Inhibition of LpxC Protects Mice from Resistant *Acinetobacter baumannii* by Modulating Inflammation and Enhancing Phagocytosis

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ABSTRACT

New treatments are needed for extensively drug-resistant (XDR) Gram-negative bacilli (GNB), such as *Acinetobacter baumannii*. Toll-like receptor 4 (TLR4) was previously reported to enhance bacterial clearance of GNB, including *A. baumannii*. However, here we have shown that 100% of wild-type mice versus 0% of TLR4-deficient mice died of septic shock due to *A. baumannii* infection, despite having similar tissue bacterial burdens. The strain lipopolysaccharide (LPS) content and TLR4 activation by extracted LPS did not correlate with *in vivo* virulence, nor did colistin resistance due to LPS phosphoethanolamine modification. However, more-virulent strains shed more LPS during growth than less-virulent strains, resulting in enhanced TLR4 activation. Due to the role of LPS in *A. baumannii*

virulence, an LpxC inhibitor (which affects lipid A biosynthesis) antibiotic was tested. The LpxC inhibitor did not inhibit growth of the bacterium (MIC > 512 μg/ml) but suppressed *A. baumannii* LPS-mediated activation of TLR4. Treatment of infected mice with the LpxC inhibitor enhanced clearance of the bacteria by enhancing opsonophagocytic killing, reduced serum LPS concentrations and inflammation, and completely protected the mice from lethal infection. These results identify a previously unappreciated potential for the new class of LpxC inhibitor antibiotics to treat XDR *A. baumannii* infections. Furthermore, they have far-reaching implications for pathogenesis and treatment of infections caused by GNB and for the discovery of novel antibiotics not detected by standard *in vitro* screens.

IMPORTANCE Novel treatments are needed for infections caused by *Acinetobacter baumannii*, a Gram-negative bacterium that is extremely antibiotic resistant. The current study was undertaken to understand the immunopathogenesis of these infections, as a basis for defining novel treatments. The primary strain characteristic that differentiated virulent from less-virulent strains was shedding of Gram-negative lipopolysaccharide (LPS) during growth. A novel class of antibiotics, called LpxC inhibitors, block LPS synthesis, but these drugs do not demonstrate the ability to kill *A. baumannii* in vitro. We found that an LpxC inhibitor blocked the ability of bacteria to activate the sepsis cascade, enhanced opsonophagocytic killing of the bacteria, and protected mice from lethal infection. Thus, an entire new class of antibiotics which is already in development has heretofore-unrecognized potential to treat *A. baumannii* infections. Furthermore, standard antibiotic screens based on *in vitro* killing failed to detect this treatment potential of LpxC inhibitors for *A. baumannii* infections.

Footnotes

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