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Minireview

Vaccine development against *Neisseria meningitidis*

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Summary

Meningococcal disease is communicable by close contact or droplet aerosols. Striking features are high case fatality rates and peak incidences of invasive disease in infants, toddlers and adolescents. Vaccine development is hampered by bacterial immune evasion strategies including molecular mimicry. As for *Haemophilus influenzae* and *Streptococcus pneumoniae*, no vaccine has therefore been developed that targets all serogroups of *Neisseria meningitidis*. Polysaccharide vaccines available both in protein conjugated and non-conjugated form, have been introduced against capsular serogroups A, C, W-135 and Y, but are ineffective against serogroup B meningococci, which cause a significant burden of disease in many parts of the world. Detoxified outer membrane vesicles are used since decades to elicit protection against epidemic serogroup B disease. Genome mining and biochemical approaches have provided astounding progress recently in the identification of immunogenic, yet reasonably conserved outer membrane proteins. As subcapsular proteins nevertheless are unlikely to immunize against all serogroup B variants, thorough investigation by surrogate assays and molecular epidemiology approaches are needed prior to introduction and post-licensure of protein vaccines. Research currently addresses the analysis of life vaccines, meningococcus B polysaccharide modifications and mimotopes, as well as the use of *N. lactamica* outer membrane vesicles.

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Introduction

Neisseria meningitidis, the meningococcus, is a Gram-negative bacterium belonging to the β-proteobacteria. The species' natural habitat is the human nasopharynx. Animal or environmental habitats are unknown. Asymptomatic colonization of the retropharyngeal wall and the tonsils is observed at high frequency in the second decade of life with a maximum in young adulthood (Claus *et al.*, 2005; Caugant *et al.*, 2007). Carriage frequencies have been scarcely studied in elder individuals. In one Norwegian study, carriage rates of male, but not of female subjects in the third and fourth decade of life were comparable to the high rates in adolescents (Kristiansen *et al.*, 1988). Transmission is dependent on close contact between individuals or exposure to droplet aerosols.

Neisseria meningitidis is closely related to the pathogenic species *N. gonorrhoeae*, a sexually transmitted organism, and to the commensal *N. lactamica*, which colonizes the same niche as meningococci and exchanges DNA therewith (Linz *et al.*, 2000). *Neisseria lactamica* may provide protection against invasive meningococcal disease (IMD) (Coen *et al.*, 2000).

Genome sequences have been published for several strains of *N. meningitidis* (Parkhill *et al.*, 2000; Tettelin *et al.*, 2000; Bentley *et al.,* 2007; Peng *et al.*, 2008; Schoen *et al.*, 2008). In total, the NCBI genome resource lists 38 neisserial genome projects, which are either in progress or already completed. Genome sequences provide an invaluable repository for phylogenetic analyses, studies on the evolution of virulence, and of course for mining for vaccine targets.

The major pathogenicity factor of meningococci is the polysaccharide capsule. The ecological role of capsule expression is unclear, as unencapsulated strains thrive well in the nasopharynx. Furthermore, IMD is an accident during the bacterium's life cycle and may be considered as an evolutionary dead-end implying costs only acceptable to very fit lineages of the species (Buckee *et al.*, 2008). The capsule possibly provides protection against desiccation during aerosol transmission. Furthermore, one might suggest that it protects the bacteria during colonization of inflamed mucosal tissue. However, the identification of

apathogenic capsule null locus (*cnl*) meningococci proofs that meningococci might well proliferate in the population without capsule expression (Claus *et al*., 2002).

There are 12 biochemically distinct capsular polysaccharides. Serogroups B, C, W-135 and Y, which are frequently observed in IMD, contain N-acetyl-neuraminic acid (Neu5Ac, sialic acid) (Bhattacharjee *et al*., 1975; 1976). Serogroup A, a major cause of epidemics in Africa, expresses a capsule of (→6)-α–D-Man*p*NAc-(1→OPO_3→) (Bundle *et al*., 1974). The α(2–8) linked sialic acid homopolymer of serogroup B is identical to a modification of the mammalian neural cell adhesion molecule (NCAM) (Toikka *et al*., 1998), which explains why the serogroup B polysaccharide is poorly immunogenic. The serogroup B polysaccharide is structurally related to the serogroup C polysaccharide, an α(2–9) linked sialic acid homopolymer (Bhattacharjee *et al*., 1975). This polysaccharide and those found in serogroup A, W-135 and Y meningococci are highly immunogenic. W-135 and Y meningococci express heteropolymeric polysaccharides composed of disaccharide units of sialic acid with either galactose or glucose respectively. The capsule polymerases of serogroups W-135 and Y are more than 99% identical (Claus *et al*., 1997) with a single amino acid determining substrate specificity (Claus *et al*., 2009). Serogroup A, C, W-135 and Y polysaccharides can be modified by O-acetylation (Jennings *et al*., 1977; Michon *et al*., 2000; Claus *et al*., 2004; Gudlavalleti *et al*., 2004). O-acetylated polysaccharide formulations of serogroup C were shown to elicit slightly lower antibody responses than de-O-acetylated ones, but this may also be an effect of the carrier protein of the polysaccharide, the conjugation chemistry and the length of the oligosaccharide (Richmond *et al*., 2001). O-acetylation is mandatory for immunogenicity of serogroup A polysaccharide (Berry *et al*., 2002). Serogroup X meningococci have recently emerged in Africa (Djibo *et al*., 2003), but do not yet play a global role.

Serogroup distribution varies on a global scale (Stephens, 2007), which makes it necessary to adapt vaccine strategies to regional needs. Whereas in Europe serogroups B and C dominate, serogroup Y plays an additional role in Northern America. Devastating epidemics due to serogroup A meningococci are observed in the African Meningitis Belt. Figure 1 demonstrates the serogroup distribution in Germany as an example. Of note, in contrast to several other European countries, meningococcus C (MenC) conjugate vaccination has been implemented in Germany quite late in 2006 and without a catch-up campaign including adolescents. Therefore, serogroup C still plays a significant role.

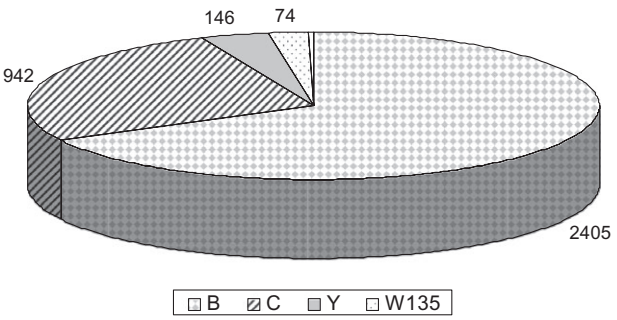

Fig. 1. Frequency of serogroups in invasive meningococcal disease in Germany (2002–09). Data were obtained from the database of the German Reference Laboratory for Meningococci at the University of Würzburg.

Incidences of meningococcal disease, i.e. sepsis and meningitis, in Europe and Northern America are low with values undulating around 1 per 100 000 inhabitants per year. Peak incidences are seen in infants, toddlers and adolescents, explaining the need for childhood vaccination programs. Figure 2 exemplifies age-specific incidences using Germany as an example. The figure highlights differences between serogroup B and C. The low incidences of meningococcal disease provide a challenge for vaccine evaluation, because vaccine efficacy cannot be established in clinical trials and surrogates of protection need to be relied on before licensure.

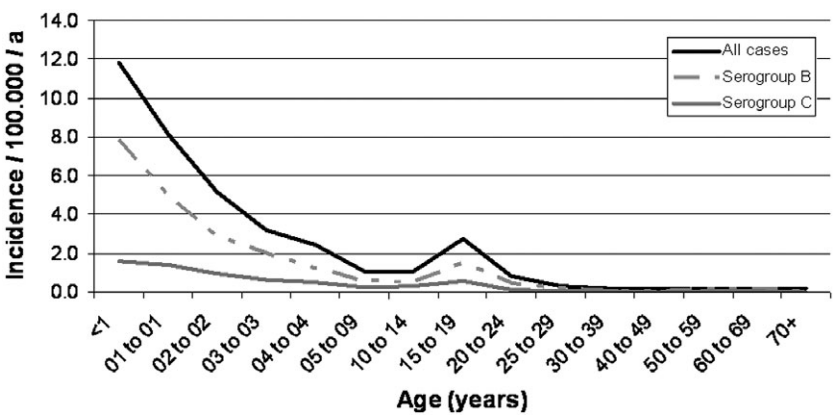

Fig. 2. Age-specific incidence of IMD in Germany (2001–09). Data were obtained from the Robert Koch-Institute, Berlin: Surv*S*tat@RKI, http://www3.rki.de/survstat, data status as of 13 January 2010.

Meningococci exchange DNA by genetic transformation and homologous recombination. Recombination and selection shape clonal complexes, which are groups of related genotypes (Achtman and Wagner, 2008). Successful clonal complexes have been shown to be distributed internationally and to persist for decades (Caugant *et al*., 1986). Members of a clonal complex tend to share alleles of immunogenic outer membrane proteins (Urwin *et al*., 2004). Nevertheless, immune selection drives forward an extensive microevolution of surface antigens (Thompson *et al*., 2003; Russell *et al*., 2004; Brehony *et al*., 2009), which must be considered a major obstacle for the development of protein based vaccines, as vaccines need to cover a major share of variants and immune-escape needs to be monitored.

Surveillance of meningococcal disease in many countries relies on statutory notification and a complementary active or passive laboratory surveillance program. Representative strain collections assembled by reference laboratories are a major resource for evaluation of protein based vaccines, because they can be used to study antigenic variability as well as susceptibility of strains towards bactericidal antibodies elicited by vaccines.

General problems faced in vaccine development

A universal meningococcal vaccine is lacking (Table 1). Due to molecular mimicry, serogroup B capsular polysaccharide is poorly immunogenic, and manufacturers have been deterred from polysaccharide vaccine development by theoretical considerations of autoimmunity and potential fetal damage. A recent population based study from Denmark, however, failed to exhibit evidence for autoimmunity elicited by natural meningococcus serogroup B (MenB) infection (Howitz *et al*., 2007).

Vaccine development against MenB is hampered by variability of surface antigens. Between 2002 and 2005 in Germany alone 33 and 69 variants of the PorA variable regions (VR) 1 and VR2, respectively, were observed (Elias *et al*., 2006). The meningococcal population is versatile and dynamic. There are emerging clones and lineages, and even neglected serogroups such as X may rise as a new problem as observed in Africa (Boisier *et al*., 2007).

Specific aspects need to be addressed during meningococcal vaccine development. Licensure of modern meningococcal vaccines is mostly based on safety data, serological correlates of protection, and – for MenB vaccines – strain coverage. Efficacy studies are mostly conducted after vaccine introduction due to the low incidence of disease. Strain coverage is assessed by the analysis of protein expression in a representative strain panel and by determination of the allelic diversity (Bambini *et al*., 2009; Lucidarme *et al*., 2009; Murphy *et al*., 2009). Serum bactericidal assays are a major effort for MenB vaccines, as in contrast to polysaccharide vaccines, several strains have to be tested. If proteins are used, which are not expressed under routine culture conditions, assay formats have to be adapted, which complicates assay standardization.

Furthermore, one has to consider that asymptomatic carriage is a double edged sword in light of vaccine development. The meningococcal serogroup C conjugate (MCC) vaccine campaign in the UK resulted in a reduction of carriage of a limited subset of strains (Maiden *et al*., 2008), which probably will not affect natural boosting massively. Broadly active meningococcal vaccines, however, might also reduce carriage of apathogenic meningococci, such as *cnl* meningococci (Claus *et al*., 2002), and of *N. lactamica* due to cross reactive antigens (Gold *et al*., 1978; Gorringe *et al*., 2009). Since it is likely that apathogenic strains contribute to natural immunity against disease, an ideal vaccine would rather not touch these variants or species. On the other hand, the vaccine should strongly impact carriage of pathogenic variants to provide for herd immunity as evidenced for polysaccharide conjugate vaccines (Trotter *et al*., 2008).

Meningococcal polysaccharide conjugate vaccine development

Several MCC vaccines have been licensed, which differ with regard to O-acetylation of the polysaccharide, protein conjugate, conjugation chemistry and adjuvant. The vaccines are considered as safe (Pollabauer *et al*., 2005). The UK in 1999 introduced MCC vaccines, which was highly successful and as an added value provided striking scientific knowledge. The efficacy of the vaccines was about 90%. However, there was an age-dependent decline of efficacy over time following vaccination (Snape *et al*., 2005; 2006; Borrow and Miller, 2006). This finding led to the recommendation of a booster vaccination in the infant immunization schedule (Trotter *et al*., 2004). Maintenance of protective titres seems to be essential as the time required for an effective booster response elicited by acquisition of a MenC strain probably exceeds the incubation period (Auckland *et al*., 2006). The success of the vaccination campaign was in large parts due to the fact that herd immunity was elicited (Ramsay *et al*., 2003; Trotter *et al*., 2006). Herd immunity depended on mucosal immunity towards MenC carriage in the UK, which was considerably reduced (Maiden and Stuart, 2002; Maiden *et al*., 2008). Vaccination of adolescents is most effective in this regard, as carriage rates of meningococci increase in the second decade of life (Claus *et al*., 2005). One might have speculated that rates of serogroup switching (genetic alteration of a clone) or replacement (increased occurrence of a variant not expressing the vaccine antigen), which have been shown for meningococci on

Table 1. Summary of vaccine concepts discussed.

Vaccine composition	Indication	Status	Advantage	Disadvantage
Polysaccharide vaccines				
Plain polysaccharide	Epidemic control	On the market	Low cost	Uneffective in small children
	Travel medicine		Efficacy	For some serogroups booster doses are ineffective. No herd immunity.
	Lab workers			
Protein conjugated polysaccharide	Routine toddler/infant/ adolescent vaccination	MenC: on the market	Elicit herd immunity (proven for MenC)	Cost (for most preparations with the exception of the meningococcal A conjugate (PsA-TT) vaccine).
	Epidemic control	MenACWY: on the market/ close to be marketed/in clinical trials	Efficacy in infants and toddlers	Waning immunity in young vaccinees
	Travel medicine Lab workers	MenA: in clinical trials	Memory response	
OMV approaches				
Tailor made (OMVs of epidemic clones)	Epidemic control	Programs have been introduced on several occasions	Effective control of MenB outbreaks and epidemics	Several doses required Immunogenicity in small children may be unsatisfying. Lack of cross-reactivity Time consuming pre-clinical and clinical trials. Poor antibody persistence.
Multivalent PorA vaccines	Broad protection against meningococci	In clinical trials	Theoretically covers most strains	Some PorA variants are poorly immunogenic
OMV from GMO expressing one or more recombinant minor antigens, in some cases in a PorA negative background	Broad protection against meningococci	Pre-clinical	May confer protection against a large panel of strains May avoid dominant effect of PorA	Pre-clinical and clinical assessment of protection may be a difficult issue with regard to *in vivo* expression of antigens
OMV from *N. lactamica*	Broad protection against meningococci	In clinical trials	May confer protection against diverse meningococcal lineages, however, probably by mechanisms independent of bactericidal antibodies. Avoids dominant effect of PorA.	*N. lactamica* carriage in early childhood, which is likely to confer protection against meningococci, might be affected.
Subunit vaccines				
Genome derived recombinant multicomponent vaccine	Broad protection against MenB	In clinical trials	Combination of several targets ensures targeting of many lineages Self-adjuvating effects of OMVs	Complex design
Factor H binding protein presented in two allelic variants as lipoprotein	Broad protection against MenB	In clinical trials	Two antigenic variants for broad coverage Application of lipoprotein with self adjuvating effect	Depends on expression of factor H binding protein and the presence of cross-reactive alleles
Meningococcal secretome	Broad protection against meningococci	Animal models	Many components may ensure broad coverage	Secretome yet not completely deciphered
MenB capsule derived approaches				
N-propionylated polysaccharide	Broad protection against MenB	In clinical trials	Independent of antigenic variability	Theoretically possible induction of autoantibodies Poor induction of bactericidal antibodies in human volunteers

Table 1. *cont.*

Vaccine composition	Indication	Status	Advantage	Disadvantage
de-N-acetylated polysaccharide	Broad protection against MenB	Animal models	Independent of antigenic variability Induction of memory response	Theoretically possible induction of autoantibodies?
Mimotope	Broad protection against MenB	Animal models	Independent of antigenic variability Polysaccharide purification no longer needed	Theoretically possible induction of autoantibodies?
Live vaccine carriers				
S. gordonae expressing NadA and NhhA/Hsf	Broad protection against meningococci	Animal models	Use of attenuated or commensal organisms Induction of colonization Induction of IgA response towards NadA Natural immunization route	Regulatory issues: Release of GMOs NadA not present in every meningococcal lineage
Attenuated unencapsulated *N. meningitidis* strains (ΔsiaDΔrfaF or ΔsiaDΔmetH)	Broad protection against meningococci	Animal models	Protection of mice against heterologous strains Natural immunization route	Regulatory issues: release of GMOs

OMV, outer membrane vesicle; GMO, genetically modified organism.

several occasions (Vogel *et al*., 2000), will increase under selective pressure induced by vaccination campaigns (Maiden and Spratt, 1999). However, the UK disease surveillance did not evidence for increased serogroup switching and replacement (Balmer *et al*., 2002; Maiden *et al*., 2008). The occurrence of serogroup switch variants was reported for Spain (Cano *et al*., 2004). It is unclear whether this observation was due to a different vaccine introduction strategy.

The success of MCC stimulated the introduction and development of novel MenACWY conjugate vaccines (Keyserling *et al*., 2005; Snape *et al*., 2008; Ostergaard *et al*., 2009) and also of combinations of conjugated meningococcal polysaccharide with other components, such as the *H. influenzae* type b polysaccharide (Borrow *et al*., 2010). The serological data published until now for tetravalent polysaccharide vaccines are promising; instruments to control MenACWY disease seem to be available now. However, data are needed on the induction of herd immunity. Of interest is further the development of a serogroup A conjugate vaccine by the Meningitis Vaccine Project (MVP) and collaborating agencies (Kshirsagar *et al*., 2007; LaForce *et al*., 2007), which hopefully will provide a solution to the devastating serogroup A epidemics in the African meningitis belt.

Subcapsular vaccine targets

There is a variety of highly immunogenic structures in the outer membrane, which serve as possible vaccine components. A catalogue has been published recently (Feavers and Pizza, 2009) that categorizes possible candidates as major outer membrane proteins, iron uptake proteins, adhesins, other virulence factors, antigens with unknown function or those involved in membrane architecture, and enzymes. Major outer membrane proteins such as porins are expressed at high amounts (Frasch and Gotschlich, 1974). Other proteins are repressed under *in vitro* culture conditions, but can be observed, e.g. after iron depletion (Grifantini *et al*., 2003). Outer membrane proteins frequently are hard to express or purify in native conformation, so that alternative strategies have to be employed such as the production of outer membrane vesicles (OMVs) (Bjune *et al*., 1991; Sierra *et al*., 1991; de Moraes *et al*., 1992). A variety of lipidated proteins have been described as possible meningococcal vaccine antigens (Fletcher *et al*., 2004; Delgado *et al*., 2007; Hsu *et al*., 2008), which are attractive due to their self-adjuvanting activity.

Besides the induction of bactericidal antibodies, which activate serum complement resulting in bacterial cell death, antibodies against some targets block their function. Some monoclonal antibodies against the meningococcal factor H binding protein (FHBP) bind in close proximity to the factor H binding site and block factor H binding (Beernink and Granoff, 2009). Although the structure of FHBP has been resolved (Cantini *et al*., 2009; Mascioni *et al*., 2009; Schneider *et al*., 2009), precise binding sites of antibodies elicited in vaccinees have not been reported to our knowledge.

Proteins involved in iron uptake have been investigated intensively for their vaccine potential. The transferrin binding protein TbpA and even more so the TbpB are immunogenic and protect mice from meningococcal challenge, e.g. when delivered as recombinant antigen (West *et al*., 2001). Antigenic variability has to be considered for

Tbps, and it has been shown for TbpB that there are two families or isotypes with isotype I being shared between sequence type 11 meningococci and apathogenic species (Harrison *et al*., 2008).

Proteins abundantly present on the meningococcal cell are easily accessible by bactericidal antibodies. However, harsh immune selection may trigger immune escape variants, generated by meningococci through horizontal gene transfer (Feavers *et al*., 1992). It has been suggested that overexpression of poorly expressed minor proteins in a PorA negative genetic background has a synergistic effect by augmenting the topical concentration of bactericidal antibodies on the bacteria above threshold values critical for complement activation (Weynants *et al*., 2007).

Besides protein antigens, lipopolysaccharide (LPS) has been considered a possible immunogen active against MenB (Weynants *et al*., 2009). LPS has self-adjuvant activity, but is toxic. Assays have been developed to study the biological effects of LPS in OMVs (Stoddard *et al*., 2010). Detoxification of LPS in OMV preparations is delicate, as detergent extraction also reduces the amount of immunogenic lipoproteins. LpxL1 mutants impaired in lipid A acylation were shown to retain adjuvant effects while being less toxic (van der Ley *et al*., 2001). Lipid A acylation mutants therefore provide a solution to the toxicity of OMVs.

To combat a regional epidemic, tailor-made meningococcal vaccines have been developed, which are OMVs derived from the epidemic strain (Holst *et al*., 2005). The immunodominant antigen of OMVs is the porin A (PorA). Tailor-made vaccines have been used in Norway, Chile, Cuba and New Zealand (Bjune *et al*., 1991; Sierra *et al*., 1991; de Moraes *et al*., 1992; Oster *et al*., 2005). These vaccines in general have to be delivered in three to four doses. The antibody response increases with age, as does the cross-reactivity towards strains with differing antigens. It is debatable whether strain-specific OMV vaccines have the practical potential to be developed in a flexible fashion in analogy to annual influenza vaccine preparations. Nevertheless, they have proven to be effective in New Zealand (Kelly *et al*., 2007) recently and have been introduced in the Normandie to combat an outbreak of MenB disease (Rouaud *et al*., 2006).

Up to nine different PorA variants are included in OMV preparations by recombinant technology in order to increase the theoretical strain coverage (van Alphen and van den Dobbelsteen, 2008). Outer membrane vesicles have also been used as vehicles to present other recombinant antigens in native conformation (Hou *et al*., 2005; Weynants *et al*., 2007). Finally, OMVs are added to the present formulation of the Novartis investigational vaccine (Rinaudo *et al*., 2009).

Outer membrane vesicles of *N. lactamica*, which lacks the immuno-dominant PorA protein, are in development (Oliver *et al*., 2002; Gorringe *et al*., 2009). Interestingly, these vaccines seem to only poorly elicit bactericidal antibodies, but protect mice from bacterial challenge, probably by augmenting opsonophagocytosis (Finney *et al*., 2008). In a phase I clinical trial the vaccine as well displayed a stronger effect on opsonophagocytosis than on bactericidal antibody concentrations, which was rather low (Gorringe *et al*., 2009).

Subunit vaccines containing recombinant meningococcal proteins now play a prominent role in the field of investigational vaccines for meningococci. The groundbreaking approach of 'reverse vaccinology' demonstrates how genome research results in potential products (Rinaudo *et al*., 2009), and along the way enhances the understanding of meningococcal pathogenicity (Pizza *et al*., 2000; Comanducci *et al*., 2002; du-Bobie *et al*., 2004). Predicted surface exposed proteins were tested for immunogenicity. Consecutively, it was investigated whether the proteins elicited bactericidal antibodies. Finally, a broadly reactive subunit vaccine was designed of recombinant proteins partly presented as fusion proteins (Giuliani *et al*., 2006). One of the proteins is FHBP, previously referred to as GNA1870 (Madico *et al*., 2006), a regulator of the complement cascade. Recruitment of factor H on the bacterial surface blocks consecutive complement activation. Wyeth, also identified FHBP as a vaccine target using biochemical approaches (Fletcher *et al*., 2004; Pillai *et al*., 2005; McNeil *et al*., 2009). The protein was designated LP2086 and is included in two antigenic lipoprotein variants in the investigational vaccine.

Alternative concepts

The MenB polysaccharide, a poor antigen eliciting low affinity antibodies, has been modified by N-propionylation to reduce cross-reactivity towards human glycosylated proteins and augment immunogenicity (Jennings *et al*., 1987; Ashton *et al*., 1989; Fusco *et al*., 1997). Unfortunately, N-propionylated polysaccharides elicited antibodies cross-reactive with human α-2,8-linked polysialic acid, the glycosylation of the neural cell adhesion molecule NCAM (Granoff *et al*., 1998). Furthermore, a human trial with an N-propionylated MenB capsular polysaccharide conjugated to tetanus toxoid was dissatisfying, because the vaccine did not elicit functional antibodies (Bruge *et al*., 2004). Another MenB polysaccharide modification currently tested in animal models is de-N-acetyl MenB polysaccharide (Moe *et al*., 2009). Removing N-acetyl groups at the non-reducing end of the polysaccharide obviously stimulates T cell help and supports the induction of IgG in mice. The search for MenB polysaccharide modifications further stimulated the investigation of polysaccharide mimotopes, e.g. by screening phage display libraries with group B specific monoclonal antibodies

lacking affinity to human polysialic acid (Shin *et al*., 2001; Park *et al*., 2004). Vaccination of mice with mimotopes resulted in bactericidal antibodies (Lo Passo *et al*., 2007).

Another concept pursued is the vaccination of animals with secreted proteins of meningococci obtained from cell- and vesicle-free supernatants (Li *et al*., 2009). The secretome of meningococci has been reviewed recently (van Ulsen and Tommassen, 2006). Li and colleagues (2009) suggest that secreted proteins partly stick to the outer membrane, thereby serving as targets for bactericidal antibodies.

Of interest is the investigation in animal models of attenuated meningococcal strains as live vaccines such as unencapsulated strains with the genotype Δ*siaD*Δ*rfaF* or Δ*siaD*Δ*metH* (Li *et al*., 2004). Furthermore, Ciabattini and colleagues (2008) reported *Streptococcus gordonii* strains expressing the adhesin NadA (Comanducci *et al*., 2002) and the putative serum resistance modulator NhhA (Sjolinder *et al*., 2008), which induced the mucosal secretion of specific IgA in mice (Ciabattini *et al*., 2008). NadA is probably not the best choice for broad protection against meningococci, as it is not expressed in a variety of IMD-associated strains (Comanducci *et al*., 2004; Elias and Vogel, 2007; Lucidarme *et al*., 2009). Nevertheless, vaccination with live bacteria is an interesting issue to pursue. We suggest to consider also *cnl* meningococci, which are constitutively unencapsulated and widely present among healthy carriers (Claus *et al*., 2002; 2005). IMD caused by *cnl* meningococci is extremely rare. We reported one case in a severely immunocompromised patient (Vogel *et al*., 2004), who was the only *cnl* IMD case among more than 3400 cases investigated by the reference laboratory between 2002 and 2009. There are two further case reports of invasive disease due to *cnl* meningococci with one fatal case from Canada and three cases from Burkina Faso (Hoang *et al*., 2005; Findlow *et al*., 2007). There is evidence that *cnl* meningococci represent ancestors of meningococci (Schoen *et al*., 2008). Uptake of the capsule locus is possible in the laboratory (own unpublished observation), but unlikely to occur in nature based on genetic analysis of carrier isolates and theoretical consideration taking into account the size of the DNA fragment harbouring the capsule locus. Data are needed for the persistence of carriage of capsule null locus meningococci and the induction of bactericidal antibodies during carriage. Furthermore, the effect of live immunization practices on the population structure of the bacteria and natural boosting would need to be addressed.

Conclusions

The next years will see the introduction of conjugated meningococcus A vaccines in Africa, of new conjugated tetravalent vaccines, and hopefully also of the first broadly cross-reactive MenB vaccines. A variety of MenB protein vaccine candidates with possibly broad cross-reactivity also among other serogrups are under investigation, with the ones using the FHBP being advanced farthest. It is difficult to provide an estimate of the potential coverage of FHBP vaccines due to geographic effects, the unknown impact of carriage, and the unknown velocity and effectiveness of immune escape once effective herd immunity has been established. Many questions will only be answered after licensure, and one may assume that the

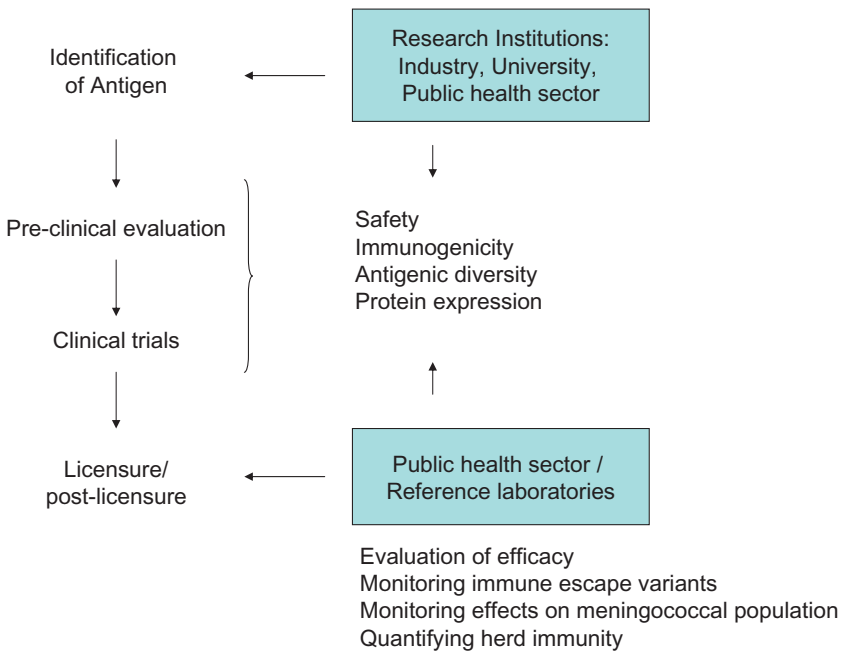

Fig. 3. Development of meningococcal vaccines and depiction of the interaction between research institutions, industry and the public health sector.

first generation of universal MenB protein vaccines will be a starting point of continuous development. For sure, MenB vaccines might affect the population structure of the bacteria and consequently the development of natural immunity. Therefore, carriage studies are necessary, as well as an effective post-licensure surveillance of disease. The integration of various players during MenB vaccine development and introduction is summarized in Fig. 3.

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