The Non-transcriptional Function of IRF3 Dynamically Regulates Immune Cell Populations in Acute on Chronic Ethanol in Mice

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Introduction: Interferon regulatory factor 3 (IRF3) is a transcription factor mediating anti-viral responses, yet recent evidence indicates that IRF3 also has critical non-transcriptional functions, including activating RIG-I-like receptors-induced IRF-3-mediated pathway of apoptosis (RIPA) and restricting activity of NFκB. Using a novel murine model expressing only non-transcriptional IRF3 activity (*Irf3*$^{S1/S1}$), we tested the hypothesis that non-transcriptional functions of IRF3 modulate innate immune responses in the Gao-binge (acute on chronic) model of alcohol-related liver disease.

Objective: To prove that non-transcriptional functions of IRF3 modulate innate immune responses in the Gao-binge (acute on chronic) model of alcohol-related liver disease.

Material and Methods: C57BL/6, *Irf3*$^{-/-}$ and *Irf3*$^{S1/S1}$ were exposed to Gao-binge ethanol-induced liver injury. IRF3-mediated RIPA was investigated in cultured macrophages.

Results: Phospho-IRF3 and IRF3-mediated signals were elevated in livers of patients with alcoholic hepatitis. In C57BL/6 mice, Gao-binge ethanol exposure activated IRF3 signaling and resulted in hepatocellular injury. Indicators of liver injury were differentially impacted by *Irf3* genotype. *Irf3*$^{-/-}$, but not *Irf3*$^{S1/S1}$, mice were protected from steatosis, ALT/AST and inflammatory cytokine expression. In contrast, neutrophil accumulation and ER stress were independent of genotype. Protection from Gao-binge injury in *Irf3*$^{-/-}$ mice was associated with an increased ratio of Ly6C^{low} (restorative) to Ly6C^{high} (inflammatory) cells compared to C57BL/6 and *Irf3*$^{S1/S1}$ mice. Reduced ratios of Ly6C^{low}/Ly6C^{high} in C57BL/6 and *Irf3*$^{S1/S1}$ mice were associated with an increased apoptosis in the Ly6C^{low} population in response to Gao-binge. Activation of primary cultures of macrophages with Poly (I:C) induced translocation of IRF3 to mitochondria, association with Bax and activation of Caspases 3 and 9, processes indicative of activation of the RIPA pathway.

Conclusions: Taken together, these data identify important contributions of the non-transcriptional function of IRF3 in modulating the innate immune environment in response to Gao-binge ethanol exposure via regulation of immune cell apoptosis.

Keywords: etanol, IRF3, innate immune responses, Gao-binge model, alcohol-related liver disease.