Vaccine 32 (2014) 2722–2731

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Variability of genes encoding surface proteins used as vaccine antigens in meningococcal endemic and epidemic strain panels from Norway

Johan Holst[a,*], Maurizio Comanducci[b,1], Stefania Bambini[b], Alessandro Muzzi[b], Sara Comandi[b], Jan Oksnes[a], Lisa DeTora[c], Mariagrazia Pizza[b], Rino Rappuoli[b], Dominique A. Caugant[a,d]

[a] Division of Infectious Disease Control, Norwegian Institute of Public Health, Oslo, Norway
[b] Novartis Vaccines and Diagnostics, Siena, Italy
[c] Novartis Vaccines and Diagnostics, Cambridge, USA
[d] Section for International Health, Faculty of Medicine, University of Oslo, Oslo, Norway

ARTICLE INFO

Article history:
Received 2 December 2011
Received in revised form 5 February 2014
Accepted 25 February 2014
Available online 12 March 2014

Keywords:
Neisseria meningitidis
Meningococcal disease
MenB vaccine
Vaccine evaluation

ABSTRACT

Surface-expressed protein antigens such as factor H-binding protein (fHbp), Neisserial adhesin A (NadA), Neisserial heparin-binding antigen (NHBA) and Porin protein A (PorA); all express sequence variability that can affect their function as protective immunogens when used in meningococcal serogroup B vaccines like the recently-approved 4CMenB (Bexsero®). We assessed the sequence variation of genes coding for these proteins and two additional proteins ("fusion partners" to fHbp and NHBA) in pathogenic isolates from a recent low incidence period (endemic situation; 2005–2006) in Norway. Findings among strains from this panel were contrasted to what was found among isolates from a historic outbreak (epidemic situation; 1985–1990). Multilocus sequence typing revealed 14 clonal complexes (cc) among the 66 endemic strains, while cc32 vastly predominated in the 38-strain epidemic panel. Serogroup B isolates accounted for 50/66 among endemic strains and 28/38 among epidemic strains. Potential strain-coverage ("sequence match") for the 4CMenB vaccine was identified among the majority (>70%) of the endemic serogroup B isolates and all of the epidemic serogroup B isolates evaluated. Further information about the degree of expression, surface availability and the true cross-reactivity for the vaccine antigens will be needed to fully characterize the clinical strain-coverage of 4CMenB in various geographic and epidemiological situations.

1. Introduction

Invasive meningococcal disease causes death and permanent disability, most frequently in otherwise healthy infants and young children [1,2], often with a secondary incidence peak among adolescents, associated with an increased case-fatality rate [3,4]. Serogroups, A, B, C, W and Y, cause the vast majority of meningococcal disease globally [5]. Glycoconjugate vaccines are widely available against serogroups A, C, W, and Y; however, the serogroup B (MenB) capsular carbohydrate is an ineffective vaccine candidate [6–9]. MenB strains cause a high proportion of invasive meningococcal disease cases in many industrialized countries where conjugate vaccines are routinely used [5,10–14].

The development of effective MenB vaccines has been based on surface exposed proteins capable of eliciting protective immunity [8,9,15]. Until December 2012, the only approved MenB vaccines were so-called wild-type outer membrane vesicle (wtOMV)

Abbreviations: GNA, genome-derived Neisserial antigen; fHbp, factor H-binding protein; NadA, Neisserial adhesin A; NHBA, Neisserial heparin-binding antigen; cc, clonal complex; MenA, serogroup A meningococci; MenB, serogroup B meningococci; MenC, serogroup C meningococci; MenW, serogroup W meningococci; MenY, serogroup Y meningococci; PorA, porin protein A; FetA, ferric transporter-protein A; ST, sequence type; 4CMenB, four component meningococcal serogroup B vaccine; MATS, meningo antigen typing system.

* Corresponding author at: Department of Bacteriology and Immunology, Division of Infectious Disease Control, Norwegian Institute of Public Health, P.O. Box 4404 Nydalen, NO-0403 Oslo, Norway. Tel.: +47 915 36 782/21 07 64 23; fax: +47 21 07 65 18.
E-mail addresses: johan.holst@fhi.no (J. Holst), comandi80@gmail.com (S. Comandi), lisadetora@hotmail.com (L. DeTora).
[1] Present address: Crucell BV, Leiden, The Netherlands.

http://dx.doi.org/10.1016/j.vaccine.2014.02.068
0264-410X/

vaccines, mainly effective against outbreaks caused by one bacterial clone and/or strains with the same PorA antigen [16,17]. The Norwegian OMV vaccine (MenBvac®) and other similar wtOMV vaccines, made for particular epidemics in Cuba and New Zealand, have proven effective against clonal outbreaks [18–21]. The dominant functional immune response to these vaccines, especially in infants and young children, is against the sero-subtype defining epitopes on PorA, which limits their ability to provide protection in situations where many different pathogenic MenB strains are circulating in the population [16,22].

Various approaches have been used to discover vaccine candidates capable of eliciting protective immunity against the diversity of circulating pathogenic MenB strains [8,9,15,23]. Currently, the multi-component vaccine 4CMenB is approved in Europe, and a bivalent factor H-binding protein (fHbp) based vaccine has entered advanced clinical trials [24–27]. Given the dynamic epidemiology of meningococcal strains and the high variability among surface proteins and their diversity in expression level, it is vital to assess the degree of strain-coverage these protein-based vaccines offer against disease causing strains. The first step in any such analysis is to consider the sequence variation of the vaccine antigens in question among representative strain panels.

Both the investigational bivalent vaccine and 4CMenB employ fHbp [25,26]. The 4CMenB (Novartis) [27–30] is a multi-component vaccine that contains fHbp fused with genome-derived Neisserial antigen (GNA) 2091, Neisserial adhesin A (NadA), Neisserial heparin-binding antigen (NHBA) fused with GNA1030, and the OMV from the New Zealand outbreak strain, which harbours PorA P1.4 [20,21,31]. The four major protein components, fHbp, NadA, NHBA, and PorA, exhibit different degrees of genetic and phenotypic variation (often substantial).

The current study examined genetic information in endemic and epidemic strain panels from Norway as an adjunct to the clinical development program and further evaluations of the strain-coverage for the 4CMenB vaccine (Bexsero®); such as the meningococcal antigen typing system (MATS) [32–34].

2. Materials and methods

2.1. Bacterial isolates and serogroup determination

The National Reference Laboratory for Meningococci at the Norwegian Institute of Public Health (NIPH) receives nearly all *Neisseria meningitidis* pathogenic isolates recovered through the national mandatory reporting system. Nearly all cases are laboratory confirmed by culture, with few cases confirmed only by PCR or, very rarely, cases diagnosed solely by clinical symptoms with or without microscopic findings. We used two strain panels, one representing the epidemic period and one accounting for all strains submitted to NIPH during 2005 and 2006 (endemic period). All 66 isolates received by the reference laboratory in 2005–2006 and included in our analysis represent 89% of the 74 reported cases of meningococcal disease in Norway during these two years (www.msis.no). The epidemic panel comprises 38 isolates collected at Ullevål University Hospital in Oslo from 1985 to 1990. These isolates are similar and representative for those isolates collected across Norway during this period [35–38], although they may not cover all epidemiological developments during the lengthy epidemic [35,36]. The rationale for selecting this "historic" panel was to represent a period with low epidemiologic variation; as a contrast to the more low-incidence and heterologous situation that is of greater challenge to a sub-capsular (protein based) vaccine.

Serogrouping was determined upon receipt of isolates by slide agglutination using Remel antisera (Remel Europe Ltd., UK) and/or a dot blot method with monoclonal antibodies [39].

2.2. Bacterial growth and isolation of genomic DNA

Bacteria were grown overnight (18–20 h) at 37 °C in a humidified atmosphere with 5% CO_2 on chocolate agar plates or on Colombia agar plates (Oxoid Ltd., Hampshire, UK) with 5% horse blood.

One loop of bacterial growth was harvested and boiled (>95 °C) for 10 min in a 10 mM Tris/HCl buffer with 1 mM EDTA, pH 8.0. Cell debris was removed by centrifuge and the supernatant was transferred to an Eppendorf tube. DNA concentration was adjusted (if necessary) to approx. 10 ng/μl, by checking the absorbance at 260 nm. In instances where gene amplification for an isolate proved difficult, the ZR Fungal/Bacterial DNA Kit, (Zymo Research, Orange, USA) was used following the manufacturer's instructions.

2.3. Standard genotyping

All strains were genetically classified using Multilocus Sequence Typing (MLST) and *porA*- and *fetA*- type were defined following procedures available at http://pubmlst.org/neisseria/.

2.4. PCR amplification and DNA sequencing of vaccine antigen genes

The entire gene sequence for the five proteins GNA2091, GNA1030, NadA, NHBA and fHbp was amplified using the primers listed in Table 1 under the conditions specified below.

About 10 ng of chromosomal DNA was used as template for gene amplification. Either AccuPrime Taq DNA Polymerase (Invitrogen, USA) or GoTaq DNA Polymerase (Green Master Mix, Promega, Wisconsin, USA) was used; no significant difference was observed between the two, although the elongation temperature was 68 °C for AccuPrime, and 72 °C for GoTaq. The PCR cycle, including 30 s denaturation at 95 °C and 30 s annealing at various temperatures (see below); 1 min elongation was repeated 30 times regardless of the enzyme used.

The annealing temperatures were: 55 °C for *gna1030*, 63 °C for *nadA*, 56 °C for *nhba* and 58 °C for *fHbp*; while for *gna2091*, 52 °C was used for five cycles, followed by 65 °C for 30 cycles.

PCR products were purified using QIAquick PCR purification kit (QIAGEN) and sequenced using an ABI 377 automatic sequencer (Applied Biosystems Inc., Foster City, USA). The primers for sequencing are in Table 1.

Sequences were assembled and manually edited with Sequencher v4.7 (Gene Code Corporation, Ann Arbor, USA).

2.5. Phylogenetic analysis and representation

Nucleotide sequences were aligned using ClustalX v2.0 [40] then manually curated. Molecular relationships were inferred using the Maximum Parsimony (MP) [41] phylogeny as implemented by MEGA and using protein sequences [42,43].

2.6. Alleles and proteins nomenclature

Nucleotide and/or peptide sequences of the five gene loci were assigned according to the *Neisseria* Sequence Typing Database (http://pubmlst.org/neisseria). New allelic or protein variants were submitted to be assigned a numerical identifier. NadA and fHbp were classified based on the major variants identified in the phylogenetic clades [32]. Regarding NadA there have been identified five main variants named 1 through 5 and a number of sub-variants within each main variant giving a nomenclature like NadA-1.1 and 3.1. In this current work we did only sequence a subset of the various PCR-products for NadA; the classification into main variants