Novel NOMV-based vaccine against Neisseria meningitidis serogroup B

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**Neisseria meningitidis** is a Gram-negative diplococcus bacterium that causes a great number of deaths worldwide. The most common form of meningococcal disease is meningitis, which often occurs as epidemics. Despite appropriate treatment, the case-fatality rate is around 10%.

The highest incidence rates are found in the "meningitis belt," which extends from Senegal to Ethiopia (Fig. 1). In this area, A, C, W135 and X are the major disease causing meningococcal serogroups. The development of capsular polysaccharide-conjugated vaccines has allowed the prevention of disease caused by these serogroups.

On the other hand, there is a great meningococcal serogroup B incidence in Europe and the USA. Its capsular polysaccharide is poorly immunogenic due to its antigenic structure, which mimics the cell surface glycoproteins of human neurological tissue. For this reason, developing a group B vaccine has supposed a true challenge.

Reverse vaccinology has allowed identification of new vaccine candidates. The first meningococcal serogroup B vaccine based on this technique (Besserer) was approved in Europe and Australia in 2013.

In this work, another approach to a vaccine development against Neisseria meningitidis serogroup B is proposed.

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**Factor H and the complement system**

The role of the complement system against N. meningitidis is well established. Individuals deficient in components of the alternative (AP) and terminal complement pathways are highly predisposed to meningococcal infections.

Factor H (FH) is a regulatory protein of the alternative complement pathway that can inhibit the complement system action. This protein binds to host cells due to its glycosaminoglycans (GAGs) affinity. Because of GAGs are not found on the bacterial cell surface, FH can inhibit the complement system attack in host cell. Interestingly, N. meningitidis use several mechanisms to evade killing by human complement. Capturing host FH permits meningococci to disarm the AP and downregulate its action. Expression of surface proteins that bind to FH increases bacterium resistance to complement system attack.

**Antigens that interact with factor H**

Three of the most important meningococcal proteins that bind to FH are:

- **fHbp**: N. meningitidis strains with a fHbp gene deletion lose binding capacity to FH and are more susceptible to complement attack. This antigen is a clear candidate for a meningococcal serogroup B vaccine; it is cell surface exposed, it is present in more than 99% of all invasive strains, it is expressed during infection and it generates protective bactericidal antibodies.

- **Nspa**: Recombinant Nspa expressed in E. coli and purified does not have the same conformation as the protein present in the meningococcal outer membrane. For this reason, the first vaccine approximation does not elicit a bactericidal antibody response. Protective antibodies may be directed against conformational epitopes of the native protein.

- **PorB**: It is one of the most abundant outer membrane proteins in the bacterium. Because of its low affinity interaction with FH, its expression downregulates the AP of complement.

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**Vaccine design**

Four strains are appropriately chosen to be genetically modified. Each strain naturally expresses the antigen which will be overexpressed (Table 1).

Three genes are inactivated to improve vaccine safety and to reduce the possibility of inducing autoimmunity: A: fHbp, A:ΔfHbp expressed outer membrane vesicle (OMV) (blocks sialic acid synthesis), A:Δapf (blocks lacto-N-neotetraose expression) and A:ΔlipL2 (reduces LOS endotoxicity).

**Table 1.** Vaccine strains with their genetic modifications.

<table>
<thead>
<tr>
<th>Vaccine strain name</th>
<th>Derived from</th>
<th>ΔafHbp</th>
<th>Δapf</th>
<th>Over-expression of</th>
<th>ΔlipL2</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>PM81745</td>
<td>Yes</td>
<td>Yes</td>
<td>fHbp AOS</td>
<td></td>
</tr>
<tr>
<td>S2</td>
<td>AOS373</td>
<td>Yes</td>
<td>Yes</td>
<td>fHbp A05</td>
<td></td>
</tr>
<tr>
<td>S3</td>
<td>A44/76</td>
<td>Yes</td>
<td>Yes</td>
<td>NspA</td>
<td></td>
</tr>
<tr>
<td>S4</td>
<td>2996</td>
<td>Yes</td>
<td>Yes</td>
<td>PorB</td>
<td></td>
</tr>
</tbody>
</table>

Enhanced expression of the desired antigen is achieved by inserting a second copy of the corresponding gene in the nass locus (Fig. 3).

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**Objectives**

The main objective of this work is to develop a novel meningococcal serogroup B vaccine. This vaccine consists in NOMVs (Native Outer Membrane Vesicles) over-expressing these proteins:

- **fHbp (AOS and B01 variants)**
- **NspA**
- **PorB**

**Serological assays**

![Diagram of serological assays](image)

- **SDA assay** (against 18 different meningococcal serogroup B strains)
- **ELISA assay**

**S1, S2, S3 and S4 strains purified outer membrane proteins (3 µl)**

<table>
<thead>
<tr>
<th>Vaccine strain</th>
<th>3 doses (weeks 0, 4 and 8)</th>
<th>10 mice</th>
<th>Aluminium hydroxide (0.17 mg/dose)</th>
<th>Serum is taken at week 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1, S2, S3, S4</td>
<td></td>
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</tbody>
</table>

**Possible benefits**

- Antigens included in this vaccine fulfil the same function. Binding to FH. Moreover, these molecules present low variability between meningococcal strains.

- Achieving bactericidal antibodies against these proteins would produce loss of bacterial virulence and an increased susceptibility of these bacteria towards elimination by the complement system.

- The technique used in this work could be applied to a universal vaccine design against the main meningococcal serogroups, trough the expression of the appropriate antigens.

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**Relevant bibliography**