Review

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Quest for a broad-range vaccine against *Neisseria meningitidis* serogroup B: implications of genetic variations of the surface-exposed proteins

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Despite the development of new vaccine formulations using new biotechnology resources to combat emerging and re-emerging diseases, serogroup B meningococcal disease is still a worldwide burden, accounting for many deaths and disabilities every year. The successful approach of coupling a polysaccharide (PS) with a carrier protein in order to increase long-lasting immunity could not be exploited against *Neisseria meningitidis* B because of the limitations of using the capsular PS of serogroup B meningococci. Tailor-made vaccines based on exposed proteins were shown to be a promising approach to overcome these flaws. However, the continuous adaptation of surface meningococcal structures to the external environment has led to genetic shifts of potential vaccine-target epitopes, hampering the quest for a broad-range vaccine that could be used against all serogroups, especially against serogroup B.

Introduction

Neisseria meningitidis is a global pathogen responsible for endemic, epidemic and pandemic outbreaks of meningococcal disease (MD) [World Health Organization (WHO) Initiative for Vaccine Research – *Neisseria meningitidis*, http://www.who.int/vaccine_research/diseases/soa_bacterial/en/index2.html]. Epidemic meningitis can be a devastating medical emergency with socioeconomic and public-health implications. The vast majority of cases of MD are caused by one of five serogroups: A, B, C, W135 and Y. Currently, there are 500 000 cases of MD per year worldwide with 50 000 deaths. Serogroup B meningococci are responsible for 20 000 to 80 000 cases per year worldwide, accounting for 2000 to 8000 deaths annually according to WHO reports (WHO, 1998). The disease is potentially fatal, and even in affluent countries with well-organized health care, 5–10 % of patients die and about 20 % of the survivors suffer from a number of significant sequelae.

Pathogenicity and disease

N. meningitidis, the meningococcus, is a harmless commensal of the human nasopharynx and, for reasons that are still poorly understood, occasionally causes septicaemia and meningitis. The major outer-membrane components, capsular polysaccharide (CPS), outer-membrane proteins (OMPs) and lipooligosaccharide (LOS) (endotoxin) have been implicated in meningococcal virulence (Stephens *et al.*, 2007). The CPS mediates resistance against complement-mediated lysis and opsonophagocytosis. The severity of MD is related to the level

of endotoxin in the plasma and cerebrospinal fluid, which in turn determines the intensity of the host's proinflammatory response (Unkmeir *et al.*, 2002). Disease presentation can involve a variety of symptoms resembling other infectious diseases, culminating in meningitis or systemic disease (meningococcaemia). The disease is characterized by an abrupt onset of fever and a petechial or purpuric rash, which may progress to purpura fulminans, and is often associated with the rapid onset of hypotension, acute adrenal haemorrhage (Waterhouse–Friderichsen syndrome) and multiorgan failure (Rosenstein *et al.*, 2001). This scenario is mainly due to bacterial endotoxin release into the bloodstream, which is a crucial part of the inflammatory reaction. LOS is also important in adherence and colonization, as well as in the pathogenesis of fulminant sepsis and meningitis. To decrease the odds of fatality, patients with suspected cases of MD are treated with antibiotics and, despite the severity of the disease, the micro-organism is susceptible to most antimicrobial drugs. However, early administration of adequate antibiotics frequently is not associated with a successful outcome and may also compromise disease diagnosis by conventional laboratory methods (Rosenstein *et al.*, 2001). Moreover, release of LOS into the bloodstream is increased after bacterial lysis with potentially severe consequences. In those cases, earlier antibiotic therapy would be helpful to stop bacterial growth and halt additional release of endotoxin into the bloodstream. The reversion of damage caused by the immune system reaction to endotoxin relies solely on patient health and appropriate medical management (Stephens *et al.*, 2007; Brandtzaeg & Van Deuren, 2002).

Prevention

MD is a global concern. In developed, and in many developing countries, diagnosis of a case of MD leads health surveillance authorities to initiate prophylactic antibiotic administration to all primary contacts of the index case patient. Humans are the only host for meningococci, but as at least 10 % of healthy individuals carry the micro-organism in their nasopharynx, prompt administration of antibiotics to contacts may halt the spread of virulent strains. However, vaccination has been the most effective disease prevention strategy among susceptible populations. The immunogenicity of the polysaccharide (PS) exposed on the capsule of the meningococcus has been used for vaccine development against the most common meningococcal serogroups; however, the protection provided by immunization with PS alone is not T-cell-dependent, leading to poor immunological memory. Also, the immunogenicity of PS in infants, who are the main target of the disease, is low. In order to increase the immunogenicity and duration of protection of PS-based vaccines, meningococcal PS has been chemically conjugated to carrier proteins, inducing a T-cell-dependent response, and resulting in an improved immune response in infants, increased immunological memory, and leading to a booster response to subsequent doses (Rosenstein *et al.*, 2001).

PSs can be converted to T-cell-dependent immunogens when covalently coupled to highly immunogenic proteins, such as diphtheria toxoid (Frasch, 2005). The PS is recognized and processed by B cells, which present the carrier protein to CD4⁺ T-cells, resulting in a typical immune response of a T-cell-dependent immunogen (Suker *et al.*, 2004).

These vaccines are expected to provide long-lasting immunity when given as a series of doses in infancy, and they may provide herd immunity through protection from nasopharyngeal carriage, thereby interrupting the transmission of *N. meningitidis* (Gardner, 2006). With this new approach, a number of capsule vaccines based on PS conjugated to a protein have been developed against *N. meningitidis* serogroups A, C, Y and W135, and other bacterial species (Frasch, 2005). The most successful example is the commercial development and licensure of several Hib conjugate vaccines resulting in the near eradication of Hib disease (Bisgard *et al.*, 1998).

Vaccines against serogroup B meningococci

An apparently unsolved problem remains, however: the design of a broadly effective vaccine against serogroup B MD. The PSs exposed on the surface of these bacteria resemble polysialylated glycoproteins, which are expressed mainly in fetal brain tissue, but are also expressed in adults, and its immunogenicity is extremely poor. While infection with other serogroups of *N. meningitidis* (e.g. A, C, Y, W135) stimulates antibody responses against their CPSs,

infection with serogroup B *N. meningitidis* fails to stimulate an anti-capsular antibody response (Wyle *et al.*, 1972). The tentative enhancing of immunogenicity by using the conjugation strategy with serogroup B meningococci is not recommended by medical and researcher communities, since immune response against structures similar to human cells may lead to auto-immune diseases (Perrett & Pollard, 2005).

Outbreaks of serogroup B meningococci have been reported worldwide, and in some countries health authorities have supported the production of type-specific vaccines in order to contain outbreaks caused by specific genotypes. The identification of novel virulence determinants by a range of genetic approaches, most of which require highly characterized isolate collections, provides the prospect of new approaches to vaccination. Thus, strategies have focused on non-capsular antigens such as OMPs and vesicles.

N. meningitidis has a number of different proteins embedded into the outer membrane of the bacterial cell called OMPs: porins, coiled-coil proteins, lipoproteins, factor-H-binding proteins and others are attached to the outer membrane exposing immunogenic epitopes to the host immune system (Rappuoli, 2000). Additionally, meningococci naturally and frequently release outer-membrane vesicles (OMVs) formed by circular pieces of the outer membrane carrying a number of different OMPs. This feature allowed the design of type-specific vaccines based on variants of such OMPs involved in outbreaks (Stephens *et al.*, 2007). The most successful examples of OMP-based vaccines are those based on the protein PorA. As a porin, this protein has a circular structure exposing eight loops crossing the outer membrane. The tips of these surface-exposed loops present a variety of different amino acids that interact with the host immune system eliciting T-cell-dependent bactericidal antibodies. Single amino acid variations of these epitopes, however, may be sufficient to modify the tertiary structure of the protein loops, interfering in the antigen-immune system interaction (Martin *et al.*, 2000). A recent example of a successful vaccine using this strategy is the MenZB vaccine, which was used between 2001 and 2003 in New Zealand to contain an extended epidemic of MD caused by a serogroup B PorA variant P1.7-2,4 strain. This outbreak resulted in almost 6000 cases of MD since 1991, with over 200 deaths. The outbreak was caused by a single subtype, which accounted for 86 % of all serogroup B MD during that time (Sexton *et al.*, 2004a). The efficacy of the vaccination is estimated to have reached 75 % (Oster *et al.*, 2005; Sexton *et al.*, 2004b). Other countries have experienced outbreaks of serogroup B MD caused by different serosubtypes and this has been a problem for the design of an effective comprehensive serogroup B vaccine (Thomas, 2004; Urwin *et al.*, 2004). There are also several uncertainties about the long-term effects of a vaccine that will protect against only a proportion of those meningococci strains responsible for disease. For example, could the use of type-specific

vaccines result in the increase of disease caused by meningococci expressing antigenic variants that are not part of the vaccine formulation? This has been observed when type-specific vaccines are used against other species such as *Haemophilus influenzae* and *Streptococcus pneumoniae* (Hammitt *et al.*, 2005; Gardner, 2006; Tsang *et al.*, 2007). However, this was not observed in the UK after the broad immunization of children with a serogroup C meningococcal conjugate vaccine (Gardner, 2006).

Other OMPs that have an important role in the immunity elicited by OMV-based vaccines are the porin PorB and the iron-regulated FetA. Both show good levels of immunogenicity, but like PorA, elicit bactericidal antibodies only against homologous strains. The high degree of genetic diversity found in the variable regions of the exposed loops is an obstacle for the use of these targets in a more comprehensive vaccine (Thompson *et al.*, 2003; Dyet & Martin, 2005).

New targets for new vaccines

The costs for the production of new meningococcal vaccines are high and the production of bespoke vaccines for each outbreak is not a good option for health authorities. The quest for broad range serogroup B vaccines has been the research focus of many groups worldwide (Vermont & Van den Dobbelsteen, 2003; Giuliani *et al.*, 2006; Perrett & Pollard, 2005; Mitka, 2005; Bernardini *et al.*, 2007). In the specific case of serogroup B *N. meningitidis*, the advent of a new strategy for the design of new vaccines called reverse vaccinology (RV) (Rappuoli, 2000) has been an important development in the prevention of MD. This computer-based approach has identified a number of potential protein targets for the production of a vaccine that could prevent MD caused by any serogroup. Such comprehensive vaccines would be the 'holy grail' for health authorities worldwide. The production of millions of doses of a single vaccine, to be used in the five continents, could reduce costs to developing countries like those in the meningitis belt in Africa where outbreaks of serogroup A, C and W135 are frequent (Jóðar *et al.*, 2003; Hodgson *et al.*, 2008). Some of the OMPs identified by RV have been shown to be immunogenic and elicit bactericidal antibodies (Giuliani *et al.*, 2005; Jacobsson *et al.*, 2006; Beernink *et al.*, 2007).

NspA

Neisserial surface protein A (NspA) is one of the OMPs believed to be a good target for a new vaccine formulation (Halperin *et al.*, 2007). NspA is a small highly conserved protein (18 kDa, 174 aa), which has been shown to be highly immunogenic, eliciting bactericidal antibodies (Norheim *et al.*, 2005; Moe *et al.*, 2002; Hou *et al.*, 2003). According to the two basic rules for the perfect vaccine component, genetic conservation and immunogenicity, this could be a good target for a new comprehensive vaccine. However, studies in

animals show that NspA loops appear to be small, resulting in poor surface accessibility to NspA epitopes for the immune system, especially in the presence of abundant CPS, thus allowing the epitopes to escape the immune cells (Wedge *et al.*, 2007). On the other hand, because of its high conservation, the *nspA* gene may be considered as a good molecular diagnostic marker since it is present in 99 % of meningococci (de Filippis *et al.*, 2005). Other proteins such as the adhesin NadA, the genome-derived neisserial antigens (GNA) and the factor H binding protein GNA1870 (fHbp) also have been suggested as potential targets because of their high conservation and are still under investigation (Jacobsson *et al.*, 2006; Beernink *et al.*, 2006, 2007; Koeberling *et al.*, 2007, 2008; Comanducci *et al.*, 2004).

NadA

NadA shows a high degree of genetic conservation, and basically can be subdivided into three genetic variants or genotypes based on small blocks of insertions and deletions within strains isolated from different countries (unpublished observations). As an adhesin its role is likely to be related to virulence. This might be the reason why several authors have described the presence of this protein among certain hypervirulent clones, and thus, it would not be a good target since anti-NadA bactericidal antibodies would not protect against non-hypervirulent clones and carrier strains (Beernink *et al.*, 2007). However, new information has arisen from a set of isolates still under analysis showing that the *nadA* gene was present in more than 73 % of the strains analysed, belonging to different hypervirulent and non-hypervirulent clonal complexes (unpublished observations). In addition, whether or not this protein is present among other neisserial serogroups is not clear (Comanducci *et al.*, 2004).

fHbp (GNA1870)

The exposed lipoprotein fHbp is an important component of the neisserial outer membrane, since its role is to bind factor H, which is a key regulatory protein of the alternative complement pathway. For this reason this protein has been found in all the strains and serogroups so far (Jóðar *et al.*, 2003; Giuliani *et al.*, 2005). It has been divided into three genotypes I, II and III. Genotype I is present in ~80 % and genotype II in ~10 % of the strains investigated according to recent studies (Giuliani *et al.*, 2005; Koeberling *et al.*, 2007; unpublished observations). Since this protein has been shown to elicit bactericidal antibodies, the combination of genotypes I and II in a vaccine could, in theory, protect against more than 90 % of the strains, including hypervirulent and non-hypervirulent clones, carrier strains and all serogroups (Beernink *et al.*, 2006).

GNA33

GNA33 is a less well characterized lipoprotein associated with membrane architecture. It has been described as a

potential new target for vaccine development since it appears to be highly conserved, but a recent study showed that this protein actually elicits protective antibodies to meningococci as a result of mimicking an epitope on loop 4 of PorA. Although there are no studies relating the level of genetic diversity of GNA33, an ongoing analysis of this gene shows that it is highly conserved with minor amino acid changes on specific regions (unpublished observations).

Other approaches

Other approaches to find a universal vaccine to prevent MD are under investigation and include the following.

- (i) Wild-type and recombinant OMVs – these have good immunogenicity and immunological memory after booster doses (Roupe van der Voort *et al.*, 2000).
- (ii) Immunization with vaccines based on commensal *Neisseria* species (*Neisseria lactamica*, *Neisseria cinerea* and *Neisseria flavescens*) – this is expected to induce a protective antibody response against all pathogenic meningococci; however, recent studies (Oliver *et al.*, 2002; O'Dwyer *et al.*, 2004) showed that mice immunized with *N. lactamica* OMVs presented good antibody titres against heterologous *N. meningitidis* isolates, but without bactericidal activity.
- (iii) Immunization with LOS – this has the potential to offer cross-protection against antigenically diverse meningococci; recent studies showed that although immunization with preparations containing LOS elicited high anti-LOS antibody titres, there was no serum bactericidal activity observed against meningococcus (Tiwana *et al.*, 2005).
- (iv) Serogroup B PS – in order to improve immunogenicity and prevent the induction of autoimmune antibodies, the PS is conjugated to a carrier protein and chemically modified; promising results have been achieved with the induction of bactericidal antibodies in monkeys, but these vaccine have not been tested in humans (Granoff *et al.*, 1998; Moe & Granoff, 2001).

OMP genetic shifts

The search for a comprehensive vaccine for broad protection against the bacterial pathogen *N. meningitidis* has been complicated by the antigenic diversity of the organism. Meningococci exhibit marked genetic plasticity in the face of immunological selection and readily exchange antigen genes. Over periods of months to years, *N. meningitidis* can import foreign genes at extremely high frequencies, presumably by transformation with DNA released from other neisseriae during mixed colonization of the nasopharynx. Recombination after the import of DNA from other strains of *N. meningitidis* has been

described several times (Maiden *et al.*, 1996; Morelli *et al.*, 1997; Feil *et al.*, 1999). This recombination would facilitate the escape of strains from the immune system. The likelihood of such switches occurring would be minimized by vaccination against multiple serogroups, but the absence of an effective vaccine against serogroup B may lead to an increase in pathogenic clones bearing the B serogroup antigen (Morley & Pollard, 2001).

A solution seems to be simple and within reach: the use of highly conserved genome-derived proteins should overcome this problem, and the presence of such proteins with the same amino acid sequence among all serogroups of meningococci is an additional positive factor toward the use of these targets.

The extreme response specificity against the most common OMPs such as PorA, PorB and FetA, is a concern. When only small modifications are made to the PorA OMP, representing a specific serosubtype, serum bactericidal antibody activity may be abolished (Martin *et al.*, 2000). Another concern with the widespread use of OMV vaccines, and with OMPs found among epidemic strains, is that the increase in positive selection may lead to a number of new genetic variants arising, as we have found for the three OMPs mentioned above by analysing data in the neisseria.org database (<http://neisseria.org>). So far, PorA has 694 reported variants with the differences distributed among two variable regions; PorB has 127 reported variants with the differences distributed among six loops, which can lead to a fantastic number of different variant combinations within the loops; FetA has 290 reported variants with the differences in a unique variable region. This scenario emphasizes the need for careful selection of different OMPs to cover the present circulating strains and for frequent reviews of the vaccine constituents to account for the frequently changing epidemiology of meningococcal OMPs.

Conclusions

When we look at NspA, which is the most conserved OMP studied so far for vaccine production, we should ask the following questions:

- (i) Why are some exposed proteins highly conserved while others are not?
- (ii) Are these conserved proteins interacting with the immune system?
- (iii) If they are interacting with the immune system, why is the selection pressure over these proteins not as strong as we see with PorA, PorB and FetA, which have hundreds of different variants according to the neisseria.org database?

These questions still need to be answered, but the following statements appear to be valid at this point:

- (i) If an OMP is highly conserved it is likely that it will not interact with the immune system to elicit

satisfactory levels of protective antibodies; thus it might be a comprehensive target, but probably it would not show sufficient immunogenicity to be used in a new vaccine.

- (ii) If an OMP is highly immunogenic it is likely that it has an active interaction with the immune system, but its genetic conservation can be rapidly compromised by natural positive selective pressure, which would be a disadvantage against vaccine coverage.

The overall outcome to this dogma, at least for meningococci, could be the following formula: 'if an OMP is conserved it is not immunogenic, if it is immunogenic it is not conserved'.

These are the obstacles that hamper the quest for a comprehensive vaccine against all meningococci serogroups. Through genomic mining and RV, we have found the path to reach the perfect target, but we have not found, so far, conservation and immunogenicity together in the same protein. In fact, the advent of RV has led to the discovery of a number of new exposed proteins that have been the subject of several studies to determine their genetic diversity and immunogenicity. Based on such studies, the use of fHbp as a broad-range vaccine seems to be a real possibility. Time will answer our major question: are we on the right path? Is this the time to change our views and look for a new strategy? If a fHbp-based vaccine was released in the next 2 years, we could have an idea of its efficacy within the next 5 years. So far, the more answers we find, the more questions we have.

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