

Acquisition of the microbiome drives maturation of the hepatic innate immune response
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Purpose: Neonatal vulnerability to sepsis is thought to be due to an impaired innate immune response. A key component of protection in adults is a robust hepatic innate immune response. The murine neonatal hepatic innate immune response is impaired compared to the adult. The microbiome is key to innate immune development, and acquisition of a mature microbiome correlates with resistance to systemic infection. However, it remains unclear whether the microbiome influences maturation of hepatic innate immunity. We hypothesize that wild-type mice, through microbiome-driven maturation of the hepatic innate immune system, mount a robust innate immune response to stimuli, whereas gnotobiotic mice exhibit an impaired response characterized by reduced expression of [innate inflammatory markers].

Methods: P7 C57BL/6 WT and GF mice were exposed to LPS (5 mg/kg IP, 1hr). Exposures were performed at P7 to correlate with previously reported dynamic developmental changes in murine microbiome. Hepatic tissue was collected and protein and mRNA isolated. Evidence of innate immune signaling was assessed with WB for nuclear translocation of NFkB subunits (p65, p50), STAT-3 activation (pSTAT/STAT ratio). Expression of primary response genes *Tnfa*, *NfKbia*, *Icam*, *il1a*, *il1b*, *il10*, *il6* was assessed using RT-qPCR.

Summary of Results: Hepatic tissue collected from LPS exposed p7 WT mice showed significant increases in nuclear translocation of the NFkB subunits (p65, p50) and STAT-3 activation (increased p-STAT/STAT ratio) compared to unexposed controls. Activation of these innate immune signaling pathways was significantly attenuated in the GF mice (n=3-7, p<0.05). Consistent with attenuated activation of innate immune signaling, LPS exposed GF p7 mice had significant attenuation (n=3-8, p<0.05) in the induction of key innate immune genes including *Tnfa*, *NfKbia*, *Icam*, *il1a*, *il1b*, *il10*, *il6* when compared to similarly exposed WT. **Conclusions:** At a timepoint when WT mice are developing resistance to pathogen exposure and an increased innate immune response, they show a more robust response compared to GF mice. These results are consistent with our hypothesis, demonstrating an attenuated inflammatory milieu and blunted activity of key innate immune regulators. Continued work is needed to determine whether this reflects intrinsic defects in immune cell function, altered hepatocyte signaling, or both. Defining the specific cell types and pathways affected in GF mice may reveal therapeutic targets to reduce neonatal susceptibility to sepsis.