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A Review of Research of Vaccine against Meningococcal Disease

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ARTICLE INFO	ABSTRACT
<p>Article type: Review Article</p> <p>Article history: Received: 10 Apr 2018 Revised: 11 Jun 2018 Accepted: 16 Jul 2018 Published: 06 Oct 2018</p> <p>Keywords: Capsular polysaccharide, Meningitis, <i>Neisseria meningitides</i>, Vaccines.</p>	<p>Background: Meningococcal disease as a worldwide health problem causes approximately 1.2 million cases of bacterial meningitis, annually. <i>Neisseria meningitidis</i> a major cause of bacterial meningitis and serious diseases such as sepsis and bacteremia is fatal, and despite antibiotic treatments, the mortality rate of about 135 thousand cases has been reported. Meningococcal pathogen has been detected in nasopharynx of about 10-40% of the healthy people. There are several vaccines against six major groups of bacteria A, B, C, W135, X and Y. Although the bivalent (C-B), trivalent (A-C-Y) and quadrivalent (A-C-Y-W135) vaccines are used these days, there are yet significant rates of the disease in different geographical areas.</p> <p>Conclusion: Although the polysaccharide capsule conjugate vaccine that have been developed against meningococcal serogroups A-C-Y and W135 are successful, but serogroup B because of the similarity with human polysialic glycoproteins is poorly immunogenic and to be cross-reactions. Thus, vaccines based on outer membrane vesicles have been designed for them</p>

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Introduction

Two very serious and deadly diseases, meningitis and sepsis, still cause significant mortality in children and adults. The main factor causing this disease is known as gram-negative non-capsulated pathogen and. It is transmitted through the air and is colonized in 10-40% of healthy people. Meningococcal disease can develop quickly and results in deaths or serious problems in less than 24 hours even in people treated with antibiotics (1). Approximately, 1.2 million cases of meningococcal infection occur throughout the world. In spite of intense antibiotic therapy, about 135 thousand annual deaths in children under 5 years, pilgrims, residents of the dormitories and huge number of people at high risk places have been reported. *Neisseria meningitidis* is divided into 13 serogroups based on the differences in the structure of the polysaccharide capsule (2), that are pathogens in a human and geographical spread in different areas, where serogroup A in Asia and Africa, serogroups Y and W135 serogroup C in Europe and America resulted in the spread of epidemics were recently in New Zealand serogroup B disease is universal. The importance of sporadic, sudden onset, antibiotic resistance, particularly to ciprofloxacin and rapid disease progression is the need for vaccination as a useful tool in the management of the disease caused by this bacterium (3).

Pathogenesis

Type 4 pili, LOS, Opa proteins and glycolipid are main adhesions for primary attachment of non-capsulated meningococci. These proteins facilitates their function. These pili are polymeric filaments that could be found in many of Gram-negative bacteria and formed. The structure of these pili is composed of multiprotein complexes and pili filaments. This complex is encoded by *pilE* gene. Mutant *PilE* strains of Non-capsulated meningococci that are deficient in pili can interact incompletely with host cells. Pilin proteins in filaments could be changed by glycosylation and

this modification is part of phase variation of meningococci but affects insignificantly on type 4 pili function. Recently, PilX protein has been identified and extracted along with pili type 4 and is essential for clumping and attachment of bacteria. PilC1 also has an important role in the adhesion and it has been detected that its up-regulation in CSF has led to the expansion of meningitis. Another component of pili is PilT that drives assembly of pili and is important for twitching and pathogenesis reactions in host cells. The receptor of pili type 4 on host human cells is CD46 or membrane cofactor protein. This receptor is part of a superfamily of resistant proteins to complement and 16 isoforms of these proteins have been identified. Non-capsulated meningococci use several other proteins including Opa and Opc for adhesion. Opa proteins can bind to CD66/CEACAM. CD66 proteins can conduct cell-cell binding and encode by distinct genes followed by different posttranscriptional processes. Different responses may develop depending on CD66 and Opa type. Opc proteins, however, have not been detected in all strains of meningococcus. Opc interacts with vitronectin and facilitates the entry of bacteria into the cells. Opc also is effective on the entrance of bacteria into the brain cells by the interaction of serum fibronectin followed by $\alpha\gamma\beta3$ integrins. The early binding of bacteria results in elongation of microvilli that engulf and internalize the bacteria. Disseminated binding of bacteria occurs after 8 to 16 hours of meningococci reaction in host cells. The bacteria spread over the surface of the cells and lose their pili and then attach firmly to cells. Disseminating is affected by two important processes. First is spreading of bacteria on the surface of the cells and second, complete attachment which is controlled by down-regulation of PilC1 and capsule. The bacteria divide in phagosomes. Importance of intracellular multiplication is not clear, but it perhaps plays a significant role in pathogenesis. The intracellular developer of bacteria requires Iron for growth (4-9).

Epidemiology

Meningococcal infection is a global health problem and occurs in many countries, worldwide. However, it is epidemiologically variable and dynamic (10). Meningococci serogroup A can dramatically develop extent epidemics and the high prevalence of this disease is related to this serogroup. In the sub-Saharan regions of Africa serogroup A has been isolated more than other serotypes. Meningitis can spread rapidly with high frequency in these regions. Serogroups X and W135, also mainly develop meningitis in Africa (11). Since surveillance systems and diagnostic methods for meningococci are not sufficient in some countries, there are failures in epidemiological information for meningococcal disease in Asia. High prevalence of meningococci serogroup A has been reported in China, Nepal, and Mongolia from the 1960s to 1970s (12). Meningococci serogroup A has been the main cause of epidemics in India in 2005. This serogroup has been also reported responsible for a meningitis outbreak during 2004 to 2005 (13). Mortality of meningitis cases in Europe has demonstrated as sporadic and caused by serogroups B and C (14). In endemic cases, however, the main agent of this disease has been reported as serogroup C (10).

Prevention and control

Invasive meningococcal infection only occurs in people who are deficient in bactericidal, specific and opsonizing antibodies. Therefore, the introduction of such antibodies by vaccination can prevent meningococcal infection. Main studies about meningococcal vaccines have emerged after the development of resistance against sulfonamides and penicillins, and then polysaccharide vaccine which is based on polysaccharide capsule was designed for serogroups A and C in 1960 (15).

Polysaccharide vaccines

The first vaccine for prevention of meningococcal infection was generated in 1912 from killed cells of *Neisseria meningitidis*. Skerp and Richard purified capsular polysaccharide of serogroup A and provided them for usage as polysaccharide vaccine in 1935, however, its antigenic depolymerization during distinct steps of purification delayed making of polysaccharide group A vaccine for years (16). Some researchers purified polysaccharide capsule of serogroup A without antigenic destruction in 1969. Then, the first polysaccharide vaccine for serogroups A and C was developed when meningitis epidemics were detected between American military groups. This vaccine was highly effective in prevention of serogroups C infection among American troops, control of serogroup A epidemics in Africa and significant lowering frequency of sporadic cases in Brazil and France (17). However, this vaccine is a poor immunogen in neonates and cannot induce immunological memory between them. The tetravalent polysaccharide vaccine against serogroups A, C, W135 and Y was designed in 1981. Divalent A, C and tetravalent (A, C, Y, and W135) polysaccharide vaccines are produced massively by well-known companies around the world (likely, Pasteur institute, Biomerieux and other corporations). These vaccines were presented to the global market (18). These vaccines consist some disadvantages such as, low levels of antigenicity between children less than 2 years old and inability for induction of immunological memory and repetitive booster injections (18). Vaccination with meningococcal capsular polysaccharides has no effect on microorganism colonization. Despite such disadvantages, cocktail of divalent and tetravalent capsular polysaccharide vaccines is used in many developing countries (19, 20).

Polysaccharide conjugated vaccines

The first monovalent conjugated vaccine against serogroup C, developed by Chiron Wyatt. The conjugated or carrier protein in this vaccine was CRM197 (21, 22). Baxter simultaneously designed similar vaccine with Tetani toxoid (23). Monovalent conjugated vaccine of serogroup C was used in UK as the routine immunization approach for infants in 1999 (24). The infants acquired three doses of vaccine in second, third and fourth months of their lives. All infants older than 4 months and people below the 18 years old were vaccinated by this vaccine in UK during 1999-2000 (25, 26). Carriers of serogroup C also were reduced in healthy communities after vaccination with this type of vaccine. Non-expensive monovalent conjugated vaccine for serogroup A was designed in 2001 for developing countries (21, 28). A divalent conjugated vaccine against serogroups A and C also has been designed with more responsiveness and immunological memory for serogroup C. The tetravalent conjugate vaccine against serogroups A, C, Y, W135 complemented with diphtheria toxoid as carrier designed by Sanofi Company, was approved in the USA and authorized for human usage (29). Then, another tetravalent conjugated vaccine against four serogroups A, C, Y and W135, was provided by Novartis Company in 2010 (30). Both of these vaccines are safe, and are useful in all age groups, develop long prevention, and reduce the nasopharyngeal carriers and bacterial transmission (30, 31).

Side Effects of Meningococcal Vaccine

Polysaccharide vaccines

The side effects of MPSV4 are usually mild and often include local reactions at the injection site, such as pain and redness of the skin. These reactions take 1-2 consecutive days and can be seen in 48% of the recipients of the vaccine and systemic reactions such as a headache and weakness and fatigue in the first week after

vaccination are observed in 60% of cases. In less than 3% of cases, these systemic reactions may be severe.

Conjugate vaccines

The side effects of the MCV4 vaccine are similar to those reported for the MPSV4 vaccine. Most cases included injection site reactions (59%), fever 37.5 to 38.39 °C in the first week after vaccination (5%), systemic reactions such as a headache and fatigue in the first week after vaccination (60%) and severe systemic reactions (Less than 3%). The incidence of mild side effects with conjugate vaccines is higher than that of polysaccharide vaccines, but in general, conjugate vaccines are preferable because of greater efficacy, memory retention in the immune system, increased immune response, response times, and longer duration of immunity.

Vaccines against serogroup B

As mentioned, serogroup B is not immunogenic in humans and results in unwanted responses because, due to the similarity of the polysaccharide capsule it interacts with glycoproteins on the surface of the human nervous cells, and therefore, immunosuppression is weak and lead to induction of the autoantibody. Therefore, there are various researches on breaking immunological tolerance, preventing production. Autoantibodies and its replacement by induction of bactericidal and opsonizing antibody synthesis to serogroup B polysaccharide capsule, which did not yield favorable results. So today, the researchers focus on other parts of the wall, and especially the outer membrane vesicles (OMV) (32). Researchers took the first steps for eliminating the immunological tolerance for group B capsule during 1987–1986. They replaced N-propionyl for N-acetyl in polysaccharide capsule and conjugated it with Tetani toxoid as protein carrier, therefore emerged high immunogenicity in animal models (33, 34). This vaccine led to bacteriolysis mediated by complement system and was preventive in animal

challenge with serogroup B (35). This vaccine was studied in the primary clinical trial in adult volunteers and researchers found out that produced antibodies were nonfunctional (35). The second generation of these vaccines contained modified K1 capsule of *E. coli* K1 capsule is structurally similar with serogroup B capsule.(36) Polysialic acid of K1 capsule was propionylated and then it fused with PorB of meningococcal outer membrane as a protein carrier. This type of vaccine produced high immunogenicity in primates except humans (37), but triggered vast studies in Europe and America. This vaccine is safe that somehow is due to the N-propionyl group that partially prevents the autoimmune reactions. The main disadvantage of this vaccine is anti-capsular antibodies that cross-react with host Polysialic acid (38).

Non capsular vaccines

Components of cell wall such as LOS and OMP. Since there are some disadvantages for serogroup B vaccines, studies focused on other

LOS – based vaccines

The scientists noticed on Lipo-oligosaccharide of meningococci because it has a significant role in meningococcal pathogenesis (39). Plested et.al. 1999, demonstrated that prevented epitopes in inner core of LOS, produces preventive immunity in experimental animals (40). Anderson et.al. In 2002, studies demonstrated that LOS causes cross responses between distinct serogroups of meningococci. Nevertheless, using these vaccines not to be advised of their side effects and unwanted reactions in vaccinated people (41). If LPS or LOS is considered as vaccine candidates, Lipid O in their structure should be detoxified. Detoxification could be performed by removing or modification of O-acetyl or N-acetyl or incorporation of LOS inside the liposomes (42). After these modifications, toxicity in these vaccines would be lowered. Another method for lowering the toxicity and consistency of adjuvant character of LOS is the

mutation in a lpxL gene that is responsible for adding the second fatty acid chain to 2' position in Lipid A(39, 43).

OMV-based vaccines

Outer membrane vesicle (OMV) of meningococci derived from bacterial membrane and including OMPs and some of LOS molecules (44, 45). OMVs could be purified with or without detergents or reducing the LOS content from the cell wall (46). The first OMV-based vaccine against meningococci and outbreak of meningitis due to serogroup B designed by Cuban Institute and Norway National Institute of public health. These vaccines were used in countries such as Norway, Cuba, Brazil, and Chile for epidemic control and their efficacy was investigated by those countries (47). Clinical studies showed in volunteers, persons that their efficacy was in the range of 50%-80%. These vaccines are not preventive in children less than 4 years old and specific antibodies are generated against homologous strains (48). Although extended responses are developed against heterologous strains in children and adults. Responses of bactericidal antibodies due to these vaccines are largely against PorA protein and weakly against OPC. Since, however serogroup B epidemics mainly generated by individual strain and continue for decades, OMV-based vaccines are appropriate for control of epidemics and clonal prevalence (49, 50). In 1998 a vaccine based on strain B: 4:p1.7b, Including PorB, PorA and LOS developed by Chiron assisted by the health ministry of New Zealand. This vaccine was successfully studied in children and infants during primary and second phases of trials and was able to develop bactericidal Antibodies in 75% of 6-24 months infants and 8-12 years old children (51). In the middle of 1990s, Poolman et.al. Designed recombinant OMV-based vaccines with six or nine distinct subtypes of PorA in Netherland, which named Hexamer and Nonamer vaccines respectively (52). Hexamer vaccine was induced OMV that provided from two different strains of

meningococci. Each strain in this vaccine expresses three variants of PorA. This vaccine was used as three doses in adult people in the primary clinical trial and infants and children in the second phase of the trial. The significant bactericidal responses from antibodies after the last dose were developed and indicated that this vaccine could eliminate 75% of serogroup B strains (53). Studies indicate that PorA could be chosen as a proper agent candidate against serogroup B in Vaccine. There are many evidences indicate that PorA induces preventive immune responses in human more than other surface proteins (54). E. de Kleijn et al. In 2001 demonstrated that PorA easily is presentable in a live bacterium and stimulates the bactericidal antibodies (55). Vermont et al. Also studied cross-reactions of antibodies against PorA following immunization with monovalent and hexavalent OMV vaccines against serogroup B by the bactericidal function of serum (SBA) method. They pointed that hexavalent OMV vaccine generated cross-reactive response against serogroup B (56). Humphries et al. In 2004 studied the immunogenicity of recombinant PorA in a rat model and they indicated that rPorA (recombinant PorA) induces high titres of bactericidal antibodies against PorA in homologous strains (57). Tamara et al. In same year studied the interaction between rPorA and dendritic cells and role of rPorA in stimulation of innate and adaptive immune responses. They demonstrated that rPorA causes maturing of dendritic cells and increases the production of chemokines such as RANTES, and the other factors like IL8, MIP1 β and MIP1 α . rPorA can also activate dendritic cells and effects on nature of immune responses generated by Th cell. All the results from studies on OMV-based vaccines during several years showed that OMPs could induce preventive antibodies against serogroup B of meningococci. Thus, the scientists searched for the presented proteins of outer membranes. They studied these proteins by genome-based analysis or reverse vaccinology. They identified 600 antigens that 350 out of them were expressed successfully as recombinant proteins in *E. coli* and then these recombinant

proteins were injected into rats for assessment of immune responses after their purification (58). Twenty-nine out of 91 identified surface proteins can induce bactericidal antibodies. These proteins were called Genome Derived Antigens (GNAs) and their identification methods named as Reverse Vaccinology. The scientists assessed these proteins as probable candidates of vaccines following detection of partially prevented proteins during in silico studies. One of the detected surface proteins is GNA1870 or LP2086 that generated antibodies against it are high bactericidal and produces the prevention against several non-capsulated strains of meningococci. LP2086 is a surface protein and 28KDa that recognized as fHbp or factor H binding protein (59). Zhu et al. in 2006 showed that intranasal immunization of rats by recombinant protein rLP2086 with CT adjuvant induces generation of bactericidal IgG and IgA antibodies. These antibodies inhibit the primary colonization of bacteria in nasopharynx (60). NM Caesar et al. In 2007 demonstrated that intranasal immunization of rats with *Streptococcus jordanii* expressed surfaced protein NadA or GNA1994, can stimulate the bactericidal systemic IgG and mucosal IgA and is considered as an appropriate vaccine candidate against serogroup B (61). Kotelnikova et al. In 2005 studied the preventive properties of peptide fragments in conserved areas of outer membrane proteins OpaB, NspA and PorA of serogroup B in experimental rat models. They indicated the preventive effects of these peptides in rats infected by homologous and heterologous strains of serogroup B (62).

Recently, several vaccines based on outer membrane proteins produced as recombinant vaccines and assessed in human volunteers. One of these vaccines is a pentavalent vaccine made by Novartis Company, which evaluated in 2008. This vaccine consists proteins NadA, GNA2091, GNA1030, GNA2132, GNA1870 and OMV (63). Immunization of rats with these compounds along with Freund's adjuvant induces bactericidal responses of antibodies against 97% of study strains. Adjuvant properties of Meningococcal OMV as a modifying of Immunity is fully

determined. Some commercial companies such as Merck, Durham, Sharp Presented useful conjugate bivalent, vaccine that produced by Thio-Ether coupling method for linking of purified serogroup B meningococcal OMV and capsular polysaccharide of Hib (64). Therefore, meningococcal OMV is one of the significant alternatives to polysaccharide vaccines for prevention of meningococcal infections, which the of its protein nature, this fragment has conformational characteristics in the shape and even after it's purification, can maintain epitopes and motifs and considered as a powerful immunogen. There are controversial about purification methods of cell wall proteins of Bacteria. Scientists believe that repetitive modifications result in damage to the conformational structure, effective epitopes that induce immune responses and finally purified protein contains only primary structure or primary amino acid sequence. Thus, the mild procedure for purification should be used because this method can maintain native conformation of proteins (65, 66).

Conclusion

Although the polysaccharide capsule conjugate vaccine that have been developed against meningococcal serogroups A-C-Y and W135 are successful, but serogroup B because of the similarity with human polysialic glycoproteins is poorly immunogenic and to be cross-reactions. Thus, vaccines based on outer membrane vesicles have been designed for them. The first OMV-based vaccines have been produced in Cuba and Norway and are now being used in some countries. The problem with these vaccines requires a high injection and therefore a long time to create of protection. Therefore, vaccination against serogroup B is still continuing because the vaccine in epidemic can affect to do while does not have the ability to endemic conditions.

Conflict of interest

No potential conflicts of interest were disclosed.

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