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The Molecular and Cellular Underpinnings of Cirrhosis of the Liver

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ABSTRACT

Cirrhosis is among the leading causes of death worldwide, making up 3.5% of global deaths. It is characterized by the replacement of the liver parenchyma with fibrotic tissue and progressively leads to complete failure of the organ. It is classified into 2 types – compensated and decompensated based on the complications such as esophageal varices, hepatic encephalopathy, asterixis, and ascites. The 2 main contributors of the disease include infection and alcohol use and these, in turn, affect 4 main cell types- Hepatic Stellate Cells, Hepatocytes, LSECs, Kupffer Cells. Commonly observed in patients with cirrhosis, stellate cells are characterized by a change in phenotype often seen in wound healing mechanisms and high proliferation whereas the apoptosis of hepatocytes is a key feature leading to inflammation. The polarization of Kupffer Cells leading to the secretion of proinflammatory cytokines is another key cause of cirrhosis and the change from a fenestrated to a smooth, capillarized phenotype is observed in Sinusoidal Endothelial Cells. The role of cirrhosis on the immunology of the liver is another important topic where changes in the concentration of complement proteins, cytokines can affect the number of dendritic cells and in turn, cause pro- and anti-inflammatory responses. Modern treatment for cirrhosis has been shaped based on these cells, complications, and molecules. In this review we shall discuss all these topics in greater detail along with interesting ideas such as the reversibility of fibrosis as well as the future research on treatments, based on the current findings.

Introduction

Cirrhosis in its simplest terms is the late-stage fibrosis of the liver, often a result of chronic liver disease and can eventually lead to the complete failure of the organ. It accounts for half (1 million) of the total deaths due to liver disease worldwide, and in 2018, it ranked as the 11th leading cause of death, accounting for 3.5% of all deaths worldwide.[1]

There are many causes of cirrhosis, but the main causes include chronic infection of Hepatitis B and C Virus (HBV and HCV), Non-Alcoholic Steatohepatitis (NASH), Chronic Autoimmune Hepatitis and Alcohol Use Disorder. Currently – Infection of HBV and HCV and alcohol use are the largest contributors to this disease. But it is expected in the near future, that with a growth in the number of people with fatty liver disease (steatosis) due to an alarming increase in the rates of obesity (51% of the population of the United States is predicted to be obese by 2030)[2], rates of cirrhosis due to NASH are expected to surpass rates of cirrhosis caused by HCV.[3]

Cirrhosis of the liver can be further broken down into two major types: compensated and decompensated. It is common to notice a progression from compensated cirrhosis to decompensated cirrhosis with the transition being estimated to occur at a rate of 5%-7% per year.[4]The risk of death of a patient with compensated cirrhosis is 4.7 higher than the general population whereas decompensated cirrhosis is 9.7 times as high.[39] The average life span post diagnosis of a patient with compensated cirrhosis is 10-13 years whereas that of patients with decompensated cirrhosis is 2 years.[3]

The key distinguishing factor between the two types lies in the damage to the liver and the surrounding organs. Compensated cirrhosis is generally associated with minimum varices and ascites, whereas decompensated cirrhosis is associated with esophageal varices, hepatic encephalopathy, asterixis, and ascites. These symptoms will be discussed in much greater detail in the next section, specifically delving into some of the major complications of cirrhosis, primarily portal hypertension, ascites spontaneous bacterial peritonitis, hepatic encephalopathy, hepatocellular carcinoma and variceal hemorrhages.

Portal hypertension

Portal hypertension refers to the elevated pressure of the hepatic portal vein which carries blood to the liver. In cirrhosis, scar tissue is formed because of fibrosis. This restricts the blood flow by decreasing the ability of the blood vessel to expand in relation to greater blood flow, which increases the blood pressure. CT scans are commonly used for imaging to check for the presence of enlarged veins that may indicate portal hypertension.[1]

Ascites

Ascites is the build-up of fluid within the peritoneal cavity, resulting in abdominal swelling. It has multiple etiologies including circulatory, vascular, functional, biochemical, and neurohormonal abnormalities. As one explanation, in cirrhosis, the liver is not able to function normally which leads to decreased protein production. This alongside, portal hypertension, causes fluid to leak out of capillaries, and accumulates in the peritoneal cavity, causing ascites.[1]

Spontaneous bacterial peritonitis

This is often a result of ascites that is complicated by bacterial infection of the peritoneal fluid. Infection of the Gram-negative *Escherichia coli* (*E. coli*) is the most common cause of this complication. Common symptoms include fever, abdominal pain, and eventually sepsis- a life-threatening condition in which the body's immune reaction against a particular infection can cause multi-organ damage.[1]

Hepatic encephalopathy

The liver is the primary site of deamination, the process by which the amine group is removed from the carbon exoskeleton in a molecule of amino acid, forming the toxic waste ammonia (NH_3) as a byproduct. During cirrhosis, the damage of the liver, affects the ability of the liver to properly carry out the urea cycle. NH_3 concentration in the blood increases and travels to the brain where it accumulates in the astrocytes. Here it affects the glutamine-glutamate synthesis cycle causing symptoms such as asterixis (flapping hand motion), sleep disturbance, and altered mental status. As it progresses it can worsen and lead to a coma or even death.[1]

Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is also the most common form of liver cancer, often driven by the cirrhotic disease process. At its earliest stages it is asymptomatic, but as the disease advances, it can lead to abdominal pain, reduced food consumption and jaundice. Palpable masses can also be formed and when ruptured may cause fatal peritoneal hemorrhages.[1]

Variceal haemorrhage

Esophageal varices are enlarged veins that develop when blood flow to the liver is blocked by a clot or scar in the liver parenchyma. Blood flow is diverted around these clots to narrower vessels that do not support the larger input of blood volume.[1] This can cause the vessels to rupture and result in internal bleeding, which can be detected by abdominal CT scans.

Fibrosis

Fibrosis is a pathological condition that is caused when collagen-1 fibers get deposited within the sinusoidal region of the liver parenchyma as a response to cell injury. These collagen fibres also tend to replace healthy liver cells with fibrotic tissue and cause scarring, ultimately leading to the formation of nodules in the liver. Fibrosis can be classified into 5 well-defined stages: F0—no fibrosis, F1—portal fibrosis, F2—periportal fibrosis, F3—bridging fibrosis, F4—cirrhosis[5]. Ultrasound elastography is a technique commonly used to detect the levels of fibrosis within a liver.

Cirrhosis as a Hypermetabolic State

Cirrhosis is often described as a hypermetabolic state. Herein, the liver carries out metabolic functions at an increased rate and more energy (high Resting Energy Expenditure) than usual. This means that it requires more glucose and as a result, the glycogen reserves are depleted at a faster rate. Muscles throughout the body face a shortage of glucose and 20-60% of patients with cirrhosis suffer from malnutrition. Although a cirrhotic liver is associated with reduced functioning of the liver and thus, inefficiency to break down amino acids, it is not exactly the case. In fact, it was noticed in patients with acute hepatic encephalopathy that a high protein diet had an improvement in mental status. As a result, protein restriction is avoided in patients and official guidelines recommend a daily protein intake of 1.0 to 1.5 g per kg of dry body weight.[3,6] Late evening or nocturnal meals have also been proven to improve the nitrogen balance in patients without worsening their status of hepatic encephalopathy.[7]

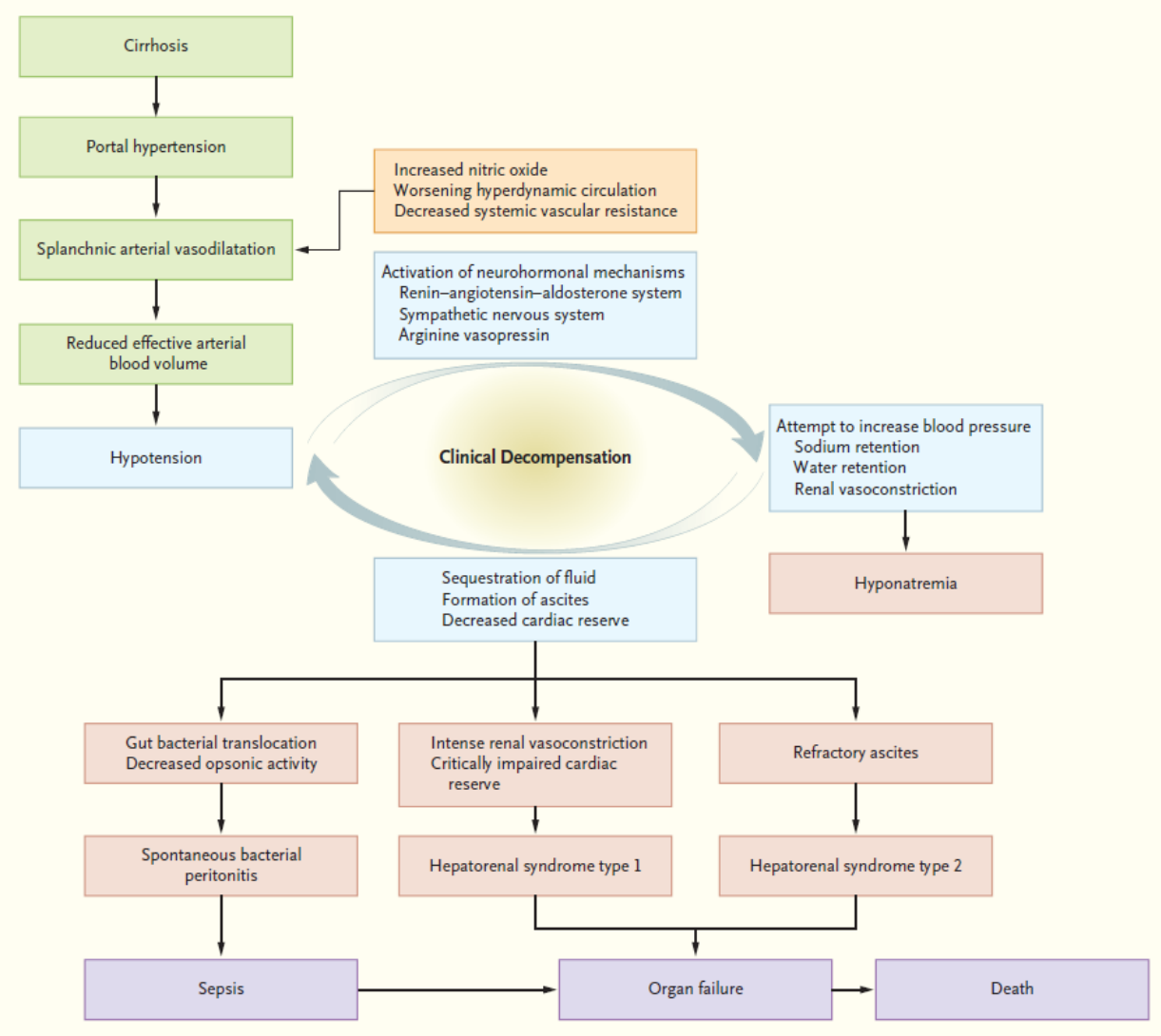

Figure 1. A list of the major complications associated with cirrhosis of the liver [3]

Our next goal is to discuss how various cells in the liver contribute to symptoms of cirrhosis on a molecular level. In this review article, four cell types shall be discussed in particular. These include

- Hepatic Stellate Cells
- Hepatocytes
- LSECs
- Kupffer Cells

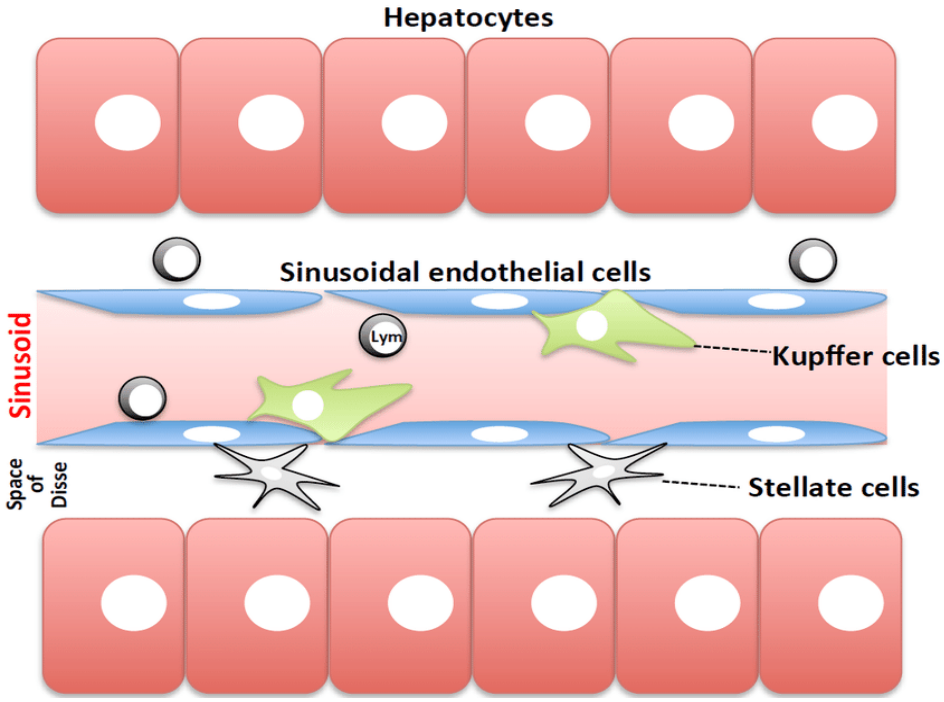

Figure 2. The four main cell types located around the sinusoid that are involved in cirrhosis. Hepatocytes are located directly above the liver sinusoidal endothelial cells (LSECs); LSECs are located on the walls of the sinusoid, Kupffer Cells are located within the sinusoid and Stellate Cells are located in the Space of Disse just below the sinusoid [41]

We will also be discussing immune dysfunction as it relates to cirrhosis pathology on a molecular level, as well as treatments for cirrhosis, and where the future of cirrhosis treatments lies.

Discussion

Hepatic Stellate Cells

Residing in the space of Disse a region between the Sinusoidal Endothelial Cells and hepatocytes containing blood plasma Hepatic Stellate Cells (HSCs) in a normal, healthy liver play an important role in the sequestration of Vitamin A. HSCs are fibroblasts which are cells that secrete collagen fibres to maintain cell structure and are essential in wound healing that are usually in a quiescent (inactive) state.[9]

However, during the initial stages of fibrosis, HSCs have the ability to undergo changes and transform into an activated α-smooth muscle actin (α-SMA) positive myofibroblast phenotype[8] – cell types that are essential for the process of wound healing and wound contraction caused by liver injury. This new phenotype raises the proliferative index of the cells, causing them to multiply more rapidly and become the primary source of collagen fiber production in the liver. Tissue Inhibitors of Metalloproteinases (TIMP) are also produced by the HSC which work by replacing the Extra Cellular Matrix (ECM) with collagen fibres, increasing the rate of fibrosis. By doing so, it disturbs the normal processes within liver parenchymal cells and induces HSC apoptosis.[9]

These cells can also moderate blood flow in the sinusoid and cause the release of pro-inflammatory and profibrogenic cytokines including IL-6, IL-8, MCP-1, and ICAM-1.[9]

The ECM has been found to have a very important role in regulating HSC survival. Collagen-1 fibres are required for HSC proliferation. A degradation in these fibres has been noticed to induce HSC apoptosis. The αvβ3 integrin (receptors by which HSCs bind to the ECM) is also essential for HSC activation.

Growth factors of HSC

Tumour necrosis factor-alpha (TNF-α) shows both anti-apoptotic and anti-proliferative properties for HSC. It can inhibit the expression of the p53 gene (a tumor suppressor protein that is essential to induce cell cycle arrest and apoptosis) thus causing the cells to multiply rapidly and also eventually leading to hepatocellular carcinoma. TNF-α expresses its HSC apoptotic -properties when it reacts alongside cycloheximide (a chemical that can disrupt protein synthesis).[9,10]

Transforming growth factor-beta (TGF-ß) is another important growth factor for the activation +and proliferation of HSC and works in the same way as TNF-α in its anti-apoptotic effect.[10]
Interferon-α (IFN- α) also has been proven to have anti-apoptotic effects in HSCs. It causes a reduction in caspase 3 and 8 activity (proteases essential for triggering apoptosis) and thus prevents G1 arrest.[11]

Leptin is a profibrogenic cytokine (hormones that lead to fibrosis) that is released when HSCs get activated and has been found to mediate HSC survival and proliferation.[12] It works by increasing the number of cells in the S, G2, and M phase of the cell cycle as well as increasing cyclin D1 expression which is essential for the transition between the M and G1 phase.

Transcription factors in HSC apoptosis

NF-κB (Nuclear Factor kappa-light-chain-enhancer of activated B cells) is an important proinflammatory transcription factor (a protein that regulates the conversion of DNA to mRNA). It is made up of different Release factors (Rel factors) which is a protein that helps in ending the process of translation (production of amino acid sequence based on the sequence of mRNA) by reading the sequencing of codons. This in turn helps to prevent the production of proteins that cause an apoptotic effect

NF-κB in activated HSCs is linked to the release of proteins such as cytokines that cause inflammation and are thus described as proinflammatory. NF-κB is also said to have anti-apoptotic effects in HSCs. When cells get apoptotic or proliferative signals, MAPK (MAP kinases; these are proteins that regulate a range of cellular functions, based on extracellular signals). These activated MAPk in turn activate NF-κB which releases cytokines that can be pro-inflammatory and anti-inflammatory. These also help to recruit immune cells to elicit an immune response.

Hepatocytes

Hepatocytes are the main liver parenchymal cells. They carry out most of the important functions of liver metabolism, glycogen storage, drug detoxification, and bile secretion. These cells face the sinusoids that are lined with LSECs and the HSCs. Hepatocytes also work to elicit an innate immune response against microorganisms by secreting innate immune proteins.[13] Hepatocytes also have a long lifespan and multiply for progenitor cells.

Being the site of actions for most of the liver's functions, they are also the target to most of the cellular damage due to hepatotoxic agents such as HCV viruses, alcohol metabolites, and bile acids.[14] This can induce pressure on the regular function of the hepatocytes and can promote apoptosis or cause compensatory regeneration of hepatocytes. The apoptosis of hepatocytes is the key cause of inflammation, fibrosis, and cirrhosis

When treated with CCl4, the free radicals (highly reactive molecules which when accumulated in a cell can lead to a change in DNA sequence) begin to damage the hepatocytes. These hepatocytes produce Reactive Oxidation Species and Damage Associated Molecular Patterns (DAMPs) to cause inflammation. The DAMPS alert nearby macrophages- primarily the Kupffer cells which secrete interleukin-6 (IL-6) to induce proliferation of the remaining hepatocytes. This inflammation stimulates nearby HSCs to secrete Hepatocyte Growth Factor (HGF). Fibrogenic mediators are also secreted by hepatocytes to activate HSCs and cause myofibroblasts to begin producing an ECM of collagen fibres to act as a platform for hepatocyte proliferation. LSECs are also activated by the inflammation and

secrete HGF and Wnt2 to promote liver regeneration. Sema3e produced by the damaged hepatocytes caused contraction of LSECs.[15] necrosis of hepatocytes in mice liver cells was noticed to be induced and hepatocytes began to proliferate for 3 days, reaching its peak on the second day and the sinusoid remodelling is initiated.

Regeneration of hepatocytes

In acute liver injury stem cells are not required for the restoration of damaged hepatocytes. Pro- regenerative factors (hormones to support hepatocyte proliferation) such as IL-6, HGF, Wnt, and TNF are released by Kupffer Cells to cause hepatocyte proliferation in order to replace the lost hepatocytes.[15] But in chronic liver injury, Liver Progenitor Cells (LPCs) are required for liver regeneration.

Kupffer Cells (KCs)

Kupffer Cells (KCs) are a type of macrophage present in the sinusoidal region of the liver that are essential for innate immune response, which is the first defensive response against pathogens. They remove foreign and toxic substances from blood in the portal vein.[16]

KCs also display cell plasticity- they can change their phenotype through polarization as a response to the cellular microenvironment. As a result, they can serve a variety of different functions. Two types of polarization have been noticed -M1 to produce proinflammatory cytokines such as TNF-α; and M2 to express anti-inflammatory mediators such as interleukin 10 (IL-10). [17]

Polarization of KCs

KCs are plastic cells that need to be polarized to a specific activation state and can have different functions as a response to the environment they are in. 2 polarization phenotypes have been observed – M1 polarization (classically activated macrophage) and M2 polarization (alternatively activated macrophage). [17]

M1 polarized KCs have been observed to link to increased expression of proinflammatory cytokines like TNF- α.[18] Whereas, M2 polarized KCs are linked to an increased expression of anti-inflammatory mediators like IL-19. [4,17]The M1 polarization can be elicited due to stimuli such as microbes, damaged tissues, and activated lymphocytes. This can be cancelled out by M2 polarized KCs, which can often cause inflammation resolution and tissue repair.

Binge drinking can lead to increased translocation of LPS which causes M1 polarization in KCs. Levels of M1 and M2 polarized KCs need to be balanced. Otherwise, it can lead to the overproduction of proinflammatory cytokines which can cause hepatocyte injury.

During cirrhosis, especially caused due to Alcoholic Liver disease (ALD), KCs play an important role in leading to Autoimmune Hepatitis. It can affect both the parenchymal and non-parenchymal cells, thus hastening the process of pathogenesis and fibrosis of ALD. Long-term exposure to alcohol can cause intestinal hyperpermeability, resulting in increased levels of the endotoxin – Lipopolysaccharide (LPS). KCs are activated by LPS. As a result, they can release chemicals such as reactive oxygen species (ROS), tumor necrosis factor α (TNF -α), and interleukins to elicit an immune response which works by halting the functions of the adjacent cells, in turn – leading to liver injury. [17]

In patients suffering from ALD, it was observed that the number of activated KCs in the portal tract increased due to the high levels of LPS. LPS is the main component of the cell membrane of Gram-negative bacteria.[19] Chronic alcohol exposure can increase intestinal permeability by removing tight junctions, letting LPS through the intestinal epithelium in very small amounts. Chronic ethanol consumption can also cause the liver to favor the overgrowth of

the Gram-negative bacteria and also increase the levels of LPS as well as putting the patient at risk for Spontaneous Bacterial Peritonitis (SBP).

LPS activates KCs by activating the Toll-Like Receptor 4 (TLR-4) by forming a complex (a group of molecules that work together to serve one function) between LPS-binding protein (LBP), cluster of differentiation 14 (CD14), and myeloid differentiation factor 2 (MD-2). LPS is transported to CD14 by LBP and then binds with TLR-4 .[17] After undergoing oligomerization Tlr-4 triggers the Myeloid differentiation primary response gene 88 (MyD88) and toll-interleukin-1 receptor – both are proteins used for cell-to-cell signaling – producing proinflammatory cytokines and type 1 interferon (IFN).

Chronic alcohol use can increase the amount of LPS within the sinusoidal blood. This causes the KCs to become activated and secrete more TNF-α and other pro-inflammatory cytokines than normal levels. Chronic alcohol usage can cause oxidative stress (a disturbance in the balance between the production of reactive oxygen species and antioxidant defences)[20] which results in an increased production of reactive aldehydes such as malondialdehyde (MDA) which can react with proteins forming protein adducts leading to apoptosis

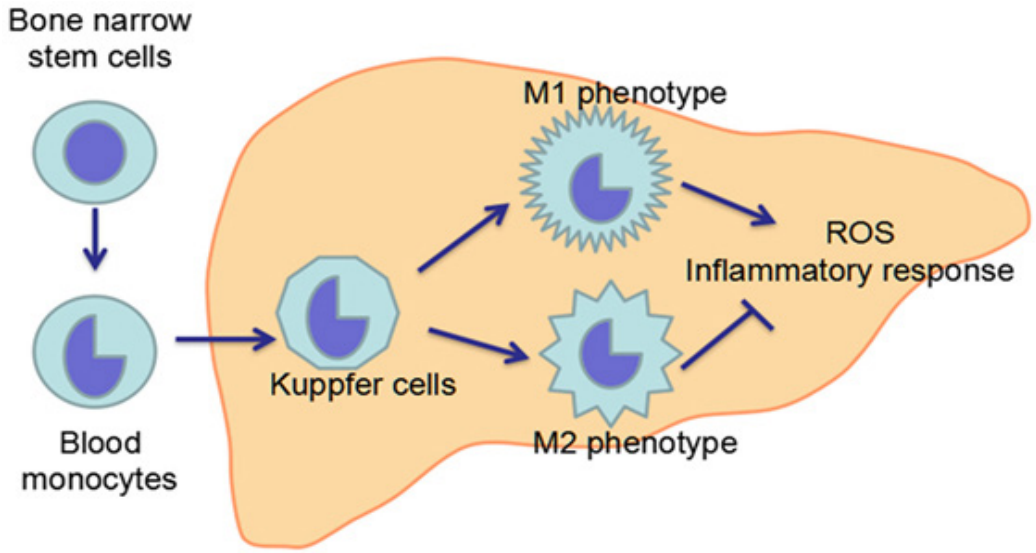

Figure 3. The polarization of Kupffer Cells (KCs) [40]

Inflammasome and IL-1β in relation to KCs

Inflammasomes are multiprotein complexes within a cell which when activated can elicit cell inflammation. They are only activated during infection and activate caspase-1 and proinflammatory cytokines. Inflammasome activates pro-caspase-1 generating IL-1 β which leads to inflammation through the IL-1 receptor. It was observed that in patients with ALD, there was an increased level of IL-1β than normal. This activates more KCs to produce an inflammatory response which is key in cirrhosis. [21]

Role of KCs in Liver Damage due to binge drinking

Some experiments have shown that KCs may play a crucial role in liver injury induced due to binge drinking (acute ethanol consumption). Binge drinking occurs when 5 and 4 drinks are consumed by men and women respectively in 2 hours, eventually leading to a blood ethanol level of over 80 mg/dl or 6g/kg body weight. [17]

Binge drinking then leads to an increase in the serum endotoxin (LPS) levels in the blood. The Endotoxin Neutralizing Protein released as a response caused an increase in TNF- α level. In mice livers treated in ethanol at 6g/kg of their body weight at intervals of 12 hours, the mice developed hepatic steatosis and a hepatic enterobacteria level. [17]

LSECs

Liver Sinusoidal Endothelial Cells (LSECs) line the wall of the sinusoid and are separated from hepatocytes by the space of Disse. They are described as partially permeable barriers to filter out viruses and other foreign particles as well as waste macromolecules and particulates from the blood in the sinusoidal region. They are able to achieve this function due to the presence of fenestrae found on sieve plates, making them unique from capillary endothelial cells. The number and size of sieve plates are dependent on the chemical agents and the liver disease. They also play an important role in controlling lymphocyte adhesion and HSC quiescence.[22,23]

During the initial stages of fibrosis, eventually leading up to cirrhosis, the LSECs undergo a major phenotypic change that restricts most of their functions. They undergo "capillarization" wherein they lose their fenestrations and form a continuous basal membrane- in short, they start having a phenotype similar to ordinary capillaries. LSECs also begin to develop pro-inflammatory and pro-fibrotic features.

Capillarization has only been observed in the S2 stage of fibrosis due to HCV infection in livers. This is caused due to the exclusion of the liver X receptor alpha (LXRα) protein. [24]

TEM

The ultrastructural features of LSECs can be studied through Transmission Electron Micrographs (TEM), which provide a detailed view of the internal cellular features. In a study conducted on liver samples collected from patients infected with HCV, fenestrations were rarely observed. Along with this, the presence of fibrins and cellular debris was noted in the sinusoid alongside an increased activity of phagocytes. Cellular inflammation was also noticed in HCV-positive liver samples, leading to discontinuities (gaps) in the lining.[22]

CD31, CD32 staining

The phenotype of LSECs can also be recognized through the process of immunohistochemistry by using the protein markers - CD31 and CD32. [22]

CD 31 is noted as a protein marker of cells with a vascular phenotype whereas CD32 was a marker of LSECs. In normal liver samples, it is noted that fenestrated LSECs express CD31 on their cell surface membrane and CD 32 was often found in the cytoplasm. However due to the vascular phenotype as a result of capillarization, it was noticed that CD 31 showed a positive reaction i.e., it was found within the cytoplasm in liver samples infected with HCV and CD 32 was located on the cell membrane. The same result can also be observed sometimes in normal LSECs. [22]

Caveolin-1 (CAV-1)

CAV-1 is a cholesterol-binding protein present in the walls of fenestrae in LSECs and thus laser scanning confocal microscopy can be used to localize it. In normal liver cells, its presence was mainly restricted to the sinusoidal walls and was spread uniformly. But in the liver sample of HCV-positive patients, CAV-1 maintained its positivity, however, it was lower and irregular along the endothelial layer due to the morphological changes in the cells. An overexpression of CAV-1 has also been observed in late-stage cirrhosis. A possible explanation could be due to the high levels of cholesterol. The presence of CAV-1 was detected only in the cytoplasm or the nucleus. The localization of CAV-1 in the nucleus could be so, in order to serve the function of gene regulation. [22]

Treatments

Multiple treatments have been focused on reducing the disruption in function of the aforementioned cell types and lessening the further complications of cirrhosis. As a result modern methods are available in the form of medications and surgeries. Recent research has also helped to find out the reversibility of fibrosis

Medications

Analgesic Agents: Low dose aspirin can be used in patients with cirrhosis who have acute cardiovascular diseases in order to prevent the formation of blood clots due to the increased levels of thrombin and factor VIII (procoagulant factor) with reductions in protein C (anticoagulant factor) levels in the blood.[2]However, overconsumption of analgesic agents or aspirin in patients with cirrhosis can lead to aggravated acute renal failure and gastrointestinal bleeding. Thus, they must be carefully selected.

Statins: Statins are relatively safe to consume for patients with cirrhosis as well as cardiovascular ailments caused as a result of Non Alcoholic Steatohepatitis.[1] They are also important to prevent the buildup of cholesterol in blood vessels- whose adverse effects can lead to conditions such as atherosclerosis.

Vaptans and diuretics: Vasopressins are antidiuretic hormones that are important for maintaining the osmotic levels within the body by reabsorbing water into the body in the kidney tubules. Vasopressin V2 receptor antagonists (vaptans) are used to prevent this from happening. They work by increasing the number of aquaporins on the walls of the Loop of Henle, increasing water reabsorption. Thus, these drugs are specifically used to treat ascites.[1] Diuretics are also used for the expulsion of water in the form of urine. They act on the loop of Henle and inhibit the Na-K-2Cl ion channel. Loop diuretics are used to treat hyponatremia (low sodium levels) by preventing the reabsorption of sodium (Na+) and chloride (Cl-) ions, which helps maintain the osmotic potential of the blood. As the concentration of ions in the Loop of Henle is high, water remains and passes through the nephrons and collects as urine in the bladder.

Curcumin: Curcumin is a popular chemical found in the spice turmeric and is used to induce apoptosis on activated HSCs. Peroxisome proliferator-activated receptor- γ (PPAR- γ) is one of the primary receptors involved in HSC activation. Curcumin works by downregulating the function of the PPAR- γ receptor and reducing the production of collagen-1 fibres.[9] Curcumin also works by inhibiting TGF-ß receptor expression which induces apoptosis. By inhibiting HSC activation, the production of proinflammatory cytokines and chemokines to activate immune cells is reduced, thus reducing the effects of cirrhotic inflammation.

Lactulose: Lactulose is an oral drug that works on the large intestine of a patient and is used to treat Hepatic encephalopathy (HE). It is metabolized in the colon by bacteria to form organic acids which in turn reduces the pH. The ammonia (NH_3) produced by the colonic bacteria can be absorbed by cells. The low pH, however, converts the ammonia (NH_3) to ammonium ion (NH_4^+). Being an ion, NH_4^+ cannot be absorbed into the colon cells due to the absence of ammonium ion channels, and as such is excreted in feces.[27]

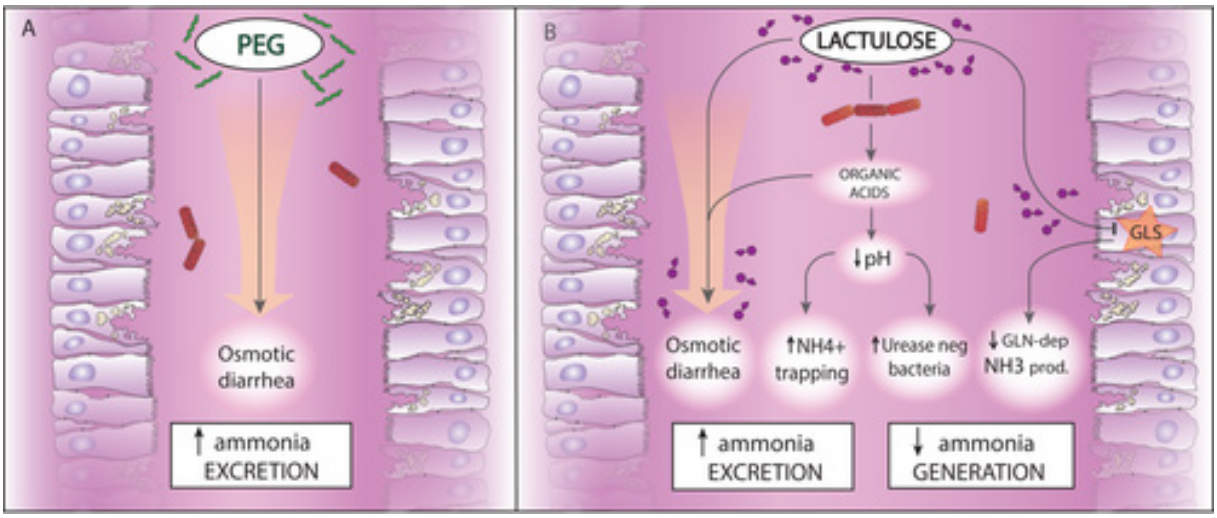

Figure 4. The difference in the action of PEG (polyethene glycol) and lactulose. (A) PEG, is a nonabsorbable chemical that increases the total amount of ammonia eliminated through the feces by directly working on the osmolarity of water whereas (B) Lactulose is also a nonabsorbable chemical that increases ammonia excretion by working on lowering the pH to trap NH4+. Note that in many cases PEG was found to be more useful at ammonia excretion than lactulose. [27]

Rifaximin: Rifaximin is an antibiotic that has been specifically targeted for the treatment of Irritable Bowel Syndrome (IBS) as well as HE. It is poorly absorbable into the bloodstream and thus remains in the gut.[28] It binds to bacterial markers and kills gut bacteria, reducing ammonia production.

Surgeries

Cirrhosis (mainly decompensated) acts as an obstacle for carrying out intra-abdominal and other invasive surgeries. The risks and benefits of a procedure should be carefully calculated before recommending surgery.
Liver transplants cannot be recommended for every single patient due to the high probability of mortality and morbidity as well as the shortage of readily available and suitable donor livers. They are only recommended at the stage of complete liver failure. Thus, non-transplantation routes must be recommended to the majority of patients with cirrhosis.

The Model End-Stage Liver Disease (MELD) score is used to calculate the chances of mortality of a patient undergoing non-transplantation surgeries.[29] The MELD score is on a scale from 6 to 40 with an increase in the scores suggesting a more advanced stage of liver disease.

Endoscopy: Endoscopic procedures are among the most common and safe procedures for treatments of patients.[1] This can include a simple screening of the damages to the liver to Rubber Band Ligation Systems to bond esophageal varices. However, in patients with ascites, percutaneous endoscopic gastrostomy (PEG) – a procedure through which a feeding tube is passed to the stomach directly through the abdominal surface as a means to pass food -is avoided.[34]
Paracentesis: Paracentesis is another relatively safe procedure that is especially useful for patients with ascites. The procedure broadly involves the extraction of ascitic fluid from the peritoneal cavity. This is especially useful to detect the neutrophil count in ascitic fluid and give an appropriate diagnosis of bacterial peritonitis. It can also be useful in the removal of ascitic fluid to help prevent further complications.[1]

Embolization: Embolization is a procedure used during the rupture of hepatocellular carcinoma. In this procedure gelatin sponges and beads are used to block the ruptured blood vessels.

Liver Transplantation: This is a very risky procedure due to the chance of post-transplant rejection. As mentioned before, this procedure is only reserved for end-stage liver failure. Several ethical considerations need to be taken to assign a patient (from a list of thousands) for liver transplantation. When approved, the procedure can take place. Post operation, the patient is placed under immunosuppressors to inhibit the action of the immune system- to reduce the chances of organ rejection.[31]

Treatment of HSC for the reversibility of fibrosis

For many years fibrosis of the liver had been considered to be an irreversible process. But with an increase in experimentation, evidence has been collected to prove the reversibility of the disease. Liver biopsies of patients with Hepatitis C treated with pegylated interferon and ribavirin have been used as evidence to prove the reversibility of the condition.[9]

Another experiment conducted on rat livers treated with carbon tetrachloride (CCl_4) (a chemical that induces similar levels of fibrosis as that of a cirrhotic liver), showed that after ending the treatment with the chemical, the livers were able to return to normal histology. This experiment thus proved that HSC apoptosis was important for the treatment of this condition.[25]

The inhibition of the αvβ3 integrin using antibodies or inhibitory RNA results in a lower Bcl2/Bax ratio (a chemical ratio to determine the susceptibility of a cell to undergo apoptosis) and an increase in caspase-3 activity which in turn induces HSC apoptosis. This is mainly because integrins are essential for the transmission of survival signals in activated HSCs. The recombinant Matrix Metalloproteinase-9 (MMP-9) causes HSC apoptosis by damaging the ECM.[9]

The Molecular Immunology of Cirrhosis

The innate immune system of the liver is composed of complement proteins, Pattern Recognition Receptors (PRRs), dendritic cells, and Natural Killer (NK) cells. These are the components present in the body upon birth and the first line of defense from pathogens. The main function of the innate immune system is to present processed antigens to the adaptive immune system to attack pathogens and elicit an immune response. Adaptive immunity is composed of B and T lymphocytes and are able to retain a memory of the antigen-antibody response and is thus, long-lasting.[32,33] Liver cells have both membrane-bound and cytoplasmic innate immunity components that recognize Pathogen Associated Molecular Patterns (PAMPs)[34] and Microbial Associated Molecular Patterns (MAMPs)[35] released by microorganisms; and Danger Associated Molecular Patterns (DAMPS)[36] released during cellular damage to elicit an innate immune response.[32] PRRs are present in immune and liver parenchymal cells and serve the purpose of recognition of pathogens and in turn activate complement proteins, cytokines, and dendritic cells. This as a result causes pro- and anti-inflammatory responses.

Kupffer Cells (KCs) are the main macrophages present in the liver and are antigen-presenting cells that involve the Major Histocompatibility Complex-II (MHC-II) which is the method by which processed antigens are presented to T–lymphocytes of the adaptive immune system. As discussed previously, KCs when activated release cytokines and Reactive Oxidation Species (ROS). KCs express TLR2 which concentrates at the hepatocytic plasma membrane. KCs have a flexible plasma membrane that is adapted for the phagocytosis of pathogens, and also have a role in taking antigens and presenting them to immune cells.[33]

Immunological dysfunction in cirrhosis

Cirrhosis affects both the adaptive and innate immune system and is termed as Cirrhosis-Associated Immune Dysfunction (CAID) which can lead to immunodeficiency and systemic inflammation. It is often linked to an increase in bacterial translocation.[32]

Innate immune dysfunction

Toll-Like Receptors are a form of PRRs and play a major part in innate immune dysfunction. In cirrhosis, due to prolonged exposure to bacteria, and as a result of their related PAMPs, TLRs can undergo activation and is ultimately linked to a loss of tight junctions (proteins between cells that are used to create an impermeable barrier) and a widening of intercellular spaces through which but flora can enter and enhance Alcoholic Liver Disease.[32] TLR-2 and -4 (components required for the activation of NF-kB)are impaired during cirrhosis caused due to Hepatitis B infection. In the case of Hepatitis B infection caused due to the presence of the hepatitis B e antigen (HBeAg), the has been a decrease in TLR-2 expression whereas the opposite is seen in Hepatitis B tested negative for HBeAg.[34] Decreased TLR-2 function has only been observed in the early stages of cirrhosis. But as the disease progresses to advanced cirrhosis, the TLR-4 expression also decreases.[32]

Adaptive immune dysfunction

Alcoholic Liver Disease (ALD) is said to damage T and induce hyperactivity in B-Lymphocytes, both of which are a key part of the adaptive (acquired) immune system. Th1 lymphocytes express antifibrotic cytokines and Th2 lymphocytes express profibrotic cytokines (Th- Helper T lymphocytes).[32] In order to maintain a healthy liver, there needs to be a balance in the Th1/Th2 lymphocyte ratio. In cirrhosis, Th2 lymphocytes are expressed more and as a result, $CD8^+$ levels increase, decreasing the $CD4^+/CD8^+$ ratio, thus causing the production of fibrotic bands.[38] Increased levels of $CD4^+$ and $CD8^+$ lymphocytes increase the level of apoptosis markers such as $CD95^+$, causing more cell death than normal patients.

Cirrhosis on the Complement system

The complement system comprises complement proteins that are mainly found in hepatocytes. They play an important role in innate and adaptive immunity. Opsonin is an important antibody produced by B lymphocytes. In cirrhosis, opsonin levels are reduced and the complement protein C3 also faces a lowered expression, increasing the risk of bacterial infection.[32] In an experiment, the role of the complement proteins C5 and C5aR also has been proven to be key in the process of fibrosis. Normally, the complement protein C5 is broken into C5a which attaches itself to the C5aR (R - receptor) on the pathogen which tags it and alerts immune cells to destroy it. In cirrhosis, the C5 protein has a lower expression, thus the binding of C5a and C5aR1 was reduced, causing an increase in the risk of bacterial infection.[38]

Future directions

Reversal of fibrosis

Fibrosis as defined previously is a pathological condition wherein collagen I fibers are produced by HSCs and get accumulated in the sinusoidal region.

Collagen is the most abundant protein found in animals. It forms the essential basis for the formation of hair, bones, and the binding component of the skin tissue. At a cellular level, it also forms the backbone of the ECM thus,

helping to provide support to tissues and allow intercellular communication. They are rope-like proteins essentially made up of three polypeptide chains wound together. There are four different types of collagen fibres (aptly named – Collagen I, Collagen II, Collagen III, and Collagen IV), but for the purpose of this research paper, we shall primarily focus on Collagen I.[9]

Collagen I is also the most abundant type of collagen found in the body. The most dominant isoform of collagen I is the heterotrimer (a biological molecule made up of 3 different monomers) of two α1(I) and one α2(I) chains. But in the case of lesions formed as a result of fibrosis, the collagen present is a homotrimer of (a biological molecule made up of 3 chains of the same monomer) three α1(I) chains. The formation of these collagen fibres is harmful as it can Alter the liver's structure and can restrict blood flow to the liver.

Collagenases are a type of enzyme that specifically target the digestion of different types of collagen proteins. Although forming an essential component of the cell structure of the liver parenchymal cells as a whole, collagen fibers need to be broken down to prevent the progression of fibrosis and further damage to the liver. Research on the usage of collagenase 1 to digest collagen I fibres could be done in the future to specifically target the breakdown of the harmful collagen component in fibrotic bands in the liver, taking caution not to destroy or disrupt the rest of the cells or the ECM.

Targeted Therapy

Targeted therapy has been widely used for the treatment of cancer in general. In this procedure, chemotherapeutic drugs such as alkylating agents, mitotic inhibitors, and antimetabolites are taken in intravenously through injections or are delivered directly to the site of the tumor via surgery to help suppress its growth

In a similar fashion, antifibrotic components can be directed to specifically target the damaged or injured parts of the liver. For instance, gliotoxins that are essential for triggering HSC apoptosis can be directly delivered to the site of injury. Gliotoxins, due to their high apoptosis-inducing character, can be encapsulated lipoprotein complexes with an affinity to bind to the TNF-alpha receptor of an activated HSC and in turn trigger HSC apoptosis. By doing so, only the activated HSCs undergo apoptosis and the other quiescent HSCs are unaffected by it. Note that intra-abdominal surgeries are not a viable option for patients with ascites. Endoscopic treatments have been found to be relatively safe for most patients with cirrhosis and thus can be used to deliver drugs to the liver.

Immunology

The complement system is an important part of the immunology of the liver which needs to be given the focus on for the future treatment of cirrhosis. Opsonin, which was mentioned previously is an important antibody that binds to the surface receptors of bacteria and other pathogens to alert immune cells for phagocytosis.[32] However, in studies conducted on ascitic fluid, a reduction in opsonin was discovered and as a result, cirrhosis becomes a proliferative state for bacteria, causing conditions such as Spontaneous Bacterial Peritonitis and Ascites. It is necessary to research treatments that link to the opsonization of bacteria. One such method that was discovered showed that opsonic activity was enhanced on heating and on freezing and thawing. Integration of such methods in treatments can be key to the resolution of SBP.

Conclusion

In this review, we had discussed the causes of cirrhosis at a cellular level paying attention to HSCs, LSECs, KCs and hepatocytes and their relation to fibrosis and other complications of cirrhosis such as SBP, Ascites, and HE. We also discussed the role of the immune system on the progression of the disease paying attention to the complement system,

PRRs and other lymphocytes. Future directions such as possible factors to revert fibrosis; opsonization of bacteria and targeted therapy, were mentioned.

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References

1) Asrani SK, Devarbhavi H, Eaton J, Kamath PS. "Burden of liver diseases in the world." *J Hepatol*. 70, no. 1 (2019): 151-171. doi: 10.1016/j.jhep.2018.09.014.
2) Finkelstein, E. A., Khavjou, O. A., Thompson, H., Trogdon, J. G., Pan, L., Sherry, B., & Dietz, W. "Obesity and Severe Obesity Forecasts Through 2030.". *American Journal of Preventive Medicine*. 42, no. 6 (2012): 563–570. doi: 10.1016/j.amepre.2011.10.026.
3) Ge PS, Runyon BA. "Treatment of Patients with Cirrhosis". *N Engl J Med*. Aug 2016: 767-777. doi: 10.1056/NEJMra1504367.
4) Samonakis, Dimitrios N et al. "Clinical outcomes of compensated and decompensated cirrhosis: A long term study." *World journal of hepatology* 6, no. 7 (2014): 504-512. doi: 10.4254/wjh.v6.i7.504
5) Axley Page, Mudumbi Sandhya, Sarker Shabnam, Kuo Yong Fang, Singal Ashwani "Patients with stage 3 compared to stage 4 liver fibrosis have lower frequency of and longer time to liver disease complications." . *PloS One* . 13, no. 5 (2018): Article No. e0197117. doi: 10.1371/journal.pone.0197117
6) Plauth M, Cabré E, Riggio O, et al. "guidelines on enteral nutrition: liver disease" . *ESPEN.Clin Nutr* 25 (2006): 285-294. doi: 10.1016/j.clnu.2018.12.022
7) Córdoba J, López-Hellín J, Planas M,et al. "Normal protein diet for episodic hepatic encephalopathy: results of a randomized study". *J Hepatol* 41 (2004): 38-43.
8) Pinzani M, Rombouts K. "Liver fibrosis: From the bench to clinical targets". *Dig Liver Dis* 36 (2004): 231–242. doi: 10.1016/j.dld.2004.01.003
9) Elsharkawy AM, Oakley F, Mann DA. "The role and regulation of hepatic stellate cell apoptosis in reversal of liver fibrosis". *Apoptosis*. 10, no. 5 (2005): 927-939. doi: 10.1007/s10495-005-1055-4.
10) Saile B, Matthes N, Knittel T, Ramadori G. "Transforming growth factor beta and tumor necrosis factor alpha inhibit both apoptosis and proliferation of activated rat hepatic stellate cells". *Hepatology*. 30, no. 1 (1999): 196-202. doi: 10.1002/hep.510300144.
11) Saile B, Eisenbach C, Dudas J, El-Armouche H, Ramadori G. "Interferon-gamma acts proapoptotic on hepatic stellate cells (HSC) and abrogates the antiapoptotic effect of interferon-alpha by an HSP70-dependant pathway". *Eur J Cell Biol.* 83, no. 9 (2004): 469-476. doi: 10.1078/0171-9335-00409.
12) Saxena NK, Titus MA, Ding X, Floyd J, Srinivasan S, Sitaraman SV, Anania FA. "Leptin as a novel profibrogenic cytokine in hepatic stellate cells: mitogenesis and inhibition of apoptosis mediated by extracellular regulated kinase (Erk) and Akt phosphorylation" . *FASEB J.* 18, no. 13 (2004):1612-1614. doi: 10.1096/fj.04-1847fje.
13) Zhou, Z., Xu, MJ. & Gao, B. "Hepatocytes: a key cell type for innate immunity". *Cell Mol Immunol* 13, (2016): 301–315. doi: 10.1038/cmi.2015.97
14) Zhou WC, Zhang QB, Qiao L. "Pathogenesis of liver cirrhosis". *World J Gastroenterol*. 20, no. 23 (2014): 7312-7324. doi: 10.3748/wjg.v20.i23.7312

15) Tanaka M, Miyajima A. "Liver regeneration and fibrosis after inflammation". *Inflammation and Regeneration*. 36, no. 1 (2016):19-24. doi: 10.1186/s41232-016-0025-2.
16) Zeng T, Zhang CL, Xiao M, Yang R, Xie KQ. "Critical Roles of Kupffer Cells in the Pathogenesis of Alcoholic Liver Disease: From Basic Science to Clinical Trials". *Frontiers in Immunology*. 7, no. 10 (2016): 1-14. doi: 10.3389/fimmu.2016.00538
17) Gordon S, Taylor PR. "Monocyte and macrophage heterogeneity". *Nat Rev Immunol* 5, no. 12 (2005): 953–964. doi: 10.1038/nri1733
18) Dixon LJ, Barnes M, Tang H, Pritchard MT, Nagy LE. "Kupffer cells in the liver".*Compr Physiol* 3, no. 2 (2013):785–797. doi: 10.1002/cphy.c120026
19) Hersoug LG, Moller P, Loft S. "Gut microbiota-derived lipopolysaccharide uptake and trafficking to adipose tissue: implications for inflammation and obesity". *Obes Rev* 17, no. 4 (2016): 297–312. doi: 10.1111/obr.12370
20) Betteridge DJ. "What is oxidative stress?" *Metabolism*. 49, no. 2 (2000):3-8. doi: 10.1016/s0026-0495(00)80077-3.
21) Petrasek J, Bala S, Csak T, Lippai D, Kodys K, Menashy V, et al. "IL-1 receptor antagonist ameliorates inflammasome-dependent alcoholic steatohepatitis in mice" . *J Clin Invest* 122, no. 10 (2012): 3476–3489. doi: 10.1172/JCI60777
22) Baiocchini A, Del Nonno F, Taibi C, *et. al.* "Liver sinusoidal endothelial cells (LSECs) modifications in patients with chronic hepatitis C." *Scientific reports* 9, no. 8760 (2019): 1-10. doi: 10.1038/s41598-019-45114-1
23) Shetty S., Lalor P. F., Adams, D. H. "Lymphocyte recruitment to the liver: molecular insights into the pathogenesis of liver injury and hepatitis". *Toxicology* 254 (2008): 136–146. doi: 10.1016/j.tox.2008.08.003
24) Xing Y., Zhao T., Gao X., Wu Y. "Liver X receptor α is essential for the capillarization of liver sinusoidal endothelial cells in liver injury," *Scientific. Reports*. 6, no. 21309 (2016): 1-11. doi: 10.1038/srep21309
25) Yang F, Yang XP, Liu YH, *et al.* "Ac-SKDP reverses inflammation and fibrosis in rats with heart failure after myocardial infarction". *Hypertension* 43, no. 2 (2004): 229–236. doi: 10.1161/01.HYP.0000107777.91185.89
26) Tripodi, A et al. "Hypercoagulability in cirrhosis: causes and consequences." *Journal of thrombosis and haemostasis* 9, no. 9 (2011): 1713-1723. doi: 10.1111/j.1538-7836.2011.04429.x
27) Vaquero Javier, and Bañares Rafael. "A gut solution for hepatic encephalopathy." *Hepatology* 61, no. 6 (2015): 2107-2109. doi: 10.1002/hep.27784
28) Koo Hoonmo L, and DuPont Herbert L. "Rifaximin: a unique gastrointestinal-selective antibiotic for enteric diseases." *Current Opinion in Gastroenterology* 26, no. 1 (2010): 17-25. doi: 10.1097/MOG.0b013e328333dc8d
29) Northup PG, Wanamaker RC, Lee VD, Adams RB, Berg CL. "Model for End-Stage Liver Disease (MELD) predicts nontransplant surgical mortality in patients with cirrhosis". *Ann Surg* 242 (2005): 244-251. doi: 10.1097/01.sla.0000171327.29262.e0
30) Fernández J, Ruiz del Arbol L, Gómez C, *et al.* Norfloxacin vs ceftriaxone in the prophylaxis of infections in patients withadvanced cirrhosis and hemorrhage. *Gastroenterology* 131, no. 4, (2006): 1049-1056. doi: 10.1053/j.gastro.2006.07.010
31) Mellinger, Jessica L et al. "Misconceptions, preferences and barriers to alcohol use disorder treatment in alcohol-related cirrhosis." *Journal of Substance Abuse Treatment* 91 (2018): 20-27. doi: 10.1016/j.jsat.2018.05.003
32) Noor Mohd Talha, and Manoria Piyush. "Immune Dysfunction in Cirrhosis." *Journal of Clinical and Translational Hepatology* 5, no. 1 (2017): 50-58. doi: 10.14218/JCTH.2016.00056

33) Albillos, Agustín et al. "Cirrhosis-associated immune dysfunction: distinctive features and clinical relevance." *Journal of Hepatology* 61, no. 6 (2014): 1385-1396. doi: 10.1016/j.jhep.2014.08.010
34) Sipeki N, Antal-Szalmas P, Lakatos PL, Papp M. "Immune dysfunction in cirrhosis". *World J Gastroenterology* 20 (2014):2564–2577. doi: 10.3748/wjg. v20.i10.2564.
35) Neish AS. "Microbes in gastrointestinal health and disease". *Gastroenterology* 136 (2009):65–80. doi: 10.1053/j.gastro.2008.10.080.
36) Newton K, Dixit VM. "Signaling in innate immunity and inflammation". *Cold Spring Harb Perspect Biol* 4 (2012). doi: 10.1101/cshperspect. a006049.
37) Thompson AJ, Locarnini SA, Lau GK, Naoumov NV, Desmond PV, Mommeja- Marin H, et al. "Quantitative HBeAg levels and patterns of TLR2 and TLR4 expression on CD14+ monocytes during potent antiviral therapy for chronic hepatitis" *B. J Gastroenterol Hepatol* 20, no. A83 (2005). doi: 10.14218/JCTH.2016.00056
38) Hillebrandt S, Wasmuth HE, Weiskirchen R, Hellerbrand C, Keppeler H, Werth A, *et al*. "Complement factor 5 is a quantitative trait gene that modifies liver fibrogenesis in mice and humans". *Nat Genet* 37 (2005):835–843. doi: 10.1038/ng1599.
39) Fleming KM, Aithal GP, Card TR, West J. "All-cause mortality in people with cirrhosis compared with the general population:a population-based cohort study". *Liver Int* 32 (2012): 79-84. doi : 10.1111/j.1478-3231.2011.02517.x
40) Zeng, Tao et al. "Critical Roles of Kupffer Cells in the Pathogenesis of Alcoholic Liver Disease: From Basic Science to Clinical Trials." *Frontiers in Immunology* 7 (2016): 538. doi:10.3389/fimmu.2016.00538
41) Tsutsui, Hiroko, and Shuhei Nishiguchi. "Importance of Kupffer cells in the development of acute liver injuries in mice." *International Journal of Molecular Sciences* 15, no. 5 (2014): 7711-7730. doi:10.3390/ijms15057711