dose, confirming the difficulty of achieving long-term protection.[76] A nonavalent PorA outer membrane vesicle vaccine has been developed, adding a third outer membrane vesicle containing three further PorA proteins. This vaccine elicited serum bactericidal antibody in mice against most of the targeted PorA variants[77] and is in clinical trials. The six PorA variants included in the hexavalent vaccine represent 60–70% of European meningococcal disease isolates overall, and use of the nonavalent vaccine would result in a slight increase in potential vaccine coverage to 70–80%.[78]

An alternative approach to increase breadth of protection is combining outer membrane vesicles, and two such vaccines have been investigated.[42,43] In teenagers

and adults, 42–87% of vaccine recipients achieved serum bactericidal antibody responses against homologous strains (ie, expressing the same PorA), with similar results for both vaccines (table 1). Minor outer membrane proteins and genetically modified lipopolysaccharides have also been manipulated in outer membrane vesicle vaccines. Outer membrane vesicles without PorA and with overexpression of the conserved proteins TbpA, Hsf, NspA, or Omp85 only induced a serum bactericidal antibody response in mice when combined.[79] This suggests that a key density of bactericidal antibodies is needed on the bacterial surface to mediate bacteriolysis, which can be done either by a single major protein, such as PorA, or multiple minor outer membrane proteins. More recently, a vaccine combining outer membrane vesicles from three different genetically modified strains, each expressing two PorA variants and different detoxified lipopolysaccharide immunotypes, with overexpression of the outer membrane proteins fHbp, NadA, or Opc, elicited serum bactericidal antibody in mice and rabbits.[80] All three sets of antigens were involved in the bactericidal response, and a phase 1 study is in progress.

Outer membrane vesicle vaccines are useful in the control of serogroup B outbreaks, but a number of hurdles remain. The conventional method of detergent extraction for removal of potentially toxic lipopolysaccharide from these vaccines might also remove other desirable surface antigens.[81] This could be avoided using native outer membrane vesicles (extracted without detergent) derived from strains containing genetically modified lipopolysaccharide. The problem of serosubtype-restricted responses has yet to be overcome to produce a vaccine with wide coverage. Lack of persistence of immune responses suggests that multiple doses throughout childhood would be needed to maintain protection;[17,36,37,74,82] this is already necessary with vaccines against tetanus and diphtheria, which have been acceptable for decades worldwide. Most outer membrane vesicle vaccines have shown limited efficacy in younger children, although estimated effectiveness of the New Zealand vaccine was higher than in previous studies.[35–37,68,70]

## Other vaccines in clinical trials

Several vaccines based on other subcapsular antigens are in development (table 1). One promising candidate, 5CVMB, is based on three proteins—NadA, factor H binding protein (fHbp; also known as GNA1870 or LP2086), and neisserial heparin-binding antigen (NHBA, also known as GNA2132)—combined with the New Zealand outer membrane vesicle.[83] The inclusion of multiple antigens reduces the risk of escape variants, which might readily happen with single antigen vaccines given the high rate of phase variation, recombination, and mutation in *N meningitidis*.[84] This vaccine was developed by use of so-called reverse vaccinology—570 new putative surface-exposed or secreted proteins were identified from the *N meningitidis* genome sequence,[85] and 350 of the encoded proteins were used to immunise mice.[86] 29 proteins induced bactericidal antibodies and the most immunogenic were included in 5CVMB. fHbp combined with GNA2091 and NHBA with GNA1030 are present as fusion proteins. Immune responses elicited by the fusion proteins were more potent than those induced by the individual antigens, and they also allow large-scale industrial manufacturing.[83] Although GNA2091 and GNA1030 did induce protective immunity in mice, they elicited lower bactericidal activity than the other proteins and are not thought to be major vaccine antigens.

Sera from mice immunised with 5CVMB (without the New Zealand outer membrane vesicle) killed 66 (78%) of 85 representative worldwide isolates,[83] suggesting that this vaccine has the potential to provide broad coverage against serogroup B disease. In phase 2 studies of a vaccine including the outer membrane vesicle component, three doses resulted in serum bactericidal antibody titres of 1/4 or greater in 63–100% of young infants and 96–100% of children 6–8 months old, against homologous strains.[44,45] All target strains expressed one of the vaccine antigens, so no comment can be made about the breadth of protection based on these data. A phase 3 study in infants is in progress across Europe.

fHbp has also been studied as an independent vaccine candidate. Different classification systems have divided fHbp into either three variants (1, 2, and 3)[87] or two subfamilies (A and B).[88] A vaccine containing proteins of subfamilies A and B, which therefore has the potential for broad coverage, elicited serum bactericidal antibody responses in 22–100% of adults in a phase 1 trial in Australia, depending on dose and target strain.[46] Data from completed phase 1 trials in toddlers and teenagers are awaited.

The genes encoding fHbp and NHBA seem to be present in all meningococci, whereas only 38–46% contain *nadA*.[89–92] More recently, NadA, fHbp, and NHBA have been subdivided into 14, 3, and 5 variants, respectively, and how broad the protection is against strains containing more genetically distant variants is unclear. Variant 1 (subfamily B) of fHbp is present in 5CVMB and is the most common, but data from animal studies suggest that immune responses are restricted to strains expressing the vaccine variant.[87]

fHbp is an outer membrane lipoprotein responsible for recruiting fH to the bacterial surface, resulting in dysregulation of the complement pathway and increased bacterial survival.[93] fHbp also provides protection for bacteria against killing by the antimicrobial peptide LL37.[94] Anti-fHbp antibodies might therefore mediate beneficial effects by multiple mechanisms. Inhibition of binding between fHbp and fH would increase bacterial susceptibility to killing via the alternative complement pathway, and directly elicit bactericidal activity via the classical pathway. One potential problem is that surface

expression of fHbp varies between meningococci, and isolates expressing low fHbp concentrations might not be targeted by anti-fHbp antibodies. Antisera from immunised mice killed bacteria expressing low concentrations of fHbp in studies with rabbit complement.[87] Studies with human complement, however, found that monoclonal antibodies recognising different epitopes that were not bactericidal individually were bacteriolytic when combined.[95,96] This synergy was thought to happen because the overall antibody density on the bacterial surface was sufficient to allow complement deposition and activation of the classic pathway. Whether this happens with antibodies generated in vivo is unknown.

fHbp consists of two domains of antiparallel β-strands connected by a five aminoacid linker.[97–99] Variable residues are in the surface-exposed part of the molecule, fully accessible to the immune system, and correspond to known antibody epitopes.[100,101] These regions also seem to bind to fH, suggesting that vaccine-induced antibodies could block this interaction, even if they are not bactericidal. The bactericidal epitopes of variants 1 and 2 are only partly overlapping, suggesting that a recombinant protein containing epitopes from both could increase breadth of coverage. Moreover, recombinant chimeric fHbp molecules and outer membrane vesicles with overexpression of different fHbp variants did elicit bactericidal antibodies in mice against strains of meningococci expressing different variants of fHbp.[81,102] A potential difficulty is that the high affinity binding between fHbp and fH might mask relevant epitopes during immunisation. Future vaccines might reduce fH binding sufficiently to allow exposure of all immunogenic epitopes to the immune system.[103]

NadA aids meningococcal adhesion and invasion into epithelial cells,[104] activates human monocytes or macrophages in vitro, and might have a role in stimulating the inflammatory cascade that happens during invasive meningococcal disease.[105] Vaccines that elicit anti-NadA antibodies might have the added benefits of improving outcome from disease or inducing herd immunity via blocking of nasopharyngeal attachment.

NHBA is a surface-exposed lipoprotein that is a target of both meningococcal and human proteases and binds to heparin at an arginine-rich region, suggesting a possible role in serum resistance.[106,107] The genes encoding GNA2091 and GNA1030 were found in all 95 meningococcal isolates in one collection,[90] but they are thought to be accessory proteins in the 5CVMB vaccine and there have been no studies describing their structure or function.

A potential problem with these outer membrane proteins as vaccine components is variation in expression between strains, which some studies have considered for fHbp. Furthermore, immunodominant surface structures such as PorA, which are exposed to the immune system in vivo, are under immune selection pressure and therefore show sequence variability. The conservation of NadA, fHbp, and NHBA might suggest that they are not naturally immunologically exposed (during colonising infection), and as such might not be as immunogenic as more variable outer membrane proteins.

Several other vaccines have been tested in clinical trials (table 1). The protein NspA is highly conserved across most strains of *N meningitidis*, but it did not induce bactericidal antibodies in human beings after promising mouse studies and is not being pursued.[47,108] Transferrin-binding proteins elicited serum bactericidal antibody in mice and rabbits,[109,110] and there have been trials in people,[111] but data have not been published. Intranasal outer membrane vesicle vaccines have been tested in a way that mimics the natural immunising effect of colonisation. Nasal IgA concentrations did increase in most people vaccinated in phase 1 trials, but only 43–75% had significant serum bactericidal antibody responses.[49–51] More data are needed, in particular to establish whether mucosal IgA has a role in protection against colonisation with virulent meningococci. *Neisseria lactamica* is a commensal carried in the nasopharynx of young children and has been implicated in the

| | Serum bactericidal antibody in mice | Serum bactericidal antibody in rabbits | Passive protection in rats or mice |
|---|---|---|---|
| **Outer membrane vesicle vaccines** | | | |
| Nonavalent PorA outer membrane vesicle[77] | Yes | .. | .. |
| Outer membrane vesicle with overexpression of fHbp[81] | Yes | .. | .. |
| Outer membrane vesicle with overexpression of TbpA, Hsf, NspA, Omp85[79] | Yes | .. | .. |
| Hexavalent PorA, trivalent lipopolysaccharide outer membrane vesicle with overexpression of fHbp, NadA, Opc[80] | Yes | Yes | .. |
| Outer membrane vesicle with overexpression of LbpA and LbpB[113] | Yes | .. | .. |
| Outer membrane vesicle with genetically detoxified lipopolysaccharide[114] | Yes | .. | .. |
| *N lactamica* outer membrane vesicle [112] | No | No | Yes |
| **Outer membrane protein preparations** | | | |
| Chimeric fHbp[102] | Yes | .. | .. |
| Transferrin-binding proteins[109,110] | Yes | Yes | Yes |
| FetA (FrpB)[115] | .. | Yes | |
| Opa[116] | Yes | .. | .. |
| NMB0606[117] | Yes | .. | .. |
| NMB0928[117] | Yes | .. | .. |
| NMB0873[117] | Yes | .. | .. |
| NMB1163[117] | Yes | .. | .. |
| NMB0938[117] | Yes | .. | .. |
| **Other vaccine candidates** | | | |
| Capsule mimetics[118,119] | Yes | .. | Yes |
| Lipopolysaccharide[120] | .. | Yes | .. |
| Lipopolysaccharide inner core[121] | Yes | .. | Yes |
| Live attenuated *N meningitidis*[122,123] | Yes | .. | .. |
| DNA vaccines[124] | Yes | .. | .. |

*Table 2:* **Preclinical studies of serogroup B meningococcal vaccines**

development of natural immunity to the meningococcus. A *N lactamica* outer membrane vesicle vaccine was developed on the basis that it would avoid PorA-dependent strain restriction because it does not express PorA. No bactericidal responses were elicited after immunisation in mice or rabbits,[112] and only 8–31% of adults (dependent on the target strain) showed a four-fold or greater rise in serum bactericidal antibody after three doses during a phase 1 trial.[48] Antisera from immunised rabbits, however, did mediate opsonophagocytosis, suggesting this as a mechanism for the protection against meningococcal bacteraemia seen in mice. With the limited data available at present for alternative assays to the serum bactericidal antibody, whether vaccines that do not induce serum bactericidal antibody could be licensed is unclear.

## Vaccines in preclinical studies

Several approaches have provided promising data in animal models, in addition to those involving fHbp (table 2).[77,79–81,102,109,110,112–124] Iron-regulated proteins (eg, FetA, LbpA, and LbpB) are upregulated in conditions where the concentration of iron is low, so the amount present in outer membrane vesicle vaccines is dependent on growth conditions during manufacture. FetA induced serum bactericidal antibody in rabbits,[115] and an outer membrane vesicle vaccine with high expression of FetA is in development, aiming to overcome strain restriction by eliciting antibodies against both PorA and FetA. Other proposed candidates include outer membrane vesicles with overexpression of LbpA, purified LbpB, and recombinant Opa, which have all induced bactericidal antibodies in mice.[113,116] Although these vaccines are immunogenic, antibodies are usually directed against variable regions of the outer membrane proteins and have limited cross-reactivity. The additional problem of variable expression on target bacteria must also be overcome if these vaccines are to be developed further. In addition to these antigens, analysis of meningococcal genome sequences, similar to the methods used during the development of 5CVMB, has revealed five new putative surface proteins that have elicited bactericidal antibodies after immunisation of mice and which need further assessment.[117]

Lipopolysaccharide has been implicated in the immune response to natural infection,[125] but must be detoxified before inclusion in a vaccine. Meningococcal strains that have a disrupted *lpxL1* gene produce the less toxic penta-acylated instead of the usual hexa-acylated lipopolysaccharide. These strains have been used in the preparation of native or partly detergent-extracted outer membrane vesicles, including the outer membrane vesicles with overexpressed fHbp and a vaccine based on outer membrane vesicles enriched with different immunotypes of genetically detoxified lipopolysaccharide. Both of these vaccines elicited bactericidal antibodies in mice, and avoid loss of antigens caused by the detergent used in conventional outer membrane vesicle production.[114] Different approaches with lipopolysaccharide have shown that oligosaccharides conjugated to tetanus toxoid induced immunotype-specific serum bactericidal antibody in rabbits[120] and the conserved lipopolysaccharide inner core elicited serum bactericidal antibody in mice.[121] An alternative function of lipopolysaccharide could be as an adjuvant. Outer membrane vesicle vaccines without lipopolysaccharide resulted in lower immune responses, whereas an *lpxL1* mutant lipopolysaccharide retained adjuvant activity similar to wild-type with reduced toxicity in mice;[126] although more recent in-vitro studies using human dendritic cells suggested this adjuvant property of the mutant lipopolysaccharide might not happen in human beings.[127]

## From clinical trials to immunisation schedules

Multiple vaccines are at present competing for introduction into routine immunisation schedules. A successful serogroup B vaccine would, at the very least, have to show immunogenicity against some pathogenic strains using the serum bactericidal antibody assay. Induction of antibodies against different variants of included antigens would provide further supporting evidence that such a vaccine might be broadly protective. On the one hand, vaccines containing multiple antigens, such as 5CVMB and some of the modified outer

***Panel:* Timeline showing the development of serogroup B meningococcal vaccines**

**1972**
B polysaccharide not immunogenic in human beings

**1978**
Outer membrane protein vaccines do not elicit serum bactericidal titres in human beings

**1979**
First trials of outer membrane vesicle vaccines in human beings

**1991**
Outer membrane vesicle vaccine introduced into routine immunisation schedule in Cuba

**1991–96**
Efficacy trials of outer membrane vesicle vaccines in Cuba, Norway, Brazil, and Chile

**1999**
Hexavalent PorA outer membrane vesicle vaccine immunogenic in clinical trials

**2004**
Mass vaccination campaign in New Zealand with outer membrane vesicle vaccine

**2008**
5CVMB and fHbp vaccines immunogenic in clinical trials

**Search strategy and selection criteria**

Data for this Review were identified by searches of Medline and PubMed, references from relevant articles, and the reports of health agencies of relevant countries. Search terms were "menb", "vaccination", "immunisation", "neisseria", "meningitidis", "meningococcal", "serogroup b", "GNA 1870", "GNA1870", "LP2086", "factor H binding protein", "fHbp", "GNA2132", "NadA", "genome-derived neisseral antigen", "5CVMB", and "reverse vaccinology". English, Spanish, Portuguese, and French language papers were reviewed. The date limits of the searches were February, 2008, to November, 2009.

membrane vesicle vaccines, have a lower likelihood of escape variants. On the other hand, those that include multiple variants of the same antigen, such as the fHbp subfamily A and B, and nonavalent PorA outer membrane vesicle vaccines, reduce the problem of antigen diversity. A vaccine with 50% strain coverage that induced a response in 80% of recipients and had 90% uptake would have an effectiveness of 36%, which is similar to the contribution of MenC to the prevention of overall invasive meningococcal disease in the UK. Data on diversity and expression of all new vaccine antigens among circulating strains is therefore urgently needed to provide confidence that clinical trial data derived with a small number of target strains can be translated into broad protection. It would be useful to add antigens from any vaccine used in the future to routine typing, including assays of surface expression levels, to aid postlicense monitoring.

Clinical trials are focusing on young children as the target population because they have the highest incidence of disease. Persistence of antibody after immunisation in infancy, however, is poor so one approach would be to give multiple doses throughout childhood to provide long-term protection. An alternative approach exists if there is a herd immunity effect, similar to that seen for MenC.[128] Studies examining the effect of herd immunity should be included in the development plan for serogroup B vaccines given the potential for herd immunity to complement and enhance direct protection. If herd immunity was induced, fewer doses could be given in early childhood, and population protection might be achieved by reducing transmission via immunisation of teenagers and young adults, who have the highest rates of meningococcal carriage.

These vaccines need to be economically viable. Potential cost of the hexavalent PorA outer membrane vesicle vaccine in the Netherlands was estimated at €21415 per life-year saved and €15721 per quality adjusted life-year gained, compared with £6529 per life-year saved for MenC in the UK.[129] The threshold for recommendation of new treatments by the National Institute for Health and Clinical Excellence in the UK is usually £20000–30000 per quality adjusted life year gained.[130] Strain coverage, vaccine efficacy, cost per dose, and number of doses needed will be the key factors in determining cost-effectiveness.

## Conclusion

Lack of immunogenicity of the serogroup B polysaccharide was shown in 1972 and since then, despite studies involving almost 6 million people, the search for a vaccine that is immunogenic in all age groups, and provides broad protection against serogroup B disease, continues (panel). During the past 40 years there has been some success, with outer membrane vesicle vaccines contributing to disease control in Cuba and New Zealand. However, these vaccines elicit only limited protection against PorA heterologous strains and are poorly immunogenic in young children; moreover, multiple doses are needed to provide long-term immunity. They are therefore useful for epidemics caused predominantly by a single strain, but not for endemic disease. Vaccine candidates in late phase development are based on subcapsular proteins and, if successfully implemented, could have an effect on other serogroups. Recent clinical trials have produced encouraging initial results, leading to media interest, and hope that substantial control of meningococcal disease is within reach. The major outstanding issue is that the persistence and breadth of protection that these vaccines induce remains unknown. Countries with a high incidence of invasive meningococcal disease, such as the UK, might be in a position to prevent more of the burden of meningococcal disease beyond serogroup C in the near future, but policy makers will have the challenge of deciding how much protection is enough to justify the use of such a vaccine.

**Contributors**

MS reviewed published work and drafted the paper. AJP assisted with selection and interpretation of included studies and with preparation of the paper. Both authors read and approved the final version.

**Conflicts of interest**

AJP acts as chief investigator for clinical trials done on behalf of Oxford University, sponsored by vaccine manufacturers (Novartis Vaccines, GlaxoSmithKline, Sanofi-Aventis, Sanofi-Pasteur MSD, and Wyeth Vaccines). Industry sourced honoraria for lecturing or consultancy, travel expenses, and grants for education and attendance at scientific meetings are paid directly to an educational/administrative fund held by the Department of Paediatrics, University of Oxford. AJP does not receive any personal payments from vaccine manufacturer. MS has no conflicts of interest to declare.

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