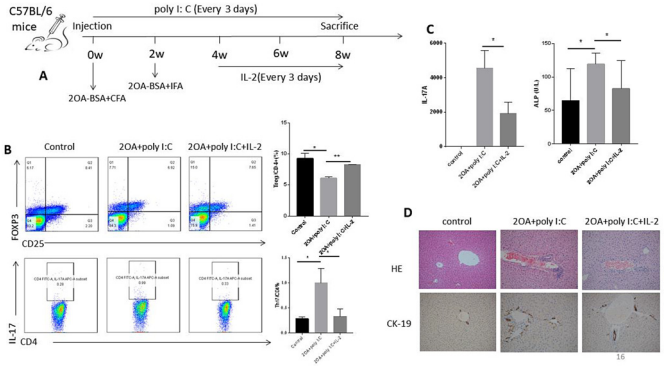

Conclusion: The PBC mouse model was successfully induced by the combination of 2OA-BSA and poly I:C and it can model human disease early status. Low-dose IL-2 inhibited PBC by augmenting Treg and decreasing Th17 numbers, which paly important role in the pathogenesis of the PBC. The improvement of biochemical indexes and liver histopathology, suggesting that low-dose IL-2 treatment may be considered as novel therapy for PBC in the future.

OS021

Cholangiocytes cleave surface CD100 from biliary infiltrating T cells and mediate pathogenic Th17 differentiation

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Background and aims: Chronic inflammation surrounding bile ducts contributes to the disease pathogenesis of most cholangiopathies, but the mechanism enabling pathogenic immune cells to adapt and survive in the biliary environment remains largely unknown. We have recently reported a variant of CD100 to be the causal mutation for a familial form of primary sclerosing cholangitis (PSC). Herein, we investigate how CD100 participates in the biliary local inflammation and its relevance to the differentiation of pathogenic immune cells.
Method: CD100 expression was assessed by spatial transcriptomics (10x Genomics) and *in situ* immunohistochemistry (IHC) in explanted livers of patients with PSC (n = 4–6) and other cholangiopathies (Ctrl, n = 4–5). Soluble CD100 (sCD100) was measured by ELISA in paired serum, plasma, and bile samples (n = 11–19). Biliary infiltrating immune cells were collected from endoscopic retrograde cholangiopancreatography brush samples (Ctrl, n = 6; PSC, n = 6) and surface expression of CD100 was evaluated by flow cytometry. To model pathogenic interactions between immune cells and cholangiocytes, splenic cells isolated from C57BL/6 wild-type (WT) and CD100 mutated mice were co-cultured with small or large cholangiocytes. Altered gene expression was assessed with RNA sequencing of purified cell subsets after co-culture.
Results: Spatial transcriptomics revealed *SEMA4D/CD100* RNA expression in all examined livers and demonstrated the localization in *KRT19*$^{+}$ bile duct regions (Ctrl, 7–40%; PSC, 11–94%). In contrast, CD100 protein expression measured by IHC was nearly undetectable in diseased periductal areas of the PSC livers. Moreover, surface expression of CD100 on biliary infiltrating immune cells was reduced and accompanied by increased sCD100 in plasma and bile from PSC patients, suggesting that CD100 is cleaved from the surface of immune cells in regions adjacent to the bile ducts. In co-culture experiments, we observed that activated immune cells adhered to cholangiocytes and correlated with the death of large but not small cholangiocytes. T cells were dominant (49.9–64.4%) in the adherent immune population and lost their surface CD100 expression. RNA sequencing data showed increased *Adamts4* in co-cultured cholangiocytes which appeared to cleave CD100. Genes involved in anti-apoptosis and T-helper 17 (Th17) differentiation were enriched in adherent T cells and further upregulated in T cells with mutated CD100.
Conclusion: Cholangiocytes induce cleavage of CD100 on biliary infiltrating T cells that facilitates persistent inflammation and local Th17 differentiation. These findings highlight a novel pathway in the liver with cholangiocyte-driven Th17 cell differentiation that is associated with CD100 cytoplasmic signaling. Targeting this pathogenic pathway serves as an attractive target for mitigating bile duct inflammation in PSC.

OS022

Novel approach combining whole liver single-cell RNA sequencing and spatial gene profiling using Nanostring GEOMX enables identification of specific cell sub-populations and pathways regulated by CCL24

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Background and aims: CCL24 (Eotaxin-2) is a chemokine that regulates profibrotic and proinflammatory activities through the CCR3 receptor. We previously demonstrated that CM-101, a CCL24 blocking antibody, improves liver inflammation, fibrosis and cholangitis in the Mdr2 knockout (Mdr2 -/-) mouse model. Characterization of CCL24 expression in relation to the different cell populations in Mdr2-/- mice and its inhibition effect on cholangiocytes and immune response was studied using two gene expression methods.
Method: Identification of cell population, including immune cells and CCL24 expressing cells in livers of Mdr2-/- mice was done by scRNA seq from whole liver. NanoString's technology was utilized for gene expression studies, focusing on the bile-duct injury sites. In this system segmenting of cholangiocytes (Pan-CK+) and immune cells (Pan-CK–) cells was done in liver sections from Mdr2-/-mice non-treated or treated with CM-101.
Results: Mdr2-/- mice treated with CM-101 resulted in reductions in: serum ALP, liver inflammation, liver peribiliary collagen deposition and cholangiocytes proliferation. Using whole liver scRNA seq we demonstrated that CCL24 is expressed in macrophage cells. This analysis identified a few specific disease relevant immune sub-populations, however it is missing crucial information that relates to cell localization. To overcome this, we used NanoString to characterize the alternations in peribiliary transcriptome following CM-101 treatment. Spatial profiling separated cholangiocytes (PanCK+) and non-cholangiocytes (PanCK–) peribiliary cells, distinguishing cholastatic, proinflammatory and profibrotic effects. Gene set enrichment analysis showed reduction in proliferation and senescence pathways in PanCK+ cells, whereas PanCK- cells showed reduction in ECM remodeling pathways and increased metabolic pathways. Cell deconvolution of the heterogeneous PanCK- population revealed alternation in the peribiliary immune cells, marked by reduction in macrophages and monocytes following treatment with CM-101.