Brucella mutants in outer membrane molecules for biophysical studies

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The members of the genus *Brucella* are α-*Proteobacteria* causing brucellosis, an important disease affecting livestock and wild life as well as human beings. These bacteria are imperfectly detected by innate immunity and follow a stealthy behavior that allows them to reach sheltered intracellular niches before effective immunity activation. The outer membranes of brucellae are of critical importance in this strategy. The outer membrane of *B. abortus* is unusually resistant to antimicrobial peptides (AMPs). The *Brucella* lipopolysaccharide (LPS) is implicated in this property and there is evidence that other lipid molecules also contribute. *Brucella* outer membranes contain large amounts of phosphatidylcholine (PC), a typically eukaryotic phospholipid, and a blockage of the synthesis of PC with the subsequent replacement by phosphatidylethanolamine generates attenuation. Ornithine lipids (OL) and some other uncharacterized amino lipids are present in relatively large amounts in *Brucella* and can be predicted to stabilize the outer membranes against AMPs by virtue of their positively charged amino group. Furthermore, these bacteria have acyl chains of average number of carbon units longer than those of the γ-*Proteobacteria* members, and an effect of the predicted increase in hydrophobicity has been proposed as a biophysical factor underlining AMPs resistance and pathogenicity in these bacteria. Thus, the brucellae are bacteria that provide a model of resistance to AMPs that relays largely on the LPS and free lipid composition.

Thus far, only the properties of the *Brucella* LPS have been studied at biophysical level. Recently, we have constructed a series of mutants blocked in PC and OL synthesis as well as others with various degrees of defects in the LPS. The latter include two mutants (*BABΔwadB* and *BABΔwadC*) that while keeping the O-polysaccharide lack core sugars and mutants (*BMEΔlptA*) defective in ethanolamine substitutions in the lipid A. The phenotype of these mutants is described in this communication. The next aim of our work is to characterize the interactions between AMPs and reconstituted lipid membranes using the lipids and LPS of these various *Brucella* mutants and to characterize the interactions at biophysical level. It is hoped that these studies will lead to a better understanding of the interaction of this important pathogen with innate immunity.