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Deletion of Chemokine Receptor 6 Augments Ductular Reaction and Fibrosis in a Mouse Model of Cholestatic Liver Injury

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3305 | DELETION OF CHEMOKINE RECEPTOR 6 AUGMENTS DUCTULAR REACTION AND FIBROSIS IN A MOUSE MODEL CHOLESTATIC LIVER INJURY

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Background: The chemokine CCL20 is expressed by activated cholangiocytes implicating its' receptor, CC-chemokine receptor 6 (CCR6) in immune cell trafficking to the liver during cholestatic liver injury. In particular, the innate immune cells macrophages and neutrophils have been identified in cholestatic liver injury. Therefore, our aim was to investigate the effect of CCR6 deletion on a mouse model of 3,5 -diethoxycarbonyl-1,4-dihydrocollidine (DDC) diet-induced cholestatic liver injury. **Methods:** Age and gender matched WT and *Ccr6*$^{-/-}$ mice were placed on an DDC or a control diet for 21 days. Serum biochemistry, liver injury and the ductular reaction (DR) were examined by standard approaches. Gene expression by nanostring analysis and immunophenotyping of intrahepatic leukocytes (IHL) by mass cytometry were performed. **Results:** Unexpectedly, liver weight/body weight percentage (6.026 ±0.13 vs 5.18 ±0.23), serum alkaline phosphatase (646.8 ±48.88 vs 357.7 ±39.4 U/ml), alanine aminotransferase (2058 ±377.2 vs 1140 ±151.6 U/mL), total bilirubin (2.91 ±0.66 vs 1.0 ±0.12 mg/dL) were significantly greater in *Ccr6*$^{-/-}$ mice compared to WT mice on DDC diet. *Ccr6*$^{-/-}$ mice also had a more extensive DR compared to WT mice on DDC diet (5.32 ±0.52 vs 3.44 ±0.37 %). Sirius red (1.82 ±0.17 vs 0.54 ±0.13 %), desmin (3.62 ±0.41 vs 1.63 ±0.59 %) and alpha-smooth muscle actin (4.44 ±0.41 vs 2.13 ±0.27 %) stainings indicated that fibrosis was significantly increased in *Ccr6*$^{-/-}$ mice as compared to WT mice on DDC diet. Immunophenotyping of IHL revealed no difference in the total number of macrophages recruited into the liver during DDC administration. For contrast, there was a two-fold increase in the accumulation of neutrophils in the *Ccr6-/-* mice reared on the DDC diet as compared to the WT mice. This observation was supported by the significantly increased gene expression levels of *Ltb4r1, Cfp, Cfb, Myd88, Hfe,* and *Stat5b* suggesting an increased recruitment and survival of neutrophils in the *Ccr6*$^{-/-}$ mice. **Conclusion:** Genetic deletion of *Ccr6* causes extensive DR, fibrosis, and upregulation of proinflammatory genes responsible for recruiting neutrophils during DDC diet-induced cholestatic liver injury in mice. These data provide novel insights into the role of the CCR6-CCL20 signaling axis in restraining neutrophil accumulation in the liver during cholestatic liver injury.

Disclosures:
The following people have nothing to disclose: Begum Ozturk, Anuradha Krishnan, Gregory J. Gores
Disclosure information not available at the time of publication: Kaiyel Cutshaw

3306 | THE ABSENCE OF FUNCTIONAL PEROXISOMES IN MOUSE HEPATOCYTES IMPAIRS AUTOPHAGY AND CAUSES CHOLESTASIS DURING FASTING

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Background: Hepatic pathology is one of the cardinal features of peroxisomal disorders, and mice with a hepatocyte-selective depletion of peroxisomes during their first postnatal week (e.g., alb-Pex5 null mice; These mice were generated by inbreeding *Pex5-loxP* and albumin-*Cre* mice) develop a broad array of liver abnormalities (e.g., steatosis, fibrosis, and hepatocarcinogenesis) at later age. In this study, we document the liver changes in 4-week-old alb-Pex5 null male mice under both feeding and fasting conditions, with a focus on autophagy. These mimic the situation of newborn Zellweger syndrome patients. **Methods:** 4-week-old control (CT) and alb-Pex5 null male mice were either fed normal chow ad libitum or fasted for 24 h (n=4). Liver tissues were collected for immunoblotting or fixed for histological analyses. Statistical significance ($p < 0.05$) between the two groups was evaluated by using the unpaired t-test. **Results:** At the macroscopic and microscopic level, the following changes were observed in 4-week-old alb-Pex5 null mice versus CT mice (i) the body weight was significantly decreased, (ii) under feeding conditions, the livers exhibited excessive lipid accumulation and hypertrophy, and (iii) after 24h fasting, the livers displayed a yellowish color, with disordered hepatic cords, cellular hypertrophy, and severe sinusoidal dilatation. In addition, the gallbladder was increased in size and the color of the intestinal content was strongly darkened. Furthermore, immunoblotting experiments demonstrated that, compared to the CT mice, the protein level of the classical autophagy receptor SQSTM1 was significantly enhanced in the alb-Pex5 null male mice, while the LC3B-II/LC3B-I ratio, an often-used marker for autophagy, was clearly decreased in both feeding and fasting conditions. Finally, also the protein levels of (i) mTOR, a well-known negative regulator of autophagy, (ii) its active isoform phospho-S2448 mTOR, and (iii) phospho-S757 ULK1, an inactive isoform of the autophagy activator ULK1 whose activity