

EDITORIAL

Mast cells in the liver

The studies of Collins and co-workers described in this issue [1] suggest an interesting mechanism whereby mast cell mediators released into the liver following allergen challenge are delivered by the bile ducts to the intestinal lumen where they cause appreciable damage to the villous epithelium. This 'action at a distance' of mast cell mediators represents an interesting paradigm shift from conventional views emphasizing the influence of mast cell mediators on targets in their immediate vicinity. It also poses important questions regarding other potential roles of mast cells in the liver, an organ which has received relatively little attention from mast cell biologists. Within the normal liver, mast cells are relatively scarce with a density of $\approx 1.2\text{--}3.9$ mast cells per square millimetre in humans [2,3], although they are more abundant in rat liver ($\approx 1.8\text{--}12/\text{mm}^2$) [3,4]. Immunostaining using antibodies against mast cell proteases or metachromatic dyes has shown that hepatic mast cells are mainly associated with the connective tissues adjacent to hepatic arteries, veins and bile ducts of the portal tracts in both human and rat liver [2–4]. They are therefore ideally positioned to deliver their products into bile ducts in accordance with the proposal of Collins and co-workers. Mast cells are virtually absent from the sinusoids and the liver parenchyma in rats [3,4] but up to 10% of liver mast cells have a perisinusoidal location in the human liver [2] and, as discussed below, this proportion increases under conditions of mast cell hyperplasia in liver fibrosis. The subtype of mast cells, tryptase only (MC_T) or tryptase/chymase containing (MC_{TC}), present in normal human liver has not been reported, although the majority of the expanded mast cell population in fibrotic human liver are of the MC_{TC} subtype [3]. In rat liver, staining of mast cells in liver sections with alcian blue/safranin indicate that these cells are predominantly of the mucosal subtype [4], with Collins and co-workers reporting that 7% of hepatic mast cells had the staining characteristics of connective tissue mast cells. Whether mature mast cells in adult liver derive primarily from precursors normally resident in this organ, or are recruited from circulating precursors, is uncertain. Bardadin *et al.* [5] have identified two different types of mast cell in livers from patients with acute hepatitis: periportal mast cells morphologically similar to those in connective tissues in other organs and sinusoidal mast cells which appeared to be derived from myeloid cells. The existence of a resident liver population of mast cell progenitors is supported by reports from a number of workers that dispersed populations of human fetal liver cells are a rich source of mast cell precursors which can be induced to

differentiate following several weeks of incubation with purified stem cell factor (SCF) or co-culture with fibroblasts [6–8]. Following incubation with SCF, precursors matured to mast cells predominantly c-kit and tryptase positive, with 31% showing weak chymase immunoreactivity. However, co-culture with fibroblasts predominantly resulted in the development of the MC_T subtype (94%). This contrasts with mast cells derived from fetal cord blood mononuclear cells co-cultured with fibroblasts where chymase is expressed in almost half of the mast cell population; this suggests that precursors in liver and cord blood differ at an early stage in their commitment to protease expression in their mature phenotype [8].

Information about the subtype of mast cells in liver is important as numerous studies have established that rat mucosal and connective tissue mast cells differ both qualitatively and quantitatively in mediator content and therefore their potential to influence targets within the liver. Collins and co-workers [1] consider the rapid appearance of $\text{TNF}\alpha$ in bile following allergen challenge to support the concept that this cytokine derives from preformed stores within mast cells. Rat intestinal mucosal mast cells are known to store this cytokine, although contain approximately six times less than connective tissue mast cells [9]. However, the effects of mast cell degranulation on other liver cells is uncertain, and the possibility remains that mast cells could induce $\text{TNF}\alpha$ release from other cells. Indeed, bile duct epithelial cells themselves have the capacity to produce $\text{TNF}\alpha$ in inflamed liver [10,11] and this cytokine increases permeability of biliary epithelial tight junctions thereby enhancing bile duct leakiness and movement of macromolecules into bile [12]. The liver also has a substantial capacity for leukotriene production in anaphylaxis, and hepatic mast cells may be the main source of anaphylactic leukotrienes in the liver, being the predominant cell type in rat liver expressing 5-lipoxygenase [13]. This finding provides some support for allocation of liver mast cells to the mucosal subtype, as LTC_4 is the major eicosanoid produced by these cells. Mast cell products may exert effects at extrahepatic sites not only via entry into bile but also by release into the hepatic circulation. Sufficient quantities of mediators are apparently released from hepatic mast cells during liver disease to achieve systemic effects: plasma histamine is raised almost twofold in chronic cholestatic liver disease, being highest in patients with pruritis which is a frequent symptom of this disorder [14].

Collins and co-workers have examined an acute response by hepatic mast cells, but there is accumulating evidence

that mast cells may regulate chronic pathological responses within the liver. Several recent studies support a role for mast cells in the liver's fibrotic response to chronic inflammation and parasitic infection. It is well established that mast cell numbers are increased in tissues undergoing fibrotic changes, and expansion of the hepatic mast cell population is a consistent feature of liver fibrosis in humans and in animal models following liver damage by autoimmune reactions, chemical toxins, viral infections and cholestasis [2–4,14–17]. The magnitude of the mast cell response varies widely, with relatively modest changes of one–twofold increase observed in liver fibrosis resulting from cholestasis in rats [4] whilst in human cholestatic liver disease (primary biliary cirrhosis) increases of more than eightfold were observed [2]. In fibrotic liver, mast cells are most consistently observed within the developing fibrous septae around the portal tracts and the degree of tissue mast cell infiltration correlates with the extent of collagen deposition [2].

To date, studies showing a causal link between mast cell activity and fibrosis development in liver are lacking, although this hypothesis is supported by the finding that portal fibrosis is a frequent complication in systemic mastocytosis where mast cells are dramatically increased in liver [18,19]. Although the roles of mast cells in a wide variety of biological responses have been examined using mast cell-deficient mice, the development of liver fibrosis in these animals has not yet been examined. Nevertheless there is a good theoretical basis for considering mast cells as being profibrogenic as *in vitro* studies have shown that mast cell mediators histamine, heparin and IL-4 enhance fibroblast proliferation and/or collagen production (reviewed in [20]). Human mast cell tryptase induces proliferation, migration and type I collagen synthesis by fibroblasts [21,22] and we have shown that this protease has similar effects on hepatic stellate cells, the major matrix-producing cells in liver fibrosis [23]. Mast cells themselves also synthesize a limited spectrum of extracellular matrix (ECM) components [24]. A recent study [3] has shown that mast cells in fibrotic human liver showed strong immunoreactivity for a variety of serpins (serine protease inhibitors). These may protect deposited collagens from degradation by metalloproteinases such as stromelysin and interstitial collagenase by inhibiting serine proteases required for metalloproteinase processing and activation. Evidence for ongoing mast cell degranulation in fibrotic rat liver has been obtained [4]. The degranulating stimulus has not been identified, although stem cell factor derived from activated liver stellate cells or fibroblasts is a possible candidate. Release of vasoactive mediators such as histamine and eicosanoids from the expanded mast cell population may contribute to the disruption of microvascular blood flow which accompanies hepatic fibrosis. Reilly *et al.* [25]

examined the responses of hepatic microvasculature to topical application of the mast cell degranulator compound 48/80 and observed constriction of sinusoids and central venules which suggests that resident cells may regulate microvascular circulation.

In considering the factors which drive the hyperplasia of mast cells during development of liver fibrosis, interest has not unexpectedly centred around SCF, as this protein is an important maturation factor for liver mast cells and is also a mast cell chemoattractant and secretagogue (reviewed in [26]). Our studies have shown that SCF protein is increased three–fourfold in cirrhotic human liver relative to normal [23]. Fibroblastic cells in various tissues are a rich source of SCF and we have shown that activated hepatic stellate cells, perisinusoidal myofibroblastic cells which are the major producers of the ECM which accumulates in liver fibrosis, produce SCF when activated to overproduce collagens *in vitro* [23]. Although mast cells principally lie within portal connective tissue in the fibrotic liver, stellate cell-derived SCF might contribute to the expansion of hepatic mast cells into sinusoids which occurs in cholestatic liver disease [2]. This is supported by our recent findings that human mast cells rapidly adhere to monolayers of stellate cells in culture and adherence is disrupted by neutralization of SCF. Rat stellate cells also produce IL-10 during development of fibrosis in bile duct ligated rats [27]. This cytokine promotes development of rodent mast cells *in vitro* [28] and may act with other growth factors such as SCF in the liver. Transforming growth factor beta is markedly upregulated in fibrotic tissues including the liver, where activated stellate cells are an important source of this profibrogenic cytokine which is also a potent mast cell chemoattractant [29]. Stellate cells may in turn become targets for mast cell mediators such as tryptase which induces their proliferation [23]. Mast cells are also a rich source of TNF α which induces proliferation, collagen production and SCF production in these cells [30]. The profound quantitative and qualitative changes in the liver matrix in fibrosis may itself influence mast cell activities. Mast cells derived from fetal liver cultures and other sources express a variety of integrin adhesion molecules [31–33]. Mast cell development is known to be influenced by surrounding stroma, and recent reports show that matrix/integrin interactions may regulate mast cell secretory responses to soluble stimuli [34].

The accumulation of mast cells in fibrosis under conditions of matrix accumulation seems at odds with their suspected role in connective tissue degradation in sites of inflammation, such as in rheumatoid synovium. This raises the possibility that mast cells may accumulate in the liver as a tissue response to limit and constrain the deposition of the matrix. This idea is supported by findings that mast cells accumulate relatively late in fibrogenesis when matrix

deposition is already underway. Mast cell proteases have *in vitro* activities which would encourage matrix degradation. Human tryptase, chymase and cathepsin G degrade matrix, activate metalloproteinases and generate plasmin [35–37]. The net effect of mast cell activation on matrix turnover may be complex, depending on the presence or absence of metalloproteinases, tissue inhibitors of metalloproteinases and urokinase, factors which regulate matrix homeostasis in the liver and other tissues [38,39].

Mast cells may also promote collagen deposition in granulomas following infiltration of microbes or parasites into the liver. Schistosomiasis is a common cause of liver fibrosis. Toluidine blue staining of liver sections from patients with liver granulomas showed that mast cell numbers correlated with granuloma volumes, being highest in schistosomiasis [40]. Brito *et al.* [30] have proposed a co-operation between mast cells and hepatic stellate cells in the formation and chronic persistence of schistosomal granulomas in mice. They showed that co-culture of mast cells with stellate cells induced SCF production in the latter which was blocked by neutralization of TNF α . They proposed a self-sustaining mechanism in which TNF α from mast cells maintained HSC proliferation, collagen synthesis and SCF production, the last acting to maintain mast cell numbers.

In conclusion, there is an increasing awareness that mast cells may contribute to liver disease. In particular, a number of recent studies have started to examine the molecular basis whereby mast cells interact with resident liver fibroblastic cells to promote fibrogenesis. There are a number of liver disease models in rodents which mimic human liver pathology, and use of mast cell deficient mice in such models should help us assess the contribution of mast cells, although with the caveat that rodent mast cells differ in many respects from their human counterparts. Studies of the effects of mast cells and their mediators on individual liver cell subtypes, like those performed using stellate cells, may also give insights into their roles in liver physiology and pathology.

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