Vaccine 36 (2018) 3926–3936

Contents lists available at ScienceDirect

Vaccine

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

Immunization with recombinant truncated *Neisseria meningitidis*-Macrophage Infectivity Potentiator (rT-Nm-MIP) protein induces murine antibodies that are cross-reactive and bactericidal for *Neisseria gonorrhoeae*

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ARTICLE INFO

Article history:
Received 20 February 2018
Received in revised form 14 May 2018
Accepted 16 May 2018
Available online 24 May 2018

Keywords:
Macrophage Infectivity Potentiator
Neisseria gonorrhoeae
Neisseria meningitidis
Bactericidal antibody
Human complement
Liposomes

ABSTRACT

Neisseria meningitidis (Nm) and *N. gonorrhoeae* (Ng) express a Macrophage Infectivity Potentiator (MIP, NMB1567/NEIS1487) protein in their outer membrane (OM). In this study, we prepared independent batches of liposomes (n = 3) and liposomes + MonoPhosphoryl Lipid A (MPLA) (n = 3) containing recombinant truncated Nm-MIP protein encoded by Allele 2 (rT-Nm-MIP, amino acids 22–142), and used these to immunize mice. We tested the hypothesis that independent vaccine batches showed similar antigenicity, and that antisera could recognise both meningococcal and gonococcal MIP and induce cross-species bactericidal activity.

The different batches of M2 rT-Nm-MIP-liposomes ± MPLA showed no significant ($P > 0.05$) batch-to-batch variation in antigenicity. Anti-rT-Nm-MIP sera reacted equally and specifically with Nm-MIP and Ng-MIP in OM and on live bacterial cell surfaces. Specificity was shown by no antiserum reactivity with *Δmip* bacteria. Using human complement/serum bactericidal assays, anti-M2 rT-Nm-MIP sera killed homologous meningococcal serogroup B (MenB) strains (median titres of 32–64 for anti-rT-Nm-MIP-liposome sera; 128–256 for anti-rT-Nm-MIP-liposome + MPLA sera) and heterologous M1 protein-expressing MenB strains (titres of 64 for anti rT-Nm-MIP-liposome sera; 128–256 for anti-rT-Nm-MIP-liposome + MPLA sera). Low-level killing ($P < 0.05$) was observed for a MenB isolate expressing M7 protein (titres 4–8), but MenB strains expressing M6 protein were not killed (titre < 4–8). Killing ($P < 0.05$) was observed against MenC and MenW bacteria expressing homologous M2 protein (titres of 8–16) but not against MenA or MenY bacteria (titres < 4–8).

Antisera to M2 rT-Nm-MIP showed significant ($P < 0.05$) cross-bactericidal activity against gonococcal strain P9-17 (expressing M35 Ng-MIP, titres of 64–512) and strain 12CFX_T_003 (expressing M10 Ng-MIP, titres 8–16) but not against FA1090 (expressing M8 Ng-MIP).

As an alternative to producing recombinant protein, we engineered successfully the Nm-OM to express M2 Truncated–Nm-MIP, but lipooligosaccharide-extraction with Na-DOC was contra-indicated. Our data suggest that a multi-component vaccine containing a select number of Nm- and Ng-MIP type proteins would be required to provide broad coverage of both pathogens.

1. Introduction

Neisseria meningitidis (Nm, Men, meningococcus) is a major causative organism of meningitis and sepsis contributing significantly to mortality and morbidity worldwide [1], and *Neisseria gonorrhoeae* (Ng, gonococcus) causes the sexually transmitted disease gonorrhoea [2]. Capsular polysaccharide-conjugate vaccines to prevent infections by MenA, MenC, MenW and MenY meningococci are widely available, routinely used and effective [3,4], and two new vaccines Bexsero and Trumenba, have been licensed for MenB infection [5,6]. Bexsero has shown a vaccine efficacy of 83% against all MenB cases in vaccine-eligible infants in the UK since 2015, equivalent to ~94% efficacy against the 88% predicted vaccine-preventable MenB strains [7]. A 50% reduction in MenB cases was observed in the vaccine eligible

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https://doi.org/10.1016/j.vaccine.2018.05.069
0264-410X/

age-group, which seems to have persisted [8]. By contrast, there are no gonorrhoea vaccines and infection control has relied on antibiotics, but this is being severely compromised by the emergence of antibiotic-resistant gonococci worldwide [9]. Thus, new vaccine technologies have led to renewed interest in developing prophylactic gonococcal vaccines [10,11].

The impact of new MenB vaccines on the levels of protection, the epidemiology of circulating meningococcal strains, the potential for selection of new antigenic variants and variation in protein expression, needs to be monitored. Next-generation MenB vaccines may see the incorporation of additional antigens capable of inducing serum bactericidal antibodies, the accepted correlate of protection. A possible candidate for inclusion in new vaccines is the Macrophage Infectivity Potentiator protein (MIP, NMB1567/NEIS1487), which is a member of the FK506-binding protein (FKBP)-type peptidyl prolyl cis/trans isomerase (PPIase) family of proteins [12,13]. Expression of the *nm-mip* gene was important for meningococcal survival in the blood [13,14] and inhibition of Nm-MIP prevented meningococci from adhering, invading and/or surviving in epithelial cells [15]. Nm-MIP is highly conserved and expressed by all meningococcal strains reported to date, is outer membrane (OM)-located, surface exposed and capable of inducing cross-protective bactericidal antibodies [13,16,17]. *N. gonorrhoeae* also produces a FKBP-type PPIase and expression of the surface-exposed 30 kDa Ng-MIP lipoprotein appeared to be important for bacterial persistence within macrophages and protected gonococci from the bactericidal activity of immune effector cells [18,19]. Sera from patients with urethritis or disseminated gonococcal infections recognized purified Ng-MIP, suggesting that this antigen is expressed during infection *in vivo* and is immunogenic [13,18,19]. Ng-MIP is also highly conserved across all reported strains of *N. gonorrhoeae*, although the vaccine potential has not been reported.

Recently, we reported that Nm-MIP and human FKBP2 PPIase protein shared ~48% similarity of amino acids (AA) located in region AA166-252. The C-terminal globular domain of Nm-MIP covers AA143-272 and contains the PPIase FKBP-type domain [16]. Molecular mimicry between Nm-MIP with hFKBP2 protein was obviated by generating a recombinant truncated protein (rT-Nm-MIP, AA22-143), which induced murine bactericidal antibodies against meningococci that did not recognise human FKBP protein [16]. A baby rabbit complement Serum Bactericidal Assay (BRC-SBA) demonstrated that antibodies to rT-Nm-MIP Type I protein were bactericidal for MenB bacteria expressing different MIP (Type I, II and III) proteins and for MenA, MenC, MenW and MenY bacteria expressing the same MIP protein. Antisera to rT-Nm-MIP appeared also to show bactericidal activity against a gonococcal strain P9-17, although the BRC-SBA showed high levels of background killing by sham-immunized sera. For examining bactericidal activity of antisera raised to recombinant proteins, it is preferable that MenB and gonococcal SBAs use a human complement/human serum (HC/HS) source rather than BRC, which tends to inflate bactericidal titres due to the presence of IgM antibodies. It has been reported also that the HC-SBA is conservative with a high rate of false-negatives, which makes the assay less sensitive, but more specific, that the BRC-SBA [20–22].

In the current study, we extended our studies of the vaccine potential of rT-Nm-MIP by (i) examining the antigenicity of independent batches of experimental vaccines against meningococci and gonococci, (ii) using HC/HS-SBA assays to quantify bactericidal activity against meningococci and gonococci expressing different MIP Type proteins and (iii) attempting to engineer the MenB OM to express truncated MIP.

2. Materials and methods

2.1. Bacteria, growth conditions and preparation of outer membranes (OM)

Bacteria used in this study are listed in Table 1. Wild type and mutant strains were grown on supplemented GC agar plates, incubated at 37 °C, 5% (v/v) CO_2 [23]. For human complement-mediated serum bactericidal assays (HC-SBA), *N. gonorrhoeae* strains MS11 and 12CFX_T_003 were grown on supplemented GC agar plates with the addition of Cytidine-5′-MonoPhospho-N-Acetylneuraminic Acid to impart resistance to human serum (HS) [24]. *Escherichia coli* DH5α (cloning) and BL21(DE3) pLysS strains (protein expression) were grown at 37 °C on Luria-Bertani (LB) agar, LB or SOB broths.

OM of MC58, MC58Δm*ip* and MC58Δm*ip::t-nm-mip*, P9-17 and FA1090 bacteria were prepared as described previously [25,26]. Treatment with sodium deoxycholate (Na-DOC) was done as described previously [26].

2.2. Construction of Neisseria meningitidis nm-mip gene and Neisseria gonorrhoeae ng-mip gene knock-out mutants

Construction of MC58Δm*ip*, FA1090Δm*ip* and P9-17Δm*ip* mutant strains was done as described previously [14,16] using primers listed in Supplementary Table 1. Transformed colonies were screened by PCR and confirmed by western blotting using cross-reacting rabbit anti-rNm-MIP sera [17].

2.3. Complementation of MC58Δnm-mip strain with c-term truncated nm-mip

For chromosomal complementation of MC58Δm*ip* strain, the 3′-end truncated *nm-mip* gene (encoding for AA1-143) under transcriptional regulation of a strong and constitutive *PorA/NadA*

Table 1
Neisseria organisms used in this study. NIPH, Norwegian Institute of Public Health, Norway. ATCC, American Type Culture Collection. PHE, Public Health England. CDCP/FDA – Centre for Disease Control and Prevention/Food and Drug Administration Antibiotic/Antimicrobial Resistance Isolate Bank (https://www.cdc.gov/drugresistance/resistance-bank/currently-available.html).

Organism	Strain	Serogroup	Provenance
Neisseria meningitidis	Z1534	A	NIPH, Norway
	Z1092	A	NIPH, Norway
	MC58	B	[16]
	MC168	B	[16]
	MC90	B	[16]
	MC54	B	[16]
	M15 240139	B	PHE, Manchester
	M15 240337	B	PHE, Manchester
	M15 240973	B	PHE, Manchester
	M16 240169	B	PHE, Manchester
	MC173	C	[23]
	M11 240441	W	PHE, Manchester
	M12 240717	Y	PHE, Manchester
	M15 240043	Y	PHE, Manchester
	M16 240363	Y	PHE, Manchester
Neisseria gonorrhoeae	P9-17	–	[39]
	FA1090	–	ATCC700825
	MS11	–	ATCC BA1833
	12CFX_T_003	–	CDCP/FDA - AR Isolate Bank