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Review

The Space of Disse: The Liver Hub in Health and Disease

Carlos Sanz-García [1], Anabel Fernández-Iglesias [2,3], Jordi Gracia-Sancho [2,3,4], Luis Alfonso Arráez-Aybar [5], Yulia A. Nevzorova [1,6,7] and Francisco Javier Cubero [1,7,*]

1 Department of Immunology, Ophthalmology & ENT, Complutense University School of Medicine, 28040 Madrid, Spain; csanz17@ucm.es (C.S.-G.); yulianev@ucm.es (Y.A.N.)
2 Liver Vascular Biology Research Group, IDIBAPS, 08036 Barcelona, Spain; AFERNANDEZI@clinic.cat (A.F.-I.); jordi.gracia@idibaps.org (J.G.-S.)
3 Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD), 28029 Madrid, Spain
4 Hepatology, Department of Biomedical Research, University of Bern, 3012 Bern, Switzerland
5 Department of Anatomy and Embriology, Complutense University School of Medicine, 28040 Madrid, Spain; arraezla@med.ucm.es
6 Department of Internal Medicine III, University Hospital RWTH Aachen, 52074 Aachen, Germany
7 12 de Octubre Health Research Institute (imas12), 28040 Madrid, Spain
* Correspondence: fcubero@ucm.es; Tel.: +34-394-1385

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Abstract: Since it was first described by the German anatomist and histologist, Joseph Hugo Vincenz Disse, the structure and functions of the space of Disse, a thin perisinusoidal area between the endothelial cells and hepatocytes filled with blood plasma, have acquired great importance in liver disease. The space of Disse is home for the hepatic stellate cells (HSCs), the major fibrogenic players in the liver. Quiescent HSCs (qHSCs) store vitamin A, and upon activation they lose their retinol reservoir and become activated. Activated HSCs (aHSCs) are responsible for secretion of extracellular matrix (ECM) into the space of Disse. This early event in hepatic injury is accompanied by loss of the pores—known as fenestrations—of the endothelial cells, triggering loss of balance between the blood flow and the hepatocyte, and underlies the link between fibrosis and organ dysfunction. If the imbalance persists, the expansion of the fibrotic scar followed by the vascularized septae leads to cirrhosis and/or end-stage hepatocellular carcinoma (HCC). Thus, researchers have been focused on finding therapeutic targets that reduce fibrosis. The space of Disse provides the perfect microenvironment for the stem cells niche in the liver and the interchange of nutrients between cells. In the present review article, we focused on the space of Disse, its components and its leading role in liver disease development.

Keywords: chronic liver disease; hepatic stellate cells (HSCs); liver sinusoidal endothelial cells (LSECs); fibrosis; Kupffer cells (KCs); extracellular matrix (ECM)

1. Introduction

In the normal liver three major compartments are found: (i) Hepatocytes, which represent over 70% of total liver cells, (ii) the biliary system, that communicates within and outside with the adjacent organs and, (iii) a highly complex vascular system. Oxygen-rich blood from the hepatic artery and nutrient-rich blood from the portal vein mix in a very specialized capillary, called the sinusoidal capillary. The hepatic sinusoids are formed by liver sinusoidal endothelial cells, highly fenestrated cells that allow flow of plasma from the sinusoids throughout the space of Disse, a thin perisinusoidal area of contact with the hepatocytes. Blood flowing through the sinusoidal lumen carries its contents of

oxygen, nutrients, hormones among other substances like inflammatory factors, toxins and neoplasms; this makes the space of Disse a unique area for the bidirectional interchange between cells. The space of Disse was first described by the German anatomist and histologist Joseph Hugo Vincenz Disse [1] in 1880, as an area where several cell types are located: Pit cells, a liver-associated natural killer (NK), mesenchymal stem cells (MSC) and a multifunctional cell-type known as hepatic stellate cells (HSC) [2]. NK cells patrol from the sinusoids to the hepatocytes, looking for virus-infected cells and tumor cells, which they kill by using cell-to-cell cytotoxic activity [3]. Quiescent hepatic stellate cells (qHSCs) store vitamin A in lipid droplets, but after activation, hepatic stellate cells (HSCs) proliferate, and progressively lose vitamin A storage and start the deposition of extracellular matrix (ECM) in the injured liver. Progressive liver fibrosis contributes to the scarring of the liver, which can progress to cirrhosis, a critical pathology considered as end-stage liver disease. Although the liver is an organ with an extensive regenerative power, an impairment of regeneration is associated with the progression of a fibrotic liver [4,5]. Lately, several studies showed that activated HSCs (aHSCs) have a mesenchymal stem cell (MSC)-like phenotype, a heterogeneous group of multipotent stem cells that exemplifies an important element of the hematopoietic stem cell niche [6,7]. In this review, we discuss the Space of Disse from an anatomo-physio-pathological point of view comparing Prof. Disse's observations and the current knowledge.

2. The Hepatic Sinusoids

Sinusoids are low pressure vascular channels that receive blood from terminal branches of the hepatic artery and portal vein at the periphery of lobules and deliver it into central veins. Sinusoids are lined with endothelial cells and flanked by plates of hepatocytes. The hepatic sinusoids house an important part of the phagocytic system, Kupffer cells (KCs), a type of fixed macrophages, but also other active immune cells like T lymphocytes [2]. Blood flows full of nutrients, oxygen, but also bacteria and debris from our organism through the sinusoids. An imbalance in the uptake or activation of the different cells may result in physically altered sinusoids. HSCs, localized in the space of Disse, are the major contributor to the perisinusoidal fibrosis. Upon activation, HSCs transdifferentiate and proliferate, acquiring a contractile and fibrogenic myofibroblast-like phenotype during liver injury [8].

Liver sinusoidal endothelial cells (LSECs) are highly specialized endothelial cells in the human liver. Under normal physiological conditions, they mediate the exchange of plasma, nutrients, lipids, and lipoproteins between hepatic sinusoids and hepatocytes through a filtration system that consists of fenestrae, non-diaphragmed pores that traverse the endothelial cytoplasm. However, in pathological conditions, their structural and functional features markedly change [2]. Defenestration—reduction in the amount and diameter of fenestrae—and formation of a continuous basement membrane of LSECs is characteristic of chronic liver diseases (Figure 1). This mechanism can protect the liver from continuous damage by restricting toxins to a specific area, and also alters the physiological structure of hepatic sinusoids. The lack of nutrients flow from the blood to the hepatocytes modifies the hepatic physiology and finally leads to the development of liver injury [9]. LSECs have also the highest endocytic capacity of the human body, capable of eliminating large material (MW > 20,000) previously associated with the phagocytic function of KCs [10]. This endocytic capacity combined with a strong lysosomal activity provides another level of protection to the liver against toxins and waste from the blood [11]. Specialized LSECs, called scavengers endothelial cells (ScaECs), have a specific function of endocytosis called "pinocytosis" with very specialized receptors: scavenger receptors (SR-A, SR-Band SR-H), mannose receptor and Fc gamma-receptor-mediated endocytosis FcγRIIb2, the latter involved in the clearance of blood-borne small IgG immune complexes [11–13]. While phagocytosis denotes cell eating of large particles and internalization (mainly associated with macrophage function), pinocytosis represents cellular uptake of soluble molecules without internalization [10]. Several reports demonstrated the lack of internalization of ScaECs with red blood cells coated with IgG or chondroitin sulfate particles [14,15].

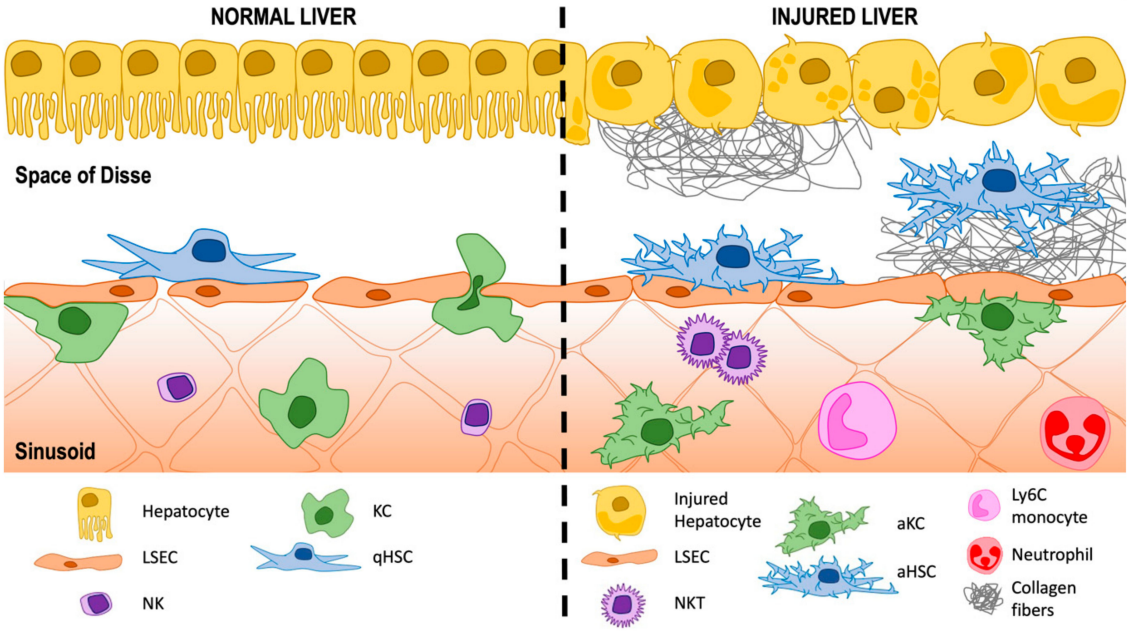

Figure 1. Schematic representation of a normal and an injured liver. While in a healthy liver, hepatocytes with microvilli, resident macrophages (Kupffer cells, KC), quiescent hepatic stellate cells (qHSCs), natural killers (NK) and liver sinusoidal endothelial cells (LSEC) maintain their structure and function, in the injured liver the modification of its architecture is associated with the activation of KCs (aKC) and HSCs (aHSC). Furthermore, the infiltration of immune cells, like neutrophils and monocytes (Ly6C), to the injured area recruits other cells like natural killer T-cells (NKT) and alters the normal morphology of the liver. Thus, hepatocytes lose their microvilli and finally their function, associated with the increased extracellular matrix deposition (ECM) from aHSCs and changes in the balance between the hepatic artery and the portal vein blood flow. The excessive ECM and vascularized portal tracts favor the progression to cirrhosis and finally hepatocellular carcinoma (HCC).

While LSECs change after pathological situations, KCs and T lymphocytes also adapt to the new state. KCs are key factors for the initiation and progression of fibrosis (Figure 1). In response to hepatocyte injury, KCs can be induced into an activated state in which they secrete a wide variety of proinflammatory cytokines, such as IL-6, IL-10, IL-13, TNF-α, and TGF-β [16]. The balance between M1 (proinflammatory) and M2 (anti-inflammatory) KCs regulate the liver inflammation and may lead to further activation of HSCs. Not only do KCs induce inflammation in the liver, but they also activate the innate immune response. For example, Th17 cells, producers of IL-17, have a strong profibrogenic effect through two independent mechanisms. While IL-17 stimulates KCs, promoting the expression of pro-inflammatory cytokines; IL-17 also activates HSCs differentiation into myofibroblasts via the STAT3 pathway [17]. Another population, the NKT cells, have two subtypes that can be pro-inflammatory (type I) or more protective (type II) based on different models of liver injury [18]. However, not all the innate immune system contributes to inflammation and fibrosis. The regulatory T cells (Treg) ($Foxp3^{+}CD4^{+}$), with a more anti-inflammatory profile, modulate the immune system and prevent autoimmune diseases. TGF-β expression is necessary for the maturation and homeostasis of Treg [19] and have been related with metabolic disorders, providing a novel therapeutic strategy for NASH/MASH [16]. Furthermore, NK cells, up to 50% of the liver lymphocyte population in humans, increase their killing activity after HSCs activation due to hepatocyte damage. aHSCs promote the expression of different compounds that trigger the death of aHSCs by NK cells (Figure 1). In addition, the inhibition of the major histocompatibility complex-I (MHC-I) in HSCs results in reduced engagement of inhibitory NK cell receptors and enhanced killing [20,21].

3. A Historic Perspective of the Space of Disse: Home for Hepatic Stellate Cells (HSCs)

The unusual anatomical configuration described by Disse, born in Brakel, North Rhine Westphalia, but with a Germanized French last name, has not been given proper credit. Once he finished the studies in Medicine, Joseph Disse went on to do postgraduate work in Anatomy at the University of Strasbourg. Later, he accepted a position as Professor of Anatomy at the University of Tokyo, where first described that the liver fills up particularly well when you inject ink into the subcutaneous lymphatic cavity of the abdomen, and the existence of perivascular spaces around the capillaries of the liver is obvious in reptiles. After returning to Germany, Disse spent a brief period in Berlin but accepted a position of *Privatdozent* (Assistant Professor) on the faculty of the Anatomical Institute of the Georg-August University in Gottingen. However, by special dispensation of the "Königliche Kurator," he was exempted from having to submit the customary "Habilitationsschrift" (professorial dissertation). In Gottingen, he continued to investigate whether the perivascular spaces observed in Tokyo were also found in the liver of mammals and published his *magnum opus* "*über die Lymphbahnen der Saugethierleber*" (Regarding the Lymphatic Tracts of the Mammalian Liver) and was promoted to Associate Professor [22]. After a short stay in University of Halle, he moved to Marburg becoming full Professor of Anatomy and Director of the Anatomical Institute, where he spent 16 years of fruitful career until he passed away of tuberculosis.

The existence of a system that envelops the blood vessels in the liver in mammals was first claimed by Mac Gillavry [23] and later modified by Frey and Irminger. E. Hering [24] also mentioned star-shaped cells, that leaves the capillaries lying on the outside usually referred to as connective tissue bodies, whereas there are no mention of these cells in larger anatomical manuals, for example in Henle [25] and W. Krause [26]. Moreover, Kupffer [27], during his career in research, published over 60 original articles, reviews and books, out of which only two are dedicated to "Sternzellen" [27,28]. In 1876, he published the work entitled "On the stellate cell in the liver" (in German), in *Archiv fur Mikroscopische Anatomie* [27] where he first described the KCs. Kupffer believed that these stellate cells belong to pericytes or an adventitial group. He described under the name "star cells" the capillaries of the liver lobules, which show several extensions of different lengths and often encompass the capillary vessel in a ring. Sometimes the processes of these star cells (HSCs) penetrate between the liver cells. Rothe [29] also mapped these cells for various types of mammals, in normal and pathological conditions [30–32]. The distinction between different cells of the sinusoid wall was not easy, and gave rise to many controversial discussions. They came to an end in 1970, when Eddie Wisse in Leiden, using electron microscopy, clearly discriminated between liver macrophages, endothelial cells, and fat storing cells [33]. All these observations are the bases of the anatomy and physiology of the perisinusoidal space nowadays.

Inflammation and activation of the innate immune system due to hepatocyte injury leads to liver fibrosis mediated by HSCs (Figure 2). In the space of Disse, qHSCs store retinoids (vitamin A) and produce glial fibrillary acidic protein (GFAP). After activation, HSCs transdifferentiate into proliferative, migratory, and contractile myofibroblasts, manifesting pro-fibrogenic transcriptional and secretory properties [34]. Paracrine factors such a connective tissue growth factor (CTGF), platelet-derived growth factor (PDGF) and/or TGF-β combined with other cytokines, including TNF-α or IL-1β, promote HSC proliferation, migration and secretion of ECM molecules that accumulate and form scar tissue in the space of Disse leading to sinusoidal capillarization characterized by loss of endothelial fenestrations [9,16]. Molecules such as collagen type I and III as well as other proteins contribute to the hardening of the liver and pathologic fibrous tissues [34] (Figure 2).

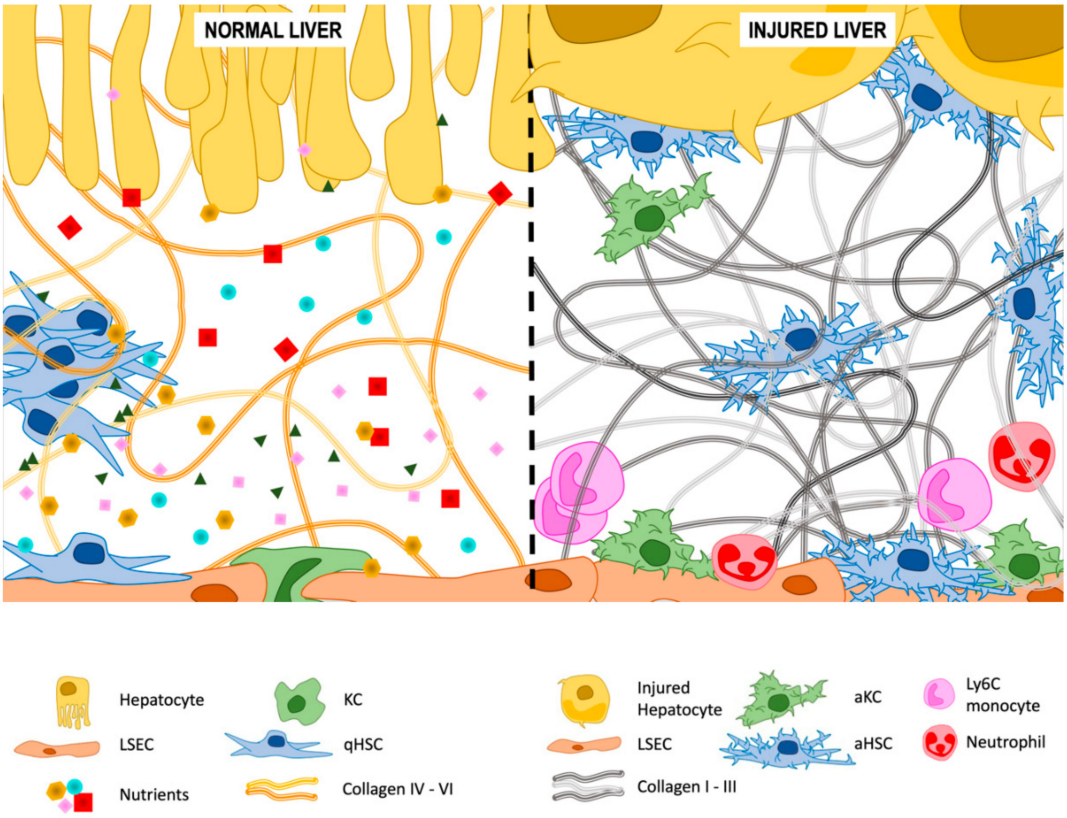

Figure 2. Space of Disse, home for hepatic stellate cells (HSCs). In healthy liver, collagen IV and VI fibers provide the perfect scaffold for architecture and function of the space of Disse, where nutrients flow between the sinusoids and hepatocytes. Recently, new discoveries showed that qHSC complex in niches provides the maintenance of the stem cell characteristics in the liver. However, after activation of HSC (aHSC), there is a replacement of collagen fibers between IV and VI for I and III. (fibrotic fibers), impairing the exchange of nutrients and activation of the immune system. Thus, hepatocytes lose their microvilli and finally their function; activated KC (aKC) express inflammatory mediators to recruit neutrophils and monocytes to the injured area, that try to resolve the problem. When the injury persist, immune system is unable to repair the damage with an increase in ECM deposition by HSCs, cell death and finally, loss of liver function.

3.1. Activation of HSCs

In a normal liver, HSCs maintain a non-proliferative, quiescent phenotype. After an injury or culture in vitro, HSCs become activated, transdifferentiating from vitamin A-storing cells to myofibroblasts, which are proliferative, contractile, inflammatory and chemotactic. HSCs are associated with fibrogenesis and characterized by enhanced ECM deposition. Several years ago, mechanisms regulating stellate cell activation were organized into "core" or "regulatory" [35]. While core pathways are defined as those that contribute to fibrosis across tissues and disease contexts, regulatory pathways are largely tissue restricted.

3.1.1. Intracellular Pathways Involved in HSC Activation

Fibrogenic and proliferative pathways are the most important pathways for HSC activation. For example, PDGF, CTGF and vascular endothelial growth factor (VEGF) induce proliferation, migration and angiogenesis [36–41]. TGF-β, the most important fibrogenic cytokine, binds its receptor providing a intracellular phosphorylation cascade that activated SMAD proteins, particularly SMAD3 that induces the expression of type I and III collagen [42,43] and activates mitogen-activated protein

kinases (MAPK) including extracellular signal-regulated kinase (ERK), c-jun N-terminal kinase (JNK) and p38 [44,45], promoting HSC activation.

Additionally, the Sonic hedgehog (Hg) pathway is also very important for HSC activation. Gli2, a primary transcription factor that drives the transcription of Gli1 and its target genes, one of them osteopontin (OPN). OPN controls cell survival, migration, proliferation and differentiation [46,47]. Studies showed that blocking Hg signaling in activated HSCs not only inhibited liver fibrosis, but also prevented accumulation of liver progenitor cells [48–51].

In the last years, a plethora of evidence showed the association of different pathways with HSC activation [52]. Processes like autophagy and endoplasmic reticulum (ER) stress [53–56]; oxidative stress [57,58], cholesterol stimulation [59], adipokine expression [60–62], retinol metabolism [63,64], cytokines and immune system [65–70] or epigenetic modifications, including miRNA (small non coding RNAs) which regulate post-transcriptional gene expression by altering RNA degradation [71], methylation of the DNA [72,73] or histone modifications [74].

3.1.2. Extracellular Interactions Involved in HSC Activation

The transition from HSCs to myofibroblasts may be caused by different levels of activation. The microenvironment created in the space of Disse, between the sinusoids and the hepatocytes, allows the HSCs to be in contact with biomolecules between portal blood flow from gastrointestinal tract and hepatocytes. Diet, alcohol and toxic compounds lead to the activation of KCs, T lymphocytes, LSECs and hepatocytes, and the expression and release of pro-inflammatory cytokines or damage-associated molecular patterns (DAMPs) that trigger the activation of HSCs [8,34,75]. Herein, we define the activation of HSCs associated to other cell types.

HSCs and Liver Sinusoidal Endothelial Cells (LSECs)

Defenestration is the main characteristic for the activation of LSECs in the liver and occurs earlier than fibrogenesis. While in a normal liver, differentiated LSECs prevent HSCs activation and promote reversion to quiescence through nitric oxide (NO) production and stimulation by VEGF; defenestration and capillarization of LSECs due to liver injury promotes the activation of HSCs, thereby inducing liver fibrosis through loss of VEGF-stimulated NO production [76] (Figure 1). Additionally, LSECs express α4β1-integrin and vascular adhesion protein (VAP)-1, two cell surface markers use by Th1 and Th2 cells, respectively, to adhere to liver sinusoids during liver fibrosis [77].

HSCs and Macrophages—Both Resident KCs and Infiltrating Cells

Macrophages play a central role in liver inflammation and fibrosis and their activation mechanism has been studied for decades. KCs are resident macrophages that localize within the lumen of the liver sinusoids, accounting for about 30% of sinusoidal cells [75]. After being exposed to nutrients and gut-derived bacterial products, also called pathogen-associated molecular patterns (PAMPs), they sense and remove pathogens and dangerous molecules via pattern-recognition receptors (PRRs). The toll-like receptors (TLRs) recognize gut microbiota-derived bacterial products such as LPS and peptidoglycan. KCs respond to LPS through TLR4 to produce various inflammatory cytokines including TNF-α, IL-1β, IL-6, IL-12, IL-18, and chemokines [16].

After both acute and chronic liver injury, bone marrow-derived monocytes are recruited into the liver and are involved in the development of liver disease (Figures 1 and 2). Depending on the sensed signals, macrophages are classified in a pro-inflammatory profile (M1), expressing molecules including nitric oxide (NO) by inducible nitric oxide synthase (iNOS) or anti-inflammatory profile (M2) expressing arginase-1 (ARG1) [78]. However, recent transcriptomic studies suggest that the M1/M2 paradigm is more complicated than these two subtypes of monocytes and may be an oversimplification for hepatic macrophages exposed to various pro- and anti-inflammatory stimuli [79]. Studies using acetaminophen (APAP)- and EtOH (alcohol)-derived liver injury showed that infiltrated monocytes are $Ly6C^{hi}$ (pro inflammatory) and become $Ly6C^{low}$ (restorative) once they reach the injured area [80,81].

Viral infection, hepatic toxins, metabolic disorders, and autoimmune diseases trigger various modes of cell death (e.g., apoptosis, necrosis, necroptosis, and autophagic cell death) and their death responses in parenchymal cells (hepatocytes and cholangiocytes). The release of the intracellular content of the dying cells and the phagocytic activity of macrophages exposed to DAMPs that combined with PAMPs and compounds derived from the gut activate KCs and infiltrated monocytes via TLRs. For example, lipopolysaccharide (LPS) binds TLR4 and activates an intracellular cascade of kinases triggering the expression of inflammatory cytokines and chemokines like TNFα, IL-1, IL-6, MCP-1, CCL5 or TGF-β1 [82,83]. Activated HSCs, that also express TLR4 [70,84], induce chemokines (MCP-1, MIP-1α, MIP-1β, RANTES, KC, MIP-2, and IP-10) and adhesion molecules (ICAM-1, VCAM-1, and E-selectin) to recruit KCs and circulating macrophages [70]. A feedback loop of activation is created between KCs/infiltrated monocytes and HSCs triggering the expression of TGF-β, a well-known profibrogenic molecule.

HSCs and T Lymphocytes

The microenvironment created by the release of pro-inflammatory cytokines attracts and activates other cells to the injured area. Thus, Th17 cells produce IL-17A and IL-22; while IL-17A stimulate KCs and HSCs activating STAT3 pathway and inducing expression of TNF-α, IL-1β, IL-6, and IL-17A, and collagen I expression in HSCs [17], IL-22 upregulates STAT3 pathway and p53 expression attenuating fibrosis mediated by HSCs [68]. Treg ($Foxp3^+CD4^+$) cells also regulate fibrosis by the expression of IL-8 and activation of HSCs in viral hepatitis [85].

Although some populations of lymphocytes have a more fibrotic role in liver disease, several studies demonstrated the anti-fibrotic role of NK cells (Figure 1). The activation of HSCs and the release of retinoic acid (RA) leads to the expression of RAE-1, a ligand for the NK cell receptor NKG2D that combined with MHC class I–related protein (MICA) generates killing of aHSCs by NK cells [86]. Other NK cell receptors (NKp46 and NKp30) are also involved in this process [87,88]. After activation, MHC-I is downregulated in HSCs leading to a reduced engagement of inhibitory NK cell receptors and enhanced killing [89]. The expression of NK cells-derived cytokines also contributes to ameliorate fibrosis. The expression of IFNγ and IFNα triggers HSCs apoptosis and cell cycle arrest, and increased expression of TRAIL in NK cells, thereby enhancing NK-mediated HSC death, respectively [90,91]. Nevertheless, TGF-β1, the major pro-fibrotic cytokine, suppresses the anti-fibrotic function of NK cells via the downregulation of surface markers' expression [92].

HSCs and Other Cell-Types

Although neutrophil infiltration is clearly related with inflammation, the relationship between neutrophils and fibrosis remains elusive. Some studies indicated that neutrophils contribute to the development of metabolic syndrome, and specifically to MASH [93,94]. Dendritic cells (DCs) are professional antigen-presenting cells (APCs) that capture and process antigens, migrate to lymphoid organs, and secrete cytokines to initiate both innate and adaptive immune responses. DCs contribute to inflammation, by activation of KCs, HSCs and NK cells but also DCs are involved in regression of fibrosis via the expression of matrix metalloproteases (MMP), specifically MMP-9 [95].

3.1.3. Crosstalk Cells-Stimulus Involved in HSC Activation

As mentioned above, several inputs, pathways and cells are involved in the activation of HSCs. However, this process is a complex mechanism whereby each part plays a role in disease development [4]. While in a healthy liver, every cell type has a specific function (e.g., LSECs allow the permeability of macromolecules and lipoproteins from the blood through the space of Disse to hepatocytes for absorption and storage or KCs, that control and patrol to prevent infection or tissue damage) [9,30]. However, in an injured liver, each and every cell changes their transcriptional program to restore the balance and return to a normal state. Loss of fenestration in LSECs affect directly to hepatocytes that undergo apoptosis due to the lack of nutrients and oxygen [9]. DAMPs, from hepatocytes, and PAMPs,

from the gut, activate KCs via TLRs, thus inducing the expression of inflammatory mediators, cytokines and chemokines that will serve as chemoattractant to other immune cells including neutrophils, T-helper lymphocytes or monocytes to the injured area [96]. The microinflammation caused will activate the immune system as well as HSCs, inducing the expression of deposition of ECM [97]. Both the interchange between collagens, and the loss of fenestration of LSEC create a microenvironment without nutrients, whereby more hepatocytes die triggering the activation of the immune system and chronic inflammation. When balance cannot be restored, chronic inflammation leads to fibrosis, and even to irreversible cirrhosis or end-stage hepatocellular carcinoma (HCC) [98].

3.2. Extracellular Matrix (ECM) Deposition

The ECM is an intricate macromolecular structural network which forms a scaffold for adhesion, providing a signaling platform: interchange of cytokines, anchoring processing enzymes and activation of integrins of hepatocyte surface (Figure 2). Activation of HSCs and deposition of extracellular matrix (ECM) in the space of Disse is a complex mechanism orchestrated by several cell types and crosstalk between populations. In a normal liver, collagens IV and VI are present in the space of Disse but after fibrogenesis, aHSCs begin to proliferate, contract and deposit elevated amounts of collagen fibers and extracellular matrix molecules in the hepatic parenchyma. Molecules like glial fibrillary acidic protein (GFAP), nerve growth factor receptor (p75), desmin, lecithin-retinol acyltransferase (LRAT), integrin $\alpha v\beta 3$, collagen type I, collagen type VI receptor (CVIR), α-smooth muscle actin (αSMA), PDGFRβ, vimentin, and cytoglobin contribute to the stiffening of the organ and the perturbation of all cellular functions [4,16,34,98]. Progressive deposition of ECM proteins triggers increased density and stiffness of the ECM that may contribute to the loss of endothelial fenestrations of LSECs and to the activation of HSCs [99] aggravating hepatic fibrosis; a self-perpetuating cycle between collagen-producing activated HSC and capillarized LSEC stimulate each other, further contributing to liver fibrosis [34,98].

3.2.1. Type IV and VI Collagen

Prior to the progression to a fibrotic liver, type IV and type VI collagen (Col) function as a barrier between tissue compartments [100,101]. Col IV is the specialized sheet-like ECM of multicellular tissues that exists around certain cell types (e.g., skeletal muscle cells, smooth muscle cells, heart muscle cells, and adipocytes) and is the major structural scaffold of basement membranes. Col IV is a trimer of α chains (six different: α1(IV)–α6(IV)) that is ideally suited for the close incorporation of laminin and heparan sulfates [101]. Col VI is a beaded filament that forms a unique microfibrillar network, with many binding partners. It is composed of three different chains: α1–α2–α3 and the C-terminal pro-peptide is a hormone called endotrophin, associated with the metabolic syndrome; thus, type VI COL is both a structural and signaling protein [101] (Figure 2).

3.2.2. Type I and III Collagen

Hepatic fibrosis is the result in excessive production of ECM, mostly type I and III Col deposition. These fibers are usually surrounding non-specialized and non-polarized cells like fibroblasts, the aHSCs in the liver. TGF-β expression enhances the dependent and independent SMAD signaling pathways, where nuclear members of the Sp family, among others, bind the promoters of COL1A2, COL1A1 and COL3A1 and regulate their transcription [42,43,102–104]. Col I plays a key role in changing the mechanical characteristics of tissue and the stiffness of the ECM in the progression of liver fibrosis [100,105] (Figure 2).

3.2.3. Other Components of the ECM

ECM deposition is highly characterized for the expression of several different fibers that modify the tissue environment and function of cells. Proteins like proteoglycans, fibronectin, fibrin and laminin are also expressed in the perisinusoidal space after HSCs activation [100]. Pro-inflammatory cytokines,

mechanical changes and other factors are sensed by myofibroblast, that initiate a transcriptional program for the expression and release of these proteins [4].

The balance between deposition and remodeling of ECM is the key for the progression to a fibrotic liver. A family of degradative enzymes, the MMPs, is responsible for the turnover of the ECM. MMPs are zinc metallo-endopeptidases that are secreted to the extracellular milieu, sometimes anchored to the plasma membrane, where they bind and break collagen fibers. While a normal liver has more basement membrane-like matrices, an injured liver switches to a fibrillar and contractile matrix. The progression from normal to a stiffer ECM may be related with the beginning of liver fibrogenesis, as previously described [106].

3.3. Microvilli

Hepatocyte differentiation is highly determined by the chemical and physical properties of ECM. Hepatocytes are not attached to a tough basal lamina; they are surrounded by a low-density ECM that contains hepatocyte-secreted components. The absence of a basal lamina allows the exchange of macromolecules between the sinusoid and the space of Disse through the endothelial cell fenestrae and the perfect functioning of the hepatocytes. Excess deposition of ECM in a fibrotic liver results in alteration of the liver architecture and loss of hepatocyte function [107]. Compounds such as laminin are very important for hepatocyte differentiation in hepatic development and regeneration [108].

LSECs provide a sieve able to separate plasma from portal blood, allowing macromolecules of different sizes and lipoproteins, like cholesterol and retinol, and avoiding large triglycerides-rich pattern chylomicrons. Disruption of the porosity of the sieve will have a strong influence in the metabolism of lipoproteins and other molecules like vitamins, cholesterol or macromolecules and will interfere with the balance between healthy and injured liver [109]. Hepatocytes are organized in hexagonal lobules around a central vein and are the first cells exposed to everything we ingest and absorb from our gut, whether it is nutritious or toxic [110]. Several channels, receptors and surface proteins in their basolateral (sinusoidal) membrane allow hepatocytes to collect molecules and distribute them based on a gradient. However, the apical (canalicular) membrane also contains ATP-binding cassette (ABC) transporters and other bile acid efflux transporters predominate [110]. The disposition of the localization of these transporters and channels is associated with microtubule cytoskeleton polarization [111,112].

Hepatocytes excrete lipids, salts and degraded proteins from their apical plasma membranes into small channels that feed bile contents through a complicated ductular system called the intrahepatic "biliary tree". Bile is then drained from the liver into the gall bladder for storage and next injected into the intestinal lumen during feeding [107,110]. In fact, Disse's interest in the lymphatics of the liver was kindled by a casual observation he and his coworker Tiegel made in snakes and lizards that had been injected with India ink [22]. They found that the liver cells not only produce bile draining into the biliary system but also are the fountainhead of the hepatic lymph system that eventually abuts the venous system. In this context, there is a theory of the space of Disse as part of the lymphatic system, being capable of collecting and redirecting fluids into the lymphatic tract [113]. Fluids primarily flow through the space of Mall, a space between the stroma of the portal tract and the outermost hepatocytes, into the interstitium of the portal tract and then into lymphatic capillaries. Some portion of the fluid in the space of Disse flows into the interstitium around the central vein, which is located in the center of the liver acinus and connected to the hepatic vein, or underneath the hepatic capsule [113,114].

3.4. HSCs as Stem Cells

Mesenchymal stem cells (MSC) are a heterogenous group of somatic, multipotent stem cells that secrete immunomodulatory and trophic factors. Although MSC were first described in bone marrow, recent findings suggested that MSC are in all organs where they are associated with blood vessels [115]. Recent studies showed that HSCs are the hepatic pericytes, multipotent cells that share similar characteristics with MSC; indeed, it seems that MSC originate from pericytes [6,7,116].

qHSCs displayed similar transcriptome and secretome to another pericytes or MSC from other organs [7]. Thus, HSCs represent a quiescent state of MSC in liver sinusoids. Although aHSCs display a myofibroblast-like phenotype, that could be reversible [117]. The space of Disse provides a niche for HSCs, allowing the maintenance of the stem cell characteristics in the liver (Figure 2). The stiffness and composition of ECM may alter the integrity of the stem cells niche and subsequently, in the maintenance of HSCs [116].

4. Involvement of the Space of Disse in Liver Disease

Liver fibrosis is a common outcome generated as result of chronic liver injury including viral hepatitis infection, alcohol abuse, metabolic disorders, metabolic-associated fatty liver disease/metabolic-associated steatohepatitis (MAFLD)/MASH and other rare diseases including autoimmune hepatitis (AIH) [4]. The self-protective behavior of the body allows fighting pathogenic factors that can limit damage, regressing early-stage fibrosis when its origin is eliminated. However, advanced fibrosis can progress into more severe stages, like cirrhosis, with irreversible damage to the liver and end-stage HCC [4,9,75]. Due to the relevance in the number of deaths associated to cirrhosis worldwide, affecting between 1% and 2% of global population with more than 1 million deaths per year [118,119], many studies focused on understanding the molecular mechanisms that drive HCC, but also establishing efficient diagnostic and therapeutic strategies. Animal models have been used combined with diets, chemical compounds, surgical approaches or viral infections to mimic the stages in an injured liver, although efforts to relate those stages with the human pathologies are still far from complete. Several reports classified the different in vivo models based on the compound used for the treatment [4,120,121].

4.1. Hepatotoxicity

4.1.1. Drug-Induced Liver Injury (DILI)

DILI remains the most common cause of acute liver failure in the Western world, associated with drug abuse and herbal medicines or other xenobiotics that lead to liver failure.

After uptake by hepatocytes, drugs are metabolized by phase I and phase II enzymatic reactions. After phase I reactions, the metabolites have minor modifications but still can have very different pharmacological actions [122]. Phase II metabolism involves the conjugation of a drug or metabolite with endogenous molecules such as glucuronic acid, sulfate or glutathione resulting in a more polar product that usually does not have pharmacological activity. Drugs and metabolites efflux from hepatocytes into the bile or back into the sinusoidal blood for subsequent renal excretion, which is mediated mainly by ATP-binding cassette (ABC) transporters such as multidrug resistance protein 1 (MDR1), also called P-glycoprotein, which is encoded by ABCB1, and anion exchange mechanisms [122]. The mechanism of action of DILI is a complex interplay between different organelles: mitochondrial dysfunction and endoplasmic reticulum (ER) stress associated with immune cell-derived inflammation. Mitochondrial oxidative stress and membrane permeability transition (MPT) combined with inhibition of the mitochondrial electron transport lead to cell death and release of DAMPs to the milieu. Furthermore, the metabolization of drugs increases reactive oxygen species (ROS) production that causes dysregulation of Ca^{+2} and activation of the unfolded protein response (UPR). If the programmed mechanisms in the cell cannot alleviate ER stress, the cell is programmed for apoptosis. Cell death and DAMPs induce infiltration of immune cells, expression of pro-inflammatory cytokines, activation of HSCs via TGF-β, and deposition of ECM in the space of Disse.

4.1.2. Alcoholic Liver Disease (ALD)

Alcohol consumption is a worldwide cause of chronic liver disease and results in approximately 3 million deaths each year (5.3% of all deaths) with most of them associated with ALD [123]. ALD starts with hepatic steatohepatitis that can progress into fibrosis and later cirrhosis. Perivenular fibrosis

that extends outward along the sinusoids and accumulation of ECAM is primarily observed in the space of Disse. Because this pericellular or perisinusoidal fibrosis extends outward, it shows a classic chicken-wire fence pattern, sometimes all the way to the portal tract. Chronic ethanol consumption upregulates cytochrome P450 2E1 (CYP2E1); thus, ROS are generated triggering a proinflammatory response and activation of HSCs. However, alcohol also disrupts the microbiota in the gut, leading to an increase in the bacterial products to the portal circulation and activation of KCs by the TLR4 and expression of inflammatory mediators like TGF-β, that also activates HSCs via SMAD pathway [124]. These mechanisms lead to hepatocytes apoptosis, inflammation and ECM deposition by HSCs.

4.1.3. NAFLD/MAFLD and NASH/MASH

Obesity is a strong risk factor for the development of metabolic syndrome (MS) and is associated with insulin resistance (IR) and type 2 diabetes (T2D) as well as non-alcoholic fatty liver disease (NAFLD)/non-alcoholic steatohepatitis (NASH), recently re-termed as metabolic-associated fatty liver disease (MAFLD)/metabolic-associated steatohepatitis (MASH) [125]. Dietary lipids are stored in hepatocytes leading to loss of function of the hepatocytes and protein unfolding thus activating the ER stress pathways. As a result, hepatocytes are unable to function properly and undergo cell death. The release of intracellular content to the milieu, DAMPs, recruits immune cells to the space of Disse and expression of pro-inflammatory cytokines. TGF-β activates HSCs via SMAD2/SMAD3/SMAD4 inducing the deposition of ECM [126]. Importantly, in adult steatohepatitis-related fibrosis, ECM is deposited primarily in the zone three perisinusoidal space of Disse, and then spreads to surround hepatocytes and thicken the space of Disse; forming characteristic "chicken-wire" fibrosis (see ALD section). Eventually, the pericentral fibrosis forms septa to isolate regenerating nodules [127,128].

4.1.4. Portal Hypertension

During the development of chronic liver disease, hepatic cell types suffer intense modifications in their phenotype that ultimately lead to liver microvascular dysfunction, increased intrahepatic vascular resistance (IHVR) and portal hypertension. It appears to have two major mechanisms for IHVR progression: a profound alteration in liver architecture (structural component) and a pathological increase in the hepatic vascular tone (dynamic component) [120]. The structural component greatly contributes to fibrogenesis (exaggerated ECM deposition), disorganized regenerative nodules (non-neoplastic nodules with surrounding fibrosis), vascular occlusion and sinusoidal capillarization (de-fenestration of the LSECs). For the dynamic component, contractile elements influencing the hepatic vascular bed include sinusoidal and extra-sinusoidal cells, such as HSCs and vascular smooth muscle cells, which compress sinusoids, regenerative nodules and venous shunts in response to vasoactive molecules [120]. Furthermore, LSECs and KCs, actively contribute to the dynamic component of IHVR by promoting the production of vasoconstrictors and having reduced capacity to produce or respond to vasodilators. These changes profoundly affect the hepatic vascular tone of the fibrotic liver. However, there is an extrahepatic contributor as well, the splanchnic vascular bed [129]. Several reports showed that elevation in splanchnic blood flow and reduced splanchnic arteriolar resistance lead to chronic elevations in portal pressure and hyperdynamic systemic circulation with high cardiac index and low systemic arterial resistance [120,130–135].

4.1.5. Chronic Cholestatic Liver Diseases

Chronic cholestatic liver diseases including primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC) are associated with active hepatic fibrosis, and ultimately cirrhosis. The progressive structural damage of the intrahepatic biliary three leads to cholestasis, which has been traditionally considered an important pro-fibrogenic factor [136]. In experimental models of cholestasis, fibrogenic markers like TIMP-1, α-SMA, collagen 1 and TGF-β, and accumulation of B-cells and T-cells in the portal tracts generate ROS and liver damage [137].

4.2. Liver Regeneration

The liver is the only visceral organ that possesses the capacity to regenerate after surgical removal or chemical injury. Regeneration is a complex process that relies on the proliferation of hepatocytes and non-parenchymal cells after loss of liver mass, although hepatic progenitor cells (HPCs) appeared to have an important function in regeneration too. HPCs differentiate into bile duct cells and hepatocytes after a severe liver injury. However, the origin and function of HPCs after liver injury is not well-established and their ability to participate in liver regeneration is far from clear [138]. Traditionally, regeneration is an orchestrated mechanism that combines three phases: the priming phase, where HPCs activate more than 100 genes in response to cytokines like TNFα and IL-6, the proliferation phase, where HPCs respond to growth factors (TGF-α) moving to mitosis and termination phase, with inhibition of proliferation of HPCs controlled by TGF-β and activin. Several pathways are involved in the activation and proliferation of HPCs like Wnt pathway, Notch pathway, NFκB pathway and PI3K/AKT pathway among others (reviewed in [139]). Vascularization is very important for liver regeneration, and HSCs seem to be a major role for this phenomenon. Proliferation of HSCs and their interaction with LSECs allow neovascularization during regeneration [140].

ECM degradation is another step crucial for regeneration; while Col I and III do not change their expression, Col IV, fibronectin and laminin increases their expression after partial hepatectomy (PHx). Several models showed that deficient and uncontrolled HSCs activation impairs liver regeneration. Therefore, a precise HSCs response may be an important factor to guarantee a satisfactory regeneration [140].

However, the conditions after PHx are not the same as in an injured liver. Inflammation, activated immune cells, hepatocyte death and fibrosis are some of the characteristics after chronic liver disease. It seems that liver fibrosis progression is related with liver regeneration failure and subsequently, hepatocyte proliferation impairment. Several mechanisms (cytokine production [141] or deficiency EGFR pathway [142]) tried to explain this lack of proliferation in a NAFLD/MAFLD model, but hepatocytes had abnormal oxidative stress that was rescued when mice were treated with antioxidants [143].

4.3. Progression from Fibrosis to Cirrhosis

Chronic liver disease is associated, usually, with injury and death of hepatocytes among other cell types, and activation of an immune response leading to inflammation, also called hepatitis. While this stage is reversible, progression to further stages like cirrhosis is not. In the last decade, a lot of effort has been made in order to develop novel anti-fibrotic strategies to minimize the progression of liver fibrosis and accelerate fibrosis resolution. All the strategies are based on the inactivation or death of HSCs, the main source of ECM deposition. TRAIL-mediated and TNFα-mediated apoptosis of HSCs, expression of MMP by restorative Ly6C^{low} monocytes and interferon (IFN)γ by NK cells or ER stress are some directions that seem to improve fibrosis resolution [4,5,16]. Nevertheless, cells stay in a stage that predisposes them to reactivate into myofibroblast, after a local stimulus, developing a more severe stage of fibrosis [144]; thus, full recovery cannot be achieved and more studies have to be developed to address this issue.

A fibrotic liver may progress to cirrhosis, irreversible stage that is characterized for hardening of the liver, where normal tissue is replaced by scar tissue and nodule formation of the liver. The normal flow of blood through the liver is impaired, leading to an increase in death of hepatocytes and finally, a loss of function of the liver. Vascularized fibrotic septa links portal tracts with central veins surrounding by hepatocytes islands, increasing intra-hepatic resistance (portal hypertension) and the development of HCC [145]. Although the mechanism of action is different for every disease, the impairment of resolving fibrosis and the excessive production of ROS and pro-inflammatory cytokines lead to an overactivation of HSCs that finally triggers higher ECM deposition [145].

4.4. Hepatocellular Carcinoma (HCC)

HCC is the fourth most common cause of cancer-related death worldwide and the chance of potentially curative treatment and surveillance is based on early detection; however, incidence and cancer-specific mortality still continue to increase in many countries. Early-stage HCC can be treated curatively by local ablation, surgical resection or liver transplantation although the majority of HCC patients still present at an advanced stage in many parts of the world [146].

Development of HCC is a multifaceted process that involves continued inflammatory damage, hepatocyte death and lack of regeneration, associated with ECM deposition. HCC has an enormous molecular heterogeneity due to the accumulation of somatic genomic alterations in passenger and driver genes in addition to epigenetic modifications. Risk factor like tobacco, diabetes or infection with HIV are associated with development of HCC; although the promotion to healthy life habits may reduce the risk of progression to HCC, it is increased when cirrhosis is established [147].

4.5. Hemochromatosis

Hemochromatosis is a clinical condition associated with an abnormal deposition of iron causing several organ dysfunctions. Although iron absorption in the body is quite controlled, the excess of iron accumulation inside the cells disrupts their function, leading to an organ failure. Hereditary hemochromatosis is the most common autosomal recessive disorder in whites and is associated with mutations of: hemochromatosis (HFE) gene, hepcidin, the hormone associated with iron absorption in the cells, transferrin transporter-2 or ferroportin. However, there is another second type of hemochromatosis that appears to be related with iron deposition in damaged tissue or due to the presence of excessive iron in the body because of continuous transfusions or iron administration in disease like anemia or thalassemia. Basically, the excess of iron deposition inside the cells is controlled by hepcidin that binds up and induces degradation of ferroportin transporter. Thus, hepcidin concentrations are inversely correlated with iron absorption. Co-regulators of hepcidin synthase are related with SMAD4, a member TGF-β superfamily. TGF-β activates HSCs leading to ECM deposition and fibrosis, and, finally, liver failure [148,149].

4.6. Other Diseases Related with the Space of Disse

Hepatic sinusoidal obstruction syndrome (SOS), also known as veno-occlusive disease (VOD), is an obliterative venulitis of the terminal hepatic venules, which in its more severe forms imparts a high risk of mortality. This pathogenic event leads to the destruction of the LSECs, with sloughing and downstream obstruction of terminal hepatic venules. Glutathione and NO depletion, increased expression of MMPs and vascular endothelial growth factor (VEGF) and expression of clotting factor are some features that contribute to SOS. Hematopoietic stem cells transplantation has become the most important and frequent cause of SOS [150].

Several mice models, with diets and drug treatments, studied the progression of SOS in the liver. The sinusoid is eventually obstructed and aggregation of LSECs, red blood cells and adherent monocytes. KCs are replaced by phagocytic infiltrated monocytes which accumulate in the injured centrilobular area. Increased expression of MMP-9 into the space of Disse leads to a breakdown of ECM and further loss of LSECs fenestrae. Absorption of oxygen and nutrients from hepatocytes is impaired and leads to cell death. Thus, inflammation activates HSCs that start the deposition of ECM, hardening the liver and impeding its function [151].

5. Therapeutic Intervention in the Space of Disse

Liver inflammation and fibrogenesis are controlled by complex immunologic pathways that implicate many possible therapeutic targets that are being investigated extensively during recent years. Targeting either core or regulatory mechanisms each has advantages in developing antifibrotic strategies. For core pathways, therapies active in one organ could be relevant to other organs as

well, but carry an increased risk of off-target effects in unaffected organs. In contrast, regulatory or tissue-specific pathways offer the advantage of restricting therapeutic activity only to the organ of interest. This distinction is not entirely validated experimentally, but informatics-based approaches, such as those used to define an HSC-specific gene signature, could be employed to further support this notion. Many studies focused on targeting TGF-β signaling, oxidative stress pathways, cell death and MMPs expression in order to reduce fibrosis and avoid the loss of liver function.

Due to the major role of TGF-β in liver fibrogenesis, several studies focused on its inhibition to develop effective antifibrotic therapies. For example, the use of small molecule kinase inhibitors such as LY2157299 (also known as galunisertib) and LY2109761 to inhibit TβRI and TβRI/TβRII, respectively [102]. The use of galunisertib in preclinical animal models of liver fibrosis showed promising results for interrupting intracellular downstream signaling [152,153]. Furthermore, inhibition of TGF-β signaling including interference of the SMAD pathway: preventing the formation of the complex SMAD3/SMAD4 [154], inhibiting the translocation of SMAD3 to the nucleus [155] or increasing the expression of SMAD7, that interferes with TGF-β/Smad signaling [156,157]. In addition, there are still several ongoing clinical trials, including AVID200 in patients with diffuse cutaneous systemic sclerosis and myelofibrosis (Identifiers: NCT03801438 and NCT03895112). AVID200 represents a computationally designed highly potent trap for TGF-β1 and TGF-β3 [103].

Inflammation and ROS-mediated oxidative stress represent two major fibrogenic factors that have been also well studied. Recent studies from Lin and colleagues showed that the use of polydatin, had hepatoprotective and antifibrotic properties in a murine model of liver fibrosis [158], and a nano-carrier inside of ROS-sensitive polymeric particles reduced oxidative stress of liver fibrosis. The chemical microenvironment allows ROS to react with the nano polymer, triggering the release of the drug but also consuming ROS to reduce oxidative stress [159]. Therefore, a highly effective therapy with minimal effects may be provided loading antifibrotic drugs into ROS-sensitive particles.

Apoptosis of hepatocytes is another trigger of inflammation and HSC activation in the evolution of liver fibrogenesis [5]. Two randomized placebo-controlled trials investigated Emricasan, a pan-caspase inhibitor, in NASH/MASH patients with F1-F3 fibrosis [160] or cirrhosis with severe portal hypertension [161]. Data from another trial with Emricasan in post-transplant HCV-induced fibrosis patients after sustained virological response (NCT02138253) are ongoing. Another approach to reduce cell death is to inhibit stress signals, like inhibition of apoptosis signal-regulating kinase (ASK1), kinase related with apoptosis, inflammation and fibrosis [162]. Although studies with ASK1 inhibitor, Selonsertib, have been reported with patients with F2-3 fibrosis [163], new data from patients with F3-4 fibrosis are also ongoing. Activation of the inflammasome may also lead to cell death. Constitutive activation of NLR family pyrin domain-containing protein 3 (NLRP3), a major component of the inflammasome, triggered cell death, inflammation and fibrosis [164]. Therefore, NLRP3 inhibition has been proposed as a plausible anti-inflammatory and anti-fibrotic therapy in several models of fibrosis [164–166].

Activation of HSCs and deposition of ECM leads to a modification of the space of Disse, altering the properties of the liver. Due to an overproduction of new collagen fibers, the liver becomes stiffer and more compact; this mechanism may be reduced by degradation of ECM by MMPs. Thus, MMPs are another therapeutic target for the treatment of fibrosis [167]. In the last decade, several groups made the effort to understand the mechanism underlying the expression and function of the specific MMPs in liver disease. The utilization of adeno-viral vectors with MMP1 in rat models and cells showed an improvement in hepatoprotection and reduction in fibrosis after the treatment [168,169]. Another study associated the inhibition of miR-222 and expression of MMP1 [170]. In the same direction, adeno-viral vectors with MMP8 exhibited great results in reducing fibrosis and inducing cell proliferation and regeneration in vitro and in vivo [171,172]. Other targets are MMP2 and MMP9, where treatments with rosmarinic acid or mutants for TIMP1 reduce the expression of these MMPs and finally cause a decrease in the deposition of ECM [173,174].

Furthermore, changes in the architecture of the liver, by abundant ECM deposition and activation of different hepatic cells, may lead to portal hypertension. There are several therapeutic approaches to resolve IHVR, but statins and arachidonic acid pathway inhibitors are the most important [120,175]. Statins were developed to reduce intracellular cholesterol synthesis, but they showed a vasoprotective function. Many studies with rats showed the beneficial of the statins reducing fibrosis and improving microvascular function and portal hypertension [176–185]. Inhibition of the arachidonic acid is another way to improve IHRV. Selective inhibitors of enzymes of the pathway, like ciclooxygenase 1 (COX1), improved portal hypertension and the vasodilatory capacity of the intrahepatic microcirculation [120,186]. Moreover, antioxidant, anticoagulant and antidiabetic agents, caspase and RHOA-RHO-associated protein kinase (ROCK) inhibitors or FXR agonists are other therapies used for portal hypertension [120].

Migration and proliferation are mechanisms closely associated with HSC activation. As we discussed previously, recognition of PAMPs and DAMPs by TLRs triggers the expression of inflammatory cytokines and chemokines in the injured area. Thus, the inhibition of the expression or release of these pro-inflammatory mediators could be an anti-fibrotic approach. In this context, Cenicriviroc is an oral dual CCR2-CCR5 antagonist that displayed great anti-fibrotic results in different mouse models [187–189]. Currently, Cenicriviroc is being tested in clinical trials for patients with NASH/MASH and fibrosis in Europe and USA.

Although there are several options for the treatment of fibrosis, more studies have to be accomplished to really understand the mechanism of action and to be able to treat patients with the best approach based on gender, ethnicity and age.

6. Conclusions

The space of Disse is delimited by the hepatocytes and the sinusoidal space, allowing the reversible flow of nutrients between cells. After activation, HSCs are the major contributor to ECM deposition in the space of Disse, with a total remodeling of collagen fibers. Loss of fenestration of LSECs combined with deposition of Col I and III, make the flow of nutrients more difficult and lead to lose of hepatocytes microvilli and finally, hepatic death. Infiltration of immune cells and persistent activation of HSCs lead to fibrosis of the liver, an irreversible stage that can evolve to cirrhosis and finally HCC. Although some studies appear to elucidate some insights about the reversible state of fibrosis and new therapies for its treatment, it is still not well understood and more studies should be focused on understanding the mechanism of fibrosis resolution.

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