The Emerging Role of Mast Cells in Liver Disease

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Abstract

The depth of our knowledge regarding mast cells has widened exponentially in the last 20 years. Once thought to be only important for allergy-mediated events, mast cells are now recognized to be important regulators of a number of pathological diseases. The revelation that mast cells can influence organs, tissues, and cells has increased interest in mast cell research during liver disease. The purpose of this review is to refresh the reader on the development, type, and location of mast cells and to review recent work that demonstrates the role of hepatic mast cells during diseased states. This review will focus primarily on liver diseases and mast cells during autoimmune disease, hepatitis, fatty liver disease, liver cancer and aging in the liver. Overall, these studies demonstrate the potential role that mast cells have in disease progression.

Mast Cell Biology, Development, and Classification

Dr. Paul Ehrlich first described mast cells in 1878 in his doctoral thesis, and in 1908 he was awarded the Nobel Prize for his discoveries. These immune cells were once thought to only mediate allergic reactions and stimulate wound healing; however, it has been revealed that mast cells may also be players in many autoimmune, inflammatory, infectious, and other disorders (8, 12). Mast cell research during disease progression is critical because mast cells have been found to potentiate negative and positive effects on tissues and organ function (5, 8, 28).

In both humans and rodents, mast cells originate from CD34+ hematopoietic stem cells stimulated by various stem cell factors and interleukins; these factors further regulate the development of mast cell subtypes (29, 109). Unlike other immune cells, mast cells do not mature before leaving the bone marrow; instead, the immature progenitors circulate the lymphatic and vascular system and complete their development peripherally (80, 109). Mast
cells are distributed throughout the body in areas close to nerves, blood vessels, and lymphatic vessels (80, 109). Stem cell factor (SCF) plays a crucial role in the development, migration, growth, survival, and location of mast cells (52, 80). Other factors that determine mast cell growth and survival including various interleukins, chemokines, cytokines, transforming growth factor-β (TGF-β), and nerve growth factor (28, 80, 109).

Mast cells can be activated in a receptor-dependent or independent manner, and the high-affinity IgE receptor (FCεRI) is highly expressed on their surface (28, 45, 53, 80). FCεRI-dependent activation results in a variety of specific signaling cascade mechanisms that lead to intracellular calcium influx, activation of certain transcription factors, mast cell degranulation, and cytokine production (105). Apart from FCεRI, mast cells also express other surface markers such as complement receptors, FcγR, β2-integrin, intracellular adhesion molecule-1 (ICAM-1), serotonin receptor, and toll-like receptors which allow them to respond to diverse stimuli (45).

Regardless of whether degranulation is through classic FCεRI, novel receptors, or is receptor-independent, upon activation, mast cells release newly synthesized (lipid mediators and cytokines) and stored (histamine, heparin, proteases) bioactive substances that are contained in cytoplasmic lipid bodies and granules into the surrounding tissue (5, 12, 28, 105). The release of these mediators is dependent on numerous factors, such as which protease the mast cells express and the location at the time of activation (28, 45).

The classification of mast cells is dependent on their phenotypic characteristics and their anatomic locations. Reber, et al. recently summarized the classification of mast cells in both mice and humans along with a description regarding their phenotypic characteristics (80). Similar to mice, human mast cells are subcategorized into tryptase-positive, MC_T, and tryptase- and chymase-positive, MC_TC. MC_TC have an affinity for the small intestinal submucosa and
muscularis mucosa, whereas MC\textsubscript{T} have a tendency to inhabit the mucosa of the stomach, small intestine, and colon. Within rats, mast cells are distinguished by staining for rat mast cell protease (RMCP)-1, which identifies connective tissue-derive mast cells, or RMCP-2, which identifies mucosal-derived mast cells (14, 114). The specific mast cell subtypes will determine the anatomical residency and the positioning of mast cells also secures them as one of the first cells in the line of defense in the immune system.

Liver Biology and Mast Cells

The liver has many roles in the maintenance of systemic function and overall organismal homeostasis. The location and specific anatomy of the liver make it a primary leader in immune responses against infectious pathogens. These responses are performed via interactions between parenchymal cells (hepatocytes), antigen-presenting cells, and effector cells of the innate and adaptive immune systems (13, 26). Mast cells are mainly associated with the connective tissue that is found near hepatic arteries, veins, and bile ducts of the portal tracts in both human and rat livers(52, 53, 57). In normal rodent and human livers, mast cells have been shown to accumulate in small numbers along the portal tracts, indicating that mast cell presence is not solely based on liver injury (52, 53, 57). However, increases in mast cell number have been noted during different hepatic injuries. Specifically, Johnson, et al. found that during human cholangiocarcinoma, the number of chymase-positive mast cells totaled approximately 70 per portal area and tryptase-positive mast cells were numbered at approximately 30 per portal area (52). Following injury, hepatic mast cell number increases and they degranulate to release numerous growth mediators such as histamine, heparin, tryptase, TGF-β1, TNFα, interleukins, cytokines, and basic fibroblast growth factor (bFGF) (13, 31, 45).
Hepatic inflammation is characterized by the migration of inflammatory cells to the damaged area (26). Resident Kupffer cells release chemical messengers that draw inflammatory cells, such as mast cells, to the surrounding area where they mediate immunoregulatory events. The similarities between mast cells and Kupffer cells are further illuminated when the integral role that mast cells play during inflammation is examined. Surrounding the hepatic sinusoids are the parenchymal cells of the liver, hepatocytes, whose major function is the production of bile. Hepatocytes are in direct contact with the blood supply, which makes them vital in the development and progression of liver pathophysiology (28, 32). As previously stated, mast cells circulate the body via the vascular system and are found in close proximity to blood vessels; since hepatocytes are in contact with the blood supply, it seems plausible that mast cells closely regulate hepatic injury through crosstalk with hepatocytes. Hepatocyte-secreted bile is modified by cholangiocytes, which are the cells lining the biliary tract and the target of cholangiopathies, such as primary sclerosing cholangitis (PSC) and primary biliary cholangitis (PBC) (34, 79). Mast cells have been found in close proximity to bile ducts during various cholangiopathies, indicating that mast cells and cholangiocytes may regulate one another via paracrine signaling. Figure 1 depicts mast cell interactions with various liver cell types, including cholangiocytes (biliary ductal cells) and hepatocytes (modified and reprinted with permission from Grizzi, et al. (39)).

**Autoimmune Cholangiopathies and Obstructive Bile Duct Injury**

**Primary Biliary Cholangitis (PBC)**

PBC is an autoimmune biliary disease characterized by the injury of small and medium sized bile ducts, which gradually progresses to liver cirrhosis and eventually death (49, 111). As
these ducts are damaged, bile builds up in the liver over time and damages the surrounding tissue. The pathogenesis of PBC is related to autoimmunity as indicated by cell-mediated responses against self-antigens. Studies have found that PBC predominantly affects women (female to male ratio of 10 to 1), and the incidence of PBC is higher in patients who have a relative afflicted with PBC or any other autoimmune disorder (64).

One of the first studies regarding mast cells and PBC came from Nakamura, et al. This study found that mast cell number was increased around the portal tract in patients suffering from PBC (140 ± 25 cells/mm²; p<0.05) when compared to those with chronic hepatitis (72 ± 10 cells/mm²; p<0.05) (71). This work identified that mast cell activation may regulate the pathogenesis of eosinophilia in PBC progression due to the fact that mast cells secrete mediators which are critical for eosinophil differentiation, chemotaxis, and activation; such as, interleukin (IL)-3, IL-5, granulocyte-macrophage colony-stimulating factor, and platelet-activating factor (71). Furthermore, PBC patients often present with increased circulating bile acid pools and it has been demonstrated that specific bile acids can alter mast cell activation, in vitro (78, 108).

It has been demonstrated that mast cells are in close contact with nerve fibers and the liver is innervated by both the sympathetic and parasympathetic nervous system, thus supporting the concept that mast cells may influence or be influenced by nerve fibers. According to Matsunaga, et al., mast cells may be stimulated by innervation and this can increase the release of fibrogenic factors in patients with PBC (68), suggesting that the role of mast cells in this disease is not passive. The authors found that there was a significant increase in the number of chymase- and tryptase-positive mast cells that were in close proximity to S-100 positive nerve fibers. The density of mast cells in contact with nerve fibers was 12.0 ± 10.1 chymase-positive mast cells/mm² (p<0.0005) and 10.1 ± 10.7 tryptase-positive mast cells/mm² (p<0.000001) in
PBC versus 3.4 ± 0.9 chymase-positive mast cells/mm² and 4.1 ± 0.7 tryptase-positive mast
cells/mm² in a normal liver. Furthermore, their study revealed that there is a significant
relationship between both chymase- and tryptase-positive mast cell density and stromal fibrosis
during PBC. The authors concluded that increased nerve stimulation induces mast cell migration
and activation, thus releasing pro-fibrogenic factors into the liver and increasing fibrosis (68).

Similarly, a recent study indicated that mast cells were located in the portal areas and
sinusoidal walls in patients with PBC, and that these mast cells expressed increased chymase
(85). Specifically, the amount of hepatic chymase in PBC livers was 11.67 ± 9.96 ng/mg.
Furthermore, the authors deduced that chymase-positive mast cells co-localized in areas that
contained large amounts of hepatic fibrosis. From these findings it is apparent that chymase-
positive mast cells increase fibrosis in patients with PBC. Currently, there is limited work
regarding the role of mast cells in both human PBC and in rodent models of the disease.
However, based on these few studies there may be a strong correlation between mast cell and
PBC progression that warrants further examination (67, 70, 77, 84, 107). While these studies
demonstrate the increased presence of mast cells, their causal effect remains to be fully
examined.

Primary Sclerosing Cholangitis (PSC)

Primary sclerosing cholangitis (PSC) is a chronic disease that damages both intra- and
extra-hepatic bile ducts. During PSC, bile ducts become inflamed which leads to scarring and
narrowing of the affected ducts. Eventually, blockages may occur causing bile to become trapped
within the liver resulting in fibrosis, cirrhosis, and potentially liver failure (44, 61).
In 1995 a 75-year-old woman was found with extensive sclerosing cholangitis coupled with a massive infiltration of mast cells. This was the first case to demonstrate that mast cell presence may correlate with PSC, but this was attributed to systemic mastocytosis (6). Approximately 10 years later a separate study found four patients with PSC (class 2 or 3) with increased expression of SCF within bile ducts and enhanced c-Kit positive mast cell presence near portal tracts (124.8 ± 62.1 mast cells per area of portal tract) (50). Both of these studies further opened the window to investigate the role that mast cells play in PSC development and progression.

A study by Tsuneyama, et al. evaluated mast cell infiltration and bFGF expression in patients with PSC. (98). In their examination, the authors found mast cells surrounding bile ducts during the early stages of PSC, but were located in fibrous septa in late stage PSC (98). Sclerosing areas in both regions were marked by intense bFGF expression, a factor that is also secreted by activated mast cells (77).

Similarly, another study demonstrated that numerous mast cells positive for c-Kit were found within periductal and ductal fibrotic areas around intrahepatic large bile ducts, and also surrounding the proliferative peribiliary glands (97). Infiltrated mast cells expressed bFGF and/or TNF-α, components that are known to act as promoters of fibrosis. Interestingly, in PSC samples the aberrant expression of SCF was found on biliary epithelia of dilated and stenotic bile ducts that displayed periductal fibrosis and inflammation, while there was no expression in non-affected bile ducts in normal livers (97). It may be conceivable that the aberrantly expressed SCF found on biliary epithelial cells accumulates and attracts/stimulates mast cells via the c-Kit receptor, and these activated mast cells induce progressive periductal and portal fibrosis found during PSC.
More recently, a study from Jones and Hargrove, et al. examined the role of mast cells in PSC using both human PSC samples and the multidrug resistant knockout mouse, Mdr2\textsuperscript{-/-}, which histopathologically mimics human PSC (53). In this study, the authors found that mast cell number and mast cell markers are increased in both Mdr2\textsuperscript{-/-} mice and PSC patients compared to controls (Figure 2, modified and reprinted with permission from Jones and Hargrove, et al. (53)). Further, when Mdr2\textsuperscript{-/-} mice were treated with cromolyn sodium, a mast cell stabilizer that blocks the release of histamine, mast cell indicators and PSC-associated fibrosis were both significantly reduced (53). In this study the authors also examined the effects that mast cells and mast cell-derived histamine have on bile flow, total bile acid pool and bicarbonate excretion. Interestingly, treatment with cromolyn sodium decreased all of these parameters in Mdr2\textsuperscript{-/-} mice demonstrating for the first time that mast cells and their mediators may influence the function of cholangiocytes and hepatic bile production and flow (53). This is the first study to demonstrate that mast cell-derived histamine may regulate biliary proliferation and hepatic fibrosis in Mdr2\textsuperscript{-/-} mice and human PSC.

The majority of studies examining PSC and mast cells demonstrate an upregulation of mast cell number and activation, but do not examine direct, causal effect; however, there is an indication that inhibition of mast cells has significant causal effect on PSC damage as shown by Jones & Hargrove et al(53).

Human and Rodent Bile Duct Obstruction

Biliary obstruction is characterized by a blockage of the bile ducts. Upon obstruction, bile begins to build up causing abdominal pain, itching (pruritus), nausea, and jaundice. If left untreated, bile duct obstruction can lead to chronic liver disease or an increased buildup of bilirubin, which can also be life-threatening.
In a study utilizing surgical biopsy specimens obtained from fifty patients with secondary cholangitis caused by obstruction of the common bile duct, mast cells positive for tryptase, chymase, vasointestinal polypeptide (VIP), and substance P (SP) were detected (42). Patients who presented with combined exacerbated cholangitis (CEC) and chronic sclerotic cholangitis (CSC) showed significantly higher numbers of mast cell subtypes compared to the controls. In patients with CEC, the number of mast cell subtypes was 11.3 tryptase-positive mast cells/mm², 62.9 tryptase- and chymase-positive mast cells/mm², 26.7 VIP-positive mast cells/mm², and 24.7 SP-positive mast cells/mm²; and in patients with CSC, the number of mast cell subtypes was 2.6 tryptase-positive mast cells/mm², 23.3 chymase- and tryptase-positive mast cells/mm², 3.7 VIP-positive mast cells/mm², and 3.9 SP-positive mast cells/mm². This is compared to controls with 0.6 tryptase-positive mast cells/mm², 5.4 chymase- and tryptase-positive mast cells/mm², 1.0 VIP-positive mast cells/mm², and 0.7 SP-positive mast cells/mm². The authors also detected nerve fibers positive for SP and VIP, and serotonin-positive endocrine cells in close proximity to mast cells (42). This study demonstrates the existence of heterogeneous mast cells, nerve structures, and endocrine cells in patients with bile duct obstruction and the potential for mast cells to influence the pathology of obstructive cholangitis.

The rodent model of bile duct ligation (BDL) has been used over the years to study the effects of bile duct obstruction on biliary and hepatic damage (35, 100). Further, this model has been used to study the role that mast cells play in bile duct obstruction. Zhang, et al., used the BDL model to investigate the role of the anti-fibrotic agent, N-acetylseryl-aspartyl-lysyl-proline (AcSDKP) on liver damage, fibrosis, and mast cell presence (113). The authors measured mast cell presence by Giemsa staining and visualized numerous mast cells around
the portal tract following BDL, whereas mast cell number was reduced in the BDL animals treated with AcSDKP. Further, the authors found that AcSDKP ameliorated BDL-induced damage and fibrosis (113); however, the authors failed to demonstrate if this amelioration was due to the lower mast cell numbers or if it was a consequence of treatment.

In a more direct study, Lu et al. studied the effect of the mast cell tryptase inhibitor, APC 366, and its influence on hepatic fibrosis induced by BDL (65). Mast cells are known to contribute to fibrosis via the release of tryptase (30) and by increasing fibrogenic factors like collagen and laminin (26). The authors found that treatment with APC 366 inhibited hepatic fibrosis, reduced collagen content by 2 fold (p<0.01) when compared to the BDL group, and lowered serum liver enzymes (65). Inhibition of mast cell tryptase successfully reduced BDL-induced hepatic damage, demonstrating the potential of APC 366 to prevent fibrosis in patients suffering from bile duct obstruction or chronic liver injury.

A study focusing on isolation of mature hepatic mast cells from rats also examined the migration of mast cells into the liver following BDL (47). Mast cell migration and counts were started 6 hours following BDL and continued through 14 days. The authors report that total mast cell numbers increased at 3 days following BDL (~500 mast cells) and peaked at 14 days (~700 mast cells) (47). The average number of mast cells per lobe at 14 days was ~200 and mast cells were a varied mix of both chymase- and tryptase-positive (47). A similar study using BDL-induced liver damage found that inhibition of mast cell-derived histamine decreases biliary proliferation and hepatic fibrosis. Kennedy, et al. demonstrated that mast cells infiltrate the liver following BDL and that there is an increase in mast cell marker expression (c-Kit, chymase, and tryptase) in total liver compared to normal rats subjected to sham surgeries (57). When BDL rats were treated with the compound cromolyn sodium (mast cell stabilizer), biliary proliferation,
mast cell number, and hepatic fibrosis all significantly decreased. The authors found that mast cell number also significantly correlated with bile duct mass that was increased in BDL rats, but decreased in rats treated with cromolyn sodium (Figure 3, modified and reprinted with permission from Kennedy, et al. (57)). This study also found that cromolyn sodium acts solely on mast cells, but not cholangiocytes, demonstrating that these are mast cell-specific events (57) and the potential exists for cromolyn sodium treatment in patients suffering from complications related to bile duct obstruction.

A recent publication describes the effects of BDL on mast cell-deficient mice (KitW-sh); in this study, the authors found that, in mast cell-deficient mice, there is reduced biliary hyperplasia, hepatic fibrosis and vascular cell activity (48). Further, the authors found that, when cultured mast cells were reintroduced into mast cell-deficient mice, all of these parameters were significantly upregulated compared to injections with saline (48). These studies are the first to demonstrate that mast cells directly impact biliary response following injury and highlight the importance of mast cells during biliary obstruction and their key role in mitigating hepatic damage and fibrosis.

A serious complication/side effect of cholestatic liver injury induced by obstruction is chronic pruritus (102). There has been a constant mystery surrounding the origin of hepatic pruritus and numerous signaling pathways have been investigated to better understand this phenomenon and offer treatment for patients suffering from this debilitating symptom. While it has been shown that the histamine and serotonin pathways play only a minor role (69), mast cells may contribute significantly to pruritus. A study performed to detect itch response in BDL rats found that there was increased activation of protein activated receptor 2 (PAR2) receptors that can be activated by mast cells (10), which are known to be upregulated in BDL-
induced liver injury (57). Patients with cholestasis and pruritus also presented with increased peripheral neuroinflammation that is documented by an increase in dermal mast cells (101) again suggesting that mast cells play a role in promoting pruritus.

In contrast to this study, O'Keefe, et al. found that cutaneous mast cells had no contribution in cholestatic-induced pruritus (74). The authors analyzed skin biopsies from patients with cholestatic liver disease with pruritus, as well as patients with cholestatic liver disease without pruritus, and found that there was no difference in mast cell number between these groups (74). However, the findings of this study are limited since it analyzed a small sample number (n=5 or 6 patients per group). Due to the debilitating effects that are induced by chronic pruritus, further studies are warranted to understand and develop sophisticated tools to combat this condition.

Speculation on the role that mast cells may play in promoting pruritus is further enhanced when examining the role that bile acids have in chronic itch induced by chronic cholestatic injury. It is well known that bile acids are increased during liver injury, and therapy using ursodeoxycholic acid (UDCA) can somewhat ameliorate pruritus during PBC (33). It has also been demonstrated that UDCA can inhibit the release of histamine from mast cells (unpublished observations, Francis, et al. 2016), so while traditional anti-histamines that block histamine receptors have had only mild success in treating pruritus, UDCA therapy may act on mast cell-derived histamine thus offering some true relief from chronic itching.

**Hepatitis and Alcohol-Induced Liver Injury**

In very basic terms, hepatitis is inflammation of the liver that can lead to a broad spectrum of diseases from cirrhosis to liver cancer (7). Hepatitis viruses (A, B, C, D, and E)
are the leading activators of hepatitis in the world (with B and C leading to chronic hepatitis) but other infectious modes can also induce hepatitis including toxins (e.g. alcohol, certain drugs) and autoimmune diseases (7).

The interplay between mast cells and hepatitis has been highlighted in several studies. Using liver blocks from patients with hepatitis C virus (HCV), Amiot, et al. found an increase in HLA-G positive cells, and their presence correlated with increased fibrosis (Spearman’s test, r=0.6102, p<0.05). Based on co-staining for HLA-G and CD-117, the authors deduced that these HLA-G-positive cells were mast cells (1). In vitro, the authors found that mast cell secretion of HLA-G was increased following stimulation with IL-10 (7.8 ng/ml versus 4.0 ng/ml in unstimulated controls), further confirming their findings (1). A separate study using a larger cohort of samples from patients with chronic HCV found that as fibrosis increased in the liver, so did the number of mast cells in portal areas (60). Specifically, the number of mast cells per portal area was 0.87 ± 0.86 in chronic HCV. In contrast, no correlation was found between the degree of fibrosis and the number of mast cells in the sinusoids. Further, the increase in the number of portal mast cells correlated with an increase in liver steatosis suggesting that mast cells can manifest in HCV and contribute to fibrosis and steatosis associated with this chronic disease (60).

In work by Li, et al. it was noted that mast cell c-Kit and hepatic SCF expression were enhanced in rats that were given subcutaneous injections of carbon tetrachloride (CCl₄) and a diet high in cholesterol and alcohol and low in protein and choline to induce chronic hepatitis (63). Expression of c-Kit and SCF were significantly upregulated in livers from the chronic hepatitis rats compared to the controls. C-kit expression was 2.783 ± 0.577 in the control versus 12.86 ± 3.126 in rats with chronic hepatitis (t = 9.511, P less than 0.05), and
SCF expression was $3.383 \pm 1.583$ in the control versus $15.58 \pm 6.431$ in rats with chronic hepatitis ($t = 9.625$, $P$ less than 0.05). Plasma tryptase and hyaluronic acid levels were also increased in the hepatitis rats along with increased fibrosis associated with numerous degranulating and degranulated mast cells located around the liver blood vessels and in fiber-intervals (10).

In the United States, two-thirds of the adult population drinks alcohol, but only a minority will develop alcoholic-related liver diseases, including alcoholic steatosis, hepatitis, cirrhosis and, hepatocellular carcinoma (83). Alcoholic steatosis is the earliest and most common liver disease that occurs during abuse alcohol (59, 95). This condition is usually asymptomatic and resolves within 6-8 weeks of abstinence. Alcoholic liver disease is distinguished from non-alcoholic steatosis and non-alcoholic fatty liver disease (NAFLD) because it is not associated with metabolic syndrome, unlike NAFLD (83). Serum tryptase levels are often used when diagnosing alcohol-induced injury and baseline serum tryptase concentrations are typically used in the diagnosis and monitoring of mast cell disorders and obesity. Additionally, related metabolic syndromes are found to be associated with increased total tryptase concentrations in adults (37). Beceiro, et al. studied the level of mast cell tryptase in heavy drinkers and found that heavy alcohol drinkers have a low concentration of serum tryptase levels (9). The authors found that heavy drinkers had median serum tryptase levels of $2.23 \, \mu g/l$ versus $3.25 \mu g/l$ in healthy controls. While many patients present with abnormally high AST and ALT levels, the levels of tryptase were significantly decreased and not detectable in some patients. To explain these findings, it has been noted that serum tryptase levels are dependent upon mast cell burden and mast cell activation (87).
There are a number of reports on the effects of alcohol on mast cell degranulation including work that demonstrates that rats fed ethanol have increased numbers of both total and degranulated mast cells (70). *In vitro*, ethanol treatment inhibits mast cell viability by increasing apoptosis, which might offer an explanation of the immunosuppression that is seen with alcohol abuse (73). Furthermore, high-doses of ethanol may induce a toxic release of histamine by mast cells that induces damage to surrounding tissues (82). Further, acetaldehyde, which is the metabolite produced in the liver when ethanol is broken down, induces mast cell degranulation and histamine release from isolated rodent mast cells (82). Acetaldehyde-induced mast cell activation in the gut could be partly responsible for excessive endotoxin passage (25) and subsequent systemic immunomodulatory effects. Taken together, these findings may indicate that low serum tryptase concentrations in heavy drinkers could reflect mast cell exhaustion after chronic alcohol consumption. From a clinical standpoint, these findings may be of importance considering that serum tryptase levels are commonly used as a diagnostic tool. The impact that heavy drinking has on mast cell degranulation should be considered when analyzing tryptase concentrations (37).

In a recent publication, the effects of an antioxidant ginger extract, zingerone, was used to evaluate the damage induced by ethanol treatment in rats (67). The authors found that in rats treated with ethanol there was a significant increase in mast cell presence and the expression of inflammatory markers like NF-κB, COX-2, TNF-α, and IL-6 within the liver. When ethanol fed rats were treated with zingerone all of these parameters decreased, including mast cell presence (67). Due to the antioxidant and anti-inflammatory properties of zingerone it is likely that mast cell presence was reduced via the reduction of inflammatory mediators and this study contributes to the concept that natural therapies may be an
alternative strategy to treating patients suffering from ethanol-induced hepatotoxicity.

Finally, alcohol intake may not directly target mast cells, but act via other cellular signaling pathways and thereby influence mast cell function. Ferrier, et al. demonstrated that alcohol induces dysregulation of the intestinal barrier, causing the activation of mast cells (25). This activation may be due to increased endotoxin blood levels, which have been noted in 20% of chronic drinkers. To evaluate if blocking mast cell degranulation could influence alcohol-induced intestinal permeability, Ferrier, et al. used the mast cell membrane stabilizer, Doxantrazole, in combination with ethanol treatment. The authors found that stabilization of mast cells decreased ethanol-induced intestinal barrier permeability (25). Although this study was not directed specifically at the liver, it does highlight the importance of paracellular reactions and permeability of the intestinal barrier via activation of mast cells.

Since alcohol consumption induces profound changes on immune function (9) it is conceivable that mast cells could be involved in these changes; therefore, further studies should be performed to examine this potential interaction.

Steatosis and Steatohepatitis

Obesity and metabolic syndrome are increasing at alarming rates worldwide, and this escalation directly impacts the development of obesity-related illness including diabetes, heart disease, and liver disease. Fat deposition in the liver can develop into a mild disease like steatosis which can develop into a more progressive and severe pathology, non-alcoholic steatohepatitis (NASH) (55). These diseases can lead to liver fibrosis, cirrhosis, and hepatocellular carcinoma (81). The understanding of the progression from steatosis to
steatohepatitis is still largely unknown. One potential theory that has been investigated is the “two-hit” theory (51). During the “two-hit” theory, the “first hit” is thought to be driven by insulin resistance, which leads to hepatic lipid accumulation. The “second-hit”, made decidedly worse due to the “first hit”, causes hepatocyte injury which increases inflammation and fibrosis (51). Many factors have been suggested in the initiation of the “second hit,” such as pro-inflammatory cytokines and adipokines, mitochondrial dysfunction, oxidative stress, and endoplasmic reticulum stress and some of these factors can be released from mast cells (76, 91).

Mast cells have been implicated in the development of steatosis and are associated with different liver etiologies, including HCV. Steatosis is frequently associated with chronic HCV, although it is unclear if steatosis is associated with the virus or with host factors. A study by Franceschini, et al. evaluated mast cell markers and fibrotic reaction in patients diagnosed with HCV with or without steatosis and found that mast cell density was increased in patients with HCV coupled with steatosis; however, the degree of fibrosis between the two groups was not significantly altered (27). This study sheds light on the role that mast cells may play during fatty liver disease (in this case, induced by HCV) (19, 96). Due to the inflammatory environment found around adipose tissue it is not surprising that mast cells contribute to the pathogenesis of steatosis.

In a study by Smith et al. authors evaluated the role of mast cells on the progression of hepatic steatosis using the apolipoprotein E-deficient (ApoE−/−) and ApoE−/−/mast cell-deficient (KitW-sh/W-sh) mouse models fed a high-fat diet (90). ApoE−/−/KitW-sh/W-sh mice developed significantly less hepatic steatosis than ApoE−/− mice after 3 months of high fat diet feeding. Mast cell numbers were detected by Giemsa staining and were significantly reduced in the ApoE−/−/KitW-sh/W-sh mice compared to the ApoE−/− mice fed high fat diet. The analysis of Th1/Th2/Th17
cytokine profile in the sera revealed a significant reduction of interleukin IL-6 and IL-10 in ApoE-/-/KitW-sh/W-sh mice compared with ApoE-/- mice (90). The IL-6 level was 11.0 ± 1.4 pg/ml and the IL-10 level was 74.9 ± 14.2 in ApoE-/-/KitW-sh/W-sh mice versus 17.3 ± 1.5 pg/ml and 111.9 ± 14.4 pg/ml respectively in ApoE-/- mice. These results demonstrate the direct involvement of mast cells in the progression of hepatic steatosis following high fat diet feeding.

Mast cells may also be involved in the pathogenesis of nonalcoholic steatohepatitis (NASH), which is the progressive form of non-alcoholic fatty liver disease (NAFLD). The mast cell protease, chymase, contributes to the formation of angiotensin II and matrix metalloproteinase (MMP)-9, factors that contribute to liver fibrosis. Hamsters were fed a methionine- and choline-deficient diet (MCD) for 8 weeks, to induce fatty liver disease, coupled with treatment of a chymase inhibitor, TY-51469 (92). The authors report that treatment with TY-51469 ameliorated serum liver enzymes that were increased following MCD diet and reduced liver steatosis. Fibrosis that is associated with fatty liver was also decreased in animals treated with TY-51469. Toluidine blue-positive mast cells were increased following MCD diet and these numbers were reduced in animals treated with TY-51469 (92). This study pinpoints mast cells in the progression of NASH as well as demonstrating the importance of inhibiting mast cell proteases. In a separate but similar study, hamsters were treated for 12 and 24 weeks with the same chymase inhibitor and MCD diet. Similar parameters were measured and hepatic steatosis and fibrosis were more prominent in the placebo-treated hamsters fed the MCD-diet for 24 weeks versus 12 weeks. TY-51469 ameliorated fibrosis and decreased the gene expression of collagen I, collagen III, and α-SMA. These findings demonstrate that blocking mast cell mediators may alter the progressive and damaging course of fatty liver disease.
Hepatic Fibrosis: Congenital and Noncongenital

In the generation of liver fibrosis there are three common phases that follow injury: inflammation, synthesis of collagennous and non-collagenous extracellular matrix (ECM), and tissue remodeling due to dynamic fibrogenesis (26). Mast cells play an important role in the response to liver fibrosis, which develops as a result of chronic inflammation (60). Fibrosis is the accumulation of interstitial or “scar” extracellular matrix after liver injury (66, 103). Mast cells are thought to play an intricate role in the development and progression of liver fibrosis given their participation in sinusoidal capillarization (26). Sinusoidal capillarization occurs when hepatic sinusoids transform into continuous capillaries, which contributes to liver fibrosis by depositing collagen in extravascular spaces.

In terms of cellular involvement, once hepatic injury has occurred, hepatocellular necrosis leads to the recruitment of various inflammatory cells and platelets, activation of Kupffer cells, and the release of cytokines and growth factors. During the establishment of liver fibrosis, levels of collagen type IV, entacin, and laminin may increase and form continuous basement membrane-like structures, a process that is accompanied by a decrease in the number of fenestrated capillaries (26, 107). Specifically, Franchecchini, et al. demonstrated that mast cells may be associated with hepatic fibrosis through the activation of fibroblast growth and collagen synthesis and may inhibit ECM degradation through the means of enzymes called tissue inhibitors of metalloproteinases (TIMPs). Their study shows that mast cells may be the primary contributor in the transition from sinusoidal to capillary-type endothelial cells, which leads to the development of sinusoidal basement membrane (26).

Another protease that plays a key role in the development and progression of fibrosis is chymase. Chymase is found in mast cell granules and is thought to provoke the development
of fibrosis by aiding in the differentiation of connective tissue, and through the production of angiotensin II (AII) from angiotensin I (AI). AII is able to promote fibrotic reaction by inducing hepatic stellate cell proliferation and ECM production in autoimmune hepatitis and PBC (85). Furthermore, blocking AII signaling through the use of the AII receptor antagonist, Losartan, is able to decrease and even reverse fibrosis associated with chronic HCV (84). Since chymase, which is produced in large quantities by mast cells, is able to produce AII that promotes hepatic fibrosis, it may be of interest to block mast cell degranulation to prevent the release of chymase, the production of AII, and subsequent hepatic stellate cell activation and ECM production.

Aside from non-congenital hepatic fibrosis there are also forms of hepatic fibrosis that are congenital in nature. Congenital hepatic fibrosis (CHF) is a developmental disorder of the biliary tree and portal veins that is present at birth (43, 89). CHF is histopathologically identified by ductal plate malformation, abnormal branching of the biliary tree, and extensive hepatic fibrosis generally found surrounding the portal tracts (43). Considering CHF is characterized by progressive portal fibrosis, and mast cells are found in abundance near portal tracts during hepatic injury (4) and are able to increase hepatic fibrosis (24, 53, 57), it seems intuitive that there may be a link between the degree of CHF-associated portal fibrosis and the number of mast cells present. In a manuscript by Ozaki, et al. the authors noted that patients suffering from CHF tended to have an abundant number of mast cells (21.2 ± 9.7 absolute number of mast cells; p<0.001) in the portal fibrous areas when compared to those with other chronic liver diseases (chronic viral hepatitis (CVH) F1/F2 4.1± 2.7; CVH F3/F4 8.5± 4.1; alcoholic fibrosis/cirrhosis 7.7± 7.2; extrahepatic bile duct obstruction 3.7± 2.6; non-specific reactive change 2.7± 2.3; p<0.001) (75). Based on these findings the authors concluded that
the numerous portal mast cells might be a strong regulator of CHF-related portal fibrosis (75).

This is the only publication to date that evaluates the possible role that mast cells play in CHF progression and only looks at CHF-related fibrosis and not biliary-related characteristics. Certainly, more studies are necessary to identify significant findings on the role that mast cells play in CHF.

Liver Cancer

Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide and is generally associated with a poor prognosis and high level of recurrence following resection (86). One major side effect associated with tumor development is tumor-associated inflammation, which negatively impacts immune response and therapeutic efficacy (38). Cell types that can influence tumor-associated inflammation include macrophages, dendritic cells, lymphocytes, neutrophils, eosinophils, and mast cells (28). Specifically, mast cells have been found to be integral in the progression of HCC by promoting angiogenesis and tumor growth (40).

Previous studies have found the high expression of IL-17 and IL-17-producing cells to be a poor prognostic factor for HCC (46, 99, 110). A manuscript by Zhang, et al. indicated that the density of IL-17-producing cells within the HCC tumor environment correlated with high mortality and reduced survival (112). The median overall survival in patients with higher density of intratumoral IL-17 producing cells was 34 months versus 49 months in those with lower density. Furthermore, mast cells have also been indicated as an IL-17-producing cell (22, 56). In another study Tu, et al. evaluated the role of mast cell-produced IL-17 in the progression of HCC (99). The authors found that mast cells compromised the majority of IL-7-producing cells within
HCC, and increased numbers of mast cells were associated with increased angiogenesis. Furthermore, this study demonstrated that the number of IL-17-positive mast cells were primarily located in peritumoral areas and positively correlated with a poor prognosis (99). This corroborates the study completed by Ju, et al. that further characterized the negative impact that peritumoral mast cells have on HCC prognosis via inflammatory processes (54).

While mast cells do express and secrete IL-17 (16) they are also able to release many other preformed mediators, such as histamine (20, 94). One study found that the human HCC cell line, HA22T/VGH, expressed both the H1 and H2 histamine receptors (HR), and treatment with mast cell-derived histamine showed a significant increase in cell growth (62). However, this study also showed that mast cell-derived histamine reduced cell proliferation and viability in the human HCC cell line, HuH-6, even though these cells expressed both the H1 and H2 HR (62).

The difference in cell response to mast cell-derived histamine may be due to the fact that each of these cell lines has different characteristics of differentiation, biological behavior, and genetic defects. Recently, a study found that patients suffering from HCC who exhibited increased density in tryptase-positive mast cells positively correlated with microvascular density, showing that mast cells may contribute to increased angiogenesis during HCC (2). This finding is further supported by a manuscript by Goffredo, et al. demonstrating that patients with HCC have increased serum tryptase levels (36). Considering that mast cells can secrete a wide variety of mediators following degranulation, these studies only scratch the surface of the impact that mast cell-derived factors may have on HCC growth and angiogenesis. Further studies are necessary to identify the characteristics of HCC tumoral mast cells, the direct role they have on HCC pathology, and the efficacy of manipulating these mast cells as a therapeutic for patients with HCC.
Aside from their individual contribution to HCC progression, mast cells may also interact with other cell types in the tumor microenvironment to influence HCC stage and prognosis. In the same study by Ju, et al. that highlighted the pro-inflammatory role that mast cells have in HCC tumors, the authors found that peritumoral Foxp3(+) T-regulatory cells (Tregs) positively correlated with mast cell density (54). This study further clarified that mast cell presence in combination with Tregs had a better prognosis than tumors that contained mast cells alone. From this study one can indicate that there is cross-talk between mast cells and Tregs that influences the tumor environment in a positive way; considering Tregs have been deemed as anti-inflammatory (58) it is possible that Tregs are working to counteract the pro-inflammatory nature of mast cells during HCC.

Hepatic tumors are not always locally derived and may form from various metastatic cancers from other tissue types. In a study by Gulubova, et al. the authors evaluated the numbers of tryptase-positive (MC_T) and tryptase- and chymase-positive (MC_TC) mast cells in livers containing metastases from either colorectal, gastric, or pancreatic cancer (41). This study found that the number of MC_TC and MC_T were significantly higher in extra- and peritumoral regions of higher grade metastases when compared to lower grade. The mean number of MC_T and MC_TC was 7.9 ± 5 and 26.4 ± 15.1 respectively in moderate/high grade metastases and 3.9 ± 1.4 and 11.0 ± 2.6 in low-grade metastases. Furthermore, the number of MC_TC was greater than the number of MC_T in both extra- and peritumoral regions. Mast cells positive for SP and VIP were not seen (41). This study highlights the potential role that heterogeneous mast cells may play in the progression of hepatic metastases.

Cholangiocarcinoma
Cholangiocarcinoma (CCA) is a rare form of cancer that comprises 2% of all primary neoplasms; however, it is the second most common hepatic malignancy after hepatocellular carcinoma (18). This form of cancer originates from the perihilar, intrahepatic, or extrahepatic bile duct epithelium. The risk factors for developing CCA include chronic inflammation from liver fluke infestation, hepatitis B and C infections, PSC, fatty liver disease, cholelithiasis, and inflammatory bowel disease (17, 106). At the time of diagnosis, only 10-15% of patients with CCA are amenable to potentially curable surgery as a majority present at an advanced stage. Even with resection, CCA has high rates of recurrence; the five year overall survival rate is 30% (17).

Considering mast cells play such a prevalent role in other cholangiocyte-specific diseases (i.e. PBC and PSC) and in HCC, it seems relevant to review whether mast cells play a role in CCA. Johnson, et al. evaluated mast cell numbers, SCF/c-Kit signaling, angiogenesis, and epithelial-mesenchymal transition (EMT) in CCA tumors excised from athymic mice and human patients (52). The authors found that both mouse and human tumors had increased mast cell numbers when compared to normal livers (numbers reported above), and this was accompanied with increased angiogenesis and EMT markers. In vitro, the link between mast cell-secreted factors and CCA angiogenesis and EMT was shown by treating human CCA cells with supernatants from mast cells treated with control, cromolyn sodium (to block mast cell degranulation) or an SCF inhibitor, ISCK03. From this study the authors noted that CCA cells treated with mast cell supernatants had increased angiogenesis and EMT markers, and these factors were decreased in CCA cells treated with supernatants from mast cells that were pretreated with cromolyn sodium or the SCF inhibitor (52). This strongly implicates mast cells in the progression of CCA via increased angiogenesis and metastatic potential. To evaluate whether
these findings could be verified in vivo, athymic mice receiving CCA xenografts were treated with control or cromolyn sodium before evaluating the same parameters. Tumors from mice treated with cromolyn sodium had decreased size, mast cell number (Figure 4, modified and reprinted with permission from Johnson and Huynh, et al.(52)), angiogenesis, and EMT when compared to control treated (52). To determine the pathway regulating mast cell recruitment to CCA tumors, the authors performed a migration assay in vitro with human CCA cells, treated with either control or SCF inhibitor, and murine hepatic mast cells and found that inhibition of SCF was able to block mast cell migration. Overall, this study described how mast cells are able to migrate into the CCA tumor microenvironment via SCF/c-Kit signaling and increase tumor progression, angiogenesis, and EMT (52). Furthermore, the authors showed that blocking mast cell migration and degranulation by inhibition of SCF/c-Kit signaling or treatment with cromolyn sodium decreases these parameters.

Another study examined the number of mast cells in human intrahepatic cholangiocarcinoma (ICC) and identified whether they were MC_T or MC_TC subsets (93). In this study, it was noted that in normal livers mast cells were located near portal tracts; however, in ICC mast cells were present within the tumor. The authors also noted that there were significantly more mast cells in ICC tumors when compared to normal livers, and approximately 20% of the mast cells were MC_T while the other 80% were MC_TC (93). These findings further support the study by Johnson, et al. that show that mast cells are increased in CCA and may regulate tumor progression (52). Furthermore, this study mimics the findings of Gulubova, et al. that showed that HCC tumors were largely infiltrated by MC_TC versus MC_T (41). These findings help to highlight the significant role that mast cells can play during tumorigenesis, and assist in identification of the specific mast cell subsets, which will help future researchers in developing a
potential therapeutic target. These studies are highly valuable as they shed light on a largely understudied field of research; however, more studies are warranted to further prove the pathological features of mast cell infiltration in CCA and to further unveil possible mast cell manipulative therapies.

Senescence and Aging in the Liver

Aging, initially considered an inexorable product of time, is actually related to post-maturation processes that lead to diminished homeostasis and decreased immune responses (39). The aging process is associated with many physiological changes, and is generally linked to cellular senescence. Cellular senescence has been deemed the link that connects the visible consequence of aging to its molecular cause, cellular damage (11, 21). Recent studies have shown that senescence can lead to remodeling of the immune system including changes in cell population, number, migration, and function (21). These senescence-related changes in the immune system may help to explain why aging is associated with weakened immune function. Consequently, the incidence of liver disease increases with age and the ability to overcome a hepatic insult decreases with each decade (39). Though the liver goes through a minor aging process compared to other organs it still undergoes aging; mast cells have been found to have an important relationship in this aging process. However, few studies have been performed to look at the role that aging and senescence have on hepatic mast cell populations.

One study evaluated whether age-related senescence was able to affect mast cell development in bone marrow. In this study researchers performed myeloablation in normal and senescence accelerated mice (SAMP1, exhibit accelerated senescence at 30 weeks of age) that were either young (8-12 weeks) or old (30-36 weeks) (15). The authors then evaluated levels of
SCF, a positive regulator of mast cell development, and TGF-β, a negative regulator of mast cell development, as well as the number of mast cell progenitors. Overall, the authors found that the SCF/TGF-β ratio was increased in both young and old mice following myeloablation, but this ratio quickly and drastically decreased in the old myeloablated mice (that exhibit accelerated senescence) but remained high in the young myeloablated mice (that exhibit low senescence). As a consequence of altered SCF/TGF-β ratios, old mice that underwent myeloablation had a lower recovery of the number of mast cell progenitors, unlike young mice that had a high recovery (15). This study highlights the impact that age-related senescence can have on regulators of mast cell development. This lowered ability to generate mast cells may have an impact on age-related altered immune responses to disease.

To study the impact that aging has on mast cell response during injury, one very early study evaluated mast cell numbers in young (2 month) and old (19 month) rats following carbon tetrachloride (CCl₄)-induced liver damage (104). This study found that following CCl₄ administration, the younger rats had considerably increased numbers of hepatic mast cells at 24 hours after CCl₄ intoxication (6.5 ± 1.0 cells/mm²; p = 0.000076); however, the number of infiltrating mast cells was greatly diminished in the older rats treated with CCl₄. No significant changes were noted in hepatic mast cell number between the young and old rats treated with control (104). This suggests that the older rats have a reduced immune response, which may be due to age-related immune remodeling. When comparing this study to the one highlighted above (15), it is probable that these older rats have decreased mast cell response to injury due to senescence-related impairments in mast cell generation. Further studies are necessary to pinpoint the direct impact that senescence and aging have on mast cell numbers, generation, and response.
Three principle features of acute liver allograft rejection are portal inflammation, bile duct damage, and venular endothelitis. Chronic rejection is characterized by specific damage and loss of small bile ducts and/or foam cell obliterative arteritis (88). As stated earlier, mast cells in normal hepatic tissue are found in low numbers near portal tracts, and following injury, infiltrating hepatic mast cells are found in large numbers near portal tracts and damaged bile ducts. This information may be useful in understanding the pathogenesis of chronic rejection.

A study by Nakano, et al. evaluated the role that mast cells may have on immune response to liver transplantation in rats (72). The authors noted that tolerated livers had increased levels of SCF and c-Kit (integral for mast cell migration), mast cell presence, and histamine release, which may be indicative of mast cell degranulation when compared to the rejected livers (23). Considering mast cells can interact with other cell types to influence hepatic response to injury, Nakano, et al. immunohistochemically evaluated whether mast cells co-localized with other cells following liver transplantation. Based on staining, the authors found that mast cell co-localization with Tregs, γδ T cells, and hepatic progenitors was largely noted in tolerated livers when compared to rejected livers (72). In vitro, the authors used co-culture techniques to evaluate whether crosstalk between these cell types was occurring, and noticed that mast cells were able to increase the γδ T cell population by mitogen stimulation and increase hepatocyte proliferation. These changes in γδ T cell and hepatocyte numbers were accompanied by mast cell degranulation, showing that mast cell-secreted factors, such as histamine, can induce proliferation in various cell types (72). Overall, this study highlights the beneficial role that mast cells may have during immunotolerance of transplanted livers.
In contrast, a study by Arikan, et al. looked at portal mast cell presence in pediatric livers that were normal, acute rejected (AR), and chronic rejected (CR) and found that the density of portal tract mast cells was increased in AR with a mean number of mast cells of 5.7 ± 4.4 compared to control with a mean number of mast cells of 0.4 ± 0.54, and further increased in CR with a mean number of mast cells of 34.2 ± 26.2 compared to AR. Based on their findings, the authors deduced that portal areas that are mast cell-rich may indicate the potential for CR during allograft liver transplantation (3). In terms of c-Kit expression, El-Refaie, et al. demonstrated that there was significantly elevated levels of MC_T and c-Kit-positive mast cells in the livers of both acute and chronic rejection patients (23). The results of their study mimicked the results of the study by Arikan, et al. and showed that mast cells may play a detrimental role in both acute and chronic allograft rejection through modulation of inflammatory processes. However, neither of these studies evaluated whether mast cells were co-localizing with other cell types, which could potentially influence the hepatic immune response. The role of mast cells during liver tolerance/rejection is controversial and mostly unknown. Further research is necessary to uncover the role that mast cells may have on cell proliferation and immune response following transplantation.

**Conclusion**

In summary, studies on mast cells and liver disease progression have clearly pinpointed these immune cells as important regulators of pathogenic processes. Mast cells contribute to hepatic fibrosis, cholangiopathies, liver cancer, senescence-impaired aging, alcoholic liver injury, fatty liver disease, and allograft rejection. Since mast cells are recruited in high numbers during injury but reside in normal tissues in low numbers, targeting the migration or activation of
mast cells may offer alternative strategies for patients suffering from liver diseases. To date, no specific studies have demonstrated the role of mast cell subtypes in liver diseases; however, the implication that mast cells are present during liver disease is evident. Agents that block mast cell histamine release, such as cromolyn sodium or histamine receptor blockers, are compounds that are frequently employed in the treatment of other illnesses; these tried and true compounds could be easily implemented into patient therapy for those suffering from liver disease with minimal negative consequences. It is evident from this litany of potential treatment options that further studies are warranted to explore the full magnitude that mast cells have on liver disease.

References:


30. Frungieri MB, Albrecht M, Raemsch R, and Mayerhofer A. The action of the mast cell product tryptase on cyclooxygenase-2 (COX2) and subsequent fibroblast proliferation involves activation of the extracellular signal-regulated kinase isoforms 1 and 2 (erk1/2). *Cell Signal* 17: 525-533, 2005.


91. **Tariq Z, Green CJ, and Hodson L.** Are oxidative stress mechanisms the common denominator in the progression from hepatic steatosis towards non-alcoholic steatohepatitis (NASH)? *Liver Int* 34: e180-190, 2014.


Legends:

**Figure 1** Mast cell interactions with liver cells. The parenchyma of the liver is a complex structure with different cell types having various functions in normal liver function and disease response. These include: macrophages, or Kupffer cells, hepatic stellate cells, sinusoidal endothelial cells, vascular endothelial cells, fibroblasts and pit cells. It has been ascertained that all these cells (in addition to biliary cells) play definitive roles in liver pathophysiology and determine complex interactions with hepatic MCs. Modified and reprinted with permission(39).

**Figure 2** Mast cells and mast cell markers are upregulated in human PSC. Mast cell presence was assessed in human liver biopsy samples from control (no disease) and PSC patients (late and advanced PSC) by real-time PCR, toluidine blue staining and immunohistochemistry for mast cell markers (chymase and tryptase). (A) The gene expression of c-Kit, FCεR1, chymase and tryptase increased in samples from advanced and late stage PSC when compared to normal, non-diseased tissues. (B) By immunostaining (toluidine blue) and immunohistochemistry (chymase and tryptase), there is an infiltration of mast cells found surrounding damaged bile ducts in PSC patients compared to normal tissue (red arrows depict mast cells). Data are expressed as mean ± SEM of at least 6 experiments for real-time PCR.
*p<0.05 versus control. Images are 20x magnification. Modified and reprinted with permission(53). PSC = primary sclerosing cholangitis

**Figure 3** Mast cell numbers increase following bile duct ligation. Evaluation of hepatic mast cell infiltration and correlation with intrahepatic bile duct mass (IBDM). By toluidine blue staining the number of mast cells significantly increased in bile duct ligation (BDL) compared to normal rat (NR), but were reduced in BDL + cromolyn [A and B]. Data are mean ± SE of 6 experiments. *p<0.05 versus normal rat (NR); #p<0.05 versus BDL. Modified and reprinted with permission (57).

**Figure 4** Evaluation of mast cells in human cholangiocarcinoma. In tumors from mice treated with saline, histamine, the histidine decarboxylase (HDC) inhibitor, \( \text{\textalpha}-\)methyl or cromolyn sodium, mast cell presence was measured (red arrows = mast cells). Histamine treatment increased mast cell infiltration shown by toluidine blue staining compared to saline-treated mice (A). Mast cell infiltration is not seen in tumor sections from mice treated with \( \text{\textalpha}-\)methyl or cromolyn sodium (A). By immunohistochemistry in tumors from saline treated mice, there was an increase of c-kit-positive mast cells, whereas the number of c-kit positive mast cells was lower in cromolyn sodium treated tumors (B). By immunohistochemistry and semi-quantitative analysis, we found that the number of chymase-positive mast cells is decreased in tumors from mice treated with cromolyn sodium compared to saline (C) and similar results were found for tryptase-positive mast cells (D). Data are expressed as mean ± SEM of 9 experiments. *p<0.05 versus saline (NaCl) treatment. Representative images are shown for immunostaining. Original images x40. Modified and reprinted with permission (52).
MAST CELL

BILIARY DUCTAL CELL
- Increased density around inflamed ductal cells (PSC, PBC, CCA)

KUPFFER CELL
- Recruitment of inflammatory mediators

HEPATOCYTE

SINUSOIDAL ENDOTHELIAL CELL
- Contribute to the sinusoidal capillarization
- Recruitment of inflammatory mediators

ITO CELL
- Recruitment of inflammatory mediators

Increased density as hepatocellular carcinoma advances
**Figure 2**

- **c-Kit** = Mast/stem cell growth factor receptor
- **FCεR1** = high-affinity IgE receptor
- **PSC** = Primary Sclerosing Cholangitis

### Graph:

- Relative gene expression (x-fold)

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### Images:

#### A

- **Control**
  - Toluidine blue
  - Chymase
  - Tryptase

- **PSC**
  - Toluidine blue
  - Chymase
  - Tryptase
Figure 3

BDL = Bile Duct Ligation

A

Sham-operated
BDL
BDL + cromolyn

Mast cells
Bile ducts

B

Avg. # of mast cells

Sham-operated
BDL
BDL + cromolyn

*

* #
Figure 4

A

Saline

Histamine

B

c-Kit

Saline

Cromolyn sodium

C

Chymase

Saline

Cromolyn sodium

D

Tryptase

Saline

Cromolyn sodium

* Indicates significant difference.