P2Y2 PURINERGIC RECEPTOR-MEDIATED METABOLIC REPROGRAMMING AND ACTIVATION OF AEROBIC GLYCOLYSIS CONTRIBUTES TO SEPSIS-INDUCED LIVER INJURY IN MICE.

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Background: Sepsis is a major life-threatening condition caused by maladaptive host response to infection. Metabolic shift from oxidative phosphorylation to aerobic glycolysis and NLRP3 inflammasome activation are hallmarks of sepsis. Whereas, Pyruvate kinase M2 (PKM2) is a rate-limiting enzyme in glycolysis and elevated lactate levels have been associated with increased mortality, the molecular basis of sepsis-associated enhanced glycolysis and lactate production and their role in liver injury is currently unknown. Infection and cell injury, known mediators of elevated ATP levels in the extracellular milieu, have the potential to activate P2Y2 purinergic receptors expressed in hepatocytes and immune cells. Following upon our previous observations that sepsis-induced liver injury is attenuated in P2Y2-/- mice, the purpose of this study is to test the hypothesis that P2Y2 purinergic signaling, via induction of PKM2-mediated metabolic reprogramming and inflammasome activation, contributes to the pathogenesis of sepsis-induced liver injury.

Materials/Methods: Polymicrobial sepsis was induced by cecal ligation and puncture (CLP) in adult wild type (WT) and P2Y2-/- (KO) mice, both on C57BL6J background. Total protein extracts from livers harvested at 21 hr post-CLP were analyzed by Western blotting for PKM2, NLRP3, and IL1-beta. Separately, AML-12 hepatocytes grown in serum free conditions (24 hr) were treated with 100 uM ARC-118925xx, (P2Y2 antagonist) prior to treatment with ATP (100 uM; P2Y2 agonist). Total protein extracts were analyzed by Western blotting. Fold changes (FC), calculated based on the densitometry of band intensity, were analyzed by Student's t-test ($p<0.05$)

Results: CLP led to a robust induction of PKM2 protein expression and inflammasome activation in the WT livers, which was attenuated in KO livers (WT vs KO, FC: PKM2, 3.6 v 1.4, NLRP3, 9.0 v 2.9; IL1-beta, 1.7 v 1.4; $p<0.05$). Suggesting a role for P2Y2 purinergic signaling in the activation of glycolysis and lipogenesis in hepatocytes, ATP treatment alone was sufficient to induce PKM2 (1.7), SREBP1c (1.3) and Plin2 (1.3) protein expression in AML12 cells, as compared to controls (1.0) and these effects were not observed in AML12 cells pre-treated with ARC-118925xx.

Conclusions: P2Y2 purinergic receptors, expressed in hepatocytes and liver-resident macrophages, have a unique and untapped potential to influence both metabolic and immunologic responses, and modify the outcomes of sepsis-induced liver injury.

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