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A Versatile Role of Outer Membrane Protein A in Pathogenesis of *Acinetobacter baumannii*

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Bacteria of the genus *Acinetobacter* is an important hospital-acquired pathogen associated with a wide spectrum of human diseases, particularly among immunocompromised patients. Among the *Acinetobacter* species from clinical specimens, *Acinetobacter baumannii* is the most prevalent species and is of great concern in a clinical setting because of multiple antibiotic resistance and high mortality of the infected patients. Despite a great deal of published data regarding human infections caused by *A. baumannii*, the specific virulence factors or pathogenic mechanisms of *A. baumannii* remains unclear. We investigated the role of a outer membrane protein A (AbOmpA) in pathogenesis of *A. baumannii*.

Adherence and invasion of microorganisms to epithelial cells is an important virulence factor as it is the initial step of the colonization and infectious process. *A. baumannii* adhered to human bronchial epithelial cells and two types of adherence were observed, dispersed adherence of bacteria to the cell and adherence of clusters of bacteria at localized areas of the cells. Bacteria with dispersed adherence interacted with epithelial cells through the fimbrial-like elements, but were also entrapped by protrusions extending from the epithelial cells. *A. baumannii* invaded epithelial cells by a zipper-like mechanism, which was associated with microfilament- and microtubule-dependent uptake mechanism. Internalized bacterium was separately located in the cytoplasmic membrane-bound vacuoles. Recombinant AbOmpA (rAbOmpA) directly bound to the surface of epithelial cells and competitively inhibited 87% of adherence and 69% invasion of *A. baumannii* to human bronchial epithelial cells. An isogenic AbOmpA knock-out mutant showed a significant inhibitory effect on invasion of epithelial cells compared with wild-type strain. These results suggest that AbOmpA is mainly responsible for adherence and invasion of epithelial cells by *A. baumannii*.

Next, we determined the ability of *A. baumannii* to induce cell death. Live *A. baumannii* and culture filtrates, but not formalin-killed bacteria induced the apoptosis of host cells, suggesting that the released or secreted bacterial products are responsible for apoptosis. Of the transposon-inserted mutants, AbOmpA knock-out mutant was not as able to induce apoptosis than that of the wild-type strain. Purified AbOmpA

from *A. baumannii* ATCC19606^T and rAbOmpA directly translocated into and intimately interacted with mitochondria. The release or secretion of proteins from bacterium and their binding to cell surfaces are necessary for the subcellular targeting of bacterial proteins on host cells. AbOmpA was detected in the culture supernatant as early as 3 h after the culture and was maintained during the exponential and stationary phases of the growth. The binding of AbOmpA to the cell surface was in a dose-dependent manner, but was partly saturable at 9 μg/ml. After the binding to the cell surface, AbOmpA was internalized by host cells as early as 30 min. Immunoprecipitation assay showed that AbOmpA bound to voltage-dependent anion channel (VDAC) located in a outer membrane of mitochondria and changed mitochondiral transmembrnae potential, which led to mitochondrial swelling and a release of proapoptotic molecules such as cytochrome *C* and apoptosis inducing factor (AIF). The activation of caspase-3, which is activated by caspase-9, degraded DNA with the size of approximately 180 bp, which resulted in an appearance of a characteristic DNA ladder. AIF degraded chromosomal DNA with the size of approximately 50 kb, which resulted in an appearance of large-scale DNA fragmentation. Our results provide the molecular mechanism of AbOmpA to induce apoptosis of host cells in *A. baumannii* infection.

In addition to the mitochondrial targeting, AbOmpA translocated to the nucleus by a novel monopartite nuclear localization signal (NLS). A putative NLS region (KTKEGRAMNRR) was identified between residues 320 and 330 (GenBank accession number AY485227). The introduction of rAbOmpA into the cells or a transient expression of AbOmpA-EGFP caused the nuclear localization of these proteins, while the fusion proteins of AbOmpAΔNLS-EGFP and AbOmpA with substitutions in residues lysine to alanine in the NLS sequences were exclusively cytoplasmic distribution. Nuclear localization of rAbOmpA induced apoptosis of host cells, but not necrosis. The microinjection of rAbOmpA into the nucleus of *Xenopus laevis* embryos failed to develop normal embryogenesis and led to embryonic death. Thus, we propose a novel pathogenic mechanism of *A. baumannii* regarding the nuclear targeting of the bacterial structural protein AbOmpA.

In conclusion, we have clearly demonstrated that the bacterial bound form of AbOmpA acts as adhesin and invasin to promote colonization and infection of *A. baumannii* at mucosal sites. The secreted form of AbOmpA is responsible for apoptosis of the host cells through the mitochondrial and nuclear targeting. We suggest that AbOmpA plays a versatile role in the onset of *A. baumannii* infection and establishment of infection.