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Vaccination with a LPS-deficient inactivated whole cell provides protection against experimental *Acinetobacter baumannii* infection

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Introduction

Acinetobacter baumannii **is a Gram-negative coccobacillus that can cause different types of infections as a nosocomial pathogen including pneumonia, bacteremia, meningitis and skin and soft tissue infection, among others. The number of multidrug and pandrug resistant strains has increased alarmingly in recent years. In this context, the development of an efficient vaccine against *A. baumannii* could contribute to reducing morbidity and mortality in certain patient populations.**

The experimental vaccines described for *A. baumannii* can be classified into two broad groups, vaccines that consist of a single purified antigen, and multicomponent vaccines. Within the first group, survival experiments after active immunization have only been reported for OmpA, which showed partial protection, and Bap, whose expression in strains that do not form biofilms is unclear. The strategies employing multicomponent vaccines have included outer membrane complexes, outer membrane vesicles, and formalin-inactivated whole cells. Each of these vaccines induced protection against *A. baumannii* infections in a murine model, however, their use in humans is complicated by the elevated endotoxin content due to the high levels of lipopolysaccharide (LPS) present in these preparations.

LPS consists of the O-antigen, a core polysaccharide and lipid A, the moiety responsible for the endotoxin activity of LPS. A recent report demonstrated that *A. baumannii* can acquire resistance to the peptide antibiotic colistin via mutation in the genes involved in the first steps of lipid A synthesis *lpxA*, *lpxC* and *lpxD*, resulting in strains completely deficient in LPS. The objective of the present study was to develop an LPS-deficient inactivated whole cell (IWC) vaccine against *A. baumannii* and to characterize the immune response to immunization and its efficacy in a murine sepsis model.

Conclusion and Discussion

Immunization with an IWC LPS-deficient *A. baumannii* strain is capable of providing protection after infection with two LPS-containing strains, producing a robust antibody response based on IgG antibodies and a significant decrease in bacterial loads and pro-inflamatory cytokines compared to control mice in a mouse model of disseminated *A. baumannii* infection.

To date, no other survival studies have been reported using LPS-deficient strains as vaccines. Deficiency of LPS in *N. meningitidis* reduces the immunogenicity of outer membrane proteins in mouse models, underscoring the adjuvant activity of LPS. Our results indicate that the elicited immune response is sufficient for recognizing antigens in their native conformation in LPS-containing strains.

Methods and Results

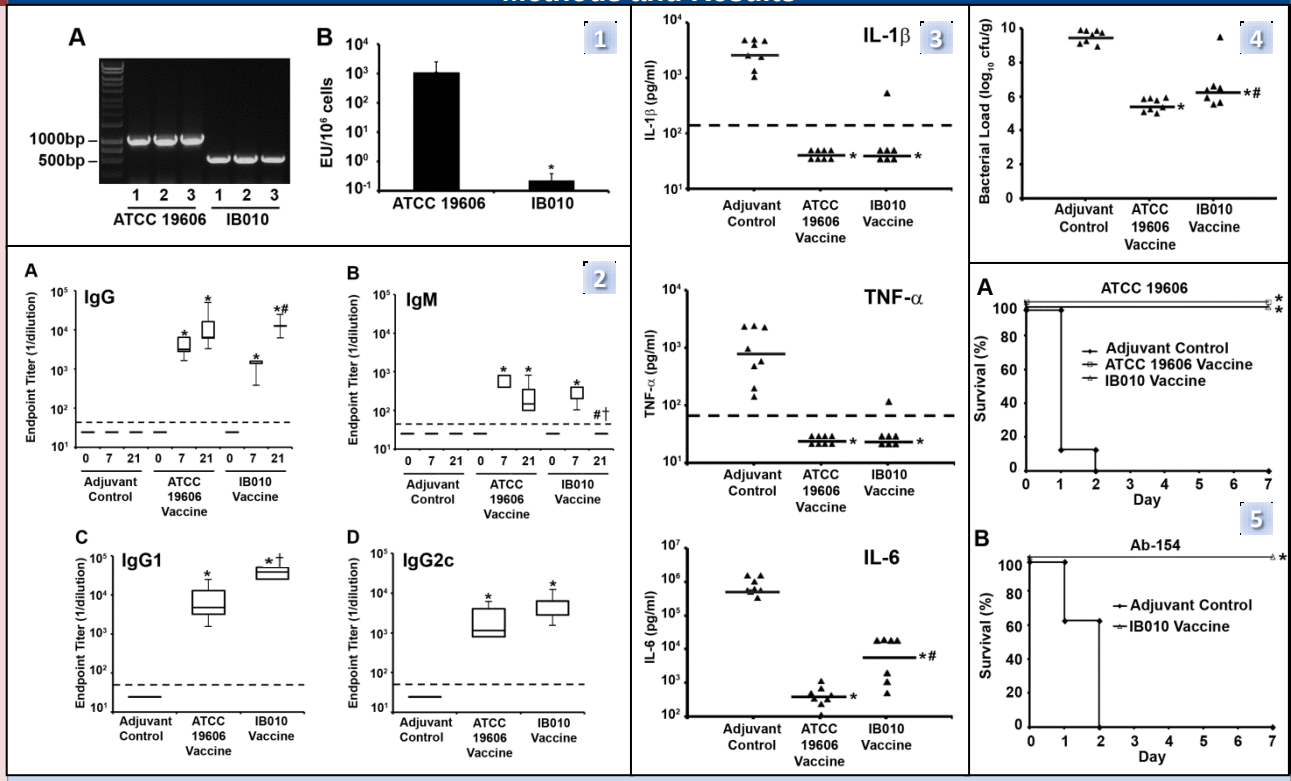

Figure 1. Selection of an LPS-deficient strain for vaccine development. Growth of ATCC 19606 in the presence of 10 mg/L colistin resulted in numerous colistin-resistant derivatives. One of these strains, IB010, contained a large deletion of 462 nucleotides in the *lpxD* gene (nucleotides 104-565) (A) and was chosen for vaccine studies. Reduction in endotoxin levels were characterized by the Limulus Amebocyte Assay (B). Bars represent the means of three independent cultures with the standard error. EU; endotoxin units. **Figure 2. Antibody response.** Serum samples were collected from C57BL/6 mice before intramuscular vaccination in aluminum phosphate adjuvant (day 0) and at day 7 and 21 after the first immunization, and levels of total IgG (A) and IgM (B) were measured by ELISA. IgG1 (C) and IgG2c (D) levels were measured in 21-day serum. Box and whisker plots represent the interquartile ranges and ranges, respectively. Horizontal lines represent median values. * $p < 0.05$ compared to control mice at the same time point, # $p < 0.05$ compared to 7-day samples from the same experimental group, † $p < 0.05$ compared to 21-day samples in ATCC 19606 vaccinated mice. **Figure 3. Effect of vaccination on post-infection pro-inflammatory cytokine levels.** Immunized and control mice were infected with 2.0×10^6 cfu ($300 \times LD_{50}$) of the ATCC 19606 strain and IL-1β, TNF-α, and IL-6 were determined. Data points represent cytokine levels from individual mice. Horizontal lines represent median values from groups of mice. * $p < 0.05$ compared to control mice, # $p < 0.05$ compared to ATCC 19606 vaccinated mice. **Figure 4. Effect on tissue bacterial loads.** Immunized and control mice were infected with 2.0×10^6 cfu of the wild type strain and spleen bacterial loads were determined 12 hours post-infection. Data points represent bacterial loads from individual mice, and horizontal lines represent median values from groups of mice. * $p < 0.05$ compared to control mice. **Figure 5. Effect on survival in a mouse model of disseminated *A. baumannii* infection**. Vaccinated and control mice were infected with 2.25×10^6 cfu ($340.9 \times LD_{50}$) of the ATCC 19606 strain (A) or 1.05×10^6 cfu ($2.18 \times LD_{50}$) of the *A. baumannii* clinical isolate Ab-154 (B), and survival was monitored over the following 7 days (n=8 mice/group). * $p < 0.05$ compared to control mice.