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Review Article

Alcoholic liver disease and mast cells: What's your gut got to do with it?☆

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ABSTRACT

Alcoholic liver disease (ALD) remains one of the leading causes of liver injury and death when left untreated. The gut microbiota has been recognized as a key regulator of a number of pathologies, including ALD. The role of mast cells (MCs) during liver disease progression has been demonstrated in a number of animal models and in human liver diseases. The interaction between the gut microbiota and MCs has been investigated, and links between the gut and these immune cells are being uncovered. The interplay between the gut microbiota and MCs during ALD has been evaluated and studies suggest that there could be an important link between MCs, their mediators and gut inflammation during the progression of ALD.

1. Introduction

Alcoholic liver disease (ALD) is one of the primary causes of liver injury and death with no true treatment except for abstaining from alcohol use.[1] The number of patients diagnosed with ALD has risen sharply in the last 20 years and continues to rise predominantly in the veteran's population of the United States. The pathology of ALD includes steatosis, which is one of the earliest symptoms of ALD and is characterized by an enlarged liver. Furthermore, biopsies showing inflammation in the liver tissue indicate the progression to a more serious state of the disease.[1] In addition, ALD contribute to the development of more dire situations, including cirrhosis and hepatocellular carcinoma (HCC), and there are no Food and Drug Administration (FDA) approved therapies.[2]

The gut microbiota regulates the homeostasis of the majority of organ function, but also can be dysregulated during various pathologies, including ALD. Alcohol, especially excessive alcohol consumption, can alter the delicate equilibrium of the gut microbiota by inducing overgrowth, dysbiosis and increasing gut permeability, affecting not only the structure and function of the gastrointestinal (GI) tract, but also dysfunction of other organ like the liver.[3,4] Certain features of microbiota dysfunction during ALD include decreased gut epithelial junctional tight barriers, mucin production and antimicrobial levels.[5,6] All of these contribute to the destruction of the gut barrier and disrupt the homeostatic presence in the microbiota.

The link between mast cells (MCs) and liver damage/injury has been demonstrated in a number of animal models and human liver diseases including ALD. MCs are regulatory cells that migrate to damaged tissues and/or organs to either be helpful or damaging, depending upon the mediators that they release.[7,8] During ALD, inflammation is dramatically increased and the contribution from MCs (via their release of histamine) may be a key player in this event. Since MCs release a number of factors that can contribute to inflammation,[7,8] blocking MC migration and/or activation can prevent the initiation of this inflammatory response and may be key in alleviating damage to the liver during ALD.

The interaction between the gut microbiota and MCs has been investigated, and links between the gut and these immune cells are

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being uncovered. The interplay between the gut microbiota and MCs has been evaluated during ALD and studies suggest that there could be an important link between MCs, their mediators and gut inflammation during the progression of ALD. In this review, we have described ALD, the gut microbiota and MCs along with highlighting numerous works over the past 5–10 years that emphasize the critical roles of MCs and the gut microbiota in the development of ALD. These studies and future studies are likely to impact the treatment of ALD patients with altered gut microbiota.

2. ALD

2.1. ALD epidemiology and treatment

Alcohol abuse is the fifth leading cause of morbidity and mortality in the world and is the primary contributor to ALD.[2] ALD is caused by excessive alcohol intake and characterized by steatosis (fatty liver), alcoholic steatohepatitis (ASH) and progressive fibrosis.[2,9] Fig. 1 shows hematoxylin and eosin (H&E) staining, and sirius red/fast green staining for healthy liver versus ASH/ALD (with cirrhosis) patient livers.[10] Steatosis, one of the earliest signs of ALD, is primarily characterized by an enlarged liver; biopsies showing inflammation in the liver tissue indicate progression to a more serious disease.[1] ALD can progress to dire complications, including cirrhosis and HCC, which have no FDA approved therapies.[2] The current therapies to ameliorate ALD and/or alleviate its symptoms include abstinence from alcohol consumption, nutritional support, and corticosteroids; however, these treatments do little to combat the disease overall.[9,11] Liver transplantation is an alternative, invasive and costly treatment after all other methods have failed to alleviate the disease and transplants are typically only conducted after the patient alcohol consumption has ceased.[5,12]

2.2. Progression of ALD

Although steatosis is common in ALD, progression to ASH is only seen in 20–40% of ALD patients and 10–15% of those patients develop cirrhosis.[2,13–15] Despite alcohol consumption being the primary inducer of ALD, environmental factors and genetics can lead to the development of ALD.[16–18] Additionally, alcohol consumption patterns are thought to be important in alcohol-induced damage; specifically, it is important to identify the difference between acute and chronic alcohol consumption and their effects on ALD prognosis.[1,19]

2.3. Models of ALD

Animal models are commonly used to mimic and study human ALD.[2] Mouse models of ALD include the chronic *ad libitum* ethanol (EtOH) feeding (Lieber-DeCarli liquid EtOH diet), chronic intragastric (IG) EtOH administration (Tsukamoto-French model), and the Bin Gao chronic-plus-binge EtOH administration model.[20]

In the Lieber-DeCarli liquid EtOH diet, there is progression to hepatic steatosis and inflammation with limited liver injury.[2] While the chronic IG EtOH administration causes severe steatosis, inflammation, and mild fibrosis, it is a labor-intensive and complicated model to adhere to reference 2. The Bin Gao chronic-plus-binge EtOH model simulates the drinking patterns of heavy drinkers and induces neutrophil-mediated liver injury.[2,21] Moreover, this model synergistically upregulates interleukin (IL)-1β and tumor necrosis factor (TNF)-α in hepatocytes and induces neutrophil accumulation in the liver compared to the stand-alone chronic or binge models.[21] This model further displays an increase in proinflammatory cytokines and E-selectin. During the Bin Gao chronic-plus-binge EtOH model, hepatic neutrophil infiltration and injury and elevated E-selectin play an important role, which may

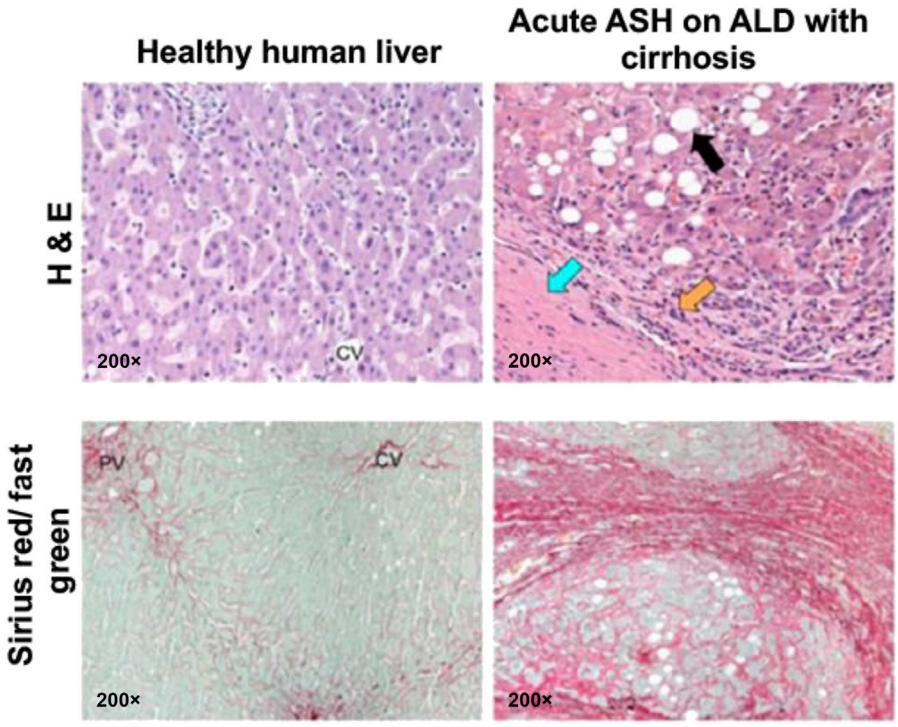

Fig. 1. Liver biopsies from patients with ASH superimposed on ALD and cirrhosis. H&E staining shows significant steatosis (black arrow), inflammation (orange arrow), and cirrhosis (blue arrow) in ASH patients compared to healthy human liver, and sirius red/fast green staining shows total collagen (red) in healthy human lobectomy specimens and liver needle biopsies from patients with clinically proven acute ASH superimposed on ALD and cirrhosis. Adapted from reference 10. Abbreviations: H&E, hematoxylin and eosin; ASH, alcoholic steatohepatitis; ALD, alcoholic liver disease.

contribute to the pathogenesis of early stages of ALD.[21] These models coupled with various gene knockout/overexpression, high fat diets, and other external factors can reproduce the phenotypes seen in humans from alcohol consumption or ALD.[2] Since no FDA approved drugs exist for the treatment of ALD, new therapies are urgently needed.

3. MCs

3.1. MC development

MCs are known to be involved in allergic and immune responses; however, their detrimental effects during liver disease are becoming an area of interest. At their inception, MCs are derived from hematopoietic stem cells found in bone marrow.[22] While in the bone marrow, MCs do not fully mature, but instead circulate throughout the body as immature progenitors.[23] MC development is completed once they have reached connective or mucosal tissues.[23] Stem cell factor (SCF) bound to c-Kit receptors on MC surfaces regulates MC development and *de novo* proliferation.[22] SCF bound to c-Kit on MC surfaces not only regulates the development, but also promotes MC migration.[23] In addition, certain cytokines such as IL-3, IL-4, IL-6, IL-9, and IL-10 can influence MC differentiation.[24]

3.2. MC activation

Involved in the innate and adaptive immune responses, MCs can be activated by toxins, mediators, including proteins from eosinophils and neutrophils, and immunoglobulin E (IgE)-independent and IgE-dependent mechanisms.[25] For example, when IgE antibodies bind to high-affinity FcεRI receptors on the surfaces of MCs, also known as IgE receptors, readjustment of the cytoskeleton occurs, which influences MC degranulation.[26] In addition to FcεRI, MCs bear a large variety of surface receptors allowing them to effectively detect pathogens, and MC response is based on the intensity and combination of the activation of these receptors (Fig. 2).[24]

MCs express Fcγ receptors, and receptors for complement components, cytokines, chemokines and hormones, as well as receptors for pathogen-associated molecular patterns (PAMPS).[24] Toll-like receptors (TLRs) are pattern recognition receptors (PRRs) expressed on human MCs that elicit a spectrum of responses from priming the MC to full activation, triggering release of an array of cytokines and chemokines.[24] NOD-like receptors (NLRs) are another PRRs that interact with cytoplasmic microbial products promoting inflammation and cytokine (IL-1β) secretion via the activation of inflammasome, NLR protein (NLRP). Finally, MCs express cluster of differentiation (CD) 40L, OX40L, CD86, and CD80 which are costimulatory molecules that can inhibit or enhance T cells and B cells.[24]

3.3. MCs and innate immunity

In general, MCs participate in both innate and adaptive immune responses, but they are the main effector cells in the innate response.[24] Since MCs reside at body surfaces (skin, respiratory passages, GI tract, and urogenital tract) that are exposed to the environment, they are usually the first immune cells to encounter antigens. MCs can directly kill the pathogens via phagocytosis or by

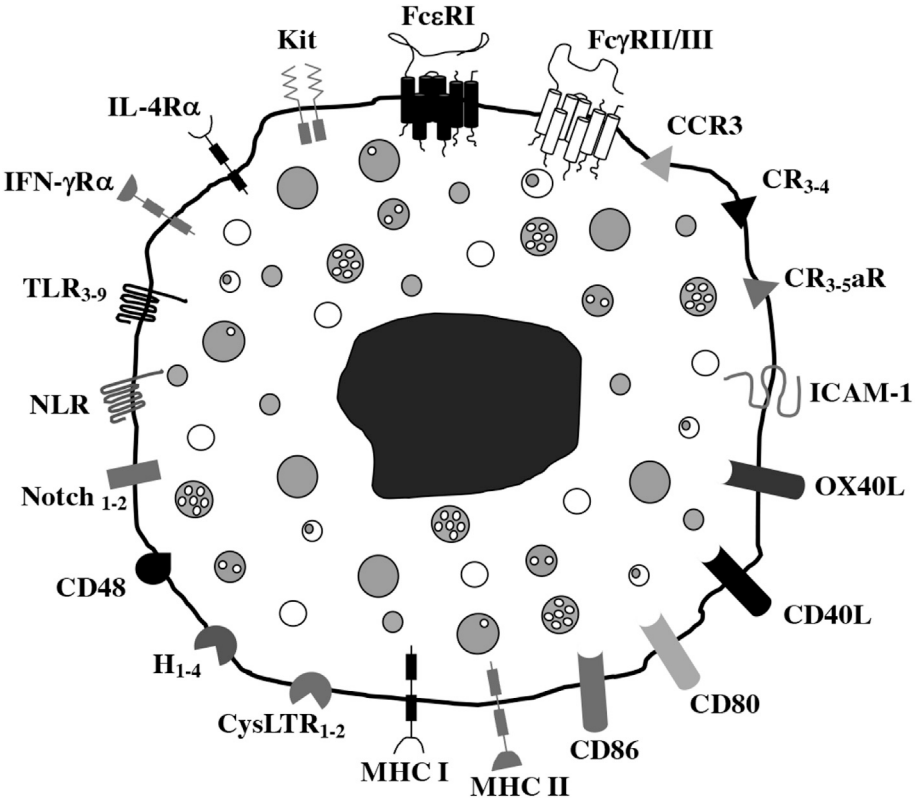

Fig. 2. Human MC surface receptors. Receptors on MCs respond to bacterial, viral, fungal products, Igs, complement components, cytokines and chemokines. When these substances bind to the respective receptors, MCs are activated and recruited to peripheral tissues during infection. Adapted from reference 24. Abbreviations: MC, mast cell; Ig, immunoglobulin; $C_{3\text{-}5}$aR, $C_{3\text{-}5}$ anaphylatoxin chemotactic receptor; CCR3, C-C chemokine receptor type 3; $CR_{3\text{-}4}$, complement receptor type 3–4; CysLTR, cysteinyl leukotriene receptor; H, histamine receptor; MHC, major histocompatibility complex; NLR, NOD-like receptor; TLR, toll-like receptor; IL, interleukin; ICAM-1, intercellular adhesion molecule-1.

weaving extracellular traps (MC extracellular traps (MCETs)) during infection, using deoxyribonucleic acid (DNA), histones, and granule proteins which ensnare the pathogens.[27] The specific trigger for MCET formation is still unknown; however, MCs are the most vital for the recruitment of other immune cells including neutrophils, eosinophils, macrophages, T helper (Th) cells, cytotoxic T cells, and regulatory T cells.[24] MCs accomplish this through the immediate release of histamine and other preformed mediators (such as proteoglycans, serotonin, lipid mediator prostaglandin D2 (PGD2), leukotrienes (LTs), and platelet-activating factor (PAF), the neutral proteases, chymase and tryptase, and pre-stored cytokines like TNF-α followed by a slower secretion of newly synthesized mediators including cytokines, chemokines, and angiogenic factors that regulate the recruitment (Table 1).[24]

While MCs contribute to a physiologically normal immune response, they can contribute to chronic allergic and inflammatory conditions.[22] In such conditions, MCs release the paramount proinflammatory mediators, TNF-α and IL-4.[28] Kim *et al.*[28] investigated the effects of elaeocarpusin (EL), an ellagitannin isolated from elaeocarpus sylvestris leaves, on MCs. This study showed that EL inhibits calcium responses induced by FcεRI, thereby inhibiting MC degranulation, histamine release, and inflammatory response.[28] Based on these findings, EL could act as a therapeutic target for allergic and inflammatory conditions.

3.4. MCs and adaptive immunity

After phagocytosis, MCs can present class I and II major histocompatibility complex (MHC) molecules to T cells and B cells, contributing to memory and adaptive immunity.[20,23] Upon activation via FcεRI receptors, MCs release TNF-α which activates T cells. MCs assist in B cell activation by contributing to the production of IgE, IgM, and IgG_4. However, MC-derived IL-6 could be crucial for B cell growth in B cell neoplasms. In this regard, MC surface molecules have been shown to activate tumor cells such as in Hodgkin lymphoma and Waldenström macroglobulinemia.[24]

4. MCs in liver pathology

4.1. Hepatic MC markers

MCs contribute to liver disease progression via a number of traditional and unconventional receptor subtypes.[29] Using a novel technique for isolating pure hepatic MCs from cholestatic livers, Hargrove *et al.*[29] concluded that isolated hepatic MCs exhibit a similar morphology compared to cultured, activated MCs. Both hepatic and cultured MCs express typical markers: c-Kit, FcεRI, chymase, tryptase, and histamine receptors H1–H4.[29] However, hepatic MCs also express vascular endothelial growth factor receptors (VEGF-R2/R3) and angiopoietin receptors (TIE1/2), which are not traditionally found in cultured MCs.[29]

Table 1
MC-derived mediators and their target cells of innate and adaptive immunity.

Mediators	Target cells
CXCL8, CXCL10, TNFα, GM-CSF	Neutrophils
CCL5, CCL11, LTC_4, PAF, $sPLA_2$	Eosinophils
Histamine, GM-CSF, IL-4, $sPLA_2$	Macrophages
CCL3, CCL4, CXCL9, CXCL10	Th1 $CD4^+$ cells
CCL5, CCL11	Th2 $CD4^+$ cells
CCL2, CCL20	Th17 $CD4^+$ cells
CCL4, CCL5, CXCL10, LTB_4	$CD8^+$ cells
Histamine, TGF-β1	Regulator T cells

Adapted from reference 24. Abbreviations: CXCL, C-X-C motif ligand; TNF-α, tumor necrosis factor α; GM-CSF, granulocyte-macrophase colony-stimulating factor; CCL, C-C motif chemokine ligand; LTC_4, leukotriene C_4; PAF, platelet-activating factor; $sPLA_2$, secreted phospholipase A_2; IL, Interleukin; LTB_4, leukotriene B_4; TGF-β1: transforming growth factor-β1; Th, T helper; CD, cluster of differentiation.

4.2. MCs and cholestatic liver injury

Primary sclerosing cholangitis (PSC) and primary biliary cholangitis (PBC) are cholangiopathies characterized by abnormal growth or loss of cholangiocytes, which are the cells that line the intra- and extra-hepatic ducts of the biliary tree.[8] During cholangiopathies, MCs promote hepatic fibrosis but do not necessarily act alone; in fact, there is evidence suggesting that MCs interact with hepatic stellate cells (HSCs), portal fibroblasts (PFs), vascular cells, and cholangiocytes to promote liver damage.[7,8,22,30]

During proliferation, large cholangiocytes release VEGF and histamine.[31] Indeed, while MCs are the traditional source of histamine, Meng *et al.*[31] showed that cholangiocytes are capable of producing histamine to sustain increased ductal mass. Furthermore, in human cholangiocarcinoma (CCA), increased SCF recruited c-Kit positive MCs.[32] Jones *et al.*[33] found that MCs are recruited to proliferating cholangiocytes in PSC to further promote fibrosis, but the pathway regulating MC migration is unknown. According to Thomson *et al.*,[22] MCs and cholangiocytes work together to contribute to the activation of HSCs, *in vitro*, which contribute to fibrosis by secreting collagen. Furthermore, Hargrove *et al.*[30] reported that collagen deposition is drastically reduced in bile duct ligation (BDL, an experimental model of cholestasis), MC-deficient mice compared to BDL wild-type mice, further supporting the conclusion that MCs are important for HSC activation. To support the hypothesis that MCs promote hepatic fibrosis, when MC-deficient mice are injected with cultured MCs, collagen deposition is increased and fibrosis is further exacerbated.[30]

In the progression of cirrhosis, fibrosis, hepatitis, and different cholangiopathies, hepatic MC number increases.[22] Following BDL, the number of infiltrating hepatic MCs increased almost 10-fold according to Kennedy *et al.*,[8] and the number of MCs positively correlated to increased intrahepatic bile duct mass (IBDM). Similarly, when MC-deficient mice were injected with MCs, by Hargrove *et al.*[30] these MCs were found in close proximity to bile ducts, and IBDM and biliary proliferation increased in these mice when compared to mice injected with 1 × PBS control.

Enhanced MC infiltration increases serum histamine levels, especially during carbon tetrachloride (CCl_4)-induced hepatic fibrosis.[34] The hepatotoxin, CCl_4, induces necrosis in hepatocytes coupled with functional damage (apoptosis) of large cholangiocytes.[34] Consequently, this damage is associated with *de novo* proliferation and the acquisition of large biliary phenotypes by small cholangiocytes to compensate for the loss of large cholangiocyte function. Johnson *et al.*[34] found that after BDL concomitant with CCl_4 treatment, biliary expression of histidine decarboxylase (HDC) was decreased, which also decreased histamine levels. However, when BDL rats or cultured cholangiocytes were treated with CCl_4 and histamine both *in vivo* and *in vitro,* histamine restored CCl_4-induced cholangiocyte damage. Johnson *et al.*[34] concluded that histamine can behave as a trophic and restorative factor in these conditions.

4.3. Histamine signaling

Once histidine is converted into histamine by HDC, it is stored in MCs to later induce stimulatory and inhibitory effects on cellular function via G protein-coupled receptors: H1 histamine receptor (H1HR), H2HR, H3HR, and H4HR.[31,35] Francis *et al.*[35] proposed that histamine stimulates small cholangiocyte proliferation by interacting with H1HR coupled to $G\alpha_q$ mobilizing inositol-1,4,5-

trisphosphate (IP_3)/Ca^{2+} signaling, and histamine promotes large cholangiocyte proliferation through 3′,5′-cyclic adenosine monophosphate (cAMP) transduction pathways mediated by H2HR.

The paracrine function of MCs on biliary proliferation is evidenced by experiments done by Kennedy *et al.*,[8] who reported that the inhibition of MC-derived histamine with cromolyn sodium decreases IBDM and biliary proliferation. Cromolyn sodium is known for its anti-histamine and MC-stabilizing properties, and it specifically affects MCs with no effect on cholangiocytes.[8] Furthermore, Jones *et al.*[33] found that the inhibition of MC-derived histamine decreases biliary fibrosis in *multidrug resistance-2* knockout mice (*Mdr2*$^{-/-}$ mice, an experimental model that mimics human PSC progression). Similarly, according to Johnson *et al.*,[32] blocking MC-derived histamine leads to a decrease in CCA tumor growth, for which patients with PSC and other diseases that lead to chronic biliary obstruction and inflammation are at a greater risk of developing. Further, when H1HR and H2HR (alone or combined) were blocked in *Mdr2*$^{-/-}$ mice, Kennedy *et al.*[7] observed that biliary damage and liver fibrosis were decreased, as well as CCA tumor growth, serum histamine levels, angiogenesis, and epithelial-mesenchymal transition.

4.4. MCs and human leukocyte antigen G (HLA-G)

Non-resident hepatic MCs are involved in tissue remodeling, wound healing, and the progression of hepatic fibrosis.[22] HLA-G is a non-classical MHC class Ib molecule and a Th2 cytokine that promotes hepatic fibrosis.[36] HLA-G is present in both adult and fetus livers, and its best-known function is to protect the fetus from its mother's immune system.[37] However, HLA-G plays roles in certain liver diseases. For example, HLA-G express in liver tumors and high soluble HLA-G levels in HCC inhibit the properties of immune cells (T8 lymphocytes, natural killer cells, B cells, and dendritic cells), thus allowing tumor cells to evade the immune response.[37] Furthermore, Amiot *et al.*[36] reported that in hepatitis C virus (HCV) fibrotic areas, MCs are a source of HLA-G production, regulated by interferon-α signaling. Amiot *et al.*[36] hypothesized that HLA-G could reflect the number of MCs, as well as act as a fibrosis marker.

5. Gut microbiota

5.1. Compositions of gut microbiota

The gut microbiome is a diverse and complex population of microorganisms found within the GI tract. The microbiota colonizing the GI tract is composed mainly of bacteria, dominated by the phylum Bacteroidetes and Firmicutes, and other microbes such as fungi, archaea, viruses, and unicellular eukaryotes.[38–40] These population of microorganisms can interact with one another and have a symbiotic relationship with the host. Microorganisms like those found in the gut microbiome can either be pathogenic or non-pathogenic. Invasive, pathogenic microorganisms are typically recognized and eliminated by the host's immune system. However, the majority of the gut microbiome are non-pathogenic and exist in a homeostatic relationship with the host.[41,42]

5.2. Gut microbial variability and dysbiosis

Gut microbial populations are host specific with interindividual variability due to age, diet, host genetics, antibiotics, health, stress, lifestyle, and alcohol.[20] Despite this variability, a healthy individual can maintain a stable community of microorganisms needed for optimum function. The gut microbiome in a well-balanced host-microbial symbiotic state provides crucial signals that promote maturation of immune cells, leading to protection from infections.[6] The dysbiosis of the gut microbiome, that is the disruption of this stable and diverse community, is associated with many diseases including inflammatory bowel disease and metabolic diseases, such as obesity and diabetes, non-alcoholic fatty liver disease, ALD, and metabolic diseases.[41,43–46] The gut microbiome plays a significant role in not only maintaining the health of the GI tract but also in maintaining overall human health with its contribution to metabolism, intestinal architecture, immune responses, and protecting against the development of inflammatory diseases.[43] Alcohol, especially excessive alcohol consumption, can alter the delicate equilibrium of the gut microbiota by inducing overgrowth, dysbiosis and increasing gut permeability, affecting not only the structure and the function of the GI tract, but also the dysfunction of other organs like the liver.[3,4]

6. The interaction between gut microbiota and MCs

6.1. Role of MCs

The interactions between microbiota and MCs are quite complex. MCs, like the gut microbiota, are involved in the regulation of the health of an individual with its involvement in allergic and immune responses.[44] MCs react to their environment responding to signals that directly and indirectly affect one's health by either inducing or regulating inflammation.[45] As stated previously, MCs play a role in innate and acquired immunity, autoimmunity, as well as a modulator in bacterial infections.[46–48] There are a range of signals to which MCs respond and react, which include signals from the body's microbiota.[49]

6.2. Alcohol, gut homeostasis and MCs

Alcohol suppresses one of the intestine's main lines of defense against harmful bacteria, such as Paneth cells, which secrete antibacterial compounds including α-defensins, lysozyme, secretory phospholipase A2 (sPLA2), angiogenin-4 (Ang4), RegIIIγ, and α1-antitrypsin.[50] The suppression of Paneth cells leads to less secretion of antibacterial compounds, affecting mucosal immunity and leading to bacterial overgrowth, dysbiosis, and increased permeability of the intestinal mucosal barrier.[3,51] A study by Tang *et al.*[51] elucidated the mechanisms of alcohol-induced disruption of tight junction proteins that regulate intestinal permeability by studying the role of both micro ribonucleic acid 212 (miRNA-212 or miR-212) and inducible nitric oxide synthase (iNOS) in alcohol-induced disruption of the intestinal mucosa. Excessive alcohol consumption causes the overexpression of miR-212 and intestinal hyperpermeability.[51] iNOS is upregulated in the intestine by alcohol, and iNOS signaling is required for alcohol-induced miR-212 overexpression, ZO-1 (tight junction protein) disruption, increased intestinal permeability, and steatohepatitis.[51]

The growth and maintenance of the gut microbiota, and subsequently its end products, like short-chain fatty acids, are known to be influenced by alcohol consumption. Alcohol alters the delicate equilibrium of the gut microbiota and can trigger a series of mechanisms that lead to inflammation and organ dysfunction throughout the body, particularly in the liver and the brain.[4] Alcohol can also activate the immune system and upregulate molecules that promote the release of inflammatory immune cells, such as leukocytes and MCs.[18]

Studies have shown increased intestinal permeability in both humans and animals after alcohol consumption by assessing the permeability of the gut and endotoxin plasma concentrations.[52–54] Findings from these studies associated increased intestinal permeability with alcohol-induced endotoxemia and liver injury.

A study by Renga *et al.*[55] identified an interaction between a member of the gut flora, *Candida albicans*, the cytokine IL-9, and MCs. This study showed that the activity of IL-9 and MCs might go beyond host immunity to include the regulation of the microbiota, especially that of *Candida* at mucosal surfaces. The excess bacteria that occur as a result of dysbiosis and their byproducts, endotoxins, triggering the release of proinflammatory cytokines (by the intestines).[3,55] Increased permeability of the intestinal mucosa allows endotoxins and cytokines to enter the bloodstream, be transported to the liver, and interact directly with hepatocytes and hepatic immune cells.[56]

TLRs expressed by cells in the liver detect the endogenous danger signals produced by the gut-derived endotoxin, triggering inflammatory responses.[57,58] As previously stated, MCs are equipped with TLRs, which recognize PAMPs, including lipopolysaccharide (LPS) (endotoxin; recognized by TLR4) and lipopeptides (recognized by TLR1, TLR2, and TLR6) and activate the production of local cytokines (*e.g.* TNF-α and IL-1β), chemokines and lipid mediators that promote inflammation and fibrosis of the liver.[15,59,60]

A study by Szabo *et al.*[60] confirmed the relationship of gut-derived endotoxin and its recognition by TLR4 expressed on innate immune cells and liver parenchymal cells. The study found that mice deficient in TLR4 expression are protected from alcohol-induced liver steatosis, inflammation and hepatocyte injury. Endotoxins also activate Kupffer cells via TLR4 receptors to produce inflammatory cytokines and chemokines (all of which are also released by MCs), which attract neutrophils and monocytes to the liver, promoting damage.[61–63] While these studies do not focus specifically on MCs, it could surmise that the roles of MCs in ALD may be regulated via TLRs.

7. Interactions between the gut microbiota and the liver during ALD

7.1. Alcohol metabolism and intestinal injury

Through oxidative conversion, the body breaks down alcohol to acetaldehyde via the enzyme alcohol dehydrogenase (ADH). Acetaldehyde dehydrogenase (ALDH) converts the acetaldehyde to acetate.[64] The pathway of alcohol, once absorbed, is through the upper intestinal tract and then to the liver through the portal vein.[65] Once in the liver, alcohol metabolism occurs in hepatocytes, which are the target cells in ALD.[65]

The microsomal ethanol oxidizing system (MEOS) is another pathway that metabolizes alcohol, inducing the production of oxygen free radicals, which can lead to cellular damage. The enzymes involved in the oxidative metabolism of alcohol are present in the intestinal mucosa as well as hepatocytes.[64,66] Alternatively, non-oxidative metabolism occurs in the intestines as the alcohol reacts with membrane phospholipids and free fatty acids.[67] Human studies have shown that people with alcohol use disorders have increased intestinal permeability.[66] Overconsumption of alcohol can alter the original and benign microbiota in the gut, increase the permeability of the intestinal wall as well as lead to dysbiosis of gut microbiota contributing to the establishment of ALD. Alcohol disrupts the epithelial cells and the spaces between the epithelial cells in the gut, causing mucosal ulcerations and cell death.[68,69]

As the first line of contact with digested materials, the GI tract is susceptible to damage and toxins, including those caused by alcohol. Overconsumption of alcohol can lead to inflammation of the whole body, and organ dysfunctions and chronic disorders are thought to be a symptom of intestinal-induced inflammation.[70] Metabolism of alcohol can lead to an increased amounts of reactive oxygen species (ROS), epigenetic changes, and inflammatory responses, which are products of this metabolic process.[71] Unfortunately, inflammation is often used as a host defense against infection and injury that can also contribute to increased damage.[72] Fig. 3 depicts the effects of chronic alcohol consumption on the intestinal barrier and subsequent interaction with the liver, as well as the potential role of MCs.[73]

7.2. Alcohol and gut dysbiosis

The effects of alcohol on the gut microbiota are both quantitative and qualitative; small intestinal bacterial overgrowth (SIBO) and dysbiosis are common developments in ALD and overconsumption of alcohol can disrupt the naïve microbiota in the gut.[74,75] These alterations can decrease GI motility and contribute to SIBO and immune responses in the host, causing downregulation of anti-microbial peptides leading to ASH.[75,76] Bacterial overgrowth contributes to the functional and morphological abnormalities of the small intestine found in patients with chronic alcohol abuse.[74] Reg3b and Reg3g are common bactericidal C-type lectins that are down-regulated during alcohol feeding. When treated with probiotics, Reg3g protein levels were slightly restored, which led to a reduction of bacterial overgrowth and ASH.[76]

A study was conducted to determine the changes associated with ALD in the intestinal microbiota. Intestinal bacterial overgrowth was noted after 3 weeks of being chronic IG EtOH administration compared to control liquid diet fed mice. The authors concluded that intestinal antimicrobial molecules are dysregulated after 3 weeks of chronic IG EtOH administration, which contributes to the changes seen in the enteric microbiome and ASH.[76] In another study that investigated intestinal hypoxia-inducible factor (HIF)-1α on ALD, the authors found that HIF-1α induced intestinal dysbiosis and barrier dysfunction and increased serum alanine transferase (ALT) and LPS levels following chronic alcohol consumption when compared to non-treated mice.[77] Gram-negative bacteria are characterized by LPS, and its presence is elevated in patients with ALD.[78] A metagenomic analysis from this study displayed decreased *Bacteroidetes*, a dominant member of the microbiota, and increased gut dysbiosis. The study sought to evaluate the role of HIF-1α and its adaptation to alcohol in the intestinal microbiota.[77] HIF-1α has been shown to protect the intestinal epithelial layer and is essential for adaption to alcohol-induced changes in intestinal microbiota.[77] In addition to alcohol-related mediators, LPS contributes to the overall development of ALD, particularly through increased inflammation and sepsis.[71] The reduction of alcohol-induced steatosis, liver injury, and inflammation has been shown when alcohol-fed rats were treated with antibiotics.[79]

During ALD, the liver is one of the first organs to receive altered bacteria from the gut through the portal venous blood.[65] Rifaximin's effects on the gut-liver axis are currently being evaluated, since this axis has been shown to be increasingly relevant in the progression of ALD.[80–82] Rifaximin is an antibiotic that has a significant impact on gut microbiota when used in animal models. As a broad-spectrum antibiotic, it acts on GI bacteria where it binds to the β-subunit of bacterial DNA-dependent RNA polymerase.[81,82] Currently, no human studies have been done to measure this antibiotic effects on human gut composition, but a clinical trial seeking to use Rifaximin to display its effects on the liver is underway and is due to be completed in 2020.[80]

8. The effects of MCs on ALD

8.1. MCs and liver diseases

Since MCs are known to be involved in liver diseases, their role in ALD could be significant. In the liver, MCs normally reside in low numbers, but during injury MCs increase in number.[22] As previously stated, an increase in MC presence is often accompanied by

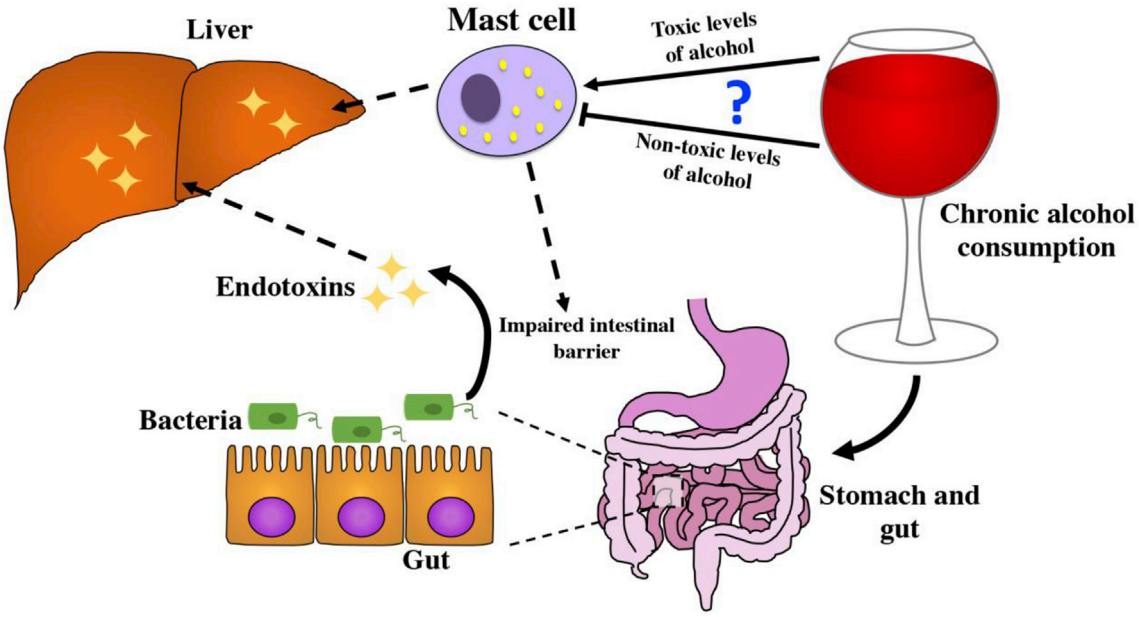

Fig. 3. Gut-liver axis. The hepatic portal system connects the gut and liver. Alterations in intestinal epithelial barrier follow chronic alcohol consumption and expose the liver to structural (endotoxins) and non-structural (toxins) bacteria-derived factors, causing disturbances in hepatic homeostasis. Chronic alcohol consumption may mediate mast cell activation, based on quantity consumed, which can impact intestine and liver damage. Adapted from reference 73.

inflammation and has been shown to contribute to liver pathologies, such as cholangiopathies and fibrotic-driven liver diseases.[29] While cellular action during inflammation can benefit the host in the elimination of pathogens, chronic inflammation can be detrimental since it can lead to fibrosis. It has been proposed that ASH involves the migration of MCs and other immune cells to the damaged liver tissue.[83] Furthermore, chronic activation of the immune response can lead to fibrosis and possibly progress to cirrhosis, chronic liver disease, and eventual liver failure.[83]

8.2. MCs and EtOH

Inflammatory cytokines play a role in the development and progression of ALD. The immune response in ALD is abnormal and imbalanced; for example, pro- and anti-inflammatory cytokines, chemokines, and other cytokines are irregularly released.[84] A recent study exposed bone marrow-derived MCs (BMMCs) to nontoxic concentrations of EtOH *in vitro*.[85] Draberova *et al.*[85] concluded that even a short 15-min exposure to nontoxic concentration of EtOH had an inhibitory effect on the production of the cytokines, TNF-α, IL-6, and IL-3 in BMMCs. Consequently, it can be hypothesized that toxic concentrations of EtOH alter MC immune response leading to detrimental tissue damage. However, lacking current studies on this topic indicate that more investigation needs to be done to support this statement.

Comparatively, Draberova *et al.*[85] and Toivari *et al.*[86] concluded the nontoxic concentration of EtOH inhibited FcεRI-mediated MC degranulation *in vitro*. Toivari *et al.*[86] also determined that after 7 days of exposure to EtOH, there was no change in the amount of MC Fcε-mediated degranulation when compared to MCs exposed to EtOH for only 1 h. After 7 days of EtOH exposure, some of these cells were moved to media without alcohol and their degranulation ability started to resemble that of normal cells.[86] This suggests that the inhibitory effect of EtOH on Fcε-mediated MC degranulation could be reversible.[86]

MCs release inflammatory mediators, such as histamine, from their granules upon the activation of Fcε receptors.[26] While EtOH has been generally assumed to induce histamine release, these studies diverge from this assumption in concluding that when present at nontoxic levels, EtOH inhibits Fcε-mediated degranulation.[26,86] Toivari *et al.*[86] witnessed a modest increase in histamine release and presented two possible explanations for this trend. First, MCs may not have been activated by EtOH itself, but rather by another activating factor possibly increased by EtOH, such as neuropeptide substance P or vasointestinal peptide, two robust MC activators. Second, acetaldehyde may be involved in MC activation.[86] In alcohol-induced asthma, bronchoconstriction induced by acetaldehyde is accompanied by histamine release, suggesting that acetaldehyde induces the release of histamine in MC.[86]

8.3. MCs and hypomagnesemia

It has been shown that chronic alcohol intake frequently induces hypomagnesemia.[87] Takemoto *et al.*[88] found that rats fed with a magnesium-deficient diet had an increase in MCs near the portal triads of the liver compared to the control diet fed rats. These findings demonstrate that hypomagnesemia can induce MC migration to the liver.[88] Thus, EtOH-induced hypomagnesemia could induce MC migration to the liver. However, more experimental data are needed to support this statement. In conclusion, MC migration and activation in ALD have been proposed, but the precise EtOH-induced molecular mechanisms need to be investigated.

9. Conclusions

These studies are just the beginning of what is sure to be an important line of work regarding MCs and the gut microbiota, specifically the role these two play during ALD development and progression. Since inflammation is a key driving force behind ALD, and inflammation of the gut has been shown to regulate liver disease, MCs are likely an important target and potential therapy for patients with ALD. Future work might include studies to target specific MC mediators, including histamine, as target therapies for ALD patients.

Conflict of interest

The authors declare that they have no conflict of interest. This material is the result of work supported with resources and the use of facilities at the Central Texas Veterans Health Care System, Temple, Texas. The content is the responsibility of the author(s) alone and does not necessarily reflect the views or policies of the Department of Veterans Affairs or the United States Government.

Authors' contributions

J. A. Tolefree: study design, researching articles, writing the manuscript, acquiring republished figures; A. J. Garcia: study design, researching articles, writing the manuscript, acquiring republished figures; J. Farrell: study design, researching articles, writing the manuscript, acquiring republished figures; V. Meadows: writing the manuscript, researching articles, editing final product; L. Kennedy: researching articles, manuscript preparation; L. Hargrove: researching articles, manuscript preparation, editing final product; J. Demieville: researching articles, manuscript preparation; N. Francis: manuscript preparation; J. Mirabel: manuscript preparation; H. Francis: study design, researching articles, acquiring republished figures, manuscript preparation, project funding. J. A. Tolefree, A. J. Garcia and J. Farrell contributed equally to this work.

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