

Single-Cell Profiling Reveals Previously Unrecognized Intrahepatic Neutrophil States in Cholestatic Liver Disease

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Introduction: Neutrophils exhibit functional plasticity during chronic inflammation, but their role in cholestatic liver disease remains undefined. In primary sclerosing cholangitis (PSC), injured cholangiocytes establish a pro-inflammatory niche that recruits neutrophils to the bile duct; however, their functional heterogeneity and contribution to disease are unclear. We hypothesized that cholestatic injury induces temporally dynamic neutrophil states that contribute to PSC pathogenesis.

Methods: Single-cell RNA sequencing was performed on intrahepatic leukocytes from wild-type controls, 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC)-diet model (2-days and 10-days exposure), and *Mdr2*^{-/-} mice. Neutrophils were identified using canonical markers and analyzed by subclustering and differential gene expression. Immunofluorescence (IF) was performed to validate neutrophil heterogeneity and evaluate markers.

Results: Neutrophils constituted a distinct intrahepatic immune population across models. Initial subclustering identified inflammatory/pro-survival neutrophils (52.25%) expressing *Il1b*, *Cxcl2*, *Ptgs2*, *Tnfaip3*, and *Bcl2a1b*, and terminally mature/degranulating neutrophils (46.87%) enriched for *Retnlg*, *Mmp8*, *Ngp*, *Cd177*, and *Padi4*. Higher-resolution analysis resolved stress-responsive (45.74%), tissue-adapted (36.25%), and terminally mature (14.39%) neutrophil states. Acute DDC injury enriched stress-responsive and tissue-adapted populations, whereas terminally mature neutrophils were predominantly derived from *Mdr2*^{-/-} mice (60.47%). Early (2-day) DDC neutrophils demonstrated increased *Cd74* and MHC-II-associated gene expression across all subclusters compared with 10-day DDC injury, suggesting activation of immune-interacting programs early in cholestatic injury. IF confirmed distinct CD177⁺ and CD177⁻ neutrophil populations. Variable CD74 expressions within the peribiliary niche supported distinct neutrophil activation states.

Conclusions: These findings identify previously unrecognized neutrophil states that emerge during distinct stages of cholestatic liver injury. Early *Cd74*/MHC-II-associated programs and accumulation of terminally mature CD177⁺ neutrophils reveal candidate immune pathways linked to disease initiation and progression. Targeting stage-specific neutrophil programs may represent a novel therapeutic strategy for PSC.

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