

Title: IRF3 Deficiency Protects from CCl₄-Induced Liver Fibrosis in Mice by Modulating Neutrophil Responses

Authors and affiliations:

Yingting Zhang¹, Christina K. Cajigas-Du Ross¹, Megan R. McMullen¹, Emily Huang¹, and Laura E. Nagy¹

¹Department of Inflammation and Immunity, Cleveland Clinic, Cleveland OH

Introduction: Interferon regulator factor 3 (IRF3) executes multiple transcriptional and non-transcriptional functions in the development of metabolic liver diseases. While *Irf3*-deficient mice are protected from CCl₄-induced liver fibrosis, the specific cell types mediating its effects remain unclear. Liver fibrosis is characterized by excessive extracellular matrix accumulation and involves recruitment and activation of innate immune cells. Neutrophils play a dual role in fibrosis, promoting fibrosis through cytokines and granule proteins while also degrading the matrix. Yet regulation of neutrophils by IRF3 is largely unstudied in liver. Therefore, we investigated the role of neutrophils in liver fibrosis and assessed the contribution of IRF3 to neutrophil responses.

Methods: C57BL/6J (WT), *Irf3*-deficient (*Irf3*^{-/-}), and mice expressing only non-transcriptional IRF3 function (*Irf3*^{S1/S1}) were exposed to CCl₄-induced liver fibrosis. To determine the functional role of neutrophils, mice were treated with anti-Ly6G antibody to deplete neutrophils. Primary neutrophils were isolated from control WT, *Irf3*^{-/-}, and *Irf3*^{S1/S1} mice to characterize their functional activities.

Results: Liver bulk RNA-seq showed that chronic CCl₄ triggered neutrophil-associated response, which was attenuated in *Irf3*^{-/-} compared to WT mice. NET formation, assessed by citrullinated histone staining, was also reduced in *Irf3*^{-/-} livers. Interestingly, NIMP-R14 staining revealed greater hepatic neutrophil accumulation in *Irf3*^{-/-} than in both WT and *Irf3*^{S1/S1} mice, without a corresponding increase in neutrophil chemoattractants. Primary neutrophil degranulation and NETosis were induced by challenge with PMA. Despite intact ROS production, NET formation and release of granules were restricted in primary *Irf3*^{-/-} neutrophils compared to neutrophils isolated from WT and *Irf3*^{S1/S1}. Finally, neutrophil depletion in mice abolished the protective effect of *Irf3* deficiency on CCl₄-induced liver fibrosis.

Conclusions: *Irf3*-deficiency impaired suicidal NETosis both after CCl₄ treatment *in vivo* and in stimulated primary neutrophils from *Irf3*^{-/-} mice. However, it enhanced hepatic neutrophil retention and protected against CCl₄-induced fibrosis. Importantly, normal neutrophil responses were restored in *Irf3*^{S1/S1} mice, indicating that the non-transcriptional function of IRF3 contributes to critical neutrophil NET formation, likely facilitating in the progression of hepatic fibrosis.