Neutrophil Infiltration via STING Signaling Promotes Inflammation in Primary Sclerosing Cholangitis

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Introduction:

Primary Sclerosing Cholangitis (PSC) is characterized by a pro-inflammatory environment that contributes to liver fibrosis. Human PSC bile ducts are enriched with myeloid cells, indicating a potential role for the innate immune system. The STING (stimulator of interferon genes) signaling pathway is critical in regulating innate immune responses through the release of cytokines. Our goal is to investigate the mechanisms and consequences of peribiliary neutrophil infiltration observed in PSC.

Method:

Primary cholangiocytes isolated from WT and mouse models of PSC (3,5-Diethoxycarbonyl-1,4-Dihydrocollidine (DDC)-fed mice and Mdr2-/- mice) were analyzed by RNA-sequencing. Intrahepatic leukocytes (IHL) isolated from WT and Mdr2-/- were evaluated by flow cytometry and RT-PCR. Mdr2-/- mice injected with Ly6G antibody to deplete neutrophils were analyzed by immunofluorescence (IF), histology, and cytometry by time-of-flight (CyTOF). Autoimmune nCounter analysis was performed on human PSC tissues to interrogate immune signatures. Cholangiocytes exposed to TNF (inflammatory phenotype) were analyzed for chemokines upon genetic knockdown and pharmacological inhibition of STING.

Results:

Primary cholangiocytes from PSC mouse models demonstrated enrichment in inflammatory and neutrophil degranulation pathways. Congruently, flow cytometry analysis on $CD45^+$ IHL revealed an increase in the $Ly6G^+$ $Cd11b^+$ neutrophils in Mdr2-/- mice compared to WT (8.7% vs 2.5%, FC=3.48, *p<0.0001*). These neutrophils displayed an activated phenotype with increased expression of Cxcr1 and Cxcr2. Anti-Ly6G-mediated peripheral depletion of neutrophils in Mdr2-/- mice alleviated liver injury (57.6% and 59.2% reduction in ALT and AST, respectively). IF and histology revealed a substantial reduction in peribiliary neutrophil infiltration and reduced bridging fibrosis with Ly6G treatment in Mdr2-/- mice. CyTOF on IHL revealed an attenuation in cytotoxic CD8 T cells upon neutrophil depletion. nCounter analysis showed an upregulation of STING-associated genes in human PSC tissues. Mechanistically, TNF-induced upregulation of neutrophil chemoattractants, CXCL1 and IL8, was abolished by pharmacologic and genetic inhibition of STING.

Conclusion:

Our findings suggest the STING pathway in activated cholangiocytes triggers an immune response resulting in peri-portal neutrophil infiltration. The sustained presence of these activated neutrophils perpetuates the inflammation seen in PSC.