VACCINES AGAINST Neisseria meningitidis: *PAST, PRESENT AND FUTURE*

REVISIÓN

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SUMMARY

The ultimate goal in meningococcal vaccine research is the development of an ideal vaccine which is safe, offers long lasting immunity to all age groups, cross-protects against all meningococcal serogroups, serotypes and serosubtypes, be given orally or nasally and be easily incorporated into the World Health Organisation's Expanded Program on Immunisation. So far no such vaccine has been developed. Vaccines based on the capsular polysaccharides are available against serogroups A, C, W135 and Y which offer good, but relatively short-lived, protection against their respective serogroups. These vaccines do not cross-protect against serogroup B meningococci. Various alternative approaches have been now explored, including improved capsular polysaccharides and preparations of outer membrane proteins (constitutively expressed or iron-regulated). Over the past few years there has been considerable activity in the field and a number of clinical trials were conducted on various vaccine preparations with varying successes. We may witness research breakthroughs in the foreseeable future, however, it may be some time before a broadly or universally cross-protective vaccine becomes available.

Key words: **Meningococci, vaccines, iron, iron-regulated proteins, transferrin, transferrin-binding proteins, outer membrane proteins, capsule, polysaccharide, immune response**

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RESUMEN

La vacuna antimeningocóccica ideal debe ser segura, conferir una inmunidad duradera en todos los grupos etarios, proteger contra todos los serogrupos, serotipos y serosubtipos de *Neisseria meningitidis*, administrarse oral o nasalmente, y poder ser incorporada con facilidad dentro del Programa Ampliado de Inmunización de la Organización Mundial de la Salud. Hasta ahora tales objetivos no se han alcanzado. Existen vacunas basadas en el polisacárido capsular contra los serogrupos A, C, W135 y Y, las cuales ofrecen una buena, aunque relativamente corta, protección contra sus serogrupos respectivos. Éstas no ofrecen protección cruzada contra el serogrupo B. Para lograrlo se han explorado varios enfoques alternativos, incluyendo polisacáridos capsulares mejorados y preparaciones de proteínas de membrana externa (de expresión constitutiva o reguladas por hierro). Ha habido una considerable actividad en este campo durante los últimos años y varias preparaciones vacunales han sido llevadas a pruebas clínicas con diferentes grados de éxito. Aunque es posible que presenciemos adelantos cardinales en este campo en el futuro cercano, puede pasar algún tiempo antes de que dispongamos de una vacuna antimeningocóccica de amplio espectro de protección.

Palabras claves: **Meningococci, vacunas, hierro, proteínas reguladas por hierro, polisacárido, transferrina, proteínas de membrana externa, proteínas de unión de transferrina, cápsula, respuesta inmune**

Introduction

Neisseria meningitidis (meningococcus) is the commonest cause of pyogenic meningitis and is the only bacterium that is capable of generating epidemic outbreaks of meningitis. The pathogenesis of the disease remains unknown, however, it is known that the organism colonises the nasopharynx by adhering to the non-ciliate columnar cells. It will then reach the subepithelial cells and finally the blood stream where, if survived, it may cause bacteræmia and cause a number of different clinical syndromes depending on the host's immunity and a number of other unknown factors. The syndromes can vary in severity from a transient mild flu-like illness to fatal meningitis or septicæmia. Mortality can vary between 10-30 % depending on socio-economic factors *e.g.* the standard of health care.

However, in Europe and North America up to 14 % of the patients die despite the high standard of living and health care in these two continents and despite the sensitivity of the organism to many antibiotics. Furthermore, of those patients who recover, a significant number will develop permanent neurological sequelae, such as cranial nerve deficits.

Epidemiology

Currently, we are experiencing a world-wide epidemic with a clear increase in the number of cases reported in the recent years. In the United Kingdom, *e.g.*, more than 1 300 cases/year were reported in the past few years and around 60 % of these occurred in children under five years of age, 40 % of which were children aged less than one year (1). In the United States, approximately 2 600 cases of meningococcal disease have occurred annually over the past few years with a case-fatality rate of 12 % (2) and 46 % of the of cases affect those of two years of age or younger. In third-world countries, it is estimated that more than 0,3 million cases occur each year with up to 30 % fatality. In the *meningitis belt*

of Savanna Africa, attack rates reach up to 1 000 cases per 100 000 population In this region, epidemics occur every five to ten years and last for approximately 2-4 years (3), but, in contrast to other areas, cases occur mainly in the hot dry months. Large scale epidemics have also occurred in many countries of Asia (*e.g.* Pakistan, India and China), and Central and Latin America (*e.g.* Cuba, Chile and Brazil). In Cuba, the attack rate reached levels of more than 50 cases per 100 000 population in children younger than 6 years (4).

Based on the antigenic differences in their capsular polysaccharide (CPS), 13 serogroups of *N. meningitidis* have been identified, with Groups A, B and C responsible for 90 % of the cases. Group A meningococci are now rare in the more developed countries, but are the major pathogen in the *meningitis belt* of Africa and a number of Asian countries. In Europe, South Australia and the New world (North, Central and Latin America), Group B is responsible for the vast majority of cases followed by Group C (4, 5, 6, 7, 8).

N. meningitidis is further classified immunologically into serotypes, serosubtypes and immunotypes, based on antigenic differences in class 2/3 outer membrane proteins, class 1 outer membrane protein and lipooligosaccharides respectively. Many of these antigens are considered as vaccine candidates.

It is interesting that within one geographical location there is a trend for each serotype and subtype, particularly among serogroups B and C, to change with time. For example, Group B:15:Pl.7.16 strains have been found responsible for infections in England and Wales. However, there are currently more serologically non-typable strains isolated in these two countries than any individual types identified (1, 9), reflecting the emergence of new strains. These changes clearly will have important implications for the design of vaccines based on serotype and subtype outer membrane proteins. In this context, it is interesting to note that the class 1 protein, product of the *por*A gene (10), is known to be expressed by most but not all meningococcal isolates (11, 12, 13). Furthermore, with increased international travel, global dissemination of outbreak-associated strains is common.

Correlates of Protection

Despite extensive studies over the past few decades, the mechanisms responsible for the development of natural immunity against meningococci remains unclear. Protection has been correlated with the presence of bactericidal antibodies (14) and following the study reported by Goldschneider and colleagues (15) bactericidal assays have become established as the best available test to determine the protective ability of specific antisera raised against vaccine candidates.

However, it is not certain to what extent the *in vitro* experimental conditions reflect events occurring *in vivo* nor whether the above data apply to infections with all serogroups. The data linking bactericidal antibodies with protection relate primarily to the Group A and C polysaccharides but have been extended to include bactericidal antibodies against outer membrane proteins.

While the great majority of the studies have focused on the role of serum bactericidal activity in the host's defense against meningococcal disease, much less attention has been given to the cellular immune response (*e.g.* helper T-cell) and killing of bacteria by phagocytosis, and therefore little is known about the importance of phagocyte-mediated killing of meningococci as compared to serum bactericidal activity.

The Capsular Polysaccharides (CPS)

Group A and C capsular polysaccharide

The first successful vaccines produced were against N. *meningitidis* Group A and C using high molecular weight (100 kDa) CPS of these strains (15). A series of large scale field trials were conducted in the 1970s among different age groups in different parts of the world, including Europe, Africa and Latin America (16, 17, 18, 19) which showed that the CPS-vaccine is effective in controlling epidemics of Group A disease in almost all age groups. It soon became clear that antibody responses among infants to the Group A and C CPS vaccines depended on a number of factors including the age of the infant, the molecular weight of the antigen, the number of doses of antigen, and the prior experience of the infant with naturally occurring antigens cross-reactive with the meningococcal CPS. It became evident that children under the age of two years do respond, particularly to A CPS, with small increases in specific antibodies.

The strength of the response and its duration increased with age and in adults 100 % seroconversion was achieved which lasted longer than in children (19, 20, 21, 22, 23, 24). For example, Reingold *et al.* showed that the Group A CPS vaccine efficacy in children vaccinated at less than four years of age almost disappeared over the following three years, whereas those who were four years of age or older when vaccinated showed evidence of vaccine-induced clinical protection for three years after vaccination (24).

The Group C CPS vaccine when administered routinely among recruits of the United States army to prevent severe outbreaks, has virtually eradicated the Group C meningococcal disease in this population. Subsequent trials and antibody response studies among children confirmed this success among age groups above two years but not among children under two years of age (25, 26, 27, 28). It is interesting that more recent data show that although anticapsular antibodies and bactericidal activity in adults decline substantially by two years following vaccination, both persist at a level significantly above prevaccination levels for up to 10 years (29).

On the basis of these results, C CPS vaccines are now recommended for general use in epidemics except for children under the age of 2 years. In contrast, A CPS vaccines are recommended to be given during epidemics in all age groups including infants with a booster dose given to those under the age of 18

months. However, it is important that, due to significant shortcomings, these CPS vaccines are not useful for routine immunisation of infants. Production of improved CPS vaccines, particularly against Group A and C meningococcal infections, is still a research priority. A vaccine capable of inducing protective immunity among infants is needed so that it can be added to the routine childhood immunisation as part of the Expanded Program on Immunisation.

The success of linking Hib CPS to a protein carrier which results in a vaccine which induces a thymus-dependent (T-cell dependent) IgG response in young children, and thus immunological memory, has encouraged the development of CPS-protein conjugate vaccines for serogroups A and C meningococci. Considerable work has therefore been done to couple A and C CPS to proteins so as to change the character of the antigen from thymus independent to thymus dependent (30). These conjugates are generally strong immunogens and capable of inducing memory (30, 31).

Conjugates of Group A and C CPS conjugated to tetanus toxoid or a non-toxic mutant of diphtheria toxin have been shown to be highly immunogenic in mice and rabbits. Conjugate vaccines against Group A and C meningitis have been evaluated in toddlers by the NIAID in the USA, and in Gambian infants by the Medical Research Council (28, 32, 33). Results from these trials are expected in the near future. The World Health Organisation's Steering Committee on Encapsulated Bacteria: Program for Vaccine Development, is now coordinating and facilitating the development of conjugate vaccines against *N. meningitidis*, particularly serogroup A, and has encouraged manufacturers to produce conjugates for evaluation (34). The program is also coordinating projects to standardise immunoassays to enable proper assessment of the antibody responses to CPS vaccines, the evaluation of various clinical trials and to allow comparisons of different vaccines, different vaccination schedules, and different populations.

Group B capsular polysaccharide

Group B meningococcal CPS consists of repeated residues of α-(2-8)-linked oligomers of sialic acid, 2-8-α-N-acetylneuraminic acid, which serves as an important virulence factor and protective antigen for the organism. Candidate vaccines based on the native Group B polysaccharide (B CPS) induce a transient antibody response of predominantly IgM isotype. This poor immunogenicity of the Group B CPS, could be due to sensitivity to neuraminidases or immunotolerance of the host due to its similarity to sialic acid moieties in human brain tissues (35) which has caused considerable concern regarding the possible induction in humans of adverse autoimmune consequences by administration of B CPS based vaccines. Nevertheless, attempts are on-going to produce a CPS-based Group B vaccine.

It has been proposed that conformational determinants on the Group B CPS as presented on the intact organisms, raise antibodies that do not cross-react with the linear a-(2-8) linked determinants. The conformational structure is therefore seen to be more important in the generation of protective immunity than the primary polysaccharide structure. Various ways of stabilising the molecule in order to present an appropriate conformation, have therefore been investigated. These include the formation of non-covalent complexes of B CPS with OMPs, the binding to $Al(OH)_3$ and the conjugation of the polysaccharide to carriers such as tetanus toxoid (36, 37, 38, 39), and at best only transient bactericidal B CPS specific antibodies of mostly IgM class were detected.

Another approach to generate T-cell dependent protective IgG responses has involved attempts to modify the structure of B CPS itself prior to conjugation. By replacing the N-acetyl groups of the sialic acid with N-propionyl groups, Jennings and colleagues produced a highly immunogenic chemically modified form of Group B CPS (40). The propionylated CPS when conjugated with tetanus toxoid yielded T-cell dependent IgG response. It is interesting that two populations of antibodies were generated in mice when tested, a population reactive with isolated *native* B CPS with little bactericidal activity, and another population non-reactive with B CPS but with bactericidal activity against live organisms (41, 42). The latter population of antibodies seems to recognise a conformational epitope on the surface of the organisms which is probably formed by a combination of more than one cell-wall element, including the B CPS and, therefore, not present when the purified CPS is used alone. Clearly considerable pre-clinical safety evaluations will be necessary before such vaccines go into humans.

Outer Membrane Proteins (OMPs)

In view of the problems associated with the currently available CPS vaccines and the poor immunogenicity of Group B CPS much of the attention has been focused on non-capsular antigens, including constitutively expressed and iron-regulated outer membrane proteins. It is important to know that recurrence of meningococcal disease is extremely rare in the absence of immunodeficiencies, irrespective of the serogroups of the infective organisms. This indicates that non-capsular antigens can generate long-lasting cross-protective immunity.

Constitutively Expressed OMPs

Among the constitutively expressed OMPs, the class proteins (especially class 1, 2 and 3 proteins) have attracted most of the attention. These proteins show considerable interstrain antigenic variation, hence used as a basis for the serotyping and subtyping scheme for characterizing strains of *N. meningitidis.*

However, they are still considered attractive candidate vaccine antigens because within a particular epidemiological setting the majority of strains causing disease belong to only a limited number of types and sub-types. Antibodies against the class I OMP as well as the mutually exclusive class 2 and 3 OMPs have been detected in both immunized and infected individuals, however, the presence of antibodies does not neces-

sarily correlate with protection (43, 44, 45). The class 4 OMP appears to be highly conserved between meningococcal strains, however, it is thought to generate antibodies that might block the effect of bactericidal antibodies directed against other surface antigens (46, 47). Although details of the blocking action, or its relevance *in vivo* in humans, remain unclear, it has been proposed that future vaccines should not contain this protein. Some have attempted to produce OMP vaccines from class 4-mutants (46), and others have attempted to clone and express the other class proteins in heterologous expression systems such as *E. coli* or *Bacillus subtilis* (48, 49). The class 5 proteins, which have also been considered as vaccine antigens, are known to be surface exposed and induce antibodies in humans, however, they undergo phase variations and, therefore, the protective value of their antibodies is questionable.

An obvious drawback of vaccines based entirely on serotype and serosubtype antigens is the fact that the predominant types and subtypes associated with the disease in any one area change from time to time. The immune-pressure created by these vaccines may also contribute to such change. Therefore, it may be necessary to include several serotype/subtype proteins in one vaccine. Attempts have been made to address this for the class 1 protein by constructing a vaccine strain of *N. meningitidis* capable of expressing more than one subtype epitope of this protein (50, 51).

Another approach, adopted by McCarvil *et al*, includes expressing variable but surface-exposed epitopes of the class 1 protein on the surface of *Escherichia coli* (52). They have cloned in frame the identified sequences into the *lamB* gene of the *E.coli* expression vector pAJC 264. *E. coli* carrying these constructs expressed hybrid lamB proteins containing the surface loops of *N. meningitidis* class 1 protein. If serotype/subtype based vaccines are indeed type specific in humans then whichever way is chosen to overcome the problem there will be a need for continuous detailed epidemiological surveillance of disease-associated organisms in order to predict the optimal vaccine composition for any given time and place.

In the past decade, a number of serogroup B meningococcal vaccines based on serotype/subtype protein-enriched outer membrane proteins were developed and tested in clinical trials (4, 7, 53). Following large scale placebo-controlled, randomised double-blind trials, only the vaccines produced in Norway and Cuba showed significant protective efficacy. The Norwegian vaccine consists of 25 μg/dose lipooligosaccharide-depleted outer membranes from the Norwegian epidemic strain of *N. meningitidis*, B: 15:P1.7. 16, with only traces of meningococcal CPS. Each dose of the Cuban vaccine consists of 50 g of Group C CPS mixed with 50 μg of lipooligosaccharide-depleted outer membranes from a Cuban epidemic strain, B:4:Pl.15. In addition, the Cuban vaccine has been described as containing other higher molecular proteins, as well as class proteins (4). The Norwegain vaccine, given to children aged 14-16 years, produced point estimate of protective efficacy of 57 % after a 30 month follow up and was, therefore, considered insufficiently effective for general use (53). The Cuban vaccine, given to children aged 10-16 years, offered an estimated point efficacy of 83 % after 16 months of follow-up (4) and, as a result, the vaccine is now incorporated into the routine childhood vaccination programme in Cuba. Although the Cuban trial did not directly address efficacy in children aged less than 10 years, follow up studies of the mass vaccination have suggested that the overall protective efficacy based on vaccine coverage and incidence of disease in children under six years old is about 93 % (4). However, when the Cuban vaccine was tested in a case-control study in Brazil, protective efficacy was reported to vary with age. The vaccine was effective in children aged 4 years and older, but not in younger children (8).

In order to address some of the unresolved issues, such as differences in efficacy between the Cuban and Norwegian vaccines, the duration of protection versus the number of doses given, and in an attempt to establish a sounder basis for the evaluation of new candidate vaccines, as well as to accelerate the development of more effective preparations, a multinational collaborative study, sponsored by the World Health Organization, was undertaken in Iceland in 1992-1993. This prospective, randomised, double-blind study compared the reactogenicity, immunogenicity and serum bactericidal activity elicited in 408 young adults by 2 or 3 doses of either the Cuban or Norwegian vaccines.

A polysaccharide serogroup A/C meningococcal vaccine was used as a control. Results showed that the Cuban and Norwegian vaccines were similar but not identical in protein composition, containing class 1, 3 and 4, Opc and FrpB proteins. Class 5.5 protein was identified in the Norwegian vaccine only (54, 55). Differences were also noted between the stabilities of the two vaccines both prior to and after adsorption onto carrier. The overall results of the Icelandic study were disappointing (56). Despite extensive studies, during and after the Iceland trial, the issues of discrepancy between the Cuban and Norwegian vaccine trials have not yet been resolved. Therefore, it is concluded that efforts to understand the mechanisms by which these vaccines confer protection should now be intensified and other tests for evaluating possible immunological correlates of protection explored. Detailed analysis of these aspects has been recently reviewed in greater depth by Ala'Aldeen and Griffiths (1995) (57).

Transferrin receptors and other iron regulated outer membrane proteins

This exciting field has expanded very rapidly over the past few years and iron-regulated proteins have attracted considerable attention as possible vaccine candidates. When grown under iron-restriction, meningococci express several proteins which appear to be suppressed (partially or totally) under iron-sufficient growth conditions. Many of these proteins, which vary in terms of their molecular mass and cellular localisation, are believed to be directly related to iron-acquisition from the host's iron binding pro-

teins and other iron sources. These iron-regulated proteins include two transferrin-binding proteins (Tbpl and Tbp2) (58, 59), a 105 kDa lactoferrin-binding protein (60), a 37 kDa periplasmic iron-binding protein (Fbp) (61), an 85 kDa haemoglobin-haptoglobin utilisation protein (Hpu) (62), two RTX cytotoxin-related proteins (a 120 kDa FrpA and a 200 kDa FrpC) (63, 64) and a 70 kDa protein (FrpB) of uncertain function (65, 66).

Among the iron-regulated proteins, the Tbps (Tbpl and Tbp2) have attracted most of the attention as vaccine candidates. It is now clear that the transferrin receptor is formed, partly or wholly, by the transferrin binding proteins Tbpl and Tbp2. Ala'Aldeen *et al.*, produced the evidence linking the Tbps with biologically functional transferrin receptors in live meningococci (67). Rabbit antisera containing antibodies against the Tbps inhibited the specific binding between transferrin and live meningococci. Theoretically, it is possible that antibodies against Tbps might interfere with the chelation and uptake of iron from transferrin, and thereby inhibit the survival and growth of the organism *in vivo*. Recently, Lissolo *et al.*,(1995) (68) demonstrated the ability of such antibodies to inhibit meningococcal growth *in vitro* when transferrin was used as the only source of iron. More recently, Pintor *et al.* demonstrated the ability of anti-Tbp antibodies to inhibit the acquisition of radioactive iron from transferrin by live meningococci (unpublished results). It is not clear whether this effect is entirely mediated by the blocking of transferrin-binding, iron-internalisation or both.

Tbpl is a c. 98 kDa transmembranal protein which varies in molecular weight only marginally (+ c. 5 kDa) between meningococcal strains (59, 69). This protein loses its biological and much of its immunological properties when exposed to denaturing conditions, such as those used for SDS-PAGE, and hence it is not visualised on Western blots. Tbp2 (c. 65-90 kDa) shows considerable molecular and antigenic heterogeneity amongst different strains of *N. meningitidis* (59, 70, 71) and retains its transferrin-binding activity and strong immunogenic properties following SDS-PAGE. This protein is now believed to be a lipoprotein anchored to the outermost layer of the cell membrane (72).

Both Tbpl and Tbp2 proteins generate widely heterogeneous immune responses *in vivo*, depending on the host species, Tbp2 isotype, the vaccine preparation, the route of administration and other less well understood factors. The available data suggest that Tbp2 possesses strain-specific and cross-reactive epitopes, as determined by Western blots (59, 73). It has been demonstrated that mice, infected with live organisms or vaccinated with natively purified Tbps generate strain-specific anti-Tbp2 antibodies, whereas similarly treated rabbits generate broadly cross-reactive anti-Tbp2 antibodies (59). It is interesting that humans recovering from natural infection responded with fully cross-reactive anti-Tbp2 antibodies (59).

Rokbi *et al.*, raised rabbit anti-Tbp antibodies using gel-extracted Tbps obtained from two different strains, representing strains with low (F kDa) and high (70 kDa) molecular weight Tbp2 molecules (69). They clearly highlighted the presence of mutually exclusive epitopes which divided the Tbp2 molecules into two different families (groups, isotypes). This antigenic heterogeneity correlated well with molecular and genetic heterogeneity. The majority of the examined strains expressed high molecular weight Tbp2 and showed full cross-reaction between them, but failed to cross-react with the low molecular weight Tbp2 isotypes, Ferreiros *et al.*,(1994) (74), using human convalescent sera, studied immunoreactivity of Tbp2 molecules of different strains and obtained reaction patterns which supported this isotype classification.

Danve *et al.*,(1993) (75) have shown that mice, whether actively immunised with purified Tbps or passively immunised with rabbit polyclonal anti-Tbp antiserum, protect mice from meningococcal challenge with the homologous strain. They also showed that rabbit anti-Tbp antibodies are bactericidal against some (not all) heterologous test strains, irrespective of the strain identity in terms of serogroup, serotype and serosubtypes. More recently, Lissolo *et al.*, (1995) (68) demonstrated that mice and rabbits immunised with purified Tbp2 produced bactericidal antibodies capable of protecting mice from lethal challenge. However, they failed to purify native Tbpl and, therefore, failed to define the role of this polypeptide in generating protective immunity.

More recently, Ala'Aldeen and Borriello have shown that murine and rabbit anti-Tbp antisera (raised to *natively* purified Tbps) kill homologous and heterologous meningococcal strains with no obvious correlations between the bactericidal activity of the antisera and the molecular mass or the Western blot profile of Tbp2 (76). Animal antisera were able to kill strains which expressed Tbp2 molecules of either higher or lower molecular weight isotypes. Conversely, even strains expressing Tbp2 molecules of almost identical mass showed some variation in susceptibility to the sera. Also, they killed strains which showed no cross-reactivity on Western blots and, conversely, some strongly cross-reactive strains were not killed by these sera. These observations indicated that the bactericidal antibodies were not restricted to those generated against linear epitopes. While most of the generated anti-Tbpl antibodies are directed to discontinuous epitopes, antibodies to Tbp2 are to continuous epitopes, though some might also be directed against conformational epitopes.

More recently, Bishop *et al.*, have raised murine monoclonal anti-Tbpl antibodies, one of which shows bactericidal activity and recognises a conformational epitope, and another one reactive with a linear epitope failed to kill the organism (77). Therefore, the Tbps seem to possess a combination of important epitopes which can generate protective antibodies capable of killing the organism by complement mediated bactericidal activity and/or nutritional starvation. It is possible that a mixture of more than one Tbp2 isotype (depending on the prevalent meningococcal strains in any one country) with stabilised native Tbpl molecules

will be required in order to enhance and broaden the protective efficacy of any Tbp-based vaccines.

Thus, it is now increasingly evident that both Tbps are surface-exposed and immunogenic in humans and animals, and antibodies to their native structure are bactericidal to homologous and many heterologous strains. This suggests that a meningococcal vaccine based on, or enriched with, undenatured Tbps from one or more strains in combination with conventional CPS-based vaccines might increase the spectrum of strains against which protection can be achieved to include serogroup B strains.

Conclusions

It is evident that there has been considerable activity in the meningococcal vaccine field over the past few years and we may see breakthroughs in the foreseeable future. However, it may be another decade or so before safety and efficacy data from large scale clinical trials become available. It may take even more time before a broadly or universally cross-protective vaccine becomes available and/or incorporated into childhood immunisation programmes. It is unfortunate that no reliable serological correlates of protection exist, nor an universally accepted animal model for estimating protecting potency. Measurements of serum bactericidal activity, which proved useful as a correlate of protection assay for CPS vaccines, may not be as useful in assessing the protection afforded by OMP-based vaccines. Furthermore, studies with murine monoclonal antibodies, Western blot analysis and current so called *animal models* may be extremely misleading. Accumulating data suggests that mouse and man respond differently to different meningococcal antigens (59). No doubt, there is a greater need than ever before, for more appropriate parameters for correlates of protection to be found. Furthermore, in order to develop vaccines of consistent composition and efficacy, more detailed analysis of future vaccines will be required.

The OMP-based vaccines which have undergone clinical trials, so far, are extremely complex preparations and it is not easy to establish which of the numerous components really contribute to protection in vaccinees. Also, greater knowledge on cellular immune response (particularly, B-cell and T-cell responses) to meningococcal antigens is required before T-cell dependent protective antigens are tested and the ideal vaccine is developed.

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