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Vaccine development

VACCINATION WITH A LIPOPOLYSACCHARIDE-DEFICIENT INACTIVATED WHOLE CELL VACCINE PROVIDES PROTECTION AGAINST EXPERIMENTAL ACINETOBACTER BAUMANNII INFECTION

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Objective: Over the last two decades theincidence of hospital-associated infections caused by multi-drug resistant *Acinetobacter baumannii* has increasedsignificantly, requiring the development of novel approaches for controlling theseinfections. Immunization with an inactivated whole cell vaccine has previously beenshown to induce protection in experimental models of infection. However, thereare concerns over the toxicity of this vaccine due to the high LPS content ofwhole bacterial cells. The objective of the present study was to develop aprophylactic vaccine for the prevention of *A.baumannii* infection using inactivated whole cells lacking LPS due tomutation of the genes involved in LPS biosynthesis.

Methods: An LPS-deficient derivative of the *A. baumannii* ATCC 19606 strain was obtainedby selecting for growth in the presence of colistin. Mutation was confirmed bysequencing *lpx* genes and confirmationof no LPS was performed using the Limulus Amebocyte Assay. LPS-deficientbacterial cells were inactivated with formalin and combined with an aluminiumhydroxide adjuvant for intramuscular vaccination of C57BL/6 mice. Levels ofantigen specific antibodies in serum were quantified by ELISA for total IgG,IgG1 and IgG2c, and Western blotting was performed in order to characterize theantibody response against bacterial antigens. After immunization, vaccinatedand control mice were challenged with the ATCC 19606 strain and a clinicalisolate using a disseminated sepsis model, and the following parameters weremeasured: i) bacterial loads in tissues, ii) serum levels of the pro-inflammatorycytokines IL-1 beta, IL-6, and TNF-alpha, and iii) mortality.

Results: The mutant strain harboured a deletionof 462 nucleotides in *lpxD* gene. Endotoxinlevels of the LPS-deficient mutant decreased 4830 fold compared with the parentalstrain. Two doses of the vaccine two weeks apart elicited high levels of antigenspecific total IgG, IgG1 and IgG2c 7 days after the second immunization, andproduced antibodies against multiple outer membrane protein antigens. Inchallenge experiments, at 12 hours post-challenge vaccinated mice had fewerbacteria than control mice in spleens (6.52 vs. 9.55 log10 cfu/g; p=0.004).Vaccinated mice had lower serum levels of the pro-inflammatory cytokines IL-1beta (88.86 vs. 2903.74 pg/ml, p<0.001), TNF-alpha (31.33 vs. 767.0 pg/ml; p<0.001),and IL-6 (5718.22 vs. 767876.55 pg/ml; p<0.001) than control mice 12 hourspost-infection. Vaccinated mice had increased survival over control mice afterchallenge with the ATCC 19606 strain (100% vs. 0% survival; p<0.001), and aclinical isolate (100% vs. 0% survival; p<0.001).

Conclusion: Immunization with an inactivatedwhole cell vaccine lacking LPS protects against infection with *A. baumannii* in a mouse model ofdisseminated sepsis, and may represent a viable strategy for preventinginfections caused by this pathogen.