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ORIGINAL ARTICLE

Recombinant outer membrane protein A fragments protect against *Escherichia coli* meningitis

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bacterial meningitis;
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Background: Although the mortality rates have decreased over the past few decades, neonatal meningitis is still a severe disease with high morbidity. Moreover, approximately 40% of survivors exhibit neurological sequelae. *Escherichia coli* is the major Gram-negative bacterial pathogen in neonatal meningitis. The N-terminal β-barrel domain of the outer membrane protein A (OmpA) of *E. coli* is essential for effective protein conformation and function and contains four surface-exposed hydrophilic loops. In this study, we expressed different fragments of the four ring structures of the N-terminal domain, and investigated whether these recombinant OmpA fragments can protect mice from death after *E. coli* infection.
Methods: We expressed the recombinant proteins of the following OmpA fragments by using molecular cloning of Loop 1—2, Loop 1—3, Loop 1—4, Loop 2—3, Loop 2—4, and Loop 3—4. Animal experiments were subsequently performed to investigate the effects of these recombinant *OmpA* fragments on the survival of C57BL/6 mice after intracerebral *E. coli* RS218 administration.
Results: This study demonstrated that the recombinant Loop 1—3, Loop 2—3, and Loop 2—4 fragments of *OmpA* can protect mice from intracerebral *E. coli* infection.

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Conclusion: In bacterial meningitis, although antibiotic therapy is the first choice for management, neurological complications can seldom be averted. Based on the results of the present study, we intend to establish an effective therapeutic application for *E. coli* meningitis.

Introduction

Despite the availability of advanced antibiotic therapy and patient care for bacterial meningitis, it is still associated with a relatively high morbidity and mortality. Nearly 40% of surviving patients experience various neurological complications.[1–3] The pathological complications of bacterial meningitis include cerebritis, brain abscess, empyema, and ventriculitis in the acute phase and the sequelae of cerebral atrophy, hydrocephalus, seizure, and hearing impairment. Seizures are the most common complication, followed by hydrocephalus and hearing impairment.[3,4] A neuronal injury associated with bacterial infection of the central nervous system involves multiple microbial and host factors; *Escherichia coli* strains with the K1 capsular polysaccharide are the most predominant Gram-negative bacteria associated with neonatal bacterial meningitis.[5] Severe bacteremia and invasion through brain microvascular endothelial cells (BMECs) are the determining factors contributing to central nervous system infection.[6] Several K1-associated components participate in BMEC binding and invasion, including Fim H, K1 capsule, and outer membrane protein A (OmpA).[7–11] OmpA is a major outer membrane protein of *E. coli* and is essential in maintaining the integrity of the outer membrane and in bacterial conjugation.[12–14] This protein is also the receptor for several bacteriophages.[15–18] OmpA is encoded using a 1,038-bp open reading frame consisting of a 21-amino acid leader peptide and a mature 325-amino-acid protein. Moreover, the N-terminal membrane-anchoring domain of OmpA forms an antiparallel β-barrel, which has eight transmembrane β-strands connected by three short periplasmic turns and four relatively large surface-exposed hydrophilic loops; the OmpA C-terminal domain interacts with the peptidoglycan layer in the periplasm to maintain outer membrane integrity.[19] Furthermore, OmpA is highly conserved through the evolution of Gram-negative bacteria and is crucial for *E. coli* binding and the invasion of BMECs and astrocytes.[20,21] We previously reported that the recombinant full-length OmpA protein can protect mice from death after *E. coli* infection.[21] This has therefore encouraged study into a new therapeutic approach to improve the prognosis of bacterial meningitis. In this study, we expressed different fragments of the four ring structures of the N-terminal domain that were exposed on the surface of the OmpA protein, including Loop (L) 1–2, L1–3, L1–4, L2–3, L2–4, and L3–4 (Fig. 1), and investigated whether these recombinant OmpA fragments can protect mice from death after *E. coli* infection.

Materials and methods

Chemicals, bacteria, and culture media

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA), unless otherwise indicated. The *E. coli* strains used in the present study were kindly provided by Dr K.S. Kim (Division of Pediatric Infectious Diseases, School of Medicine, Johns Hopkins University, Baltimore, MD, USA); RS218 (O18:K1:H7) was isolated from the cerebrospinal fluid of a neonate with meningitis.[20] *E91* is an RS218 mutant that lacks the entire *ompA* gene. The bacteria were grown in brain–heart infusion broth with appropriate antibiotics (Difco Laboratories, Detroit, MI, USA). For infection experiments, overnight cultures were expanded in brain–heart infusion broth and incubated at 37°C for 2–3 hours to the midlog phase. The bacteria were centrifuged at 10,000 × g for 5 minutes and resuspended in a cell culture medium without antibiotics.

Mouse strain

C57BL/6 mice were obtained from the National Laboratory Animal Center of Taiwan, Nangang, Taipei, Taiwan and maintained under pathogen-free conditions. All animal procedures were performed according to the approved institutional protocol (LAC-99-0009) of Taipei Medical University, Taipei, Taiwan.

Expression and purification of OmpA fragments

The DNA fragments of the *ompA* L1–2, L1–3, L1–4, L2–3, L2–4, and L3–4 were amplified using polymerase chain reaction with restriction enzyme sites containing primers. These fragments were subsequently digested with SacI and XhoI and ligated into the pET-21a expression vector (Novagen, Darmstadt, Germany). The resultant plasmid was

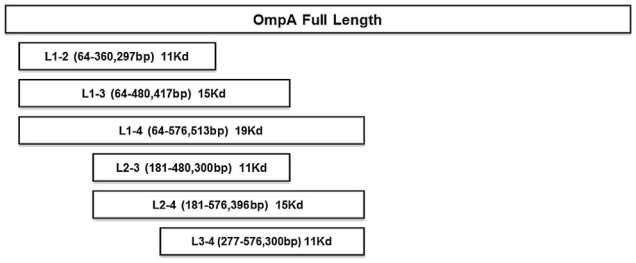

Figure 1. N-terminal, surface-exposed, OmpA fragments: Loop 1–2, Loop 1–3, Loop 1–4, Loop 2–3, Loop 2–4, and Loop 3–4.