Prediction and characterization of T-cell epitopes for epitope vaccine design from outer membrane protein of *Neisseria meningitidis* serogroup B

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**Abstract:**
*Neisseria meningitidis* serogroup B (MC58) is a leading cause of meningitis and septicemia, principally infecting the infants and adolescents. No vaccine is available for the prevention of these infections because the serogroup B capsular polysaccharide is unable to stimulate an immune response, due to its similarity with polysialic acid. To overcome these obstacles, we proposed to develop a peptide based epitope vaccine from outer membrane protein contained in outer membrane vesicles (OMV) based on our computational analysis. In OMV a total of 236 proteins were identified, only 15 (6.4%) of which were predicted to be located in outer membrane. The major requirement is the identification and selection of T-cell epitopes that act as a vaccine target. We have selected 13 out of 15 outer membrane proteins from OMV proteins. Due to similarity of the fkpA and omp85 with the human FKBP2 and SAMM50 protein, we removed these two sequences from the analysis as their presence in the vaccine is likely to elicit an autoimmune response. ProPred and ProPredl were used to predict promiscuous helper T Lymphocytes (HTL) and cytotoxic T Lymphocytes (CTL) epitopes and MHCPred for their binding affinity in *N. meningitidis* serogroup B (MC58), respectively. Binding peptides (epitopes) are distinguished from nonbinding peptides in properties such as amino acid preference on the basis of amino acid composition. By using this dataset, we compared physico-chemical and structural properties at amino acid level through amino acid composition, computed from ProtParam server. Results indicate that porA, porB, opc, rpmM, mtrF and rnsA are more suitable vaccine candidates. The predicted peptides are expected to be useful in the design of multi-epitope vaccines without compromising the human population coverage.

**Keywords:** Outer membrane vesicles, epitope vaccine, epitopes, *Neisseria meningitidis* serogroup B.

**Background:**
Meningococcal disease is a major health problem worldwide. *Neisseria meningitidis* is a major cause of meningitis and septicaemia globally, predominantly affecting children and adolescents. Disease develops rapidly and often difficult to distinguish from other febrile illnesses. Meningococcal meningitis and sepsis are devastating diseases that kills children and young adults within hours despite the availability of effective antibiotics. Mortality and permanent disability rates are high, even under optimal health care conditions [1]. Therefore, any further reduction in morbidity and mortality is most likely to be achieved through prophylactic vaccination. The sole ecological niche of *Neisseria meningitidis* is mucosa of the oropharynx of humans. Meningococcal colonization of the respiratory tract, a phenomenon commonly referred to as carriage, represents a successful commensal relationship between the host and the bacterium, with the host experiencing no detectable pathology. On the other hand disease represents a failed or dysfunctional relationship with the host. Acquisition of *Neisseria meningitidis* demands person to person transmission via direct contact or through dispersion of respiratory droplets from an infected to a susceptible individual. Although, often protected by a polysaccharides capsule, meningococci are particularly sensitive to desiccation; thus spread from one individual to another requires close contact [2].

The causative agent, *Neisseria meningitidis*, is a Gram-negative encapsulated bacterium classified into different groups according to the chemical composition and immunogenic properties of the capsular polysaccharide. Serogroups A, B, C, W135, and Y account for >95% of infections. Effective vaccines consisting of capsular polysaccharide or capsular polysaccharide-cojugates are available for the prevention of infections caused by serogroup A, C, Y, and W135 strains [3, 4]. However, no capsule-based vaccine is available for the prevention of infections caused by *N. meningitidis* serogroup B, which is highly prevalent in industrialized countries. This problem is caused by the poor immunogenicity of the serogroup B capsular polysaccharide. It is likely that the immune system tolerates the serogroup B capsular polysaccharide because of its similarity or mimicry to the widely distributed human carbohydrate α(2→8)N-acetyl neuraminic acid or polysialic acid, both consisting of repeating units of two to eight linked sialic acid [5]. Therefore, for the development of an effective vaccine against serogroup B meningococci, researchers have focused on the proteins of the outer membrane (OM).

Alternative vaccine candidates have been sought, which are based on protein components being at the most advanced stage of development. These are commonly presented as outer membrane vesicle (OMV) formulations prepared by detergent extraction of meningococcal broth cultures [6]. OMV vaccines have been used with some success in Norway and Cuba [7, 8] and have recently been granted a provisional license in New Zealand [9]. Outer membrane vesicles (OMVs) are released from the outer membrane of *N. meningitidis*, which is a characteristic of many...
Gram-negative bacteria [10]. It has been demonstrated that they contain outer membrane and periplasmic proteins and in some cases DNA or cell-

...this indicates the potential of OMVs to deliver membrane active

virulence factors into the host. OMV vaccine is not only composed simply

This clearly indicates the potential of OMVs to deliver membrane active

transport hydrophobic compounds like membrane proteins into the host.

...cell signaling molecules

outer membrane and periplasmic proteins and in some cases DNA or cell-

The antigenicity of the proteins is identified by the VaxiJen server

compositions were computed by using the Expasy's ProtParam server

Vaccine candidate characterization:

Theoretical isoelectric point (pI), molecular weight and amino acid

sequences of

number of proteins detected

A set of the 15 OM protein complements have been selected from the

Target protein sequence retrieval:

Methodology:

Target protein sequence retrieval:

The antigenicity and non-allerginicity of OM proteins shows that they are

predicting the proteasomal cleavage sites were incorporated in algorithms

The IC₅₀ value of corresponding peptides is deduced by MHCPred

The physicochemical properties of proteins are given in (Table 1 see

Discussion:

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The pI values of all these OM proteins

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This information is useful for the proteomics study

especially for the 2D PAGE and is also useful for cataloguing OMV

proteins with emphasis on 15 OM proteins used in this study. Outer

membrane proteins are notoriously difficult to resolve by 2D PAGE. By

their nature, hydrophobic proteins are often difficult to solubilise and are

least soluble when focused at their pI. The antigenicity of all the OM

proteins predicted using VaxiJen server is given in (Figure 1 see

Table 2 see supplementary material). The allergenicity of all OM proteins are

predicted by AlgPred server and all 15 OM proteins found non-allergens.

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The antigenicity and non-allerginicity of OM proteins shows that they are
human FK506 binding proteins 2 (FKBP2) and AIP aryl hydrocarbon receptor interacting proteins, and omp85 (NMB0182) has limited similarity with human SAMM50 sorting and assembly machinery component 50 homolog, this give rises the possibility of immunological cross reactivity between vaccine and host cell proteins, which could lead to autoimmunity. Therefore 13 out of 15 OM proteins are being selected for the epitope prediction.

The summary of the results of the prediction tools ProPred and ProPred1 used for the HTL and CTL epitope identification respectively in the present study are being made available at (http://www.bioinfoindia.org/epitopes/HTL_CTL_Table.pdf). According to the prediction results of MHCPred, peptides with the best predicted binding affinities of HLA_A*0101 for MHC class I and HLA_DRB*0101 for MHC class II molecules are also presented therein. The predicted output is given in units of IC50 nM. A lower value of peptide IC50 indicates higher affinity towards MHC molecules. Peptides with IC50 values < 5nM, < 50nM, and <500nM are considered having high affinity, intermediate affinity and low affinity towards MHC molecules respectively (HTL_CTL_Table.pdf). In OMV 236 nonredundent proteins have been identified out of which 15 (6.4%) proteins were predicted to be located in the outer membrane. From BlastP results it is established that 13 out of 15 OM proteins are the most suitable for the vaccines and they contain maximum promiscuous epitopes at many HLA alleles as these 13 OM proteins didn’t show any significant similarity with human proteins and neglect the chances of autoimmunity response. A multiple promiscuous target epitope peptides would overcome the genetic restriction, low immunological responsiveness and parasite evasion of the immune response and provide support for developing a multistage, multivalent, universal, safe, stable and effective meningococcal vaccine.

The OM proteins contain a porins, porA and porB, which are used for serotyping and serosubtyping of meningococci. The most abundant of these included proteins with important biological function, including the porA and porB porins, opc is involved in the adhesion of the meningococci to host cell-surface proteoglycan and therefore plays a part in colonisation and invasion by bacterium. This protein associated with invasion of epithelial and endothelial cells [26]. The rmpM protein, which may protect against complement-mediated bactericidal attack [27], mtrE is one of four proteins encoded by the multiple transferable resistance mtrE locus and forms outer membrane component of multidrug efflux pump [28], outer membrane protein npaA and pilQ which is essential for pilus assembly [29]. Two hypothetical proteins NMB0345 and NMB0088 are identified in the outer membrane protein constituent of OMV. Some other major OM proteins are also present like hap for adhesion and penetration, IptD involved in the assembly of LPS in the outer leaflet of the outer membrane, determines N-hexane tolerance and is involved in outer membrane permeability, essential for envelope biogenesis, thpB acts as a transferrin receptor and is required for transferrin utilization.

The peptides of 13 antigenic proteins are classified into binding and non-binding groups. The binding peptides signify the predicted epitopes and non-binding peptide signifies the protein sequence of OM protein minus binding sequence. Both dataset constructed for the MHC class I and class II individually. Then amino acid composition is computed and results are evaluated in three category of amino acid: hydrophobic, charged and polar and other small amino acid. Our data illustrate (Figure 2, 3) that hydrophobic amino acids are found more frequently in binding group (epitope) than in the non-binding group. The non binding group have more polar amino acid compared to other amino acids. This condition is found in both CTL as well as in the HTL binding and non binding dataset (Table 3 see supplementary material). Based on our analysis a computational protocol (Figure. 4) has been proposed which will work as a model for such further studies. The predicted epitopes in OMV proteins would be useful for the earlier identification of meningitis and septicaemia, and will be helpful used for secure vaccine development against meningococcal diseases.
Hypothesis

Conclusion:
Predicted promiscuous HTL and CTL epitopes from the genome/proteome sequences of the pathogens would greatly reduced the time as well as cost and is useful for experiment planning in development of peptide-based epitope vaccines. We have predicted numerous epitopes in OMV proteins which would be useful for the earlier identification of meningitis and septicaemia, the predicted epitopes may be used for safe vaccine development against meningococcal diseases. Results indicate that porA, porB, opc, mmpL, mtrE and nspA are more suitable vaccine candidates. These six proteins are selected due to their smaller size and most important thing is that they are present most abundantly in the OMV proteins as an outer membrane proteins. We frequently found large number of hydrophobic amino acid, certain polar and charged amino acids in binding group. Hydrophobic protein regions may tend to be highly conserved because of their importance for correct folding they may show epitopes particularly effective for protective host immunity. Also they have variable pl which is important for 2D study for separation of proteins. These results have important propositions for the development and use of vaccines based on epitopes of OM proteins after in vitro study and experimental validation.

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Hypothesis

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### Table 1: Physicochemical properties of proteins

| Protein designation                          | Gene name    | Accession No. | Size aa | Expected MW kDa | pI  |
|----------------------------------------------|--------------|---------------|---------|-----------------|-----|
| Major outer membrane protein P.IA (PIA)     | porA         | Q52140        | 392     | 42.1            | 9.13|
| Major outer membrane protein P.IB (PIB)     | porB         | P30690        | 331     | 35.7            | 7.14|
| Class 5 outer membrane protein              | Opc          | Q7DDI3        | 272     | 29.9            | 9.71|
| Type IV pilus biogenesis and competence pilQ| pilQ          | Q70M91        | 769     | 82.5            | 9.43|
| Probable FKBP-type peptidyl-prolyl cis-trans isomerase fkpA (PPIase) (Rotamase) | fkpA NMB1567 | Q9JYI8        | 272     | 28.9            | 5.72|
| Outer membrane protein class 4              | ompS5        | P070A3        | 242     | 26.2            | 6.97|
| Putative cell-binding factor                 | NMB0345      | Q7DDR0        | 288     | 31.5            | 9.23|
| Outer membrane protein omp85                | mtrE NMB1714 | Q9JY68        | 467     | 50.4            | 8.34|
| Multidrug efflux pump channel protein       | mtrE NMB1714 | Q9K1M2        | 466     | 50.5            | 9.37|
| Putative outer membrane protein NMB0088     | NMB0088      | Q9K187        | 802     | 88.7            | 8.50|
| Adhesion and penetration protein            | Hap NMB1985  | Q9K187        | 1457    | 159.9           | 9.19|
| LPS-assembly protein lptD                    | lptD NMB0280 | Q9K187        | 802     | 88.7            | 8.50|
| Outer membrane protein NspA                  | nspA NMB0663 | Q7DDM2        | 174     | 18.4            | 9.54|
| VacJ-related protein                        | NMB1961      | Q9JXN3        | 275     | 29.5            | 4.79|
| Transferrin-binding protein 2 (TBP-2)       | tbpB NMB0460 | Q9K0V0        | 712     | 77.4            | 5.79|

### Table 2: Computational prediction of Antigenicity (in ascending order) of the proteins by VaxiJen server at threshold 0.4

| Proteins | Probable Antigenicity | Prediction score |
|----------|-----------------------|------------------|
| pilQ     | Antigen               | 0.49             |
| NMB1812  | Anitigen              | 0.53             |
| NMB0345  | Anitigen              | 0.55             |
| porB     | Anitigen              | 0.57             |
| NMB2039  | Anitigen              | 0.61             |
| mtrE     | Anitigen              | 0.62             |
| Opc      | Anitigen              | 0.65             |
| NMB0182  | Anitigen              | 0.67             |
| NMB0382  | Anitigen              | 0.68             |
| NMB0088  | Anitigen              | 0.68             |
| NspA     | Anitigen              | 0.70             |
| NMB1567  | Anitigen              | 0.77             |
| NMB0663  | Anitigen              | 0.81             |
| fkpA     | Anitigen              | 0.82             |
| NMB0460  | Anitigen              | 0.90             |
Table 3: Amino acid preference of Binding and Non Binding group for CTL MHC class I and HTL MHC class II.

| Amino Acid | CTL MHC class I (%) | HTL MHC class II (%) |
|------------|---------------------|-----------------------|
|            | Binding             | Non Binding           | Binding | Non Binding |
| Ala, A     | 13.3                | 9                     | 6       | 12.4        |
| Ile, I     | 4.1                 | 4.3                   | 6.1     | 3.7         |
| Leu, L     | 16.2                | 6.1                   | 11.6    | 6.7         |
| Met, M     | 0.4                 | 0.9                   | 1.5     | 0.7         |
| Phe, F     | 3.4                 | 4.2                   | 4.9     | 3.2         |
| Pro, P     | 3.3                 | 3.7                   | 3.6     | 3.9         |
| Val, V     | 7.1                 | 6.3                   | 11      | 5.1         |
| Arg, R     | 3.9                 | 5.4                   | 8.2     | 4.2         |
| Asp, D     | 2.9                 | 6                     | 3.3     | 6.2         |
| Glu, E     | 4.8                 | 4.9                   | 2.7     | 6.2         |
| His, H     | 1                   | 1.9                   | 1.1     | 1.5         |
| Lys, K     | 6.9                 | 6.8                   | 5.8     | 6.7         |
| Asn, N     | 3.9                 | 6.6                   | 4.9     | 6.1         |
| Cys, C     | 0.1                 | 0.3                   | 0.1     | 0.4         |
| Gln, Q     | 2.2                 | 5                     | 4.4     | 4.9         |
| Ser, S     | 8.5                 | 7.8                   | 8.7     | 8.2         |
| Thr, T     | 6.1                 | 6.4                   | 2.8     | 7.1         |
| Trp, W     | 0.9                 | 1.2                   | 0.8     | 0.9         |
| Tyr, Y     | 2.2                 | 4.1                   | 5.6     | 2.3         |
| Gly, G     | 8.5                 | 9.2                   | 6.9     | 9.8         |