Inhibition of LPS-mediated inflammation by intravenous administration of a cationic antimicrobial peptide in mice

Justin Curtis[1], Joud Mulla[1], Zachary Secunda[1], Bashar AlMatour[1], Berthony Deslouches[2], Melanie J. Scott[1]

1. Department of Surgery, University of Pittsburgh, Pittsburgh, PA 15213

2. Department of Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA 15213

Introduction: Cationic antimicrobial peptides (AMPs) have long been observed to eliminate pathogenic microorganisms, such as gram positive and negative bacteria, through their ability to disrupt cell membranes and induce bacterial killing. E35, an engineered cationic AMP, is highly effective against multi-drug-resistant bacteria and can also bind lipopolysaccharide (LPS). We examined whether E35 was able reduce LPS-mediated inflammation in a mouse model *in vivo.* **Methods:** Male C57BL/6 (WT) mice were randomized to the following experimental groups (n=3-6/expm.gp): 1. intravenous (IV via tail vein) E35 peptide alone (either 5mg/kg or 10mg/kg); 2. intraperitoneal (IP) LPS (5mg/kg or 10mg) alone; 3. IV E35 directly followed by IP LPS; 4. Control (no treatment). Blood and liver were collected after 4h. Plasma TNFα, IL1β and IL6 were measured by ELISA. MAPK (JNK, p38MAPK, ERK) activation/phosphorylation, and caspase-11 expression/activation were measure in liver whole tissue lysates by Western blot. **Results:** LPS significantly increased all measured inflammatory cytokines at 4h at both 5mg/kg and 10mg/kg compared with controls. Pretreatment with E35 just prior to LPS injection significantly reduced TNFα (p=0.03) and trended towards reduced IL1β (p=0.36) compared to LPS alone. When TNFα was measure, LPS induced mice had a mean of 79.02 pg/mL (+/- 16.57) compared to 12.88 pg/mL (+/- 5.51) for mice given E35 treatment before LPS injection. Interestingly, E35 did not reduce IL6 levels in LPS-induced inflammation (p=NS). E35 peptide alone had no effect on systemic cytokine levels, but did significantly increase JNK and ERK1/2 activation/phosphorylation in the liver (Fig.1&2). However, pretreatment with 10mg/kg E35 in LPS-treated mice did reduce JNK activation (Fig.1). **Conclusions:** These data suggest that E35 peptide is able to reduce systemic LPS-mediated inflammation in a mouse model. The mechanism of liver MAPK activation with E35 administered alone, is interesting and this finding is being explored further. Overall, our results suggest that cationic AMPs may be able to reduce LPS-mediated inflammation, which may be important in regulating response to bacterial sepsis.

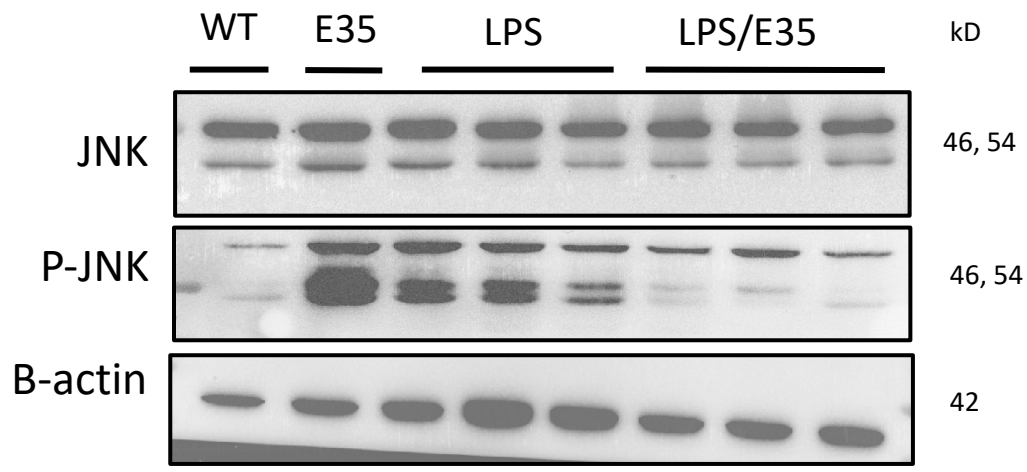

Figure 1: Pretreatment with 10mg/kg E35 regulates LPS-induced JNK activation in vivo. E35 alone shows increased MAPK signaling of P-JNK in liver lysate.

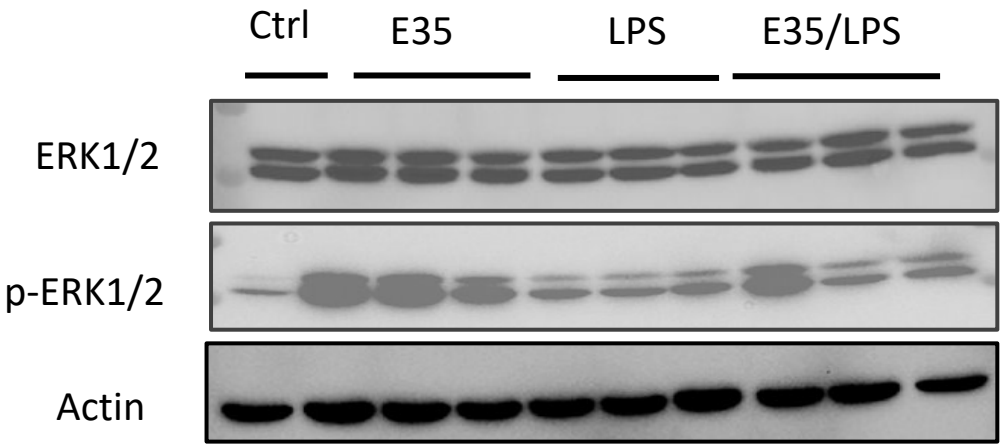

Figure 2: E35 administered alone shows increased MAPK signaling of P-ERK1/2 in liver lysate.