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THE ROLE OF MENINGOCOCCAL PORIN B IN PROTEIN-PROTEIN INTERACTIONS WITH HOST CELLS

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ABSTRACT

***Neisseria meningitidis* is a Gram-negative diplococcus responsible for bacterial meningitis and fatal sepsis. Ligand-receptor interactions are one of the main steps in the development of neuroinvasion. Porin B (PorB), neisserial outer membrane protein (ligand), binds to host receptors and triggers many cell signalling cascades allowing the meningococcus to damage the host cells or induce immune cells responses via the TLR2-dependent mechanisms. In this paper, we present a brief review of the structure and function of PorB.**

Key words: *Neisseria meningitidis*; PorB; protein; Toll-like receptor

INTRODUCTION

N. meningitidis is an exclusive human pathogen, and the leading worldwide cause of meningitis and fatal sepsis. In the development of the meningococcal disease an interaction of several pathogen's ligands with receptors located on the endothelial cells surface in the brain microvesicles is essential. In this paper we have focused on Porin B (PorB), one of the important ligands of Neisseria, that binds to the brain microvascular endothelial cells. Unfortunately, there is a lack of consistent information available on the function of the porin of *Neisseria meningitidis*. Thus, in this paper we review the role of PorB of Neisseria and presented it in a consistent manner. Reviewing the multifaceted functions of PorB, (e.g. selective sugar binding, role in nonselective ions translocation, role in the activation of human B cells, induction of the release of cytokines, etc.) we will show that this protein is one of the major ligands of *Neisseria* that govern its crucial role in pathogenesis.

Porin B (PorB)

Approximately 60% of the outer membrane proteins of *Neisseria* species consist of porins, which belong to the Gram-negative porin superfamily [14, 18]. In the case of gonococcus, porins are termed protein IA (PIA, PorBIA, 35 kDa) or IB (PIB, PorBIB, 37 kDa), while in the case of meningococcus they are designated as PorA (class 1 pro-

tein, ~45 kDa) or PorB (class 2 or 3 protein, ~33 kDa) [9, 12, 18]. Porins from *N. meningitidis* and *N. gonorrhoeae* share 60—70 % amino acid sequence homology [7, 27] and moderate antigenic variability, which is the basis of the *neisseria*l serotyping system [9].

Structure of PorB

PorB is in trimeric form (Figure 1). Each monomer (~ 35 kDa) contains a high proportion (~36 %) of β-pleated sheets [7, 28]. Each unit forms a 16-stranded (S) β-barrel with eight short periplasmic turns connecting the strands on the periplasmic side of the channel, and eight long interstrand loops (L1-L8) on the extracellular side of the channel (Figure 2). L2 was located as an interface among monomers, and contributes to the trimeric formation. In particular, the extracellular loop 3 (L3) protrudes into the pore and forms a α-helix, which constricts the pore to 8 Å by 10 Å at its narrowest point. This region is often referred to as a constriction zone for controlling the pathway for solute transport. This topology is similar to that observed for other outer membrane proteins (OMPs), with sequence insertions and variability in the region exposed to the host immune system. The β-barrel regions share a high level of sequence homology among the different strains, while the amino acid sequence variability characterizes the surface-exposed loops [15, 39, 40].

Function of PorB

Neisserial porins act as pores and are essential for bacterial survival because they modulate the ion exchange between the bacteria and its surrounding environment [44]. The physiological function of porins is to bind sugars selectively. It has been shown that PorB transports small sugars more quickly than larger sugars [28]. On the extracellular side of the channel, the funnel approaching the pore is strongly electronegative, whereas on the periplasmic side of the channel, the funnel is strongly electropositive. Although PorB has previously been characterized as a nonselective channel, these electrostatic charges differ from those of other nonselective porins of known structure, which have consistent, intermediate charge [6, 29]. It was found that glucose has the highest rate of substrate translocation. Similarly, galactose and arabinose showed fast transport through PorB, whereas sucrose and maltose displayed much slower transport rates [40]. Co-crystallization of PorB with sugar substrates revealed a specific binding site for both galactose and sucrose at the same location within the positively charged funnel. This indicates that PorB contains multiple selective mechanisms for substrate selection [40].

The second function involves putative non-selective translocation pathways. PorB translocate cations since it has been electrophysiologically characterized as weakly an-

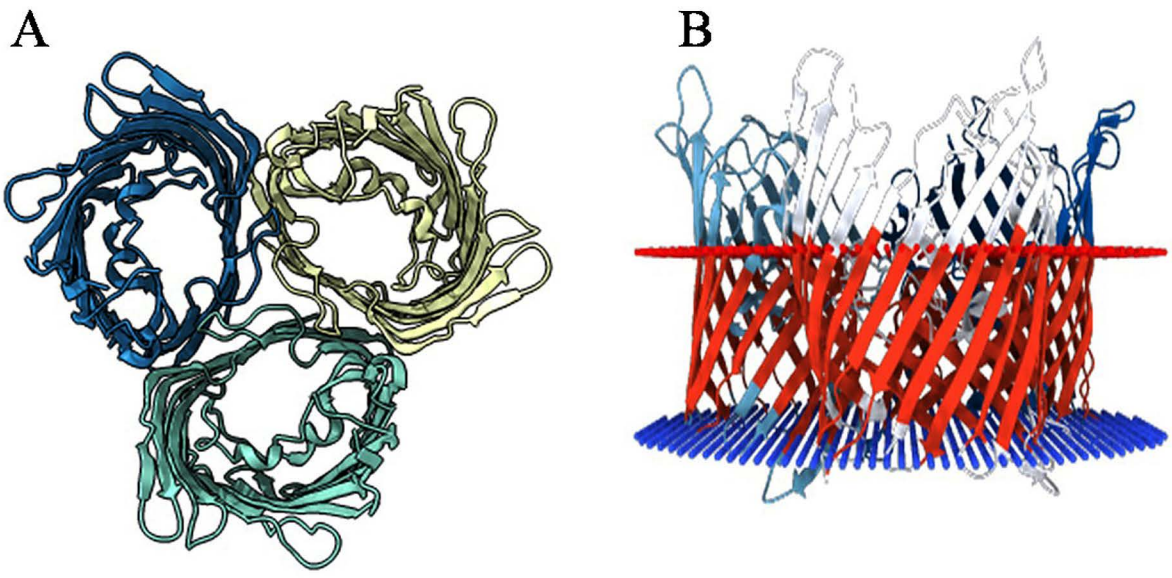

Fig. 1. Transmembrane regional views of the trimeric form [40]: structure of the PorB trimer (A); cartoon model of the PorB trimer (B).

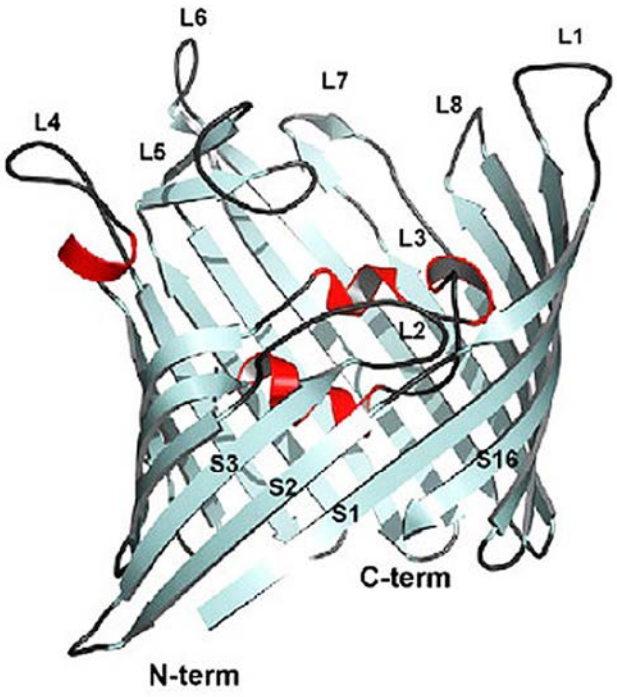

Fig. 2. A cartoon model of the lateral view of the PorB monomer structure. S1—S16 represent strands of amino acids which form the beta barrel, L1—L8 are loops of amino acids (grey), and L3 forms a helical structure (red) that lies across the pore [40].

ion selective [38], suggesting that an anion pathway must be available. Examination of the electrostatic surface representation identifies a putative pathway on the pore face nearest to the crystallographic 3-fold axis that is lined with positively charged residues. A similar feature has been identified in the *E. coli* OmpF [5, 13], the Delfita acidovorans Omp32 [45, 46], and the *E. coli* OmpC [3], which share structural similarity to PorB in the transmembrane β-barrel region of the protein [40].

The regulation of conductance by purine nucleotide triphosphates (pNTP) is a characteristic of PorB, which likely occur physiologically during infection when the channel is inserted into the host mitochondrial membrane. The binding site for pNTP is surrounded by the side chains of Lys9, Lys42, Arg77, Asn96, Lys100, Arg130 and it is adjacent to L3 near to the pore constriction [40]. A previous hypothesis claimed that 16- stranded β-barrel OMPs is non-specific [45]. However, current research shows that porins contain multiply selective and sophisticated mechanisms for substrate selection [40].

PorB as a ligand

Neisserial porins play a role in: the induction of inflammation; activation of human and murine B cells with a mitogenic effect; release of cytokines from human cells; and stimulation of platelet-activating factor production by human endothelial cells [10, 41]. However, the effect on T cells has not been directly observed. Moreover, neisserial porins have host cell-associated functions, including those that facilitate bacterial/host cell interaction, modulation of host cell survival, and induction of immune stimulation [24, 42]. It was found that porins interact with host cell receptors associated with bacterial adhesion/invasion processes (e.g. the laminin receptor LamR, the gp96 and Scavenger receptor SREC) [1, 33]. Porins promote epithelial cell invasion, especially their critical residues in the surface-exposed loops result in invasion of epithelial cells and direct the interaction of PorB with host cell receptors. Then, they are able to react with host receptors involved on complement activation [21, 32] and with members of the Toll-like receptor family (TLR2 and TLR1) [22, 25]. Toll-like receptors are a set of innate immune receptors that recognize common structures to many different pathogens and to some endogenous molecules. Currently, 11 human TLRs have been discovered, of which TLR4 was the first described mammalian TLR [2, 34].

Neisserial porins are potent immune adjuvants and induce antigen-presenting cell (APC) activation, increase MHC II and CD86 surface expression, which leads to the induction of the B cells proliferation, maturation of dendritic cells (DCs) and activation of macrophages [37, 43]. This immune adjuvant effect is due to the induction of CD86 surface expression [19]. It was found that *Neisseria meningitidis* PorB induces signal transduction events in murine B cells such as upregulation of CD86 and proliferation. Consequently, PorB can induce protein tyrosine kinase (PTK) activity, phosphorylation of Rrk1, Erk2 (serine/threonine kinase) and IκB-α, leading to nuclear factor (NF)-κB nuclear translocation in B cells in a TLR2-dependent manner. PorB-induced NF-κB nuclear translocation was not dependent on either PTK or Erk1/2 activities. Moreover, it was demonstrated that PorB acts through TLR2 as a B-cell mitogen [20]. PorB is able to activate other cell types, including the induction of CD86 expression, cytokines production and other signal transduction events involved in such phenomena [42]. Increased expression of CD86, MHC class I and II and induced DC maturation are caused by PorB. In addition, PorB stimulates T cells in an antigen-specific dependent manner through the activated DC. During *N. meningitidis* infection, many cytokines, mainly IL-6, are produced. Regularly, IL-6 is involved in the inflammatory process observed during infection or disease and is secreted by PorB-maturated DCs [37]. It has been observed that the maturation of DCs, macrophages activation, participation of TLR2 and myeloid differentiation primary response gene 88 (MyD88) are important for PorB-induced B cells [22]. The direct binding of PorB to TLR2 described by Wetzler in 2010, is directly related to cellular activation [42]. This activation via TLR2 occurs in association with TLR1 [11]. It has been described that murine B cells from WT mice respond to PorB by upregulating of co-stimulatory surface molecules CD86, CD40 and MHC II [22]. IL-6 failed to be induced by PorB in the absence of TLR2, although the co-receptors TLR1 and TLR6 were expressed on B cells [42]. Therefore, the induction of cytokine IL-6 production by PorB requires the presence of TLR2 but it maybe does not depend on the presence of TLR1. It is still unclear whether a TLR2 homodimer or a TLR2 heterodimer connected with another TLR for PorB-induced IL-6 is needed [42]. In general terms, PorB interacting with TLR2 and TLR1 is able to induce immune stimulation because of the evidence of the actual adjuvant activity of PorB and its ability to enhance the humoral immune response against bacterial capsular polysaccharide (CPS) has been already been described [19]. An increased CD86 expression of APCs, the presence of TLR2 and MyD88 are required for the enhancement of PorB immune activity [19, 22, 43]. *In vivo* it was confirmed that TLR2 plays a role in the adjuvant activity of PorB [42].

A closer similarity in the surface charges of L1, L2, L4, L6 and L7 was observed when electrostatic surface charges of PorB from invasive meningococci serogroups B and C were analysed, finding that all of them were negatively charged. Surface charges of L5, L6 and L7 could be important for mediating TLR2-dependent activation of intracellular signalling cascades that regulate host immune responses. It is still not clear however, whether PorB variants from different strains may modulate TLR2-dependent host cell responses [39].

Many processes of the apoptotic cascade take place in mitochondria in response to several pro- apoptotic signals. PorB binds to the mitochondrial porin (i.e. a voltage dependent anionic channel, VDAC), which is part of the mitochondrial permeability transition pore (PT) and triggers the induction of apoptosis in which mitochondrial depolarization and opening of the PT with cytochrome c releasing into the cytosol are carried out [16, 31, 36]. PorB and VDAC, two different classes of porins, share common functional and structural characteristics such as a high proportion of β- sheets, a β-barrel 3-D structure and regulation of pore size by nucleotides [4, 7, 35]. It seems that modulation of the mitochondrial potential is caused by its association with PorB [23].

In the case of N. gonorrhoeae, PorBIA possesses the ability to interact with the scavenger receptor expressed on endothelial cells (SREC-I). This connection is important for the mediation of bacterial uptake into endothelial or epithelial cells in a phosphate sensitive manner [17, 33]. The interaction between PorBIA and SREC-I leads to the re-localization of SREC-I to membrane rafts, caveolin-1 activation and the recruitment of signalling molecules PI3 kinase (PI3K) and phosphoinositol phospholipase C gamma 1 (PLCy1). Activation of PI3K and PLCy1leads to the phosphorylation of polycystin1 (PKD1) and to the activation of Rac-1, which finally triggers cytoskeletal rearrangements and bacterial uptake [8]. PKD1 is an integral membrane protein that regulates calcium permeable cation channels and the intracellular calcium homoeostasis. Moreover, PKD1 plays

a role in cell-cell/matrix interactions and may modulate G-protein-coupled signal-transduction pathways. Members of Rac-1 superfamily regulate a diverse array of cellular events, including the control of cell growth, cytoskeletal reorganization, and activation of protein kinases.

Laminin receptor (LamR), capable of interacting with PorBIA, allows cell adhesion to the basement membrane and it is also involved in tumour cell metastasis [26]. Furthermore, LamR plays a role in the intracellular signalling, ribosomal activity and cell viability [1]. Interestingly, many neurotropic bacteria and viruses use LamR in the binding process to human brain microvascular endothelial cells [30]. It was discovered the LamR-binding domain of PorA lies within the amino acids 171—240 and was localized on the L4. These findings provide an opportunity to produce antibodies recognizing this sequence or a peptide corresponding to LamR residues 263-282, which could inhibit bacterial binding to microvascular endothelial cells [1].

CONCLUSIONS

All these findings show that neisserial porins play an important role in the stimulation of B-cell proliferation and following an increase of immunoglobulin secretion, as well as they can bind to several described receptors and thereby initiate cascades causing cells reorganization and bacterial uptake.

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