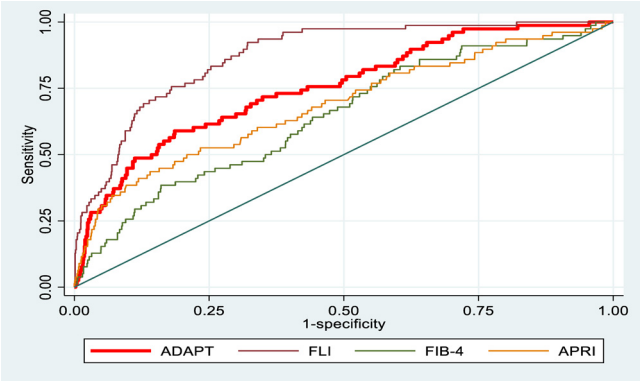

Figure: ROC curves of non-invasive fibrosis scores for prediction of significant fibrosis.

Conclusion: In this population-based study, the ADAPT composite score that includes PRO-C3 offers superior predictive accuracy for the diagnosis of significant liver fibrosis as compared to other recommended screening tools such as FIB-4 and APRI. The individual components of ADAPT are easily accessible clinical parameters and as such could be used as a tool for the early detection of liver fibrosis in the general population, particularly in a sequential-type approach.

Immune-mediated and cholestatic: Experimental and pathophysiology

OS019

Novel anti-cholestatic treatment strategies by combining inhibition of the Apical sodium-dependent bile acid transporter with stimulation of urinary bile salt excretion or lowering bile salt synthesis

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Background and aims: The apical sodium-dependent bile acid transporter (ASBT) is primarily expressed in the small intestine and kidney, where it prevents bile salts from being excreted in respectively feces and urine. Intestine-restricted drugs that inhibit ASBT are currently clinically explored to reduce toxic accumulation of bile acids during cholestasis. Intestine-restricted ASBT inhibitors (ASBTi) may be less effective in severe cholestasis and also yield gastrointestinal side-effects in case of high bile salt load in the colon. Here, we test two ASBT-targeting treatment strategies in pre-clinical models with cholestasis-induced liver injury. First, systemic ASBT inhibition, to increase renal bile acid excretion and second a combination treatment with obeticholic acid (OCA) to limit bile salt synthesis and reduce colonic bile acid load.
Method: Systemic ASBT inhibition was tested by performing a bile duct ligation (BDL) in adult ASBT knock-out (KO) mice (129P2/OlaHsd background, Jackson) and wild-type littermates to induce severe cholestasis. In our second strategy, BDL was performed in adult wild-type C57Bl/6 mice after 2 days oral gavage pre-treatment with OCA and ASBTi. In a different model, wild-type C57Bl/6 mice were fed a 0.1% 3, 5-diethoxycarbonyl-1, 4-dihydrocollidine (DDC) diet while receiving daily treatment with either placebo, OCA, ASBTi or both (OCA + ASBTi). After sacrifice, liver injury was determined by plasma liver enzymes, RT-qPCR and liver histology, while HPLC analysis was used to quantify bile salt concentrations in plasma, liver, small intestine and feces.
Results: ASBT KO mice had reduced liver necrosis, reduced bilirubin and alkaline phosphatase (ALP) levels compared to wild-type mice after BDL. ASBT KO mice also showed a trend to reduced bile salt pool size, and increased urinary bile salt excretion. OCA + ASBTi treatment reduced the total bile salt pool size before cholestasis-onset and resulted in reduced bilirubin, ALP and a ~60% reduction in liver necrosis compared to placebo control in a BDL model. Besides, OCA + ASBTi treatment decreased fecal bile salt excretion compared to monotherapy with ASBTi.
Conclusion: Systemic ASBT inhibition effectively reduces BDL-induced liver damage. Combined OCA + ASBTi treatment lowers the bile salt pool size and improves liver health after BDL-induced cholestasis, while it also shows therapeutic potential by reducing fecal bile salt excretion.

OS020

Low-dose IL-2 alleviates drug-induced primary biliary cholangitis in mice by improving Treg and Th17 balance

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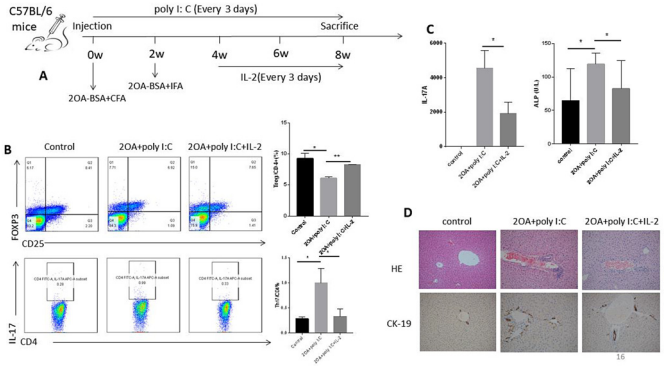
Background and aims: The imbalance of regulatory T (Treg) and T helper 17 (Th17) cells correlates with increased risk of autoimmune diseases. Their imbalance was also reported in primary biliary cholangitis (PBC) patients. Previous studies have suggested that low-dose IL-2 can alleviate disease severity through modulating $CD4^+$T cell subsets in patients with autoimmune diseases. However, the efficacy of low-dose IL-2 in PBC remains unexplored. Hence, the present study aimed to examine effects of low-dose IL-2 in PBC mouse models.
Method: PBC was induced in female C57BL/6 mice by two immunizations with 2-nonynoic acid (2OA-BSA) at two-week intervals. Besides, polyinosinic polycytidylic acid (poly I:C) was injected i.p. every three days. The control group was injected with PBS instead of 2OA-BSA and poly I:C. PBC mice were divided into the treated and untreated groups, and low-dose IL-2 was injected s.c. every three days after four weeks from modeling in the treated group (Fig. A)and the untreated group was replaced with saline. The serum was isolated from blood sampled by eyeball extirpating for biochemical detection. Th17 and Tregs were analyzed by flow cytometry, and the related cytokines were analyzed by ELISA. Liver histopathology was examined by HandE and immunohistochemical staining. The experimental data were analyzed by SPSS 24.0 software. $P < 0.05$ indicated statistical significance.
Results: Eight weeks after modeling, the serum AMA was positive and the ALP was significantly increased in PBC mice compared with control group. The pathology showed lymphocyte infiltration in the portal area, and damage and reactive proliferation of small bile duct, and $CD4^+$ and $CD8^+$ T cells were infiltrated around the bile duct. Flow cytometric examination of spleen cells revealed recovery of reduced Tregs and increased Th17 after low-dose IL-2 treatment (Treg/CD4%:9.26 ± 0.50 vs 6.10 ± 0.14 vs 8.24 ± 0.04; Th17/CD4%: 0.29 ± 0.20 vs 1.00 ± 0.17 vs 0.33 ± 0.09) ($p < 0.05$) (Fig. B). Low-dose IL-2 treatment inhibited IL-17A levels (0 vs 4549 ± 597.5 vs 1928 ± 387) ($p < 0.05$) and improved serum biochemical index (ALP: 119.1 ± 6.20 vs 82.36 ± 12.6 U/L) (Fig. C). Histopathological examination of liver revealed the improvement of portal area inflammation and reactive bile duct hyperplasia and damage after low-dose IL-2 treatment (Fig. D).



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.