

Protective immune response against *Neisseria meningitidis* serogroup B after immunization with peptides mimicking a capsular polysaccharide epitope

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Introduction

The Gram-negative bacterium *Neisseria meningitidis* is an important cause of meningitis and septicemia worldwide. Meningococcal disease is a life threatening illness that may progress to death even after medical intervention. Approximately 500 000 - 1 000 000 cases of invasive meningococcal disease occur annually worldwide with a 10% mortality rate. Significant sequelae, including neurological damage, limb loss and hearing loss, occur in up to 20% of survivors. Meningococcal disease is more common among infants and young children [1]. Prevention through vaccination remains the most effective approach to control invasive meningococcal disease.

The most relevant mechanism for antibody-mediated protection against *N. meningitidis* seems to be the antibody-mediated direct complement lysis [2]. The *in vitro* measurement of complement-dependent serum bactericidal activity (SBA) is currently considered the immunological surrogate of protection against meningococcal disease [3].

N. meningitidis is classified in 13 serogroups, based on chemical composition and antigenic differences of the capsular polysaccharide (CPS). Serogroups A, B, C, Y and W-135 strains account for most cases of meningococcal infections [1]. Effective plain or conjugated CPS-based vaccines are available against serogroups A, C, Y and W-135 [1]. However, a vaccine inducing protection against most of the circulating variants of serogroup B meningococcal strains is not yet available.

Up to now, all field-applied anti-B vaccines have been based on outer membrane vesicles (OMV), which vary in composition among strains. Therefore, OMV-based vaccines induce an immune response mainly protective against the strain used for vaccine production and control the disease in specific regions with similar circulating strains. The vaccine VA-MENGO-BC[®], developed at the Finlay Institute (Havana, Cuba), is an OMV-based vaccine that uses the Cuban type strain CU385 (B:4:P1.19,15; ST=33). The vaccine showed an efficacy of 83 % in clinical trials and its introduction into the Cuban Immunization Program keeps low the incidence of meningococcal disease in Cuba [4].

The poor immunogenicity of purified MenB CPS, even after conjugation [5] has precluded the formulation of vaccines based on this compound. MenB CPS is a linear homopolymer of α (2-8)-linked N-acetylneuraminic acid (polysialic acid, PSA) [6]. The mam-

malian neural cell adhesion molecule (NCAM) is glycosylated with identical sialic acid residues [7]. The replacement of N-propionyl (Npr) groups for N-acetyl (NAc) moieties in MenB CPS greatly increases immunogenicity and allowed the discovery of two classes of antigenic determinants in the MenB CPS, which can be found on the intact bacterial surface: human non-cross-reactive and human cross-reactive determinants [8-10].

The identification of peptide mimetics of MenB CPS-specific antigenic determinants is a strategy aimed to further the development of vaccine formulations to cover all meningococcal serogroup B strains. In the present work we investigated the possibility to identify peptides able to mimic the properties of a unique capsular polysaccharide epitope in MenB that could be proposed as meningococcal serogroup B vaccine candidates.

Results

To identify mimetic peptides of meningococcal serogroup B capsular polysaccharide, a collection of random peptides was screened, exposed in the surface of filamentous phages [11] using the bactericidal and protective IgG_{2a} mAb 13D9 [10] as bait. Four peptides, able to bind mAb 13D9 in competition with MenB CPS, were identified and named 4L-5, 2L-4, 2L-17 and 2L-27.

The alignment and comparison of the four sequences (Figure 1) revealed the presence of a 7-residue stretch in each of them displaying some of the common residues found in other carbohydrate-mimicking peptides, with coinciding relative positions [12, 13]. One of these features is the presence of W (tryptophan) residues. Here a W residue was found in 3 out of the 4 peptides in a very similar sequence context. W, and aromatic amino-acids in general, are hypothesized

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> 4L - 5   R G D K S R P P V M Y V E G E
> 2L - 4   P A G R Q A R R P A T V P E G
> 2L - 17  - - - - - P E M W L E P P F I S S G K
> 2L - 27  - P V Q R G A W R M P V V T N E
  
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Figure 1. Sequence alignment of peptide mimetics of *Neisseria meningitidis* serogroup B capsular polysaccharide identified in the present work. A stretch characterized by the presence of some amino-acids frequently found in carbohydrate-mimicking peptides such as W, P and E residues, was identified in all the sequences (highlighted in a red box).

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to mimic monosaccharide moieties due to similarities in cyclic shape and volume. Another signature of carbohydrate-mimicking peptides is the presence of P (proline) residues, which are thought to function by introducing turns in the peptide backbone that allow the adoption of an adequate spatial disposition by the voluminous side chains of aromatic residues, resembling the carbohydrate they mimic. There is more than one P residue in all of the sequences identified here, and in three cases a P residue appears in a very similar sequence context. E (glutamic acid) is also present in the four peptides. An E residue in the same sequence context can be found for peptides 4L-5, 2L-4 and 2L-17, but is apparently missing in peptide 2L-27. However, even in this case the C-terminal E might be considered to be present in a similar sequence context, since it is preceded by a turn-inducing sequence (TNE) that should bring the E into close spatial proximity to the consensus stretch. Generally, the presence of acid residues in carbohydrate-mimicking peptides guarantees charge similarity with negatively charged polysaccharides, as is the present case with PSA.

It was hypothesized that this 7-residue region in each peptide could be the target for mAb binding, and that probably the E plays a role in such binding. The 4L-5 sequence was selected as a model to test this hypothesis. Using collections of overlapping peptides covering the 4L-5 sequence it was defined that the sequence WYVE, contained in the afore mentioned 7-amino-acid-residue stretch, sufficed for mAb binding. A synthetic peptide containing three copies in tandem of the minimal sequence necessary for mAb 13D9 binding in peptide 4L-5 inhibited mAb 13D9 binding to MenB CPS. The importance of the E residue for mAb binding was studied with synthetic peptide libraries containing amino acid changes in the 4L-5 sequence. It was found that the E residue is essential for mAb binding since its replacement by other amino acids abolished the reactivity with the mAb. These experiments also revealed the role of the two prolines located at positions -3 and -2 counting from W in WYVE, because their presence increased the reactivity of peptide 4L-5 with mAb 13D9, even though they are not directly involved in binding.

To assess the potential of the identified peptides as vaccine candidates, we started immunizing groups of mice directly with the viral particles displaying such sequences. For three of the phage-displayed peptides (4L-5, 2L-17 and 2L-27), more than half of the mice from the corresponding group elicited bactericidal specific anti-peptide IgG antibodies (Table 1). The highest bactericidal titers were measured in the group immunized with phage-displayed 4L-5 and passive transfer of pooled sera from this group conferred protection to infant rats challenged with live MenB.

The 4L-5 sequence was selected as a model to develop vaccine formulations, based on synthetic peptides, that could be used in humans. Various forms of sequence presentation to the immune system were evaluated (Figure 2). The structures L/4L-5; MAP 4L-5 and D/MAP 4L-5 were conjugated to the carrier protein P64K. Three conjugation methodologies were assayed for MAP 4L-5. The antigens were characterized by chromatography, spectrometry and amino acid analysis. All antigens reacted in ELISA with

Table 1. Serum bactericidal activity (SBA) against meningococcal strain CU385 (B:4:P1.19,15) in sera from mice immunized with the phage-displayed peptides 4L-5, 2L-4, 2L-17 and 2L-27

Group	Inmunogen ^a	Responders (%) ^b
1	Phage 4L-5	7/7 (100%)
2	Phage 2L-4	2/7 (28.5%)
3	Phage 2L-17	4/7 (57.1%)
4	Phage 2L-27	5/7 (71.4%)
5	Npr-MenB CPS/HSA	6/7 (85.7%)
6	Control phage	0/7 (0%)

^aAnimals received three injections of 10¹¹ viral particles (groups 1-4 and 6) or 5 µg of N-propionylated MenB CPS conjugated to human serum albumin (Npr-MenB CPS/HSA, group 5).

^bThe individual sera of mice from each group were evaluated. The animals with SBA titers ≥8 were considered as responders and the percentage fraction of responders in each group was computed.

mAb 13D9. Plain antigens and antigens coupled to P64K were used to immunize BALB/c mice. Of those variants giving immunogenic results, MAP-TT 4L-5 rendered the highest levels of specific anti-peptide IgG antibodies and serum bactericidal activity. The structure MAP-TT was selected as the platform structure for further studies.

Similar structures called MAP-TT 2L-17 and MAP-TT 2L-27, with sequences 2L-17 and 2L-27 respectively, were also synthesized and simultaneously with MAP-TT 4L-5, their antigenicity and immunogenicity in mice were studied. The three structures reacted in ELISA with mAb 13D9 and inhibited binding of mAb 13D9 to purified Npr-MenB CPS. MAP-TT 4L-5 and MAP-TT 2L-27 inhibited 80% of binding, while MAP-TT 2L-17 caused approximately 50% of inhibition. The structures were immunogenic in mice. Protective SBA titers against MenB were measured in groups of mice immunized with MAP-TT 4L-5 and MAP-TT 2L-27, in group pooled sera and in more than half of the individual mice from these two groups (Table 2). The serum antibodies elicited against both antigens adsorbed to MenB CPS. Additionally, pooled sera from mice group immunized with MAP-TT 2L-27 passively protected from bacterial challenge in the infant rat model. The assessment of serum bactericidal activity of anti-MAP-TT-2L-27 sera against two other strains of serogroup B, yielded

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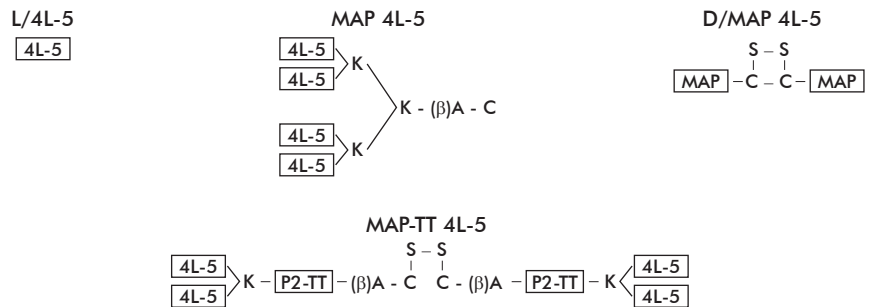


Figure 2. Synthetic antigens containing the peptide mimetic of MenB CPS 4L-5 with sequence RGDKS RPPVWYVEGE. L/4L-5: Linear 4L-5 peptide, MAP 4L-5: multiple antigen peptide containing four copies of the 4L-5 sequence linked through K residues, D/MAP 4L-5: disulfide dimer of MAP, MAP-TT 4L-5: a disulfide dimer, with each monomer consisting of two copies of the MenB CPS 4L-5 sequence and a copy of a T-helper cell epitope P2 of tetanus toxoid (P2-TT, residues 830-844, sequence QYIKANS KFIGITEL). The structures L/4L-5, MAP 4L-5 and D/MAP 4L-5 were conjugated to the carrier protein P64K. Three conjugation methods were assayed for the structure MAP 4L-5.

positive results. Pooled sera from mice immunized with MAP-TT 4L-5 and MAP-TT 2L-27 did not react with a homogenate of brain from neonatal BALB/c mice in an ELISA performed to evaluate the reactivity of anti-peptide antibodies with mammalian NCAM.

Conclusions

The peptide sequences 4L-5 and 2L-27 mimic the properties of a unique epitope of meningococcal serogroup B capsular polysaccharide. They induce antibodies with bactericidal activity against *N. meningitidis* B after mouse immunization.

The peptides identified in this study could be considered for the design of immunogenic, safe and universal vaccines against *N. meningitidis* serogroup B. This work has also contributed to the knowledge of properties of peptides reacting with an anti-carbohydrate antibody [14]. The studies of immunization with synthetic peptides emphasized the paramount importance of the presentation form of a peptide sequence to the immune system [15].

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Table 2. Serum bactericidal activity (SBA) against meningococcal strain CU385 (B:4:P1.19,15) in sera from mice immunized with the MAPs-TT 4L-5, 2L-17 and 2L-27

Group	Inmunogen ^a	SBA titers (in pooled sera from each group) ^b	Responders (%) ^b
1	MAP-TT 4L-5	64	4/7 (57.2 %)
2	MAP-TT 2L-17	4	-
3	MAP-TT 2L-27	512	5/7 (71.4 %)
4	Npr-MenB CPS/HSA	256	-
5	Control (MAP-TT P64K)	4	-

^aAnimals received three injections of 50 µg of each MAP-TT (groups 1, 2, 3 and 5) or 5 µg of N-propionylated MenB CPS conjugated to human serum albumin (Npr-MenB CPS/HSA, group 4).

^bTiters of SBA were measured in pooled sera from each group of mice. Negative sera were assigned a titer of 4.

^cThe individual sera of mice from groups 1 and 3 were also evaluated. Animals with SBA titers ≥8 were considered responders and the percentage fraction of responders in each group was computed.

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