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Outer membrane protein A and OprF – Versatile roles in Gram-negative bacterial infections

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Abstract

Outer membrane protein A (OmpA) is an abundant protein of *Escherichia coli* and other enterobacteria with a multitude of functions. Although the structural features and porin function of OmpA were well studied, its role in the pathogenesis of various bacterial infections has been emerging for the past decade. The four extracellular loops of OmpA interact with a variety of host tissues for adhesion, invasion and evasion of host-defense mechanisms. This review describes how various regions present in the extracellular loops of OmpA contribute to the pathogenesis of neonatal meningitis induced by *E. coli* K1 and for many other functions. In addition, the function of OmpA like proteins such as OprF of *Pseudomonas aeruginosa* is also discussed herein.

Introduction

Bacterial infections contribute to significant mortality throughout the world. Globally important diseases such as tuberculosis kill millions of people every year. Other infections such as pneumonia, which can be caused by *Streptococcus* and *Pseudomonas* and food borne illnesses due to *Shigella*, *Salmonella* and *Campylobacter*, tetanus, typhoid, diphtheria, syphilis leprosy and meningitis contribute to high morbidity rates. In the USA, 71% of urinary tract infections, 65% of pneumonia episodes, 34% of surgical site infections, and 24% of bloodstream infections were due to Gram-negative bacilli. The rates of nosocomial infections caused by Gram-negative bacteria are even higher in many parts of the world. Since the emergence of antibiotic resistant strains is problematic to treat these infections, understanding the pathogenesis of various diseases by these bacteria would yield information to develop new preventative or therapeutic strategies. Bacterial pathogens have several surface structures that potentially interact with host tissues for attaching to and invasion, which include pili or fimbriae, outer membrane proteins (OMPs) and various secretion systems. Gram-negative bacteria contain several classes of OMPs that form monomeric or trimeric barrels to facilitate the transport of nutrients into the cell.

Outer membrane protein A (OmpA) is a multi-functional major outer membrane protein of *Escherichia coli* and other *Enterobacteriaceae* family. OmpA is a highly conserved protein among Gram-negative bacteria throughout evolution. In addition to its role as a structural protein, OmpA serves as a receptor for several bacteriocins namely colicin U, colicin L and bacteriocin 28b. It also serves as a receptor for several bacteriophages such as K3, O_x2 and M1. Furthermore, OmpA interaction with F-plasmid encoded outer membrane protein TraN is required for conjugal mating. The current understanding of the structure and assembly of OmpA is reviewed in previous sections and elsewhere [1,2]. OmpA is a beta-barrel protein

that contains four extracellular loops and, a comparison of sequence similarities in the extracellular portions of various Gram-negative bacteria is shown in Fig. 1. Recently, OmpA has been shown to exist as two different allelic forms, *ompA1* and *ompA2*. These two alleles have specific differences in the amino acids especially in loop 2 and 3 [3]. Here, the function of OmpA in the pathogenesis of various diseases by different bacteria is reviewed.

Pathogenesis of *E. coli* K1 induced neonatal meningitis – All round action of OmpA

Bacterial meningitis in the neonatal population is a serious central nervous system (CNS) infection distinguished by a severe inflammation of the meninges and the subarachnoid space. The mortality rates associated with this disease range from 20 to 30% of infected infants and reaches 100% if untreated. Though antibiotic treatment reduces mortality rates, 50% of survivors suffer neurological sequelae that include mental retardation, cortical blindness and hearing impairment. *E. coli* K1 is one of the prominent pathogens that cause meningitis in neonates. The bacterium colonizes nasopharyngeal or gastrointestinal sites initially and penetrates into circulation in which it multiplies to reach high-grade bacteremia. Subsequently, *E. coli* K1 interacts with brain microvascular endothelial cells, which comprises the lining of the blood-brain barrier (BBB), to bind to and invade. During these steps, the bacteria must evade host-defense mechanisms for which *E. coli* K1 has developed a variety of strategies for survival.

E. coli K1 OmpA role in evasion of complement attack

The first report for the role of OmpA in virulence was demonstrated in a neonatal rat model of *E. coli* K1 meningitis and by far the most extensively studied. *E. coli* K1 lacking OmpA expression were inefficient in inducing bacteremia in neonatal rats and embryonated chick embryo [4]. Serum survival is an important initial step for *E. coli* K1 to reach a threshold level of bacteremia and for subsequent crossing of the BBB. Several bacterial pathogens have developed strategies to evade complement mediated killing by interfering with complement activation [5]. Our studies have demonstrated that the serum resistance of *E. coli* K1 is due to OmpA binding to C4b binding protein (C4BP), which is the predominant serum inhibitor of C3b activation via the classical pathway. C4BP that bound to *E. coli* K1 acts a cofactor in Factor I-mediated cleavage of C4b to C4d and increases the dissociation of the classical pathway C3 convertase. The N-terminal extracellular loops of OmpA interact with complement control protein module 3 (CCP3) found in the alpha chain of C4BP to prevent complement-mediated killing [6]. Of note, synthetic peptides that represent sequences of CCP3 when pre-incubated with *E. coli* K1 increased serum killing. Logarithmic phase (LP) cultures of OmpA+ *E. coli* were more efficient in serum resistance than post-LP cultures. Increased binding of LP OmpA+ *E. coli* to C4BP is responsible for this resistance to serum killing due to concomitant decrease in C3b formation and other downstream proteins required for bacteriolysis [7].

Recent studies have demonstrated that mutations in loops 1, 2 and 4 of OmpA to alanines, three amino acids at a time in different positions of each of the loops, are involved in resisting serum bactericidal activity by *E. coli* K1 as these mutants were unable to bind C4BP efficiently (Fig. 2). Of note, mutation of three residues in Loop 4 increased the virulence of *E. coli* in the newborn mouse model of meningitis. Loop 4 mutant *E. coli* K1 caused high-level bacteremia in relatively shorter time than the wild type *E. coli* K1, indicating that this mutant is surviving efficiently during the initial stages of infection. In addition, Loop 4-mutant also induces greater production of TNF- α , IL-1 β , IL-6, and IL-12, and recruited more microglia, B cells, macrophages and granulocytes compared to *E. coli* K1 to the sites of infection. Therefore, it is possible that the binding of a serum protein that

initiates serum killing depends on Loop 4 sequence during the initial stages of infection. Once the bacteria survive in serum, evasion of immunocyte-mediated killing is the next step for survival.

Role of OmpA in *E. coli* interaction with immune cells

Neutrophils or polymorphonuclear leukocytes (PMNs) provide first line of defense against invading microorganisms. Binding of microorganisms to PMN surface-receptors generates signals that regulate the response of the PMNs for killing of the microbial intruder, which requires delivery of the cellular bactericidal arsenal to the prey [8]. Killing may be achieved through production of reactive oxygen species (ROS) generated by NADPH oxidase [9]. However, pathogenic microbes develop strategies to avoid PMN killing by suppressing ROS generation [10]. OmpA of *E. coli* K1 is shown to interact with gp96, a heat shock protein, expressed on the surface of neutrophils for entering and survive in the cells. The interaction of OmpA with gp96 increases the expression of TLR2 and suppresses the expression of TLR4 and CR3 in neutrophils. In addition, OmpA+ *E. coli* downregulates the expression of gp91^{phox}, Rac1 and Rac2, the components of NADPH oxidase, thereby avoid killing by PMNs. Mutation of three amino acids in the extracellular loops of OmpA and their interaction with PMNs revealed that two regions in loops 1 and 2 are important for interaction. Loop 1 and Loop 2 mutants' interaction with PMNs significantly increased expression of TLR4 and CR3, thereby increasing the phagocytic ability of PMNs. Of note, depletion of neutrophils or gp96 in newborn mice renders them to be resistant to *E. coli* K1 infection, indicating that PMNs are initial safe havens for *E. coli* for survival and multiplication.

The expression of OmpA also influences the entry and survival of *E. coli* K1 in macrophages. Opsonization with antibody or complement was not required for bacterial entry, indicating that OmpA interaction with a specific receptor is critical for entry into macrophages. Invasion of macrophages was also dependent on microfilament and microtubule condensation [11]. Although macrophages after infection with certain bacteria undergo apoptosis to limit the spreading of the pathogen, many bacterial pathogens developed anti-apoptotic mechanisms to extend the life of these cells for their own advantage. In a similar fashion, *E. coli* K1 prevents macrophage apoptosis by inducing the expression of anti-apoptotic protein Bcl_{XL}. Surprisingly, OmpA+ *E. coli* K1 infected macrophages were resistant to staurosporine-induced apoptosis, indicating that the bacterium takes over the control of macrophage function [12]. An intriguing aspect of *E. coli* K1 interaction with macrophages is that OmpA was shown to bind the alpha chain of Fcγ receptor I (CD64a) in macrophages for entry and survival (Fig 3A and B). OmpA interaction with CD64a also prevented the association of the γ-chain and induced a tyrosine phosphorylation pattern distinct from Ig2a induced phosphorylation. The OmpA binding to CD64a enhanced the expression of additional CD64 and TLR2 molecules on the surface, whereas it suppressed the expression of TLR4 and CR3. Interestingly, CD64^{-/-} mice were not susceptible to infection due to accelerated clearance of the bacteria as a result of increased CR3 expression (Fig. 3C) [13]. OmpA mediated interaction with macrophages suppressed the expression of TNF-α, IL-1β and other pro-inflammatory cytokines by preventing the phosphorylation of IκB, which in turn responsible for the suppression of NF-κB activation. Macrophages infected with OmpA+ *E. coli* were unresponsive to LPS induced NF-κB activation and pro-inflammatory secretion [14].

Dendritic cells (DCs) are vital for initiation and modulation of specific immune responses and are the most potent antigen presenting cells (APCs). Immature DCs sample the external environment and capture various antigens including whole bacteria in the periphery and submucosa. After ingestion of bacterial pathogens, DCs migrate to secondary lymphoid tissue where they present processed antigen to stimulate antigen-specific T cells. The

activation of DCs relies on the up-regulation of costimulatory molecules and abundant surface expression of MHC class II molecules resulting in so-called mature DCs, which are potent stimulators of naive T cells. *E. coli* K1 has been shown to invade and survive in dendritic cells (DCs), for which the OmpA expression again is critical. OmpA+ *E. coli* promotes its survival in DCs by suppressing DC maturation markers like CD40, HLA-DR and CD86 [15]. OmpA+ *E. coli* also induced the interaction of CD47 and thrombospondin-1, which has a negative regulation on the markers for DC maturation [16]. Previous studies have demonstrated that recombinant OmpA from *Klebsiella pneumoniae* interacts with TLR2. However, this interaction induced the maturation of DCs and production of IL-12, which is distinctly different from OmpA interaction when present in intact *E. coli*.

***E. coli* K1 OmpA interaction with brain endothelial cells for invasion**

After reaching a certain threshold level of bacteremia, *E. coli* K1 interacts with the blood-brain barrier (BBB) to invade and enter the central nervous system. Our studies demonstrate that OmpA expression is crucial for *E. coli* K1 to invade human brain microvascular endothelial cells (HBMEC), which is an *in vitro* culture model for the BBB. The studies using OmpA- *E. coli* K1 showed it invades 25- to 50-fold less than OmpA+ *E. coli* K1 in HBMEC. Of note, anti-OmpA antibodies that recognize the N-terminal portions of OmpA significantly prevented the invasion of *E. coli* K1. In agreement with the role of N-terminal regions of OmpA, two synthetic peptides that represent the sequence of Loops 1 and 2 of OmpA significantly inhibited bacterial invasion of HBMEC [17]. Interestingly, one of these peptides had sequence homology to the Loop 2 of OmpA2, encoded by *ompA* allele 2, found commonly in pathogenic bacteria. In addition, *E. coli* K1 OmpA was found to interact specifically with GlcNAc1, 4GlcNAc (chitobiose) moieties present on the surface of HBMEC. The chito-oligomers also prevented the onset of meningitis in a rat model of hematogenous meningitis further substantiating the role of these sugars in *E. coli* K1 pathogenesis [18]. Computer modeling studies demonstrated that the extracellular loops are highly mobile, which upon interaction with chitobiose moieties initially stabilizes the three dimensional structure of OmpA for subsequent interaction with peptide backbone of the receptor [19](Fig. 4). Modeling studies also revealed that the chitobiose binds in two different grooves formed by Loops 1 and 2, and Loop 4 [20].

The receptor for OmpA on HBMEC was identified as Ecgp96, a homolog of gp96 belonging to the Hsp90 heat shock protein family [21,22]. OmpA interaction with Ecgp96 induces a cascade of cellular signals in HBMEC that aid in the successful invasion of the bacterium - [23–33]. It was demonstrated that the purified N-terminal portion of OmpA directly bound HBMEC while a mutant peptide lacking loop regions could not [34]. A recent study also employed *E. coli* K1 OmpA mutants that completely lacked the loop regions and showed that Loops 1 and 2 were critical for Ecgp96 binding [35]. However, complete deletion of loop regions might severely impair OmpA folding and localization in the outer membrane. A more logical model where selective amino acids in the loop regions were mutated to alanines was used in two different studies recently. One study showed that mutating specific residues in Loops 1, 2 and 4 to alanines significantly prevented bacterial binding and invasion of HBMEC [20]. Although lack of OmpA expression in *E. coli* K1 modulates the expression of type 1 fimbriae, some of the loop mutations did not affect expression of other surface structures including type 1 fimbriae, suggesting that OmpA interaction with Ecgp96 is truly required for HBMEC invasion. In addition, we have recently demonstrated that Ecgp96 interacts with TLR2 more efficiently upon infection with OmpA+ *E. coli*, not OmpA- *E. coli* and that the C-terminal portions of both these molecules are important to induce signaling events in HBMEC for invasion (unpublished data).

OmpA was also shown to be important for *E. coli* K1 invasion of astrocytes. OmpA expression induced actin re-arrangements in astrocytes. Intra-cerebral injection of *E. coli* K1 pre-incubated with recombinant OmpA (rOmpA) in C57BL/6 mice had a protective effect while those infected with bacteria alone died within 36 hours. rOmpA pre-incubation also prevented astrocyte activation and neutrophil infiltration in the brain [36].

Pathogenesis of meningitis and necrotizing enterocolitis by *Cronobacter sakazakii* – twisting the signal by OmpA

Cronobacter sakazakii (CS), earlier known as *Enterobacter sakazakii* is an emerging pathogen in neonates that causes necrotizing enterocolitis (NEC) and meningitis. NEC is an inflammatory intestinal disorder that affects 2%–5% of all premature infants and is associated with high mortality rates. OmpA of CS was required for bacterial invasion of INT407 intestinal cell line. Microtubule and microfilament condensation was also shown to be important for the invasion process [37]. Another porin OmpX along with OmpA was later shown to promote basolateral invasion of CS in epithelial cells [38]. OmpA of CS was also important for invasion of HBMEC but the invasion process required only microtubule condensation and not microfilaments [39]. In agreement with the requirement of OmpA expression, only OmpA+ CS induced meningitis in newborn mouse model of meningitis and was accompanied by neutrophil infiltration, gliosis and hemorrhage (Fig. 5A). CS has also been shown to induce NEC in newborn rat model under hypoxia conditions and in a mouse model. The binding of CS to enterocytes induced apoptosis of the cells by increasing the production of IL-6. Preceding to apoptosis, CS infected enterocytes produced significant quantities of inducible nitric oxide, which in turn is responsible for the disruption of tight junctions (Fig. 5B). Feeding newborn rats with *Lactobacillus bulgaricus* prior to infection with CS completely prevented the onset of NEC. Subsequent studies have shown that OmpA expression in CS is critical for the binding of the bacterium to enterocytes both *in vitro* and in animal models.

CS OmpA binds DC-SIGN (DC specific ICAM non-integrin) for survival in DCs but is not required for DC invasion. OmpA+ CS also induced the anti-inflammatory cytokines TGF- β and IL-10 to prevent DC maturation. OmpA+ CS could not induce DC maturation and the infected DCs could not present antigens to T-cells despite pretreatment with LPS [40]. Intestinal epithelial cell monolayers pretreated with supernatant from OmpA+ CS/DC culture markedly enhanced membrane permeability and enterocyte apoptosis, whereas OmpA– CS/DC culture supernatant had no effect. Analysis of OmpA+ CS/DC co-culture supernatant revealed significantly greater TGF β production compared to the levels produced by OmpA– CS infection. TGF- β levels were elevated in the intestinal tissue of mice infected with OmpA+ CS. Co-cultures of CaCo-2 cells, and DCs in a “double layer” model (CaCo-2 cells on the top of the filter and DCs at the bottom in transwell inserts) followed by infection with OmpA+ CS significantly enhanced monolayer leakage by increasing TGF β production. These studies thus revealed a correlation between DCs and intestinal epithelial cells mediated by TGF- β .

Elevated levels of inducible nitric oxide synthase (iNOS) were also observed in the double-layer infection model and suppression of iNOS expression inhibited the CS-induced CaCo-2 cell monolayer permeability even in the presence of DCs or OmpA+ CS/DC supernatant. Blocking TGF β activity with a neutralizing antibody suppressed iNOS production and prevented apoptosis and monolayer leakage. Depletion of DCs in newborn mice protected against CS-induced NEC while adoptive transfer of DCs rendered the animals susceptible to infection. Therefore, CS interaction with DCs in intestine increases intestinal epithelium damage and the onset of NEC due to increased TGF β production. In agreement with the role of DCs, infection of newborn mice at Day 3 with CS induced the recruitment of significantly

greater number of DCs to the lamina propria in the intestine without change in the recruitment of other immune cells during the pathogenesis of necrotizing enterocolitis (NEC). However, OmpA⁺ CS only survived in intestine and attached to enterocytes, indicating that CS interaction with DCs is a critical step in the pathogenesis.

***Pseudomonas aeruginosa* pathogenesis – OprF, the ortholog of OmpA**

Pseudomonas aeruginosa (PA) is a ubiquitous Gram-negative bacterium that causes ventilator-associated pneumonia, chronic lung infections in CF patients, skin and soft tissue infections of burn victims, and bacteremia and sepsis in cancer patients. Flagella, pili, and lipopolysaccharide initially help PA to bind to the cell surface glycolipid asialo-GM1 during the lung damage. Subsequently, PA uses type III secretion system to inject virulence factors into the cytoplasm of eukaryotic cells. So far, four effector proteins have been identified in PA: ExoU, ExoS, ExoT, and Exo Y.

OprF of PA is widely considered as an ortholog of OmpA with significant amino acid similarity in their C-terminal domains [41]. It is a general porin that non-specifically allows diffusion of ionic particles and small polar nutrients. Recent advances in OprF porin biology has been reviewed in this series [42]. Being described as an anchoring protein and expressed on the cell surface, OmpA's role in host-pathogen interaction has been recently getting more attention. OprF has been shown to be responsible for the adhesion to pulmonary epithelial cells, glial cells and Caco-2 cells. OprF also contributes to host pathogenicity in two metazoans models namely, the plant *Cichorium intybus* and the nematode *C. elegans*. The H103 strain of PA induced necrosis in the leaves of *C. intybus* 8 days after inoculation in the middle vein of its leaves while an OprF mutant of the same strain could only induce limited necrosis. PA was also able to kill *C. elegans*, which required ingestion of the bacteria by the worm and subsequent proliferation in the worm gut.

OprF surprisingly does not express in tandem with other adherence factors during colonization of cystic fibrosis (CF) lung. Pili and flagella expression are completely down regulated during this stage. OprF is essential for microaerobic growth of PA [43]. OprF binding to gamma interferon has been shown to upregulate another adhesin, LecA through quorum sensing. Activation of gamma interferon also increased expression of pyocyanin, which is another quorum sensing related virulence product. Activation of LecA and pyocyanin leads to the disruption of epithelial cell function [44]. OprF expression is also imperative for the formation of anerobic biofilms [45]. OprF mutants are deficient in adherence to animal cells and lack the ability to secrete ExoT and ExoS toxins via the type III secretion system. An OprF mutant was deficient in the production of signal molecules *N*-(3-oxododecanoyl)-L-homoserine lactone and *N*-butanoyl-L-homoserine lactone, both of which regulate the timing and production of pyocyanin, elastase, lectin PA-1L and Exotoxin A. OprF is considered as a sensor and modulates quorum sensing to enhance bacterial virulence [46]. A recent study demonstrates the efficacy of two different vector types (Adc7 and Ad5 non-human primate vectors) cloned with *oprF* gene and their effect on anti-*P. aeruginosa* systemic and lung immunity in mice. Administration of AdC7OprF to the respiratory tract directly resulted in an increase of OprF-specific IgG and IgA in lung ELF, OprF-specific INF- γ in lung T-cells and OprF-specific IgG in serum compared to immunization with Ad5OprF. Furthermore, challenge with a lethal dose of *P. aeruginosa* to these animals increased the survival rates [47].

***Acinetobacter baumannii* – AbOmpA**

Acinetobacter baumannii (Ab) is an emerging nosocomial pathogen that causes very severe to fatal pneumonia. The bacterium requires OmpA (AbOmpA) and invades epithelial cells in a zipper-like mechanism by a process with requires both microtubule and microfilament

condensation. Presence of OmpA in *A. baumannii* induced severe lung pathology in a murine model of infection and bacterial burden in the blood while the OmpA⁻ strain could not [48]. AbOmpA was further shown to localize to mitochondria and induce apoptosis in epithelial cells [49]. Transient expression of AbOmpA in epithelial cells led to the localization of the protein in the nucleus and required a novel monopartite nuclear localization signal (NLS). Mutation of NLS led to cytoplasmic localization of AbOmpA [50]. AbOmpA also induced apoptosis of DCs by targeting mitochondria and inducing production of reactive oxygen species (ROS) [51].

Enterohemorrhagic *E. coli* – EHEC-OmpA

Enterohemorrhagic *E. coli* (EHEC) strains are responsible for frequent food- and waterborne outbreaks causing non-bloody diarrhea to bloody discharge in humans. A number of diarrheagenic intestinal pathogens including enteropathogenic *E. coli*, enterohemorrhagic *E. coli* and *Citrobacter rodentium* induce a characteristic adherence pattern upon binding to epithelial cells. An isogenic OmpA mutant of the non-invasive enteric pathogen EHEC showed reduced adherence on cultured HeLa cells, and anti-OmpA serum prevented EHEC binding to HeLa cells [52]. Further studies on EHEC adherence on alfalfa sprouts showed that OmpA was important for binding [53]. EHEC OmpA also induced murine dendritic cells (DCs) to secrete IL-1, IL-10 and IL-2. However, EHEC OmpA could induce DC migration across polarized epithelium [54].

***Pasteurella multocida* – PmOmpA**

P. multocida is a small, Gram-negative, frequently found as a commensal in the upper respiratory tracts of many animals, especially cats and dogs. Humans often infected with *P. multocida* infection due to animal bite, scratch, or lick. *P. multocida* OmpA (PmOmpA) binds extracellular matrix proteins like heparin and fibronectin [55]. However, immunization of mice with recombinant PmOmpA did not protect the animal against bacterial challenge in spite of inducing potent serum IgG responses [56].

***Klebsiella pneumoniae* - KpOmpA**

Klebsiella pneumoniae is a capsulated Gram-negative pathogen that causes community-acquired and nosocomial pneumonia. *K. pneumoniae* OmpA (KpOmpA) binds and activates DCs and macrophages and triggers cytokine production and DC maturation [57]. However, in airway epithelial cells KpOmpA modulates the inflammatory responses and is responsible for immune evasion. A mutant lacking OmpA triggers higher level of cytokine responses and is attenuated in a pneumonia animal model [58]. OmpA mutants activate IL-8 induction via NF- κ B-dependent and p38- and p44/42-dependent pathways. In addition, OmpA mutants of *K. pneumoniae* engage TLR2 and -4 to activate NF- κ B. These strategies utilized by KpOmpA may facilitate pathogen survival in the hostile environment of the lung. OmpA gene showed a protective effect against *Klebsiella pneumoniae* when administered as a DNA vaccine [59].

***Salmonella typhimurium* – Sal-OmpA**

The genus *Salmonella* contains over 2,000 serotypes and is one of the most important pathogens in the family Enterobacteriaceae, which cause diarrhea, fever, and abdominal cramps up to 72 h post-infection. *Salmonella typhimurium* is one of the common *Salmonella* serovars that causes Salmonellosis. *S. typhimurium* OmpA (Sal-OmpA), although does not play a role in bacterial adherence or invasion, has been shown to be a potential vaccine candidate in various studies. Immune response during typhoid fever is predominantly directed against OmpA [60–62]. *S. typhimurium* OmpA also induces DC maturation via

TLR4, p38 and ERK1/2 activation and further enhances Th1 polarization [63]. Sal-OmpA also induces DC maturation and profound activation of MHC II molecules. It also induces a CD4 and CD8 specific response when pulsed along with E7 and PADRE peptides and shows a promising, long term protective anti-tumor effect in mice [64].

Other bacteria and OmpA

Bacteroides vulgatus strains derived from ulcerative colitis (UC) patients revealed the presence of 2 OmpA variants. Tissue adherence of UC- derived strains was stronger than non UC- derived strains. Adherence of DH5- α transformed with OmpA of UC derived strains were also better than non-UC strains [65]. *Reimerella anatipestifer* OmpA has also been recently shown to be important for binding and invasion of Vero cells in vitro. The bacterium is a major disease causing agent in farm ducks [66].

Conclusion

OmpA has been the subject of many studies to unravel its structural features and porin activity. Its role in the pathogenesis of various bacterial infections is gaining importance lately. Despite its conservation throughout evolution among pathogenic and non-pathogenic bacteria, OmpA interacts with specific receptors for initiating pathogenesis in some Gram-negative bacterial infections. Computer modeling studies have indicated that OmpA extracellular loops are highly mobile, and thus a variety of three-dimensional structures have been proposed. In reality, the membrane architecture of OmpA in spatial arrangement may depend on the presence of other molecules of that particular pathogen, and thereby enhancing its virulence of certain pathogens. An OmpA homologue, OprF has been emerged as a virulence factor for *Pseudomonas aeruginosa* for its binding to eukaryotic cells and/or the production of other virulence factors. In addition, OprF is a target for gamma interferon to act as a sensor for quorum sensing leading to activation of virulence factors when in contact with the host. However, due to the emergence of antibiotic resistance strains, the treatment options for reducing Gram-negative bacterial infections will be limited in the future. Therefore, a greater understanding of the pathogenesis mediated by outer membrane proteins, such as OmpA and OprF may lead to development of novel anti-virulence drug targets.

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Fig. 1. Sequence homology of OmpA from various Gram-negative bacteria

Representative pathogens for which OmpA has been shown to play a role in virulence were subjected to protein sequence alignment using the online ClustalW2 alignment tool (www.ebi.ac.uk/ClustalW2). The 4 loops of OmpA are shown within boxes and difference in amino acid sequences are highlighted in yellow. Cleavage site of the mature protein is indicated by arrowhead.

Fig. 2. C4b-binding protein interaction with *E. coli* K1 that express mutant OmpA
 Two dimensional structure of OmpA depicting regions of three or four amino acids mutated to alanines (A). *E. coli* K1 expressing either wild type or mutated OmpA were incubated with C4bp for 30 min, washed and the bound proteins were evaluated by flow cytometry. Previously published in J. Biol. Chem. DOI: 10.1074/jbc.M110.178236

Fig. 3. OmpA interaction with CD64 is critical of *E. coli* K1 survival in macrophages
 (A) RAW 264.7 macrophages were infected with OmpA+ *E. coli* or OmpA- *E. coli* for various times, washed, fixed and extracellular and intracellular bacteria were stained with anti-S-fimbria antibody. (B) RAW 264.7 macrophages were transfected with control short hairpin RNA (shRNA), FcγRI or CR3 shRNA and the cells were used for bacterial binding and invasion assays. Both the binding and entry of *E. coli* were significantly reduced in FcγRI^{-/-} macrophages. (C) FcγRI^{-/-} infant mice at Day 3 were infected with 10³ cfu of *E. coli* K1, the brains were collected after 72 h, paraffin embedded and tissue sections stained with H and E. Wild type mice showed gliosis, neuronal apoptosis and neutrophil infiltration, whereas FcγRI^{-/-} newborn mice showed no such pathology. Previously published in PLoS Pathogens, DOI: 10.1371/journal.ppat. 1001203.

Fig. 4. Simulation snap shots of OmpA interaction with GlcNAc1, 4 GlcNAc moieties
Wild-type OmpA binds to two GlcNAc1, 4GlcNAc moieties (chitobiose), one at the tips of loops 1 and 2, and second one near the membrane region at the base of loops 2 and 4. The simulations studies showed that OmpA structure stabilizes by 10 ns with more favorable free energy. In contrast, mutation of three amino acids in loop 2 (aa 61–64) to alanines renders the chitobiose unable to bind and exhibited less favorable energy. Previously published in J. Biol. Chem. DOI: 10.1074/jbc.M110.122804.

Fig. 5. OmpA+ CS induces NEC in newborn mice by disrupting the tight junctions

(A) Newborn mice were infected with OmpA+ CS or OmpA- CS and intestines were processed for H and E staining. Arrow indicates the disruption of villi structure. (B) Confluent monolayers of Caco-2 cells were infected with OmpA+ CS or OmpA- CS for 4 h, washed, fixed and stained with anti-ZO-1 antibody followed by secondary antibody coupled to Cy3. Stained cells were imaged with confocal microscope LSM710. Previously published by The American Association of Immunologists, Inc. in *J. Immunology*, DOI: 10.4049/jimmunol.1100108