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Outer membrane protein OmpW of *Escherichia coli* is required for resistance to phagocytosis

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Abstract

Eight-stranded β-barrel outer membrane proteins can confer bacterial virulence via resistance to host innate defenses. This resistance function of OmpW, which was recently identified as an eight-stranded β-barrel protein, was investigated in this study. Our results demonstrated that upregulation of OmpW correlated with increased bacterial survival during phagocytosis. Bacterial mutants harboring a deletion of *ompW* exhibited a significantly increased phagocytosis rate. Both observations suggest that the OmpW protein protects bacteria against host phagocytosis. In addition, expression of *ompW* is regulated by iron, which implies that the resistance provided by OmpW may be an important factor in iron-related infectious diseases. Furthermore, OmpW has been identified as a protective antigen that protects mice against bacterial infection and is therefore a promising target for vaccine development against infectious diseases.

Keywords: Outer membrane protein; β-barrel; OmpW; Resistance to phagocytosis; Protective antigen; Iron

1. Introduction

The outer membrane (OM) of Gram-negative bacteria is the most critical component for bacterial survival in a wide range of environments, providing functions such as serving as a protective barrier and allowing for the uptake of nutrients (Maiti et al., 2009). The outer membrane proteins (OMPs) are the major components of the OM and include anchoring lipoproteins and transmembrane β-barrel proteins (Ruiz et al., 2006). The transmembrane β-barrel proteins are characterized by the number of anti-parallel β-strands, ranging in number from eight to twenty-four (Fairman et al., 2011). These proteins participate in processes that are important for adaptation functions, such as the active transport of large molecules, membrane anchoring, enzyme activity, signal relaying and adhesion to host cells (Naveed et al., 2012).

Moreover, certain β-barrel proteins, such as OmpA, OmpX, Ail and NspA, have been found to be important for bacterial colonization and invasion (Choi et al., 2008; Kim et al., 2010; Lewis et al., 2010; Prasadarao et al., 1996). OmpA, PagC, Rck and OmpX are required for resistance to serum complement-mediated killing (Ho et al., 2011; Kim et al., 2009; Nishio et al., 2005; Prasadarao et al., 2002; Weiser and Gotschlich, 1991), while OmpA and PagC contribute to bacterial survival in macrophages (Bartra et al., 2012). Interestingly, all of these β-barrel proteins belong to a small family of proteins with β-barrels consisting of only eight anti-parallel β-strands (Fairman et al., 2011).

Another outer membrane protein, OmpW, belonging to the same family, was recently described (Albrecht et al., 2006; Hong et al., 2006). Although this protein has been identified in many different bacterial species, the biological function of OmpW remains largely uncharacterized. However, previous studies had demonstrated that OmpW may be involved in

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protection of bacteria against various forms of environmental stress, such as osmosis (Xu et al., 2005), oxidation (Gil et al., 2007), temperature and the unavailability of nutrients and oxygen (Nandi et al., 2005). In addition, proteomic methods demonstrate some correlation between the presence of OmpW in cells and bacterial resistance to antibiotics, including ampicillin, tetracycline and ceftriaxone. The upregulation of OmpW is also correlated with an increase in bacterial virulence (Goel and Jiang, 2010). In addition, an increase in OmpW expression has been associated with reactive arthritis (ReA) (Singh et al., 2007).

In the present study, we constructed an *ompW* deletion mutant from *Escherichia coli* K-12 K99+ and investigated the role of OmpW in the pathogenesis of this bacterium. Using in vitro and in vivo assays, we demonstrated that OmpW is required for resistance to phagocytosis. Thus, we provide the first direct evidence that OmpW promotes bacterial resistance to innate immunity. Moreover, we identified OmpW as an efficient protective antigen that may be an ideal target for developing vaccines.

2. Materials and methods

2.1. Bacterial strains, plasmids and media

The bacterial strain of *E. coli* K-12 K99+ was from the Institute of Microbiology, Chinese Academy of Sciences, Beijing, China. The strain was a derivative of *E. coli* K12, in which the K99 plasmid from strain B41 was introduced by conjugation. Strains of K99-positive *E. coli* can be more efficiently phagocytized by macrophages (Ferreiros and Criado, 1984). *E. coli* BL21 and plasmids pKD46, pCP20 and pET-28a were from our laboratory collection. The bacteria were grown in Luria Broth (LB) medium or Mueller–Hinton (MH) medium. When required, the medium was supplemented with ampicillin (100 mg/l), kanamycin (50 mg/l) and arabinose (2 mg/l). The solid media included 15 g/l agar. Iron limitation was conducted by the addition of 300 μM 2,2′-dipyridyl and 300 μM desferrioxamine.

2.2. Preparation of mouse peritoneal macrophages

Mouse macrophages were harvested by peritoneal lavage, as described previously (Hanazawa et al., 1985). Briefly, BALB/c mice, aged 7–10 weeks, were injected intraperitoneally with 6% (w/v) starch broth and sacrificed under ether anesthesia 24 h post-injection, and peritoneal exudate macrophages were collected by lavage. The collected cells containing the macrophages were washed and maintained in RPMI-1640 medium containing 10% fetal calf serum.

2.3. Bacterial infection, extraction of RNA and real-time PCR

The mouse peritoneal macrophages were incubated with *E. coli* strain K12 K99+ at an MOI of 100 for 1 h. Unbound extracellular bacteria were removed by washing the cells six times with phosphate-buffered saline (PBS) and adhering bacteria were detached from the cells by treatment with a trypsin–EDTA solution, as described previously (Sjölinder et al., 2008). The intracellular bacteria were released from the cells by permeability lysis. Bacteria grown to exponential phase in LB medium were used as a control. Total bacterial RNA was extracted using a total RNA Purification Kit (Haogene Biotech) and cDNA was synthesized using Superscript™ II RTase (Invitrogen). Quantitative real-time PCR was processed by real-time PCR amplification systems (Bio-Rad) using a SYBR Premix Ex Taq™ kit (Takara). The primer sets for *ompW*-rt-F (GCGCATGAAGCAGGCGAATTTTT) and *ompW*-rt-R (CCCAGACTTCCTAACGTACCAC) were used for amplification of *ompW*. 16S RNA of *E. coli* was amplified simultaneously using primers 16S-F (CTTCGGGAACCGTGAGACAG) and 16S-R (GCGCTCGTTGCGGGACTTAA) and served as an internal control.

2.4. Construction of ompW knockout mutant and complementation strains

An *ompW* knockout mutant (Δ*ompW*) was generated using a one-step method as described previously (Datsenko and Wanner, 2000), with a modification. The first primer pair, consisting of pKD13-F (TATAACCATAACGACGGAGCGGATATGGTGTAGGCTGGAGCTGCTTC) and pKD13-R (GGAATTAAAAACGATATCCTGCTGAATTCCGGGGATCCGTCGACC), which contains sequences flanking the *kan* gene of plasmid pKD13, was used to amplify the *kan* gene using PCR. The PCR products were used as a template to generate recombinant DNA fragments containing sequences upstream or downstream of *ompW* and *kan* by PCR with the second primer set *ompW*-ko-F (TGTAGGTATATTCGTCACGTTTTTATAACCATAACGACGGAGCGG) and *ompW*-ko-R (GTATATTACGGGGTCGTTTTTGTGCGGAATTAAAAACGATATCC). The resulting products were then integrated into the genome of *E. coli* K12 K99+, which had been pretransformed with the pKD46 plasmid by homologous allelic replacement. The mutant strain was selected on Amp^R and Km^R agar plates and deletion of the *ompW* gene was verified using PCR with primers *ompW*-ko-F and *ompW*-ko-R. The resulting positive Amp^R and Km^R mutants were transformed with pCP20. The ampicillin-resistant transformants were selected at 30 °C, after which a few colonies were purified once non-selectively at 42 °C and then tested for elimination of the antibiotic resistance gene.

To generate complementation strains, the complete *ompW* open reading frames were amplified using the primer pair *ompW*-c-F (5′-CGCGGATCCTGCCCTAACACCACAGTGATAAA-3′) and *ompW*-c-R (5′-CCCGGATCCTCCTGCTGAGAACATAAACACCC-3′), harboring restriction enzyme BamHI recognition sites (underlined sequences). The PCR products were cloned into a pACYC184 vector. The plasmid was then transformed into the mutant cells. The complementation strain was designated +*ompW*.

To confirm deletion or complementation of the mutants, bacterial membrane proteins of both strains were extracted by an ultracentrifuge method and analyzed by SDS-PAGE and western blotting. Protein bands that differed between the wild-type and mutant strains in electrophoresis gels were finely