

REVIEW

A combined approach to assess the potential coverage of a multicomponent protein-based vaccine

G. BOCCADIFUOCO, B. BRUNELLI, M.G. PIZZA, M.M. GIULIANI
Novartis Vaccines & Diagnostics, Research Serology, Siena, Italy

Key words

Neisseria meningitidis • Correlates of protection • ELISA

Summary

Meningococcal disease caused by Neisseria meningitidis serogroup B is a public health concern even in developed countries. Despite glycoconjugate vaccines against the other invasive serogroups (A, C, W135, Y) are already available and successfully introduced in many countries, no vaccine is currently in use for prevention of serogroup B meningitis.

A protein based, multicomponent vaccine (4CMenB) has been developed and proposed for prevention of invasive serogroup B meningococcal disease (MenB). This novel vaccine has been tested in clinical trials and shown to be well tolerated and immunogenic, inducing bactericidal antibodies in infants, adolescents and adults. The high level of genetic and antigenic variability observed in MenB clinical isolates, requires a suitable method to assess the

ability of the 4CMenB vaccine to cover genetically diverse meningococcal strains and to estimate the potential public health impact. To this purpose the Meningococcal Antigen Typing System (MATS) has been developed and recently described. This method provides a quick and reproducible tool to estimate the level of expression and immunoreactivity of each of the vaccine antigens, in any meningococcal isolate, and it is related to the likelihood that the isolate will be killed by sera from immunized subjects. A multi-laboratory study involving several European countries, demonstrates that the 4CMenB has the potential to protect against a significant proportion of menB strains circulating in Europe.

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Introduction

Neisseria meningitidis is an encapsulated, Gram-negative bacterium, obligate human commensal and occasional pathogen. In the majority of cases it colonizes the upper respiratory tract with no consequence, spreading directly from host to host. Carriage prevalence in Europe and in other countries with a similar epidemiologic pattern is low in young children, reaches the peak in 19 years old (23.7%) and decreases in adults [1, 2]. In a small number of cases it causes invasive disease, entering the bloodstream, crossing the blood-brain barrier and causing sepsis and meningitis. Young children under 5 years are the most exposed age group to invasive disease, as this age group shows the highest incidence [3].

N. meningitidis strains have been divided into 13 serogroups based on differences in the capsular polysaccharide structures. Five of these serogroups (A, B, C, Y, W 135) cause the majority of endemic, epidemic and pandemic outbreaks of meningitis. Serogroups are distributed worldwide: serogroup A has been associated with epidemic outbreaks in sub-Saharan Africa, and to a lesser extent in Asia; serogroup B is the main cause of prolonged outbreaks in Europe, America and New Zealand; serogroup C strains are spread worldwide and caused local outbreaks in Western Europe and North America, mainly in adolescents and young adults [4]. In the last twenty years, serogroup Y has emerged as an important cause of disease in North America and Israel

while local outbreaks of serogroup X strains have been reported in sub-Saharan Africa [5, 6].

Capsular polysaccharide (CPS)-based vaccines against meningococcal serogroups A, C, Y & W135 have been available for several years, and more effective conjugated vaccines have been introduced, in which CPS components are conjugated to carrier proteins such as CRM197, a non-toxic mutant of the diphtheria toxin [7]. MenACWY-CRM has been shown to be immunogenic in all age groups, including infants [8]. The introduction of routine vaccination programs have significantly reduced the incidence of serogroup C disease in many countries including Italy, UK, Australia, Brazil and Argentina [9].

Despite the successful prevention campaign against meningococcal disease, serogroup B meningitis is still an important concern in industrialized countries. For example, approximately 90% of the cases in England and Wales are currently due to serogroup B meningococci [10] while the overall proportion of serogroup B cases has been 71% among 9615 cases reported in Europe during 2008-9 [11].

The group B polysaccharide is poorly immunogenic, even when conjugated to a carrier protein; furthermore, the capsular polysaccharide chemical composition, which is similar to glycosylated structures in human cells, could facilitate the onset of auto-immune disease [12, 13].

Many efforts have been made over the last years to find broadly protective antigens against group B meningococci, but the high sequence variability of candidate pro-

tein antigens has been a serious challenge for vaccine developers [6]. Outer membrane vesicle (OMV) protein-based vaccines have been developed based on the ability of meningococcus to produce and release vesicles composed of outer membrane proteins, lipids and periplasmic components. The OMV vaccines used in Norway, Cuba, Chile and New Zealand to address local epidemic outbreaks have all been prepared from the wild-type strains causing the outbreaks [14-17]. OMV-based vaccines induce antibodies in all age groups and although effective in protecting against epidemic disease, they generally elicit protection only against the homologous strain [18]. This is mainly due to the high sequence variability of the PorA antigen, which is the immunodominant and most abundant membrane protein. Based on these observations, each single OMV vaccine is not applicable for universal use and effective menB vaccines based on different surface exposed proteins have therefore been investigated.

4CMenB vaccine

The introduction of reverse vaccinology has changed the concept of vaccine development facilitating the identification of promising candidate antigens, starting from genome sequence as an alternative to microbial culture purifications [19]. The whole genome sequence of a virulent strain (MC58) was used to identify candidate vaccine antigens, among the potential surface-exposed proteins. Using this approach, 28 Genome-derived Neisseria Antigens (GNA) were found to induce bactericidal antibodies [20].

The antigens selected by reverse vaccinology have been further investigated by means of Serum Bactericidal Assay (SBA) and passive protection assays in animal models for their ability to induce broad protection. Three surface exposed proteins were selected as components of the vaccine: GNA1870 lately named factor H binding protein [fHbp; 21-22-23], GNA2132 lately named Neisserial Heparin Binding Antigen [NHBA; 24] and GNA1994 lately named Neisseria adhesin A [NadA; 25]. To facilitate large-scale manufacturing and to increase immunogenicity, two antigens were fused with meningococcal accessory proteins GNA1030 and GNA2091 to create the fusion protein antigens GNA2091-fHbp and NHBA-GNA1030. These two fusions were combined with NadA and formulated with aluminium hy-

droxide to produce a novel recombinant MenB vaccine (rMenB). The sera obtained by immunizing mice with rMenB vaccine were tested by SBA against a panel of 85 globally distributed menB isolates and showed killing of 78% of the strain [26].

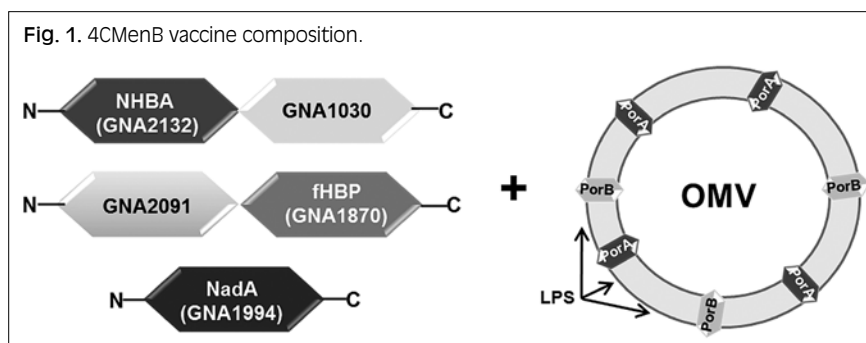
The rMenB vaccine has been also tested in Phase I and Phase II clinical trials alone or in combination with OMVs derived from the New Zealand strain NZ 98/254 [27, 28], expressing PorA serosubtype P1.4 (rMenB + OMV) which was shown to be safe and efficacious in controlling a clonal meningococcal serogroup B epidemic in New Zealand [17]. The final vaccine formulation, which includes also OMVs (Fig. 1), was named four component meningococcal serogroup B vaccine (4CMenB). The reason for using all the three main antigens combined with OMV, was to provide a broader strain coverage and reduce the risk of escape mutants.

Vaccine immunogenicity

Assessing the efficacy of a protein-based vaccine for a large panel of strains representative of the global MenB disease burden is a serious challenge for both vaccine manufacturers and regulatory agencies. Human complement (hSBA) measures the ability of human sera antibodies to kill bacteria by activating the classical complement pathway. Acquisition of a 1:4 SBA titer threshold measured using hSBA has been acknowledged as the appropriate correlate of protection to assess the efficacy of candidate vaccine [29].

However, hSBA is labor intensive and requires adequate volumes of serum sample, a particularly limiting parameter in infants. Therefore, the testing of numerous strains by numerous subjects quickly becomes untenable. The hSBA is nevertheless essential to screen potential human complement sources lacking endogenous anti-meningococcal antibodies against the test isolates. Moreover, a high number of assays can be required to establish cross protection of bactericidal antibodies across antigens variants or subvariants, and to understand whether observed differences in SBA are due to sequence variability or to the level of protein expression [30]. When a SBA assay is performed using immune sera from humans vaccinated with the 4CMenB vaccine, the assay will not discriminate between the effects of different antigens and bacteria are killed by combinations of antibodies elicited by multiple antigens.

In order to assess the immunogenicity of each of the 4CMenB vaccine components, it is necessary to measure antigen-specific bactericidal antibodies against *N. meningitidis* strains in which killing is directed primarily against a single antigen represented in 4CMenB [31]. Using this approach, a minimal strain panel can be used to assess whether a sero-



response to the vaccine has occurred. Sera from subjects belonging to different age groups immunized with 4CMenB have been tested in the bactericidal assay with human complement to measure vaccine specific bactericidal responses using this panel of strains. The data showed that most subjects elicited bactericidal antibodies against each of the major antigens, demonstrating that each component is able to induce protective bactericidal response in humans [27, 28, 32-35].

However, assessing the potential strain coverage of 4CMenB is a challenge due to heterogeneity of vaccine antigens across MenB strains which can impact antibody-mediated complement-dependent killing.

Conventional typing methods used for *N. meningitidis*, such as serotyping and serosubtyping or multilocus sequence typing, are not necessarily relevant to predict bactericidal killing in the SBA assay and to estimate 4CMenB vaccine strain coverage.

A quick and reproducible tool to estimate vaccine coverage

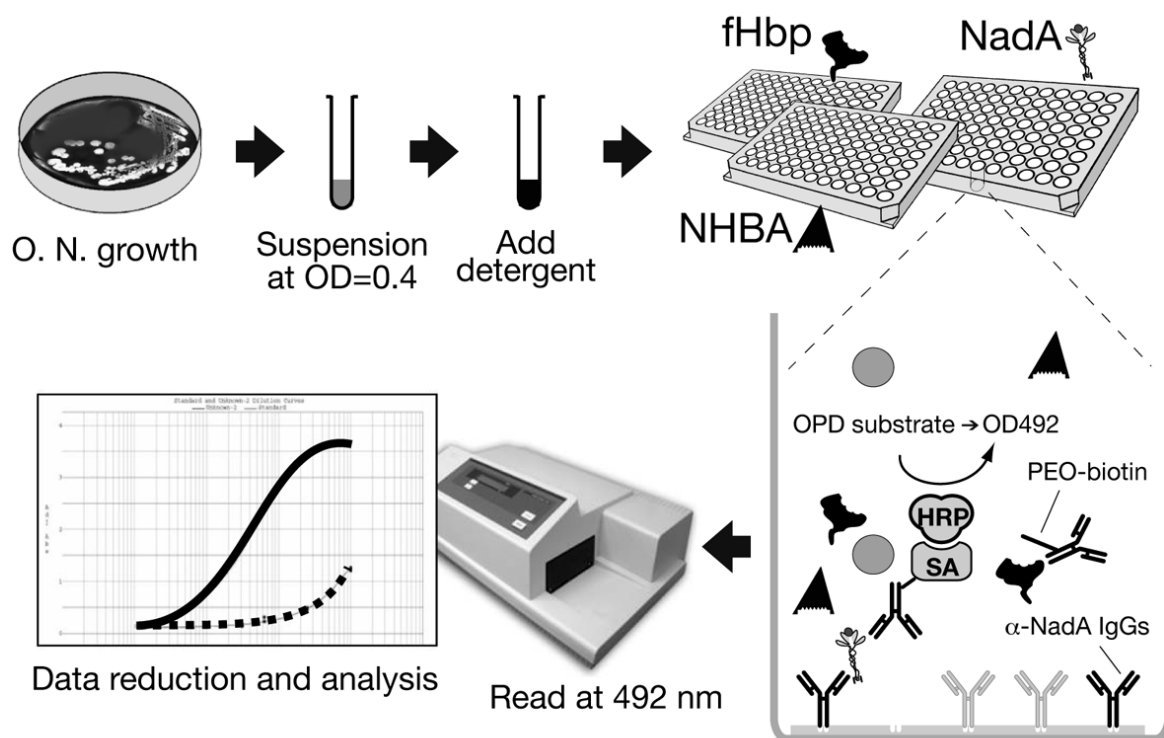
Laboratory tests intended as a surrogate of the serum bactericidal assay should be able to relate levels of antigen expression and sequence similarity to the ability of vaccine-elicited antibodies to kill bacteria. A robust esti-

mation of strain coverage would thus require a bridge between the observed immunity against a restricted strain panel in clinical trials to protection from broadly diverse endemic MenB strains. To address this, the Meningococcal Antigen Typing System (MATS), a rapid and reproducible binding assay, was developed to predict the susceptibility of MenB isolates to vaccine-elicited bactericidal killing. MATS is capable of measuring both the level of sequence relatedness and expression of antigens on a given meningococcal strain [36].

MATS combines modified ELISA assays to measure the antigenic cross-reactivity and expression of fHbp, NadA and NHBA on bacterial lysates and sequencing of the dominant OMV immunogen. A strain that matches the PorA serosubtype (PorA 1.4) is considered covered by the vaccine.

The MATS ELISA has been previously described (Fig 2). Antigenic reactivity and level of expression are measured in test samples by capturing the antigens of lysed bacterial suspensions in 3 different ELISA plates, coated with polyclonal rabbit antibodies raised against the vaccine components fHbp, NHBA, and NadA. To evaluate whether the relative antigen content measured by MATS correlates with bactericidal activity, 57 serogroup B isolates with known antigen genotypes and MATS values have been tested by hSBA using pooled sera from infants immunized with the 4CMenB.

Fig. 2. Schematic representation of the MATS ELISA method [36]: bacteria from overnight cultures on Agar Chocolate plates, are suspended in Mueller-Hinton broth; lysed with a detergent (EmpigenBB 5%) added to a final volume of 1/11 and inactivated for 1 hr at 45°C in water bath; bacterial lysates are added to three different ELISA microwell plates coated with polyclonal rabbit antibodies raised against the single vaccine components fHbp, NHBA, and NadA, the antigens are captured from the suspension to the plate; plates are then incubated with biotinylated rabbit polyclonal antibodies against each of the antigens for 1 hour at 37°C, washed, incubated with streptavidin-HRP and developed with the OPD substrate; Relative Potency is calculated by interpolating the regression curve of the unknown sample vs. that of a reference strain added to each plate.



By comparing the MATS relative potency values with SBA results, threshold values (positive bactericidal thresholds, PBTs) were defined for each of the three antigens, specifically 2.1, 29.4 and 0.9% for fHbp, NHBA and NadA respectively. A strain with a relative potency above the PBT for at least one of the 3 antigens is predicted to be killed in SBA at a $\geq 80\%$ probability [36].

The multi laboratory study

The MATS method has been transferred to five European reference laboratories: Health Protection Agency (HPA) for England & Wales, Norwegian Institute of Public Health (NIPH) in Norway, Institut Pasteur in France, Wurzburg University in Germany, Istituto Superiore di Sanità in Italy; Center for Disease Control (CDC) for USA. An inter-laboratory comparison study, among these six laboratories and the Novartis laboratory in Siena, has been run by comparing repeated testing of a selected panel of strains. MATS proved to be highly reproducible and comparable across the seven laboratories [Plykaytis *et al.* submitted] and the method has been subsequently transferred in additional laboratories in Europe and Australia.

MATS was then performed by the reference laboratories on their own collection of MenB isolates, collected during the epidemiologic period July 2007 – June 2008. Strain coverage was then defined as the proportion of strains having at least one vaccine antigen above the PBT (NadA, NHBA, or fHbp) or the PorA serosubtype 1.4 [36]. Regional strain coverage based on data provided by the five European laboratories demonstrates that the 4CMenB vaccine can be broadly protective across Europe.

To provide more global information about the vaccine antigens distribution and vaccine strains coverage, datasets from other European countries, North and South America and Australia are already available or soon to be completed.

As the introduction of vaccine will have some impact on the bacterial population, the reference laboratories will use MATS also after the 4CMenB introduction. This would allow the monitoring of strains not covered by the vaccine, or the emergence of escape mutants.

Conclusions

The hSBA is the only correlate of protection accepted thus far to assess the efficacy for a multi-components protein-based MenB vaccine [29]. Since a large number of SBA tests are required because of the high genetic variability observed in MenB isolates, the need to combine SBA with other immunologic and genetic data has been raised [12, 37].

The Meningococcal Antigen Typing System provides a useful and reproducible tool to estimate the potential coverage of 4CMenB recombinant vaccine. A large part of the isolates circulating in Europe are predicted to be killed by the 4CMenB vaccine. Since PTBs have been established using infant immune sera, the coverage estimate could be potentially higher for adolescents and adults. Furthermore, the proportion of killed strains is generally increased when a collection is populated by those that express more than one antigen above the PBT [36], suggesting that antibodies against multiple antigens elicit a cooperative effect. The MATS assay may be used after the introduction of the vaccine, in order to monitor evolution in meningococcal population structure.

The introduction 4CMenB vaccine could have an impact also on other meningococcal serogroups, as the antigens in the vaccine are not exclusively expressed in MenB strains but also present in other serogroups. Measuring the level of expression and variability of 4CMenB vaccine antigens for invasive meningococcal serogroups other than B will provide information about the impact of vaccine on other meningococcal serogroups.

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■ Correspondence: Giuseppe Boccadifuoco, Novartis Vaccines & Diagnostics, Research Serology, via Fiorentina 1, 53100 Siena, Italy - Tel. +39 0577 245057 - E-mail: giuseppe.boccadifuoco@novartis.com