Brucella-Salmonella Lipopolysaccharide Chimeras Are Less Permeable to Hydrophobic Probes and More Sensitive to Cationic Peptides and EDTA than Are Their Native Brucella sp. Counterparts

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A rough (R) Brucella abortus 45/20 mutant was more sensitive to the bactericidal activity of polymyxin B and lactoferricin B than was its smooth (S) counterpart but considerably more resistant than Salmonella montevideo. The outer membrane (OM) and isolated lipopolysaccharide (LPS) of S. montevideo showed a higher affinity for these cationic peptides than did the corresponding B. abortus OM and LPS. We took advantage of the moderate sensitivity of R B. abortus to cationic peptides to construct live R B. abortus-S-LPS chimeras to test the activities of polymyxin B, lactoferricin B, and EDTA. Homogeneous and abundant peripheral distribution of the heterologous S-LPS was observed on the surface of the chimeras, and this coating had no effect on the viability or morphology of the cells. When the heterologous LPS corresponded to the less sensitive bacterium S B. abortus S19 the chimeras were more resistant to cationic peptides; in contrast, when the S-LPS was from the more sensitive bacterium S. montevideo the chimeras were more susceptible to the action of peptides and EDTA. A direct correlation between the amount of heterologous S-LPS on the surface of chimeric Brucella cells and peptide sensitivity was observed. Whereas the damage produced by polymyxin B in S. montevideo and B. abortus-S. montevideo S-LPS chimeras was manifested mainly as OM blebbing and inner membrane rolling, lactoferricin B caused inner membrane detachment, vacuolization, and the formation of internal electron-dense granules in these cells. Native S and R B. abortus strains were permeable to the hydrophobic probe N-phenyl-1-naphthylamine (NPN). In contrast, only reduced amounts of NPN partitioned into the OM of the S. montevideo and B. abortus-S. montevideo S-LPS chimeras. Following peptide exposure, accelerated NPN uptake similar to that observed for S. montevideo was detected for the B. abortus-S. montevideo LPS chimeras. The partition of NPN into native or EDTA-, polymyxin B-, or lactoferricin B-treated LPS micelles of S. montevideo or B. abortus mimicked the effects observed with intact cells, and this was confirmed by using micelle hybrids of B. abortus and S. montevideo LPSs. The results showed that LPS is the main cause of B. abortus' resistance to bactericidal cationic peptides, the OM-disturbing action of divalent cationic chelants, and OM permeability to hydrophobic substances. It is proposed that these three features are related to the ability of Brucella bacteria to multiply within phagocytes.