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Relation between the lipid composition of *Brucella* membrane and the resistance to cationic peptides

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Introduction: The members of the genus *Brucella* are α-2 *Proteobacteria* that cause brucellosis, an important zoonosis. These bacteria trigger only low proinflammatory responses during early infection that allows them to reach sheltered intracellular niches before effective immunity activation. The outer membranes (OM) of brucellae are of critical importance in this strategy. The OM of *B. abortus* is unusually resistant to antimicrobial peptides (AMPs). *Brucella* lipopolysaccharide (LPS) is implicated in this property and there is evidence that other lipids (such as phosphatidylcholine) also contribute. Furthermore, these bacteria have acyl chains of average number of carbon units which leads an increase in hydrophobicity, proposed as a biophysical factor underlining AMPs resistance.
Objective: The aim of our work is, to characterize the interactions between AMPs and reconstituted lipid membranes using the total and purified lipids of various *Brucella* mutants, characterizing the interactions at biophysical level.
Material and Methods: Antibacterial Activity. Minimum inhibitory concentrations of the peptideswere determined by the broth microdilution test in Mueller-Hinton medium.
Extraction, purification and analysis of the total lipid mixture. The extraction was performed using Bligh and Dyer, 1959, and the analysis was made by HPTLC.
Preparation of Liposomes. Lipids were solubilized in 20 mM HEPES buffer, extensively vortexed, sonicated, and subjected to several temperature cycles between 4 and 60 °C.
Fourier-transform infrared spectroscopy (FTIR). The measurements were performed on an IFS-55 spectrometer. Lipid samples were placed in a CaF2 crystal disk and temperature-scans were performed between -10ºC to 70ºC with a heating rate of 0.6ºC/min. For measurement of hydrated lipid samples, these were spread on an ATR ZnSe plate and free water was evaporated to dryness. Every 3ºC, 50 interferograms were accumulated, apodized, Fouriertransformed, and converted to absorbance spectra.
Fluorescence resonance energy transfer spectroscopy (FRET). Liposomes were doubly labelled with the fluorescent dyes N-(7-nitrobenz-2-oxa-1,3-diazol-4yl)-phosphatidyl ethanolamine (NBD-PE) and N-(lissamine rhodamine B sulfonyl)-phosphatidylethanolamine (Rh-PE). Peptides (20 µl of 0,1mg/ml) were added to liposomes. NBD-PE was excited at 470 nm and the donor and acceptor fluorescence intensities were monitored at 531 and 593 nm, respectively.
Results: Experiments performed with the whole bacteria showed that the lipid composition of membrane is implicated in the resistance of the bacteria to the cationic peptides. Preliminary results based on FRET and FTIR experiments suggest that the interaction between the peptides and the membrane, and the gel-to-liquid crystalline phase of the acyl chains are also dependent on the lipid composition.