REVIEW ARTICLE

Meningococcal disease

— a review based on a symposium held on 11 July 1992 at the Liverpool School of Tropical Medicine

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Introduction

Meningococcal disease is a worldwide problem. It occurs in epidemics across the so-called “meningitis belt” of sub-Saharan Africa every 7–10 years but recently epidemics of meningococcal disease have been reported from parts of Africa such as Tanzania and Malawi¹ which are well outside this area. In addition, high or increasing levels of endemic meningococcal disease have been reported from Cuba, Brazil, parts of the UK and Norway. In contrast, in other regions of the world such as the Congo Basin, Zanzibar, Hong Kong and Djibouti,² meningococcal disease is rare. We have little information on why epidemics occur or spread to other areas, why there are periodic rises in the incidence of sporadic meningococcal disease and why, in certain parts of the world, it is rare.

In the past decade we have begun to gain insights into the bacterial and host factors that are important in the development of meningococcal disease and its complications. Nevertheless, fulminant meningococcal septicaemia still presents a great problem of management and mortality rates of up to 30% are common.

This symposium brought together individuals with an interest in meningococcal disease across a wide spectrum of scientific and medical expertise, with the aim of providing an update on advances in understanding of the epidemiology, pathogenesis, management and prevention of meningococcal disease.

Structure and epidemiological markers

The meningococcus is one of the two major pathogenic species in the genus *Neisseria*. It is a relatively fragile and fastidious bacterium that has an affinity for certain mucous surfaces. Furthermore, it is able to enter the bloodstream to produce potentially life-threatening disease.³

Structurally, the meningococcus resembles other gram-negative bacteria (fig. 1). It has two cell membranes, one on each side of the rigid peptidoglycan

layer. Approximately 50% of the outer leaflet of the outer membrane is composed of amphiphilic lipooligosaccharide (LOS) molecules. The hydrophobic portion of the molecule is lipid A which is the active moiety of endotoxin. The structure of the hydrophilic oligosaccharide portion is variable and provides a basis for epidemiological typing (table I). The outer membrane of the meningococcus continually produces blebs which are released as vesicles rich in endotoxin (fig. 2). On the outside of the outer membrane is a capsule composed of acidic polysaccharide. This is (except for group-B strains) highly antigenic and forms the basis for the major epidemiological subdivision of meningococci, namely serogrouping (table I). Meningococci can be typed and subtyped on the basis of epitopes on class 2 or 3, and class 1 outer-membrane proteins (OMPs), respectively.⁴ These proteins are integral membrane proteins and act as porins transporting molecules in and out of the bacterial cell. These groups, types, subtypes and LOS immunotypes can vary independently and form a sensitive method for discriminating between meningococci, particularly those of group B and group C. Thus, for example, a meningococcus could be group B, type 15, subtype P1-4, immunotype LS-8 (B15, P1-4, LS-8). In addition, virulent meningococci express filamentous projections called pili (fig. 2). These enable meningococci to attach to mucosal epithelial cells and to endothelial cells, e.g. in the central nervous system.⁵ Pili may be one of two types, class I or class II,⁶ although this has not yet been widely used for epidemiological purposes.

For further discrimination between group B meningococci, assessment of restriction fragment length polymorphisms has proved useful.⁷ Another typing scheme for meningococci based on iso-enzymes called multilocus enzyme electrophoresis has been developed.^{8,9} This is based on the presence of iso-forms of cytosolic enzymes whose mol. wts vary between different strains of meningococci. It is a very powerful tool and has, for example, enabled the movement of a particular clone of group-A meningococci (clone III-1) to be tracked from China to Nepal in 1983, to India

Fig. 1. A diagram of the structure of the meningococcal cell wall indicating structures used in epidemiological typing.

Table I. Epidemiological subdivisions of meningococci

Subdivision	Variable	Nomenclature
Group	Acidic capsular polysaccharides	A, B, C, D, 29E, H, I, K, L, W135, X, Y, Z
Type	Class 2 and 3 outer-membrane proteins	1-20 (for group B)
Subtype	Class 1 outer-membrane proteins	1-7 (for group b)
Immunotype	Lipo-oligosaccharide	1-8 (for group B)
Multilocus enzyme electrophoretic types	Cytoplasmic iso-enzymes	Many

and Pakistan in 1985, to Mecca in 1987 and thence to the meningitis belt in 1989.⁹

In England and Wales, the majority (69%) of cases of infection are due to group-B meningococci followed by 28% due to group-C meningococci.¹⁰ At present there is a hyperendemic phase of meningococcal disease in the UK and the highest rates are in the north and west of England (e.g., Mersey region 4.7 cases/100 000 population, North West region 3.5/100 000).¹⁰ A complex of related electropherotypes (ET-5) of group-B meningococci have been associated with hyperendemic disease throughout the world.⁸ In England and Wales these are present as sulphonamide-resistant strains of group B15 P1.16 and B4 P1.15 which, apparently, are more likely to infect older children and adolescents than the sulphonamide-sensitive strains.¹⁰ The commonest phenotypes isolated in England and Wales were B15 P1.16 (18%), Bnt P1.15 (13%) and B2b P1.10 (13%). Amongst group-C strains, C2a (34%) and C2b (39%) were the major phenotypes in 1991.¹⁰

It is clear that the development of precise methods of grouping, typing, subtyping and electropherotyping meningococci has provided powerful tools for monitoring the spread of particular strains of meningococci both within communities and throughout the world.

CLONAL AND VARIABLE PROPERTIES OF *NEISSERIA MENINGITIDIS*

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Serogroup-A meningococci from diverse epidemics since the 1960s have been subjected to multi-locus enzyme electrophoresis (MLEE) in various laboratories.¹¹⁻¹⁴ Electrophoretic types (ETs) were grouped in fairly stable "clones", which themselves were associated in the larger so-called "subgroups".¹¹ In a recent analysis in which results obtained with reference strains from these diverse sources were integrated, elucidated 84 ETs which fell into nine subgroups designated I-III, IV-1 and IV-2 and V-VIII (fig. 3), but could not confirm the prior assignments to "clones".¹⁵ The bacteria were also tested for expression of the conserved pilin epitopes called class I, class IIa and class IIb, and for expression of sero-subtyping epitopes associated with the VR1 and VR2 variable regions of the class 1 protein.¹⁵ Bacteria within each subgroup were fairly homogeneous for both sets of antigens (fig. 3) and for expression of variable epitopes on IgA1 protease.¹⁶ Thus, most of the different ETs can best be regarded as representing the occurrence of minor and rare variation due to mutation and horizontal genetic exchange. The results also show that sero-subtyping monoclonal antibodies (MAbs) were not available for both VR1 and VR2 of

Fig. 2. An electromicrograph of a negatively-stained meningococcus showing pili (p) and blebbing from the outer membrane (b) (bar, 0.5 μ m).

numerous serogroup-A meningococci. The VR regions which did not react contain unique sequences,¹⁷ indicating the need for additional sero-subtyping MABs.

Almost all isolates from any one epidemic, epidemic wave or pandemic of serogroup-A disease belonged to a common subgroup and most bacteria from any one subgroup belonged to a single ET (fig. 3). More detailed analyses have been performed with serogroup-A meningococci isolated during (1982–1983) and after (1983–1988) an epidemic in The Gambia.¹⁸ Essentially all these bacteria belonged to a single ET of subgroup IV-1, and were uniform not only for the subgroup properties described above but also for expression of the L9 LOS immunotype and in the amount of capsular polysaccharide synthesised. After the epidemic, rare strains were isolated with altered LOS, or that did not express the class 1 protein or synthesised increased amounts of capsular polysaccharide or with any combination of these characters; three of these strains have proved impossible to kill by antibody-mediated complement activation.¹⁸

All serogroup-A meningococci tested from epidemics in West Africa in the early 1980s were also subgroup IV-1¹¹ as were most serogroup-A meningococci isolated there from endemic disease since the

early 1960s and a minor proportion of epidemic isolates in the 1960s and 1970s.^{11,19} Subgroup IV-1 strains have not been isolated outside West Africa, except in India.^{15,20} A total of eight class 5 proteins, called 5a–5h, have been distinguished in different isolates of this subgroup.^{21,22} Class 5 proteins are trimeric, heat-modifiable and highly basic OMPs which are variable in expression and correspond to the P. II opacity proteins in *N. gonorrhoeae*.²³ The class 5 proteins from subgroup IV-1 have been subdivided into seven Opa proteins encoded by *opa* genes, which are recognised by MAb 4B12/C11²¹ and the Opc protein (formerly called 5c) recognised by MAb A222.²⁴ The protein sequences and regulatory mechanisms differ markedly between Opa and Opc proteins;²⁴ the Opa proteins expressed by meningococci can vary with the subgroup and even with the locale where the bacteria were isolated²² while the Opc protein is expressed by unrelated meningococci of diverse serogroups.²¹ With rare exceptions, only three Opa proteins or Opc, or both an Opa and Opc proteins, were variably expressed by bacteria from The Gambia.²⁵ Sequence analyses have shown that Gambian subgroup IV-1 isolates possess only three *opa* genes (Hobbs, Cannon and Achtman, unpublished data) whereas a strain of ET-37 complex serogroup-C

Fig. 3. Genetic relationships and antigenic patterns of 84 ETs of meningococci.¹⁵ *Genetic distance*: the dendrogram resulting from cluster analysis shows the genetic distance at which the clusters divided. The ET numbers are indicated at the end of the braces as is the serotype (○, serotype 4; ●, serotype 21). The subgroup designations are indicated at the branching points (I, II, etc). The horizontal dotted lines are aligned with serological changes in the rest of the figure. *Pilin*: reactivity with MAbs defining different pilin epitopes is indicated by rectangles. *Serosubtype*: reactivity with MAbs to the individual serosubtypes is indicated by rectangles. *Frequency*: number of strains tested of each ET.

meningococci (see below) possesses four.²⁶ The seven Opa proteins expressed by subgroup IV-1 bacteria represent the results of horizontal genetic exchanges which have resulted in recombinational replacement of variable regions within individual *opa* genes (Hobbs, Cannon and Achtman, unpublished data). In contrast, no variation in the DNA sequence of the *opc* gene has yet been detected although some strains, such as those of the ET-37 complex, totally lack the gene.²⁷

Serogroup-A, subgroup-III meningococci have caused two pandemic waves which have originated in the Far East and have swept across the world.²² These strains caused an epidemic in Mecca, Saudi Arabia in 1987¹² from where they spread to cause epidemics which are still continuing in East Africa. Subgroup-III meningococci from both pandemic waves variably expressed Opc and the Opa proteins designated 5a, 5f, 5h and 5i. Proteins 5a, 5f and 5h were indistinguishable from the proteins with the same designation in subgroup IV-1 although we know of no direct contact between the bacterial groups between the early 1960s and the late 1980s.

The general function of class 5 proteins is not known but recent data may have elucidated part of the role of Opc in pathogenesis. Meningococci which express diminished amounts of capsular polysaccharide can adhere to and invade human endothelial and epithelial cells if they express large amounts of Opc.²⁸ Binding is inhibited by MAbs to Opc. Opc was a component of the OMP complex vaccine used to immunise 100 000 Norwegian adolescents and most reacted strongly, producing bactericidal antibodies to Opc.²⁹ Opc also seems to have been a component of the Cuban VAMengo-BC vaccine because many hybridomas generated from peripheral blood lymphocytes from immunised Cubans were specific for that protein.³⁰ Opc is also highly immunogenic during nasopharyngeal carriage and disease.²⁹ Only meningococci expressing large amounts of Opc are sensitive to bactericidal killing and only those variants are capable of adhesion and invasion. In The Gambia, meningococci isolated from the nasopharynx expressed large amounts of Opc more often than did bacteria isolated from the bloodstream or cerebrospinal fluid.²⁵ I propose that expression of Opc is needed for adhesion and passage through cells but that the bacteria evade protection immunity because, at any one time, a large proportion of the population expresses low amounts of Opc or none. It remains unclear why adhesion and invasion can be observed in the laboratory only with bacteria that express diminished amounts of capsular polysaccharide.

It may be instructive to compare the observations summarised above with those obtained for serogroup-C bacteria of the ET-37 complex. Between 1989 and 1991, most bacteria isolated from endemic disease in Mali, West Africa, were serogroup C, serotype 2a, serosubtype P1.2,y and expressed class IIb pili.³¹ These bacteria belong to the ET-37 complex³² which has caused outbreaks in South and North America as

well as being a common cause of endemic disease in Europe. The meningococci responsible for outbreaks in US army recruits which led to the development of the A+C polysaccharide vaccine³³ were of the ET-37 complex.³² Unlike the constancy described above for serogroup-A bacteria, the ET-37 complex is antigenically diverse. Serogroups B, C, Y and W-135, serotypes 2a and 2b and serosubtypes P1.2,5 and P1.2,5 have all been associated with the ET-37 complex, although most isolates have been C:2a:P1.2,5 and have expressed class IIb pilin. Whereas any one strain possesses only four *opa* genes (and no *opc* gene), 26 Opa protein variants which differed serologically or upon migration on SDS-PAGE were distinguished within the ET-37 complex.³² Within certain locales, such as the endemic isolates from Mali, the bacteria were homogeneous but it seemed that this was rather exceptional and variability was more common.

Taken together, these analyses indicate that certain genetically related bacteria have been responsible for most of the outbreaks and cases of meningococcal disease on a global basis. The stability of antigenic properties varies with the group of organisms and these groups can currently only be identified reliably by MLEE. Recombinational events can lead to changes within the bacterial genome which are most readily observed by comparisons of genetically related bacteria from diverse locales. The currently available serological tests are inadequate to recognise these bacterial groupings and will indicate variation when global analyses are performed.

PILATION IN *NEISSERIA MENINGITIDIS* AND ITS CONSEQUENCES

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The surface of *N. meningitidis* is composed of various biologically active molecules, many of which are highly variable, most notably, OMP II (opacity proteins)^{26,34-36} and pili (fimbriae).^{34,37,38} Pili are important adhesins in meningococci and isolates from patients are almost invariably pilate when observed microscopically. Piliation appears to be required for colonisation of host mucosal surfaces and for at least some stages of invasive disease caused by these bacteria.^{37,39-43} Pili are filamentous protein appendages which extend considerable distances from the bacterial surface and are probably responsible for initial interaction with host epithelial cells and subsequently with endothelial cells.^{5,42-44}

Pili produced by pathogenic *Neisseria* spp. are composed of repeated subunits of pilin polypeptide with approximately 10 000 pilin subunits assembled to form an individual pilus.^{23,45} Meningococci have been observed to produce either one of two types of pilus, class I and class II (table II).^{5,6,44,46-52} Class I pili are

Table II. Similarities and differences in class I and II meningococcal pili

Character	Class I	Class II
Reaction with MAb SM1	+	—
Reaction with MAb SM2	+	—
Subunit mol. wt of pilin (kDa)	16–23	13–18
Homology with gonococcal <i>pilE</i> gene in producing cell	+	+
Homology with PS1 (SM1 epitope) oligonucleotide probe	+	Varies with strain
Required for adhesion to epithelial and endothelial cells	+	+
Antigenic and functional variation	+	+
Adherence to HEp-2 epithelial cells	+	—
Adherence to Chang epithelial cells	+	+
Mean diameter (nm) (SD)	5.95 (0.80)	8.47 (0.88)

similar in almost all aspects to the pili produced by all strains of *N. gonorrhoeae*; both react with MAbs SM1 and SM2. In contrast, class II meningococcal pili do not react with these antibodies. However, some epitopes may be shared by the two classes of pili.^{37, 51–53} The gonococcal and meningococcal class I pilin molecule can be divided into three regions.^{48, 54–56} The 53 N-terminal amino acids of mature pilin are conserved between antigenically distinct molecules constituting the conserved (C) region. The central portion lying between amino acid 54 and the first cysteine residue (Cys 1) at *c.* residue 120, constitutes the semi-variable (SV) region. Within SV, there are five short peptide regions that are conserved, but intervening areas are subject to numerous amino-acid substitutions when different pilin variants are compared. The carboxyl-terminal end, from Cys 1 at residue –120 to the C-terminus at *c.* residue 160, is known as the hyper-variable (HV) region and contains numerous substitutions deletions and insertions of amino-acid sequence, when variant pili are compared. The sequence between the two cysteine residues has been shown to elicit the dominant antigenic response in man and animals.^{57–59} Antibodies produced by patients infected by gonococci show limited cross-reactivity with different pili produced by a single strain.⁶⁰ MAbs SM1 and SM2 react with all gonococcal pili and a high proportion of the meningococcal pili so far studied.^{46, 47, 50, 52} A slight majority of meningococcal isolates (*c.* 60%) express pili that react with MAb SM1 (and SM2) and which have been termed class I. About 40% of isolates produce class II pili that do not react with MAbs SM1 and SM2. The SM1 isotope has been localised to a pentapeptide that spans residues 49–53 at the distal end of the C domain of pilin.⁶¹ Chromosomes of strains producing class II pili are apparently unable to elicit class I pili but contain silent truncated pilin genes that are homologous to class I and gonococcal pilin sequences. This suggests that the

ability to express class I pili has been lost during evolution and may have been replaced by class II pilin-encoding genes.⁴⁶ Class I and class II pili are equally adherent to human endothelial cells, suggesting that, although the two classes of pili are genetically and structurally different, they have functional similarities.⁴⁴ Class II pilate bacteria have reduced adhesion to HEp-2 cells compared with bacteria producing class I pili, indicating that there are tissue tropism differences between class I and class II pili.⁵ Variation in the antigenic nature of class I meningococcal pili in strain MC58 seems to affect adherence to epithelial cells but not to endothelial cells. Thus, although pilus-facilitated adherence is presumably mediated through receptors on human epithelial cells, there must be quantitative or qualitative differences, or both, in the distribution of receptors for both classes of pili on epithelial cells from different tissues. Class II pili can be divided into two subclasses based on their reaction with different MAbs and a MAb that reacts with the majority of class II meningococcal pili also reacts with the pili of *N. lactamica* (M. Achtman, personal communication). Therefore, class II meningococcal pili could have a common genetic ancestry with those of *N. lactamica*. It is conceivable that inter-species gene transfer (most probably by transformation) may have permitted *N. meningitidis* to acquire pilin genes from commensal neisseriae to replace those lost as a consequence of the natural instability of the class I *pilE* locus.

Genes that code for pilin have been located and characterised in both *N. gonorrhoeae* and class I pilus-producing *N. meningitidis*.^{46, 48, 56, 62, 63} An intact pilin gene and promoter are found only at the expression site *pilE* which is present as a single copy in the genomes of meningococci. Other silent loci (*pilS*), contain variant pilin sequences, truncated at the 5' end and lacking a promoter.^{64, 65} Two genes, *pilA* and *pilB*, involved in the regulation of expression of pilin have been located downstream of the gonococcal *pilE*.^{66, 67} and presumably there are analogues in meningococci. *PilB* probably spans the outer membrane and acts as a sensor to environmental conditions, whereas *PilA* behaves as a response regulator and has been implicated in pilus biogenesis. A further pilin-associated locus called *pilC* encodes the 110-kDa *PilC* protein which has been implicated also in pilus biogenesis and, since this polypeptide co-purifies with pilin, may play a role in adhesion *per se*.

Expression of neisserial pili can be spontaneously turned on and off in a phase variation and a single cell can produce offspring that express structurally, antigenically and functionally distinct pilins through the process of antigenic variation. Both types of change arise as the consequence of alterations in expression at *pilE*, or an alteration in the relative influence of pilin accessory genes, or both. The actual mechanisms by which these changes take place remain to be resolved. Phase variation may be either reversible or non-reversible. A P-n (Pili-, non-revertible) phenotype

results from deletions in the 5' region of *pilE* that remove its promoter.⁶⁸⁻⁷⁰ As each neisserial cell usually contains only one copy of the *pilE* promoter, loss is permanent, as is the ability to produce pili.⁷¹ P-rp- (Pili-, revertible, pilin-) derivatives produce a pilin mRNA with a mutation that codes for a truncated or abnormal pilin protein whereas P-rp+ (Pili-, revertible, pilin+) cells can produce a full pilin polypeptide, containing a missense mutation in a region encoding a domain vital for pilus assembly. Reversion of these two phenotypes to P+ can be achieved by one of the *pilS* sequences recombining with the expression site in a gene conversion event. Different types of phase variation occur leading to the production of the "abnormal" pilin polypeptides; S-pilin is pilin polypeptide that has been abnormally processed at the pre-pilin stage, is secreted to the outside of the cell and cannot be assembled in the outer membrane; L-pilin results from a partial duplication of the *pil* gene at the expression locus.^{72,73}

Neisseriae can exploit their ability to produce a non-pilate, non-attaching phase to desorb from initial sites of infection and allow movement to other locations. The non-pilate state may also enable the organisms to be transmitted from one host to another; reverting to the pilate form would then be advantageous once a new infection site has been reached. Pilus antigenic variation is due to the appearance of a new nucleotide sequence at the *pilE* locus, resulting in the production of an assembled pilus with a novel subunit pilin amino-acid sequence. Variant pili may help the organism to colonise different tissues during the course of an infection and avoid the immune system of the host by presenting a succession of different antigenic stimuli.

The transfer of sequences from *pilS* to *pilE* may occur by reciprocal, homologous recombination⁷⁴ or, more frequently, by non-reciprocal homologous recombination (gene conversion).^{65,69} In gene conversion, the donating *pilS* sequence replaces the 3' portion of the *pilE* gene, which is subsequently lost from the genome with a stored copy of the donated *pilS* gene remaining. Novel *pil* sequences may be spliced into *pilE* by the process of deletion repair where deletions arising spontaneously by illegitimate recombination are repaired by using the *pilS* genes as a template.⁷⁵ Donated *pilS* gene segments could be acquired by transformation from autolysed neisserial cells.^{74,76} In contrast, some workers argue that transformation has no effect on pilus variation but may be involved in the spread of beneficial *pil* gene sequences throughout an infecting population.⁷⁷ It has also been suggested that transformation may act as a trigger for pilin gene reassortment.⁷⁸ The variable nucleotide domains within a *pilS* sequence may act as "mini-cassettes" in pilus antigenic variation, recombining with the corresponding segment of *pilE* to produce variant pilin molecules.⁶⁵ Short portions of the *pilE* gene would be replaced in the generation of a novel pilin coding sequence, rather than the whole 3' end of the gene. This notion is also crucial to the deletion-repair theory

of antigenic variation, in which short tracts of the *pilE* site flanked by short directly repeated sequences would be deleted and subsequently repaired by using the appropriate part of a *pilS* segment as a template.⁷⁵

Summary. Pili present a highly variable adhesin to the surface of *N. meningitidis*. The ability of these bacteria to switch reversibly between a pilate and non-pilate state is crucial in allowing the pathogen to absorb to and desorb from epithelial and endothelial cells. Meningococci can produce one or other of two kinds of pili, class I and class II, both of which promote binding of these bacteria to epithelial and endothelial cells. Class I and class II pili seem to possess different specificities for adhesion to different types of epithelial cell. Furthermore, it appears that the primary amino-acid sequence of the pilin molecule can influence the adhesion specificity of mature pili. The underlying mechanisms for generating pilus diversity at the DNA level confer a great degree of plasticity in the structural gene (*pilE*) for the pilin subunit of class I pilin.

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HOST RESPONSES TO MENINGOCOCCAL INFECTIONS

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Strains of *N. meningitidis* are genetically diverse and vary in their pathogenic potential.^{8,18-20} Asymptomatic carriage of even virulent strains is the most common manifestation of infection²² with only a small, relatively unpredictable minority developing disease. When disease occurs, clinical manifestations vary from transient bacteraemia⁷⁹ through relatively benign and not always easy to diagnose syndromes affecting the joints and skin,⁸⁰ to the more readily recognised meningitis or fulminant meningococcaemia (table III). In a small proportion of cases, post-infection immunemediated complications develop.⁸¹

The outcome of exposure to the meningococcus depends on a complex and poorly understood interaction between the genetic make-up of the organism, and the genetic background and past experiences of the host, probably modified by environmental—particularly seasonal⁸²—factors and co-existent or antecedent viral infection.⁸³ Nevertheless, once bacterial and environmental variables have been taken into account, it is the way in which the host is conditioned to respond either *de novo*, or as a consequence of prior antigenic stimulation, that determines clinical outcome. Although the organism must invade the mucosal surface to cause disease, little is known of the host factors that influence this process. Attention instead has been focused on systemic defences.

Table III. Meningococcal disease at Northwick Park Hospital, 1985–1992

Clinical syndrome	Number of cells
Meningitis	30
Meningitis + septicaemia	9
Septicaemia	17
Fulminant meningococcaemia	4
Benign meningococcaemia	3
Fever, polyarthritis acute	4
Fever, polyarthritis chronic	3
Septic arthritis	2
Total	72

Non-specific host responses

Normal reticulo-endothelial function is an important facet of protection against meningococcal disease as evidenced by the severe septicaemia seen in the splenectomised patient.⁸⁴ Complement activation plays a complex role. The alternative pathway appears critical, at least in the non-immune host, since properdin deficiency predisposes the fulminant infection.⁸⁵ Terminal complement cascade deficiencies are associated with recurrent neisserial infections.⁸⁶ However, excessive complement activation may have a deleterious effect and is correlated with disease severity.⁸⁷ The way in which the host handles immune complexes may determine whether immunopathological consequences ensue.

Host handling of the LOS shed by meningococci in the form of cell-wall blebs has been little explored, although LOS may be central to disease manifestations.^{87,88} High density lipoproteins readily complex with LOS molecules rendering them less toxic;⁸⁹ the acute phase response also involves the generation of LPS binding proteins.^{90,91} The genetic control of these and cytokine responses, particularly generation of tumour necrosis factor (TNF) and interleukin (IL)-6,^{92–95} may be relevant to both the development of disease and its diversity.

Specific immune responses

While the potential for variation in the non-specific defence mechanisms is speculative, the presence of, or ability to mount, a relevant antibody response is currently accepted as the major barrier to disease.⁹⁶ Natural immunity develops with age, perhaps in response to relatively avirulent meningococci or other cross-reacting antigens.^{97,98} Therefore, the identification of the antigens involved, determining the function of the antibodies generated, and developing appropriate methods for their testing are crucial to our understanding of the immune response.

Antigens involved in the immune response

The antigenic repertoire of *N. meningitidis* is summarised in table IV.

Capsular polysaccharides. Both disease and the carrier state induce antibodies to the group-A polysaccharide.⁹⁹ All age groups respond to disease, the majority producing a four-fold increase in antibody levels which may be useful diagnostically, although the strength of the response is age-dependent. A few individuals, irrespective of age, fail to respond, perhaps because of an immunological defect.¹⁰⁰ Although less well studied, a similar pattern is seen in infection with group-C strains in adults and children over 2 years old. Data on infants is scant but a 4-month-old child failed to develop anticapsular antibody.¹⁰¹ Responses to these polysaccharides are seen very early after admission to hospital;¹⁰² some individuals have high levels at the time of diagnosis.⁹⁹

Group-B polysaccharide is a poor immunogen, although young adults usually respond with a four-fold or greater increase of predominantly IgM antibody.^{101,103} The response in children is more variable. Griffiss *et al.*¹⁰¹ detected a response in only eight of 26 patients and in these the response was of a significantly lower magnitude than in adults. Anti-polysaccharide responses are probably under genetic control¹⁰⁴ but neither this nor the potential importance of antibody avidity¹⁰⁵ has been fully explored.

Sub-capsular antigens. Components of the outer membrane are immunogenic when examined by ELISA with outer-membrane complex as antigen.^{106,107} Low levels of antibody are found usually in the general population and early in disease, but IgM, IgA, IgG and IgG3 antibodies are induced rapidly in all age groups. When examined by this technique, some of these antibodies are cross-reactive across serogroups and sero-subtypes of meningococci, as well as other *Neisseria* sp.¹⁰⁸

SDS-PAGE and immunoblotting,¹⁰⁹ specific antigen purification^{110,11} and competitive antibody assays with MAbs^{110–112} have been developed to attempt to resolve the antigenic specificity of such reactions. Significant host-to-host variation is seen in the responses to class 1, 2/3 and 4 proteins and to LOS. Strong responses are usually seen to the class 5 proteins,¹¹³ which tend to be strain specific, to H8¹¹⁴—those proteins induced in the outer membrane under conditions of stress, particularly the iron-regulated proteins^{115,116}—and to other minor undefined proteins,¹¹³ many of which show cross reactivity across serogroups and serotypes.^{113,116}

Antibody functionality

While it is evident that the host may respond to a range of meningococcal antigens, it is less clear what function such antibodies effect *in vivo*. Three possible functions have been described: complement-dependent bacteriolysis;⁹⁶ complement-dependent/independent opsonisation and phagocytosis;¹¹⁷ and cell-mediated antibacterial activity of mononuclear cells.¹¹⁸ Their relative importance is currently unknown.

Table IV. Antigenic repertoire of *N. meningitidis*

Capsule	
Outer-membrane proteins (OMPs)	Major classes 1–5 Iron regulated Other stress induced Minor—ill-defined
Lipo-oligosaccharides (LOS)	
Pili	
IgA protease	

Table V. Targets for bactericidal activity

Polysaccharides	
MAB-defined epitopes on	
Class 1	MOMP
Class 2/3	MOMP
Class 5	MOMP
70-kDa iron-induced	
LOS	

MOMP, major outer-membrane protein.

However, the keynote studies of Goldschneider *et al.*,⁹⁶ which correlated the presence of bactericidal activity in pre-exposure sera with protection, and its absence with susceptibility, coupled with the predisposition of individuals with deficiencies of the terminal complement cascade to neisserial infection, have become accepted as attesting to the pre-eminence of serum bactericidal activity.¹¹⁹ Several targets for bactericidal antibody have been defined with mouse MABs (table V). However, whether the human immune system responds to the same epitopes remains controversial.¹²⁰

Anticapsular A and C antibodies are bactericidal and protective.^{99,101} Bactericidal responses to subcapsular antigens are more difficult to evaluate, because the performance of such tests is complicated by the potential variation in expression of cell surface targets. Pili, class 5 proteins and LOS are subject to phase variation.^{38,121,122} Variation in the major OMPs can be seen during the course of infection³⁸ and the expression of class 5 and class 1 proteins and LOS can vary depending on the site of isolation in a diseased individual.^{25,123}

Specific epitope expression may vary, depending on in-vitro growth conditions.¹²⁴ New or enhanced expression of outer-membrane antigens may be induced by stress or nutrient limitation.^{125,126} The environment milieu to which meningococci are exposed *in vivo* is unclear, but the variation between organisms grown *in vivo* and *in vitro* has been the subject of recent study.¹²⁷ That meningococci are subjected to iron limitation *in vivo* is suggested by strong immune responses to iron-regulated proteins.^{115,116} This has been confirmed by examination of meningococcal cells obtained directly from CSF.¹²⁸

Variability in cell-surface molecules, both within a single isolate and amongst isolates of the same clone,²⁵ or variation in their expression *in vivo*, may explain the

apparent paradox that some patients have positive blood cultures in the presence of serum bactericidal activity, and the observation that serum collected from Gambian children, prior to their developing disease, had significant bactericidal activity against a representative isolate.¹²⁹ An alternative explanation for the apparent disappearance of bactericidal activity involves the induction of IgA antibodies which block the bactericidal effects of IgG and IgM against polysaccharide¹³⁰ and LOS;¹¹⁹ or the generation of antibodies against non-protective targets on the class 4 protein,¹³¹ which would hinder the accessibility of protective antibody.

Although bactericidal activity has been most studied, it may be neither the only nor the dominant function provided by antibody. Antibodies to group-A and group-C polysaccharides have opsonising activity,¹³² but the latter may be more important in group-B infection.¹¹⁷ Opsonic activity is low early in disease, but increases rapidly,¹³³ exhibiting a broad range of activity across serogroups and serotypes, suggesting that the relevant targets are unrelated to those epitopes defined by current serotyping schemes.¹³⁴ It remains unclear whether opsonising antibody, which may be detectable at lower concentrations than bactericidal antibody,¹³² could account for the majority of individuals who are exposed to an epidemic strain becoming carriers rather than cases,²² or whether this is determined by the speed of their immune response.

Conclusions

Evaluation of the host responses to meningococcal infection has generated a number of laboratory assays that enable serodiagnosis, particularly in those who present with less common manifestations or when prior therapy precludes a cultural diagnosis. Less impact has been made in defining the relevant target for immunoprotection, particularly in group-B disease, and determining the mechanisms that allow for the high grade bacteraemia, which is the commonest cause of death. These remain important challenges.

THE RELEVANCE OF CLINICAL FEATURES AND PROGNOSTIC INDICATORS IN THE MANAGEMENT OF MENINGOCOCCAL DISEASE

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Introduction

The incidence of meningococcal disease in the Mersey Region in 1991 was 4.7/100 000 population.¹⁰ This is the highest incidence of any region in England and Wales, but underestimates higher local figures because of variation within the Region, e.g., the incidence around the conurbations of Liverpool and

Table VI. Clinical presentation of meningococcal disease in 152 consecutive children in the Mersey Region studied prospectively between November 1988 and July 1990

Clinical status	Number (%) of	
	survivors	deaths
Meningitis	17 (100)	0 (0)
Septicaemia and meningitis	86 (93.5)	6 (6.5)
Fulminant septicaemia	36 (83.7)	7 (16.3)
Total	139 (91.4)	13 (8.6)

Birkenhead in the northern part is much higher than that in rural Cheshire in the south. This high incidence has helped make research into meningococcal disease a priority for the clinicians on Merseyside, who have collaborated in several studies over the last 4 years. Data from these studies are presented here to illustrate the relevance of clinical features and prognostic indicators in the management of meningococcal disease.

Clinical features

Early recognition of meningococcal disease is essential for its successful treatment. Unfortunately there is still widespread ignorance and confusion about the presenting clinical features of the disease which is hindering early recognition, delaying urgent treatment and hampering education of the general public *via* the media. Life-threatening meningococcal disease does not present primarily as meningitis.³ During the spread of the organism *via* the blood to the meninges, the inflammatory cascade may be activated, precipitating an acute vasculitic picture which can progress to full-blown septicaemia with shock. Of 152 children studied prospectively between November 1988 and July 1990 in the Mersey Region (table VI), only 11% (17 children) had meningitis alone, and none of this group died. The remaining 89% (135 children) presented with clinical evidence of bacteraemia or septicaemia; this was accompanied by meningitis in 61% (92 children), but fulminant septicaemia was present in 28% (42 children). Mortality was 9.6% (13 of 135) in the group with a septicaemic component to the illness. Mortality in the group with fulminant septicaemia was 16.3%, which is higher than in the remaining children, though failing to achieve statistical significance, probably because of small numbers.

The clinical features of meningococcal disease in this group are shown in table VII. The main features at presentation were fever (88.8%), rash (68.4%), vomiting (67.1%) and drowsiness (54.6%). Neck stiffness was present in only 50.7% and other features of meningitis such as coma, headache, irritability and convulsions were present in $\leq 30\%$ of these children. These data emphasise that a petechial and purpuric rash in an ill febrile child is almost pathognomonic of meningococcal disease. A rash was present in 104

(68.4%) children at presentation, but evolved after admission in 28 others so that ultimately 132 (87%) children had a rash. Of these, 20 (15%) had a maculopapular rash with no purpuric elements (136), but 112 (85%) had a typical petechial or purpuric rash. This typical petechial or purpuric rash should now be familiar to all doctors who see children in primary and secondary care in the UK, but more difficult to identify early is the maculopapular rash of meningococcal disease, which cannot be differentiated from that of non-specific viral illnesses until petechial or purpuric elements appear. This makes regular re-examination of the ill child with a maculopapular rash an essential part of clinical management.

The relevance of these clinical features becomes clearer still when it is appreciated that many patients are still inappropriately labelled as having "meningitis" when in fact they have features of septicaemia on first contact with primary or secondary care. During outbreaks of meningococcal disease, general practitioners have, in the past, been advised to look for features of "meningitis" and give penicillin if these are found. Although meningococcal septicaemia is now more explicitly included in this advice, many general practitioners have never seen a case, and may not feel that the advice is relevant to them. This may remain so even during a local outbreak because of the tendency of the press to publicise meningococcal septicaemia as "meningitis"!

Doctors, the public and the press

In a local outbreak of 14 cases of meningococcal disease in South Cheshire, all 14 children had septicaemia, but only six had meningitis. Four died from fulminant septicaemia. Forty-three press cuttings (obtained from the International Press Cutting Bureau) relating to the outbreak all described the disease as "meningitis", only two (4.7%) mentioned septicaemia.¹³⁷ Advice warning about the features of the disease was given to the public in 26 (60%) of the cuttings. This is compared with the clinical features of the 14 cases in fig. 4. Comparison of the two datasets demonstrates that the media publicised potentially misleading advice, emphasising the presenting features of meningitis, and neglecting the importance of the vasculitic rash. This caused considerable confusion amongst the general public and professionals in primary care. Parents of children subsequently admitted to hospital with meningococcal disease complained that they had been misinformed by the advice, which seriously risked misdirecting vigilance and resources.¹³⁷

Advice given to the press should take into account the clinical features of the cases who have already presented to hospital, confirmation of the organism as the meningococcus, and knowledge of the usual clinical features of meningococcal disease. Clinicians (paediatricians, physicians and intensivists) and Consultants in Communicable Disease Control should

Table VII. Clinical features of meningococcal disease in 152 children at presentation

Clinical feature	Number (%) of children
Fever	135 (88.8)
Rash	104 (68.4)
Vomiting	102 (67.1)
Drowsiness	83 (54.6)
Neck stiffness	77 (50.7)
Coma	46 (30.3)
Hypotension	38 (25.0)
Headaches	34 (22.4)
Irritability	34 (22.4)
Poor feeding	28 (18.4)
Diarrhoea	17 (11.2)
Walking problems	16 (10.5)
Convulsions	15 (9.9)

confer to ensure the accuracy of statements issued to the press. This will help avoid misinforming the profession and the public. The "Meningitis Fact Sheet" prepared by the Meningitis Trust¹³⁸ can act as a useful checklist on which to base publicity and statements to the media, but the importance of the petechial or purpuric rash in the diagnosis of meningococcal disease needs to be highlighted.

Prognostic indicators

Optimal management of children with meningococcal disease is possible only with prompt identification of the most ill; prognostic indicators and scoring systems have been studied to determine their role in quantifying the severity of illness. Possible benefits of scoring systems include: identification of the patient who warrants admission to intensive care;

as entry criteria in trials of new therapies or management; and as mandatory components of protocols for use of expensive new therapies in order to manage health service resources efficiently. A further possible benefit would be the use of a scoring system to withhold treatment, but in view of the imperfect performance of all scores so far proposed this is not a realistic expectation and should be ignored.

Poor prognostic clinical features of meningococcal disease include extreme age, a spreading rash of less than 12 h duration, shock, hypotension, coma, fits before admission, absence of meningism and hyperventilation.¹³⁹ Poor prognostic laboratory features include a white cell count of $< 10 \times 10^9/l$, platelet count of $< 100 \times 10^9/l$, ESR < 20 mm/h, metabolic acidosis with pH < 7.30 , CSF white cell count $< (50-100) \times 10^6/l$, and antigenaemia.

Numerous prognostic scoring systems based upon these prognostic indicators have been proposed for use in meningococcal disease: these include several specific to the disease.¹⁴⁰⁻¹⁴² In practice, laboratory turnaround times for investigation, even if short, limit the usefulness of this group of prognostic indicators in the context of the most seriously ill patients, in whom the disease may evolve very rapidly and even a brief delay in initiating therapy should be avoided, but for whom prognostic information would be the most valuable. Ideally, scoring systems to identify the most seriously ill patients should allow therapeutic intervention to be concentrated on this group.

The Glasgow Meningococcal Septicaemia Prognostic Score (GMSPS; table VIII) was proposed as a clinically based score to facilitate the admission of the most ill children to intensive care.¹⁴³ It has been validated retrospectively,¹⁴⁴ showing sensitivity and a negative predictive value of 100%, specificity 95%,

Fig. 4. Comparison of advice to the public on features to watch for published in 26 press reports with clinical details from 14 hospital admissions (both expressed as percentages).

Table VIII. The Glasgow Meningococcal Septicaemia Prognostic Score (GMSPS) (modified from reference 143)

Clinical feature	Points
BP < 75 systolic aged < 4 years	3
BP < 85 systolic aged > 4 years	
Skin – rectal temperature difference > 3°C	3
Modified coma scale* score < 8	3
OR deterioration of ≥ 3 points in 1 h	2
Deterioration in hour prior to scoring	
Absence of meningism	2
Extending purpuric rash	1
OR widespread ecchymoses	
Base deficit (capillary or arterial) > 8.0	1
Maximum score	15

* Modified coma scale		
(i) Eyes open	spontaneously	4
	to speech	3
	to pain	2
	none	1
(ii) Best verbal response	orientated	6
	words	4
	vocal sounds	3
	cries	2
(iii) Best motor response	none	1
	obeys commands	6
	localises pain	4
	moves to pain	1
	none	0
Add scores (i) + (ii) + (iii) to give result.		

and a positive predictive value 74% for death at maximum GMSPS of ≥ 8 . The GMSPS has been used successfully as an entry criterion in a prospective, randomised double-blind treatment trial of anti-endotoxin therapy with polyvalent immunoglobulin (Pentaglobin) and polymyxin E (colistin).¹³⁵ In this prospective study 109 children had a maximum GMSPS of ≤ 7 , and 43 a maximum GMSPS of ≥ 8 . There were no deaths in the former group, but 13 deaths in the latter. The GMSPS predicted mortality with sensitivity and negative predictive value 100%, and specificity 78%, 83% and 87%, and positive predictive value 30%, 36% and 42% at thresholds of 8, 9 and 10 respectively.¹³⁵ Maximum GMSPS was achieved within 6 h of admission in 35 (84%) of 43 children with a score > 7 . The seven items in the GMSPS (hypotension, skin-core temperature difference, coma, acute deterioration, absence of meningism, extending purpura and a gross base deficit) have each been separately validated as components of the score. Other items such as tachypnoea and fluctuating conscious level are also significant, and may allow further refinement of the score. A GMSPS of ≥ 8 in a child with clinical evidence of meningococcal disease is now used in our hospitals to define fulminant meningococcal septicaemia. The GMSPS, *via* this definition, hence determines admission to intensive care and trials of new treatment. The GMSPS has been widely adopted as an aid to clinical management in district general hospitals on Merseyside. It is also gaining acceptance in areas beyond and will be used to stratify results for analysis in the current prospective study of the anti-endotoxin MAb HA-1A (Centoxin) in menin-

gococcal disease. Advantages over other scores are that: it is clinically-based (which avoids delay waiting for laboratory test results); it can be performed equally well by medical or nursing staff after minimal training; it can be repeated to develop information on the change in clinical status; and it does not require specialised equipment or expertise. Theoretical disadvantages of intra- and inter-observer variability have not proved to be a problem in practice, as long as the score is interpreted by an experienced member of the clinical staff. It has been noted that children with high scores on admission may have lower scores after resuscitation. High scores should not justify withholding treatment from the most ill patients.

Effect on management

Study of clinical features and use of prognostic indicators in clinical management has resulted in better recognition of shock and more aggressive treatment of the critically ill child. Early use of penicillin in primary care, prompt transfer to hospital, and avoidance of lumbar puncture in a clinically unstable child have been general benefits. Volume expansion with plasma to treat shock, rapid progression to inotropic therapy with dobutamine followed by dopamine for non-responders, invasive monitoring and elective ventilation for the critically ill child with a GMSPS of ≥ 8 have all been confirmed as our standard approach to fulminant meningococcal disease during the last 5 years. There has been a reduction of mortality in fulminant meningococcal septicaemia from 74% in 1977–1986 to 30% in 1988–1990 concomitant with the introduction of this more aggressive approach.

Conclusions

Recognition of the features of meningococcal disease, in particular the purpuric rash, is the key to early diagnosis in primary care. Better education of medical staff about the clinical presentation and pathophysiology of the disease will result in more accurate use of terminology, so focusing attention on the septicaemic nature of the illness. In secondary and tertiary care this should result in better management of the gram-negative shock in the severest cases.

Education of the public and the media (newspapers, television, etc.) should be a prime concern of the Consultant in Communicable Disease Control in close liaison with paediatricians and physicians. Education and publicity should avoid prominent use of the term “meningitis” which can misinform, but should emphasise the vasculitic rash.

Scoring systems that can drive certain aspects of clinical management, such as admission to intensive care, are important as entry criteria for treatment trials, and will become mandatory components of protocols for the use of costly new therapies. The GMSPS is currently the most useful scoring system for the practising clinician.

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CHEMOPROPHYLAXIS OF MENINGOCOCCAL DISEASE

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Introduction

Meningococcal disease is a life-threatening infection and even in survivors may induce lasting debility. It is a communicable disease with a propensity to spread within families and communities. The two main strategies for preventing meningococcal disease are vaccination and chemoprophylaxis. Although capsular polysaccharide vaccines are available for prevention of disease caused by meningococci of groups A and C these are not fully efficient in young children (< 2 years).¹⁴⁵ In hyperendemic areas such as Western Europe, group-B meningococci are the major causes of disease and as yet there are no uniformly effective group-B vaccines.¹⁴⁶⁻¹⁴⁸ This means that, at present, chemoprophylaxis is the only intervention available for prevention of group-B meningococcal disease. Furthermore, the current vaccines do not eliminate nasopharyngeal colonisation¹⁴⁹ and thus do not provide herd immunity.

Rationale

The pathogenesis of meningococcal disease involves initial colonisation of the nasopharynx followed by translocation to the blood stream. This may result in asymptomatic bacteraemia, fulminant meningococcal septicaemia, meningitis or meningitis with septicaemia. It has been shown that close household contacts of patients with meningococcal disease are themselves at a much higher risk of developing meningococcal disease than the general population. One study from the USA indicated that in the 28-day period following a case there was a 500-800-fold greater risk to close contacts than for the population in general.¹⁵⁰ The risk of transmission is greatest in the first week after contact.^{151, 152} In a Belgian study, 70% of secondary household cases occurred in the week following contact, 13% in the second week, 6% in the third week and 11% in the period 21-60 days after contact.¹⁵² In a study in the UK, secondary cases were reported after > 33 weeks following initial contact.¹⁵³ In the Belgian study a relative risk of > 1200 was found for household contacts in the 60 days following contact. The relative risks for day-care nursery and nursery school contacts were 76 and 23 respectively.¹⁵² Although young children are at greater risk of acquisition of disease, all age groups are susceptible. Early studies among army recruits in "boot-camps" demonstrated

that administration of sulphadiazine eliminated carriage and prevented secondary cases of meningococcal disease.¹⁵⁴

Strategy

There are two main strategies employed for preventing secondary cases in household contacts. The first is in effect pre-emptive therapy. This is based on the fact that the majority of secondary cases occur in the first week following contact. Phenoxymethyl-penicillin or amoxycillin is given for 7 days.¹⁵⁵ This has the advantage that it can be used in pregnant or lactating women. However, penicillin does not eradicate oropharyngeal carriage;¹⁵⁶ therefore, the strategy does not remove the risk of secondary cases occurring after the period of treatment. The second strategy is in more widespread use. This involves administration of antibiotics to which the meningococci are susceptible and which are excreted into the oropharyngeal mucosa. The aim of this strategy is to eliminate oropharyngeal carriage, and thereby prevent meningococcal disease.

Antimicrobial agents

The ideal antimicrobial agent for chemoprophylaxis should be highly active against meningococci, re-excreted into the oropharynx, non-toxic and have minimal effect on the normal flora. The administration regimen should be simple and involve only a short course of therapy.

Although it is stated that many antibiotics, including penicillin, ampicillin, erythromycin, tetracycline, cephalexin and chloramphenicol have failed to eliminate pharyngeal colonisation with meningococci,¹⁵⁷⁻¹⁵⁹ there are few trials demonstrating the degree of failure. For the most part, data come from studying cases of meningococcal disease who were treated with a particular antibiotic which failed to eradicate oropharyngeal carriage.¹⁶⁰ However, one trial showed that erythromycin failed to eradicate meningococcal carriage in seven out of seven carriers and penicillin (600 000 units of aqueous procaine penicillin twice daily intramuscularly for 2 days) failed in 61 of 98 carriers.¹⁵²

However, there are several antimicrobial agents for which adequate trial data are available (table IX). Sulphonamides, in particular sulphadiazine, have been used extensively for prophylaxis since World War II, and have proved extremely safe and effective.¹⁵⁴ Unfortunately, sulphonamide-resistant meningococci are now highly prevalent^{161, 162} and sulphonamides should not be used unless it is known that the isolates are sensitive.

Minocycline has been used either alone or in combination with rifampicin^{163, 164} and has proved highly effective. However, its use should be avoided since it produces an unacceptably high incidence of side effects¹⁶⁵ and it should not be used in children and pregnant or lactating mothers.

Table IX. Examples of antimicrobial agents for chemoprophylaxis

Antimicrobial agent	Number of carriers treated	Number from whom carriage was eradicated	Efficacy (%)	Reference
Penicillin	118	41	35	156
Erythromycin	7	0	0	156
Sulphadiazine	66	63	95	154
Rifampicin	48	37	77	169
	13	12	92	166
	36	27	75	172
	64	63	98	167
Ceftriaxone*	68	66	97	172
	37	34	92	167
Ciprofloxacin†	57	51	89	167
	336	326	97	173
	23	22	96	174
	12	11	92	175

* Single intramuscular dose.

† Single oral dose.

Rifampicin has been in extensive use in recent years and is currently the drug of choice. A 2-day course of rifampicin is effective in eradicating meningococcal throat carriage in 95–98% of individuals.^{166,167} Unfortunately, rifampicin-resistant meningococci are emerging^{168,169} and some have even emerged during prophylaxis.¹⁷⁰ Problems with the use of rifampicin include orange colouration of urine, orange staining of contact lenses and induction of hepatic microsomal enzymes which might render the contraceptive pill ineffective. Long-term therapy with rifampicin is contra-indicated in pregnancy, liver disease and alcoholism. Although it is unlikely that a short course would be harmful, nevertheless there is insufficient evidence to show that it is safe in pregnancy.¹⁷⁰ A further complication arises in countries where tuberculosis is highly prevalent since rifampicin is part of first-line therapy.

The third generation cephalosporin, ceftriaxone, has been found to be highly effective in eradicating throat carriage of meningococci.^{167,171,172} In controlled trials, ceftriaxone has been shown to be 97%¹⁷² and 92%¹⁶⁷ effective compared with rifampicin at 75%¹⁷² and 98%,¹⁶⁷ respectively. Ceftriaxone is given as a single intramuscular injection (250 mg for adults and 125 mg for children under 15 years), and it can be given to young children and pregnant women. Its major disadvantage is the need for an intramuscular injection which may be painful. Among the new fluoroquinolones, ciprofloxacin is effective in eradicating throat carriage of meningococci. In both open^{173–175} and comparative¹⁶⁷ trials, a single oral dose (500 mg or 750 mg) of ciprofloxacin has been shown to be 89–97% effective in eradicating carriage. The benefit of a single dose regimen is that compliance is good and side effects are few. Fluoroquinolones are contra-indicated in pregnancy and they are not licensed for routine use in children because cartilage

damage to the weight bearing joints has followed administration to young beagle dogs. However, it is unlikely that a single dose would produce such damage in children and, in a study in children in Malawi, single dose chemoprophylaxis resulted in side effects in <1% of those treated and there were no joint problems.¹⁶⁷

Who should receive prophylaxis?

This can be a difficult problem. Household contacts are usually defined as those sleeping in the same household and those likely to have exchanged saliva either by kissing or coughing. The incubation period of meningococcal disease is 2–10 days and the prophylaxis should be given to those who have been household contacts at any time during the incubation period. There is no evidence to suggest that hospital personnel are at a greater risk unless they have given mouth-to-mouth resuscitation. A more vexed problem is contacts in schools or nursery schools or play groups. From the Belgian study, the risk to contacts in nurseries was considerably less than for household contacts. Nevertheless, it was 76- and 23-fold greater than the corresponding age groups in the general population for children in day-care nurseries and pre-elementary schools, respectively.¹⁵² Guidelines have recently been revised to offer chemoprophylaxis to children in contact with a child with meningococcal disease attending the same pre-school nursery or group.¹⁷⁶ Although transmission of meningococcal disease has been documented in schools,¹⁷⁷ the degree of risk has not been estimated accurately. Current guidelines suggest that chemoprophylaxis should be considered only in boarding schools (a situation similar to household contacts) or if two or more cases occur within a 6-month period.

There is little evidence that chemoprophylaxis given during an epidemic affects its course. The circulation of meningococci during epidemics is so great that those given chemoprophylaxis may well become re-colonised. Finally it must be remembered that the index case may well require chemoprophylaxis, especially if their meningococcal disease is treated with penicillin and chloramphenicol. Also, vaccination of close household contacts is not a substitute for chemoprophylaxis since protective antibody levels will take a minimum of 2–4 weeks to be achieved and, as described earlier, the risk of transmission is greatest in the first week of contact.

Does it work?

There is little doubt that appropriate antimicrobial agents eradicate throat carriage of meningococci with success rates of 77–97% (table IX). Reasons for failure will include poor compliance, inadequate dosage^{153,178} and rapid re-colonisation following acquisition from other colonised individuals.¹⁷⁹ Since the pathogenesis of meningococcal disease involves initial colonisation

of the throat, it is assumed that eradication of colonisation prevents disease. There is no formal proof of this for household contacts, indeed cases do arise after rifampicin prophylaxis.^{153,179} In contrast, there is evidence that chemoprophylaxis does prevent disease in military recruits. For example, in one military community, 17 cases of meningococcal meningitis occurred in a group of recruits not given prophylaxis whereas only two cases occurred in a similar group of recruits with the same risk of exposure who were given sulphadiazine prophylaxis.¹⁵⁴

Concluding remarks

There does seem to be compelling evidence for giving chemoprophylaxis for contacts of cases of meningococcal disease. Several antimicrobial agents have good efficacy, varying degrees of ease of administration and little risk of side effects. However, the use of chemoprophylaxis is cumbersome, elicits anxiety among donors and recipients alike, has only short-term benefit, and is expensive.¹⁸⁰ Clearly, since meningococcal disease is an important cause of morbidity and mortality, mass vaccination would be a more useful intervention. This will require the development of new polysaccharide-protein conjugate vaccines for groups A and C meningococci and effective vaccine for group-B meningococci.

MENINGOCOCCAL VACCINES

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Introduction

The major problem in the control of meningococcal disease continues to be the lack of an effective vaccine against *N. meningitidis*. The ideal vaccine would be effective against all strains of meningococci, would induce long-term immunity and would be suitable for incorporation into the normal childhood immunisation programme. The currently available vaccines fall far short of these goals. This review will briefly describe the background to the development of the current vaccines and their limitations, the current status of experimental vaccines and some of the prospects for future developments.

Antigenic structure of the meningococcus

Following the early work of Heist in the 1920s, it has become largely accepted that natural immunity to meningococcal infection is correlated with presence in individuals of serum bactericidal activity against the meningococcus.¹⁸¹ Indeed, Heist provided a tragic demonstration of this thesis himself, lacking bactericidal activity in his own serum he contracted meningococcal meningitis and died before his seminal paper was published. Therefore, since then the de-

velopment of meningococcal vaccines has been based on attempts to understand the antigenic structure of the bacteria and in particular to identify those antigens on the surface which are recognised by the antibodies that promote complement-mediated bactericidal killing.

Virtually all isolates from invasive disease produce an extracellular, anti-phagocytic capsule composed of polysaccharide. Meningococci can be divided into a number of serogroups based on antigenic differences between the capsular polysaccharides, with most disease caused by serogroups A, B and C.¹⁸² Underlying the capsule is the outer membrane which, like that of other gram-negative bacteria, contains LOS and a restricted number of proteins, some of which are responsible for further antigenic differences between strains. The major OMPs have been divided into five structural classes (class 1, 2, 3, 4 and 5 proteins).¹⁸³ All strains possess either a class 2 or class 3 protein as the predominant protein of the outer membrane, expression of the two classes being mutually exclusive. The class 2/3 proteins show antigenic diversity between strains, which forms the basis for differentiation of strains into serotypes; similar differences in the class 1 proteins are responsible for subtype specificity.¹⁸⁴ The class 4 protein is highly conserved between strains whereas the class 5 proteins are highly heterogeneous, undergoing antigenic shift within a single strain during the course of an infection.³⁸

Capsular polysaccharide vaccines

Pioneering investigations by Gotschlich and colleagues into the basis of immunity showed that much of the bactericidal effect of serum could be ascribed to the presence of antibodies directed against the capsular polysaccharide, demonstrating the potential of these antigens as vaccine agents.⁹⁷ This work led to the purification of the capsular polysaccharides and to their ultimate incorporation into the tetravalent vaccine currently available for immunisation against serogroups A, C, Y and W135. Such vaccines have been used successfully to reduce the high incidence of meningococcal infection among military recruits and to halt the progress of epidemics caused by group-A organisms.¹⁸⁵

Despite the success of the capsular polysaccharide vaccines in such situations, they have not been able to influence the incidence of much of meningococcal infection. The purified capsules, like other polysaccharide antigens, do not promote an effective T-helper cell response. Consequently, immunological memory is not induced, the protective antibody response is short lived and there is a poor immune response in young children, the age group who are at greatest risk of infection.¹⁸² Furthermore, the current vaccines provide no protection against the group-B meningococcus, the predominant cause of infection in most temperate countries. The group-B polysaccharide is composed of repeating units of N-acetyl

neuraminic acid with 2–9 linkages. Structurally and immunologically related molecules have been identified on human cells, particularly in developing fetal brain tissue, and it would appear that the poor immunogenicity of the group-B polysaccharide is due to immunological tolerance.¹⁸⁶ Indeed, it has been suggested that caution should be exercised in any attempts to break tolerance by immunisation, because of the possibility of inducing auto-immune processes.

Outer-membrane vaccines

The problems associated with the use of vaccines based on the capsular polysaccharide led to investigation of the use of sub-capsular antigens as immunising agents. The rationale behind such studies was the observation that antibodies directed against outer membrane components promoted complement-mediated bactericidal killing of capsulate strains. Since isolated outer membranes contain LOS with its inherent endotoxic activity, detergent extraction has been used to deplete the LOS. The resulting experimental vaccines consist of membrane vesicles containing a mixture of OMPs. After extensive studies in laboratory animals, such preparations were shown to be both safe and immunogenic in human volunteers and to induce significant increases in bactericidal antibody titres against group-B meningococci.¹⁸⁵ Limited field trials confirmed these observations but could not provide sufficient data to confirm protective immunity. However, the promising results led to further large scale trials in some countries with a particularly high incidence of meningococcal disease.

Since 1974, Norway has had the highest incidence of meningococcal disease in northern Europe, with attack rates reaching epidemic proportions. The case-fatality rate was high at about 10% and a large proportion of cases occurred in teenagers.¹⁸⁷ The overwhelming majority of the cases were caused by meningococci of serogroup B, type 15 and subtype 7,16. Therefore, a vaccine consisting of LOS-depleted outer-membrane vesicles was prepared from one such isolate. The vaccine was used in a double blind, placebo-controlled trial with 170 000 secondary school students that took place during 1988–1991. The participants received two immunisations at an interval of 6 weeks. At the completion of the trial, analysis of the data showed significant protection against group-B meningococcal disease, with a calculated protection rate of 57%.¹⁴⁷ However, it was concluded that the overall level of protection was too low to justify full scale public vaccination.

A similar efficacy trial in teenagers has also been performed in Cuba with OMP-based vaccine, although the precise composition of the vaccine has not been revealed.¹⁴⁶ The observed efficacy rate was 83% and, as a result, mass immunisation of the population between 3 months and 20 years of age has been carried out. The estimated overall efficacy following mass vaccination was reported to be 93%, and the incidence

of meningococcal disease in children under 6 years old showed evidence of a dramatic decline.¹⁴⁶ However, contrasting results have been obtained after an attempt to use the same vaccine to halt an epidemic of serogroup-B disease in Sao Paulo, Brazil. A case-control study showed a considerable variation in estimated vaccine efficacy, which could be related to the age of the immunised group.¹⁴⁸ Protection was calculated at 74% for children older than 4 years while no protective effect could be demonstrated in children younger than 2 years of age. Unfortunately the overall incidence of infection in the population aged 1–6 years showed no difference before and after the vaccination campaign. It was concluded that while the vaccine might be effective in preventing epidemic serogroup-B meningococcal disease in older children and adults, its usefulness for control of epidemic disease was questionable.

A somewhat different strategy has been used by Zollinger and colleagues in the development of an OMP-based vaccine used for field trials in Chile.¹⁸⁸ In contrast to the LOS-depleted vesicles described above, the vaccine contained more highly purified OMPs present as soluble macromolecular aggregates non-covalently complexed with group-C capsular polysaccharide. The vaccine was given in two doses, 6 weeks apart, and induced antibody responses in vaccinees which were bactericidal for group-B meningococci, although antibody levels were found to have declined markedly after 6 months. Nevertheless, subsequent analysis of the incidence of meningococcal infection reported a protective effect of *c.* 70% in vaccinees aged 5–21 years, although no significant protection was detected in those aged 1–4 years.¹⁸⁹

Although the results of the vaccine trials described above show some significant differences, particularly in the reported protective effects, general conclusions can be drawn. Most importantly, it is clearly possible for OMPs to induce a bactericidal response and to produce significant protection against group-B meningococcal disease. However, it would appear that, at least with the vaccination regimens so far used, the protective effect may be of rather limited duration and that effective immunity is not induced in young children. Nevertheless, the results obtained to date are encouraging for the ultimate prospect of the development of OMP-based vaccines effective against group-B meningococci. Further work is clearly needed to address the problems of improving immunogenicity and prolonging the duration of the effective immune response.

Future developments

Improved outer-membrane vaccines. The partial success of the OMP-based vaccine will clearly stimulate further studies. In addition to improvements to the vaccination regimens, such as the inclusion of a booster immunisation to prolong immunity, improvements to the composition of the vesicles are under

investigation. One potential problem is that outer membranes from all strains of meningococci contain the highly conserved class 4 protein, which is capable of inducing cross-reacting antibodies which are not only non-bactericidal, but also block the bactericidal effect of antibodies directed against other surface antigens.¹³¹ Since class 4 negative mutants can be readily derived in the laboratory, it is likely that future OMP-based vaccines will be formulated to exclude this protein. Additional proteins will be included in the membranes by growth of bacteria under conditions, such as iron deprivation, where they produce proteins which may be expressed during infection, but only in limited amounts during normal laboratory growth.¹⁹⁰

One problem posed by the outer-membrane vesicle vaccines is that the protection induced may be serotype and subtype specific, since the proteins with these specificities are the major components of the vaccines. However, it has been claimed that the Cuban vaccine is effective against all types and subtypes tested, although full details are not yet available.¹⁴⁶ This cross-reactivity may be due to the presence of high-mol. wt proteins, the precise nature of which has not yet been defined.

One obvious strategy to increase the potential spectrum of protection would be to include outer membranes derived from a number of different strains. As an alternative, Poolman *et al.* have used recombinant DNA technology to replace the highly variable class 5 protein genes with class 1 protein genes from different strains.¹⁹¹ The recombinants produced expressed four different subtype specificities and the addition of a number of further subtypes is feasible. Since subtype-specific antibodies are highly protective in model systems, a vesicle vaccine produced from such recombinants would be expected to have protective effects against a wide variety of strains.

Improved capsular vaccines. As discussed above, the problems of the current capsular polysaccharide vaccines in control of group-A and -C infections are largely due to the T-cell independent nature of polysaccharide antigens. Similar problems encountered in immunisation against *Haemophilus influenzae*, type b have now been resolved by the development of conjugate vaccines in which the capsular polysaccharide is coupled to a carrier protein which provides T-helper cell activity.¹⁹² This ensures an effective IgG response and induction of immunological memory even in young children. Such vaccines have been used in Finland and the USA for some time and have recently been incorporated into the normal childhood immunisation programme in the UK. Similar conjugate vaccines have also been prepared by coupling the meningococcal group-A and -C polysaccharides to carrier proteins such as tetanus toxoid.^{193, 194} The conjugates elicit an immune response indicative of their conversion to T-dependent antigens. Thus it would appear that the production of considerably more effective vaccines against infections caused by groups A and C meningococci is now scientifically

feasible. It is to be hoped that vaccine manufacturers will enable this to become a reality in the not too distant future.

Studies on the development of conjugate vaccines have also introduced the possibility of inducing an immune response against the normally non-immunogenic group-B capsule. A B-polysaccharide-tetanus toxoid conjugate has been shown recently, in laboratory animals, to induce antibodies directed against the polysaccharide *via* a T-dependent immune response.¹⁹³ Such studies open the possibility of using conjugates for human vaccination but raise the question of the desirability of breaking tolerance to a "self" antigen. However, the authors argue that naturally occurring antibodies directed against the group-B capsule can be detected commonly in adults and are acquired *via* the placenta in neonates, without any neurological consequences.

By use of a similar approach, Jennings *et al.* have prepared group-B polysaccharide-tetanus toxoid conjugates, in which the N-acetyl groups on the neuraminic acid have been replaced by propyl residues.¹⁹⁴ They found that the conjugate induced high levels of antibodies in mice, which were bactericidal for group-B organisms. Of particular interest was the observation that the serum contained two distinct populations of antibodies. The first reacted with purified capsular polysaccharide and were non-bactericidal. The second group of antibodies contained the bactericidal activity and appeared to recognise conformational determinants present only on the native capsule and hence not present on human tissue. These observations raise the question of whether, in the future, it may be possible by appropriate manipulation to induce only the bactericidal, meningococcal specific, antibodies and hence avoid the possibility of auto-immune effects.

Currently, however, the possible use of group-B conjugates still faces the dilemma that only immunisation of man will be able to address the remaining concerns over possible adverse effects of breaking tolerance. It appears likely that initial immunogenicity studies will be carried out in adult males. Only then would it be possible to consider immunising females (who may become pregnant) and young children, the ultimate target group.

Subunit based vaccines. One conclusion of the partial success of the experimental OMP-based vaccines is that an improved vaccine must direct the immune response more specifically towards those antigenic determinants that can induce a protective immune response.¹⁸⁸ It is not clear from the field trials which component(s) of the vaccines are primarily responsible for the observed protective effect but evidence implicates the class 1 and class 2/3 proteins, which are the major components of the vaccines. This would be in accord with laboratory experiments which have shown that MAbs directed against these proteins, particularly the class 1 protein, are the most effective in bactericidal killing and in animal protection experiments.¹⁹⁵

Class 1 protein. The class 1 protein is, therefore, an attractive vaccine candidate in its own right. However, the presence of other components in the current experimental vaccines, combined with the difficulty of purifying a membrane protein while retaining its native form, have prevented more detailed studies with the antigen itself. Therefore, an alternative strategy is to focus on those regions of the protein which induce the protective antibodies and to attempt to direct the immune response specifically at these determinants. Work in our laboratory has led to an understanding of the immunochemical structure of the class 1 protein and to the localisation of these protective epitopes. The class 1 protein genes from a number of different strains have been cloned¹⁹⁶ and sequenced, resulting in the availability of the amino-acid sequences of the proteins.¹⁹⁷ This sequence information has been used to construct a model for the organisation of the protein within the outer membrane.¹⁹⁸ These studies predict that the class 1 protein molecule contains a series of amphipathic β -sheets which traverse the outer membrane, generating eight surface exposed hydrophilic loops. Despite antigenic differences the proteins are highly conserved with sequence variation being largely confined to loops 1 and 4. Epitope mapping experiments with synthetic peptides reveal that the protective MAbs recognise antigenic determinants at the tips of these loops, which are the longest and, hence, most accessible to the immune system.^{197, 199} This detailed knowledge of the structure and immunochemistry of the class 1 protein has permitted studies designed to target the immune response directed to these protective epitopes.²⁰⁰ Short peptides have been chemically synthesised to contain the epitope at the top of loop 4, coupled to carrier protein and used for immunisation. Despite inducing a good immune response to the immunising agent the resulting antisera reacted poorly with the native outer membranes and were non-bactericidal. In order to more closely mimic the native conformation of the class 1 molecule a larger peptide corresponding to the whole of the surface exposed loop was synthesised and conformationally restricted by cyclisation. Immunisation with this peptide produced antibodies which appeared to recognise the three dimensional structure of the protein and which promoted complement-mediated bactericidal killing of the homologous meningococcal strain. These data demonstrate the potential of synthetic peptide immunogens for inducing a protective immune response against group-B meningococci.

LOS. Antibodies directed against meningococcal LOS are bactericidal and protect against infection in animal models.²⁰¹ Therefore, LOS would represent a potential vaccine candidate were it not for its endotoxic properties. Since the antigenic and toxic determinants of LOS are distinct, one approach is to purify the antigenic oligosaccharide and to use this for immunisation after coupling to a carrier protein.²⁰² An alternative strategy has become possible with the

availability of information on the structures of the molecules associated with different LOS serotypes. Poolman *et al.* have chemically synthesised small oligosaccharides corresponding to the antigenic domain of one LOS serotype and coupled this to a synthetic peptide containing a T-cell epitope from the class 1 protein.²⁰³ However, no information is yet available on the immunogenicity of such constructs and on the biological activities of any antibodies produced.

Other OMPs. In addition to the major OMPs, other surface proteins are under investigation as potential vaccine candidates. Amongst these are proteins induced by growth conditions which may more closely resemble those sometimes found during the course of the natural infection, such as iron deprivation. It had been hoped that, because of conserved biological functions, these proteins might show less strain-to-strain variation than the major surface antigens. However, in some cases, subsequent results have shown significant antigenic differences between strains.^{204, 205} An additional problem is that some of these proteins may be present at insufficient levels in the outer membrane to provide effective targets for bactericidal killing, and it remains unclear whether antibodies directed against such proteins may protect against infection by interfering with essential, biological functions. Nevertheless, preliminary evidence suggests that it may be possible to produce a bactericidal response to one such protein which shows some degree of antigenic cross-reactivity.²⁰⁶ Much work is currently in progress in this area.

Conclusions

The current vaccines based on capsular polysaccharides are clearly unsatisfactory, since they are not effective in the youngest children, who are most at risk of infection. Recent advances in the use of polysaccharide-conjugate vaccines should lead to much more effective vaccines against group-A and -C disease but the problem of group B remains. Field trials with OMP-derived vaccines are encouraging since they clearly show that it is possible to induce protective immunity against group-B infection. This needs to be improved and may be achieved, at least in part, by modifications to the vaccine formulation or the immunisation regimens, or both. Alternative vaccines based on the individual surface antigens responsible for the observed immunity are under active laboratory investigation. Any fully effective vaccine may ultimately need to combine several approaches. Further work is clearly required but it is possible to be optimistic about the ultimate development of a much more effective vaccine against meningococcal infection.

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