Liver immunology and Cross-talk

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ABSTRACT

The primary functions of the liver include metabolic, detoxification and nutrient storage activities. In addition, liver plays a vital role in immunological functions. The human liver, due to its unique anatomical and immunological organisation, is the site of complex immunological activity mediated by diverse immune cells, as well as non-hematopoietic cell populations. Cross-talk refers to the interactions between the various cells. Cross-talk occurs between various parenchymal, non-parenchymal and immunological cells and plays an important role in determining the overall response, in homeostasis and disease. In this review, we discuss the normal homeostasis, the interactions between different immune cells and their role in pathogenesis of diseases. The modulation of these signals can help in development of therapeutic targets in the future.

KEYWORDS: Cross talk; inflammation; immune cells; T cells; B cells; kupffer cells.

Introduction

Liver is the first immunological barrier through which blood from the gastrointestinal tract passes. Apart from the parenchymal cells, the immune cell population consisting of lymphocytes and antigen presenting cells constitutes an important component of the liver and plays a major role in defence against pathogens. The interactions between various immune cells play an important role in determining the overall response in homeostasis and disease. Cross-talk usually refers to an unwanted transfer of signals between communication channels. For example, in electronics, cross-talk is any phenomenon by which a signal transmitted on one circuit or channel of a transmission system creates an undesired effect in another circuit or channel. Similarly, biological crosstalk refers to conditions in which one or more components of one signal transduction pathway affect another. The most common form of cross-talk involves proteins of signalling cascades which are often shared and can consequently interact with either pathway. In this review, we discuss the immune cell distribution in liver as well as cross-talk, and its role in homeostasis and disease.

“Now, why is the stomach surrounded by the liver? Is it in order that the liver may warm it and it may in turn warm the food? This is indeed the very reason why it is closely clasped by the lobes of the liver, as if by fingers.” -- Galen, ca. 200 A.D.
Anatomy of the liver

Even in antiquity, the liver was identified as one of the three principal organs of the body, along with the heart and the brain. The liver is the second largest organ, accounting for approximately 2% to 3% of average human body weight and weighing an average of 1500 g. Located in the right upper quadrant of the abdominal cavity beneath the right hemi-diaphragm, it is protected by the rib cage and maintains its position with the help of peritoneal reflections referred to as ligamentous attachments. The liver has a unique dual blood supply (about 1500 mL/min) both from the hepatic artery (20-40%) and from the portal vein (60-80%). Blood flows from terminal portal venules through hepatic sinusoids into the central hepatic veins. About 30% of the total blood volume passes through the liver every minute so that to approximately 100 million peripheral blood lymphocytes pass by in 24 hours. The high degree of vascularization, slow blood flow through the sinusoids and very permeable fenestrated endothelia allow interactions between the lymphocytes and antigen presenting cells and liver tissue cells, both in homeostasis and in pathological conditions.

Cellular organization of the liver (Figure 1)

Parenchymal cells constitute about 60-80% of the total cell population in liver, and include both hepatocytes and cholangiocytes. The hepatocytes are centric for various metabolic and detoxification processes in the liver.

Non-parenchymal cells consist of intrahepatic lymphocytes, liver sinusoidal endothelial cells (LSEC), Kupffer cells and hepatic stellate cells (HSC).

Lymphocytes, which are among the commonest non-parenchymal cells, are present along the portal tracts as well as throughout the parenchyma. The normal liver contains resident large granular lymphocytes (pit cells) in the sinusoids. These cells enter the liver from the circulation via the sinusoidal endothelium and are retained in the sinusoids where they provide protection against viral infections and tumour cells. The lymphocytes seen in liver include conventional and unconventional lymphocyte subpopulations of the innate (natural killer T (NKT) cells and natural killer (NK) cells) and adaptive immune systems (T and B cells).

Conventional T cells consist of CD8+ T cytotoxic and CD4+ T helper cells, with alpha/beta-chain receptors. CD8+ and CD4+ T cells recognize antigens when presented by MHC class I and CD4+ II molecules, respectively. In the liver, CD8+ T cells are more in number as compared to CD4+ cells and the frequency of effector/memory cells is higher than in the blood. CD4+ cells regulate adaptive immunity in the liver. These cells along with antigen-presenting cells and CD8+ lymphocytes initiate and promote various adaptive immune responses. CD4+ T helper cells are divided into four major subsets, on the basis of their expression profile of transcription factors and secreted cytokines- Th1, Th2, Th17 and regulatory T cells. Th1 cells are characterized by the secretion of IFN-γ, which is a pro-inflammatory cytokine necessary for the activation of macrophages, and is involved in immunity against intracellular pathogens. Th2 cells produce mainly IL-4, IL-5 and IL-13 and play an important role in allergy as well as in the clearance of various extracellular pathogens and parasites. Th17 cells are characterized by the production of their signature cytokine IL-17. They differ from Th1 and Th2 cells in both development and function. The differentiation of Th17 cells needs the combined actions of TGF-β, IL-6, and IL-21 in mice, whereas IL-6 and IL-21 can be replaced by IL-23 or IL-1β in humans. Th17 cells are involved in clearing pathogens during host defence.
reactions and neutrophil recruitment and activation— as commonly seen in alcoholic liver disease, non-alcoholic steato-hepatitis and primary biliary cirrhosis. On the other hand, Th17 cells can also induce anti-inflammatory responses and drive tissue growth and angiogenesis— as seen in hepatocellular carcinoma. Unconventional T cells include CD-1 restricted T cells, MR1-restricted mucosal associated invariant T cells (MAIT cells), MHC class Ib-reactive T cells and γδ T cells. These T cells recognize lipids, small-molecule metabolites and modified peptides. In contrast to conventional T-lymphocytes, unconventional T cells do not interact with the peptide antigens presented by MHC class I or II molecules. These T cells are categorized into two major populations— those that express NK cell markers (called NK T cells), and those that do not. Classical and non classical NK T cells constitute up to 30% of the intrahepatic lymphocyte population and are more abundant in liver than in other organs of the body.

Kupffer cells account for about 20% of non-parenchymal cells in the liver and represent the largest group of fixed macrophages in the body. These cells arise from circulating monocytes, which in turn arise from bone marrow progenitors. Inside the liver, Kupffer cells reside within the sinusoidal vascular space, predominantly in the peri-portal area. They clear endotoxins from the passing blood and phagocytose debris and microorganisms. They also pass though into the space of Disse, making direct contact with hepatocytes and phagocytose apoptotic hepatocytes.

Dendritic cells are bone marrow-derived antigen presenting cells that play a critical role in the induction and regulation of immune reactivity. These cells are primarily found in the portal regions.

Liver sinusoidal endothelial cells (LSEC) are unique endothelial cells, both morphologically and functionally. LSECs constitute around 50% of the non-parenchymal cells in the liver, which comprise about 3% of the total liver volume. These are small cells, with a diameter of around 6.5 μm when isolated. These mammalian endothelial cells are unique since they characteristically have non-diaphragmed fenestrae and lack basement membranes. This characteristic morphology of LSEC allows free bi-directional transfer of solutes between sinusoidal blood and hepatocytes. Furthermore, this also helps in the interaction of immune cells with hepatocytes. Unlike other endothelial cells, they have high endocytic capacity. LSECs represent one of the most actively endocytosing cell types in the body. LSECs are the initial target of injury for some hepatotoxic drugs and toxins and are susceptible to ischemia-reperfusion injury. They mediate clearance of soluble waste macromolecules and colloid material, including blood-borne adenovirus.

Hepatic stellate cells (HSCs) also known as perisinusoidal cells or Ito cells. These cells represent 5-8% of all liver cells and 13% of sinusoidal cells. HSCs are pericytes of the liver in the space between the parenchymal cells and the sinusoidal endothelial cells of the hepatic lobule. Pericytes are unique contractile cells that wrap around the endothelial cells of capillaries and venules throughout the body. Pericytes are embedded in the basement membrane from where they communicate with endothelial cells of the body’s smallest blood vessels by means of both direct physical contact and paracrine signalling. Under physiological conditions, HSCs are involved in the storage of vitamin A, synthesis of extracellular matrices (ECM), matrix-degrading metalloproteinases and regulation of sinusoidal blood flow. In pathological conditions, when HSCs get activated, they lose their lipid-rich granules and trans-differentiate into α-smooth muscle actin (α-SMA)-positive myofibroblasts. This leads to increased formation of ECM and proinflammatory as well as profibrogenic cytokines, thus causing liver fibrosis.

Triggers and mediators of hepatic inflammation

The maintenance of immune tolerance is mandatory during homeostasis. The liver employs mechanisms for rapid immune activation in response to infectious disease or tissue damage. Different triggers have been identified for activating the immune system depending upon the type of liver injury.

Role of Toll like receptors

Toll-like receptors (TLRs) constitute the most important class of Pattern recognition receptors (PRR). TLRs have a
single trans-membrane domain along with an extracellular leucine-rich repeat domain and an intracellular cytoplasmic domain (TIR). In total, 13 TLRs are known to be present in mammals, but only TLRs 1-11 are expressed and functional in humans. Expression of distinct TLRs with highly variable extracellular domains plays a pivotal role in recognizing different pathogen-associated molecular patterns (PAMPs). TLRs detect PAMPs such as bacterial peptidoglycans. TLRs are widely distributed throughout the body and are expressed by endothelial, epithelial, mesenchymal and various immune cells. To maintain homeostasis, it is very important that immune response is triggered only in the context of disease. For this purpose, the activation of distinct TLRs seems to be crucial, and several TLR-mediated mechanisms have been described in the context of liver diseases.

**Alarmins and Danger signals**

Danger signals, also called danger-associated molecular patterns (DAMPs) are target structures, which originate from the body and are released or induced upon damage to the tissue. These signals or mediators are also called as ‘alarmins’ to emphasize that these factors alert the immune system. High mobility group protein B1 (HMGB1) and IL 33 are regarded as classic hepatic alarmins released from injured hepatocytes. Bile acids can also act as irritants that induce inflammation in cholestatic liver diseases. Accumulation of bile acids in the liver tissue triggers production of various pro-inflammatory mediators in liver cells, leading to increased levels of numerous inflammatory cytokines like IL 1β, chemokines (CXCL1, CXCL9, CXCL10, CXCL11, CCL2, CCL5), growth factors (G-CSF, GM CSF) and adhesion molecules (ICAM 1 and VCAM-1). Accumulation of cholesterol in NASH leads to the activation of liver macrophages and HSCs, that promotes the development of steato-hepatitis and liver fibrosis.

**Role of Inflammasome**

Inflammasomes are complexes of multiple intracellular proteins that sense intracellular danger signals. Activation of these intracellular multi-protein complexes leads to pro-inflammatory responses that are commonly associated with caspase 1 activation followed by the secretion of activated IL 1β and IL 18. The most commonly activated inflammasome complex in various liver diseases is NLRP3, which (along with the purinoceptor P2X7) senses ATP81 release following necrotic cell death. Inflammasome activation has been described in several animal studies including paracetamol-mediated injury, obesity-related lipotoxicity, ischemia reperfusion injury, and sterile liver damage after laser-induced heat trauma. Removal of excess ATP and P2X7 blockage has been shown to be hepato-protective, ameliorating inflammation.

Hepatocyte pyroptosis is a specific form of cell death caused by inflammasome hyperactivation that leads to increased liver inflammation and fibrosis development in mice. To maintain normal homeostasis, certain counteracting mechanisms exist that regulate inflammasome activation. For example lactate, a metabolite commonly present in acute organ injury, negatively regulates TLR induction of the NLRP3 inflammasome and production of IL 1β in mouse and human macrophages, and in experimental acute liver injury. Inflammasome complexes mainly respond to passive signals accompanying infections or cellular stress, targeting either factors released during necrotic cell death (dsDNA, histones, ATP), harmful structures such as crystals (uric acid, cholesterol, β–amyloid, asbestos) or microbial components (flagellin, anthrax toxin, muramyl dipeptide).

**Mechanism of liver injury in various conditions**

**Immune mediated liver inflammation model**

Concanavalin A (ConA) and Lipopolysacchride (LPS) first activate immune cells in the sinusoids upon entry into the circulation and induce liver injury through massive production of inflammatory cytokines and chemokines by intra- and extra-hepatic immune cells, followed by intra-parenchymal infiltration of inflammatory cells.
ConA-induced hepatitis

Concanavalin A is a lectin which, when injected intravenously, activates intrahepatic and systemic immune cells. Upon entry into the sinusoids, ConA binds to glycoproteins on the surface of Kupffer cells and sinusoidal endothelial cells. The sinusoidal-endothelial cell barrier is disrupted by ConA, which allows access of cytokines and sinusoidal cells to HSCs and hepatocytes. Inflammatory cytokines produced in this way, along with cells thus recruited in the liver, cause massive liver injury. Among the various inflammatory mediators, TNF-alpha and IFN-gamma are produced in the liver sinusoids—first by Kupffer cells and T-cells and secondarily by HSCs in a paracrine manner. This amplified production of cytokines leads to a series of deleterious events, all of which contribute to massive liver injury. During this inflammatory cascade, PGD2 is produced by Kupffer cells which acts on DP1 of HSCs, and suppresses inflammatory responses to limit the amplification loop of liver inflammation. PGD2-DP1 system in the liver may thus serve as the brake on constitutive hepatic inflammation that would otherwise take place due to continuous external inflammatory stimuli carried into the liver via the enterohepatic circulation.

LPS/GalN-induced hepatitis

Intraperitoneally injected LPS binds to TLR4 expressed in immune cells in the circulation and the sinusoids and activates them, which causes massive hemorrhagic liver injury through enhanced production of inflammatory cytokines and chemokines. Injection of LPS is often given simultaneously with a hepatotoxic agent D-galactosamine (GalN) to inhibit the protein synthesis and to promote cell death in hepatocytes, and this model of hepatitis is termed LPS and D-galactosamine (LPS/GalN) liver injury.

HSCs in ischemia/reperfusion liver injury

Ischemia and reperfusion (I/R) injury is a major complication of liver transplantation that affects clinical outcomes. I/R injury is a biphasic phenomenon whereby cellular damage due to hypoxia and lack of a biomechanical stimulus is accentuated upon restoration of oxygen delivery. The signalling events contributing to local hepatocellular damage are diverse and complex, and involve interactions among hepatocytes, LSECs, Kupffer cells, HSCs, as well as infiltrating neutrophils, macrophages, and platelets.

Hepatocytes are primarily affected by ischemia through mitochondrial damage and altered balances in pH and electrolytes. Most early changes in the anoxic hepatocytes occur in the mitochondria. Since mitochondria are no longer accepting electrons from substrates, a reduction in pyridine nucleotides occurs, resulting in an increase in the intracellular NADH/NAD+ ratio. The abruption of oxidative phosphorylation rapidly leads to cellular ATP depletion, acceleration of glycolysis, increased formation of lactate and alterations of H+, Na+, and Ca2+ homeostasis which altogether induces serious deleterious effects on the hepatocytes. Ischemia also leads to a considerable increase in cAMP, which is an important factor in glucose metabolism. cAMP, through the action of cAMP-dependent protein kinase, leads to the phosphorylation/deregulation of key enzymes involved in the control of carbohydrate metabolism. Endothelial cell damage, disturbance of the microcirculation through up-regulation of a vasoconstrictive peptide endothelin-1 and its receptor endothelin-A receptor, and Kupffer cell activation also ensue. Reperfusion injury mainly involves reactive oxygen species generation by endothelial cells and neutrophils triggered by the re-entry of oxygen into the liver tissue. Reactive oxygen species (ROS) are produced from both intracellular and extracellular sources; mitochondria are the major source in liver cells.

Cross-talk in NASH

NASH is distinguished from nonalcoholic fatty liver by the presence of intrahepatic inflammation accompanied by hepatocyte damage with or without fibrosis on liver biopsy. Increased intestinal permeability is recently highlighted as a primary cause of liver injury in NASH. Gut microbiota can easily flow into the liver through the portal circulation and thereby activate Toll-like receptors (TLRs) in liver cells-TLR4 in particular. In patients
with NASH and in animal models of NASH, serum LPS levels are elevated suggesting the activation of TLR4. Furthermore, TLR4 signalling in HSCs promotes the interaction between HSCs and Kupffer cells by promoting the chemotaxis of Kupffer cells and up-regulating the expression of adhesion molecules in HSCs. Following liver injury, various liver resident cells such as Kupffer cells and HSCs are activated and at the same time, inflammatory cells are recruited into the liver. This leads to an amplification of intrahepatic inflammation and hepatocyte damage progresses further, leading to tissue fibrosis in some cases.

**Cross-talk in viral hepatitis**

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are major causes of chronic hepatitis and cirrhosis. Hepatitis viruses replicate mainly in hepatocytes, causing mitochondrial injury and ROS formation. Viral peptides presented by HLA elicit acquired immune responses from T cells. For example, HCV replicates mainly in hepatocytes. The viral E1 and E2 envelope proteins and viral peptides presented with HLA molecules elicit B- and T-cell host immune responses. Immune cells, particularly T cells and monocytes, are recruited to the infected liver where they secrete inflammatory and fibrogenic mediators. Pro-inflammatory mediators, including MCP-1, are secreted by immune cells, which further stimulate the recruitment of immune cells, thereby amplifying the inflammatory reaction. Myofibroblast activation leads to reduced matrix degradation and production of large amounts of collagen, leading to tissue fibrosis.

**Cross-talk in Hepatocellular carcinoma**

Approximately 90% of hepatocellular carcinoma (HCC) cases arise from an underlying cirrhotic liver. The common causes of cirrhosis include viral hepatitis (HBV and HCV), alcoholic liver disease, and NASH. Activated HSCs residing in the fibrous tissue, along with immune cells, contribute to the formation of a tumour microenvironment favourable for tumour growth. The tumour microenvironment is a mixture of tumour cells, stromal cells, inflammatory molecules and ECM produced from the stromal cells. Activated HSCs in the tumour stroma continuously produce ECM. Dysregulation of tissue inhibitor of metalloproteinases-1 (TIMP-1) which favours matrix deposition and matrix metalloproteinases (MMPs) which degrade ECM leads to increased collagen I deposition in the stroma and contributes to HCC progression. HSCs in their active state also produce soluble factors favouring tumour growth, such as hepatocyte growth factor and TGF-β, and pro-angiogenic factors such as vascular endothelial growth factor-A (VEGF-A) and MMP9. Hepatocytes, in turn, produce inflammatory cytokines and chemokines that promote the survival of activated HSCs. HCC-HSC cross talk is vital in forming a tumour microenvironment that contributes to HCC survival and progression. HCC may also emerge from a non-cirrhotic liver as well such as in a setting of NAFLD. In NAFLD, carcinogenesis has been linked to the secretion of inflammatory cytokines from adipose tissue, lipid accumulation in hepatocytes, and associated hyperinsulinemia. Unlike HCC resulting from chronic viral hepatitis, hepatoma cells in non-cirrhotic HCC are well-differentiated and tumours grow in a large nodular pattern. It has been suggested that HSCs may play only a minor role in this type of HCC, since larger liver nodules may be associated with diminished formation of fibrotic septa, and therefore attenuation of HSC activation. However, a recent study by Yoshimoto et al. associated prolonged activation of HSC and HCC development in obese mice without preceding cirrhosis. They found that obesity alters the gut microbiota and their metabolic products enter the liver by enterohepatic circulation, thus promoting hepato-carcinogenesis through sustained secretion of inflammatory mediators by HSCs.

**Cross-talk in Alcoholic liver disease**

The spectrum of alcoholic liver disease (ALD) includes fatty liver, hepatic steatosis, and the more serious entities of alcoholic steatohepatitis, alcoholic hepatitis, fibrosis, cirrhosis, and liver cancer. Alcohol-related liver cirrhosis was responsible for 0.9% of all global deaths and 47.9% of all liver cirrhosis-attributable deaths in 2010.
There is intensive cross talk among macrophages, Kupffer cells and HSCs in alcohol-related liver injury. Patients with alcohol abuse show quantitative and qualitative changes in the composition of the intestinal microbiome. Furthermore, patients with ALD have increased intestinal permeability and elevated systemic levels of gut-derived microbial products. Maintaining eubiosis, stabilizing the mucosal gut barrier, or preventing cellular responses to microbial products protects from experimental ALD. Therefore, intestinal dysbiosis and pathological bacterial translocation appears to be fundamental to the pathogenesis of ALD.

Chronic ethanol consumption leads to intestinal bacterial overgrowth and dysbiosis. Metabolic changes such as lower bacterial synthesis of long-chain fatty acids (LCFAs) results in smaller amounts of “good” bacteria such as Lactobacillus sp. Some unknown microbial metabolites or products cause intestinal inflammation. While anti-inflammatory properties of intestinal lactobacilli suppress intestinal inflammation during health, reduced amounts of lactobacilli associated with chronic alcohol administration are not able to maintain intestinal homeostasis any longer. Inflammatory cells of the intestinal lamina propria are activated and secrete tumour necrosis factor (TNF)-alpha. TNF-alpha then binds to its receptor TNFR1 on enterocytes, which results in a disruption of tight junctions, partly mediated via myosin-light chain kinase (MLCK). Ethanol and its metabolite acetaldehyde might contribute to dysfunction of the gut barrier.

Microbial products can therefore translocate from the intestinal lumen to the portal venous blood. Translocated microbial products activate hepatic stellate cells and Kupffer cells, and damage hepatocytes. This synergizes with a direct hepatotoxic effect of alcohol and its metabolites to cause progression of ALD. Macrophages critically influence inflammation in alcoholic liver disease. To identify other pathways linking the microbiota to ALD is challenging, but could be a key to better understanding of the gut-liver axis and for designing interventional trials.

Cross talk in drug-induced liver injury

Drug-induced liver injury (DILI) is a major health problem and may present with a broad spectrum of liver manifestations. DILI can be predictable and dose-dependent or can be idiosyncratic. DILI can affect both parenchymal and non-parenchymal cells of the liver, leading to a wide variety of pathological conditions, including acute and chronic hepatocellular hepatitis, fibrosis/cirrhosis, cholestasis, steatosis, as well as sinusoidal and hepatic artery/vein damage. The fundamental process in DILI is the death of hepatocytes (in some circumstances, cholangiocytes or endothelial cells) in the background of inflammation. Idiosyncratic DILI is often mediated by the adaptive immune response. Meanwhile, some drugs and metabolites can directly damage mitochondria, produce ROS and alter signalling pathways. A biochemical stress is usually initiated by drugs and their reactive metabolites, which occurs by covalent binding or direct damage to mitochondria, leading to oxidative stress, activation of stress signalling pathways, impairment of mitochondrial function or endoplasmic reticulum stress. The ultimate cell death pathways converge in the mitochondria, acting either on mitochondrial outer-membrane permeability (MOMP) or mitochondrial permeability transition (MPT). To defend against the hazards induced by drugs, hepatocytes exhibit adaptive mechanisms including upregulation of Nrf2 signaling, mitophagy and autophagy to cope with stress. Finally, the battle between hazardous and adaptive responses determines the outcome - development of severe injury, restoration of the liver after mild injury (so-called adaptation), or no injury at all.

Conclusions

The liver is a unique central immunological and metabolic organ with a high density of myeloid (such as Kupffer cells, neutrophils or macrophages) and lymphoid (such as natural killer cells, T cells or B cells) immune cells. There is extensive cross talk among these cells both in physiological and pathological conditions. As antigen-rich blood from the gastrointestinal tract and gut-derived endotoxins pass through the sinusoids, many mechanisms in homeostasis ensure suppression of innate and adaptive immune responses, resulting in tolerance. In conditions of hepatic diseases, conserved mechanisms such as molecular danger patterns (alarmins), Toll-like receptor signalling...
or inflammasome activation initiate inflammatory responses, resulting in the chemokine-mediated hepatic infiltration of circulating leukocytes. Kupffer cells and monocyte-derived macrophages adapt their phenotypes to local signals and exert critical functions in perpetuating inflammation and wound healing, but can also mediate resolution of inflammation and fibrosis. An understanding of liver immunology and cross-talk can help in developing therapeutic targets in the future.

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