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Assessment of vaccine potential of the *Neisseria*-specific protein NMB0938

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ABSTRACT

The availability of complete genome sequence of *Neisseria meningitidis* serogroup B strain MC58 and reverse vaccinology has allowed the discovery of several novel antigens. Here, we have explored the potential of *N. meningitidis* lipoprotein NMB0938 as a vaccine candidate, based on investigation of gene sequence conservation and the antibody response elicited after immunization in mice. This antigen was previously identified by a genome-based approach as an outer membrane lipoprotein unique to the *Neisseria* genus. The *nmb0938* gene was present in all 37 *Neisseria* isolates analyzed in this study. Based on amino acid sequence identity, 16 unique sequences were identified which clustered into three variants with identities ranging from 92 to 99%, with one cluster represented by the *Neisseria lactamica* strains. Recombinant protein NMB0938 (rNMB0938) was expressed in *Escherichia coli* and purified after solubilization of the insoluble fraction. Antisera produced in mice against purified rNMB0938 reacted with a range of meningococcal strains in whole-cell ELISA and western blotting. Using flow cytometry, it was also shown that anti-rNMB0938 antibodies bound to the surface of the homologous meningococcal strain and activated complement deposition. Moreover, antibodies against rNMB0938 elicited complement-mediated killing of meningococcal strains from both sequence variants and conferred passive protection against meningococcal bacteremia in infant rats. According to our results, NMB0938 represents a promising candidate to be included in a vaccine to prevent meningococcal disease.

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1. Introduction

Meningococcal disease is a significant cause of mortality and morbidity throughout the world; it is one of the most feared bacterial infections due to its rapid progression and tendency to cause epidemics. *Neisseria meningitidis*, the causative agent, is a Gram-negative bacterium which colonizes the human upper respiratory tract [1]. Occasionally, it translocates to the bloodstream causing sepsis and from there it can cross the blood–brain barrier and cause meningitis [2]. This bacterium is found only in humans and is classified into 13 serogroups on the basis of the chemical composition of the capsular polysaccharides, but only five serogroups (A, B, C, Y, and W-135) cause the majority of disease [2]. Currently, there are successful glyconjugate vaccines against four (A, C, Y, and W-135) of the five pathogenic serogroups. However, prevention of serogroup B meningococcal disease (MenB) represents a particularly difficult challenge in vaccine development. The use of capsular polysaccharide as the basis of a vaccine for prevention of MenB has been problematic, since the serogroup B capsular polysaccharide is identical to a widely distributed human carbohydrate ($\alpha(2\rightarrow 8)$N-acetyl neuraminic acid or polysialic acid), is poorly immunogenic in humans and may elicit autoantibodies [3,4].

Current research on vaccine candidates against MenB has focused on outer membrane proteins (OMP), either purified or incorporated into outer membrane vesicles (OMV) [5]. The antigenic variability of major OMP [6,7] limits the efficacy of OMV-based vaccines and, although these proteins induce protective antibodies against the homologous strain, they fail to induce cross-protection against all circulating heterologous strains [8]. Bactericidal responses to OMV vaccines are predominately directed against PorA and, to a lesser extent, to the Opc protein [9]. However, PorA shows considerable antigenic variation both between

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and during outbreaks, raising concern that protection from monovalent OMV vaccine would be PorA-specific [10].

The lack of a broadly effective vaccine against serogroup B has focused attention on the study of novel meningococcal antigens, which must be easy accessible and capable of inducing long-term protective immune response in both adults and children [10]. After completion of the MC58 genome project [11], a growing number of high-throughput approaches has been employed to screen the meningococcal genome for potentially protective antigens, which have proved to be widely conserved and might form the basis of a truly cross-protective vaccine against *N. meningitidis*. Promising results have been obtained with one of these proteins, the lipoprotein human factor H binding protein (fHbp), the sequence of which appear to be unique to the genus *Neisseria* [12,13]. fHbp is a component of two potential universal vaccines against *N. meningitidis* serogroup B: a vaccine, that was developed on the basis of five cross-protective antigens [14] and a vaccine that contains two variants of this single lipoprotein [15].

In an attempt to find novel *Neisseria*-specific lipoproteins with vaccine potential, we surveyed the MC58 genome using public software and BLAST searches [16]. Here, we report the cloning, expression, and purification of recombinant lipoprotein NMB0938 (rNMB0938). In order to study the vaccine candidacy of this outer membrane lipoprotein, we have investigated the ability of the recombinant variant to elicit broadly cross-reactive antibodies, as determined by ELISA and western blotting. To establish the conservation of the *nmb0938* gene, we analyzed the nucleotide sequence in 35 *N. meningitidis* strains. Moreover, we evaluated the utility of this protein, as immunogen, to induce a bactericidal and protective immune response against meningococci.

2. Materials and methods

2.1. Bacterial strains

A total of 35 *N. meningitidis* strains comprising serogroups A, B, C, W135 and Z, and including strains isolated from healthy carriers, were used for gene amplification. The panel included strains isolated in Cuba between 1983 and 2003, and several standard strains with a worldwide distribution. The strains belong to different serogroups, PorA types and/or MLST sequence types (Table 1). Sequence typing information was obtained as reported on the *Neisseria* MLST website (http://neisseria.org/nm/typing/mlst/). Bacteria were grown at 37 °C in an atmosphere containing 5% CO_2 on Brain Heart Infusion (BHI) agar (Oxoid, United Kingdom) supplemented with an antibiotic mixture of vancomycin, colistin and nystatin (VCN, Oxoid) at the concentration 3 μg/mL, 7.5 μg/mL and 12.5 units/mL, respectively.

The *Escherichia coli* XL1-blue (Invitrogen, USA) was used for cloning purposes and *E. coli* K12 W3110 (New England BioLabs, UK) was employed for the expression of the recombinant protein. The *E. coli* cells with plasmid were grown aerobically at 37 °C either in Luria Bertani (LB) or in expression medium supplemented with 12.5 μg/mL tetracycline and 100 μg/mL ampicillin for XL1-blue strain, and with 100 μg/mL ampicillin for strain W3110. Expression medium consists of M9 synthetic medium

Table 1
Neisseria strains used in this study[a].

NMB0938 variant	Strain	Year of isolation	ST	Clonal complex	Serogroup	PorA type	Source	Country
1	MC58	1985	74	ST-32	B	7,16-2	Disease	UK
1	CU385	1983	33	ST-32	B	19,15	Disease	Cuba
1	1/98	1998	897	UA	B	12,16-11	Disease	Cuba
1	10/92	1992	33	ST-32	B	19,15	Disease	Cuba
1	156/85	1985	5171	ST-103	B	5-1,2-2	Disease	Cuba
1	322/90	1990	33	ST-32	B	19-24,15	Disease	Cuba
1	411/86	1986	33	ST-32	B	19,15	Disease	Cuba
1	419/85	1985	33	ST-32	B	19,15	Disease	Cuba
1	52/91	1991	33	ST-32	B	19,15	Carrier	Cuba
1	99/89	1989	33	ST-32	B	19,15	Disease	Cuba
1	B16B6	Unknown	11	ST-11	B	5,2	Disease	USA
1	H355	1973	NA	ST-32	B	19,15	Disease	Norway
1	H44/76	1976	32	ST-32	B	7,16	Disease	Norway
1	1/03	2003	41	ST-41/44	B	7-2,4	Disease	Cuba
1	Z4181	Unknown	11	ST-11	C	5,2-1	Disease	Mali
1	67/91	1991	33	ST-32	ND	7-1,1	Carrier	Cuba
1	133/83	1983	6437	UA	C	18-1,34	Disease	Cuba
1	9/99	1999	33	ST-32	B	19,15	Disease	Cuba
1	159/99	1999	103	ST-103	NG	18-1,3	Carrier	Cuba
1	135/99	1999	103	ST-103	Z	18-1,3	Carrier	Cuba
1	225/88	1988	44	ST-41/44	C	22,14-6	Disease	Cuba
1	375/91	1991	11	ST-11	C	5,2	Disease	Cuba
1	67/89	1989	53	ST-53	NG	7,30-3	Carrier	Cuba
1a	M982	Unknown	3790	UA	B	22,9	Disease	USA
2	NZ98/124	1998	44	ST-41/44	B	7-2,4	Disease	New Zealand
2	053442	2004	4821	ST-4821	C	7-2,14	Disease	China
2	Z2491	Unknown	4	ST-4	A	7-2,13-1	Disease	Gambia
2	FAM18	1983	11	ST-11	C	5,2	Disease	USA
2	811/86	1986	352	ST-269	B	21,2-2	Disease	Cuba
2	131/89	1989	883	ST-41/44	C	18-1,34	Disease	Cuba
2	102/98	1998	22	ST-22	W135	18-1,3	Carrier	Cuba
2	111/02	2002	823	ST-198	NG	18,25-15	Carrier	Cuba
2	Z1127	Unknown	ND	ND	A	9	Disease	B. Faso
2	57/89	1989	53	ST-53	B	7-2,30-2	Carrier	Cuba
2	233/89	1989	53	ST-53	ND	7-2,30-4	Carrier	Cuba
3	Y92-1009	1992	3493	ST-613	N/A	N/A	Carrier	UK
3	Nl-ST640	Unknown	640	ST-640	N/A	N/A	Carrier	UK

[a] *Abbreviations used*: ND, not determined; NA, not available; N/A, not applicable; UA, unassigned; ST, sequence type; NG, non-groupable.

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