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BDL-induced biliary hyperplasia, hepatic injury and fibrosis are reduced in mast cell deficient *Kit^{W-sh}* mice

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Abstract

Activated mast cells (MCs) release histamine (HA) and MCs infiltrate the liver following bile duct ligation (BDL) increasing intrahepatic bile duct mass (IBDM) and fibrosis. We evaluated the effects of BDL in MC deficient mice. Methods: WT and *Kit^{W-sh}* mice were subjected to sham or BDL for up to 7 days and *Kit^{W-sh}* mice were injected with cultured mast cells or 1X PBS before collecting serum, liver blocks and cholangiocytes. Liver damage was assessed by H&E and ALT levels. IBDM was detected by CK-19 expression and proliferation by Ki-67 immunohistochemistry. Fibrosis was detected by immunohistochemistry, hydroxyproline content and by qPCR for fibrotic markers. Hepatic stellate cell (HSC) activation and TGF- β 1 expression/secretion were evaluated. HDC and histamine receptor (HR) expression were detected by qPCR and HA secretion by EIA. To evaluate vascular cells, Von Willebrand (vWF) and VEGF-C expression were measured. *In vitro*, cultured HSCs were stimulated with cholangiocyte supernatants and α -SMA measured. Results: BDL-induced liver damage was reduced in the BDL *Kit^{W-sh}* mice, whereas injection of MCs did not mimic BDL-induced damage. In BDL *Kit^{W-sh}* mice, IBDM, proliferation, HSC activation/fibrosis and TGF- β 1 expression/secretion were decreased. The HDC/HA/HR axis was ablated in sham and BDL *Kit^{W-sh}* mice. vWF and VEGF-C expression decreased in BDL *Kit^{W-sh}* mice. In *Kit^{W-sh}* mice injected with MCs, IBDM, proliferation, fibrosis and vascular cell activation increased. Stimulation with cholangiocyte supernatants from BDL WT or *Kit^{W-sh}* mice injected with MCs increased HSC activation, which decreased with supernatants from BDL *Kit^{W-sh}* mice. Conclusion: MCs promote hyperplasia, fibrosis and vascular cell activation. Knockout of MCs decreases BDL-induced damage. Modulation of MCs may be important in developing therapeutics for cholangiopathies.

Keywords

mast cells; fibrosis; histamine; TGF- β 1; vascular endothelial cells

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Introduction

Cholangiocytes, the epithelial cells lining the biliary epithelium, are dynamic players during liver repair (1). Diseases that target cholangiocytes have limited therapeutic options and include primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC). Patients with damage to bile ducts often have an increased risk for extensive liver damage and potential failure (2, 3). The first biliary response to the mechanical rodent model of injury, bile duct ligation (BDL), is to regain bile duct mass following injury; however, extended, uncontrolled biliary hyperplasia leads to hepatic fibrosis (2, 3). There are multiple factors that are activated during cholangiocyte proliferation following BDL, such as secretin receptor expression (4), upregulation of vascular endothelial growth factor (VEGF) (5, 6) and the escalation of histamine release (7–9). VEGF, primarily derived from the peribiliary vascular plexus in addition to other vascular cells, is associated with cholangiocyte proliferation and cholangiocytes secrete VEGF (5, 6) as well as histamine.

Traditionally, hepatic fibrosis is regulated by hepatic stellate cells (HSCs), which, once activated, transform into myofibroblasts that drive collagen content higher resulting in fibrosis (10, 11). In addition to HSCs, cholangiocytes also play a role in paracrine signaling during hepatic fibrosis and may also contribute to the activation of HSCs (12, 13). During fibrosis several growth factors are released from multiple cell types including transforming growth factor-beta 1 (TGF- β 1) (14). These factors contribute to the hyperplastic-induced fibrosis, which can eventually lead to liver failure.

In addition to HSCs and cholangiocytes, hepatic mast cells are also involved in liver fibrosis (15). While traditionally noted for their role in allergic responses, mast cells are being more recognized in a number of pathologies (16) including liver disease and our recent studies have demonstrated that mast cells play critical and detrimental roles in BDL-induced liver damage, PSC and cholangiocarcinoma (17–19). Mast cell infiltration occurs following mechanical, chemical or toxic liver injury and mast cells migrate into cholangiocarcinoma tumors where they promote tumor growth (20, 21). Once activated, mast cells release numerous preformed mediators like histamine and other growth factors including VEGF and TGF- β 1 into the surrounding tissue microenvironment (22, 23). In the BDL rat model, we have found that blocking mast cell-derived histamine release with cromolyn sodium (mast cell stabilizer) treatment decreases hyperplasia; this same compound also decreases human cholangiocarcinoma tumor growth and angiogenesis (17, 18).

Numerous models of mast cell deficiency have been developed and adapted to study allergic response, itching, cardiac function, renal response and liver disease. Different white spotting (*W*) locus (*c-kit*) mutant rodents have been developed and investigated as models for the analysis of mast cell function, including *Kit*^{W/W^{-v}} and *Kit*^{W^{-f}/W^{-f}} mice and *Kit*^{W^{-s}/W^{-s}} rats (24–27). *Kit*^{W/W^{-sh}} mice are mast cell-deficient and adult mice contain no observable mast cell populations in tissues including the gastrointestinal tract, respiratory system, heart, brain, skeletal muscle, spleen, and other anatomical sites (25). No studies have been performed to understand if the lack of mast cells contributes to or regulates BDL-induced

liver injury and hepatic fibrosis; thus, our aim was to examine the effects of mast cell depletion following BDL using mast cell deficient mice.

Materials and Methods

Reagents and other materials

Chemical grade reagents were purchased from Sigma Aldrich Co (St. Louis, MO, USA) unless stated otherwise. All mouse primers and real-time PCR materials were obtained from Qiagen (Frederick, MD, USA). Antibodies for immunohistochemistry and immunofluorescence were purchased from Abcam (Cambridge, MA). Histamine enzymatic immunoassay (EIA) kits were purchased from Cayman Chemical (Ann Arbor, MI, USA) (28, 29).

In vivo models

We utilized commercially available homozygous C57BL/6J-*Kit^{W-sh}*/J (*Kit^{W-sh}*) mice obtained from Jackson Laboratory (Sacramento, CA) along with age matched wild-type (WT). Pair breeding was utilized to generate offspring and only male mice were used in our study. WT and *Kit^{W-sh}* mice (at least 12 weeks of age) were subjected to sham or BDL (as described, without cholecystectomy (4, 30)) for up to 7 days and, in separate experiments, *Kit^{W-sh}* mice (at least 8 weeks of age) were injected with sterile, tagged cultured mast cells (MC/9 (ATCC® CRL-8306™)) or 1× PBS (control) prior to sacrifice 3 days later. Specifically, cultured mast cells were tagged with PKH26 Red Fluorescent Cell Linker prior to injection and mice received a single injection of sterile, cultured mast cells ($5 \times 10^6/0.1$ ml sterile 1× PBS) or control via tail vein injection (31). Following euthanasia the location (liver, lung and spleen) of injected mast cells was determined using confocal microscopy for the visualization of PKH26 Red Fluorescent Cell Linker. Livers were also co-stained with CK-19 to mark bile ducts and location of injected mast cells.

All mice were housed in the Baylor Scott and White Health Animal Facility and given free access to drinking water and standard chow. Animals were kept in a temperature-controlled environment with a 12:12 hr light/dark cycle, and all protocols were approved by and strictly adhered to regulations set forth by the local IACUC committee. From all animals (n = at least 6 mice from each group) we collected serum, liver blocks and isolated cholangiocytes (4, 7). Cholangiocyte supernatants were collected and incubated at 37°C for up to 6 hours and stored for *in vitro* studies (18).

Assessment of liver damage in models of mast cell deficiency

We assessed liver damage in our WT and *Kit^{W-sh}* mice subjected to sham or BDL surgery as well as the *Kit^{W-sh}* mice injected with mast cells by H&E staining as previously described (4). Slides were evaluated by a blinded board certified pathologist and scored for lobular damage, focal necrosis and inflammation. Alanine aminotransferase (ALT) activity was measured in serum from all groups of mice using a commercially available kit (Abcam, Cambridge, MA).

Evaluation of biliary mass and proliferation in mast cell deficient models

When BDL rats or *Mdr2*^{-/-} mice are treated with the mast cell stabilizer, cromolyn sodium (blocks mast cell histamine release), biliary mass and proliferation are significantly reduced (18, 19). Thus we suspect that mast cells are an important player in the regulation of biliary hyperplasia following BDL. We evaluated the effects of mast cell deficiency on intrahepatic bile duct mass (IBDM) and proliferation in both WT and *Kit*^{W-sh} mice with sham or BDL surgery. Immunohistochemistry, real-time PCR and western blotting (29, 30) were performed for the biliary marker CK-19 (18, 29) to measure IBDM and cholangiocyte proliferation was determined by Ki-67 staining (19).

Effects mast cell deficiency on hepatic fibrosis and HSC activation

We measured fibrosis progression and collagen deposition from both WT and *Kit*^{W-sh} mice with sham or BDL surgery. Fast Green/Sirius Red, Masson's Trichrome and hydroxyproline content were assessed in liver sections and snap liver, respectively (29). In total liver from all groups, we measured the following fibrotic markers by real-time PCR: α -smooth muscle actin (α -SMA), collagen type-1a and fibronectin-1 (29). Since TGF- β 1 is a known regulator of fibrosis (11) we evaluated the expression of TGF- β 1 in total liver by real-time PCR and the secretion of TGF- β 1 by EIA in serum from these mice (19). We also evaluated the effects of mast cell deficiency on HSC activation by immunofluorescence for synaptophysin-9 (SYP-9) (32). Livers were co-stained with CK-19 to also visualize bile ducts.

Evaluation of histamine signaling and secretion in mast cell deficient models

Our previous studies have demonstrated that histamine synthesis and histamine receptors (HRs) are upregulated in models of biliary damage including BDL (18, 29). Cholangiocytes secrete small amounts of histamine (28, 29); however, the main source of histamine release is activated mast cells. To further examine the role that histamine signaling has during cholestatic liver injury, we measured histidine decarboxylase (HDC) and histamine receptor (HR) expression in total liver from WT and *Kit*^{W-sh} mice with sham or BDL surgery by real-time PCR. Histamine secretion was evaluated by EIA in serum from these mice as previously described (28, 29).

Assessment of the effects of mast cell deficiency on vascular tissue and VEGF signaling

VEGF has been shown to play a role in biliary response to BDL and cholangiocytes express VEGF receptors and secrete VEGF (5, 6). However, the peribiliary vascular plexus and vascular cells are the largest source of VEGF in the liver; therefore, we evaluated these factors in our models. Evaluation of H&E staining for peribiliary glands was performed in liver sections from WT and *Kit*^{W-sh} mice with sham or BDL surgery. Immunofluorescence was performed in liver sections from WT and *Kit*^{W-sh} mice with sham or BDL surgery for von Willebrand Factor (vWF) expression (33). Liver sections were co-stained with CK-19 to distinguish bile ducts. In total liver from WT and *Kit*^{W-sh} mice with sham or BDL surgery, we measured the expression of VEGF-C and vWF by real-time PCR.

Measurement of biliary proliferation, hepatic fibrosis and HSC activation following introduction of mast cells into deficient mice

Since our previous work has demonstrated that mast cells infiltrate the liver following BDL and contribute to biliary hyperplasia and hepatic fibrosis (18), our final *in vivo* experiments were performed to determine the implications of the reintroduction of mast cells into deficient models. *Kit^{W-sh}* mice were injected with 1X PBS or sterile, cultured mast cells via tail vein (31) and bile duct mass, proliferation, hepatic fibrosis and the vascular cell response were all evaluated as described above. Localization of injected mast cells was determined by confocal microscopy in lung, liver and spleen tissues.

Evaluation of the effects of mast cell deficiency on HSC activation, in vitro

Our work demonstrates that mast cells interact with and alter cholangiocyte response, which leads to fibrosis progression (18, 19). To determine if there is a synergistic effect between mast cells, cholangiocytes, and hepatic stellate cells, we stimulated cultured human HSCs (hHSCs) (19) with isolated cholangiocyte supernatants collected from WT and *Kit^{W-sh}* mice subjected to sham or BDL surgery or cholangiocyte supernatants from *Kit^{W-sh}* mice injected with mast cells. hHSCs were stimulated with 100 μ l of cholangiocyte supernatant for 24 hours and activation was measured by α -SMA expression by real-time PCR.

Statistical analysis

Results

Hepatic injury is ameliorated in BDL *Kit^{W-sh}* mice

BDL WT mice displayed increased focal necrosis (infarct) in zone 2 (upwards of 4–5% of lobule) and portal inflammatory changes that are consistent with obstruction (indicated by black arrowheads). An increase in mostly large bile ducts is also observed following BDL (indicated by black arrows). In BDL *Kit^{W-sh}* mice the degree of focal necrosis (infarct) was absent compared to BDL WT co(Figure 1A). ALT activity was significantly upregulated in BDL WT mice, but this was completely ablated in BDL *Kit^{W-sh}* mice that demonstrate ALT levels similar to sham WT (Figure 1B). In the livers of *Kit^{W-sh}* mice injected with mast cells there were no visible areas of liver infarcts/focal necrosis or portal inflammation/damage compared to *Kit^{W-sh}* mice injected with 1X PBS (Figure 1C). Further, ALT levels were not significantly altered after mast cell injection compared to controls suggesting that introduction of mast cells does not mimic BDL-induced damage (Figure 1D).

Biliary mass and proliferation are significantly reduced in BDL *Kit^{W-sh}* mice

In BDL *Kit^{W-sh}* mice there is an overall reduction of biliary mass compared to BDL WT mice (Figure 2A). These results were further confirmed by real-time PCR (Supplemental Figure 1A), and western blotting (Figure 2B) that displayed a similar trend to the immunohistochemistry results.

As expected, biliary proliferation (indicated by red arrows) was significantly decreased in BDL *Kit^{W-sh}* mice compared to BDL WT (Figure 2C). Cholangiocyte proliferation was similar between sham WT and *Kit^{W-sh}* mice (Figure 2C).

Hepatic fibrosis and HSC activation are dependent upon mast cells

Collagen deposition is dramatically reduced in BDL *Kit^{W-sh}* mice compared to BDL WT mice as shown by Masson's Trichrome and Fast Green/Sirius Red staining (Figure 3A and 3B, respectively). Semi-quantification of Fast Green/Sirius Red staining shows a significant decrease in collagen deposition in BDL *Kit^{W-sh}* mice compared to BDL WT (Figure 3C). Hydroxyproline content was significantly reduced in BDL *Kit^{W-sh}* mice compared to BDL WT (Figure 3D). The expression of α -SMA, fibronectin-1 and collagen type-1a increased in BDL WT mice compared to sham WT; whereas, these markers were decreased in BDL *Kit^{W-sh}* mice compared to BDL WT mice (Supplemental Figure 2).

In BDL *Kit^{W-sh}* mice, there was a significant reduction in both TGF- β 1 expression (Figure 4A) and secretion (Figure 4B) compared to BDL WT. SYP-9 protein expression was upregulated in BDL WT; however, in BDL *Kit^{W-sh}* mice there was a decrease in HSC activation compared to BDL WT (Figure 4C).

Histamine signaling and secretion are decreased in BDL *Kit^{W-sh}* mice

In BDL *Kit^{W-sh}* mice there was a decrease in HRs and HDC compared to BDL WT (Supplemental Figure 3A and 3B). Similarly, histamine serum release was increased in BDL WT mice compared to sham WT; whereas, histamine release decreased in BDL *Kit^{W-sh}* mice compared to BDL WT (Supplemental Figure 3C).

Loss of mast cells reduces peribiliary gland presence and vWF and VEGF expression

Following BDL in WT mice, there is an increase in the presence of peribiliary glands (marked by black arrows) found in close proximity to large bile ducts (indicated by the white arrow) when compared to sham WT mice (Figure 5A). However, in both sham and BDL *Kit^{W-sh}* mice, there is no visible evidence of peribiliary glands (Figure 5A) compared to BDL WT. Further, VEGF-C expression is significantly reduced in BDL *Kit^{W-sh}* mice compared to BDL WT (Figure 5B). Immunofluorescence for vWF demonstrates an increase in BDL WT mice compared to WT and in the BDL *Kit^{W-sh}* mice there is a marked decrease in the expression of vWF compared to BDL WT (Figure 5C). Similarly, vWF gene expression is increased in BDL WT mice compared to WT and in the BDL *Kit^{W-sh}* mice there is a marked decrease in the expression of vWF compared to BDL WT (Figure 5D). These data indicate that mast cells not only influence the biliary tree, but also the vascular bed during BDL-induced liver damage.

Introduction of mast cells into deficient mice increases biliary hyperplasia, hepatic fibrosis and vascular cell presence

Unpublished observations from our lab have demonstrated that injection of cultured mast cells into either WT mice or histidine decarboxylase knockout mice, which have very low numbers of mast cells, increases biliary proliferation and hepatic fibrosis. In our present study using mast cell deficient mice, we found that injected mast cells were in close proximity to bile ducts (Supplemental Figure 4) and IBDM and biliary proliferation are significantly increased in *Kit^{W-sh}* mice injected with mast cells compared to mice injected with 1x PBS as shown by CK-19 and Ki67 immunohistochemistry (Figure 6A and 6B). IBDM immunohistochemistry was further confirmed by real-time PCR and western blotting

that displayed a similar trend to the immunohistochemistry results (Supplemental Figure 1B and 1C). Similar to BDL WT, there was an increase in vWF expression in *Kit^{W-sh}* mice injected with mast cells compared to 1X PBS injection as shown by immunofluorescence (Figure 6C). Further, VEGF-C gene expression increased in *Kit^{W-sh}* mice injected with mast cells compared to 1X PBS injection (Figure 6D). Taken together these data strongly suggest that the reintroduction of mast cells alters the proliferative capacity of cholangiocytes and the vascular bed in mast cell deficient mice.

In *Kit^{W-sh}* mice injected with mast cells, collagen deposition is increased as shown by Masson's Trichrome staining (Figure 7A) and collagen type-1a and fibronectin-1 expression also increased along with hydroxyproline content (Figure 7B and 7C). TGF- β 1 expression and secretion are significantly increased in *Kit^{W-sh}* mice injected with mast cells compared to 1X PBS injection (Figure 7D). These data all pinpoint mast cells as key components of biliary hyperplasia and hepatic fibrosis progression. It should be noted that this data, coupled with the H&E and ALT levels (Figure 1C and 1D), which showed no visible alterations in liver morphology and function, suggest that mast cell injection increases proliferation and fibrosis, but does not mimic the damage induced by BDL.

Effects of mast cell deficiency on biliary cells and HSCs, in vitro

In vitro, we found that mast cells and cholangiocytes work synergistically to contribute to the activation of HSCs. Specifically, when cultured hHSCs were treated with cholangiocyte supernatants collected from BDL WT mice, there was increased expression of α -SMA when compared to hHSCs treated with sham WT cholangiocyte supernatants (Figure 8) suggesting that factors released from cholangiocytes following BDL alter HSC response. In contrast, when hHSCs were treated with cholangiocyte supernatants collected from sham or BDL *Kit^{W-sh}* mice, α -SMA expression decreased (Figure 8). Lastly, when hHSCs were stimulated with cholangiocyte supernatants from *Kit^{W-sh}* mice injected with mast cells, α -SMA increased when compared to all of the other groups, including BDL WT treatments (Figure 8). These findings indicate that mast cell presence alters cholangiocyte response thus impacting HSC activation, which is a critical step in hepatic fibrosis progression.

Discussion

Our investigation of the paracrine role that mast cells play during liver injury and repair has revealed that there is a critical pathological function induced by mast cell presence that contributes to biliary hyperplasia, promotes hepatic fibrosis progression and alters the vascular bed. While BDL is an injury that directly damages bile duct and cholangiocytes, the peripheral damage that occurs to the biliary tree extends further and involves numerous components including the infiltration of mast cells to injured bile ducts where they surround reactive cholangiocytes (18). Once infiltration of mast cells occurs, there is a dynamic series of events that induces biliary hyperplasia and an increase of histamine release (from mast cells and cholangiocytes) and induction of VEGF and TGF- β 1.

Biliary hyperplasia following BDL is the liver's first attempt to repair the injured bile duct and our data suggests that mast cells are also critical to this event as there is a dramatic decrease in biliary mass and proliferation in BDL *Kit^{W-sh}* mice compared to BDL WT mice.

In addition, the reintroduction of mast cells into mast cell deficient mice resulted in large increases in both IBDM and cholangiocyte proliferation suggesting that mast cells are essential during these events. There have been no other studies demonstrating the effects of mast cell deficiency on BDL-induced biliary hyperplasia and proliferation. In support of our work, it has been demonstrated that the introduction of mast cells into mast cell deficient mice promotes muscle cell hyperplasia and hypertrophy during eosinophilic esophagitis (34) and mast cells contribute to hepatic steatosis, which was ameliorated in mast cell deficient mice (35).

The role that mast cells play in tissue fibrosis is a controversial one. Studies have demonstrated that (i) mast cells promote tissue fibrosis; (ii) loss of mast cells induces fibrosis leading to a worsening condition and (iii) mast cells play no real role in tissue fibrosis. A study using *Ws/Ws* rats that are mast cell deficient found vigorous interstitial fibrosis in the kidney compared to the control suggesting that, in this model, mast cells are beneficial to the fibrotic process (36). In support of our study, it has been demonstrated that mast cells can promote and drive cardiac fibrosis and eventual failure and that mast cell-deficient mice are protected from cardiac fibrosis and heart failure (37, 38). In contrast to our current work, an early study by Sugihara, et al., demonstrated that mast cells have no consequence on the development of liver fibrosis in rats and mice (39); whereas, our present studies indicate that mast cell introduction (shown by mast cell injection into deficient mice) induces robust hepatic fibrosis and there is decreased fibrotic progression in BDL *Kit^{W^{-sh}}* compared to the BDL WT. One glaring discrepancy between these studies include the fact that in Sugihara's study, rats and mice that underwent bile duct resection were not euthanized and tissue collected until 21 days after surgery, therefore the reaction of the biliary tree and infiltration of mast cells during early events of fibrosis were not evaluated (39). In contrast, in our study, we evaluated BDL-induced damage no later than 7 days when the biliary tree has been shown to be fully reactive to damage and mast cell infiltration is increased (18). Another obvious difference is the rodent model of mast cell deficiency used, the other authors chose *Ws/Ws* rats and male *WBB6F₁^{-/+}* and *-W/W^v* mice, where we have employed *Kit^{W^{-sh}}* that have a different phenotype from Sugihara's models.

One prominent driver of collagen production in fibroblasts and a contributor to fibrosis is TGF- β 1, which is also found within mast cells and can be released upon activation. The release of TGF- β 1 perpetuates fibroblasts to transform into myofibroblasts inducing fibrosis during autoimmune diseases. We found that ablation of mast cells significantly reduces BDL-induced TGF- β 1 expression and secretion. In addition, TGF- β 1 levels were increased in *Kit^{W^{-sh}}* injected with cultured mast cells, supporting the concept that mast cells are an important source of TGF- β 1 during fibrosis progression. To support this, it has been shown that histamine or angiotensin released from mast cells induces secretion of TGF- β 1 from fibroblasts that, in turn, increases collagen synthesis and pulmonary fibrosis induction. When mast cells were absent, these events were inhibited (40). Inflammation is an important component to the repair process in myocardial infarction and implanted mast cells (from WT mice) into mast cell deficient mice increases angiogenesis and cardiac function via augmented fibroblast activity and transformation that was TGF- β 1 dependent (41), again pinpointing mast cell-regulated TGF- β 1 as a key player in fibrosis. The full understanding of the contribution of mast cells on liver fibrosis is not completely defined, however we present

strong data herein to show that mast cells play a critical and detrimental role in hepatic fibrosis through TGF- β 1.

Mast cells release numerous factors, but the main component of mast cells is histamine, which has been recognized now to be more than just a mediator of allergic events (14, 16). We have demonstrated in numerous studies that histamine is a trophic, growth-promoting factor that induces biliary hyperplasia, hepatic fibrosis and cholangiocarcinoma progression (8, 9, 28). Cholangiocytes secrete minimal amounts of histamine and are able, through autocrine signaling, to regulate cholangiocarcinoma growth (28); however, mast cells are the major producers of histamine in the body. Stabilization of mast cells using cromolyn sodium has demonstrated that inhibition of mast cell-derived histamine decreases biliary hyperplasia, fibrosis and cholangiocarcinoma growth, pinpointing mast cell-derived histamine as a culprit in various cholangiopathies (17–19). In our current study, we measured histamine synthesis, histamine receptor expression and histamine secretion in our rodent models. As expected, all of these factors were upregulated in BDL WT, but reduced in BDL *Kit*^{W-sh} mice further suggesting that histamine itself is a driver of biliary hyperplasia and hepatic fibrosis. While our mice are mast cell deficient, they are still receiving histidine in their standard chow and since cholangiocytes secrete small amounts of histamine (28, 29) this may account for the minimal amount of histamine found in the serum and is consistent with other studies using mast cell deficient models or HDC deficient mice (29).

Besides being a growth-promoting factor, histamine also regulates other factors including VEGF during BDL-induced injury (29, 30). The peribiliary plexus is a dynamic structure that supports the nutritional needs of the biliary tree and provides the largest source of VEGF in the liver, although cholangiocytes secrete VEGF and express VEGF-R2 and VEGF-R3. Mast cells also express VEGF receptors and produce VEGF when activated (42, 43). In melanoma tumor growth, activation of mast cells by IgE induces VEGF release that promotes tumor growth demonstrating that mast cells can regulate cell growth via VEGF (43). In our study we found that BDL *Kit*^{W-sh} mice have lower levels of VEGF-C and further, the vascular bed is altered in BDL *Kit*^{W-sh} mice as shown by the diminished presence of peribiliary glands and decreased expression of vWF. In support of the concept that mast cells can influence peribiliary gland development, a study examining mammary glands in *Kit*^{W-sh} mice found that the development of glands, but not the function was altered in mast cell deficient mice compared to wild-type counterparts (44). These mice were still able to lactate and function properly even though the mammary glands were significantly altered during development. The changes demonstrated in our current study regarding IBDM and biliary proliferation coupled with the presence or absence of peribiliary glands suggests that the peribiliary glands are not the sole source of proliferating cholangiocytes. This is supported by numerous studies demonstrating that other factors including secretin, serotonin and VEGF are secreted from cholangiocytes, which can subsequently influence biliary proliferation in both normal and damaged states (4, 6, 45). Further, *Kit*^{W-sh} mice injected with mast cells had increased VEGF-C expression as well as an increase in vascular cell activation as shown by vWF expression. Ghosh, et al. found no difference in angiogenic signaling in mast cell deficient mice, WBB6F1-W/W(V) compared to controls following implantation of cotton threading to induce granulation tissue formation; however, HDC knockout mice displayed marked alterations in angiogenic

signaling (46). Our study also demonstrates no significant difference in vascular remodeling between sham WT and sham *Kit^{W-sh}* mice implying that the effect of mast cells is enhanced during pathological conditions. These findings demonstrate that there is a significant loss of biliary reaction in mast cell deficient mice that alters both proliferation and the response of the vascular bed.

Our group and others have demonstrated that cholangiocytes are important in the progression of fibrosis (13, 18, 19) and our current study also reveal that mast cells can alter cholangiocyte response as shown by IBDM and proliferation. Whether mast cells, cholangiocytes and HSCs work together to drive fibrosis is not fully known. Our data demonstrate hHSCs express increased amounts of α -SMA when stimulated with cholangiocyte supernatants collected from BDL WT mice, which is subsequently decreased in hHSCs treated with *Kit^{W-sh}* supernatants from mice subjected to sham or BDL. When hHSCs are stimulated with cholangiocyte supernatant from *Kit^{W-sh}* mice injected with mast cells there is a dramatic increase in hHSC α -SMA expression that exceeds the BDL WT treatment strongly suggesting that mast cells are key regulators of cholangiocyte response and HSC activation that together promotes hepatic fibrosis.

Clinically speaking, the depletion of mast cells throughout the whole body is not a likely healthy scenario as they are necessary during wound healing, but mechanisms to block mast cells and the mediators that they release may rescue the liver from biliary damage, hepatic fibrosis and vascular bed alteration. Cromolyn sodium is one such drug that targets mast cell-derived histamine and treatment with cromolyn sodium inhibits biliary hyperplasia and hepatic fibrosis (18, 19), but there are other factors to consider as we have described in our study including TGF- β 1 and VEGF. Potential candidates for therapy might include drugs to target and inhibit mast cell migration (stem cell factor inhibitors) in conjunction with compounds that block VEGF or TGF- β 1 release from mast cells. Further, microRNAs are being investigated as potential mechanisms to regulate mast cell activation. For example, miR-223 targets insulin-like growth factor 1 receptor (IGF-1R) in mast cells, promoting degranulation and decreasing the PI3K/Akt pathway (47).

In conclusion, we have shown that mast cells are prominent regulators of biliary hyperplasia, hepatic fibrosis and vascular bed dysfunction demonstrated by both the BDL model and through direct injection of mast cells into deficient mice (Supplementary Figure 5). Our studies reveal a synergistic interplay between mast cells, cholangiocytes and hepatic stellate cells demonstrating the importance of cellular paracrine signaling during disease progression. Since mast cells that infiltrate the liver following damage are not resident liver cells, inhibition or knockdown of mast cells or mast cell components are likely to be excellent therapeutics for patients suffering from cholangiopathies. Our study warrants the future investigation of modulation of mast cells during cholangiopathies and hepatic fibrosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BDL	bile duct ligation
VEGF	vascular endothelial growth factor
HSC	hepatic stellate cells
TGF-β1	transforming growth factor-beta 1
EIA	enzymatic immunoassay
WT	wild-type
H&E	hematoxylin & eosin
PCR	polymerase chain reaction
PBS	phosphate buffered saline
CK-19	cytokeratin 19
IBDM	intrahepatic bile duct mass
α-SMA	alpha-smooth muscle actin
SYP-9	synaptophysin 9
HDC	histidine decarboxylase
HR	histamine receptor
vWF	von Willebrand Factor

References

1. Alpini, G., Prall, RT., LaRusso, NF. The pathobiology of biliary epithelia. In: Arias, IM. Boyer, JL. Chisari, FV. Fausto, N. Jakoby, W. Schachter, D., Shafritz, DA., editors. *The Liver; Biology & Pathobiology*, 4E. Philadelphia, PA: Lippincott Williams & Wilkins; 2001. p. 421-435.
2. Eaton JE, Talwalkar JA, Lazaridis KN, Gores GJ, Lindor KD. Pathogenesis of primary sclerosing cholangitis and advances in diagnosis and management. *Gastroenterology*. 2013; 145:521–536. [PubMed: 23827861]
3. Timmer MR, Beuers U, Fockens P, Ponsioen CY, Rauws EA, Wang KK, Krishnadath KK. Genetic and epigenetic abnormalities in primary sclerosing cholangitis-associated cholangiocarcinoma. *Inflamm Bowel Dis*. 2013; 19:1789–1797. [PubMed: 23615529]

4. Glaser S, Meng F, Han Y, Onori P, Chow BK, Francis H, Venter J, et al. Secretin stimulates biliary cell proliferation by regulating expression of microRNA 125b and microRNA let7a in mice. *Gastroenterology*. 2014; 146:1795–1808. e1712. [PubMed: 24583060]
5. Gaudio E, Barbaro B, Alvaro D, Glaser S, Francis H, Franchitto A, Onori P, et al. Administration of r-VEGF-A prevents hepatic artery ligation-induced bile duct damage in bile duct ligated rats. *Am J Physiol Gastrointest Liver Physiol*. 2006; 291:G307–317.
6. Gaudio E, Barbaro B, Alvaro D, Glaser S, Francis H, Ueno Y, Meininger CJ, et al. Vascular endothelial growth factor stimulates rat cholangiocyte proliferation via an autocrine mechanism. *Gastroenterology*. 2006; 130:1270–1282. [PubMed: 16618418]
7. Francis H, Franchitto A, Ueno Y, Glaser S, DeMorrow S, Venter J, Gaudio E, et al. H3 histamine receptor agonist inhibits biliary growth of BDL rats by downregulation of the cAMP-dependent PKA/ERK1/2/ELK-1 pathway. *Lab Invest*. 2007; 87:473–487. [PubMed: 17334413]
8. Francis H, Glaser S, Demorrow S, Gaudio E, Ueno Y, Venter J, Dostal D, et al. Small mouse cholangiocytes proliferate in response to H1 histamine receptor stimulation by activation of the IP3/CaMK I/CREB pathway. *Am J Physiol Cell Physiol*. 2008; 295:C499–513. [PubMed: 18508907]
9. Francis HL, DeMorrow S, Franchitto A, Venter JK, Mancinelli RA, White MA, Meng F, et al. Histamine stimulates the proliferation of small and large cholangiocytes by activation of both IP₃/Ca²⁺ and cAMP-dependent signaling mechanisms. *Lab Invest*. 2012; 92:282–294. [PubMed: 22064319]
10. Lee YA, Wallace MC, Friedman SL. Pathobiology of liver fibrosis: a translational success story. *Gut*. 2015; 64:830–841. [PubMed: 25681399]
11. Penz-Osterreicher M, Osterreicher CH, Trauner M. Fibrosis in autoimmune and cholestatic liver disease. *Best Pract Res Clin Gastroenterol*. 2011; 25:245–258. [PubMed: 21497742]
12. Omenetti A, Syn WK, Jung Y, Francis H, Porrello A, Witek RP, Choi SS, et al. Repair-related activation of hedgehog signaling promotes cholangiocyte chemokine production. *Hepatology*. 2009; 50:518–527. [PubMed: 19575365]
13. Omenetti A, Yang L, Gainetdinov RR, Guy CD, Choi SS, Chen W, Caron MG, et al. Paracrine modulation of cholangiocyte serotonin synthesis orchestrates biliary remodeling in adults. *Am J Physiol Gastrointest Liver Physiol*. 2011; 300:G303–315. [PubMed: 21071507]
14. Balta C, Herman H, Boldura OM, Gasca I, Rosu M, Ardelean A, Hermenean A. Chrysin attenuates liver fibrosis and hepatic stellate cell activation through TGF-beta/Smad signaling pathway. *Chem Biol Interact*. 2015; 240:94–101. [PubMed: 26297989]
15. Ishii M, Iwai M, Harada Y, Morikawa T, Okanoue T, Kishikawa T, Tsuchihashi Y, et al. A role of mast cells for hepatic fibrosis in primary sclerosing cholangitis. *Hepatol Res*. 2005; 31:127–131. [PubMed: 15777700]
16. Bachelet I, Levi-Schaffer F, Mekori YA. Mast cells: not only in allergy. *Immunol Allergy Clin North Am*. 2006; 26:407–425. [PubMed: 16931286]
17. Johnson C, Huynh V, Hargrove L, Kennedy L, Graf-Eaton A, Owens J, Trzeciakowski JP, et al. Inhibition of Mast Cell-Derived Histamine Decreases Human Cholangiocarcinoma Growth and Differentiation via c-Kit/Stem Cell Factor-Dependent Signaling. *Am J Pathol*. 2016; 186:123–133. [PubMed: 26597881]
18. Kennedy LL, Hargrove LA, Graf AB, Francis TC, Hodges KM, Nguyen QP, Ueno Y, et al. Inhibition of mast cell-derived histamine secretion by cromolyn sodium treatment decreases biliary hyperplasia in cholestatic rodents. *Lab Invest*. 2014; 94:1406–1418. [PubMed: 25365204]
19. Jones H, Hargrove L, Kennedy L, Meng F, Graf-Eaton A, Owens J, Alpini G, et al. Inhibition of mast cell-secreted histamine decreases biliary proliferation and fibrosis in primary sclerosing cholangitis Mdr2^{-/-} mice. *Hepatology*. 2016
20. Akiyoshi H, Terada T. Mast cell, myofibroblast and nerve terminal complexes in carbon tetrachloride-induced cirrhotic rat livers. *J Hepatol*. 1998; 29:112–119. [PubMed: 9696499]
21. Halova I, Draberova L, Draber P. Mast cell chemotaxis - chemoattractants and signaling pathways. *Front Immunol*. 2012; 3:119. [PubMed: 22654878]
22. Beaven MA. Our perception of the mast cell from Paul Ehrlich to now. *Eur J Immunol*. 2009; 39:11–25. [PubMed: 19130582]

23. Boyce JA. The biology of the mast cell. *Allergy Asthma Proc.* 2004; 25:27–30. [PubMed: 15055558]
24. Geissler EN, McFarland EC, Russell ES. Analysis of pleiotropism at the dominant white-spotting (W) locus of the house mouse: a description of ten new W alleles. *Genetics.* 1981; 97:337–361. [PubMed: 7274658]
25. Grimbaldston MA, Chen CC, Piliponsky AM, Tsai M, Tam SY, Galli SJ. Mast cell-deficient W-sash c-kit mutant Kit W-sh/W-sh mice as a model for investigating mast cell biology in vivo. *Am J Pathol.* 2005; 167:835–848. [PubMed: 16127161]
26. Kitamura Y, Go S, Hatanaka K. Decrease of mast cells in W/W^v mice and their increase by bone marrow transplantation. *Blood.* 1978; 52:447–452. [PubMed: 352443]
27. Niwa Y, Kasugai T, Ohno K, Morimoto M, Yamazaki M, Dohmae K, Nishimune Y, et al. Anemia and mast cell depletion in mutant rats that are homozygous at “white spotting (Ws)” locus. *Blood.* 1991; 78:1936–1941. [PubMed: 1912576]
28. Francis H, DeMorrow S, Venter J, Onori P, White M, Gaudio E, Francis T, et al. Inhibition of histidine decarboxylase ablates the autocrine tumorigenic effects of histamine in human cholangiocarcinoma. *Gut.* 2012; 61:753–764. [PubMed: 21873469]
29. Graf A, Meng F, Hargrove L, Kennedy L, Han Y, Francis T, Hodges K, et al. Knockout of histidine decarboxylase decreases bile duct ligation-induced biliary hyperplasia via downregulation of the histidine decarboxylase/VEGF axis through PKA-ERK1/2 signaling. *Am J Physiol Gastrointest Liver Physiol.* 2014; 307:G813–823. [PubMed: 25169977]
30. Meng F, Onori P, Hargrove L, Han Y, Kennedy L, Graf A, Hodges K, et al. Regulation of the histamine/VEGF axis by miR-125b during cholestatic liver injury in mice. *Am J Pathol.* 2014; 184:662–673. [PubMed: 24384130]
31. Melillo RM, Guarino V, Avilla E, Galdiero MR, Liotti F, Prevete N, Rossi FW, et al. Mast cells have a protumorigenic role in human thyroid cancer. *Oncogene.* 2010; 29:6203–6215. [PubMed: 20729915]
32. Cassiman D, van Pelt J, De Vos R, Van Lommel F, Desmet V, Yap SH, Roskams T. Synaptophysin: A novel marker for human and rat hepatic stellate cells. *Am J Pathol.* 1999; 155:1831–1839. [PubMed: 10595912]
33. Xu B, Broome U, Uzunel M, Nava S, Ge X, Kumagai-Braesch M, Hultenby K, et al. Capillarization of hepatic sinusoid by liver endothelial cell-reactive autoantibodies in patients with cirrhosis and chronic hepatitis. *Am J Pathol.* 2003; 163:1275–1289. [PubMed: 14507637]
34. Niranjana R, Mavi P, Rayapudi M, Dynda S, Mishra A. Pathogenic role of mast cells in experimental eosinophilic esophagitis. *Am J Physiol Gastrointest Liver Physiol.* 2013; 304:G1087–1094. [PubMed: 23599040]
35. Smith DD, Tan X, Raveendran VV, Tawfik O, Stechschulte DJ, Dileepan KN. Mast cell deficiency attenuates progression of atherosclerosis and hepatic steatosis in apolipoprotein E-null mice. *Am J Physiol Heart Circ Physiol.* 2012; 302:H2612–2621. [PubMed: 22505639]
36. Miyazawa S, Hotta O, Doi N, Natori Y, Nishikawa K, Natori Y. Role of mast cells in the development of renal fibrosis: use of mast cell-deficient rats. *Kidney Int.* 2004; 65:2228–2237. [PubMed: 15149336]
37. Hara M, Ono K, Hwang MW, Iwasaki A, Okada M, Nakatani K, Sasayama S, et al. Evidence for a role of mast cells in the evolution to congestive heart failure. *J Exp Med.* 2002; 195:375–381. [PubMed: 11828013]
38. Kong P, Christia P, Frangogiannis NG. The pathogenesis of cardiac fibrosis. *Cell Mol Life Sci.* 2014; 71:549–574. [PubMed: 23649149]
39. Sugihara A, Tsujimura T, Fujita Y, Nakata Y, Terada N. Evaluation of role of mast cells in the development of liver fibrosis using mast cell-deficient rats and mice. *J Hepatol.* 1999; 30:859–867. [PubMed: 10365813]
40. Veerappan A, O'Connor NJ, Brazin J, Reid AC, Jung A, McGee D, Summers B, et al. Mast cells: a pivotal role in pulmonary fibrosis. *DNA Cell Biol.* 2013; 32:206–218. [PubMed: 23570576]
41. Shao Z, Nazari M, Guo L, Li SH, Sun J, Liu SM, Yuan HP, et al. The cardiac repair benefits of inflammation do not persist: evidence from mast cell implantation. *J Cell Mol Med.* 2015; 19:2751–2762. [PubMed: 26471858]

42. Garcia-Roman J, Ibarra-Sanchez A, Lamas M, Gonzalez Espinosa C. VEGF secretion during hypoxia depends on free radicals-induced Fyn kinase activity in mast cells. *Biochem Biophys Res Commun.* 2010; 401:262–267. [PubMed: 20850416]
43. Jimenez-Andrade GY, Ibarra-Sanchez A, Gonzalez D, Lamas M, Gonzalez-Espinosa C. Immunoglobulin E induces VEGF production in mast cells and potentiates their pro-tumorigenic actions through a Fyn kinase-dependent mechanism. *J Hematol Oncol.* 2013; 6:56. [PubMed: 23915951]
44. Lilla JN, Werb Z. Mast cells contribute to the stromal microenvironment in mammary gland branching morphogenesis. *Dev Biol.* 2010; 337:124–133. [PubMed: 19850030]
45. Marzioni M, Glaser S, Francis H, Marucci L, Benedetti A, Alvaro D, Taffetani S, et al. Autocrine/paracrine regulation of the growth of the biliary tree by the neuroendocrine hormone serotonin. *Gastroenterology.* 2005; 128:121–137. [PubMed: 15633129]
46. Ghosh AK, Hirasawa N, Ohtsu H, Watanabe T, Ohuchi K. Defective angiogenesis in the inflammatory granulation tissue in histidine decarboxylase-deficient mice but not in mast cell-deficient mice. *J Exp Med.* 2002; 195:973–982. [PubMed: 11956288]
47. Wang Q, Zhao DY, Xu H, Zhou H, Yang QY, Liu F, Zhou GP. Down-regulation of microRNA-223 promotes degranulation via the PI3K/Akt pathway by targeting IGF-1R in mast cells. *PLoS One.* 2015; 10:e0123575. [PubMed: 25875646]

Figure 1.

Liver damage was assessed by H&E staining in liver sections from all groups. BDL WT mice displayed increased focal necrosis (infarct) in zone 2 indicated by black arrowheads (upwards of 4–5% of lobule) and portal inflammatory changes that are consistent with obstruction. An increase in mostly large bile ducts is also observed following BDL (indicated by black arrows). In *Kit^{W-sh}* sham mice and *Kit^{W-sh}* mice subjected to BDL, the presence of necrosis/liver infarcts was absent (A). ALT activity was significantly increased in BDL WT compared to sham WT, whereas ALT activity in both sham and BDL *Kit^{W-sh}* sham mice was similar to sham WT (B). In the livers of *Kit^{W-sh}* mice injected with mast cells there were no visible areas of liver necrosis/infarcts or portal inflammation/damage when compared to *Kit^{W-sh}* mice injected with 1X PBS (C) and ALT levels remain unchanged (D). Data are expressed as mean $\pm$ SEM of at least 6 experiments for ALT.

* $p < 0.05$ versus sham WT mice; # $p < 0.05$ versus BDL WT mice. Images are 10 $\times$ magnification. PV = portal vein; CV = central vein

Figure 2.

Bile duct mass (A and B) and Ki-67 (C) were evaluated in liver sections from WT and *Kit^{W-sh}* subjected to sham and BDL surgery. We found that bile duct mass (shown by CK-19 staining (A) and western blotting (B)) and proliferation (indicated by Ki-67 staining (C)) increased in BDL WT mice when compared to sham WT mice, whereas both bile duct mass and the number of proliferating cholangiocytes were reduced in BDL *Kit^{W-sh}* mice. Semi-quantitative data are expressed as mean ± SEM of at least 10 experiments and at least 8 experiments for western blotting. * $p < 0.05$ versus sham WT mice; # $p < 0.05$ versus BDL WT mice. Images are 10× magnification for CK-19 and 20× for Ki-67.

Figure 3.

Fibrosis and collagen content was evaluated by staining in WT and *Kit^{W-sh}* mice subjected to sham or BDL surgery. (A) Staining for Masson's Trichrome and (B) Fast Green/Sirius Red demonstrate an increase in collagen deposition in BDL WT mice compared to sham WT, whereas collagen deposition is dramatically reduced in BDL *Kit^{W-sh}* mice compared to BDL WT mice. (C) The percent of collagen deposition was significantly higher in BDL WT compared to sham WT mice and these levels were decreased in BDL *Kit^{W-sh}* mice compared to BDL WT. (D) Hydroxyproline content in total liver was significantly increased in BDL WT compared to sham WT, whereas BDL *Kit^{W-sh}* mice had significantly lower hydroxyproline content when compared to BDL WT. Data are expressed as mean $\pm$ SEM of at least 6 experiments for hydroxyproline content and an average of 10 images per group analyzed for semi-quantification (% of red pixels over total pixels). * $p < 0.05$ versus sham WT mice; # $p < 0.05$ versus BDL WT mice. Images are 10 $\times$ magnification for Fast Green/Sirius Red and 20 $\times$ for Masson's Trichrome.

Figure 4.

Measurement of TGF-β1 expression and secretion and HSC activation by SYP-9 expression in WT and *Kit^{W-sh}* mice subjected to sham or BDL surgery. (A) TGF-β1 expression and (B) secretion are increased in BDL WT mice compared to sham WT mice, whereas both expression and secretion of TGF-β1 are decreased in BDL *Kit^{W-sh}* mice compared to BDL WT. (C) The protein expression of SYP-9 was increased in BDL WT mice compared to sham WT mice, and SYP-9 expression was significantly reduced in BDL *Kit^{W-sh}* mice compared to BDL WT. Data are expressed as mean ± SEM of at least 6 experiments for real-time PCR and at least 3 experiments run in triplicates for EIA. *p<0.05 versus sham WT mice; #p<0.05 versus BDL WT mice. Images are 20x magnification.

Figure 5.

Evaluation of peribiliary glands and vascular cell presence/activity in WT and *Kit^{W-sh}* mice subjected to sham or BDL surgery. (A) H&E staining reveals the presence of peribiliary glands in BDL WT mice (indicated by black arrows) that are found in close proximity to large bile ducts (indicated by the white arrow), whereas sham WT and both sham and BDL *Kit^{W-sh}* mice have no visible peribiliary glands. (B) The expression of VEGF-C is increased in total liver from BDL WT mice compared to sham WT. Following BDL in *Kit^{W-sh}* mice there is a decrease in VEGF-C expression compared to BDL WT. (C) The protein expression of vWF is shown by immunofluorescence (green) and is upregulated in BDL WT compared to sham WT mice, whereas the intensity of vWF is reduced in both sham and BDL *Kit^{W-sh}* mice. Bile ducts (CK-19) are marked by red staining. (D) The gene expression of vWF is increased in total liver from BDL WT mice compared to sham WT and is significantly reduced in BDL *Kit^{W-sh}* mice compared to BDL WT. Data are expressed as mean $\pm$ SEM of at least 6 experiments for real-time PCR. * $p < 0.05$ versus sham WT mice; # $p < 0.05$ versus BDL WT mice. Images are 10 $\times$ magnification for H&E and 20 $\times$ for immunofluorescence.

Figure 6.

Evaluation of biliary mass, proliferation and the vascular bed following the reintroduction of mast cells into mast cell deficient, *Kit^{W-sh}* mice. (A) IBDM and (B) biliary proliferation are increased in *Kit^{W-sh}* mice injected with mast cells compared to control injections as shown by CK-19 (marked with black arrows) and Ki67 (marked by red arrows). Evaluation of vascular cell activation and VEGF expression in *Kit^{W-sh}* mice injected with 1XPBS or sterile mast cells. (C) The protein expression of vWF is shown by immunofluorescence (green) and is upregulated in *Kit^{W-sh}* mice injected with mast cells compared to control (1XPBS injection). (D) The gene expression of VEGF-C is increased in total liver from in *Kit^{W-sh}* mice injected with mast cells compared to control (1XPBS injection). Data are expressed as mean $\pm$ SEM of at least 9 experiments for real-time PCR. *p<0.05 versus *Kit^{W-sh}* mice injected with 1X PBS. Images are 20 $\times$ magnification.

Figure 7.

Measurement of fibrosis following the reintroduction of mast cells into mast cell deficient, *Kit^{W-sh}* mice. (A) Masson's Trichrome staining reveals an increase in collagen deposition in *Kit^{W-sh}* mice injected with mast cells compared to 1X PBS. (B) Gene expression of fibrotic markers, collagen type-1a and fibronectin-1 are increased in *Kit^{W-sh}* mice injected with mast cells compared to control injections and (C) hydroxyproline content is increased following injection with mast cells compared control. (D) TGF-β1 expression and secretion are upregulated in *Kit^{W-sh}* mice injected with mast cells compared to control injections. Data are expressed as mean ± SEM of at least 6 experiments for real-time PCR and at least 3 experiments run in triplicates for EIA. *p<0.05 versus *Kit^{W-sh}* mice injected with 1X PBS. Images are 20× magnification.

Figure 8.

In vitro effects of mast cell, cholangiocyte and HSC interaction. hHSC gene expression of α-SMA increased following treatment with BDL WT cholangiocyte supernatants compared to sham WT treatment. α-SMA expression decreased in hHSCs treated with cholangiocyte supernatants from sham and BDL *Kit^{W-sh}* mice. HSC gene expression of α-SMA also increased following treatment with cholangiocyte supernatants from *Kit^{W-sh}* mice injected with sterile mast cells compared to all other groups. Data are expressed as mean ± SEM of at least 6 experiments for real-time PCR. *p<0.05 versus basal; #p<0.05 versus hHSCs treated with cholangiocyte supernatant from BDL WT mice; & p<0.05 versus all other groups.