Acquisition of the microbiome drives maturation of the hepatic innate immune response

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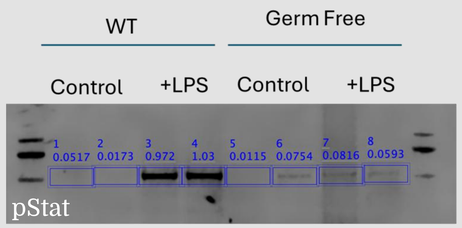
Abstract

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.

Methodology

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.

Results



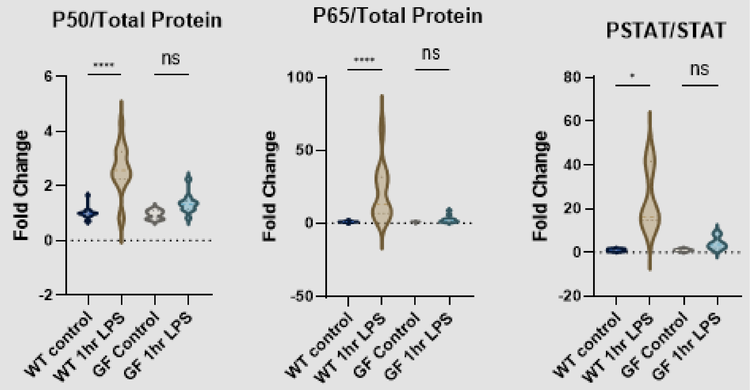


Figure 1
Hepatic tissue collected from LPS exposed p7 WT mice showed significant increases in nuclear translocation of the NFkB subunits (p65, p50) and STAT-3 activation (increased p-STAT/STAT ratio) compared to unexposed controls. Activation of these innate immune signaling pathways was significantly attenuated in the GF mice (n=3-7, p<0.05).

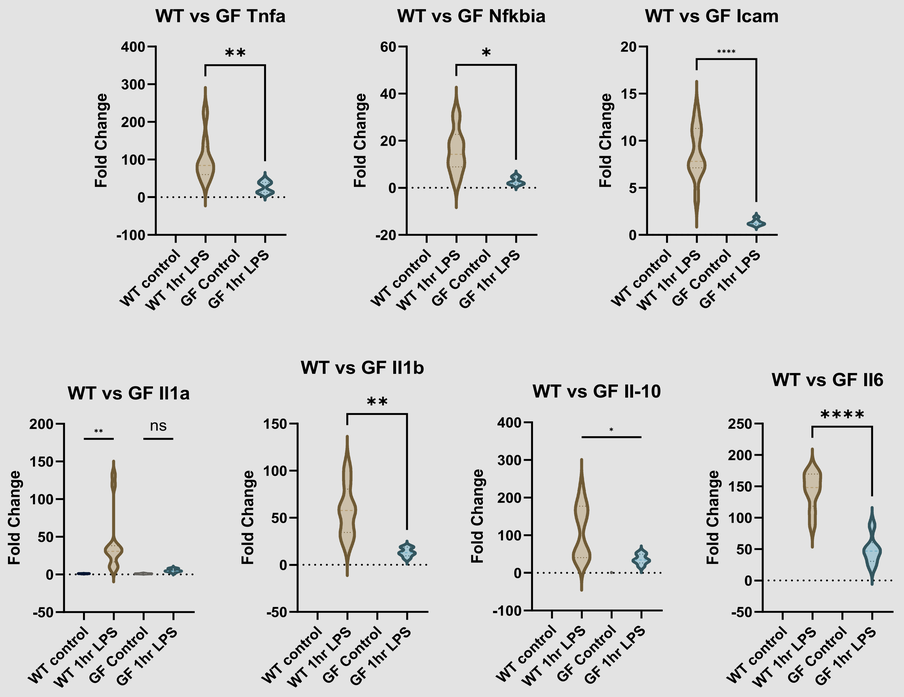


Figure 2
Consistent with attenuated activation of innate immune signaling, LPS exposed GF p7 mice had significant attenuation (n=3-8,p<0.05) in the induction of key innate immune genes including Tnfa, NfKbia, Icam, il1a, il1b, il10, il6 when compared to similarly exposed WT.

Conclusion

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.

Future Directions

Going forward I will focus on defining the contribution of Kupffer cells to hepatic innate immune maturation, with particular attention to MARCO and other key pattern-recognition receptors that may be dysregulated in GF mice. I plan to use flow cytometry to isolate Kupffer cells from WT and GF mice across additional developmental time points to determine when receptor expression and function begin to diverge. These sorted cells will then be used in in vitro stimulation assays to test whether altered MARCO-dependent signaling reflects intrinsic defects in Kupffer cell activation or results from an immature hepatic microenvironment. Expanding both the temporal resolution and receptor-level analysis of these experiments will help clarify how Kupffer cell maturation and PRR signaling shape early-life immunity and may reveal targets for microbiome-based interventions in neonatal sepsis.

Acknowledgements

All the Wright lab members
Research Track
Department of Pediatrics and the Perinatal Research Facility