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Review

The Dynamic Interplay Between Mast Cells, Aging/Cellular Senescence, and Liver Disease

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Mast cells are key players in acute immune responses that are evidenced by degranulation leading to a heightened allergic response. Activation of mast cells can trigger a number of different pathways contributing to metabolic conditions and disease progression. Aging results in irreversible physiological changes affecting all organs, including the liver. The liver undergoes senescence, changes in protein expression, and cell signaling phenotypes during aging, which regulate disease progression. Cellular senescence contributes to the age-related changes. Unsurprisingly, mast cells also undergo age-related changes in number, localization, and activation throughout their lifetime, which adversely affects the etiology and progression of many physiological conditions including liver diseases. In this review, we discuss the role of mast cells during aging, including features of aging (e.g., senescence) in the context of biliary diseases such as primary biliary cholangitis and primary sclerosing cholangitis and nonalcoholic fatty liver disease.

Key words: Senescence; Senescence-associated secretory phenotype (SASP); Aging; Mast cells (MCs); Liver diseases; Inflammation; Fibrosis

MAST CELLS

Mast cells (MCs) are one of the most widely studied cells of the innate immune system. MCs are classified as granulocytes due to the presence of peptidase-filled granules within the cell and are found in almost all tissues of the body, indicating dynamic tissue-dependent function such as angiogenesis, pathogen elimination, and vasodilation[1]. Upon activation, MCs secrete a number of peptidases and mediators including chymase, tryptase, and histamine (HA)[2]. Activated MCs also secrete a wide array of proinflammatory cytokines that mediate various downstream signaling pathways[3]. MCs develop from common myeloid progenitor cells [expressing cluster of differentiation protein 34 ($CD34^+$)] in bone marrow and mature following infiltration into tissues through various transcription factors like GATA-binding protein 1 and 2 (GATA-1 and GATA-2), microphthalmia-associated transcription factor (MITF), and CD11b[4–6]. GATA-1 is essential for the differentiation of MCs, signal transduction, MC survival, and cytokine production in mature MCs[7]. β-7 integrin is considered key for MC localization in the intestine since β-7-deficient mice show reduced committed MC progenitors (MCp) and mature MCs in the small intestine[8]. A separate study demonstrated that CD11b-deficient mice had depleted MCps in their peritoneal cavity and dorsal skin, indicating not only the importance of these factors in the maturation and localization of MCs but also the diverse role that MCs have in particular tissues[9]. Overall, mature MCs do not circulate in the peripheral blood in an otherwise healthy human[1].

Upon binding with an antigen-presenting cell (APC) or by activation via receptor/ligand interactions, MCs

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elicit type I hypersensitivity by degranulation and releasing mediators, mainly HA; however, MCs also secrete chemokines and growth factors that may influence disease progression and tissue remodeling[1,10]. For example, MCs promote the colonization of melanoma in lungs using a transgenic MC-defective murine model[11], and in osteoporotic patients, MCs were found to be increased in the bone marrow[12]. Another study demonstrated that MCs regulate osteoclast activity in a murine model of fracture-induced inflammation[13]. Following fracture and during the healing process, MCs localize on the surface of the bone and in the vicinity of the periosteum[14]. In vitro MC-derived HA was found to promote osteoclastogenesis through autocrine/paracrine signaling[14]. In cardiac tissue, MCs are involved in the inflammation of atherosclerotic arteries[15]. MCs have been shown to act as key modulators of epithelial permeability and mucosal barrier[16]. Moreover, MCs regulate the sensory function of visceral organs by interacting with nerve fibers and neuropeptides[17]. Therefore, it can be concluded that MCs have far-reaching roles in pathophysiological events other than allergen-mediated hypersensitivity.

MCp in the blood expresses KIT proto-oncogene (c-Kit), β-7 integrin, and high-affinity immunoglobulin E (IgE) receptor (FcεR1) in addition to $CD34^{+}$[18,19]. MCs also have surface receptors that establish interactions with the external milieu[20]. The principal receptor that mediates MC function is high-affinity IgE receptor FcεR1[21], which is also present on other dendritic cells and monocytes[22]. Upon binding with its ligand [the fragment crystallizable (Fc) region of IgE], FcεR1 activates the vesicular trafficking and subsequent fusion of HA-containing granules to the membrane, resulting in "degranulation" and release of HA[23]. The other traditional receptors on MCs are high-affinity IgG receptor, FcγR (binds with IgG), and major histocompatibility complex (MHC), which binds with T-cell receptor[10]. MCs express c-Kit [binds to stem cell factor (SCF)], Toll-like receptors [TLRs; binds to bacterial pathogens' lipoteichoic acid and pathogen-associated molecular patterns (PAMPs)], and neurokinin receptors (NK1R; binds to neuropeptides), among others[10], showing the various receptors capable of leading to MCs activation. A great body of research provides evidence that MCs play a critical role in immune-mediated diseases of the hepatobiliary system and gastrointestinal (GI) tract mediating the pathogenesis of these diseases[24,25], which is the key aspect of this review. Figure 1 and Table 1 show some of the receptors that play important role in the activation of MCs during liver disease.

BILIARY DISEASE/CHOLANGIOPATHIES

Cholestatic liver disease is the collective term for all pathophysiological conditions where bile secretion is impaired[26]. Under normal physiological conditions, hepatocytes secrete bile acids, which drain from the sinusoid to the extrahepatic bile duct[26]. When cholangiocytes (lining the bile ducts) are injured by T-lymphocyte-mediated autoantibodies, a plethora of events such as loss of self-immune tolerance, elevated anti-mitochondrial

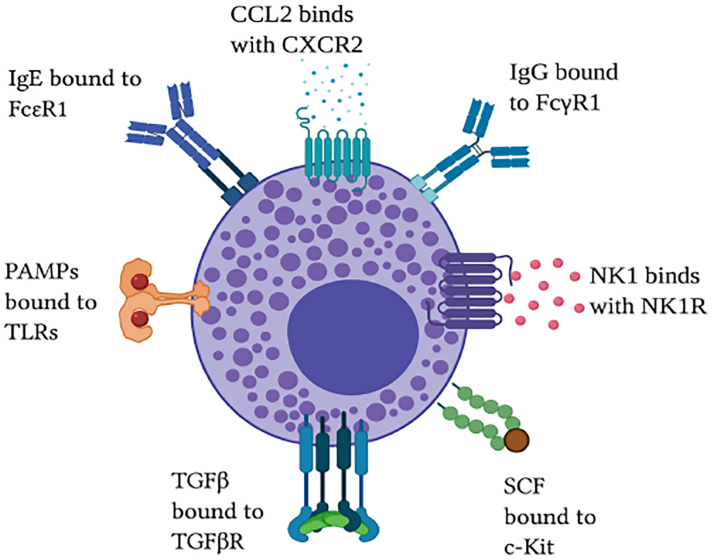

Figure 1. Diagrammatic representation of mast cells (MCs) with the receptors and the ligands that lead to activation and play an important role in liver diseases. CCL-2, C-C motif chemokine family 2 protein; CXCR2, C-C motif chemokine family 2 protein receptor; c-Kit, KIT proto-oncogene receptor tyrosine kinase; IgE, immunoglobulin E; IgG, immunoglobulin G; NK1, neurokinin 1; NK1R, neurokinin 1 receptor; PAMPs, pathogen-associated molecular patterns; TGF-β, transforming growth factor-β; TGF-βR, transforming growth factor-β receptor; TLRs, Toll-like receptors; SCF, stem cell factor.

Table 1. Summary of the Mast Cell (MC) Receptors and Activators That Play an Important Role in Liver Disease

Factors Affecting MC Activation	Receptors
Stem cell factor (SCF)	Binds to KIT receptors expressed on MCs
C-C motif chemokine family protein 2 (CCL-2)	Binds to CXCR2, a G-protein-coupled receptor leads to inflammatory response
Pathogen-associated molecular patterns (PAMPs)	Toll-like receptors (TLRs) binds and dimerizes activating the NF-κB pathway
Immunoglobulin E (IgE)	High-affinity IgE receptor, FcεR1. Activates MCs
Immunoglobulin G (IgG)	High-affinity IgG receptor, FcγR1. Activates MCs
Histamine (HA)	H1HR, H2HR, H3HR, H4HRs via autocrine loop
Neurokinin 1(NK1)/substance P	NK1R on MCs. Plays a role in sensory innervation in liver
Transforming growth factor-β (TGF-β)	TGF-β receptor. Induces production of SASP factors

antibody (AMA), ductopenia, ductular reaction, and periportal fibrosis are triggered, marking the manifestation of primary biliary cholangitis (PBC)[27]. Although the true etiology is unknown, primary sclerosing cholangitis (PSC) is suspected to be an autoimmune-mediated cholangiopathy, which differs from PBC in its pathogenesis, diagnosis, and treatment[28]. PSC is chronic and manifested by deposition of extracellular matrix (EM) around the biliary epithelium that progressively leads to fibrosis in the small-, medium-, and large-sized ducts[27,29]. As a result, the biliary lumen develops strictures resulting in cholestasis[28]. PBC is found to predominantly affect middle-aged females, while PSC has been associated with a male prevalence. The exact role of aging in these diseases is unclear; however, since both PBC and PSC are chronic conditions, it can be surmised that age-related changes in liver physiology affect disease phenotype and alter treatment response in elderly patients compared to younger patients[30].

ROLE OF MCs IN PSC AND PBC

An early study found that MCs do not alter hepatic fibrosis in MC-deficient rats subjected to acute liver injury by bile duct resection (BDR)[31]. Despite this preliminary finding, there is now substantial evidence revealing MCs play a significant role in the development of cholestatic liver diseases[24,32–34]. The presence of MCs in diseased liver was confirmed by immunohistochemical analysis of liver biopsies from PSC patients that stained positively for tryptase, an MC protease[24,35]. Additionally, in the bile duct ligated (BDL) rat model of hepatic injury, MCs promote hepatic injury including increased biliary proliferation and ductular mass along with enhanced fibrosis[32]. MCs were found to be localized near bile ducts (but not hepatocytes) and induced cholangiocyte proliferation and an upregulation of fibrotic genes like α-smooth muscle actin (α-SMA)[32]. When BDL rats were treated with cromolyn sodium, an MC stabilizer, the proliferative effects of MCs on cholangiocytes and biliary fibrosis were reversed[32]. In a separate study, it was shown in Mdr2$^{-/-}$ (multidrug resistance transporter 2/ABC transporter B family member 2 knockout) mice, a murine model of PSC, inhibition of MC-derived HA by cromolyn sodium reduced peribiliary fibrosis and cholangiocyte proliferation[34]. MC-mediated biliary fibrosis was also inhibited by the treatment of ursodeoxycholic acid (UDCA)[36]. This study is significant since UDCA is a current treatment for PBC patients[37,38], and Mdr2$^{-/-}$ mice treated with UDCA showed reduced liver injury, peribiliary fibrosis, and intrahepatic bile duct mass (IBDM) compared to controls. The authors concluded that bile acids mediate MC migration and degranulation, and specifically that UDCA reduces MC presence and their damaging effects[36]. Blocking the HA receptors, histamine 1 receptor (H1HR) and histamine 2 receptor (H2HR) using over-the-counter HR antagonists reduce hepatic stellate cell (HSC) activation, fibrosis, and biliary proliferation in Mdr2$^{-/-}$ mice[39]. Following BDL in MC-deficient mice (*Kit*$^{w\text{-}sh}$), hepatocyte necrosis, bile duct mass, and liver fibrosis were significantly reduced compared to BDL WT mice, validating that MCs play a significant role in the progression of cholestatic liver injury[33]. *Kit*$^{w\text{-}sh}$ mice injected with MCs via the tail vein displayed increased fibrosis and hepatic damage compared to sham-treated mice, further emphasizing the role of MCs in liver injury[33]. Together, these studies provide strong evidence that MCs are an important driving factor in cholestatic liver disease including liver fibrosis and biliary proliferation. Figure 2 demonstrates the localization of MCs following BDL in rats and in patients with PSC.

MCs IN NONALCOHOLIC FATTY LIVER DISEASE

Nonalcoholic fatty liver disease (NAFLD) has gained much attention in the past decade owing to its increased disease burden and multifactorial nature[40,41]. Along with hepatocytes, HSCs and Kupffer cells (KCs), cholangiocytes are affected during NAFLD progression[42]. NAFLD initiates as mild steatosis (fat deposition in the liver) and progressively deteriorates to macrovesicular and microvesicular steatosis, damaged hepatocytes, accumulation of extracellular matrix (ECM) leading to fibrosis, which manifests into non-alcoholic steatohepatitis (NASH)[43]. Although not often regarded as a cholangiopathy, it has been found that MCs interact with

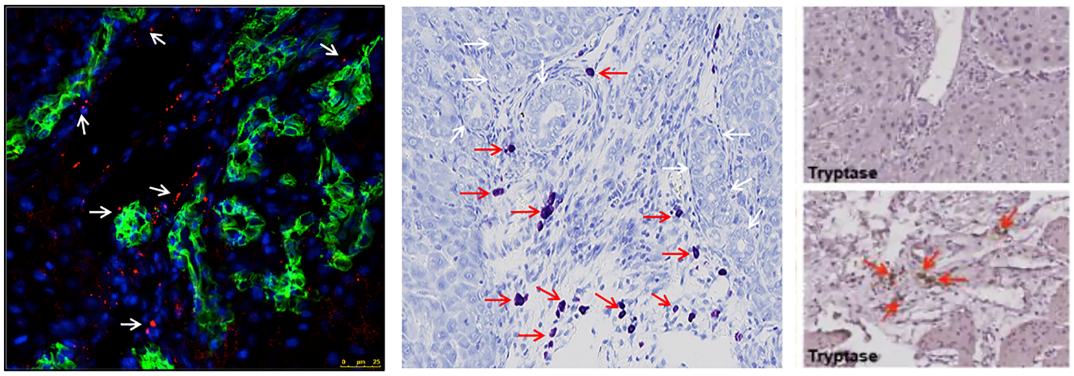

Figure 2. Localization of MCs in diseased liver. Left panel: Coimmunofluorescence staining showing MCs (white arrows) stained with FcεR1 (red) localized near the bile ducts stained for cytokeratin-19 (green). Middle panel: By toluidine blue staining, MCs (red arrows) were found near bile ducts (white arrows). Right panel: In human PSC patients, tryptase-positive MCs (red arrows) increase compared to control. Modified and reprinted with permission from Hargrove et al.[32].

cholangiocytes during the progression of NAFLD. Like other liver pathologies, hepatic fibrosis is a hallmark of advanced NAFLD, and it has been found that the presence of MCs alters the fibrotic nature of the NAFLD liver[44]. Significant correlation has been reported between the number of MCs in portal areas and the fibrosis grade of the NALFD liver[44]. Additionally, these MCs were found to be degranulating, indicating their active contribution in secreting proinflammatory cytokines[44]. Existing data indicate that in human NAFLD and NASH patients, serum HA levels are elevated compared to the healthy controls; these patients also have increased IBDM, resulting from cholangiocyte damage during NAFLD progression[42]. Although hepatocytes are the main cell population affected by senescence during NAFLD[45], in vitro data suggest that cholangiocytes also become senescent during NAFLD development and contribute to disease progression. Interestingly, the main senescence marker that is upregulated in hepatocytes during NAFLD is cyclin-dependent kinase 4 (CDK4) inhibitor 1 ($p21^{Waf1}$), and current experimental evidence suggests that CDK inhibitor 2A ($p16^{INK4A}$) is the main marker protein upregulated in senescent cholangiocytes[45,46]. Thus, both cell populations undergo senescence but through different pathways. To elucidate the role of MCs in cholangiocyte-mediated damage in high-fat diet (HFD)-induced NAFLD, liver histology of MC-deficient mice indicated amelioration of steatosis, fibrosis, and inflammation compared to WT mice, suggesting a critical role of MCs in NAFLD progression (Francis et al., unpublished data).

As the onset and progression of NAFLD mainly depend on diet and metabolic condition of an individual, cellular senescence is the main causal factor for age-dependent hepatic steatosis[47]. In a murine model of diet-induced NAFLD (Sprague–Dawley rats on high-fat high-cholesterol diet), there was no difference in the metabolic parameters (cumulative energy intake, body weight gain, and food efficacy) between aged and young groups; however, hepatic fibrosis, inflammatory, and oxidative gene expression were increased in the aged rat group compared to the young ones, asserting the effect of age on the progression and severity of NAFLD[48]. Interestingly, sex-dependent aging has a role in the progression of NAFLD. Males are more likely to be affected by steatosis than females, indicating that sex steroids play a protective role in females during NAFLD[49]. The protective nature of estrogen can be attributed to its regulation of hepatic lipid metabolism[50]. Estrogen therapy in ovariectomized rats on high-fat high-fructose diet has been shown to ameliorate steatosis in the liver, making it a promising therapeutic candidate[51]. However, there is still a need for sex-independent therapy that can be equally efficacious in NAFLD patients.

MicroRNA (miRNA/miRs) AND MCs IN AGING

miRs are short RNA sequences that play an important role in regulating gene expression by translational repression[52,53]. They are a large family of noncoding RNA sequences that are synthesized endogenously and can be secreted in extracellular vesicles (EVs). miRs have been found to bind with mRNA, along with RNA-induced silencing complex protein (RISC), initiating mRNA degradation[54]. Apart from the effectors and activators that bind MCs causing activation and degranulation, miRs too play a critical role in the physiology of MCs. In a murine model of MC-specific knockout of dicer, enzyme involved in the processing of mature miRs, there was severe deficiency of MCs in the peritoneum, GI cavity, and dermal region of the mice, signifying that global MC-miR expression is important in tissue-specific distribution of MCs in vivo[55]. miR-221-222 expression has been found to be upregulated during the activation MCs and has been implicated to affect the cell cycle in MCs[56]. miR-221 not only regulates the cell

cycle in MCs but at the same time promotes degranulation via IgE-mediated PI3K/Akt/Ca^{2+} pathway[57,58]. Thus, it can be predicted that miR-221-222 might be an important target for therapeutics in MC-related pathologies. Interestingly, miR-221 has been established as a neoplastic biomarker in the progression of hepatocellular carcinoma and plays an important role in liver regeneration[59–62]. There is not enough evidence if downregulating miR-221 in the liver would reduce MC-mediated fibrosis and damage in murine model of PSC and cholestatic liver injury. However, it can be predicted from existing experimental evidence that targeted downregulation of miR-221 in the liver might reduce MC activation and subsequent degranulation, thus alleviating fibrosis and related damage[56,58,63]. Additionally, miR-146a has been found as an active regulator for MC survival in an nuclear factor κB1 (NF-κB1) (p50)-deleted murine model, and the expression of NF-κB in MCs is important for their survival and tissue homeostasis[64,65]. miR-135a targets GATA-3 transcription factor, thus inhibiting the maturation of MCs and allergen-induced inflammation in a murine model of allergic rhinitis[66]. Spred1 (sprouty related Ena/VASP homology-1 domain containing protein), a protein that inhibits interleukin-3 (IL-3)-mediated MAP kinase activation in hematopoietic cells, is a target and modulated by miR-126[67,68]. In a Spred1-conditional knockout murine model, MC proliferation was upregulated and MCs were highly activated by FcεR and SCF stimulation, emphasizing the critical role of miR-126 in regulating MC biology[69]. Since there is mounting evidence that miRs contribute to MC proliferation, cell cycle, and life span, it can be predicted that miRs play a critical role in the age-related decline of MC regeneration in a murine model of senescence[70]. Several miRs have been identified as biomarkers of aging and aging-related diseases as their levels are significantly changed during the progression of these pathological conditions[54]. miR-151a-5p, miR-181a-5p, and miR-1248 were reported to be significantly downregulated in elderly subjects[71]. Serum analysis shows that miR-20a levels were also significantly reduced in elderly male subjects compared to the younger ones[54]. On the other hand, miR-92a, miR-375, miR-222, and miR-142-5p presence in serum was reported to be upregulated in elderly individuals, signifying their role in causing aging-related changes[72]. Therefore, miRs can be used as a therapeutic target to limit the manifestation of aging-related changes, especially with the context of liver disease.

AGING, SENESCENCE, AND RELATED HALLMARKS

Apoptosis, autophagy, and senescence impact growth and development. Previously, senescence was considered a cellular mechanism to counter the progression of tumorigenesis and regarded as a strategy to fight cancer and related stresses[73,74]. However, there is evidence that senescence is key in modulating embryonic development and the progression of aging[75]. As cells become more senescent, they begin to secrete a wide array of chemokines and enter a senescence-associated secretory phenotype (SASP)[76]. These SASP-associated chemokines are predominantly proinflammatory and accompany pathologic manifestation of many degenerative conditions[77]. The secretome from SASP cells contributes to tissue damage and disease progression, including liver disease[78].

Factors that trigger and contribute to cellular senescence include oxidative stress, oncogenic mutations, proteotoxic stress, and mitogenic signals[76,79]. Since cellular senescence occurs over the lifetime, it independently contributes to the process of aging and aging-related physiological changes[75]. The characteristic hallmark of a senescent cell is the production of senescence-associated β-galactosidase (SA-β-gal) formation in lysosomes[80], formation of senescence-associated heterochromatin foci (SAHF), as well as changes in gene expression of clusterin (SGP-2/Apo J), α1-procollagen, osteonectin, fibronectin, transgelin (SM22), cytochrome C oxidase, and guanidine triphosphate α (GTPα)[81]. These canonical manifestations of replicative senescence are initiated by telomere shortening and DNA damage pathway by tumor protein p53 activation[82]. The other pathway of cellular senescence, which occurs irrespective of DNA damage, is due to upregulation of CDK inhibitors such as $p16^{INK4A}$, $p18^{INK4C}$, and $p21^{Waf1}$. Strong activation and upregulation of $p16^{INK4A}$ promoter, in vivo, are positively correlated with the features of senescence, such as elevated expression of SASP factors, SA-β-gal, and reduced proliferation of the macrophages[83].

As mentioned, telomere shortening is a hallmark of aging and cellular senescence[84]. In chronic liver diseases like hepatitis C virus (HCV) and liver cirrhosis, telomere length was significantly reduced compared to normal liver[85]. Using quantitative fluorescence in situ hybridization (Q-FISH) as a technique to study telomere shortening, it was demonstrated that this can be a robust technique to evaluate telomere length on fixed-frozen tissues, in vivo and in cell smears, in vitro[86]. A negative correlation was found between the quantifiable fluorescence unit and patient age, demonstrating that aging patients have shorter telomeres. Almost all human tissues exhibit reductions in telomere length during aging with the exception of the brain and myocardium[87]. The length of telomeres from two different organs of the same individual is comparable, indicating that in healthy individuals, telomere shortening is a coordinated event that progresses during aging[88].

MCs AND AGING

As one of the principle mediators of innate immunity, MC functionality and population are affected by aging[89]

(Table 2). By immunohistochemical analyses, the MC population was found to increase by 27% in mesenteric lymphatic vessels (MLVs) and with a 400% increase in activated MCs in the mesentery of the aged rats (24 months) compared to the younger control (9 months)[89]. It was concluded that this increase in MC population and activation in MLVs are essential for the degradation of the lymphatic vessels that occurs with age[89]. Further, in healthy aged individuals (≥75 years), the MC population increased in the skin (papillary dermis) by 40% compared to the biopsies from younger individuals (≤30 years), and dermal MCs associated more with macrophages and nerve fibers in aged skin compared to the younger samples[90]. Interestingly, despite their increased presence in the papillary dermis, MC degranulation was significantly lower in aged skin, which might be explained by the reduced expression of the tachykinin precursor 1 (TAC1) gene encoding substance P, a neuropeptide that is also an MC activator in skin[91]. Immunohistochemical staining of the eyelid and eyeball of the murine model of atopic dermatitis, Derm1$^{NC/Nga}$ mice, revealed that MCs increased significantly in the aged, diseased mice (10 and 16 weeks) compared to the younger (4 weeks) mice afflicted with atopic keratoconjunctivitis[92]. Dexamethasone, an anti-inflammatory drug, reduced MC density in the eyeballs of these mice, demonstrating that manipulation of MCs may influence outcomes in age-related disease[90].

Immunosenescence broadly refers to senescence of immune cells and is considered to increase with age as a result of the hyperstimulation of the immune system[93,94]. In a study, elevated immune response was reported from the elderly group compared to the younger one when they were administered with *Escherichia coli* endotoxin[95]. Serum cytokine levels [tumor necrosis factor receptor (TNFR)] were measured in both the groups, and the elderly groups were reported to have higher body temperature, but at the same time, their cytokine level was dysregulated[95]. Another study found that, when compared to young rats, the aged rats showed hallmarks of immunosenescence such as fewer T cells and B cells, reduced granulocytes, and IL-6-secreting cells, including increased oxidative stress in the spleen[96]. Interestingly, the authors found an increase in apoptotic cells and MCs in the aged groups compared to the younger groups. Overall, these studies demonstrate a role for immunosenescence during aging that contributes to disease progression[96].

Table 2. Age-Related Changes in MC Physiology and Liver

Changes in the MCs
Reduced regeneration
Increased localization and activation in the skin and eye
Decreased degranulation
Changes in the Liver
Telomere shortening
Increased fibrotic damage
Cellular senescence
Reduced hepatic flow

LIVER DISEASE, AGING, AND SENESCENCE

Within the context of liver diseases, like PSC, cholangiocytes become senescent and p16^{INK4A} is the main cell cycle inhibitor that is upregulated[46]. Further, there is elevated expression of a number of proinflammatory proteins, such as IL-6, IL-8, C-C motif chemokine family 2 protein (CCL-2), and plasminogen activator inhibitor-1 (PAI-1) in senescent cholangiocytes[46]. These proteins have been characterized as SASP factors that aid in disease progression. Another factor that governs the p16^{INK4A} expression in biliary disease is secretin (Sct), a neuropeptide secreted by cholangiocytes in the liver[97] and by S-cells in the duodenum[98]. Sct/secretin receptor (SR) signaling drives liver fibrosis and biliary senescence via transforming growth factor-β1 (TGF-β1) signaling axis in BDL mice[99]. Sct is also instrumental in causing hepatic damage in early-stage PBC in Mdr2$^{-/-}$ mice lacking SR (SR$^{-/-}$/Mdr2$^{-/-}$). In this model, knockout of SR in Mdr2$^{-/-}$ mice ameliorated hepatic damage and fibrosis by reducing biliary senescence that was marked by the reduction in p16^{INK4A} expression[100]. Interestingly, although cholangiocyte senescence reduced in the absence of Sct signaling, human hepatic stellate cell (hHSC) senescence increased (marked by upregulation of p16^{INK4A} expression), indicating a differential regulation of cellular senescence within the liver. Another study demonstrated that inhibiting p16^{INK4A} expression in Mdr2$^{-/-}$ mice by Vivo-Morpholino treatment reduced biliary damage and liver fibrosis[101]. In addition, Mdr2$^{-/-}$ mice treated with the p16^{INK4A} Vivo-Morpholino had decreased SASP secretion and expression of fibrosis and senescence markers in cholangiocytes[101].

Two studies demonstrated the rate of telomere loss in the liver, but with contradicting experimental results[86,88], which can potentially be attributed to the varying sample size and the range of age of subjects for each study. Interestingly, hepatocytes and cholangiocytes did not show a significant decrease in telomere length shortening, but instead maintained their telomere length throughout aging[102], indicating this rate of telomere loss in "normal" liver was attributed to KCs and HSCs. It was also reported that HSCs, upon aging, undergo significant morphological changes as they increase in size due to lipid deposition and localize in sinusoids that restrict hepatic flow (tissue perfusion)[103]. This provides an explanation regarding how HSCs can be a major determining factor that elicits dynamic remodeling in the intrahepatic environment during aging. HSCs have also been found to be stimulated by MCs that eventually leads to hepatic fibrosis in rats[32]; thus, it can be predicted that in aged cholangiopathy

patients (PSC and PBC), altered MC presence and heightened HSC activation will produce severe disease phenotype compared to the younger patients.

Aging brings irreversible changes in the characteristics of an organ, and the liver is no exception. The liver continues to undergo standard hallmark changes during aging, and the regenerative capacity is reduced with advanced age[104]. A recent study explored the effect of age on liver volume and blood flow in healthy humans and found that liver volume reduces over the life span along with liver blood flow in healthy individuals[105]. This likely explains the reduced clearing of drugs from the hepatobiliary conduit in elderly individuals. Although diet has been indicated as an independent risk factor, it has been found that, even without HFD, aged murine models develop hepatic plasticity and steatosis over time, and hepatocytes from aged murine livers show elevated levels of triglyceride deposition[106]. Aging hepatocytes secrete more monocyte chemoattractant protein-1 (MCP-1), which facilitates the monocyte derived macrophage recruitment after hepatic injury[107]. The aging liver expresses a number of proteins that, in turn, modulate the pathobiology of many hepatic diseases. The expression of Twinfilin 1 (Twf1) increases progressively with age, and the expression of this actin-monomer binding protein is directly associated with the reduction in cell proliferation[108]. Cholangiocyte proliferation was reduced when Twf1 was knocked down in 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC)-induced disease model, thus ameliorating biliary injury. This reduction in cholangiocyte proliferation in the absence of Twf1 increased biliary senescence characterized by increased p16 and $p21^{Waf1}$ gene expression[108]. CCAAT/enhancer binding protein (C/EBP) encoded by the CEBPA gene in humans is an important transcription factor in the hepatic environment that is also affected by aging and hepatic injury. C/EBPα along with C/EBPβ are early makers for liver development[109]. If the DNA methylation of C/EBPα binding site is altered, it has been reported to cause premature aging[110]. In a model of carbon tetrachloride (CCl_4)-induced hepatic injury, C/EBP directly affected hepatic proliferation[111]. In this study, C/EBPα-S193D was knocked in in young mice that display aged phenotype of C/EBPα, and the authors reported that the aged model showed accelerated liver fibrosis. Moreover, knock-in-induced aging directly altered liver C/EBPα levels. This change in C/EBPα level favored hepatic proliferation, thereby accelerating the disease phenotype. Oxidative stress is also an important hallmark of the liver aging process, and aging hepatocytes are more prone to oxidative damage by hydrogen peroxide (H_2O_2) and do not proliferate well, even when stimulated with epidermal growth factor (EGF)[112]. Further, gadd153(chop) is critical to aging hepatocytes, and it was also found that aging hepatocytes lose the ability to respond to oxidative damage and, in turn, show induction of the proapoptotic gene, gadd153 (chop)[112].

MCs, LIVER, AGING, AND SENESCENCE

Age is a significant determinant of tissue homeostasis and physiology and can modulate signaling pathways independent of other factors. There is little published work on the direct effect of aging on MC maturation, activation, and degranulation, which may be due to contradicting experimental outcomes and tissue specificity of MCs in different models. For example, in BALB/c mice, the dermal MC population reduced in mice from 6 to 10 weeks of age, and this reduction positively correlated with the reduction in c-Kit expression[113]; however, there was no reduction in c-Kit expression in C57BL/6 mice, demonstrating a strain difference. Contradictory to the above finding, it was reported that although prostaglandin E_2 (PGE_2) is not a MC activator in younger mice, in older mice, PGE_2 acts as a potent MC degranulation mediator for dermal MCs[114]. Upon signaling through the prostaglandin E receptor 3 (EP3) pathway, PGE_2 triggers MC degranulation in an aged murine model that was deficient in MCs[114]. When MC density was assessed along with HSC activation in aged (19 months) Sprague–Dawley rats following CCl_4-induced acute liver injury, no significant increase in MC number in the aged rats was reported compared to the younger rats[115]. One probable caveat of this study was that the length of CCl_4 treatment was too short (2–24 h) to induce injury, which might not be enough to induce MC infiltration and subsequent HSC activation via TGF-β1 signaling. However, many recent works have highlighted the role of MCs in modulating the state of biliary senescence during liver disease. MC-derived HA induces biliary proliferation and liver fibrosis and models of cholestasis[116,117]. Knockout of l-histidine decarboxylase (HDC; synthesizes HA) in $Mdr2^{-/-}$ mice showed reduced biliary senescence, HSC activation, and liver fibrosis via the reduction in TGF-β1 and vascular endothelial growth factor-C (VEGF-C) signaling[118]. When HA was reintroduced in these mice, biliary senescence, HSC activation, and liver fibrosis reappeared, thus indicating the role of HA in driving hepatic damage[118]. Further, it was shown that SCF, mainly secreted by cholangiocytes in the liver, induces MC infiltration to the liver and subsequent biliary senescence in $Mdr2^{-/-}$mice[119]. This study directly shows that MCs promote biliary senescence and activation of HSCs, which are key modulators during aging and disease processes. Further, since SCF is a prime chemoattractant for MCs, this study pinpoints the role that MCs play on biliary senescence during liver injury. It has been shown that H2HR is overexpressed in patients with PSC; therefore, in a study using H2HR Vivo-Morpholino treatment in $Mdr2^{-/-}$ mice, the authors found reduced H2HR expression in cholangiocytes, ameliorated hepatic damage, and

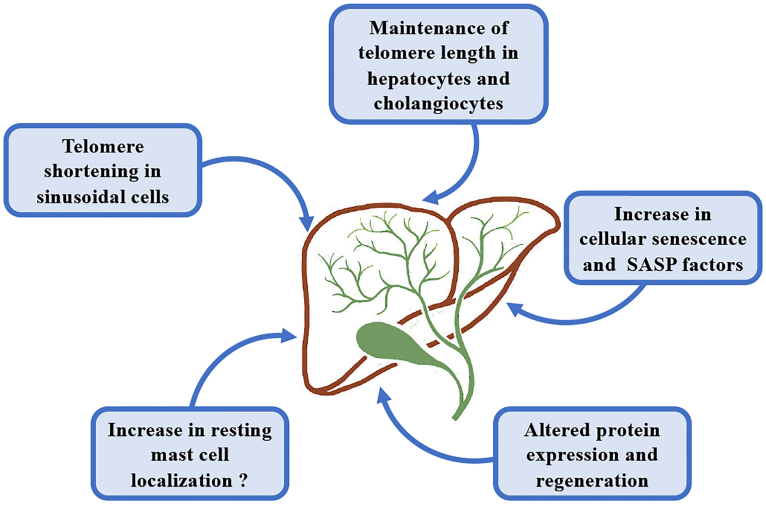

Figure 3. MCs, liver disease, and aging. Changes associated with the aging liver that indirectly lead to alterations in MC localization and the progression to cholestatic liver disease. SASP, senescence-associated secretory phenotype.

reduced biliary senescence confirmed by reduced expression of SA-β-galactosidase expression and $p16^{INK4A}$ gene expression[120]. These studies indicate that MC-derived HA drives cholangiocyte senescence in cholangiopathies that, in turn, exacerbates the disease phenotype via increased biliary SASP. Since in other organs (skin and eye) there is increased presence of MCs during aging, it can be surmised that hepatic HA expression in aged patients suffering from cholangiopathies will increase compared to younger ones. Extensive experimental evidence is required to prove whether MC-derived HA would also cause manifestation of $p16^{INK4A}$-independent senescence phenotypes including telomere shortening and SAHF formation in murine models.

CONCLUSION AND FUTURE PERSPECTIVES

MCs elicit multiple signaling processes that contribute to the pathogenesis of cholestatic liver diseases. Aging affects all organs of the body and independently acts as a risk factor and causation of progressive and degenerative diseases. It has been shown by various independent researchers that aging in the liver causes major changes in hepatic physiology, which accelerates the progression of disease. The response to damage and injury on different hepatic cell populations can be different owing to the fact that various liver cells are derived from different progenitor cells. Thus, understanding the differential responses from the resident liver cells will help us to understand the contribution of each cell type to the aging process. Upon injury, not all hepatic cells become senescent at the same time or contribute to the disease progression, thus complicating this process. However, it can be explained as a collective response that gets worse over the aging life span. The reprogramming capacity of SASPs secreted by senescent cells (and MCs) in its immediate microenvironment contributes to aging and causing morphological changes. The repertoire of present experimental data indicates that MC-induced senescence in the liver is mainly via the activation of $p16^{INK4A}$ in cholangiocytes[101]. Moreover, experimental evidence will be required to show whether hepatic MCs drive the formation of SAHF in cholangiocytes, manifesting the DNA damage, or whether it would cause telomere shortening in cholangiocytes by a putative pathway that would ultimately drive disease progression and aggravate age-related changes.

Since MCs emerge from the myeloid progenitor, elucidating its contribution to liver disease can be key to understanding the effects of immunosenescence and aging in the liver. Some of the roles of MCs in hepatic injury and disease progression have been uncovered; however, much is unknown about the effect of MCs on senescence, and more research is required (Fig. 3). Although it has been shown that the population of T cells shifts from being naïve to more primed memory T cells during aging[121], more experimental details are required to ascertain if the changes in hepatic MC populations have significant effects in altering the resident cell repertoire of the liver that may directly modulate aging.

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