Mast Cells in Immune-Mediated Cholangitis

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Mast cells (MCs) are a cell lineage produced in the bone marrow from myeloid precursors which express and retain c-kit expression throughout their developmental stages [1]. Cholestasis, which is impaired bile flow from the liver into the intestine, can be caused by cholangitis and/or bile duct obstruction. Cholangitis can arise from bacterial infections and cholelithiasis, however, immune-mediated cholangitis in primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC) is characterized by a strong immune response targeting the biliary epithelial cells (BECs). Persistent biliary inflammation further represents a risk for biliary neoplasia, cholangiocarcinoma (CCA) by driving chronic cellular stress in the BECs. Currently, immune-mediated cholangitis is considered a Th1-Th17-dominant disease, however, the presence of Th2-related mast cells (MCs) in tissue samples from PBC, PSC and CCA patients has been described, showing that these MCs are active players in these diseases.

mast cells cholangitis

1. Mast Cells in Immune-Mediated Cholangitis

Immune-mediated cholangitis in primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC) is characterized as a sustained inflammatory response targeting the biliary epithelial cells (BECs) causing an initial disarrangement and subsequent narrowing of the bile ducts due to fibrotic remodeling. Subsequently, chronic inflammation may lead to liver failure and/or malignancy. Both PBC and PSC share similarities such as diagnostic images where obliteration of the bile ducts is easily observed showing a beading pattern and histological findings unveiling both infiltrating cells and collagen fibers deposited around the bile ducts. In addition, PBC and PSC patients present biochemical and tissue architecture modifications in the gallbladder [1][2]. However, these diseases are not fully similar. In general, PBC is associated with autoimmune diseases (lupus, rheumatic and autoimmune hepatitis) and it occurs predominantly in women [3], high rates of patients show increased levels of circulating auto-antibodies including antinuclear (ANA) and antimitochondrial (AMA) [4]. Moreover, to date several identified genetic risk factors for PBC have been found in autoimmune-related loci such as HLA class II genes as well as in non-HLA alleles (e.g., *NF-κB, SOCS1, STAT4*) [5][6]. In terms of PSC, circulating auto-antibodies are much less frequent and this disease is strongly associated with autoinflammatory diseases (i.e., ulcerative colitis) rather than autoimmune counterparts [7]. Additionally, men are more affected than women. Further, unlike PBC, PSC is the strongest clinical event associated with cholangiocarcinoma (CCA).

While both PBC and PSC have been identified as Th1- and Th17-related diseases, they also share the pathological component of fibrotic reactions resulting from chronic inflammation. Fibrotic reactions are largely promoted and sustained by components of the Th2 response, including MCs. In fact, it has been long identified that

MCs release an array of pro-fibrotic mediators including IL-1β, TNFα, TGFβ and FGF, which can also be produced by other sources. Additionally, there exists several MC-specific pro-fibrotic molecules such as histamine, tryptase and chymase. It is worth noting that elevated levels of histamine are reported in chronic cholestatic diseases and are associated with the major symptom of these diseases, which is pruritus [8]. Moreover, histamine evokes collagen production by fibroblasts [9]. Therefore, evidence suggests an important role of MCs in immune cholangitis.

Both clinical and experimental evidence has emerged showing that MCs accumulate in the biliary tree and gallbladder in PBC and PSC patients in higher numbers compared to acute liver diseases and other chronic liver diseases. Clinical data have, for instance, shown that patients with PBC had increased numbers of MCs, determined by tryptase staining, as part of infiltrating cells in portal tracts [10][11][12][13]. Importantly, MCs were detected in zones of fibrosis in diverse hepatic areas such as fibrotic portal tracts and fibrotic septa [13]. Likewise, tissue samples from PSC patients were positive for tryptase and c-kit staining confirming the presence of MCs in the portal tracts, where a positive association between MC numbers and PSC severity was found [14][15][16][17]. Moreover, PSC patients had overexpression of SCF on the BECs [15], suggesting that cholangiocytes may actively be promoting activation and proliferation of MCs. Complementarily, animal studies have shown that MCs can communicate with hepatic stellate cells (HSCs) through TGFβ which induces a profibrotic program in HSCs (i.e., alpha smooth muscle actin, αSMA induction) [18]. Thus, these observations suggest that a crosstalk between BECs and MCs in chronic PBC and PSC may be inducing fibroblast and HSCs activation causing fibrotic reactions.

Despite mouse models of immune cholangitis being difficult to establish due to complexity in these diseases, relevant insight into immunopathology as well as preclinical therapeutic evaluation has been leveraged using animal models. Adding to the human studies, mouse model research has confirmed that MCs represent one important immune cell population expanding in the injured bile ducts. Using the multidrug resistant gene 2 deficient mouse model ($Mdr2^{-/-}$), Jones et al. showed that, in this spontaneous model of PSC, a significant increase in MCs paralleled the biliary injury. Moreover, when MCs were inhibited with cromolyn sodium, a significant decrease in biliary injury, cholangiocyte proliferation and collagen deposition was observed. The latter was associated with lower MC numbers and circulating histamine levels [17]. More recently, the same research group described that, amongst other mechanisms, the FDA approved treatment for cholestatic diseases ursodeoxycholic acid (UDCA) similarly attenuates MC infiltration, fibrosis, and histamine secretion [16]. Interestingly, in these studies, using samples from PSC patients complemented the mouse results, providing evidence that controlling MC activity is part of the immune cholangitis treatment highlighting the relevance of these cells.

As mentioned earlier, PBC meets the criteria to be considered as an autoimmune disease, although a mouse model reproducing the full hallmarks for this disease is not yet available. However, the OVAbil model [19], induced in an antigen-specific and cholangiocyte-restricted fashion reproduces the biliary damage observed in both PBC and PSC and is highly dependent on a progressive immune response which ultimately damages the bile ducts. Therefore, the OVAbil model closely resembles autoimmune-mediated cholangitis rather than other chemical injury-induced cholangitis models. Using this model, it can be observed that obese mice and obese mice lacking the sensor NLRP3, both presented a more severe form of the disease, had a mixed Th2/Th17 (IL-13 and IL-17)

response and IL-13, respectively [20][21]. Interestingly, other granulocytes such as neutrophils massively infiltrated in the liver. Whether Th2 downstream effectors such as MCs indeed collaborated in exacerbating cholangitis in these OVAbil obese mice remains to be tested.

Thus, convincing evidence shows that MC expansion accompanies the development of immune cholangitis (i.e., PBC and PSC) and these cells promote fibrotic reactions by communicating with other resident liver cells including HSCs and fibroblasts which worsen the course of PBC and PSC. Impaired MC infiltration and histamine release results in attenuated biliary injury. Summarized evidence showing the role of MCs in immune cholangitis is presented in **Table 1** where studies retrieved from a literature search in the PUBMED database including the keywords mouse, human, cholangitis, and mast cells were included with no time period restriction.

Table 1. Relevance of MCs in human and mouse immune cholangitis.

Host/Model	MC subtype	Location	Disease outcome	Ref.
Human PBC	Tryptase $^{+}$	Portal tracts	N.D.	[12]
Human PBC	Tryptase $^{+}$	Hepatic lobules with no significant increase Significant increase in fibrotic small portal tracts	Putative fibrosis promoters	[11]
Human PBC	Chymase $^{+}$	Fibrotic portal areas	N.D.	[13]
Human PSC	Tryptase $^{+}$	Hepatic lobules with no significant increase Significant increase in fibrotic small portal tracts	Putative fibrosis promoters	[11]
Human PSC	Tryptase^{+}	Bile ducts	Fibroplasia and inflammation	[15]
Human PSC	Tryptase^{+}	Bile ducts	Bile duct obstruction	[16]
Mouse PSC (Mdr2$^{-/-}$ model)	mMCP-1	Bile ducts	Fibrosis and biliary proliferation	[16]
Mouse PSC (Mdr2$^{-/-}$ model)	Chymase^{+}	Bile ducts	Hepatic fibrosis	[17]

Abbreviations: mMCP-1, mouse mast cell protease 1; Mdr2$^{-/-}$, multidrug resistant gene 2 deficient mice; N.D., not determined; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis.

2. Immune Cholangitis Treatment Targets Mast Cells

Up to now, the most effective FDA-approved treatment for biliary diseases such as chronic cholestatic PBC and PSC as well as in gallbladder contractility disorders is ursodeoxycholic acid (UDCA). Interest in knowing how this treatment exerts its anti-cholestatic effects has revealed that, in addition to reducing the cytotoxic effect of bile

acids, UDCA indeed evokes a concomitant anti-inflammatory immune response. UDCA treatment has been shown to diminish macrophage infiltration and MC degranulation as well as oxidative stress in patients with gallbladder cholesterol gallstones [22]. The anti-inflammatory effect of UDCA also includes control of MCs. Carotti et. al. showed that UDCA delivery also reduced the number of degranulated MCs which restored gallbladder contractility [22]. In line with this, Meng et. al. addressed the effect of UDCA on Mdr2$^{-/-}$ mice (PSC model) and PSC patients. The authors found that both Mdr2 deficient mice and patients with PSC exposed to UDCA presented reduced signs of biliary injury such as portal inflammation and necrosis as well as ductular reaction. UDCA supplementation caused lower numbers of MCs and less fibrosis, as compared to individuals without UDCA diet enrichment [16]. Interestingly, the superior effectiveness of UDCA on controlling Th2-type inflammation in liver diseases has prompted its use as a Th2 regulator in lung diseases with promising results as gauged in asthma models where UDCA attenuated lung inflammation including reduced mast cell numbers [23]. Finally, several reports have recently described that UDCA treatment indeed controlled the inflammatory cytokine storm in COVID 19 patients evidencing the role of UDCA as an immunomodulator agent [24][25].

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