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[1]. The most common form of meningococcal disease is meningitis, which often occurs as epidemics. Despite appropriate treatment, the case-fatality rate is around 10%[2].

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[1,2].

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[1].

Reverse vaccinology has allowed identification of new vaccine candidates. The first meningococcal serogroup B vaccine based on this technique (Bexsero®) was approved in Europe and Australia in 2013[1,3].

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

Figure 1. Worldwide distribution of the most prevalent Neisseria meningitidis serogroups[4].

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[5].

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 cells **preferably**[5].
N. meningitidis use several mechanisms to **evade killing** by human complement. **Capturing host fH** permits meningococci disarm the AP and downregulate its action. Expression of surface proteins that bind to fH increases bacterium resistance to complement system attack[5].

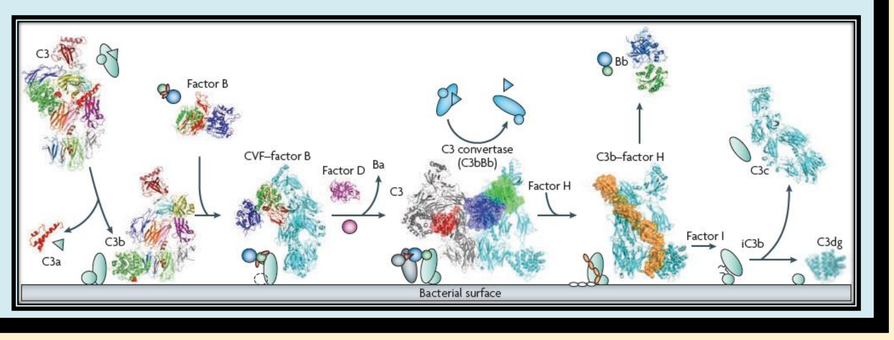


Figure 2. Molecular structure of the AP of complement components[6].

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[7].
- **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[8].
- **PorB2**: 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[9].

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 (A05 and B01 variants)**
- **NspA**
- **PorB2**

Using **NOMVs** present **several advantages** than using classic detergent-extracted OMVs[10]:

- LOS and lipoproteins are **not depleted** from the first vesicles.
- **Surface exposure** of membrane proteins is **similar** to that on the intact bacterium.
- Retaining lipoproteins allows some proteins to be present in the vaccine at **increased levels**.

Vaccine design

Four strains are appropriately chosen to be genetically modified. Each strain naturally expresses the antigen which will be **over-expressed** (**Table 1**).
Three genes are inactivated to **improve vaccine safety** and to reduce the possibility of inducing autoimmunity: Δ*synX* (blocks sialic acid synthesis), Δ*lgtA* (blocks lacto-N-neotetraose expression) and Δ*lpxL1* (reduces LOS endotoxicity)[10].

Table 1. Vaccine strains with their genetic modifications.

Vaccine strain name	Derived from	Δ*lpxL1*	Δ*synX*	Δ*lgtA*	Over-expression of
S1	PMB1745	Yes	Yes	Yes	fHbp A05
S2	M1573	Yes	Yes	Yes	fHbp B01
S3	H44/76	Yes	Yes	Yes	NspA
S4	2996	Yes	Yes	Yes	PorB2

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

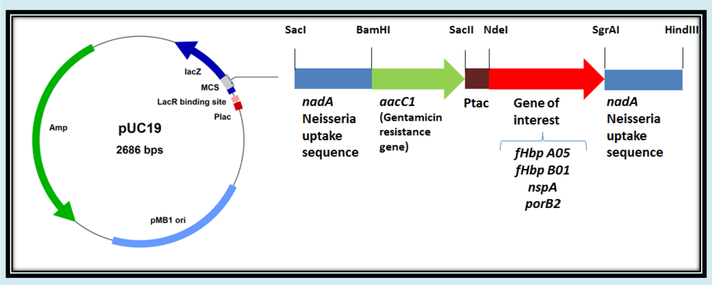
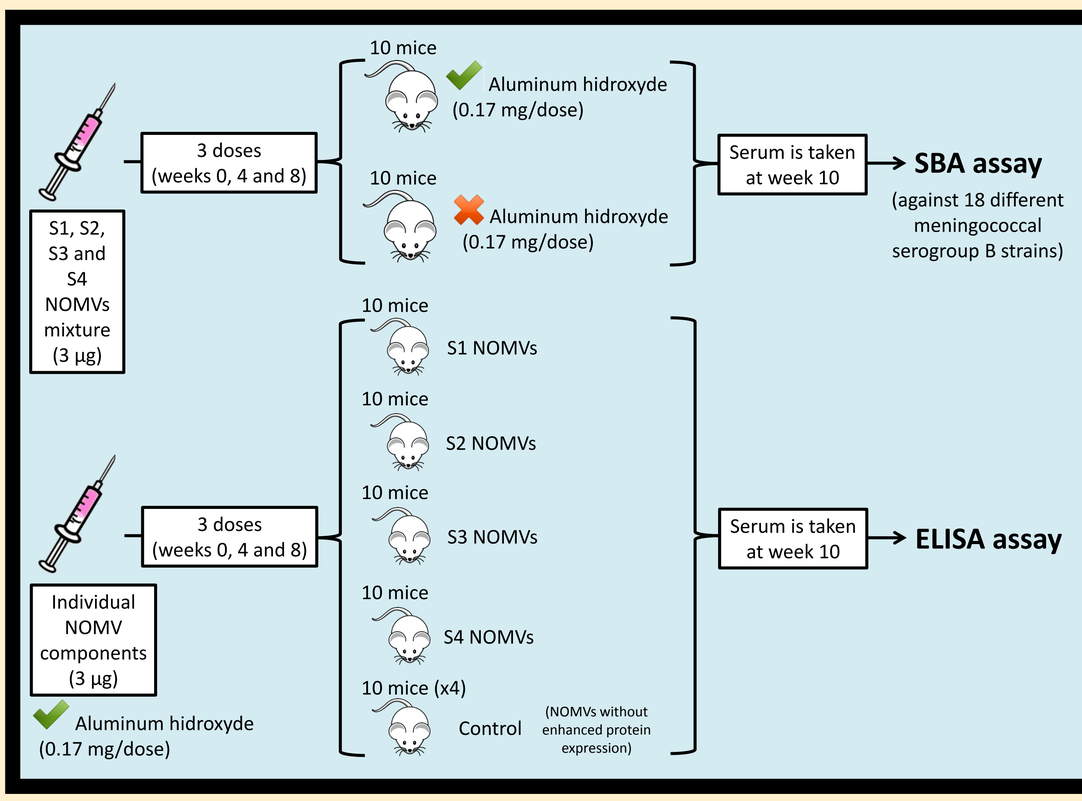


Figure 3. Plasmid used for the transformation of the meningococcal strains. Each strain is transformed with its corresponding genic construction, in which the gene of interest varies.

Expression of key epitopes and the **level** at which they are expressed are verified by **colony blotting** and **Western blotting**, respectively[10].

Serological assays



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.

Relevant bibliography

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