Bile acid metabolism and T cell responses in cholangiopathy: Not one-way traffic

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Cholangiocytes, also termed biliary epithelial cells, make a vital contribution to liver physiology and in maintaining homeostasis within the liver microenvironment.[1] They line the biliary tree and play a central role in regulating bile composition through the secretion of water, electrolytes and solutes via the expression of a range of membrane transporters. Additionally, they are involved in cross talk with other cellular populations (both resident and non-resident) and through major internal cellular pathways, such as senescence, apoptosis/proliferation, they maintain tissue homeostasis.[2] Chronic injury leads to cholangiocyte dysfunction with major pathological consequences characterised by obstructed biliary flow, progressive biliary fibrosis and, parenchymal destruction that can culminate in end-stage liver failure often requiring liver transplantation. Malignant transformation leading to cholangiocarcinoma may also occur. Cholangiopathies can be driven by genetic, environmental, and viral insults as well as idiopathic mechanisms.[3] The most common immune-mediated cholangiopathies, primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC), have quite different characteristics. PBC is characterised histologically by destruction of interlobular bile ducts with a granulomatous lymphocytic cholangitis. This disorder predominantly affects women and is associated with classical autoantibodies specific for mitochondrial antigens. PSC is characterised by a male preponderance, a strong association with inflammatory bowel disease, and inflammation of both intra- and extrahepatic ducts leading to bile duct strictures. Both diseases are associated with an inflammatory infiltrate, and while the pattern of damage is quite different, a rich T cell infiltrate is often seen in PBC and T cells can be detected in the portal infiltrates associated with PSC. A significant body of research has focused on how cholangiocyte dysfunction drives T cell infiltration with the hope of identifying novel therapeutic targets to prevent progressive tissue injury. While this process is complex and imperfectly understood, there are features that are likely to be common to all chronic cholangiopathies, including T cell recruitment from the circulation via an adhesion cascade. In the liver this occurs within the hepatic sinusoidal channels which are lined by the unique hepatic sinusoidal endothelium.[4] The mechanism by which T cell subsets are recruited appears to follow distinct pathways which can be organ and disease specific. For example, in liver sinusoids the low shear environment is associated with a lack of selectin expression and atypical adhesion molecules, which in PSC may aberrantly recruit gut-homing lymphocytes that then drive the disease.[5,6] Once T cells adhere to and migrate across liver endothelium, they localise at the site of epithelial damage and their retention leads to chronic inflammation. T cell retention is likely promoted by the direct release of cytokines and growth factors from injured cholangiocytes. Furthermore, adherence of T cells to integrin ligands such as vascular cell adhesion molecule-1 (VCAM-1) on cholangiocyte membranes has been shown to promote T cell survival and contribute to persistent inflammation.[2,7]

Bile acids have been proposed as one of the factors that may contribute to the inflammatory response in cholangiopathies.[8] Primary bile acids are synthesised from cholesterol in the liver by the rate-limiting enzyme microsomal cholesterol 7α hydroxylase (CYP7A1) and CYP8A1 in the classical pathway, and Cyp27a1 and Cyp7b1 in the alternative pathway.[9] Bile acids are then excreted into bile via the bile salt export pump (BSEP). They then undergo a highly regulated enterohepatic circulation with uptake across the terminal ileum which is mediated by the, apical sodium-bile acid transporter, and the basolateral membrane transporter, the heteromeric organic anion transporting peptide OSTα-OSTβ. Re-uptake by the liver occurs predominately via the sodium-taurocholate cotransporting peptide (NTCP). Each of these steps is tightly regulated to maintain homeostasis and the bile acid pool size. The master regulator of this process is the bile acid activated nuclear receptor, the farnesoid X receptor (FXR). Bile acid accumulation in the liver is prevented by negative feedback where bile acid activation of FXR induces the expression of the small heterodimer partner (SHP). SHP in rodents represses transcription factors which contribute to the expression of P450 enzymes which are critical to bile acid synthesis. However, in humans, bile acid synthesis is regulated in large part by ileal secretion of fibroblast growth

factor 19 (FGF-19), also an FXR target. FGF19 binds to its receptor FGFR4 on the sinusoidal membrane of the hepatocyte, activating a signal transduction pathway leading to inhibition of CYP7A1.[9] When these pathways are dysregulated during cholestatic liver injury, bile acids accumulate in the hepatocyte and result in deleterious cellular pathways involving oxidative stress, mitochondrial dysfunction, and the synthesis and release of inflammatory cytokines that in turn recruit an inflammatory response.[10,11] While these events result in parenchymal cell injury, their effects on cholangiocyte function is less clear. Yet the therapeutic use of ursodeoxycholic acid in patients with PBC and the recent findings of the beneficial role of obeticholic acid, a potent ligand for FXR, support the important contribution of bile acids in the pathogenesis of these diseases.[12] In the context of PBC, there has also been significant interest in defective biliary bicarbonate secretion. The main mediator of bicarbonate secretion in cholangiocytes is the Cl^-/HCO_3^- anion exchanger 2 (AE2). Cholangiocytes in PBC have decreased AE2 expression which leads to cholestasis, *Ae2* knockout mice spontaneously develop hepatobiliary and immunological features associated with PBC.[13] Studies have confirmed that pro-inflammatory cytokines promote microRNA driven pathways in cholangiocytes that lead to decreased AE2 expression and subsequent defective bicarbonate secretion.[14] Thus, the role of T cells in this process is currently a subject of interest.

While PBC and PSC are considered to be immune-mediated diseases, conventional anti-inflammatory therapy and effects of immunomodulators have been disappointing.[15] Nonetheless, cholangiocyte damage is often associated with T cell infiltration, although its role in the disease process remains unclear. Therefore, a better understanding of the contribution of bile acid accumulation and metabolism to inflammation might well provide novel therapeutic targets. In this edition of the Journal of Hepatology, *Glaser et al.*[16] attempt to shed new light on the relationship between T cells and bile acids in cholangiopathies. They initially use a well-recognised murine model of T cell mediated bile duct injury involving K14-OVAp mice which express an ovalbumin peptide on cholangiocytes. CD8 T cells which are antigen specific for OT-1 are then transferred intraperitoneally into these mice resulting in features of acute cholangitis. With this model they find that the transfer of T cells leads to the transcriptional downregulation of key bile acid metabolic enzymes responsible for bile acid synthesis in both the classical and alternative pathways, as well as upregulation of the BSEP. These findings appear to be directly related to the transferred T cells as the same findings are found in $Rag^{-/-}$ mice without a native adaptive immune system. To further explore what contribution T cells may have to bile acid metabolism in cholangiopathies they repeated their CD8- T cell transfer experiments in K14-OVAp which had been crossed with the $Mdr2^{-/-}$ animal model of sclerosing cholangitis.[17] Adoptive transfer of T cells once again drove down transcription of *Cyp7a1, Cyp8b1, Cyp27a1* and *Cyp7b1* in association with upregulation of SHP. Downregulation in the transcription of the basolateral bile acid transporters (*Ntcp* and *Oatp*) with a corresponding increase in *Bsep* expression were again seen. This was accompanied by a decrease in the quantity of unconjugated bile acids with an increase in conjugated bile acid in both serum and liver after T cell transfer. Mechanistically, utilising antibodies to cytokines *in vivo* and co-culture assays between T cells and hepatocytes, the authors demonstrate that these changes in bile acid metabolism were mediated by tumor necrosis factor alpha (TNFα) and interferon gamma (IFNγ), known to be major cytokine effectors of liver inflammation, which may also affect FXR signaling pathways. Direct T cell contact with hepatocytes was also required, although the mechanism remains to be explained. Finally, the relevance of these findings to human disease was explored by RNA sequencing analysis in liver tissue from patients with PBC and PSC. These studies showed a negative correlation between genes responsible for bile acid synthesis and hepatic bile acid uptake and the expression of TNFα and IFNγ.

Although it has been shown previously that cytokines influence bile acid production,[18,19] and transporter expression,[20] this study demonstrated the direct contribution of T cells to this phenomenon. Limitations include the difficulty of replicating the chronicity and pathology of human disease with the $Mdr2^{-/-}$ murine model. Additionally, the magnitude of reduction of unconjugated bile acids was minimal so that it is not clear if this was functionally important. Finally, as acknowledged, further work is required on the underlying mechanisms of the T cell contact-dependent findings on hepatocyte function. Despite this, the authors shed new light on the nexus of cholangiocyte damage, T cell infiltration and bile acid accumulation. Their results provide an insight into the complexity of T cell accumulation and progressive disease in cholangiopathies and why therapies involving broad acting immunomodulators may not have been effective in these conditions. This study suggests that novel approaches for T cell therapy in cholangiopathies need to be designed to prevent progressive tissue injury while maintaining the beneficial effects of T cells on bile acid metabolism.

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Conflicts of interest

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Supplementary data

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