The future of meningitis vaccines

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Without effective vaccines meningitis remains a substantial worldwide threat with major health-care implications. A number of advances have been made in vaccine design and implementation over the last decade, with new vaccine initiatives providing substantial promise for the future reduction of global disease burden.

Meningitis remains an important cause of worldwide morbidity and mortality despite the implementation of potent antibiotics and intensive care support. The fatality rates in industrialized countries are between 5 and 10%, being considerably higher in the developing world (Laurichesse et al, 1998; Greenwood, 1999; Peltola, 2000). A substantial proportion (10–20%) of survivors has resulting neurological sequelae, which include hydrocephalus, sensorineural deafness and seizures (Smith et al, 1988; Quagliarello and Scheld, 1993; von Reyn and Vuola, 2002).

Neisseria meningitidis and *Streptococcus pneumoniae* cause most cases of bacterial meningitis after the neonatal period. *Haemophilus influenzae* type B (Hib), once an important cause of meningitis in children under 5 years of age, has become uncommon in industrialized countries since the introduction of the Hib vaccine in 1992 (Adams et al, 1993). Group B streptococcus (GBS) is the leading cause of neonatal meningitis in the UK, other cases being caused by Gram-negative enterics and Listeria which account for a small numbers of cases overall (*Figure 1*).

Many viruses are known to cause meningitis. In England and Wales the enteroviruses (echo, enterovirus and coxsackie types A and B) have become the most common cause since the measles, mumps and rubella (MMR) vaccine was introduced routinely, resulting in the decline of mumps meningitis. Bacillus Calmette–Guérin (BCG), the only vaccine currently licensed for prevention of tuberculosis, is up to 77% effective against tuberculous meningitis. There are numerous approaches under development for new tuberculosis vaccines which include whole cell live, whole cell inactivated, subunit, DNA and prime boost vaccines. A number of vaccine candidates have been selected by extensive screening of *Mycobacterium tuberculosis* antigens in animal models and a number of human studies are currently being planned (von Reyn and Vuola, 2002).

Vaccines derived from the polysaccharide capsule of Hib, *S. pneumoniae* and *N. meningitidis* were first introduced decades ago, but they are not immunogenic in infants and have therefore not been implemented in universal immunization programmes. Since the development of protein–polysaccharide conjugate vaccines against Hib during the 1980s, which overcome the poor responses in infancy to the plain polysaccharides, the stage has been set for wider use of antimeningitis vaccines based on this technology (Hargreaves et al, 1996; Peltola, 2000).

H. INFLUENZAE TYPE B

In the late 1980s protein polysaccharide conjugate Hib vaccine became commercially available, and various combinations with other vaccines are still being released. Since 1986 at least 38 countries have included Hib vaccination in their routine childhood immunization sched-

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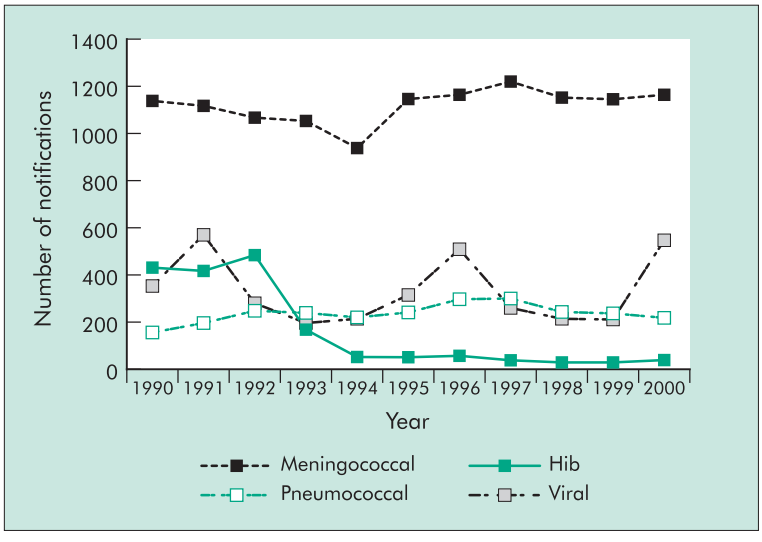

Figure 1. Statutory notifications of meningitis causes in England and Wales 1990–2000. Adapted from Public Health Laboratory Service (2000). Hib = Haemophilus influenzae type B.

ule (Wenger et al, 1999; Peltola, 2000). These countries predominantly include Europe and North America, with the vaccine being more recently introduced in Latin America. Asia and Africa have had slower uptake – The Gambia being the only country in Africa currently including the vaccine in the national immunization schedule, it having been recently withdrawn in South Africa because of cost implications. Epidemiological data from a number of countries have shown significant decreases in the reported incidences of meningitis (*Figure 1*, *Table 1*) (Peltola, 2000). In the future, wider use of affordable Hib vaccine is likely through transfer of technology to manufacturers in developing countries.

N. MENINGITIDIS

N. meningitidis, a Gram-negative encapsulated diplococcus with inner and outer cell membranes, is classified into 12 serogroups based on the polysaccharide capsule, with five serogroups (A, B, C, W135 and Y) accounting for virtually all disease (Rosenstein et al, 2001b).

In industrialized countries the incidence of invasive disease is approximately 1–5/100 000 with much higher incidences occurring in the developing world and during epidemics in affected populations (Greenwood, 1999; Cartwright et al, 2001). Serogroup B and C meningococci were responsible for the majority of endemic meningococcal disease in European countries before the introduction of serogroup C protein–polysaccaride vaccines (Morley and Pollard, 2001; Maiden and Stuart, 2002). In the United States the rate of disease caused by serogroup Y has increased during the past decade and now accounts for one third of all cases of disease. Serogroup A causes the majority of epidemic meningococcal infection in the African meningitis belt, among Hajj pilgrims and in China. Serogroup W135 has been associated with an outbreak among Hajj pilgrims as well as a large epidemic in Burkina Faso during 2002 (Riou et al, 1996; Taha et al, 2000).

N. meningitidis is carried in the nasopharynx of adolescents and adults with very low rates of carriage occurring in children under 10 years of age. Carriage is usually self-limiting and of variable duration. The peak incidence of disease is between the ages of 6 months and 2 years, with cases ranging from occult bacteraemia to fulminant septicaemia leading to death within several hours of onset. Students in dormitories, immunosuppressed individuals and those with complement deficiency or hyposplenism are at an increased risk of developing meningococcal disease (Cartwright et al, 2001; Memish and Alrajhi, 2002).

The group C glycoconjugate vaccines have been adopted in the infant immunization schedule at 2, 3 and 4 months in the UK and other European countries. Currently no booster dose is given. A catch-up target campaign was aimed at all children between the ages of 4 months and 18 years of age. This has resulted in a dramatic decline in the incidence of group C meningococcal infection with 97% effectiveness for those aged 15–19 years and 92% effective for those aged 2–3 years. In addition to the reduction in serogroup C disease, the vaccine has reduced the carriage of serogroup C meningococci in teenagers from 0.45% to 0.15% (Maiden and Spratt, 1999; Maiden and Stuart, 2002). However, overall rates of meningococcal disease in the UK have not fallen markedly, as a result of continuing increases in rates of disease caused by serogroup B meningococci (Cartwright et al, 2001).

A number of trials have shown both safety and immunogenicity of bivalent A and C conjugate vaccines in adults and children. A quadrivalent ACYW conjugate vaccine is under development by a number of manufacturers and will have a major impact on non-B meningococcal disease. It is a better vaccine for North America, where there are high rates of group Y disease and for travellers to regions of the world where there is an increased risk of A and W disease. This is the ideal vaccine for the meningitis belt of Africa where outbreaks or epidemics of disease caused by A, C and W135 have been described (Dull and Rosenstein, 2001; Morley and Pollard, 2001; Taha et al, 2002).

A majority of endemic disease in industrialized countries is caused by serogroup B meningococci and vaccines against this organism are much more difficult to develop. The B polysaccharide is not immunogenic because of

TABLE 1.
Annual number of cases of meningitis prevented by the Hib conjugate vaccine in children aged 0–4 years

Country	No. of meningitis cases before Hib immunization	No. of meningitis cases after Hib immunization
UK (1992 *vs* 1994)	920	25
USA (1987 *vs* 1995)	12 000	200
Chile (1995 *vs* 1998)	580	20
Brazil (1988–96 *vs* 1997)	29	10
Costa Rica (1992 *vs* 1994)	63	23
Uruguay (1992 *vs* 1995)	43	2
Australia (1992 *vs* 1994)	340	40

From Peltola (2000). Hib = *Haemophilus influenzae* type B

its chemical identity to human neural surface antigens. Concerns about the induction of autoantibodies that cross-react with glycosylated host antigens, most notably fetal brain tissue, has arisen (Finne et al, 1983). A number of different approaches to vaccine development have been undertaken, but no vaccine has yet proved to be highly effective.

Polysaccharide-based group B meningococcal vaccines

Despite concerns about auto reactivity, further development of polysaccharide-based serogroup B vaccines have been undertaken. In these vaccines, the native N-acetyl groups on the B polysaccharide have been substituted by N-propionyl and conjugated to a carrier protein (recombinant PorB outer membrane protein) in an attempt to overcome immunological tolerance. This vaccine has been shown to be immunogenic in animals and the conjugate vaccine has reached phase I clinical trials (Fusco et al, 1997; Tondella et al, 2000).

Outer membrane protein vesicle vaccines

Outer membrane vesicles contain a number of proteins which could potentially serve as vaccine candidates, with PorA being the most highly expressed and immunodominant antigen. Several efficacy trials using outer membrance protein vesicle (OMV) vaccines were undertaken in the 1980s in Cuba, Norway, Brazil and Chile, which showed efficacies of up to 80% (Boslego et al, 1995; Tappero et al, 1999; de Kleijn et al, 2000). However, these vaccines elicited limited protection in infants and there was only limited cross-protection to non-vaccine meningococcal group B strains. The OMV vaccines may have an advantage over recombinant protein vaccines as they present the conformational antigenic structures to the immune system and have demonstrated efficacy, at least in older children and adults. Further development of OMV vaccines, originally developed in Cuba, The Netherlands and Norway, is being undertaken and use of an OMV vaccine is planned in New Zealand to control disease caused by a single hyperinvasive clone of serogroup B meningococci (de Kleijn et al, 2000; Rosenstein et al, 2001a; Jodar et al, 2002).

A number of other potential outer membrane protein vaccine candidates have been identified which includes NspA, a conserved protein in serotypes A, B and C. Anti-NspA monoclonal antibodies have been shown to be bactericidal against meningococcal strains, however, these proteins are variably expressed in pathogenic group B meningococci resulting in inconsistent protection (Cadieux et al, 1999).

Iron binding proteins

Iron is an essential requirement for the survival of meningococci, and as a result, a number of surface proteins are involved in iron acquisition. These include the potential candidates ferric binding protein (FbpA), lactoferrin binding protein (LbpA, LbpB) and transferrin binding protein B (TbpB) (Danve et al, 1993; West et al, 2001). Animals immunized with recombinant TbpB elicited high antibody responses against most meningococcal strains, however, a phase I trial in adults showed poor bactericidal antibody production with the formulation used (Danve et al, 1993).

Lipopolysaccharide

Meningococcal lipopolysaccharide (LPS) is a major contributory factor in the induction of an inflammatory response through cytokine stimulation. A Norwegian vaccine combining LPS with outer membrane protein classes 1, 3, 4, 5 has been shown to be immunogenic in humans, but concerns have arisen about autoantibody production against erythrocytes which have a similar moiety to LPS. The advantage in using LPS as a vaccine candidate is that there are a number of LPS epitopes which are shared by all meningococci. A murine antibody directed at the core of LPS has shown high bactericidal activity as well as enhanced opsonophagocytosis (Plested et al, 1999). This vaccine has induced bactericidal antibody in animals and could be combined in a multicomponent vaccine.

Live vaccines

Development of immunity is thought to occur through cross protection from commensal meningococcal species such as *N. live* or subunit vaccines developed from *N. lactamica* species may prove to be effective (Oliver et al, 2002). Live-attenuated group B vaccines have also been suggested but may not be a realistic option for safety reasons.

A number of other potential vaccine candidates are being investigated at present and include, among others, adhesion penetration protein, FetA, FrpB, opacity associated protein, pilin, PorB (class 2/3 protein), reduction modifiable protein, OMP85, Hsf-like (NhhA) protein, PilQ, H8 and HpuAB, reviewed in Morley and Pollard (2001) and Rosenstein et al (2001a).

Genome sequencing

The genome sequence of *N. meningitidis* became available in 2000 and has allowed the identification of highly immunogenic epitopes by genome

sequencing (Jennings et al, 1999; Parkhill et al, 2000; Tettelin et al, 2000). This is promising because protein candidates, which are highly conserved among all meningococcal serogroups, may be found. A number of conserved open reading frames that code for surface exposed proteins on group B meningococci, a group A strain and a strain for *N. gonorrhoeae* have been identified, cloned and expressed in *Escherichia coli*. Twenty eight of these novel proteins were shown to elicit group B antibodies, which either had bactericidal activity or bound to the bacterial surface (Pizza et al, 2000). It is likely that in order to achieve and maintain adequate cross protection among serogroups a new vaccine will need to contain a number of epitopes, which should be both immunogenic and conserved.

Gene expression

DNA microarray technology has recently become useful in the search for vaccine targets. Labelling RNA with fluorescent dyes and hybridizing it to DNA fragments on the surface of the chip is used to monitor gene expression. Fluorescent signals are emitted upon laser beam excitation which are then quantified and which define the transcriptional activity of the arrayed genes in vivo. Microarray technology has been used to analyse gene regulation in meningococci with a number of previously unidentified genes being discovered (Grandi, 2001). Sequence tagged mutagenesis has enabled the labelling of specific meningococcal gene mutations which are crucial for survival of the organism and which may help to define particular pathogenic strategies (Sun et al, 2000).

The future should bring great reductions in disease caused by serogroups A, C, Y and W135 with the imminent availability of multivalent conjugate vaccines. However, a solution to the problem of group B disease has not been attained despite the recent explosion in possible vaccine candidates.

S. PNEUMONIAE

S. pneumoniae is a major cause of community-acquired bacterial pneumonia, otitis media and meningitis (*Figure 1*). Mortality rates from meningitis are about 25% of affected cases and are often characterized by neurological sequelae in survivors. Disease rates are particularly high at the extremes of age, in patients with underlying chronic disease and in immunocompromised individuals, particularly those with human immunodeficiency virus (HIV) infection where the incidence of disease is 50–100-fold higher (Djuretic et al, 1998; Dawson et al, 1999).

S. pneumoniae has 90 known serotypes with a limited number of serotypes accounting for a majority of invasive disease isolates in specific geographical locations. The peak rate of both colonization and invasive disease occurs during the first 2 years of life, dropping during later childhood and rising during old age. Opsonophagocytosis with specific antibodies plays an important role in the age-specific disease incidence and as expected there are increased rates of disease in individuals with antibody deficiency and hyposplenism. Antibiotic resistance has become a major hurdle in the treatment of pneumococcal infection in some regions, with widespread resistance to tetracyclines, cotrimoxazole, chloramphenicol, erythromycin and the cephalosporins being reported (Brett and Martin, 1999; Whitney et al, 2000; Sleeman et al, 2001; McIntosh and Booy, 2002).

The currently available 23-valent pneumococcal polysaccharide vaccine is poorly immunogenic in young children and its effectiveness in any age group is in doubt. In February 2000 a seven-valent pneumococcal protein–polysaccharide conjugate vaccine was licensed for use in the USA. This vaccine was shown to be effective in two trials. The first randomized controlled trial in more than 37 000 children showed that the seven-valent vaccine prevented 94% of invasive pneumococcal cases (Shinefield et al, 1999; Black et al, 2000, 2001). Both this trial and a smaller study in Finland found a reduction in cases of otitis media of about 6%.

The seven-valent pneumococcal vaccine does not cover all disease causing serotypes, prompting the development of nine- and eleven-valent vaccines, which may offer better protection in Europe and Africa. However, this protein conjugate vaccine is expensive. Two economic studies which generated Markov simulation models showed little cost-effectiveness in universal implementation (Lieu et al, 2000). The expense of the vaccine is a fundamental disadvantage in developing countries, which carry the main burden of disease. There is already some evidence of replacement of vaccine serotypes by non-vaccine serotypes in mucosal carriage (Lipsitch et al, 2000; Mulholland, 2000; Obaro, 2000; Lipsitch, 2001). Therefore, it is possible, although not certain, that other serotypes will replace invasive isolates and reduce the efficacy of these vaccines. There is also the risk that widespread conjugate vaccine use may result in increase of disease attributable to non-vaccine serotypes through genetic transformation. For

this reason development of cross-protective protein-based pneumococcal vaccines is being actively pursued.

Protein vaccines

The availability of the complete genome sequence of both virulent and non-virulent isolates of *S. pneumoniae* has provided new classes of genes as potential targets for vaccine design as well as providing insight into the mechanisms of host–bacterial interaction. Proteins within the pneumococcal cell membrane are known to be essential in pneumococcal pathogenicity. At least 69 proteins have been identified on the cell wall surface, many of which have as yet unknown function.

The most promising protein candidates thus far are the well-characterized pneumolysin, Ply, LytA and PspA proteins (Jedrzejas, 2001; Jedrzejas et al, 2001). Ply and PspA have been shown to be protective immunogens. Limited trials thus far with pneumolysin and PspA have been shown to provide partial protection against challenge with virulent pneumococci in experimental animal models (Wu et al, 1997; Berry and Paton, 2000). Specific inactivation of the genes within these proteins using insertion duplication mutagenesis significantly reduces virulence in mouse models. PspA is serologically variable among pneumococcal strains but is sufficiently conserved, in that immunization with a single PspA protects against strains with highly diverse serotypes. The antigenic relatedness of these pneumococcal proteins to those in other commensal streptococci needs careful evaluation in order to avoid disruption of the balance of harmless commensals in the nasopharynx.

Sequence scanning

The search for signature sequence motifs commonly found on surface exposed structures has identified a number of potential vaccine candidates including the choline binding repeats. CbpA is the largest and most abundant choline binding protein: it functions as a surface adhesin and plays an important role in nasopharyngeal colonization (Rosenow et al, 1997). Six novel mutants of CbpA constructs have been shown to affect nasopharyngeal carriage, the most promising as a potential candidate being CbpG which showed both loss of adherence to epithelial cells and decreased virulence in a sepsis model (Gosink et al, 2000). The Lyt proteins have also been examined as potential vaccine candidates in mouse models of sepsis and confer protection (Wizemann et al, 2001). Other proteins, with lipoprotein motifs which are thought to be important in adhesion, have been identified in the streptococcal N4 genome. Three of the four proteins identified were shown after immunization to protect mice from a number of streptococcal strains and therefore may be relevant in broad subtype protection (Adamou et al, 2001).

DNA microarrays

The complete genomic sequence of pneumococcus (Dopazo et al, 2001; Hoskins et al, 2001; Tettelin et al, 2001) has allowed the implementation of microarray technology in the search for novel vaccine candidates. Genomic variation in 20S pneumoniae isolates have been examined by using microarray technology and a variation in up to 470 genes has been detected, most notably among the choline binding proteins. Other genes implicated in virulence such as the NanA/B, LytA and Ply showed no variation which suggests that they may be better potential vaccine candidates which therefore merit further investigation (Hakenbeck et al, 2001).

Sequence tagged mutagenesis has been used in pneumococcal candidate identification and a number of novel protein candidates identified which include IgA1 protease and adhesin PavA (Polissi et al, 1998; Lau et al, 2001).

Wider use of multivalent conjugate vaccines will have enormous impact on disease caused by this organism. At present the biggest hurdle is that of cost with the vaccine remaining unaffordable for the majority of the world's children. It is unclear whether serotype replacement will hamper the long-term success of the vaccine.

GROUP B STREPTOCOCCUS

GBS is a predominant cause of neonatal meningitis. Currently prophylactic antenatal antibiotic therapy is the main preventive strategy. The development of multivalent conjugate vaccines would extend protection against invasive disease in the neonatal period. Nine serotypes of GBS have been identified. Purified polysaccharide vaccines were assessed in the late 1980s, but were shown to be poorly immunogenic. Therefore conjugate vaccines have been prepared against the most prevalent GBS serotypes in the United States (types Ia, Ib, II, III, V) and Japan (types VI and VIII). Animal studies have established their efficacy and phase I and II clinical trials undertaken in adults have shown that their administration is safe (Kasper et al, 1996; Baker et al, 2000). More recently conjugate vaccines against types iv and vii have been assessed for efficacy in a neonatal mouse model of GBS

disease (Paoletti and Kasper, 2002). These vaccines offer the potential to end perinatal GBS disease and phase III trials are eagerly awaited.

CONCLUSIONS

The impact of conjugate vaccines over the last decade has been substantial with recent data of efficacy against both pneumococcal and meningococcal disease being promising. However, the challenges of finding a safe and immunogenic vaccine against group B meningococci are considerable and the effect of mucosal pneumococcal serotype replacement on invasive disease post vaccination is yet to be determined.

Antigenic portions of outer membrane proteins of both *S. pneumoniae* and *N. meningitidis* are under strong immunological pressure but remain important vaccine candidates. The challenge in designing a protein-based vaccine is to identify candidates that will be protective against a broad range of *S. pneumoniae* serotypes and multiple antigenically variable lineages of serogroup B meningococci. The identification of potential target genes which are both immunogenic and conserved has become more feasible since the rapid advances in genomic-based approaches, although vaccine efficacy using this approach is yet to be determined. The future of vaccine development therefore remains a substantial challenge, not only because of increasing antibiotic resistance and the poor immunogenicity of polysacharide vaccines, but also because of the cost implications of conjugate vaccine programme implementation in developing countries. **HM**

Conflict of interest: Dr AJ Pollard supervises clinical trials sponsored by Wyeth Vaccines, Chiron and Aventis Pasteur within the Oxford Vaccine Group. AJP has received travel funds for attendance at scientific meetings from vaccine manufactures but has not received consultancy fees.

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KEY POINTS

- Hib conjugate vaccines have had a major worldwide impact on the incidence of *Haemophilus* meningitis.
- Disease caused by *Neisseria meningitidis* group B accounts for a substantial proportion of meningitis; however, development of effective vaccination remains a considerable challenge.
- *Streptococcus pneumoniae* conjugate vaccines are effective in preventing invasive disease but are expensive and serotype replacement may occur.
- Advances through the development of genome-based strategies will have a major impact on vaccine design.

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