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Review

Immunological Aspect of Meningococcal disease: An overview in Host- Bacteria Interaction

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ABSTRACT

Meningococcal disease remains a significant global public health and is unique among causes of bacterial meningitis and sepsis where it not only causes sporadic disease but also outbreaks. Meningococcal disease has a rapid onset with high mortality. The understanding of immunopathogenesis is crucial for development of novel therapeutic strategies and vaccines designed against meningococcal disease. In this review, immunological aspects of meningococcal disease have been discussed and the immunopathogenesis of this disease is challenged.

1. Microbiological Feature and Epidemiology

Neisseria meningitidis is gram-negative, aerobic diplococci which can be isolated on chocolate agar. It is classified into at least 13 serogroups (A, B, C, 29E, H, I, K, L, W135, X, Y and Z) according to its immunologic properties of capsular polysaccharides, which are the basis for currently employed meningococcal vaccines (Siadat and Norouzian, 2007).

These polysaccharides are specific to each serogroup and essential for pathogenicity. Group A (MenA) polysaccharide is composed of O-acetylated residues in the 3 position of mannosamine-6-phosphate linked to a (Estabrook et al., 1998; Keller and Stiehm 2000; Rosenstein et al., 2001; Siadat and Norouzian, 2007a; Siadat et al., 2007b; Rezaei et al., 2007), while group B (MenB) is a homopolymer of a N-acetylneuraminic acid (Estabrook et al., 1998; Keller and Stiehm 2000; Rosenstein et al., 2001; Rezaei et al., 2007; Siadat et al., 2007b; Siadat et al., 2007c; Siadat et al., 2007d). Group C (MenC) is more variable: it comprises O-acetylated residues in the 7 and/or 8 position (in O-acetyl-positive form), or is made up of nonacetylated (in O-acetyl-negative form)

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N-acetylneuraminic acid linked a (Estabrook et al., 1998; Keller and Stiehm 2000; Rosenstein et al., 2001; Unkmeri et al., 2002; Rezaei et al., 2007; Siadat et al., 2007b; Siadat et al., 2007c; Siadat et al., 2007d). If necessary, further typing of the different strains is possible by taking into consideration the different outer membrane proteins (Rosenstein et al., 2001; Siadat and Norouzian, 2007a).

Meningococcal disease remains a global public health problem, with a significant mortality and morbidity in both developed and developing countries, and an endemic as well as worldwide epidemic illness. Although, Five serogroups including A, B, C, Y, and W135 account for virtually all disease-causing isolates, however, over 90% of cases of meningitis are caused by serogroups A, B and C. All five serogroups cause epidemics, but most are characteristically caused by serogroup A. In Europe and Latin America, serogroup B is usually the most prevalent with causing well over 50% of cases, whereas the most common serogroup in the US and Canada is C. A virulent clone, ET-15, seems to be increasing in importance. The proportion of group B strains is especially high in Norway, The Netherlands, Germany and Denmark, while high or increasing proportions of group C strains are reported from the Czech Republic, Slovakia, Greece, Republic of Ireland, Spain, and the UK. In all countries, the incidence of group B disease is at the highest in infants, less occurs in young children. It frequently causes localized outbreaks amongst teenagers and young adults. In the USA meningococcal disease is also caused by Y strains, with each capsular group accounting for about a third of cases. Group Y disease arises in children and young adults, but has a propensity to cause disease in elderly people as well. It causes pneumonia more frequently than strains of the other groups. Once common, group A disease has been rare in the USA and Europe for more than 25 years. By contrast, group A isolates are predominant in sub-Saharan African countries with annual incidence rates for endemic disease varying considerably from country to country, and reaching up to 30 per 100,000 in countries such as Niger, where disease is hyperendemic. In other areas of the developing world, few data are available on the incidence of meningococcal disease or distribution of capsular groups (Rosenstein et al., 2001; Siadat and Norouzian, 2007a; Rezaei et al., 2007).

2. Natural Protection from Meningococcal Disease

To understand meningococcal disease and the philosophy behind the development of vaccines, after 121 years since the first isolation of *Diplococcus intracellularis* (*Neisseria meningitidis*) by Anton Weichselbaum, investigation of the nature of human immunity to the organism is still considerable and an outline of immunology needs to be given (Estabrook et al., 1998; Keller and Stiehm, 2000).

By the second decade of last century, it had been demonstrated that serum therapy would decrease the fatality of meningococcal meningitis, and it was suggested that serum antibodies would play a role in protection. The theory proved right: natural protection is achieved mainly by development of anticapsular antibodies, though less so in MenB disease. The 1960s showed an inverse relation between 'functional' bactericidal antibodies and susceptibility to disease. The disparity between carriage and disease rates is partly accounted for by the low virulence of many carrier strains but also suggests a role for naturally acquired protective immunity. Although poorly understood, natural immunization is thought to occur through prolonged or intermittent colonization at the mucosal surface by *N.meningitidis* or related organisms such as *Neisseria lactamica* (Estabrook et al., 1998; Siadat and Norouzian, 2007a; Siadat et al., 2007b). Mucosal as well as systemic immune mechanisms have been implicated in this process, with both serum and salivary antibodies demonstrated following meningococcal carriage. Complement-fixing IgG that are high in bactericidal activity (serum bactericidal antibodies) are thought to be important mediators of protective immunity against *N. meningitidis*. Since the carriage rate of virulent meningococci is too low to confer

immunity, natural immunization probably occurs through unrelated but serologically cross-reactive bacteria. *Escherichia coli* K1 has a polysaccharide structurally and serologically identical to that of MenB meningococci. From the age of 2 years onward, antibody concentrations increase, so that children have 5% more serum bactericidal activity against MenA, B and C meningococci each year. Intermittent carriage of different serotypes broadens immunity (Siadat et al., 2007b; Siadat et al., 2007c). Carriage of all meningococci is highest in young adults; military recruits develop a marked increase in bactericidal titre of immunoglobulin G (IgG), IgM and IgA antibodies within the first few weeks. Several questions still remain unanswered, especially in MenB disease. MenB polysaccharide is poorly immunogenic in humans (Rosenstein et al., 2001; Siadat and Norouzian, 2007a; Siadat et al., 2007c).

There is a fear that using this polysaccharide as a vaccine would hide risks of immunological tolerance as the homopolymer of (Estabrook et al., 1998; Keller and Stiehm, 2000; Rosenstein et al., 2001; Rezaei et al., 2007; Siadat et al., 2007b; Siadat et al., 2007c; Siadat et al., 2007d) N-acetylneuraminic acid might cross-react with polysialic acids of embryonic neural cell adhesion molecules: perhaps an autoimmune process would be triggered, and vaccine-induced antibodies might interfere with the functions of the polysialylated protein components of the brain. The relevance of the theory has been questioned. However, since it is very difficult to prove (or disprove), it has blocked much of the research on MenB polysaccharide. Nevertheless, anti-MenB polysaccharide antibodies (those few that are induced) are bactericidal in the presence of human complement (Siadat and Norouzian, 2007a; Siadat et al., 2007c; Siadat et al., 2007d).

3. Immunopathogenesis of *N. meningitides*

3.1. Colonization of N. meningitidis in nasopharynx cells

The first step during meningococcal disease is adherence of *Neisseria meningitidis* to nonciliated columnar cells of the nasopharynx, mediated by surface-associated filaments called type IV pili. A member cofactor protein (CD46) is a human cell surface complement regular, which is expressed on all human cells except erythrocytes, and has been identified as a receptor for neisserial type IV pili (Rosenstein et al., 2001; Siadat and Norouzian, 2007a).

Host–pathogen interaction is a two-step process involving first localized and then intimate adhesion. The second step is dependent on retraction of the pilus mediated by the PilT nucleotide-binding protein, which allows a closer interaction with the host cell membrane (Rosenstein et al., 2001). Pili are known to disappear when bacteria have formed intimate contacts, and this is fallowed by downregulation of capsule expression and removal of sialic acid from lipooligosaccharide (LOS) with unmasking of membrane-associated structures (Rosenstein et al., 2001; Unkmeri et al., 2002; Siadat and Norouzian, 2007a) These and other observations suggest that adhesion is a multifactorial process and that, probably, the closer range contact is maintained by interactions between outer membrane proteins and host cell surface receptors (Unkmeri et al., 2002). Several adhesins have been shown to play a role in this process, and it seems probable that interactions involving different molecules are important in determining tissue tropism and invasive versus carriage potentials. Opacity-associated proteins Opa and Opc are among the most studied of these adhesins. Opa proteins have been shown to target heparan sulfate proteoglycans receptor (HSPGs) and CD66 as well as the carcinom embryonic antigen -related cell adhesion molecules (CEACAMs) (Pollard et al., 1999) Of note, CEACAM expression is increased during inflammation. This might explain the higher rate of meningococcal disease in individuals with prior infections (Siadat et al., 2007b). Opc has been shown to bind in a complex with fibronectin and a5–b1 integrin, mediating adhesion to and invasion of human brain-derived endothelial cells (Pollard et al., 1999). Differently, interaction of this adhesin with epithelial cells occurs via binding to HSPGs receptors (Unkmeri et al., 2002). Opa and Opc do not affect the interaction with eukaryotic cells when meningococci are capsulated. Thus

downregulation of capsule synthesis seem to be essential for meningococcal interaction with host cells (Robinson et al., 2002; Fabio et al., 2006).

Recently, several findings have shed new light on the adhesion–invasion process. A new adhesin, the adhesion and penetration protein (App), was shown to mediate adherence and bacteria–bacteria aggregation of encapsulated MC on Chang human epithelial cells. By contrast, App does not appear to contribute to adhesion to human umbilical vein endothelial cells (HUVEC), suggesting that the molecule is involved in the first step of colonization at the nasopharynx mucosa. Furthermore, App is endowed with autocatalytic serine protease activity, and it has the potential to have pathogenic functions similar to the Neisseria immunoglobulin A1 (IgA1) protease (Fabio et al., 2006). Another factor shown to elicit invasion into epithelial cells is the *Neisseria* adhesin A (NadA). NadA belongs to the novel family of oligomeric coiledcoil adhesins (Oca). Conformational studies suggest that NadA is composed of a tripartite structural organization with an N-terminal globular domain, which probably binds to a cellular protein receptor (Pollard et al., 1999; Rosenstein et al., 2001; Robinson et al., 2002; Fabio et al., 2006).

Meningococcal LOS interacts with human cells, resulting in the production of proinflammatory cytokines and chemokines, including interleukin 1 (IL-1), IL-6, and tumor necrosis factor (TNF), which is important in the pathogenesis of meningococcal disease. While pili, Opa and Opc outer membrane proteins are also critical, LOS is one of the structures important in mediating meningococcal attachment to and invasion into epithelial cells (Kvalsvig and Unsworth, 2003; Fabio et al., 2006). The role of LOS in these events is further substantiated by the findings that LOS-deficient meningococcal mutants show impaired adherence and reduced induction of serum cytokines compared to the wild-type strain. The structure of *N.meningitidis* LOS has been characterized both immunologically and biochemically. Meningococcal LOS lacks the repeating O antigens of *Escherichia coli* lipopolysaccharide but maintains a conserved inner core that composed of heptose and 3-deoxy-D-manno-2-octulosonic acid. Lipid A is the active moiety through its ability to upregulate the inflammatory response. Changes in the configuration or the conformational structure of lipid A affect the biological response. Variation in the composition of meningococcal LOS is also postulated to mediate changes in host immune responses and bacterial virulence (Unkmeri et al., 2002; Robinson et al., 2002; Kvalsvig and Unsworth, 2003; Fabio et al., 2006).

A recent study reveals that bacteria reached the mucosal surfaces of the upper respiratory tract, which required expression of the meningococcal PilC1 adhesin (Fabio et al., 2006). Interestingly, PilC1 was dispensable for meningococcal growth in blood and for crossing of the blood-brain barrier, indicating that the major role of PilC1 is to interact with mucosal surfaces. This in vivo study reveals disease dynamics and organ targeting during meningococcal disease and presents a potent tool for further investigations of meningococcal pathogenesis and vaccines in vivo. This might lead to development of new strategies to improve the outcome of meningococcal disease in human patients (Estabrook et al., 1998; Kvalsvig and Unsworth, 2003; Fabio et al., 2006).

3.2. Invasion and penetration of N. meningitidis

Meningococci pass through the mucosal epithelium via phagocytic vacuoles. The bacteria are surrounded by elongated microvilli and inside intracellular vacuoles as a result of endocytosis. Further studies have shown specific molecular complexes (cortical plaques) underneath the bacteria and have suggested that elongation of the epithelial microvilli is due to polymerization of cortical actin. Cortical plaques are enriched with tyrosine phosphorylated and integral membrane proteins such as the intercellular adhesion molecule 1 (ICAM-1), the cluster of differentiation molecule 44 (CD44) and the epidermal growth factor receptor (EGFR), a protein that functions as a linker between the plasma membrane and the actin cytoskeleton (Rosenstein et al., 2001; Siadat and Norouzian, 2007a).

During invasion, several bacterial factors modulate the metabolism of the mucosal cell. Binding stimulates engulfment of the meningococci by epithelial cells, which may then traverse the mucosal epithelium through phagocytic vacuoles (Nicholson and Lepow, 1979; Scheld et al., 2002; Mairey et al., 2006).

As mentioned above, binding of pili and class 5 OMPs to their receptors transduce a signal to the host cell. PorB, a class 2/3 OMP, may translocate into target cell membranes and affect the maturation of phagosomes. Furthermore, once inside the host cell, meningococci are associated with lysosomal compartments, and it has the ability to replicate. It is proposed that intracellular survival is dependent on the activity of the *Neisseria* IgA1 protease, which seems capable of cleaving the lysosomal integral membrane protein 1 (LAMP1) and reducing the levels of several other lysosomal molecules. Although intracellular life might have evolved as a way to escape the immune attack, sepsis seems to be just a collateral effect of the invasive capacities (Rosenstein et al., 2001; Scheld et al., 2002; Fabio et al., 2006; Mairey et al., 2006).

4. Immune recognition of meningococcal disease in the blood

4.1. Complement system and the cells

Meningococci can survive and proliferate in the bloodstream by virtue of particular bacterial virulence factors or incompleteness of the host defense (Kvalsvig and Unsworth, 2003; Fabio et al., 2006; Mairey et al., 2006).

The most essential bacterial virulence factor for survival in the bloodstream is the polysaccharide capsule, which protects against complement mediated bacteriolysis and phagocytosis by neutrophils, Kupffer cells, and spleen macrophages. In brief, sialic acid residues in the group B and C capsule as well as lipooligosaccharides decrease the serum bactericidal activity by enhancing the affinity of the alternative-pathway inhibitor factor H to C3b and thus inhibiting complement activation. In addition, some class 1 OMPs impede ingestion of the meningococcus by neutrophils via downregulation of the Fcg receptor and the C1 and C3 receptor, and IgA1 proteases break IgA1 in the hinge region and liberate monomeric Faba fragments, which can block the access for intact IgG or IgM (Rosenstein et al., 2001; Fabio et al., 2006; Siadat and Norouzian, 2007a).

Host defense after meningococcal disease is performed by the innate and adaptive immune systems and each of them is strictly depended with another, by the fact that protection to invasive meningococci strongly relies on serum IgG and complement system, which trigger bacteriolysis and opsonophagocytosis. The complement is strongly activated in response to meningococcal disease and has both recognition and effector functions. Early complement activation occurs via the mannose binding lectin (MBL) and the alternative pathway (Fabio et al., 2006; Mairey et al., 2006).

The MBL pathway is activated with LPS binding protein, bactericidal permeability inducing protein (BPI), soluble CD14, and acute phase reactants, such as C reactive protein and serum amyloid P, that all of which have been implicated in recognition of meningococci. However, during complement activation, C3 and C4 split products will be deposited on the bacterial surface, and these fragments may be effective opsonins. IgG antibodies bound to bacteria are also excellent opsonins, and a synergistic opsonic effect is achieved when the target is covered with both IgG and complement split products (Scheld et al., 2002; Mairey et al., 2006).

Neutrophils and macrophages express FcγRII and FcγRIII constitutively, both with low affinity for IgG and several complement receptors (CRs) that bind to targets coated with C3 and C4 split products after complement activation (Mairey et al., 2006; Kheirandish et al., 2009). Finally, once the terminal complement cascade is activated, downstream effects include opsonization and phagocytosis, lyses of meningococci by the membrane attack complex C5-9, and further activation of inflammatory response via complement fragments. Monocytes can also phagocytose *Neisseria* species (Fabio et al., 2006; Mairey et al., 2006; Kheirandish et al., 2009). The relative importance of opsonophagocytic killing as a

defense mechanism against *N. meningitidis* has been previously demonstrated since intracellular destruction will minimize intravascular release of bacterial endotoxin and thus reduce the risk for septic shock. For this reason, opsonophagocytic assays (OPA) offer several advantages over the standard serum bactericidal assay (SBA) (Estabrook et al., 1998; Fabio et al., 2006; Mairey et al., 2006; Siadat et al., 2007b; Siadat et al. 2007d; Kheirandish et al., 2009).

4.2. Humoral immunity and antibody response

Humoral immunity has an essential role in protection against meningococcal disease, and carriage of *Neisseria* causes an increased bactericidal antibody response (Pollard et al. 1999; Fabio et al., 2006).

There have been some studies on the functional activity of the IgG1 and IgG2 subclasses from clinical materials, both concerning phagocytosis and bactericidal activity. These antibodies were mainly directed against polysaccharide and may thus not be representative for antibodies against protein antigen that may have a rather differing distribution on the microbial surface. Furthermore, IgG3 antibody, which are frequently produced after immunization with proteins antigens, were not included in these studies. There has also been very little done to compare opsonophagocytosis and bactericidal activity from IgG subclass preparations derived form the same serum sources (Mairey et al., 2006; Behzadiyannejad et al., 2008).

4.3. Cellular immunity and cytokine production: friends or foe?

The expression of cytokines among activated CD4 cells is an important event in immunopathogenesis of meningococcal disease, and has considerable bearing on the clinical course. Many studies had been shown that the T helper cell subset response was elicited by meningococcal carriage and also disease (Scheld et al., 2002; Fabio et al., 2006). During the acute phase of meningococcal disease, many proinflammatory cytokine blood levels are increased, among which the tumor necrosis factor alpha (TNF-a), the interferon-gamma (INF-γ), the interleukin-1 (IL-1), IL-6 and IL-8 play a crucial role. TNF-a is positively associated with a severe clinical outcome, mainly because of its proinflammatory and procoagulant effects (Pollard and Frasch, 2001; Mirlashari et al., 2001; Fabio et al., 2006; Mairey et al., 2006; Rezaei et al., 2007).

LOS is a potent proinflammatory stimulus. Activation of the immune response is probable to rely mainly on the interaction between LOS and the LPS binding protein (LBP) with a complex on monocytes, macrophages and other host cells formed by CD14, the Toll like receptor 4 (TLR4) and the membrane protein 2 (MD2) (Bryant et al., 2010). TLR4 and MD2 are mainly found on macrophages/monocytes, dendritic cells and other phagocytes. However, LOS deficient mutants still induce cytokines, even if at lower amounts. A central role in LOS-independent activation of inflammatory pathways seems to be played by TLR2 (Fabio et al., 2006; Bryant et al., 2010). Indeed, the nesserial porin B (PorB) has been shown to interact directly with TLR2, stimulating B cells and inducing NF-kB nuclear translocation (Fabio et al., 2006; Bryant et al., 2010). Also, LOS activates zymogens belonging to the complement system, contact system, and kallikrein-bradykinin system. Since activation of these zymogens requires only proteolytic cleavage by a serine protease, the activated factors of these systems appear immediately (Fabio et al., 2006). Similarly, neutrophils release elastase and other lysosomal proteinases instantaneously from their storage pools. Endotoxin also induces the production, expression and release of mediators such as tissue factor (TF), tissue plasminogen activator (TPA), and the pro- and anti-inflammatory cytokines. Since these latter mediators are proteins that have to be synthesized, they appear approximately 1 to 2 h later than the activated zymogens and proteins released from storage pools. Nearly all these mediators can induce shock, either alone or in synergy (Fabio et al., 2006; Rezaei et al., 2008; Bryant et al., 2010).

5. Immunological perspective of meningococcal meningitis

During the course of fulminant meningococcal sepsis (FMS), meningococci can cross the BBB and invade the brain, where they proliferate uncontrolled as the main humoral and cellular host defense mechanisms are reduced. Alternatively, meningococcal meningitis can also develop in the absence of peripheral signs of sepsis. In this case, the inflammatory response remains localized to the brain and the bacteremia are often low or even undetectable. When treated, this form of meningitis has a relatively low rate of mortality and neurological sequelae compared with other types of bacterial meningitis (Mairey et al., 2006; Kheirandish et al., 2009).

Although the ability of *N. meningitidis* to colonize the human brain is a characteristic property of this pathogen, little is known about the causes for this tropism. This is at least in part the result of human-specific adhesive properties of *N. meningitidis*, which have hampered the development of animal models (Rosenstein et al., 2001; Mairey et al., 2006; Kheirandish et al., 2009).

The mechanism for crossing the BBB still remains undetermined. In vitro experiments favor a transcellular pathway because, in these conditions, *N. meningitidis* infection does not affect tight junctions formed by the cells. Interestingly, however, local ischemia is known to lead to a temporary breach in the BBB (Scheld et al., 2002; Mairey et al., 2006).

Once in the subarachnoid space, where the principal humoral and cellular host defense mechanisms are absent, meningococci proliferate uncontrolled. The evolving endotoxin liberation elicits compartmentalized (i.e., confined to the subarachnoid space) activation of proinflammatory cytokines, by human meningeal cells, such as TNF, IL-1, IL-6, IL-8, nitric oxide, monocyte colony-stimulating factor, and platelet-activating factor and anti-inflammatory cytokines such as IL-1Ra, IL-10, IL-12, TNFsR-p55, TNFsR-p75, and IL-1sR type II (IL-1sRII) and inflammation is occurred (Voort et al., 1997; Mirlashari et al., 2001; Fabio et al., 2006; Mairey et al., 2006). By this means that molecules involved in the immune response activated by meningococci are tissue-specific, and that an important role might be played by the nucleotide-binding oligomerization domain (Nod) protein family. Among these, TNF and IL-1 enhance the permeability of the blood-brain barrier and promote the influx of neutrophils by upregulation of adherence molecules (Mairey et al., 2006). The subsequent release of neutrophil products contributes to the development of clinically overt meningitis. Furthermore, Nod1 protein has been demonstrated to function as an intracellular sensor of the meningococci peptidoglycan, mediating activation of NF-kB in epithelial cells (Massari et al., 2003; Fabio et al., 2006). Hence, an important event of the host response as yet not very clear might be triggered by intracellular bacteria in the epithelium of the pharyngeal mucosa. Evasion of the immune attack, instead, relies on several mechanisms, including production of IgA protease and of capsule, both inhibiting opsonophagocytosis, and variability of surface antigens. Finally, in meningitis the inflammatory response is localized in an extravascular compartment devoid of zymogens belonging to the complement and coagulation systems (Massari et al., 2003; Fukasawa et al., 2003).

References

Behzadiyannejad, Q., Siadat, S.D., Kheirandish, M., Tabaraie, B., Ahmadi, H., Norouzian, D., et al., 2008. Comparison among opsonic activity and serum bactericidal activity against meningococci in rabbit sera from vaccines after immunization with outer membrane vesicle of Neisseria meningitidis serogroup B. Res J Microbiol. 3(3),105-113.

Bryant, C.E., Spring, D.R., Gangolff, M., Gay, N.J., 2010. The molecular basis of the host response to lipopolysaccharide. Nature. 8(9), 8-14.

Estabrook, M.M., Zhou, D., Apicella, M.A., 1998. Nonopsonic phagocytosis of group C *Nesseria meningitidis* by human neutrophils. Infect Immun. 66(3), 1028-1036.

Fabio, B.F., Rino, R., 2006. Mechanisms of pathogenesis and prevention of meningococcal disease. Drug Discovery Today. 3(2), 273-279.

Fukasawa, L.O., Gorla, M.C., Lemos, A.P., et al., 2003. Immune response to native NadA from Neisseria meningitidis and its expression in clinical isolates in Brazil. J Med Microbiol. 52, 121-125.

Keller, M.A., Stiehm, E.R., 2000. Passive immunity in prevention and treatment of infection diseases. Clin Microbiol Rev. 13(4), 602-614.

Kheirandish, M., Siadat, S.D., Norouzian, D., Razavi, M.R., Aghasadegh, M.Ri., Rezaie, N., Farazmand, A., Izadi, M.J., Zangeneh, M., Moshiri, A., Sadat, S.M., Sharifat, S., slmani, A., 2009. Measurement of Opsonophagocytic activity of antibodies specific to Neisseria meningitidis serogroup A capsular polysaccharide-serogroup B outer membrane vesicle conjugate in animal model. Ann Microbiol. 59(4), 801-806.

Kvalsvig, A.J., Unsworth, D.J., 2003. The immunopathogenesis of meningococcal disease. J Clin Pathol. 56, 417-422.

Mairey, E., Genovesio, A., Donnadieu, E., Bernard, C., Jaubert, F., Pinard, E., Seylaz, J., Duménil, G., 2006. Cerebral microcirculation shear stress levels determine *Neisseria meningitidis* attachment sites along the blood–brain barrier. The J EXP Med. 203 (8), 1939–1950.

Massari, P., Ram, S., Macleod, H., et al., 2003. The role of proins in neisserial pathogenesis and immunity. Trend in Microbiology. 11, 87-93.

Mirlashari, M.R., Hoiby, E.A., Holst, J., et al., 2001. Outer membrane vesicles from Neisseria meningitidis: effect on cytokine production in human whole blood. Cytokine. 13, 91-97.

Nicholson, A., Lepow, I.H., 2009. Host defense against *Neisseria meningitidis* requires a complement-dependent bactericidal activity. Science. 205 (4403), 298-299.

Pollard, A.J., Frasch, C., 2001. Development of natural immunity to Neisseria meningitidis. Vaccine. 19, 1327-1346.

Pollard, A.J., Galassini, R., Voort, E.M., et al., 1999. Humoral immune responses to *Neisseria meningitidis* in children. Infect Immun. 67(5), 2441-2451.

Rezaei, N., Aghamohammadi, A., Siadat, S.D., Moin, M., Pourpak, Z., Nejati, M., Ahmadi, H., Kamali, S., Norouzian, D., Tabaraei, B,. Read, R.C., 2008. Clinical classification of common variable immunodeficiency based upon serum bactericidal antibody responses to meningococcal polysaccharide vaccination. Clin Vaccine Immunol (CVI). 15(4), 607-611.

Rezaei, N., Aghamohammadi, A., Siadat, S.D., Nejati, M., Hojat Ahmadi, H., Moin, M., Pourpak, Z., Kamali, S., Norouzian, D., Tabaraei, B., Read, R.C., 2007. Serum bactericidal antibody response to serogroup C polysaccharide meningococcal vaccination in children with primary antibody deficiencies. Vaccine. 25,5308-5314.

Robinson, K., Neal, K.R., Howard, C., et al., 2002. Characterization of humoral and cellular immune responses elicited by meningococcal carriage. Infect Immun. 70, 1301-1309.

Rosenstein, N.E., Perkins, B.A., Stephens, D.S., Popovic, T., Hughes, J.M., 2001. Meningococcal disease. N Engl J Med. 344(18),1378-88.

Scheld, W.M., Koedel, U., Nathan, B., et al., 2002. Pathophysiology of bacterial meningitis: mechanism(s) of neuronal injury. J Infect Dis. 186, 255-233.

Siadat, S.D., Behzadiannejad, Q., Tabaraie, B., Ahmadi, H., Norouziuan, D., Najar-Peerayeh, S., Nejati, M., Hedayati, M.H., Zangeneh, M., 2007. Evaluation of serum bactericidal activity specific for *Neisseria meningitidis* serogroup A and B: effect of immunization with *Neisseria meningitidis* serogroup A polysaccharide and serogroup B outer membrane vesicle conjugate as a bivalent meningococcus vaccine candidate. Res J Microbiol. 2(5), 436-444.

Siadat, S.D., Kheirandish, M., Norouzian, D., Behzadiyannejad, Q., Najar Peerayeh, S., Zangeneh, M., et al., 2007. A flow cytometric opsonophagocytic assay for measurement of functional antibodies elicited after immunization with outer membrane vesicle of *Neisseria meningitidis* serogroup B. Pakistan J Biol Science. 10(20), 3578-84.

Siadat, S.D., Norouzian, D., Tabaraie, B., Behzadiyan nejad, Q., Pirayeeh, S.N., Nejati, M., et al., 2007a. Comparative studies of conjugated capsular polysaccharide of *Neisseria meningitidis* serogroup A with outer membrane vesicle of *Neisseria meningitidis* serogroup B. Res J Microbiol. 2(4), 337-45.

Siadat, S.D., Norouzian, D., 2007b. Meningococcal vaccines: past, present, and future perspective. J Infect Develop Count. 1(2), 129-46.

Unkmeri, A., Kämmerer, U., Stade, A., et al., 2002. Lipooligosaccharide and polysaccharide capsule: virulence factors of *Neisseria meningitidis* that determine meningococcal interaction with human dendritic cells. Infect Immun. 70(5), 2454-2462.

Voort, E.R.V., Dijken, H.V., Kuipers, B., et al., 1997. Human B – and T – cell responses after immunization with a hexavalent PorA meningococcal outer membrane vesicle vaccine. Infect Immun. 65, 5184-5190.