Role of the inflammatory cytokine IL-15 in the progression of Liver fibrosis

by

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Programme de Biologie Cellulaire

Master's thesis presented to the Faculty of Medicine and Health Sciences

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RÉSUMÉ

Rôle de la cytokine inflammatoire IL-15 dans la progression de la fibrose hépatique

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Programme de Biologie Cellulaire

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L'inflammation chronique du foie provoque une fibrose, qui prédispose au cancer du foie. En effet, la plupart des cas de ces cancer surviennent dans les cas fibrotiques. Différentes cellules immunitaires, en particulier les cellules NK et les macrophages ainsi que diverses cytokines et chimiokines contribuent à la progression de la fibrose et à sa résolution. L'interleukine-15 (IL-15) est une cytokine pro-inflammatoire produite principalement par les cellules immunitaires en réponse à des stimuli immunitaires innés. Elle est nécessaire pour l'infiltration de cellules immunitaires dans le foie. Cependant, le rôle exact de l'IL-15 dans l'homéostasie du microenvironnement du foie reste à élucider. Par conséquent, l'objectif de cette thèse est de déterminer le rôle de l'IL-15 dans la progression et la pathogenèse de la fibrose du foie.

La fibrose hépatique a été induite expérimentalement soit par injection intrapéritonéale de tétrachlorure de carbone (CCl_4), soit en fournissant un régime riche en graisses et déficient en choline (CD-HFD) à des souris du type sauvage (WT) ou *Il15*$^{-/-}$. Le CCl_4 a été administré par voie intrapéritonéale deux fois par semaine pendant 7 semaines et les souris ont un accès illimité à la nourriture CD-HFD pendant 12 semaines. Après la période d'induction, les souris ont été sacrifiées et les tissus du foie ont été recueillis pour l'histologie (H&E et Sirius Red), l'immunohistochimie (α-SMA et le collagène) et l'immunofluorescence (CD68). L'expression du gène de la cytokine pro-inflammatoire a été évaluée par qRT-PCR.

Chez les souris WT, le traitement par CCl_4 a augmenté de manière significative l'expression de l'IL-15 dans le foie, ainsi que le dépôt de collagène et une proportion élevée de cellules positives pour l'actine du muscle lisse (α-SMA) dans les coupes de foie. Des observations similaires ont été observées dans le modèle de fibrose hépatique induite par CD-HFD. Les dépôts de collagène et les cellules positives pour SMA ont été significativement réduits dans le foie des souris déficientes en IL-15 traitées avec CCl_4 ou traitées avec CD-HFD. Parallèlement, l'induction de diverses cytokines pro-inflammatoires (*Il12, Tnfa, Tgfb* et *iNos*) et de chimiokines (*Ccl2, Ccl5, Cxcl9* et *Cxcl10*) après traitement par CCl_4 a été significativement réduite chez la souris *Il15*$^{-/-}$ par rapport à la souris contrôle. De plus, les foies des souris WT nourries avec CD-HFD ont également montré une expression réduite du gène *Ppara*, ce qui indique que l'inflammation médiée par l'IL-15 altère le métabolisme des lipides.

Nos résultats suggèrent que la réponse inflammatoire médiée par l'IL-15 pourrait jouer un rôle important dans la progression de la fibrose hépatique. Nos données indiquent un rôle pro-fibrogénique de l'IL-15 dans la pathogenèse de la fibrose hépatique et soulèvent la possibilité de cibler la signalisation de l'IL-15 pour le traitement de la fibrose hépatique.

Mots clés: IL-15, Fibrose hépatique, Stéatose hépatique non alcoolique (NAFLD), Régime pauvre en choline (CD-HFD), CCl4, Chimiokines, Inflammation

ABSTRACT

Role of the inflammatory cytokine IL-15 in the progression of Liver fibrosis

Bhavesh Variya

Cell Biology Program

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Chronic inflammation in the liver causes liver fibrosis, which predisposes to liver cancer. Indeed, most cases of liver cancer occur in fibrotic livers. Different immune cells specifically NK cells and macrophages and various cytokines and chemokines contribute to the progression of fibrosis as well as its resolution. Interleukin-15 (IL-15) is a pro-inflammatory cytokine produced mainly by immune cells in response to innate immune stimuli and is required for immune cell infiltration and homeostasis in the liver micromilieu. However, the exact role of IL-15 in the homeostasis of liver microenvironment remains to be elucidated. Hence, the goal of this thesis is to determine the role of IL-15 in the progression and pathogenesis of liver fibrosis.

Liver fibrosis was experimentally induced in wildtype (WT) and *Il15*$^{-/-}$ mice either by intraperitoneal injection of carbon tetrachloride (CCl_4) twice a week for 7 weeks or by providing choline-deficient high-fat-diet (CD-HFD) *ad libitum* for 12 weeks. After the induction period, mice were sacrificed, and liver tissues were collected for histology (H&E and Sirius Red), immunohistochemistry (α-SMA and Collagen) and immunofluorescence (CD68). Proinflammatory cytokine gene expression was assessed by qRT-PCR.

In WT mice, CCl_4 treatment significantly increased IL-15 expression in the liver along with increased collagen deposition and elevated proportion of α-smooth muscle actin (α-SMA) positive cells in liver sections. Similar observations were made in CD-HFD induced liver fibrosis model also. Both collagen deposition and α-SMA positive cells were significantly reduced in the livers of CCl_4-treated or CD-HFD fed IL-15 deficient mice. Concomitantly, the induction of various proinflammatory cytokines (*Il12, Tnfa, Tgfb* and *iNos*) and chemokines (*Ccl2, Ccl5, Cxcl9* and *Cxcl10*) following CCl_4 treatment was significantly reduced in *Il15*$^{-/-}$ mice compared to control mice. Additionally, the livers of CD-HFD fed WT mice also showed reduced *Ppara* expression indicating that IL-15 mediated inflammation alters lipid metabolism.

Our results suggest that IL-15 mediated inflammatory response may have an important role in the progression of liver fibrosis. Our data indicate a pro-fibrogenic role of IL-15 in the pathogenesis of liver fibrosis and raises the possibility of targeting IL-15 signaling for the management of for liver fibrosis.

Keywords: IL-15, Liver fibrosis, Non-alcoholic fatty liver disease (NAFLD), Choline-deficient high fat diet (CD-HFD), CCl_4, Chemokines, inflammation

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ABBREVIATIONS

aa	amino acid
ACAD	Acyl-CoA dehydrogenase
AFLD	Alcoholic fatty liver disease
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AMPK	AMP-activated protein kinase
ANOVA	Analysis of variance
APC	Antigen presenting cell
ApoB	Apolipoprotein B
aSMase	Acidic sphingomyelinase
AST	Aspartate Aminotransferase
BSA	Bovine serum albumin
cAMP	Cyclic adenosine monophosphate
CCl_4	Carbon tetrachloride
CDD	Choline-deficient regular diet
CD-HFD	Choline-deficient high fat diet
Chol	Cholesterol
Chyl	Chylomicrons
CoA	Acyl-coenzyme A
CPT	Carnitine palmitoyl-transferase
CRP	C-reactive protein
CYP	Cytochromes P family
DAG	Diacylglycerol
DAMP	Damage-associated molecular pattern
DC	Dendritic cells
DNA	Deoxyribonucleic acid
ECM	Extra cellular matrix
ER	Endoplasmic reticulum

FA	Fatty acid
FLD	Fatty liver disease
g	gram
GFP	Green fluorescence protein
H&E	Hematoxylin and eosin
H_2O_2	Hydrogen peroxide
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HDL	High-density lipoprotein
HIV	Human immunodeficiency virus
HMGCS	Hydroxy-methyl-glutaryl-CoA synthase
HSC	Hepatic stellate cells
i.p.	intraperitoneal
IDL	Intermediated-density lipoprotein
IF	Immunofluorescence
Ifng$^{-/-}$	Interferon gamma deficient
IHC	Immunohistochemistry
IL	Interleukin
IL-15	Interleukin 15
Il15$^{-/-}$	IL-15 deficient
IL-15R	Interleukin 15 receptor
Il15ra$^{-/-}$	Interleukin 15 receptor alpha deficient
IFN	Interferon
iNOS	inducible nitric oxide synthase
IR	Insulin resistance
JAK	Janus kinase
JNK	c-Jun N-terminal kinase
kg	kilogram
LBP	Lipopolysaccharide binding protein

LDH	Lactate dehydrogenase
LPL	Lipoprotein lipase
LPS	Lipopolysaccharides
LSEC	Liver sinusoid endothelial cells
LSP	Long single peptide
MAIT	Mucosal associated invariant T cells
MCP	Monocyte chemotactic peptide
MCSF	Macrophage colony stimulating factor
MHC	Major histocompatibility complex
mM	millimolar
MMP	Matrix metalloproteinase
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
NF-κβ	Nuclear Factor-Kappa β
NH_3	Ammonia
NK	Natural killer
NKT	Natural killer T-cell
NOS	Nitric oxide synthase
ORF	Open reading frame
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PKA	Protein kinase A
PLC	Phospholipase C
PPAR	Peroxisome proliferator-activated receptor
PUFA	Polyunsaturated fatty acid
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SEM	Standard error of mean
α-SMA	Alpha-Smooth muscle actin

SREBP	Sterol regulatory element-binding protein
SSP	Short single peptide
STAT	Signal transducer and activator of transcription
Syk	Spleen Tyrosine kinase
TACE	Tumor necrosis factor-alpha converting enzyme
TCA	Tricarboxylic acid
TCR	T-cell receptor
TG	Triglyceride
TGF	Transforming growth factor
TIMP	Tissue inhibitor of metalloproteinase
TLR	Toll-like receptor
TNFα	Tumor necrosis factor α
TNFR	Tumor necrosis factor receptor
UACC	University Animal Care Committee
UDP	Uridine diphosphate
VLDL	Very low-density lipoprotein
WBC	White blood cell
WT	Wild type

1. Introduction

1.1. Structure and function of the liver

1.1.1. Anatomy of the liver

The liver, situated between the digestive tract and the spleen in the abdominal cavity, is one of the most important and largest organs in the body, which plays a vital role including detoxification of toxins and foreign substances. Apart from detoxification, liver is the main site for carbohydrate, lipid and protein synthesis and metabolism, and thereby maintains homeostasis in the body. It weights approximately 1 to 1.5 kg and representing around 2 to 3% of average body weight but receives the highest blood supply *i.e.* about 25 to 30% of total cardiac output (Campbell, 2006; Abdel-Misih and Bloomston, 2010). Liver is also involved in vital functions such as waste metabolism, production of different proteins associated with blood coagulation, excretion of bile, hormones, drugs and xenobiotics (Ahsan et al., 2009).

Liver undergoes rapid changes in size as well as glycogen and protein content depending on nutrient supplements. It has complex blood supply; 75 to 80% of nutrients-rich but deoxygenated blood influx is supplied via portal vein, which drains alimentary tract (duodenum, small intestine, gall bladder, pancreas and a part of colon) while hepatic arteries bring remaining 20 to 25% oxygen supplemented blood (Rappaport, 1980; Campbell, 2006). These dual afferent systems merge at the sinusoids. Hepatic blood flow varies from 800 to 1500 ml/min depending on physiological state of the body (Greenway and Stark, 1971). The portal blood supply is at low pressure (6-10 mmHg in humans) while hepatic arterial blood flows at high pressure (around 90 mmHg) and this pressure is comparable to the aortic blood pressure. However, portal pressure depends on the vascularity of splenic and mesenteric arterioles as well as intrahepatic resistance.

The cells in the liver are well organized in a structural unit, termed as Lobule, consisting of peripheral hepatocytes in hexagonal shape followed with a central vein (Trefts et al., 2017). In the lobules, hepatocytes are arranged in cords leaving intercellular vascular space with a thin fenestrated endothelium along with an intermittent membrane known as sinusoids. This sinusoidal space contains tissue resident macrophages known as Kupffer cells and hepatic stellate cells (HSCs), considered as hepatic lipocytes (Vernon and Kasi, 2019). The hepatic acinus is a functional unit of the liver as is the site for solute exchange between hepatocytes and blood. Acinus is difficult to visualize as it is situated

around efferent vascular system and is of more relevance to hepatic functions (Gumucio, 1983). Based on the hepatic portal acinus, the liver could be structurally divided in 3 zones. As described in Figure 1.1, hepatocytes from zone 1 immediately surround the portal tract and are mainly involved in the oxidative energy metabolism. Zone 3 hepatocytes surround the central veins and are mainly involved in xenobiotics metabolism and biotransformation of drugs. Zone 2 hepatocytes are situated between zone 1 and zone 3 hepatocytes, and they have mixed functions (Trefts et al., 2017; Vernon and Kasi, 2019).

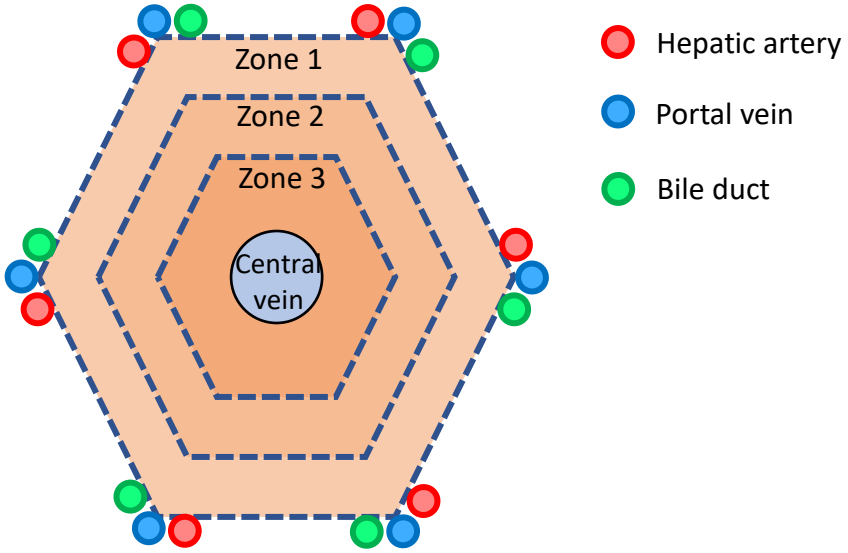

Figure 1.1. Schematic diagram of portal triad (Adapted from (Trefts et al., 2017)).

Hepatocytes in roughly hexagonal in shape, the vertices represent the portal triad area. Each portal triad contains branches of bile duct, portal vein and hepatic artery.

Structurally, liver cells could be classified into two main groups, namely epithelial cells and mesenchymal cells. Hepatocytes are the major epithelial subtype in the liver that accounts more than 90% of the liver parenchyma. Apart from hepatocytes, cholangiocytes (biliary epithelial cells), hepatic progenitor cells and intermediate hepatocyte-like cells are also part of the liver epithelial cells group (Papoulas and Theocharis, 2009). Cholangiocytes, located in the intra- and extra-hepatic biliary duct system, are involved in bile modification and transport by releasing water and bicarbonate. Cholangiocytes also respond to liver injury associated inflammation (Marzioni et al., 2002; O'Hara et al., 2013). In response to injury, cholangiocytes proliferate and maintains biliary homeostasis by inducing reparative processes associated with ductular changes (Strazzabosco and Fabris, 2012; O'Hara et al., 2013). The hepatic non-parenchymal cell group includes Kupffer cells, hepatic stellate cells, myofibroblasts

and endothelial cells (sinusoidal cell and pit cells) (Papoulas and Theocharis, 2009; Lemoinne et al., 2013).

1.1.1.1. Hepatocytes

Hepatocytes are the main parenchymal cells in the liver, and are primarily involved in the metabolism, protein synthesis and detoxification. They also release a variety of proteins such as complement component 1r/s (C1r/a), C2, C3, C4, C-reactive protein (CRP) and lipopolysaccharide binding protein (LBP), which play a crucial role in the maintenance of innate immunity (Gao et al., 2008). In the healthy liver, as part of tissue repair, dead cells stimulate mature hepatocytes replication leading to reconstitution of normal tissue function (Jou et al., 2008). However, hepatic oxidative stress, a central etiology of non-alcoholic steatohepatitis or non- alcoholic fatty liver disease (NASH/NAFLD) and fibrosis development, inhibits the replication of mature hepatocytes. This leads to expansion of hepatic progenitor cells which differentiate into hepatocyte-like cells. Total number of hepatic progenitor cells and hepatocyte-like cells is strongly correlated with stages of fibrosis, where loss of hepatocytes promotes hepatic progenitor cell proliferation followed by differentiation into hepatocyte-like cells (Roskams et al., 2003). Activation of these cells is also associated with the progression to hepatocellular carcinoma (HCC) (Roskams et al., 2003). The pathogenesis of liver fibrosis depends on the efficacy of hepatocyte regeneration, therefore impaired proliferation of hepatic progenitor cells leads to development of NAFLD/NASH and fibrosis (Jou et al., 2008; Dowman et al., 2010).

1.1.1.2. Hepatic Stellate Cells

Hepatic stellate cells are located in the space of Disse, sandwiched between the sinusoidal endothelial cells and hepatic epithelial cells (Figure 1.2). In normal physiological condition, healthy stellate cells contain abundant amount of lipid droplets for Vitamin A storage and are in a quiescent state (Blaner et al., 2009). During liver injury, viral infection, autoimmune disorder or NAFLD, damaged hepatocytes and infiltrated immune cells secrete pro-inflammatory cytokines which activate quiescent HSCs to differentiate into proliferative, contractile and fibrogenic myofibroblast-like cells (Bataller and Brenner, 2005; Friedman, 2008). In response to liver injury, activated stellate cells generate a temporary scar at the site of damage by producing collagen and other extra cellular matrix (ECM) components to protect liver from further damage. Additionally,

activated HSCs also secrete various cytokines and growth factors which promote the proliferation and regeneration of hepatic epithelial cells. However, during chronic liver injury, repetitively activated stellate cells unceasingly produce ECM components which leads to onset on liver fibrosis.

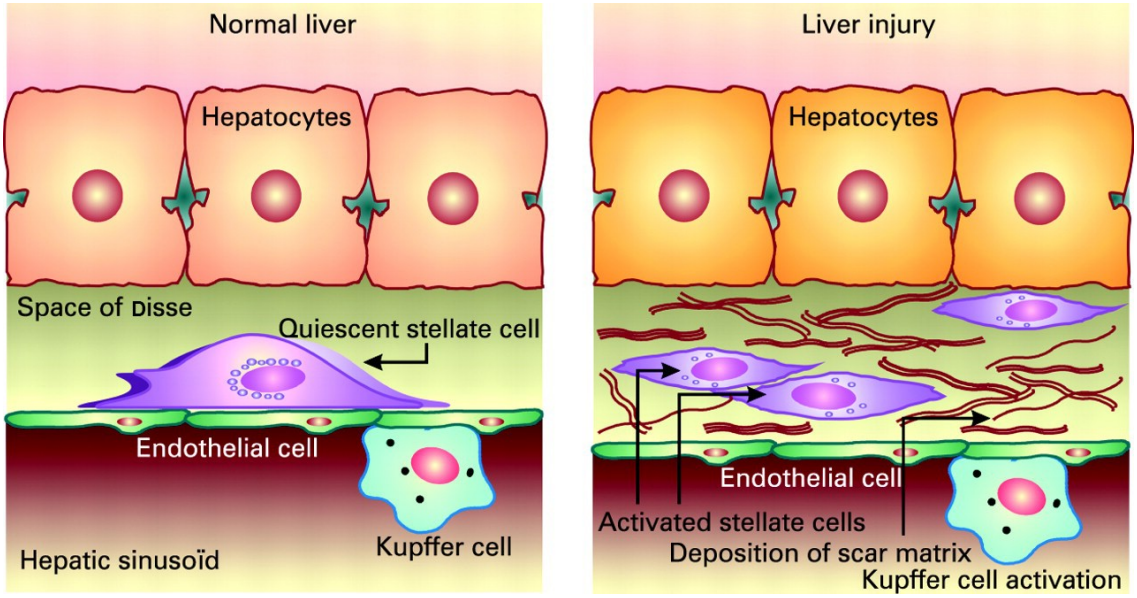

Figure 1.2. Morphology of hepatic stellate cells in normal and in injured liver (re-printed from (Asselah et al., 2009)).

Schematic diagram of hepatic stellate cells (Purple color) within the sinusoidal space in the subendothelial space.

The stellate cell activation consists of two main phases, namely initiation phase (also termed as pre-inflammatory phase) and perpetuation phase followed by resolution phase when the primary insult is resolved (Friedman, 2004). In the initiation phase, stellate cells get activated through various cytokines and other inflammatory stimuli from injury, that changes the gene expression. Moreover, products of damaged hepatocytes *i.e.* DAMPs and signals from Kupffer cell and endothelial cells also activate stellate cells leading to change in surrounding ECM-like materials. Once stellate cells get primed and activated, perpetuation occurs through the inflammatory stimuli required to maintain the activated phenotype of HSCs leading to development of fibrosis. During this phase, pro-inflammatory, pro-mitogenic and pro-fibrogenic stimuli are generated (Krizhanovsky et al., 2008; Lee and Friedman, 2011). In this phase, sustained activation of stellate cells could be observed with seven distinct features such as proliferation, fibrogenesis, contractility, chemotaxis, retinoid acid loss, matrix degradation and cytokine release (Krizhanovsky et al., 2008; Lee and Friedman, 2011).

Apart from involvement in tissue remodeling and ECM production, stellate cells also have a significant role as mediators of hepatic immunoregulation (Maher, 2001; Friedman, 2004). Immunoregulatory role of HSCs is schematically illustrated in Figure 1.3. However, interaction between immune cells and stellate cells are not yet well understood but this interaction is definitely not unidirectional. Hepatic stellate cells can augment the injury mediated inflammatory response by increasing the production of various chemokines such as CCR5, CCR7, CCL21, monocyte chemotactic peptide-1 (MCP-1) and RANTES which induce the infiltration of leucocytes (Marra and Pinzani, 2002; Bonacchi et al., 2003; Schwabe et al., 2003). Bacterial lipopolysaccharides (LPS) also stimulate the proinflammatory phenotype of stellate cells through direct interaction with toll-like receptors (TLRs) that are expressed on surface of stellate cells (Brun et al., 2005).

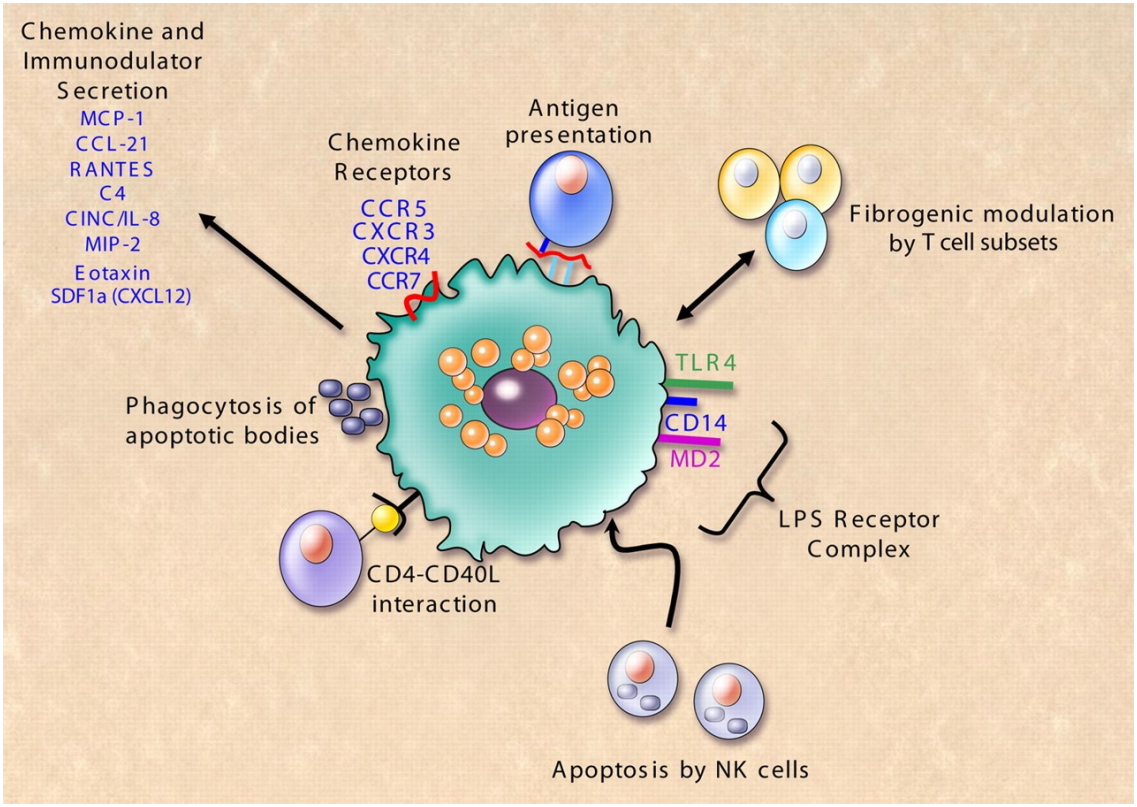

Figure 1.3. Immunoregulatory role of hepatic stellate cells (re-printed from (Friedman, 2008)).

Various mechanisms which regulate HSCs homeostasis to maintain immune tolerance in the hepatic micromilieu.

HSCs are also considered as professional antigen presenting cells (APCs) indicating that stellate cells stimulate proliferation and apoptosis of extravasated and infiltrated

lymphocytes (Kobayashi et al., 2003; Vinas et al., 2003; Unanue, 2007; Winau et al., 2007). This indicates towards a regulatory role for stellate cells in lymphocyte infiltration in the liver during the progression of fibrosis (Kobayashi et al., 2003). In addition to mononuclear cell infiltration, HSCs also produce various neutrophil chemoattractants as well as complement protein C4 which contribute to neutrophil infiltration and accumulation in liver, and liver's inflammatory response, respectively (Fimmel et al., 1996; Maher et al., 1998). Stellate cells regulate leucocyte behaviors, and are also affected by specific subsets of lymphocytes. For instance, in stellate cells, fibrotic events are promoted more by $CD8^+$ T-cells than $CD4^+$ T-cells (Safadi et al., 2004). This could in part explain, the increased fibrosis in patients with hepatitis C virus infection along with HIV coinfection, where CD8 T-cell count exceeds CD4 T-cells, compared with patients with only hepatitis C virus infection. Activated HSCs also express TLR4, CD14 and MD2, which together form the LPS receptor complex (Paik et al., 2003). Hence, LPS induces expression of chemokines and adhesion molecules in the stellate cells through activation of JNK and Nuclear Factor-Kappa β (NF-κβ). Activated stellate cells express programmed death ligand-1 (PDL-1) but quiescent stellate cells don't (Yu et al., 2004). Various immune cells including CD4 T-cells express PD1 and T-cell activation could be inhibited during the early stages with minimal activation of PD-1. However, the effect of PD1 activation depends on the degree of stimulation through TCR, stage of lymphocyte differentiation and, importantly, is context dependent. This suggests that stellate cells can suppress T-cell mediated cytotoxicity by inducing T-cell apoptosis but not proliferation or cytokine production. These could be the reasons that transplanted HSCs protect allograft from rejection, and also support transplanted cell engraftment in the liver (Benten et al., 2005; Chen et al., 2006).

1.1.1.3. Kupffer cells

Kupffer cells, the liver residential macrophages, are the crucial part of mononuclear phagocytic system and play a central role in the innate immune responses in the liver. Kupffer cells are the first line defence mechanism against pathogens that enter the liver through the elementary canal via portal circulation. Kupffer cells also exert an anti-inflammatory effect by preventing the systemic entry of gut-derived immunoreactive substances. Kupffer cells are also part of the mononuclear phagocytic system (Thomson and Knolle, 2010). The functions of the Kupffer cells are dependent on the disease status; they are protective in drug induced liver damage and toxin induced liver fibrosis (Ju et

al., 2002; Ramachandran and Iredale, 2012), while they contribute to inflammation during the progression of alcoholic and non-alcoholic FLD (Nagy, 2003; Chiang et al., 2011).

Kupffer cells that seed the liver are embryonic yolk-sac derived (Gomez Perdiguero et al., 2015). They have self-renewing capabilities and they persist into adulthood in the absence of any insult to the liver. Various growth factors including macrophage colony stimulating factor (MCSF) regulate the maturation of Kupffer cells (Naito et al., 1997). The influx of circulatory monocytes is higher in liver than in other tissues but the number of Kupffer cells is tightly regulated by complex mechanisms in the liver, the control of which is not well understood yet. The number of Kupffer cells might be regulated through apoptosis (Dixon et al., 2013). Jenkins and colleagues revealed increased Kupffer cell proliferation and expansion in response to IL-4 mediated T_H-2 inflammatory response (Jenkins et al., 2011; Dixon et al., 2013). However, during infections, if the resident Kupffer cells are lost, inflammatory monocytes from circulation seed the liver and acquire the genetic landscape of the tissue microenvironment (Lavin et al., 2014; Lai et al., 2018). Kupffer cells also play an important role in the alcohol induced hepatitis. Alcohol induces the gut permeability which leads to increase in serum endotoxin levels such as LPS. This LPS activates Kupffer cells via TLR-4 and activated Kupffer cells play a vital role in the inflammatory cascade leading to alcohol hepatitis (Suraweera et al., 2015).

The close proximity of Kupffer cells with other cells is shown in Figure 1.4. Parenchymal and non-parenchymal cells, within the sinusoid, support the functions of Kupffer cells during normal physiology and pathological state. In normal physiology, Kupffer cells exhibit tolerogenic phenotype. This helps to prevent undesired immune responses provoked by gut-derived materials as well as by antigen present on dead cells during clearance by liver (Thomson and Knolle, 2010). However, in several pathological conditions, Kupffer cells get transformed from tolerogenic to inflammatory phenotype and start producing inflammatory signals. Hence in these inflammatory conditions, due to close proximity with other cells, Kupffer cells mediated inflammation alters the functions of stellate cells and hepatocytes. In various inflammatory conditions such as NASH, NAFLD and fibrosis, Kupffer cells mediated inflammation leads to progression of the disease (Dixon et al., 2013). Hence, suggested therapeutic approach is to dampen the Kupffer cells activation rather than eliminating them as they are still critical for

minimizing inflammatory responses in the liver that can arise from the gut and systemic inflammation.

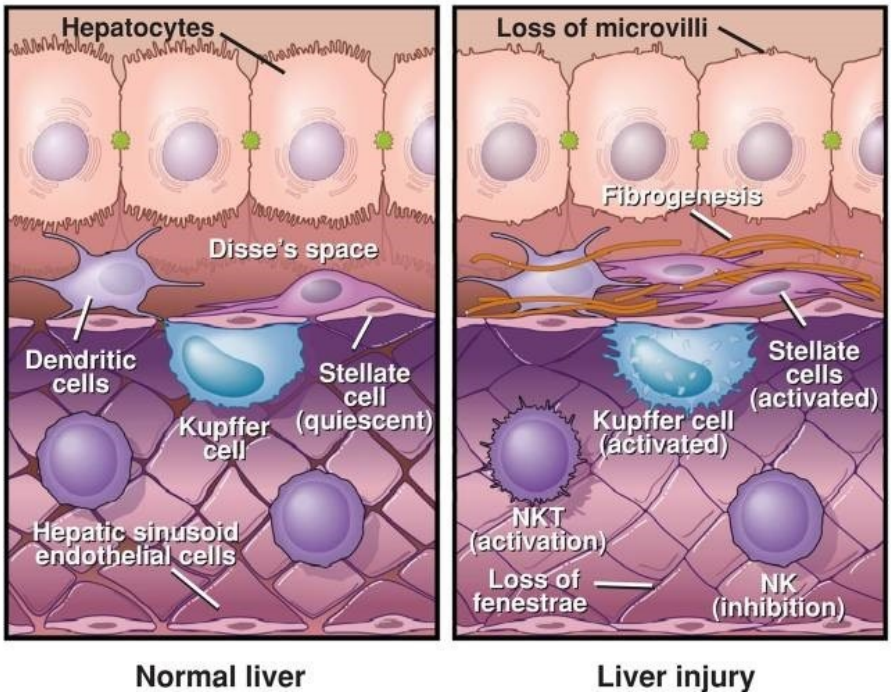

Figure 1.4. Localization of Kupffer cells in healthy and diseased liver (re-printed from (Dixon et al., 2013)).

The Kupffer cells are located in hepatic sinusoid, with close proximity to natural killer (NK), natural killer T-cell (NKT) and liver sinusoid endothelial cells (LSEC). Activated Kupffer cells produce inflammatory cytokines and chemokines which influence the activity of hepatocytes and HSCs.

1.2. Functions of the liver

The liver plays a critical role in the human body through its extremely complex functionality which include; metabolic, immunological, vascular, excretory and secretory functions (Mitra and Metcalf, 2009).

1.2.1. Metabolic function of the liver

The liver works as a central metabolic unit of the body where it performs all metabolic processes under the control of circadian rhythm (Reinke and Asher, 2016) *i.e.* detoxification of drugs and xenobiotic, metabolism of nutrients, synthesis of plasma albumin and clotting factors (Heinz and Braspenning, 2015; Trefts et al., 2017). The liver plays a significant role in metabolism of three key components of nutrients such as carbohydrate, lipids and proteins.

1.2.1.1. Carbohydrate metabolism

The liver regulates the amount of systemic glucose levels through several biochemical mechanisms, including glycogenesis, glycogenolysis, glycolysis and gluconeogenesis. After digestion of food, glucose enters the blood stream and serum level of glucose increases. The glucose that is absorbed by the intestine through the portal vein moves to hepatocytes where GLUT2 transporters are involved in its hepatic uptake (Takeda et al., 1993). The glucose surplus is transformed into glycogen by insulin via dephosphorylated active glycogen synthase (Mitra and Metcalf, 2009). After 2-3 hours of the postprandial phase once the systemic glucose level goes down to the basal state, the glycogen gets converted into glucose through glycogenolysis regulated by glucagon via adenylate cyclase/cAMP/PK-A pathway (Chiang, 2014). Next, inside the hepatocytes, hexokinase phosphorylates glucose into glucose 6-phosphate which undergoes several biotransformation steps *i.e.* isomerization into either glucose 1-phosphate or fructose 6-phosphate and oxidation processes where it is converted into gluconolactone. Glucose 1-phosphate forms UDP-glucose which serves as a predecessor of glycogen and UDP-gluconate. Fructose 6-phosphate may either initiate glycolysis pathways where it is converted into pyruvate and acetyl-Co-A or it can combine to glutamine or enter into hexosamine pathway. Pentose phosphate pathway is initiated if glucose 6-phosphate undergoes oxidation and is transformed to gluconolactone (Adeva-Andany et al., 2016).

1.2.1.2. Metabolism of lipid/fat

The liver establishes a physiological connection with intestinal tract as well as adipose tissues by which it plays a central role in the metabolism of lipids. Elevated expression of various transcription factors such as SREBP1c, ChREBP and FoxO1 regulate synthesis of fatty acids (FA). Translocation of FA takes place via transporter proteins *i.e.* FAT/CD36, FATP, FABP and caveolin-1 which enhances its uptake by hepatocytes (Canbay et al., 2007). Metabolism of fat takes place by two pathways *i.e.* exogenous pathway and endogenous pathway. In the former only dietary fat and cholesterols are absorbed as chylomicrons in the intestine. Here free fatty acid and glycerol together form triglyceride (TG) followed by esterification of cholesterol (Chol). TG and Chol unite to form chylomicrons (Chyl). The Chyl enters the systemic circulation and migrates toward peripheral tissues, where free fatty acids are separated from Chyl to be consumed as energy, transformed to TG or deposited in adipocytes. The remnants are utilized to form

High-density lipoprotein (HDL). In the later pathway the Chyl enters the liver and gets defragmented to form TG and Chol ester. Their hydrolysis by lipoprotein lipase (LPL) converts them into intermediate-density lipoprotein (IDL) or very low-density lipoprotein (VLDL) remnants. The VLDL remnants are transformed to LDL having maximum cholesterol and least TG in their composition. These LDL are again internalized via LDLR into the liver and converted into bile acid which is released into the intestine to emulsify the upcoming fat (Izem et al., 1998). The fat enters the liver and ultimately gets converted into acetyl Co-A via β-oxidation in mitochondria of hepatocytes and enters in the TCA cycle followed by either conversion into H_2O and CO_2 or transformation into ketone bodies. Acetyl Co-A may also take part in *de-novo* synthesis of cholesterol, lipoprotein and triglyceride phospholipids. Insulin retards fatty acid oxidation while glucagon augments the oxidation process (Voet et al., 2016). Insulin resistant patients are more prone to develop hypertriglyceridemia associated metabolic syndrome due to augmented level of triglyceride and elevated lipogenesis (Otero et al., 2014).

1.2.1.3. Metabolism of protein

The liver plays a prime role in metabolism of protein via production of plasma proteins, deamination, transamination and formation of urea. Liver synthesizes various clotting factors *viz*. coagulation factor II, V, VII, IX, X, XI, XII, XIII, and fibrinogen; thyroid binding globulins, albumins, VLDL ApoB-100, and several complements. The systemic circulation retains approximately 80% of proteins synthesized in the liver and further contribute to regulate oncotic pressure, lipid as well as hormonal transport and general homeostasis of the body. Net hepatic plasma protein synthesis lies between 30-60 g/day (Charlton, 1996). The deamination process consists of $–NH_2$ nicking process from the amino acid (Glutamate) in the presence of glutamate dehydrogenase which leads to the formation of ammonia (NH_3). The by-product - α-ketoglutarate - formed after this biochemical reaction, is consumed to produce energy by conversion into lipid and glucose (Mitra and Metcalf, 2009). A transaminase enzyme present in the liver promotes transamination of amino acids followed by subsequent redistribution of amino groups among amino acids. The L-glutamate/ α-ketoglutarate pair function as an amino group donor/ acceptor couple in the process of transaminase reactions. Glutamate pyruvate transaminase and alanine aminotransferase are two enzymes for diagnostic purposes. All transaminases consume pyridoxal phosphate where pyridoxal phosphate serves as a carrier of $–NH_3$ groups as well as an electron sink by enabling α-hydrogen dissociation

from the amino acid (Bhagavan, 2002). The hepatic parenchymal cells synthetize most of the coagulation factors while reticuloendothelial cells promote clearance of the released products. Together these activities regulate homeostasis. The extent of liver injury governs the level of dysfunction in coagulation process *i.e.* in case of the acute or chronic liver abnormalities there would be scarcity of the coagulation factors associated with vitamin K such as; VII, IX, and X; prothrombin; proteins C and S, while other factors would be normal (Mammen, 1992).

1.2.2. The liver and immune system

The existence of diversified immunogenic cellular environment as well as non-hematopoietic cellular systems efficiently govern the complex immunological functions of a healthy liver and make it a prime site for immunological reactions. The release of inflammatory mediators in the presence of any external component *i.e.* microbial or dietary products activates the immune system in the healthy liver which is an important factor required for several metabolic, cellular remodeling, maintenance of tolerance toward innocuous molecules and to remain vigilant towards upcoming infectious substances, tissue impairments as well as malignant tissues. In the diseased state, the regulation of inflammatory responses also helps to sustain the homeostatic state when the immune system gets activated beyond its threshold because of infections and cellular distress. If the liver fails to govern the hyperactive immune signals by eliminating such stimuli, there will be pathologically exaggerated inflammatory response which retard homeostasis of hepatic cellular environment and increases the risk of fibrosis, cirrhosis and liver failure (Robinson et al., 2016). The hepatic cells as well as Kupffer cells (described in section 1.1.1.) that reside in the liver, express pattern recognition receptors. These receptors are target for microbial associated molecular pattern as well as DAMPs present in blood entering from portal circulation and leads to neutralize them without accelerating inflammatory stimuli. This mechanism of clearance that involves systemic circulation from the intestine via portal circulation guards the remaining organs from an excessive immunogenic response (Janeway, 1992; Takeuchi and Akira, 2010; Kubes and Mehal, 2012). The hepatic microenvironment expresses an intricate cytokine system which is composed of pro-inflammatory IL-2, IL-12, IL-7, IL-15 and IFN-γ as well as anti-inflammatory cytokines such as IL-10, IL-13 and transforming growth factor beta (TGF-β) in its basal healthy state (Golden-Mason et al., 2004; Kelly et al., 2006). The innate immune

response of hepatocellular microenvironment is mediated by lymphocytes *viz*. NK cells, mucosal associated invariant T cells (MAIT), NKT cells and γδ T cells which play an important role in the production of cytokines and regulate both innate as well as adaptive immunogenic stimulus in liver. The adaptive immune system is composed of major histocompatibility complexes (MHC)-associated $CD8^+$ T-cells, $CD4^+$ T-cells as well as B-cells which is an essential part of humoral immunity. The hepatic system also comprises innate-like $CD5^+$ B-cells which get elevated during hepatotropic viral infections (Robinson et al., 2016).

1.3. Steatosis/ fatty liver

Hepatic steatosis or fatty liver is a clinical feature of many hepatic disorders including Hepatitis C virus (HCV) infection, non-alcoholic steatohepatitis, alcoholic steatosis and alcoholic steatohepatitis (El-Zayadi, 2008; Sakhuja, 2014). The liver is considered as fatty when greater than 5% fat (triglyceride) is deposited in hepatic tissues (Bedogni et al., 2014). Insulin resistance (IR) has been considered as the key factor behind hepatocellular fatty acid deposition. Furthermore, HCV infection elevates the risk of IR which promotes non-alcoholic steatosis and fibrosis (Romero-Gomez, 2006). Alcoholic fatty liver is an extremely catastrophic state which aggravates fibrosis and cirrhosis if consumption of alcohol is not stopped. The pathological condition follows multiple molecular mechanisms such as decreased PPAR-α activity, elevated $NADH/NAD^+$ ratio and elevated level of complement c3. Upregulation of SREBP-1 activity because of reduced activity of sirtuin-1, activated AMPK and/or elevated TNF-α level due to alcohol consumption also aggravate this pathological condition. Moreover, inactivation of enzymes related to peroxisomal β-oxidation as well as mitochondrial fat leads to accumulation of fat in the liver (Purohit et al., 2009).

About 25% of global, 30% of US and 24% European populations suffer from non-alcoholic fatty liver disease. By year 2020, this pathological state is expected to become a major factor for chronic hepatic disorders (Ofosu et al., 2018; Younossi et al., 2019; Younossi, 2019). Between 2015 and 2016, 4.7% US adults have been reported to suffer from alcoholic fatty liver disease. Stage 2 and stage 3 liver fibrosis was reported to affect 1.5% and 0.2% of US adults, respectively (Wong et al., 2019). Elevated level of fat in the liver induces hepatocellular damage by multiple factors *i.e.* oxidative damage, lipid peroxidation which leads to aggravated inflammatory response followed by

mitochondrial abnormality, induced cytokine activity as well as advancement of fibrosis. TNF-α secreted from active macrophages of the hepatic-fatty tissues aggravates IR and contribute to steatohepatitis. Free fatty acid promotes apoptotic events in hepatocytes and leads to liver damage. Reactive oxygen species (ROS) activates stellate cells and subsequent collagen deposition whereas by-products of lipid peroxidation upregulate nuclear factors which promote overexpression of procollagen type I which is responsible for fibrosis and ultimately cirrhosis (El-Zayadi, 2008).

1.4. Liver fibrosis

Liver fibrosis is characterised by extreme accumulation of ECM-like proteins *i.e.* collagen in disproportionate manner which in the later stage transforms to cirrhosis, hepatic failure, and aggravates portal hypertension. This catastrophic disaster rises the requirement of liver transplantation. The other mechanisms involved in the development of liver fibrosis are; oxidative stress, activation of immune cells and stellate cells; anaerobic-proinflammatory state and epigenetic modification in the hepatic microenvironment (Olsen et al., 2011; Mann, 2014; Pellicoro et al., 2014). Oxidative stress and ROS alter the structural and functional defense mechanisms which actively contribute to the fibrogenesis in liver. In general, the ROS or reactive aldehyde such 4-HNE produced by injured or nearby active hepatic tissues, can alter the functional characteristics of myofibroblasts through anomalous transcription of profibrogenic genes *i.e.* MCP1, procollagen type-I and TIMP1. Hence oxidative damage is one of the prime mechanisms behind fibrogenesis in case of chronic alcohol consumption (Pinzani, 2015).

Hepatic steatosis associated hepatocellular damage has been considered as the prime reason behind hepatic inflammation and immune cells activation. Active hepatic macrophages (Kupffer cells), T-cells, dendritic cells (DC) and neutrophils are involved in activation of inflammatory pathways (Koyama and Brenner, 2017). These inflammatory signals include fibrogenic cytokines such as leptin, angiotensin II, TGF-β1, secretion of CXCL10 by leukocytes and activation of hedgehog signaling pathway. Expression of mesenchymal snail and twist genes activate portal fibroblasts, stellate cells, and myofibroblasts which have been considered as major contributors for collagen production in the damaged hepatocellular system (Bataller and Brenner, 2005; Liu et al., 2011). Furthermore, IR is also considered as the major factor for the induction of fibrosis through several mechanisms which include; hyperleptinemia, steatosis, subsided

expression of receptor- PPARγ and extensive TNF-α production (Romero-Gomez, 2006). Involvement of various liver cells in the pathogenesis of fibrosis has been illustrated in the Figure 1.5.

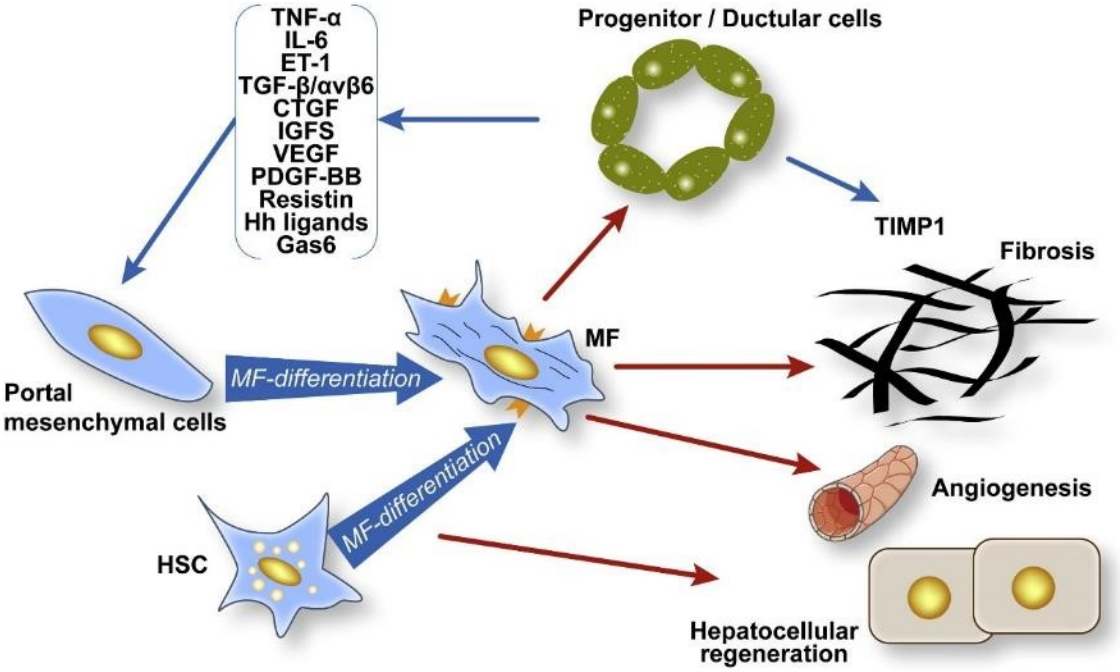

Figure 1.5. Potential mechanisms by which cholangiocytes, HSC and progenitor cells promote fibrosis (re-printed from (Lemoinne et al., 2013)).

Interaction of hepatic progenitor cells with myofibroblasts in healing response. Hepatic progenitor/ductular cells interact with myofibroblasts through various pathways which triggers pro-fibrogenic and/or pro-regenerative actions, depending on their origin and microenvironment. These inflammatory mediators act on mesenchymal cells through their respective receptors such as TNFα via TNFR, IL6 via IL6R, ET-1 via ET receptors, VEGF via VEGFR and IGFS via IGF receptors (ET1- Endothelin 1, CTGF- Connective tissue growth factor, IGFS- Insulin-like growth factors, MF- Myofibroblast, VEGF- Vascular endothelial growth factor, PDGF- Platelet-derived growth factor, Gas6- Growth arrest-specific 6, TIMP1- Tissue inhibitor matrix metalloproteinase 1).

1.4.1. Inflammation induced liver fibrosis: Role of HSCs and Kupffer cells

Chronic hepatic damage activates significantly catastrophic events: hepatocyte death, inflammation and innate immune system activation. It is evident that cell inflammation and cell death are interrelated. Infiltration of immune cells and chronic inflammation aggravate hepatic cell death during hepatitis by promoting cytolytic cytokines and ligands for TNFR. The hepatocellular death in reverse also works as the trigger factor behind infiltration of leukocytes and inflammatory process (Seki and Schwabe, 2015). These events activate hepatic stellate cells which release ECM leading to progression of fibrosis

and play a significant role in cytokines secretion which contributes to inflammation. Activation of stellate cells takes place in several ways *i.e.* elevated levels of IL-1β through the activation of inflammasome in Kupffer cells by DAMPs; activation of NF-κβ signals in Kupffer cells and recruited macrophages; and macrophage-derived TGF-β1 (Koyama and Brenner, 2017). Obese patients show leptin mediated activation of stellate cells which is further associated with increased expression of TGF-β1 and upregulation of the hedgehog pathway (Verdelho Machado and Diehl, 2018). Besides upregulation of inflammatory functions of resident Kupffer cells and immune cells, active HSCs are also regarded as a primary source of myofibroblasts which secrete and accumulate ECM proteins aggravating fibrosis. Liver of the healthy individual retains 5-10% of HSCs which remain inactive during regular physiology but gets activated through several cytokines and growth factors derived paracrine and autocrine signals initiated by infiltrating as well as resident immune cells. The transcription factors *i.e.* STAT3, SMAD3, AP-1, YAP1, SOX9, YAP1 and GL12; activate fibrogenic genes such as TGFβ, and promote trans-differentiation of HSCs to myofibroblasts (Mann and Smart, 2002).

1.4.2. Role of NK and NKT cells in control of liver fibrosis

NK cells dwell in hepatic sinusoidal space and constitute 50% of total hepatic lymphocytes. Activation of HSCs due to liver injury promotes activation of NK cells. The active HSCs produce high amount of retinoic acid which activates expression of RAE1. RAE1 serves as the ligand for NKG2D and MICA receptor on NK cells. Additionally, expression of NKp46 related ligands are also regulated by HSCs. Once the NK cells become active, they become cytotoxic for HSCs. Furthermore, interferon gamma (IFN-γ) secreted by NK cells also promotes apoptosis as well as cytostatic events in HSCs and reduces their population and retard fibrotic events (Gao and Radaeva, 2013; Fasbender et al., 2016). In many pathological conditions such as chronic liver disease, HSCs find the way to escape from cytotoxic events by NK cells and cause fibrosis and cirrhosis due to excessive expression of TGF-β by hyperactive HSCs and myofibroblasts. Here, TGF-β serves as a potent NK cell inhibitor and hinders the cytotoxic effects of NK cells upon HSCs (Gao and Radaeva, 2013). Chronic consumption of alcohol inhibits NK cells functions by inhibiting the expression of IFN-γ, NKG2D and TRAIL; aggravate HSCs activity and promote alcohol induced liver fibrosis (Jeong et al., 2008).

Human liver has fewer NKT cells. In contrast to NK cells, NKT cells aggravate liver fibrosis by upregulation of profibrotic hedgehog ligands and cytokines such as IL-13, IL-4 and osteopontin. In certain other situations, NKT cells can retard liver fibrosis by production of IFN-γ and through its cytotoxic action on HSCs (Gao and Radaeva, 2013). The complex role of NKT cells is observed in HCV infected patients. In HCV positive patients without cirrhosis, NKT secrete both anti-fibrotic (IFN-γ) as well as profibrotic (IL-4 and IL-13) cytokines. However, in cirrhotic patients, NKT cells predominantly release pro-fibrotic cytokines *i.e.* IL-4 and IL-13 and promote liver fibrosis (de Lalla et al., 2004).

1.4.3. Role of cytokines (TNF-α, IFN-γ, IL6, IL12, IL15) and ROS in liver fibrosis

Cytokines are released by nearly all kind of cells in the liver and serve as prime regulators of healthy physiology and abnormal pathophysiological state of the liver by orchestrating the signals involved in hepatocellular regeneration, inflammation, cholestasis, fibrosis, and cell death in response to liver injury. IL-6 and TNF-α are the two prototypic proinflammatory cytokines which are secreted in the preliminary phase of any liver damage and stimulate synthesis of subsequent cytokines to trigger recruitment of inflammatory cells which lead to fibrosis as a part of healing process (Tilg and Diehl, 2000). Resident macrophages and infiltrating lymphocytes produce TNFα as well as ROS at the time of liver damage (Gressner, 2001).

TNFα initiates several molecular signals involved in inflammation, proliferation as well as apoptosis. It transforms quiescent HSCs into their active fibrogenic myofibroblast phenotype which initiate fibrotic events. Fibrosis induced by carbon tetrachloride (CCl4) treatment was lower in mice lacking TNFR1 thereby demonstrating a significant role for TNFα in fibrosis (Baud and Karin, 2001; Sudo et al., 2005). Pro-TNFα is cleaved by Tumor necrosis factor-alpha converting enzyme (TACE) at Ala^{76} and Val^{7} site to generate the active TNFα. NASH induced by Choline-deficient high fat diet (CD-HFD) along with high fructose supplemented diet show high expression of TACE, subsequent higher production of TNFα, collagen, TGF-β and α-smooth muscle actin expression. Furthermore, in diabetic patients, upregulation of TACE has been documented which unambiguously points towards a role for TNF-α in the development of NASH and fibrosis (Jiang et al., 2013). TNF-α signaling through TNFR1 activates NF-κβ via phospholipase C (PLC) and endosomal acidic sphingomyelinase (aSMase) pathways.

Activation of aSMase by TNF-α is associated with DAG activity. Activated aSMase activates NF-κβ by inhibiting NF-κβ inhibitor-I (IκB) (Schutze et al., 1995). Active NF-κβ translocates to the nucleus and serves as a transcription factor for *Mmp9* and accelerates MMP9 production which in turn contributes to ECM remodeling that has been considered as one of the TNF-α associated mechanisms involved in liver fibrosis (Tarrats et al., 2011). Available data also suggest involvement of TNF-α dependent TAK1, JNK, RIP1 and RIP3 signals in the progression of liver fibrosis (Yang and Seki, 2015).

IFN-γ is mainly secreted by T-cells and NK cells that are responsible for killing tumors and infected cells (Weng et al., 2005). IFN-γ has complicated effects on fibrosis and there are several ambiguities with respect to its action on liver remodeling effects. Generally, IFN-γ has been documented to supress fibrogenesis induced by dimethyl nitrosamine and CCl_4. However, exogenous IFN-γ ameliorates fibrosis in chronic HCV infected victims through the STAT-1 pathway by killing active (but not quiescent) HSCs. Furthermore, IFN-γ also enhances expression of the NK stimulatory receptor, NKG2D, and the death activator receptor, TRAIL. Thus activation of IFN-γ in NK cells mediates the loss of HSCs and retardation of fibrosis (Horras et al., 2011). In contrast, Luo et al. observed that fibrosis associated genes such as *Tgfb, Tnfa, iNos, Il4* and osteopontin were reduced in CD-HFD fed IFN-γ deficient mice compared to CD-HFD fed WT mice. Furthermore, *Ifng*$^{-/-}$ mice were less prone to develop fibrosis when compared with the wild type mice (Luo et al., 2013).

1.5. Animal models of steatosis and liver fibrosis

Development of animal models, mimicking human alcoholic fatty liver disease (AFLD), NASH/NAFLD and fibrosis are essential to understand the underlying fundamental cytological as well as bio-molecular aspect of the human pathological state. These strategies contribute to the development of novel therapeutic approaches to cure these ailments. To date, there is no perfect pre-clinical model that mimics histopathological as well as physiological features of these pathologies, but some animal models have been created which can replicate specific features observed in human patients. Animal models for AFLD include; binge ethanol feeding/ acute ethanol gavage in rodents, chronic ethanol feeding/ Lieber-DeCarli (LDC) liquid diet model (4-12 weeks), intragastric ethanol infusion model/ Tsukamoto-French model (4-8 weeks), short-term chronic plus single binge ethanol feeding (10 days), long-term chronic plus single binge ethanol

feeding (8 weeks), chronic (4 weeks) ethanol feeding plus daily binge during the last 3 days, hybrid model of high-cholesterol, high-fat diet feeding and intragastric ethanol infusion (8 weeks); Hybrid model with weekly alcohol binge (8 weeks) (Xu and Tsukamoto, 2016; Gao et al., 2017; Bertola, 2018; Ghosh Dastidar et al., 2018; Lamas-Paz et al., 2018).

Animal models for NAFLD are induced by genetic mutation, dietary means or combination of both. The dietary models include methionine and choline-deficient (MCD) diet, choline-deficient, L-amino acid defined (CDAA) high fat diet (also termed as CD-HFD), atherogenic diet (cholesterol and cholate), high-fructose diet, western and fast food style diet (ALIOS and AMLN model), diet-induced animal model of non-alcoholic fatty liver disease (DIAMOND) model, etc. Genetic models include *ob/ob* mice, *db/db* mice, KK-A^y mice and *foz/foz* mice. Models which are developed by combined dietary as well as genetic mutation include; *ob/ob* mice and MCD diet, *ob/ob* mice and AMLN diet, *db/db* mice and MCD diet, and *foz/foz* mice and HFD models. Animal models which reveal characteristics of both alcoholic and non-alcoholic steatohepatitis include intragastric high fat overfeeding combined with ethanol infusion and high fat diet with binge ethanol feeding (Hebbard and George, 2011; Clapper et al., 2013; Matsumoto et al., 2013; Asgharpour et al., 2016; Machado and Diehl, 2016; Hansen et al., 2017; Lau et al., 2017; Van Herck et al., 2017; Bertola, 2018; Lau et al., 2018; Palladini et al., 2019).

The animal models for fibrosis developed by exposure of chronic ethanol fed mice to hepatotoxins *i.e.* lipopolysaccharide, CCl_4, dimethyl nitrosamine, thiocetamide and diethyl nitrosamine treatment have been documented to develop liver fibrosis in rats (Starkel and Leclercq, 2011). Animal models representing biliary fibrosis include common biliary duct ligation, primary sclerosing cholangitis models such as *Abcb4*$^{-/-}$ mouse, chemical and infectious agent induced cholangitis, animal model with enteric bacterial cell wall components, biliary epithelial and endothelial cell injury model; primary biliary cirrhosis models *i.e.* 2-OA-BSA-α-GalCer mice and *Ae2a,b*$^{-/-}$ mice; diet induced cholestatic liver injury *i.e.* 0.1% 3,5-diethylcarbonyl-1,4-dihydrocollidine (DDC) modified diet and α-naphtylisothiocyanate (ANIT) modified diet, d-galactosamine induced biliary fibrosis; autoimmune fibrosis model include; infection of WT FVB/N mouse with Ad-2D6, Alb-HA/CL4-TCR transgenic mouse, schistosoma mansoni/japonicum infection models and heterologous serum models (Liedtke et al.,

2013; Liu et al., 2013; Matsumoto et al., 2013; Delire et al., 2015; Yanguas et al., 2016; Kim et al., 2017; Benten et al., 2018).

1.5.1. Choline deficient high fat diet (CD-HFD) induced steatosis

NAFLD is regarded as hepatic steatosis in subjects without severe alcohol consumption (Stephenson et al., 2018). CD-HFD model has been documented as the most suitable dietary model for the development of NAFLD associated fibrosis in rodents. Scarcity of choline in diet exacerbates defects in β-oxidation of lipids and diminishes the production of VLDL which in turn accelerates hepatocellular fatty accumulation, oxidative damage, hepatocellular necrosis, alteration in adipokines and cytokines with little inflammation and fibrosis (Van Herck et al., 2017). De Lima and his colleagues fed CD-HFD (54% trans fatty acid and 35% total fat) to SD rats for 16 weeks and observed human representative histological changes with NASH along with cirrhosis, CK19 positive liver carcinoma and oval cell proliferation (de Lima et al., 2008). Wu et al. fed choline/methionine deficient (MCD)-HFD to rats for 2 weeks and observed significantly higher level of hepatic steatosis with non-significant alteration in necro-inflammatory features when compared with normal control animals (Wu et al., 2011). Recently, C57BL/6 mice maintained on choline deficient regular diet (CDD) for 8 weeks were observed to have significantly higher level of serum Lactate dehydrogenase (LDH), Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) followed by extreme histopathological alteration as seen in steatosis such as microvascular as well as macrovascular fatty accumulation and ballooned hepatocytes (Oh et al., 2019). Raubenheimer et al. fed HFD (45% calories as fat) to C57BL/6 mice for 8 weeks followed by CDD in last 4 weeks and observed significant elevation in hepatic TG level. Animals on CD-HFD showed elevated expression of *Pcyt1a* and *Dgat2* genes with reduced expression of *Pepck*. Hepatic lipid content was significantly raised without altering body weight. The CD-HFD animals also exhibited improved glucose tolerance as well as insulin sensitivity which suggested that CD-HFD doesn't aggravate type-2 diabetic symptoms (Raubenheimer et al., 2006).

1.5.2. CCl_4 induced liver fibrosis

CCl_4 has been documented to induce liver damage and fibrosis by promoting the infiltration by inflammatory cells. The study conducted by Dong et al showed that treatment of wistar rats with CCl_4 for 9 weeks reproduced the histological atypical

changes in hepatocellular region that are observed in humans, such as abnormally elevated serum AST, ALT and raised hepatic hydroxy proline levels. In brief, the study showed for the first time that CCl_4 induced oxidative stress that results in hepatic necrosis, inflammation, severe destruction of hepatic lobes due to paraplastic connective tissues and eventually severe fibrosis (Dong et al., 2016). Chronic treatment with CCl_4 leads to hepatocellular damage which in turn activates and induces the proliferation of HSCs followed by excessive release of ECM. Eventually hepatocytes are replaced by excess ECM that induces scar formation and ultimately leads to fibrosis (Duval et al., 2014; Mafanda et al., 2018). Furthermore, CCl_4 has been also reported to alter the retinol pool by impairing CYP4A3 enzymatic activity, which is one of the most significant events that initiates cirrhotic events (Lee et al., 2015). Guo et al. treated SD rats with 50% CCl_4 (1 mL/kg in peanut oil) for 4 weeks and observed upregulation of *Tgfb, Timp1* and *Col1* genes, elevated levels of collagen with a concomitant reduction in markers associated with antioxidative processes (Guo et al., 2017).

Metabolism of CCl_4 by CYP2E1 enzymes release the free radical metabolite $CCl_3^{\bullet}$. The active trichloromethyl radical interacts with nucleic acids, lipids and proteins to form adducts. Inter adduct formation due to DNA-$CCl_3^{\bullet}$ interaction leads to mutational changes and progresses to HCC. Furthermore, as $CCl_3^{\bullet}$ again undergoes oxygenation process and forms $CCl_3OO^{\bullet}$, a trichloromethyl peroxy radical, which reacts with lipids and initiates lipid peroxidation as well as destruction of polyunsaturated fatty acids (PUFA) in several lipid bilayer membranes *i.e.* plasma membrane, mitochondrial membrane and endoplasmic reticulum membrane (Scholten et al., 2015).

1.6. IL-15

The γc cytokine family includes IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21. These cytokines share the common γ-chain (also known as CD132 or IL-2Rγ) as the signalling component of their receptors. Interleukin 15 belongs to the IL-2 superfamily, and shares many activities with IL-2, including the ability to stimulate lymphocytes, to generate and maintain NK cells and cytotoxic T-cells, stimulate immunoglobulin production by B cells and T-cell proliferation (Waldmann and Tagaya, 1999). Abnormalities in IL-15 expression have been detected in various disorders including chronic inflammatory conditions and malignancy. Abnormally high level of IL-15 has been detected in adult T-cell leukemia and tropical plastic paraparesis (Azimi et al., 1998). IL-15 is over-

expressed in patients suffering from rheumatoid arthritis and inflammatory bowel disease (McInnes et al., 1996; Reinecker et al., 1996; McInnes et al., 1997). Hence, IL-15 is a potential immunotherapeutic target in inflammation induced diseases.

1.6.1. Structure

IL-15, a member of 4-alpha-helicle bundle family, is a 14-15 kDa molecular weight glycoprotein with 114 amino acids, and is mainly secreted by macrophages as well as several non-lymphoid cells (Fehniger and Caligiuri, 2001). IL-15 protein has two cystine disulphide bonds between Cys^{42} and Cys^{88}, and Cys^{35} and Cys^{85} (Grabstein et al., 1994). IL-15 gene was mapped on human chromosome 4q31, and the central region of mouse chromosome (Anderson et al., 1995). Human IL-15 has 9 exons (exons 1-8 and 4a) with eight introns (Seigel et al., 1984). IL-15 shows high homology between human, murine and simian species. Human and simian IL-15 show 97% homology while 73% homology was observed between human and murine IL-15 (Grabstein et al., 1994).

There are two alternative IL-15 precursor leader peptides; a short 21-aa (amino acid) short single peptide (SSP) and the classical 48-aa long single peptide (LSP). Both peptides share 11 amino acids encoded by exon 5 (Onu et al., 1997; Tagaya et al., 1997). Although they show structural variations, both precursors produce identical mature IL-15 protein in humans and in mice (Fehniger and Caligiuri, 2001). Intracellular localization and secretion of mature IL-15 is determined by the type of the leader peptide. SSP-IL-15 and LSP-IL-15 appear to have 2-3 log-fold less secretion than IL-2 (Tagaya et al., 1997). Analysis of IL-15, fused with green fluorescence protein (GFP), demonstrated that LSP-IL-15 selectively targets the secretory pathway (endoplasmic reticulum and Golgi apparatus) while SSP-IL-15 is restricted to the cytoplasm and nucleus (Gaggero et al., 1999; Kurys et al., 2000). SSP-IL-15 is found to be mainly expressed in thymus, heart, testis and appendix, while LSP-IL-15 is predominantly expressed in placenta, lung, kidney, liver, thymus, skeletal muscle and heart (Meazza et al., 1996; Tagaya et al., 1997). However, biological significance for differential expression of IL-15 remains to be elucidated (Gaggero et al., 1999).

1.6.2. IL-15 Receptors

IL-15 receptor, similar to IL-2 receptor, is a heterotrimer, consisting of three subunits. They share the beta chain (CD122), and gamma chain (CD132) which is shared amongst all γc family cytokines (Leonard et al., 2019). The alpha chains are unique to IL-15 and

IL-2. IL-15Rα and IL-2Rα share structural similarity with pristine GP-1 motif, also known as extra-cellular protein binding Sushi domain (Giri et al., 1995). Another similarity between these receptors is that genes for IL-15Rα and IL-2Rα have similar intron and exon organization, and they are closely linked in human (10q14-15) and murine genomes (chromosome 2 associated to *Vim2* and *Spna2*) (Takeshita et al., 1992). IL-15 binds with IL-15Rα with higher affinity having K_d equal to or greater than 10^{-11} M (Krause et al., 1996). However, like IL-2Rα, it has no role in signal transduction. Indeed, IL-15/2Rβ, along with γc, have central role in signal transduction. In the absence of their respective alpha chains, IL-2 or IL-15 signals through heterodimeric IL-2Rβγc and /IL-15Rβγc, respectively. However, the binding affinity is comparatively lower than in the presence of IL-2Rα or IL-15Rα (Armitage et al., 1995).

1.6.3. IL-15 signal transduction

IL-15, synthesized in the cell, binds to IL-15Rα intracellularly in the endoplasmic reticulum (ER) and this complex shuttles to the cell surface leading to the expression of IL-15/IL-15Rα complex on the cell surface. Thus IL-15 is not secreted and hence its detection in the biological fluid may not provide information about its bioactivity. The IL-15Rα presents the IL-15 to the responding cell that expresses IL-15Rβγc complex. This unique molecular mechanism is also termed as trans-presentation (Dubois et al., 2002; Stonier and Schluns, 2010) (Figure 1.6). In addition to the paracrine signaling, IL-15/IL-15Rα complex activates signal through IL-15βγc present on the same cell. This phenomena is termed as cis-presentation, but its physiological significance is not yet clear (Olsen et al., 2007). Soluble form of IL-15 can also be constitutively generated through defined proteolytic cleavage of IL-15Rα transmembrane receptor.

Transcript levels of full-length IL-15Rα are detected in various cell lines and tissues (Steel et al., 2010). Expression of all 8 isoforms of IL-15Rα is observed in certain tissues, for instance intestine, brain, liver, peripheral blood mononuclear cells. However, relative expression of these isoforms varies from each other tissues (Anguille et al., 2009). Free IL-15 in biological fluid is also affected by the presence of soluble IL-15Rα (Budagian et al., 2004; Bulfone-Paus et al., 2006).

Activation of IL-15 signaling leads to stimulation of Janus kinase and Signal Transduction and Activator of Transcription (STAT) pathways (Sabatti et al., 2009). Activation of IL-2/IL-15Rβ chain (CD122) recruits JAK1 leading to STAT3

phosphorylation, while gamma chain (CD132) recruits and phosphorylates JAK3 leading to phosphorylation of STAT5. Upon phosphorylation, STAT3 and STAT5 undergo dimerization and form homo- or hetero-dimer, and translocate to the nucleus where they activates transcription of certain genes (Johnston et al., 1995). Apart from STAT signaling, IL-15 activation also induces phosphorylation of Spleen Tyrosine kinase (Syk) and Lck (Ratthe and Girard, 2004; Uhlin et al., 2005).

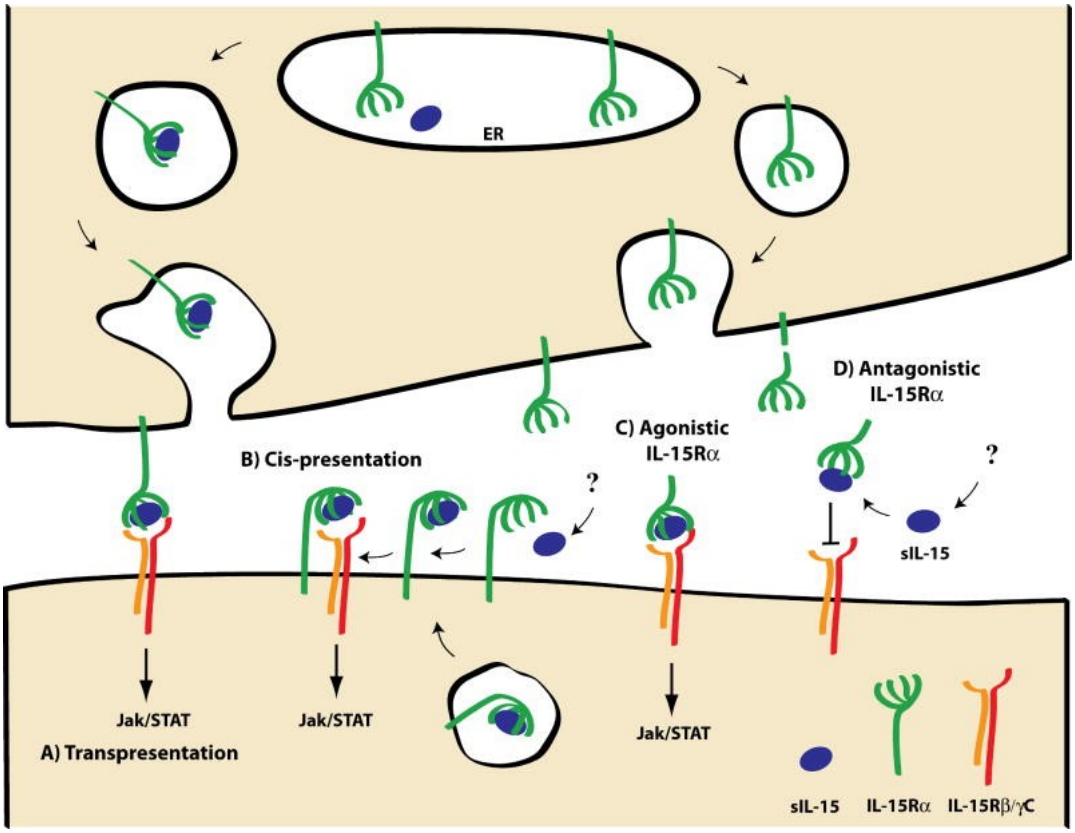

Figure 1.6. Trans-presentation of IL-15 (re-printed from (Stonier and Schluns, 2010)). *Schematic illustration of IL-15 trans-presentation.* ***A)*** *IL-15 conjugates with IL-15Rα in the ER and shuttle to the surface where this conjugate signals through IL-15Rβγc present on the neighboring cell surface.* ***B)*** *In cis-presentation, IL-15 presented by IL-15Rα on the same cell. This presentation system may operate in autocrine or paracrine manner.* ***C)*** *Artificially generated IL-15/IL-15Rα complex may act as agonist for IL-15 and stimulates signal in neighbouring cells.* ***D)*** *Cleaved empty IL-15Rα binds with soluble IL-15 and antagonize the IL-15.*

1.6.4. Immunological function of IL-15

IL-15 is produced by various tissues including kidney, skeletal muscles, placenta, heart, lung, macrophages and monocytes, and other cell types (Grabstein et al., 1994; Carson et al., 1995; Fehniger and Caligiuri, 2001). IL-15 from monocytes and macrophages play an important role in immune activation (Carson et al., 1995; Doherty et al., 1996). IL-15

upregulates the expression *Il8*, *Il12* and *McpI* (monocyte chemotactic protein-1) in macrophages to attract neutrophils and monocytes to the site of infection (Badolato et al., 1997; Budagian et al., 2006). Concurrently, IL-15 also increases the secretion of IL-6 and TNF-α (Budagian et al., 2006), while production of IL-10 from monocytes, macrophage and T-cells is observed in the absence of IL-15 (Gillgrass et al., 2014). Monocytes are identified as prototype cells having highest expression of IL-15Rα allowing trans-presentation of IL-15. IL-15 level also regulates DC differentiation. (Regamey et al., 2007; Perera et al., 2012). IL-15 priming increases the expression of CD83 and costimulatory molecules such as CD70, CD80, CD83 and CD86 (Abdel-Salam and Ebaid, 2008; Anguille et al., 2009). IL-15 also modulates adaptive immune responses through upregulation of IL-2 production, which is crucial for T-cell priming as well as DC mediated NK cell activation (Feau et al., 2005).

IL-15Rα and βγc are constitutively expressed by human neutrophils, and this constitutive expression helps neutrophils to escape apoptosis, modulate phagocytosis and to recruit other inflammatory cells to the site of inflammation (Bouchard et al., 2004; Ratthe and Girard, 2004; Perera et al., 2012). Additionally, IL-15 also increases the expression of MHC class-II, CD64 (high affinity Fc receptor for immunoglobulin G), and CD14 (lipopolysaccharide receptor) on the surface of neutrophils. This increases the ability of neutrophils to take up the pathogen through specific antibodies during gram-negative infection (Abdel-Salam and Ebaid, 2008). Mast cells can use IL-15 receptor without the requirement of IL-15Rα while T lymphocytes and NK cells cannot (Waldmann and Tagaya, 1999).

Mice lacking *Il15* or *Il15ra* are deficient for NK cells, $CD8^+$ T-cells but not $CD4^+$ T-cells (Patidar et al., 2016). IL-15 plays a critical role in the homeostasis and maintenance of lymphocytes, specifically memory phenotype $CD8^+$ T-cells, by upregulating the expression of anti-apoptotic molecule (Bcl-2). This indicates that IL-15 and IL-15Rα signals are critical for the survival of $CD8^+$ lymphocytes (Wu et al., 2002). It is also confirmed that IL-15 trans-presentation by DC is required for the maintenance and homeostasis of $CD8^+$ T-lymphocytes (Stonier and Schluns, 2010). In addition to CD8 homeostasis, IL-15 also modulates various physiological functions including proliferation, survival and T-cell responsiveness. Under normal homeostasis, $CD4^+$ T-cells require IL-7 and IL-15 for maintenance (van Leeuwen et al., 2009). However, memory phenotype $CD4^+$ cells are independent of IL-15 signals, and this is attributed to

reduced expression of IL-15Rβ chain on $CD4^+$ lymphocytes than in memory $CD8^+$ T-cells (van Leeuwen et al., 2009). Additionally, effect of IL-15 on mature $CD4^+$ cells depends on the activation status of these cells. In the absence of concomitant TCR triggering, rather than exerting growth factor activity of IL-2, IL-15 induced quiescent phenotype along with down-regulation of CD25, CD71 and CD95 expression (Dooms et al., 1998). Nevertheless, quiescent phenotype of $CD4^+$ lymphocytes is lost in the presence of concomitant TCR signaling leading to vigorous proliferation with resistance against TCR-induced death (Dooms et al., 1998). Moreover, IL-15 alone induces CD40L (CD145) on activated $CD4^+$ T-cells without concomitant signaling through CD3 and CD28 (co-stimulatory molecule), so that these cells have increased ability to interact with APCs (Skov et al., 2000). In humans, ageing induces a loss in naïve T-cells with the concomitant accumulation of CD28(null) T-cell (T-cells which lost CD28 expression), and results in reduced immune response to infections. IL-15 supplementation selectively stimulates the proliferation of $CD8^+$CD28(null) lymphocytes rather than $CD8^+CD28^+$ lymphocytes. Simultaneously, IL-15 also stimulates cytotoxicity of $CD8^+$CD28(null) lymphocytes by stimulating the expression of perforin and granzyme B (Alonso-Arias et al., 2011; Broux et al., 2015). Hence, IL-15 could be useful tool in elder persons to stimulate immune functions in order to fight against infections.

1.6.5. Functions of IL15 in liver homeostasis

Liver, as a lymphoid organ, contains abundant immune cells including $CD8^+$ T cells, NK and NKT cells and these cells require IL-15 for their development, homeostasis and maintenance (Lodolce et al., 2002; Crispe, 2009). Multiple non-hematopoietic liver cells such as HSCs (present in sinusoid space), sinusoid endothelial cells and parenchymal cells can present antigen to induce and maintain immune tolerance and to avoid autoimmunity (Crispe, 2009). Trans-presentation of IL-15 by liver associated hematopoietic and non-hematopoietic cells have been shown to maintain the IL-15-dependent immune subsets in the liver (Cepero-Donates et al., 2016b). Kupffer cells, which are the liver resident macrophages, are the major cell type that express IL-15 (Stienstra et al., 2010; Cui et al., 2014). Hepatocytes are also a major source of IL-15/IL-15Rα complex (Yokota et al., 2015). In addition, IL-15 from stellate cells has been shown to support the homeostasis of NKT cells in the liver micromilieu (Winau et al., 2007).

Previously, our lab has showed reduced lipid accumulation in *Il15*$^{-/-}$ as well as *Il15ra*$^{-/-}$ mice (Ramanathan et al., 2014; Cepero-Donates et al., 2016a; Cepero-Donates et al., 2016b). We have observed that deficiency of IL-15 reduced lipid accumulation in the liver and prevented weight gain induced by HFD. Serum cholesterol and free fatty acid levels were also found to be elevated in WT mice but not in *Il15* null mice (Ramanathan et al., 2014). Conditional deletion of IL-15Rα in macrophages and hepatocytes showed that both cell types are important for the maintenance of NK and NKT cells in the hepatic microenvironment. However, neither hepatocyte nor macrophage specific deletion of IL-15Rα is sufficient to prevent development of NAFLD (Cepero-Donates et al., 2016a; Cepero-Donates et al., 2016b). Jiao et al. observed that IL-15 and IL-15Rα reduced development of scar tissues and thereby liver fibrosis (Jiao et al., 2016). This reduced fibrosis might be attributed to hepatocyte- and HSCs- mediated IL-15 signals that maintained homeostasis of NK cells, NKT cells and $CD8^+$ T-cells. They also observed increased collagen production from the stellate cells isolated from *Il15ra*$^{-/-}$ mice liver (Jiao et al., 2016). Nevertheless, as mentioned in previous section, the role of NK cells in the pathogenesis of liver fibrosis is controversial.

1.7. Thesis premises

NASH and related liver ailments like liver fibrosis and HCC are major health problems since the past 2 decades. Liver disease can evolve from fatty liver to steatohepatitis followed by liver fibrosis, and in the long-run to cirrhosis, HCC and liver failure (Haslam and James, 2005). NASH associated with chronic inflammation leads to activation of stellate cells and ultimately fibrosis. In addition to activation of Kupffer cells, circulatory macrophages and other immune cells also get recruited in the hepatic environment. NASH/NAFLD associated inflammation promotes liver micromilieu towards a pro-inflammatory environment by promoting production of proinflammatory cytokines by damaged hepatocytes. In addition, activated stellate cells and Kupffer cells also induce the production of proinflammatory cytokines such as TNF-α, IL-6 and IL-12 (Li et al., 2003). Recently, IL-15 was also found to be up-regulated in the patients suffering from fibrosis (Jiao et al., 2016).

IL-15 is a proinflammatory cytokine, which is indispensable for the homeostasis of NK, NKT and $CD8^+$ T-cells (Kennedy et al., 2000). Infiltration of these cells promotes the progression of liver fibrosis hence it is believed to have a prominent role in the pathogenesis of liver fibrosis. However, involvement of IL-15 in the regulation of liver pathologies is not yet clear. Hence, we have addressed the role of IL-15 in the pathogenesis of liver fibrosis using *in-vivo* mouse models resembling inflammation as well as diet induced liver fibrosis and fatty liver (NAFLD).

1.8. Hypothesis

Based on above premises, we hypothesize that ***IL-15 signaling promotes development of liver fibrosis by inducing hepatic inflammation***.

1.9. Objectives

The specific aims of my research project are:

1. **To assess the role of IL-15 in the CCl_4 induced liver fibrosis**
   - **a.** Determine the degree of fibrosis in wild type and *Il15* null mice using microscopic analysis of the liver tissue.
   - **b.** Assess the severity of inflammation and macrophage infiltration by microscopic analysis of the liver tissue.
   - **c.** Evaluate inflammation, fibrosis and chemotaxis related genes expression in the liver tissues of wild type and $Il15^{-/-}$ mice after 7 weeks of CCl_4 injection.
2. **To define the role of IL-15 in the development of liver fibrosis induced by choline-deficient high fat diet (CD-HFD)**
   - **a.** Evaluate the severity of steatosis, inflammation and fibrosis induced by CD-HFD in wild type and *Il15*-knockout mice using microscopic analysis of the liver tissue.
   - **b.** Estimate the expression of series of genes involved in inflammation, fibrosis, chemotaxis and metabolism after 12 weeks of choline-deficient high fat diet.
   - **c.** Elucidate the role of IL-15 in the metabolic alteration induced by choline-deficient high fat diet.
3. **To evaluate the role of IL-15 in the development of NAFLD like condition**
   - **a.** Characterize the induction of NASH/NAFLD by CD-HFD in wild type and *Il15* null mice using microscopic and macroscopic analysis of the liver tissue.
   - **b.** Evaluate the expression of genes involved in inflammation, fibrosis, chemotaxis and metabolism in the liver tissue of WT and $Il15^{-/-}$ mice after 2 weeks of CD-HFD.

2. Materials and Methods

2.1. Experimental mice

All the experimental mice used were in C57BL/6 background and were housed in filter-topped individually ventilated cages in a specific-pathogen-free facility. Mice were fed with standard chow diet and drinking water *ad libitum* unless specified otherwise. All the experimental protocols were approved by Institutional ethics committee and were in accordance with University Animal Care Committee (UACC).

Wild type (WT) C57BL/6 mice were obtained from Charles River (Canada) and *Il15*$^{-/-}$ mice of C57BL/6 background were purchased from Taconics. The mice were bred in our facility in order to uniformize the housing conditions (Lacraz et al., 2016).

2.1.1. Induction of liver fibrosis in mice using CCl_4

CCl_4, diluted in corn oil as vehicle at 1:3 ratio, was injected to 8-12 weeks old WT or *Il15*$^{-/-}$ male mice for seven weeks (approximately 0.5 µl CCl_4/g body weight, twice a week, *i.p.*) (Mafanda et al., 2018). An equivalent volume of corn oil was injected in the control mice. Two days after the last injection, all mice were sacrificed. Part of liver tissue was fixed in 10% neutral formalin for histology and another part was snap-frozen in liquid nitrogen for RNA and protein analyses.

2.1.2. Induction of liver fibrosis in mice by Choline-deficient High-fat-diet

To induce liver fibrosis, 8-12 weeks old WT or *Il15*$^{-/-}$ male mice were maintained on CD-HFD (L-amino acid diet with 60 kcal% fat with 0.1% methionine and without choline; A06071302; Research Diet Inc.) (Matsumoto et al., 2013) while control mice were fed normal CHO diet. All mice were maintained on CD-HFD or CHO diet for 12 weeks after which they were sacrificed. Part of the liver tissue was fixed in neutral formalin for histology study and another part was snap-frozen in liquid nitrogen for RNA and protein analysis.

2.1.3. Induction of NASH in mice by Choline-deficient High-fat-diet

To induce liver fibrosis, 8-12 weeks old WT or *Il15*$^{-/-}$ male mice were maintained on CD-HFD while control mice were fed with normal CHO diet. All mice were maintained on CD-HFD or CHO diet for 2 weeks after which the mice were sacrificed. Part of the liver

tissue was fixed in neutral formalin for histological analysis and another part was snap-frozen in liquid nitrogen for RNA and protein analysis.

2.2. Tissue processing for histology

2.2.1. Hematoxylin and eosin staining of liver tissue

After fixation, liver tissue was embedded in paraffin and serial sections of 4 µm were taken. Sections were deparaffinized (placed in the xylene) and rehydrated with 100% alcohol for 1-2 min followed by 70% alcohol for 1-2 min again. Sections were rinsed in distilled water and stained with hematoxylin. Excess stain was removed by washing under running water, sections were treated with 1% acetic acid and saturated aqueous solution of lithium bicarbonate and the slides were re-washed under running water for 7-10 min. Sections were counter stained with eosin-phloxine solution for 30 seconds to 1 min and dehydrated in 70% alcohol followed by absolute alcohol, 3-5 minutes each, and mounted with Permount™ mounting medium (*SP15-500, Fisher Scientific*).

Slides were scanned using Nanozoomer 2.0 RS (*Hamamatsu Photonics*) and images were analyzed using NanoZoomer Digital Pathology (NDP, Hamamatsu Photonics). The researcher performing the analysis was blinded to the treatment protocols and the sections were analyzed.

2.2.2. Picrosirius-Red staining of liver tissue

As described earlier, liver sections were deparaffinized and rehydrated in alcohol gradient and were rinsed in distilled water. Sections were stained with eosin-phloxine solution for 30 seconds to 1 min and washed with distilled water for 5 min. The sections were kept in saturated solution of picrosirius red (1 g direct Red-80 (*365548; Sigma-Aldrich*) in 500 ml of saturated aqueous picric acid solution (approx. 14-15 g wet picric acid (*197378; Sigma-Aldrich*) in 1 l of distilled water)) for 1 hr. After 1 hr, collagen staining was intensified by immersing sections in acidified water (0.5% glacial acetic acid in water) for 10-15 sec and repeated in a fresh aliquot of acidified water. Then the sections were quickly washed in water and dehydrated in 70% alcohol followed by absolute alcohol, 3-5 minutes each, and mounted with Permount™ mounting medium (*SP15-500; Fisher Scientific*). The slides were scanned using Nanozoomer 2.0 RS and images were analyzed using NanoZoomer Digital Pathology.

2.2.3. Immunohistochemistry (IHC) of liver tissue

As described earlier, liver sections were deparaffinized and rehydrated with gradient alcohol solution and were rinsed in distilled water. Antigen retrieval was carried out by heating slides in 10 mM citrate buffer (0.96 g citric acid in 500 ml of distilled water containing 0.01% Tween-20, pH 6) for 10 min using a microwave and occasionally, the solution was allowed to cool to avoid bumping, and slides were allowed to cooldown in citrate buffer. Then slides were washed in distilled water for 3-5 min and endogenous peroxide activity was blocked by incubating slides with 0.3% H_2O_2 in PBS for 10-12 min followed by a wash in PBS. Tissue sections on slide were secured with hydrophobic pen to avoid draining of solution and blocking was performed for 90 min using 1% BSA in PBS. After BSA blocking, slides were washed in PBS and sections were incubated overnight with either α-SMA (*1:200; ab5694; Abcam*) or Collagen-I (*1:200; ab34710; Abcam*) rabbit polyclonal antibody at 4°C in humidified chamber. Excess of primary antibody was removed by washing three times in PBS containing 0.01% Tween-20, and sections were again incubated with HRP conjugated anti-rabbit secondary antibody (*1:500; LNA934V; GE Healthcare*) for 75-90 min at room temperature in humidified chamber.

After secondary antibody staining, sections were again washed three times in PBS containing 0.01% Tween-20 and incubated with peroxide-substrate solution (*DAB; D3939; Sigma-Aldrich*) for 10-15 min, meanwhile sections were observed under light microscope to verify staining, and excess of DAB was washed with PBS solution. Sections were counter-stained with eosin-phloxine solution for 30 seconds to 1 min and dehydrated in 70% alcohol followed by absolute alcohol, 3-5 minutes each, and mounted with Permount™ mounting medium. All the slides were scanned using Nanozoomer 2.0 RS and images were analyzed using NanoZoomer Digital Pathology.

2.2.4. Immunofluorescence (IF) staining of liver tissue

As described in the earlier section, liver sections were deparaffinized and rehydrated with gradient alcohol solution and were rinsed in distilled water. Antigens were retrieved by heating slides in 10 mM citrate buffer for 10 min in a microwave and slides were allowed to cooldown. Then slides were washed in distilled water for 3-5 min, tissue sections on slide were secured with hydrophobic pen and blocking was performed for 90 min using 1% BSA in PBS. After BSA blocking, slides were washed in PBS and sections were

incubated overnight with CD68 rabbit polyclonal antibody (1:200; ab125212; Abcam) at 4°C in humidified chamber. Excess primary antibody was removed by washing three times in PBS containing 0.01% Tween-20, and sections were again incubated with anti-rabbit, AF488 conjugated goat secondary antibody (1:500; A-11034; Invitrogen) for 75-90 min at room temperature in dark humidified chamber.

After secondary antibody staining, sections were again washed three times in PBS containing 0.01% Tween-20 and nucleus were counter stained with Hoechst 33342 in PBS (*1:10000; H3570; Invitrogen*) for 8-10 min. Slides were washed with PBS solution to remove excess of Hoechst, and mounted with DAKO Fluorescence Mounting Medium (*S3023; DAKO*). The slides were scanned using Nanozoomer 2.0 RS and images were analyzed using NanoZoomer Digital Pathology.

2.3. RNA isolation from liver tissue

A small piece of liver was lysed with the help of metal beads in 1 ml of TRIzol®. Samples were homogenized, 200 µl of chloroform was added and centrifuged for 15 minutes at 12000×*g* to separate the two phases. The aqueous layer was collected in a new tube containing 500 µl of cold isopropanol and allowed to stand for 10-20 min to precipitate RNA, and again centrifuged at 12000×*g* for 10 min. Supernatant was removed carefully and the RNA pellet was washed using 75% of ice-cold ethanol, and suspended in RNAse-free water. Purity of the extracted RNA was evaluated by measuring the 260/280 nm and 260/230 nm absorption ratios with Nanodrop 2000 (Thermo Fisher Scientific). 1 µg of RNA was run on a denaturing formaldehyde-agarose gel to assess RNA quality.

2.4. Quantitative polymerase chain reaction (qPCR)

Complementary-DNA were synthesized from 1 µg of total RNA using Quantitect® (Qiagen). Primers were selected by analyzing the efficiency and melting curve for each primer pair. The primers used in the study are listed in Table 2.1. Gene expression was evaluated with the MyQi5® cycler (Bio-Rad) using SYBR Green Supermix (Bio-Rad). Relative expression of the various gene targets was calculated by comparison with *Gapdh*.

Table 2.1: List of murine oligonucleotide sequences

Gene	Sense	Antisense
Col1a1	CTCCCAGAACATCACCTATCAC	ACTGTCTTGCCCCAAGTTCCG
Col3a1	AAGTCAAGGAGAAAGTGGTCG	CAGTCTCCCCATTCTTTCCAG
Acta2 / Sma	AGGGAGTAATGGTTGGAATGG	GTGTCGGATGCTCTTCAGG
Tgfb	ATACGCCTGAGTGGCTGTCT	CTGATCCCGTTGATTTCCA
Mmp2	CACCTACACCAAGAACTTCCG	GTCACTGTCCGCCAAATAAAC
Mmp9	AACTCACACGACATCTTCCAG	CCACCTTGTTCACCTCATTTTG
Timp1	TTGCACTCTGGCATCTGG	TGGTCTCGTTGATTTCTGGG
Il15	CCCATGTCAGCAGATAACCA	GAGCTGGCTATGGCGATG
Il15ra	ACTGTTGCTCCCGCTGAG	ATTCTTGACCCGGATGTCAG
Il6	AGTCCGGAGAGGAGACTTCA	TTGCCATTGCACAACTCTTT
Il12 (p40)	CATTGAACTGGCGTTGGAAG	TGAGGGAGAAGTAGGAATGGG
Il18	CTTCGTTGACAAAAGACAGCC	CACAGCCAGTCCTCTTACTTC
Ifng	CCTAGCTCTGAGACAATGAACG	TTCCACATCTATGCCACTTGAG
Tnfa	CCAAGGCGCCACATCTCCCT	GCTTTCTGTGCTCATGGTGT
iNos	AATCTTGGAGCGAGTTGTGG	CAGGAAGTAGGTGAGGGCTTG
F4/80	CTTTGGCTATGGGCTTCCAGTC	GCAAGGAGGACAGAGTTTATCGTG
Ccl2	CATCCACGTGTTGGCTCA	GATCATCTTGCTGGTGAATGAGT
Ccl5	TGCAGAGGACTCTGAGACAGC	GAGTGGTGTCCGAGCCATA
Cxcl9	AGTCCGCTGTTCTTTTCCTC	TGAGGTCTTTGAGGGATTTGTAG
Cxcl10	CCAAGTGCTGCCGTCATTTTC	GGCTCGCAGGGATGATTTCAA
Cx3cr1	GTTATTTGGGCGACATTGTGG	ATGTCAGTGATGCTCTTGGG
Ppara	CGGGAACAAGACGTTGTCAT	CAGATAAGGGACTTTCCAGGTC
Acadvl	AAAGGAACTCACTGGGCTG	ACTTAACTCTGGGTGGACAATC
Acadl	GGTGGAAAACGGAATGAAAGG	GGCAATCGGACATCTTCAAAG
Acadm	TGTCGAACACAACACTCGAAA	CTGCTGTTCCGTCAACTCAA
Acads	CCTGGGATGGGCTTCAAAATAG	GGTTCTCGGCATACTTCACAG
Acat1	AGCACACTGAACGATGGAG	CGCAAGTGGAAAATCAATGGG
Atf6	GAGGCTGGGTTCATAGACATG	GCTAGTGGTTTCTGTGTACTGG
Cyp4a10	CCCAAGTGCCTTTCCTAGATG	GCAAACCATACCCAATCCAAG
Cpt1a	GAAGAAGTTCATCCGATTCAAGA	ACACCCACCACCACGATAAG

Cd36	TTGTACCTATACTGTGGCTAAATGAG	CTTGTGTTTTGAACATTTCTGCTT
Hmgcs2	CATCGAGGGCATAGATACCAC	CACTCGGGTAGACTGCAATG
Gapdh	AACTTTGGCATTGTGGAAGG	CACATTGGGGGTAGGAACAC

2.5. Statistical analysis

Statistical analysis was performed with the GraphPad Prism 6 Software (San Diego, USA). The statistical significance (*p* value) was calculated by non-parametric one-way ANOVA (Analysis of variance) between two groups followed by Tukey's multiple *post-hoc* comparisons test. Differences were considered significant at * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$. Error bars indicate the standard error of mean (SEM).

3. Results

Previous work from this laboratory showed that inflammation contributes to the development of NAFLD and liver fibrosis (Cepero-Donates et al., 2016b; Kandhi et al., 2016; Mafanda et al., 2018). Deficiency of IL-15, a pro-inflammatory cytokine, reduced the accumulation of lipids in the liver when the mice were maintained on high fat diet. To assess whether absence of IL-15 also prevented the development of liver fibrosis, we used CD-HFD as HFD alone is not sufficient to induce fibrosis in murine models (Stephenson et al., 2018). To determine the pro-fibrotic role of IL-15, age matched WT and *Il15*$^{-/-}$ mice received CCl_4 injection or were fed with choline-deficient high fat diet to induce liver and metabolic diseases (Matsumoto et al., 2013; Mafanda et al., 2018).

3.1. CCl_4-induced liver fibrosis is reduced in the absence of *Il15*

CCl_4 was injected in wild type and *Il15* deficient mice for 7 weeks as shown in Figure 3.1.

Figure 3.1. CCl_4 was administered intraperitoneally twice a week for 7 weeks.

CCl_4 was diluted in corn oil at 1:3 ratio, and injected to 8-12 weeks old WT or Il15$^{-/-}$ *male mice for seven weeks (0.5 µl CCl_4/g body weight, twice a week)*

3.1.1. CCl_4 induced fibrotic liver showed increase in *Il15* and *Il15ra* expression

To identify pathological role of *Il15,* we evaluated *Il15* and *Il15ra* gene expression in the livers following induction of fibrosis. CCl_4 produces free radicals and induces inflammatory cascades that result in hepatocyte damage. CCl_4 treatment induced the expression of *Il15* and *Il15ra* genes expression in wild type mice (Figure 3.2). *Il15ra* expression was also increased in the *Il15*$^{-/-}$. As expected, we did not detect the expression of *Il15* in *Il15*$^{-/-}$ mice.

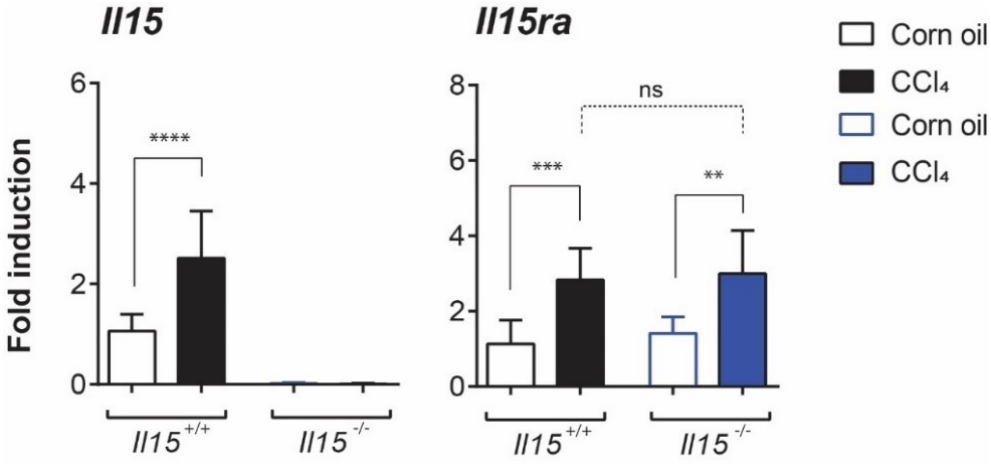

Figure 3.2. CCl_4 injection increased Il15 and Il15ra gene expression in livers from wild type mice.

*Il15 and Il15ra RNA levels were evaluated in liver samples from CCl_4 or corn oil injected wild type or Il15 null mice. The data is expressed as relative expression when compared to housekeeping gene (Gapdh). Results are shown as the mean ± SEM of two independent experiments. n = 7 to 11 mice per group. Statistical significance between treated and control groups, followed by Tukey's multiple comparisons test, is given as *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001.*

3.1.2. Loss of *Il15* reduced myofibroblast activation and decreased the expression of ECM modifying enzymes

CCl_4 induces hepatocyte damage which initiates inflammatory cascade to activate hepatic stellate cells leading to the initiation of hepatic fibrogenic response. Consequently, we observed extensive bridging fibrosis, documented by Sirius red staining of collagen fibrils, in the liver sections of CCl_4 treated wild type mice (Figure 3.3A). Liver sections of CCl_4 treated *Il15*$^{-/-}$ mice showed almost 3 times less fibrotic area as compared CCl_4 treated WT mice (Figure 3.3B). Immunohistochemical staining for collagen-I revealed significantly reduced collagen-I deposition in *Il15*$^{-/-}$ mice when compared to WT mice treated with CCl_4 (Figure 3.4).

In the presence of inflammation, HSC differentiates into myofibroblast, which starts producing extracellular matrix proteins such as SMA. Immunohistochemical staining with *α-SMA,* a myofibroblast marker, also showed substantial increase in *α-SMA* staining in the livers of CCl_4 treated WT mice when compared to control mice that received vehicle, corn oil only (Figure 3.5A). The intensity of *α-SMA* staining was significantly (p<0.01) lower in the livers of *Il15*$^{-/-}$ mice when compared to controls following CCl_4 treatment (Figure 3.5B).

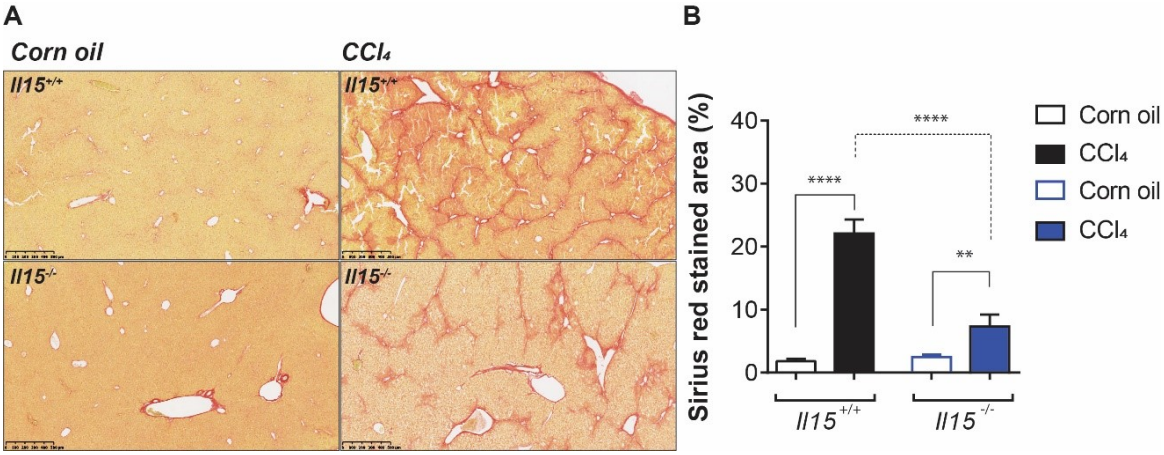

Figure 3.3. Il15 deficiency reduced CCl_4 induced collagen fibrils (Sirius red staining) in liver.

*A). Sections of liver tissues collected from wild type and Il15 deficient mice were stained with picrosirius red. Representative images from at least 4-5 mice for each group are shown. B). Quantification of the Sirius red-stained areas of collagen deposition was carried out using ImageJ software. Results are shown as the mean ± SEM of at least 4-5 mice per group. Statistical significance between treated and control groups, followed by Tukey's multiple comparisons test, is given as *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001.*

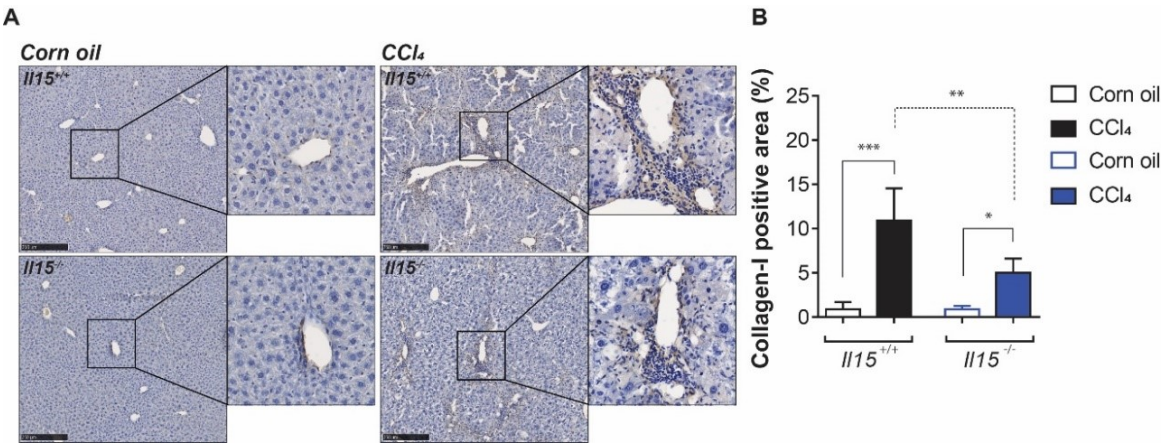

Figure 3.4. CCl_4 induced collagen deposition in the liver is decreased in $Il15^{-/-}$ mice.

*A). Sections of liver tissues collected from WT and $Il15^{-/-}$ mice were stained for collagen-I to detect collagen deposition (brown color) in liver. Representative images from 4-5 mice for each group are shown. B). Quantification of collagen-I positive area was carried out using ImageJ software. Results are shown as the mean ± SEM of at least 4-5 mice per group. Statistical significance between treated and control groups, followed by Tukey's multiple comparisons test, is given as *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001.*

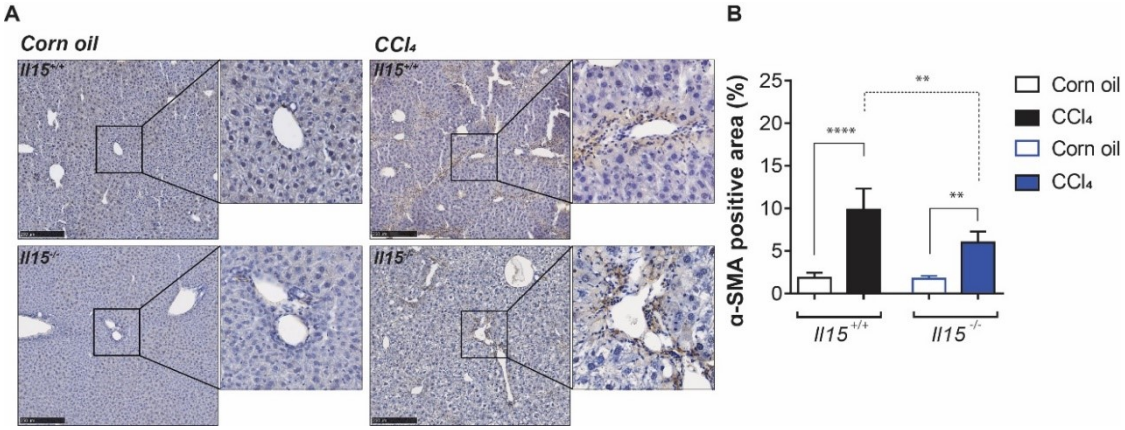

Figure 3.5. CCl_4 injection increased αSMA expression in the liver of WT mice when compared to $Il15^{-/-}$ mice.

*A). Sections of liver tissues collected from WT and $Il15^{-/-}$ mice were stained for αSMA to detect αSMA expression (brown color) in liver. Representative images from 4-5 mice for each group are shown. B). Quantification of αSMA stained area was carried out using ImageJ software. Results are shown as the mean ± SEM of 4-5 mice per group. Statistical significance followed by Tukey's multiple comparisons test is given as *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001.*

Absence of lL-15 reduces the presence of fibrogenic proteins such as collagen-I and αSMA during CCl_4 induced fibrogenic response. To assess the activation of stellate cells that are the main source of ECM proteins during the fibrotic response, we quantified the expression of various genes responsible for the production (*Col1a1*, *Col3a1, Acta2* and *Tgfb*) as well as the maintenance (*Mmp2* and *Mmp9*) of ECM. We observed significantly reduced expression of *Col1a1* and *Col3a1* (that code for collagen type 1 α1 and type 3 α1 chain, respectively) in *$Il15^{-/-}$* liver following CCl_4 treatment (Figure 3.6). Moreover, *$Il15^{-/-}$* mice also exhibited significant (p<0.01) reduction in *Acta2* gene expression (encodes αSMA), in CCl_4 treated *$Il15^{-/-}$* mice when compared to CCl_4 treated WT mice. The important profibrogenic cytokine gene, *Tgfb* that was abundantly increased in the livers of CCl_4 injected WT mice, was significantly lower in CCl_4 treated *$Il15^{-/-}$* mice. The basal level of expression of these genes were generally comparable between control WT and *$Il15^{-/-}$* mice.

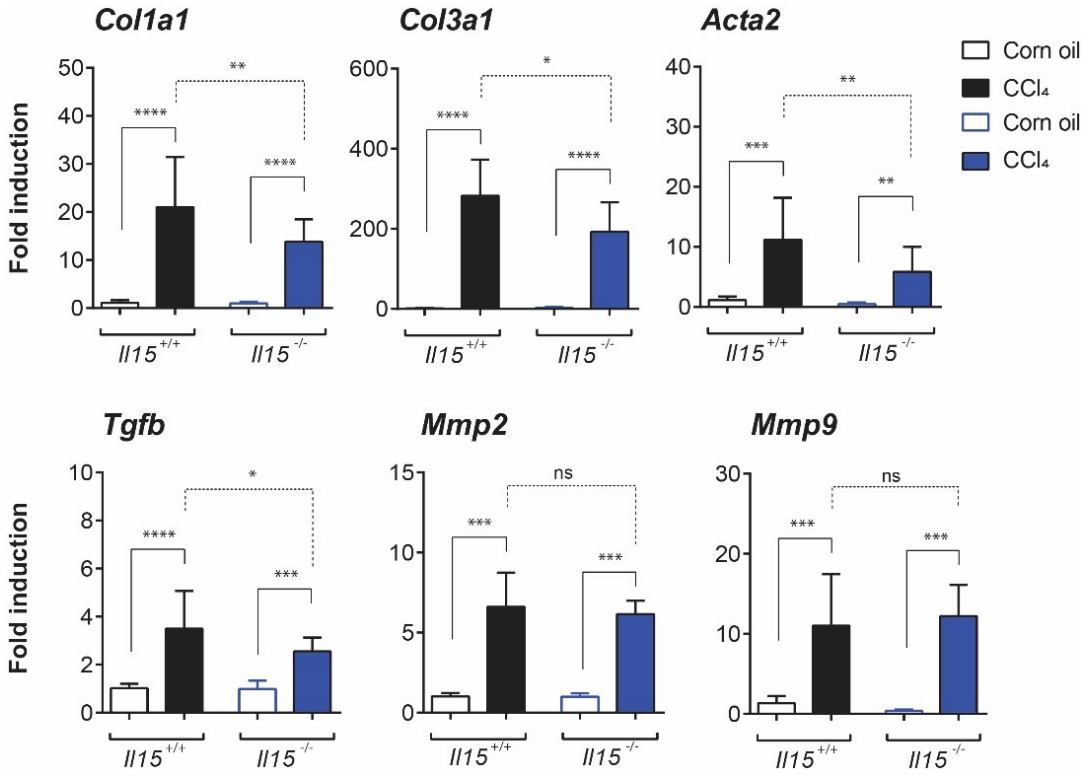

Figure 3.6. CCl_4 induced fibrogenic markers and ECM modifying enzyme expressions were reduced in the absence of IL-15.

*Col1a1, Col3a1, Acta2, Tgfb, Mmp2 and Mmp9 RNA levels were evaluated in liver samples from CCl_4 or corn oil injected WT or $Il15^{-/-}$ mice. The data is expressed as relative expression when compared to housekeeping gene (Gapdh). Results are shown as the mean ± SEM of two independent experiments. n = 7 to 11 mice per group. Statistical significance between treated and control groups, followed by Tukey's multiple comparisons test, is given as *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001.*

Matrix metalloproteinases (MMPs) are the group of enzymes responsible for maintenance and degradation of ECM during physiological and pathological conditions. To determine the presence of MMPs, we examined the expression of *Mmp2* and *Mmp9* genes in the liver tissues. CCl_4 treatment significantly augmented the expression of *Mmp2* and *Mmp9* ($p<0.001$) in the liver of WT mice (Figure 3.6). However, the magnitude of *Mmp2* and *Mmp9* was not significant difference between CCl_4 treated $Il15^{-/-}$ and WT mice. These results suggest that expression of MMPs are not directly controlled by IL-15.

3.1.3. Immune cell infiltration was reduced in the livers of CCl_4 treated *Il15*$^{-/-}$ mice

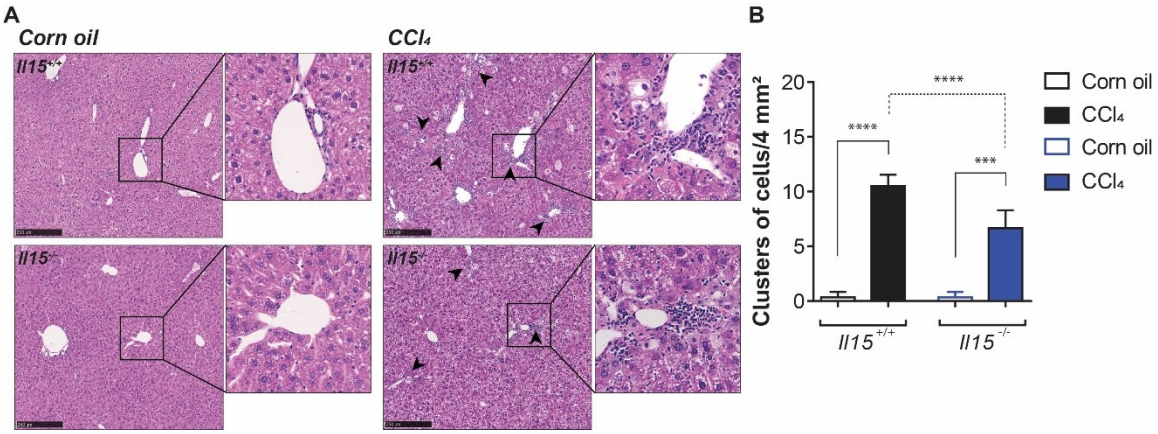

Figure 3.7. Mononuclear cell infiltration was reduced in the livers of CCl_4 treated Il15$^{-/-}$ mice when compared to WT mice liver.

*A). Sections of liver tissues collected from WT and Il15$^{-/-}$ mice were stained with hematoxylin and eosin (H&E). Representative images from at least 4-5 mice for each group are shown. B). Clusters of mononuclear cell infiltrations, indicated by arrowheads (►), were counted randomly in 4 mm^2 area of liver sections from the indicated mice group. Results are shown as the mean ± SEM of at least 4-5 mice per group. Statistical significance between treated and control groups, followed by Tukey's multiple comparisons test, is given as *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001.*

As illustrated in Figure 3.7, H&E stained liver sections from corn oil injected mice exhibited normal hepatic architecture without infiltration of mononucleated cells while CCl_4 injected mice showed increased mononuclear cell infiltration. Although the livers of CCl_4 injected *Il15*$^{-/-}$ mice showed infiltration of immune cells, the degree of infiltration was significantly reduced in *Il15*$^{-/-}$ mice when compared to controls. As shown previously, CCl_4 injection alone is a well established model to study liver fibrosis but does not otherwise resemble human NASH/NAFLD (Van Herck et al., 2017; Castro and Diehl, 2018). In our experiment, CCl_4 did not induce microvascular or macrovascular steatosis in both WT and *Il15*$^{-/-}$ mice.

3.1.4. Deficiency of *Il15* reduced CCl_4 induced proinflammatory cytokine gene expression

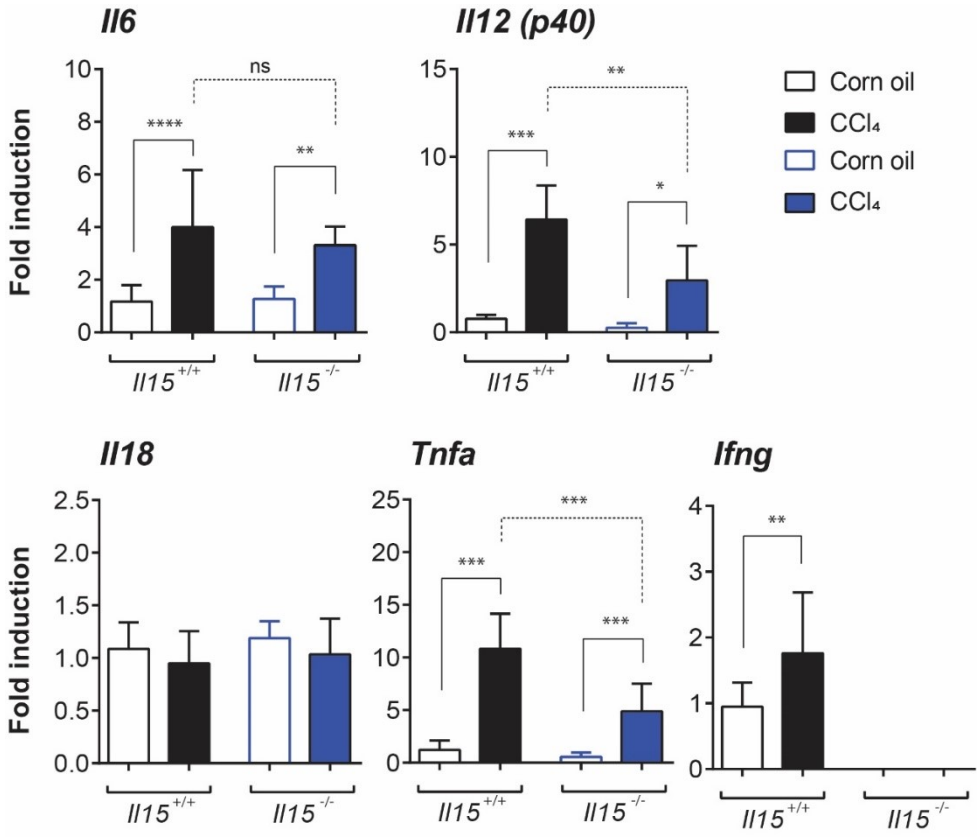

Figure 3.8. Loss of Il15 showed protection against CCl_4 induced proinflammatory cytokine expression.

*Il6, Il12 (p40), Il18, Tnfa and Ifng RNA levels were evaluated in liver samples from CCl_4 or corn oil injected WT or $Il15^{-/-}$ mice. The data is expressed as relative expression when compared to housekeeping gene (Gapdh). Results are shown as the mean ± SEM of two independent experiments. n = 7 to 11 mice per group. Statistical significance between treated and control groups, followed by Tukey's multiple comparisons test, is given as *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001.*

The above finding indicates reduced immune cell infiltration in the absence of *Il15* and suggests that the inflammation may also be reduced in CCl_4-treated $Il15^{-/-}$ mice. Hence, we examined the induction of pro-inflammatory cytokine gene expression in the liver tissue. As depicted in Figure 3.8, CCl_4 injection increased *Il6, Il12(p40)* and *Tnfa* expression in WT mice liver when compared to corn oil injected WT mice liver. Deficiency of *Il15* significantly reduced CCl_4-induced *Il12(p40)* and *Tnfa* gene expression ($p<0.01$ and $p<0.001$, respectively) when compared to CCl_4 injected WT

mice. *Il6* expression was statistically comparable between CCl_4 injected WT mice and CCl_4 injected *Il15*$^{-/-}$ mice (Figure 3.8). The expression of *Il18* remained unaltered in the CCl_4 injected wild type as well as *Il15* deficient mice. Along with this, *Ifng* gene expression was also observed to be increased in CCl_4 injected WT mice liver, but surprisingly we did not detect *Ifng* gene expression in the *Il15* deficient mice liver. These observations are in line with the requirement of IL-15 for the homeostasis of IFNγ (Strengell et al., 2003).

3.1.5. *Il15* deficiency reduced macrophage infiltration induced by CCl_4 in the liver

Macrophages are the most studied immune cells in the context of liver associated ailments including NAFLD and fibrosis. Their infiltration in different tissues correlates with the inflammatory status of the tissue. We observed a significant increase in *F4/80* expression, a marker for resident macrophages, in the livers of WT mice receiving CCl_4 (Figure 3.9) suggesting an increase in their numbers in the liver.

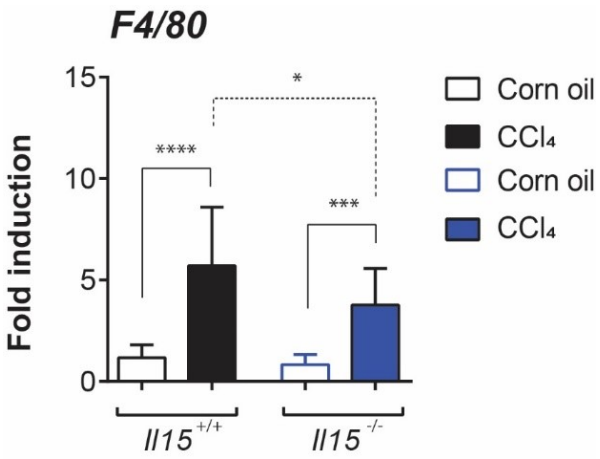

Figure 3.9. Deficiency of Il15 reduced CCl_4 induced F4/80+ macrophages in the liver.

*F4/80 RNA levels (as an indicator for the resident macrophage subset) were evaluated in liver samples from CCl_4 or corn oil injected WT or Il15$^{-/-}$ mice. The data is expressed as relative expression when compared to housekeeping gene (Gapdh). Results are shown as the mean ± SEM of two independent experiments. n = 7 to 11 mice per group. Statistical significance between treated and control groups, followed by Tukey's multiple comparisons test, is given as *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001.*

CCl_4 induced increase in *F4/80* expressing macrophages was significantly reduced in CCl_4 treated *Il15*$^{-/-}$ mice when compared to CCl_4 treated WT mice. We further evaluated the macrophage infiltration by immunofluorescence using anti-CD68 antibody. As

depicted in Figure 3.10, we observed increased frequency of macrophage infiltration in the CCl_4 injected WT mice liver that was significantly less increased in *Il15* deficient mice post CCl_4 injection.

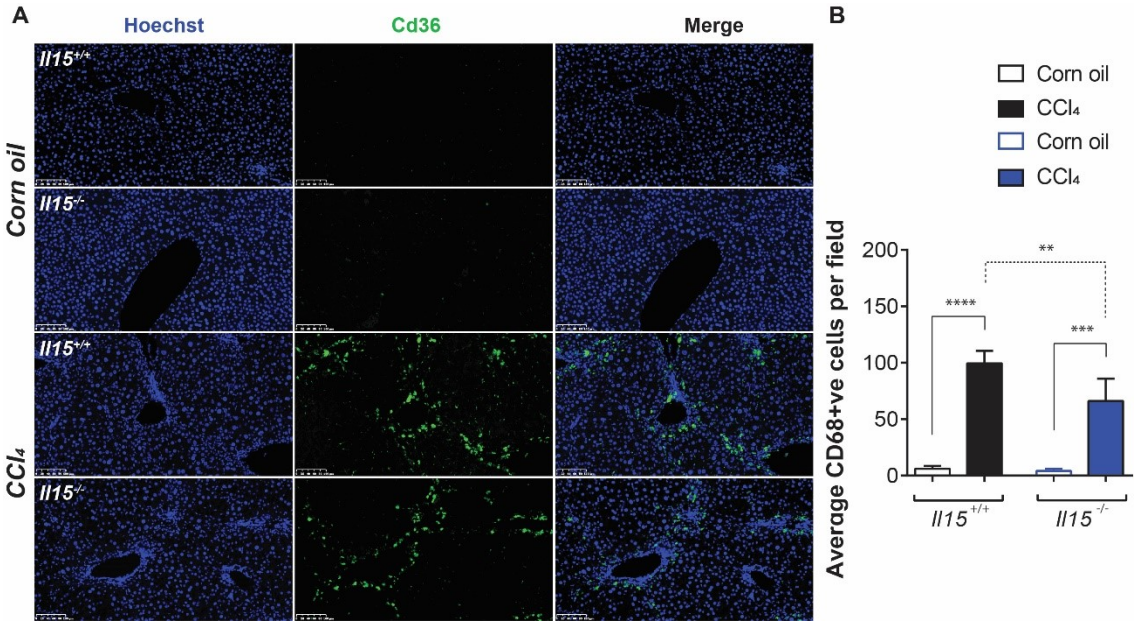

Figure 3.10. Increased presence of CD68+ macrophages in the livers of CCl_4 treated WT mice.

*A). Liver tissue sections were stained for CD68 by immunofluorescence to detect macrophages (green color). Representative images from at least 4-5 mice for each group are shown. B). Average number of CD68 positive cells per field. Results are shown as the mean ± SEM for at least 4-5 mice per group. Statistical significance between treated and control groups, followed by Tukey's multiple comparisons test, is given as *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001.*

3.1.6. CCl_4 induced chemokine expression is decreased in the absence of *Il15*

The above finding confirmed that *Il15* deficiency protected against CCl_4 induced inflammation and fibrogenic response along with reduced macrophage infiltration in liver. The infiltration by immune cells is mainly driven by chemokines, some of which recruit inflammatory immune cells, while other recruit monocytes that are responsible for the resolution of liver fibrosis (Oo et al., 2010; Wasmuth et al., 2010; Marra and Tacke, 2014). Therefore, we also examined the expression of pro-inflammatory, pro-fibrogenic chemokines (*Ccl2, Ccl5, Cxcl9* and *Cxcl10*). As shown in Figure 3.11, *Ccl2* was induced following CCl_4 treatment in both WT and *Il15*$^{-/-}$ mice. However, the expression of other chemokines such as *Ccl5* and *Cxcl9* was significantly increased

(p<0.01 and p<0.0001, respectively) in WT mice but not in *Il15* deficient mice (Figure 3.11).

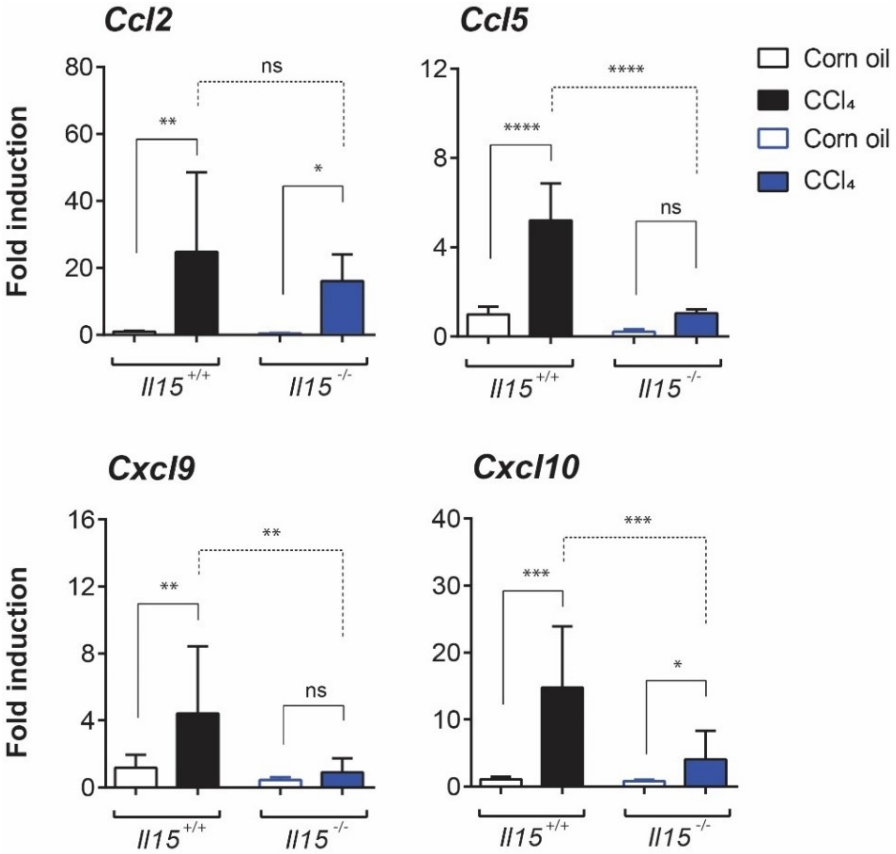

Figure 3.11. CCl_4 induced chemokine expression is reduced in the absence of Il15. *Ccl2, Ccl5, Cxcl9 and Cxcl10 RNA levels were evaluated in liver samples from CCl_4 or corn oil treated WT or $Il15^{-/-}$ mice. The data is expressed as relative expression when compared to housekeeping gene (Gapdh). Results are shown as the mean ± SEM of two independent experiments. n = 7 to 11 mice per group. Statistical significance between treated and control groups, followed by Tukey's multiple comparisons test, is given as *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001.*

3.2. *Il15* contributes to liver fibrosis induced by Choline-deficient high fat diet for 12 weeks

The experiments carried out in the previous section with CCl_4 demonstrated that IL-15 is induced during liver fibrosis and in its absence, the severity of the signs is attenuated. In metabolic syndrome, accumulation of fat in the liver leads to fibrosis later in life. However, in pre-clinical models HFD is not sufficient to induce fibrosis. When combined with choline-deficiency, high fat diet (CD-HFD) supplement results in fibrosis (Itagaki et al., 2013; Matsumoto et al., 2013; Stephenson et al., 2018). To understand the role of

IL-15 in NAFLD-associated fibrosis, we maintained WT and *Il15*$^{-/-}$ mice on CD-HFD for a period of 12 weeks.

3.2.1. CD-HFD induces the expression of *Il15* and *Il15ra*

As shown in Figure 3.12, after 12 weeks of CD-HFD supplement, the expression of *Il15* and *Il15ra* was increased in WT mice liver by 4 and 6 fold, respectively. *Il15ra* gene expression was also increased (p<0.05) in *Il15* null mice liver after CD-HFD but was significantly lower (p<0.01) when compared to CD-HFD fed WT mice liver. As expected, we did not detect the expression of *Il15* in *Il15*$^{-/-}$ mice.

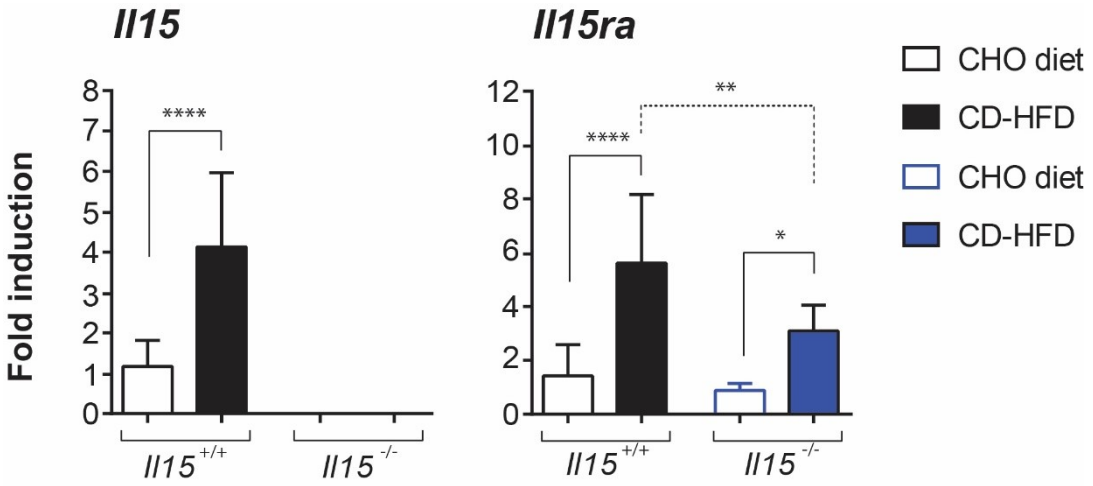

Figure 3.12. Expression of Il15 and Il15ra is increased in the livers of WT mice maintained on CD-HFD for 12 weeks.

Il15 and Il15ra RNA levels were evaluated in liver samples from WT and Il15$^{-/-}$ *mice after 12 weeks on CD-HFD. The data is expressed as relative expression when compared to housekeeping gene (Gapdh). Results are shown as the mean ± SEM of two independent experiments. n = 8 to 11 mice per group. Statistical significance between treated and control groups, followed by Tukey's multiple comparisons test, is given as *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001.*

3.2.2. CD-HFD induced steatosis was comparable in wild type and *Il15* deficient mice

H&E stained liver sections from WT and *Il15*$^{-/-}$ mice maintained on CHO diet showed normal hepatic architecture along with clear hepatic lining without triglycerides accumulation (Figure 3.13). Liver sections of WT mice on CD-HFD for 12 weeks showed

abundant fatty accumulation with microvascular as well as macrovascular steatosis along with increase in infiltration by mononucleated cells (grade 3 steatosis).

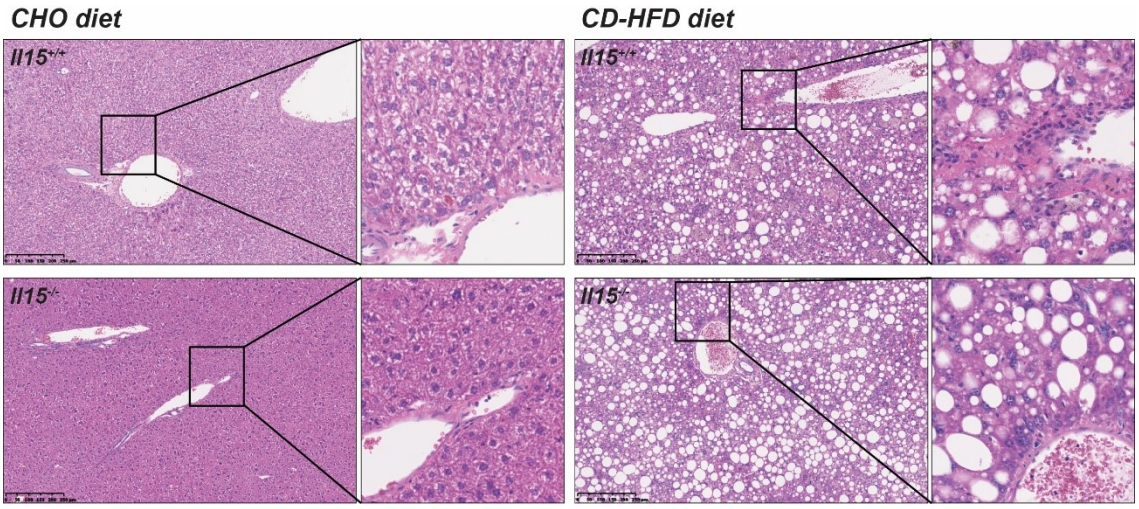

Figure 3.13. Livers from wild type or Il15 deficient mice showed identical degree of steatosis after 12 weeks of CD-HFD.

Sections of liver tissues collected from WT and Il15$^{-/-}$ mice were stained with hematoxylin and eosin (H&E). Representative images from at least 4-5 mice for each group are shown.

However, CD-HFD fed *Il15*$^{-/-}$ mice liver also depicted comparable microvascular and macrovascular steatosis with reduced infiltration of mononuclear cells. These observations suggest that *Il15* promotes progression of NAFLD associated inflammation and inflammation associated liver fibrosis but not the lipid accumulation or lipid transport.

3.2.3. *Il15* deficiency reduced collagen deposition as well as the expression of fibrosis-associated markers in CD-HFD induced liver fibrosis

As mentioned in section 3.1.4., sterile inflammation initiates hepatocyte damage leading to activation of HSCs to produce fibrogenic response. In line with previous results, livers of CD-HFD fed WT mice suggested abundant bridging fibrosis, as seen from Sirius red staining of collagen fibrils, when compared to livers of CD-HFD fed *Il15*$^{-/-}$ mice (Figure 3.14). Similar results were also observed by collagen-I immunohistochemistry (Figure 3.15). Activated myofibroblast starts producing extracellular matrix consisting of collagen-I. These cells can be stained by *α-SMA* staining (Talbott et al., 2018). As shown in Figure 3.16, *α-SMA* staining also showed extensive activation of myofibroblasts in the

livers of CD-HFD fed WT mice. However, liver sections of *Il15*$^{-/-}$ mice fed on CD-HFD showed reduced *α-SMA* staining.

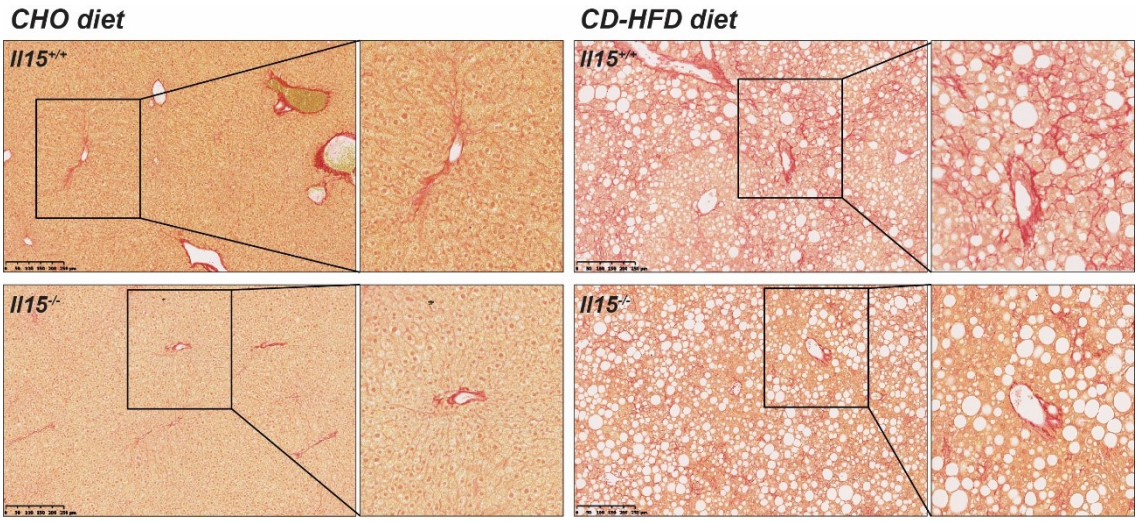

Figure 3.14. Loss of Il15 diminishes liver collagen fibril formation (Sirius red staining) induced after 12 weeks of CD-HFD.

Sections of liver tissues collected from WT and Il15$^{-/-}$ *mice were stained with picrosirius red. Representative images from at least 4-5 mice for each group are shown.*

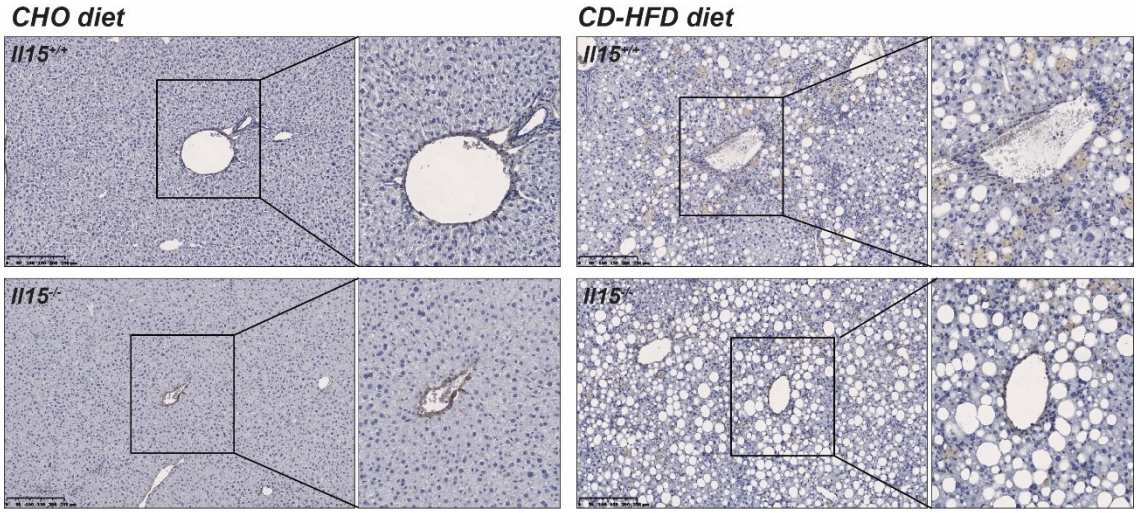

Figure 3.15. Il15 deficiency reduced collagen deposition in the livers of mice maintained on CD-HFD.

Sections of liver tissues collected from WT and Il15$^{-/-}$ *mice and Collagen-I immune-histological staining was performed using anti collagen-I antibody to detect collagen deposition (brown color). Representative images from at least 4-5 mice for each group are shown.*

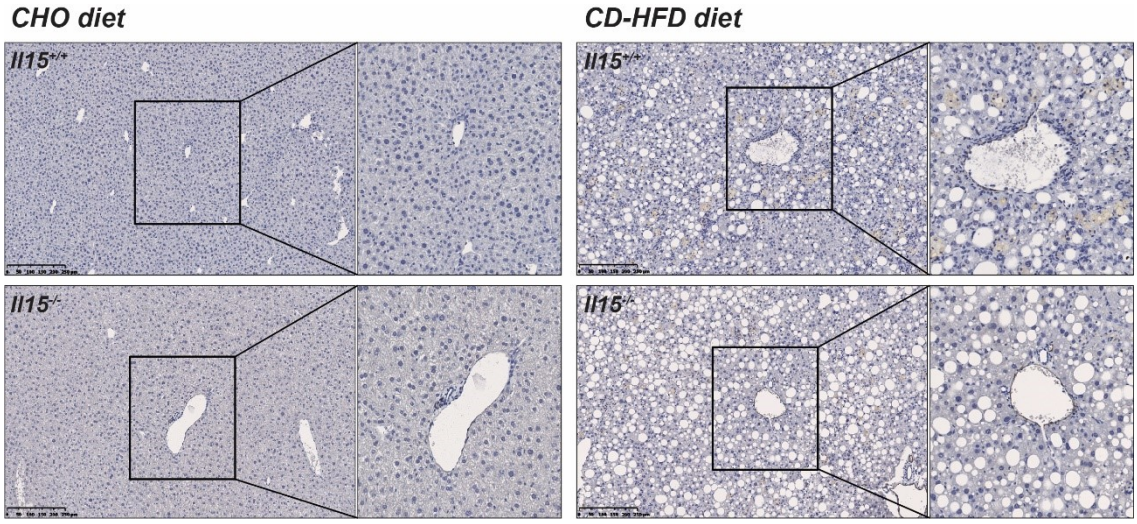

Figure 3.16. CD-HFD for 12 weeks increased α-SMA production in liver but loss of Il15 reduced α-SMA levels.

Sections of liver tissues collected from WT and Il15$^{-/-}$ mice and α-SMA immune-histological staining was performed using anti α-SMA antibody to detect smooth muscle actin production (brown color). Representative images from at least 4-5 mice for each group are shown.

Sirius red staining for collagen fibrils and collagen-I immunohistochemical staining suggested reduced fibrosis in *Il15*$^{-/-}$ mice. Further, we evaluated the expression of genes responsible for ECM production and maintenance. As shown in Figure 3.17, the expressions of *Col1a1* and *Col3a1*, gene coding for collagen, were significantly elevated in the liver of WT mice maintained on CD-HFD but the amplitude of increase was lower in *Il15*$^{-/-}$ mice livers. CD-HFD fed *Il15*$^{-/-}$ mice liver showed substantially reduced expression of *Acta2,* which encodes for *α-SMA*, as compared to choline-deficient high fat diet fed wild type mice liver (Figure 3.17). Moreover, profibrotic cytokine gene, *Tgfb,* that was significantly elevated in WT mice receiving CD-HFD showed a modest increase in *Il15*$^{-/-}$ mice. These results reflect the differences that were observed following CCl_4 treatment. Next, we assessed whether the reduced accumulation of ECM in the livers of *Il15* null mice was associated with altered expression of enzymes responsible for the regulation as well as degradation of extracellular matrix *i.e.* MMPs and TIMPs.

The expressions of matrix metalloproteinases and tissue inhibitors of MMPs varies during hepatic fibrogenic response to favor extracellular matrix production.

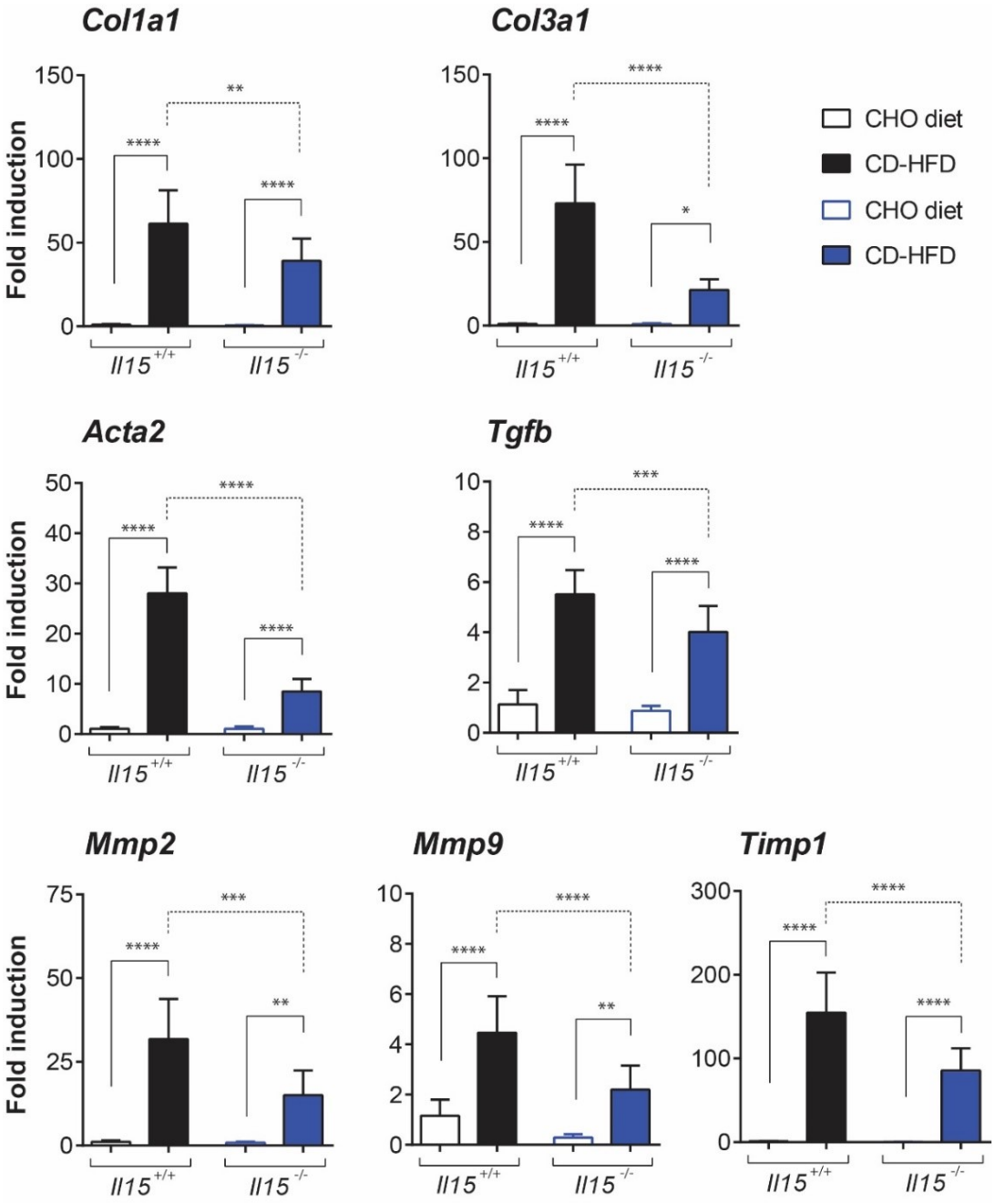

Figure 3.17. Loss of Il15 diminished the expression of fibrogenic markers and ECM modifying enzymes in CD-HFD induced fibrotic liver.

*Col1a1, Col3a1, Acta2, Tgfb, Mmp2, Mmp9 and Timp1 RNA levels were evaluated in liver samples from WT and Il15$^{-/-}$ mice after 12 weeks of dietary manipulation. The data is expressed as relative expression when compared to housekeeping gene (Gapdh). Results are shown as the mean ± SEM of two independent experiments. n = 8 to 11 mice per group. Statistical significance between treated and control groups, followed by Tukey's multiple comparisons test, is given as *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001.*

Gene expression of *Mmp2* and *Mmp9*, that both encode for matrix metallopeptidase (extracellular matrix modifying enzymes), were significantly increased (p<0.0001) in WT mice liver maintained on CD-HFD while loss of *Il15* resulted in lesser magnitude of increase in *Mmp2* and *Mmp9* (Figure 3.17). Notably, CD-HFD induced *Timp1* expression was significantly reduced (p<0.0001) by the loss of *Il15*. These results indicate that *Il15* contributes to the fibrogenic response, as observed with CCl_4 induced liver fibrosis, and that in its absence ECM deposition is reduced in the liver (Figure 3.17).

3.2.4. Loss of *Il15* protected against CD-HFD induced proinflammatory and profibrogenic cytokine expression

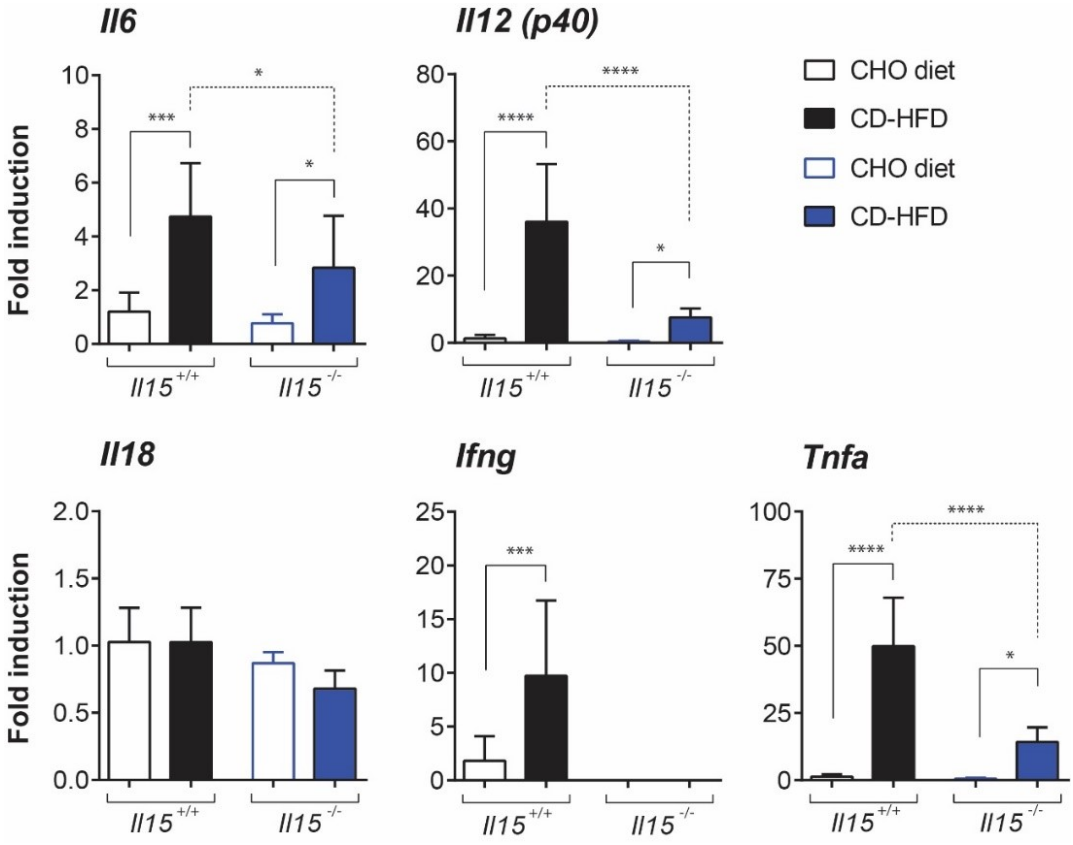

Figure 3.18. CD-HFD triggered inflammatory and profibrogenic cytokine expression were reduced in absence of I15.

*Il6, Il12 (p40), Il18, Ifng and Tnfa RNA levels were evaluated in liver samples from WT and Il15$^{-/-}$ mice after 12 weeks of dietary manipulation. The data is expressed as relative expression when compared to housekeeping gene (Gapdh). Results are shown as the mean ± SEM of two independent experiments. n = 8 to 11 mice per group. Statistical significance between treated and control groups, followed by Tukey's multiple comparisons test, is given as *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001.*

Next, we asked whether the reduction in the fibrosis associated genes was due to alteration in metabolism or, due to reduction in the expression of pro-inflammatory genes, as IL-15 promotes inflammation (Cepero-Donates et al., 2016a; Jonakowski et al., 2017). The altered production of pro-inflammatory and profibrogenic cytokine gene expression has been implicated in the fibrosis-associated complications. We examined the expression of cytokine genes in the liver tissues and observed significantly increased expressions of *Il6* and *Il12(p40)* in CD-HFD fed WT mice when compared to control mice (Figure 3.18). Loss of *Il15* significantly reduced *Il6* and *Il12(p40)* gene expressions ($p<0.05$ and $p<0.0001$, respectively) as compared to WT mice maintained on CD-HFD. As depicted in Figure 3.18, CD-HFD caused significant induction of *Tnfa* expression in control mice that was markedly reduced in the absence of *Il15*. Interestingly, the expression pattern of *Il18* and *Ifng* were identical to the CCl_4 induced liver fibrosis experiment (section 3.1.4.). The expression of *Il18* remained unaltered in WT as well as *Il15*$^{-/-}$ mice maintained on CD-HFD (Figure 3.18). While, *Ifng* was increased in the CD-HFD fed WT mice, we did not detect *Ifng* gene expression in the *Il15*$^{-/-}$ mice liver.

3.2.5. CD-HFD-induced increase in *F4/80* and *iNos* expression was reduced in absence of *Il15*

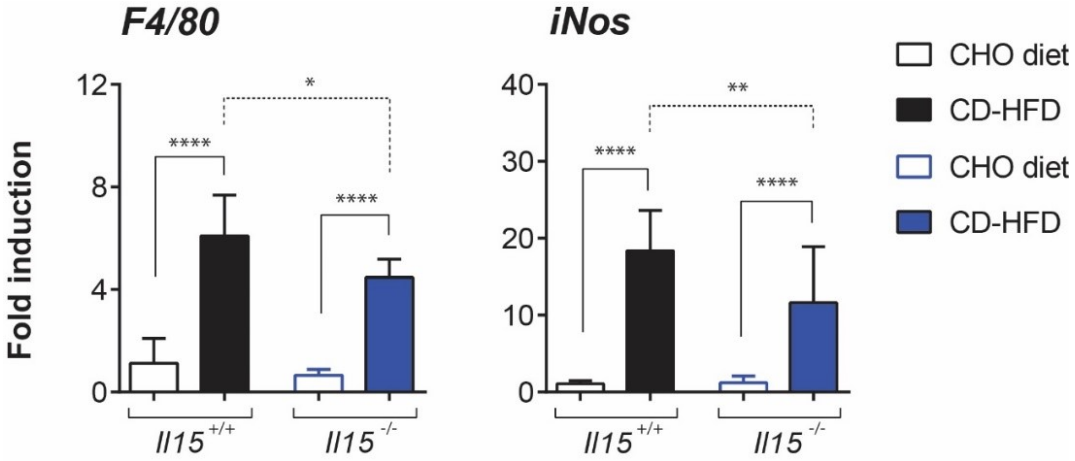

Figure 3.19. Il15 deficiency showed protection against CD-HFD induced F4/80 and iNos expressions in liver.

F4/80 and iNos RNA levels were evaluated in liver samples from WT and Il15$^{-/-}$ *mice after 12 weeks of dietary manipulation. The data is expressed as relative expression when compared to housekeeping gene (Gapdh). Results are shown as the mean ± SEM of two independent experiments. n = 8 to 11 mice per group. Statistical significance between treated and control groups, followed by Tukey's multiple comparisons test, is given as *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001.*

Macrophages respond to pro-inflammatory and profibrogenic cytokines and play a crucial role during the hepatic fibrogenic response. To observe macrophage infiltration, we evaluated the expression of *F4/80*, a marker for macrophages, and observed significantly elevated *F4/80* expression in WT mice liver fed with CD-HFD as compared to control WT mice (Figure 3.19). Loss of *Il15* reduced CD-HFD induced *F4/80* expression as compared to WT liver. As portrayed in Figure 3.19, expression of *iNos* gene was also dramatically increased in CD-HFD fed WT, while loss of *Il15* showed significant protection (p<0.01) against CD-HFD induced *iNos* expression.

3.2.6. *Il15* deficiency reduced CD-HFD induced chemokine expression

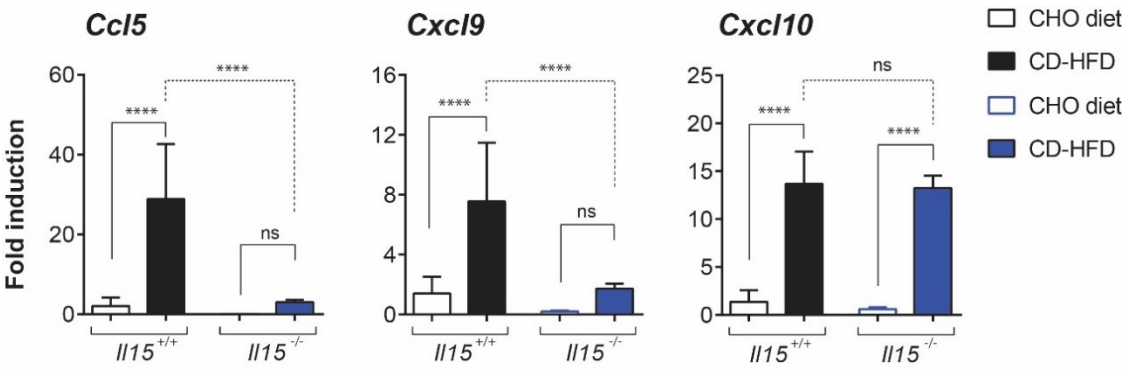

Figure 3.20. CD-HFD for 12 weeks induced chemokine expression in WT mice liver but not in Il15 deficient mice.

*Ccl5, Cxcl9 and Cxcl10 RNA levels were evaluated in liver samples from WT and Il15$^{-/-}$ mice after 12 weeks of dietary manipulation. The data is expressed as relative expression when compared to housekeeping gene (Gapdh). Results are shown as the mean ± SEM of two independent experiments. n = 8 to 11 mice per group. Statistical significance between treated and control groups, followed by Tukey's multiple comparisons test, is given as *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001.*

Various immune cells including macrophages play a vital role in this inflammatory response and these cells are recruited by various chemokines for the resolution of liver fibrosis. Therefore, we also examined the expression of pro-inflammatory chemokines *Ccl5, Cxcl9* and *Cxcl10*. The *Ccl5* chemokine that recruit inflammatory monocytes was increased in the livers of WT mice maintained on CD-HFD for 12 weeks while its expression was not increased in IL-15 deficient CD-HFD livers (Figure 3.20). Even though both *Cxcl9* and *Cxcl10* are induced by IFN-γ, *Cxcl10* can be induced independent of IFN-γ. Hence, we saw an increase in *Cxcl10* but not in *Cxcl9* that is strictly IFN-γ

dependent (Amichay et al., 1996; Farber, 1997; Qian et al., 2007; Tokunaga et al., 2018). Thus, these results suggest that IL-15 contributes indirectly to the recruitment of immune cells through upregulation of chemokines.

3.2.7. CD-HFD reduced *Ppara* expression in WT mice but not in *Il15* deficient mice

CD-HFD induces steatosis-associated inflammation which leads to fibrosis in metabolic syndrome. CD-HFD also alters cholesterol and fatty acid metabolism which increase the risk of cardiovascular events. Hence, we checked the expression of genes associated with lipid synthesis and metabolism. *Ppara* is a key regulatory transcription factor which regulates beta oxidation, fatty acid synthesis and uptake, bile acid synthesis, ketogenesis and triglycerides turnover (Liss and Finck, 2017). As depicted in Figure 3.21, CD-HFD supplement showed substantial reduction in *Ppara* gene expression in WT liver but not in *Il15* null liver. Thus, in the absence of *Il15*, CD-HFD has no effect on *Ppara* gene expression. Gene expressions of *Atf6,* a transcription factor which is involved in fatty acid oxidation by binding with *Ppara,* and *Cpt1a*, a gene that encodes carnitine palmitoyltransferase-1 enzyme, playing a critical role in translocation of long chain fatty acid through mitochondrial membrane for hepatic beta-oxidation, were significantly upregulated by CD-HFD and loss of *Il15* did not modulate increased expression of *Atf6* and *Cpt1a* (Figure 3.21).

Another important player of fatty acid metabolism is *Cd36*, which allows lipid transportation across the plasma membrane, and *Hmgcs2,* the gene that regulates mitochondrial fatty acid oxidation and controls production of ketone bodies. We also observed dramatic increase ($p<0.01$) in expression of *Cd36* in the liver of WT as well as *Il15*$^{-/-}$ mice fed with CD-HFD (Figure 3.21).

Acyl-CoA dehydrogenase (ACAD) enzymes, a family of four enzymes, catalyze the sequential steps in the mitochondrial fatty acid oxidation and produce acetyl-CoA for the tricarboxylic acid (TCA) cycle. As shown in Figure 3.21, the gene expression of *Acads,* responsible for short chain fatty acid oxidation, was significantly upregulated while *Acadvl,* responsible for very long chain fatty acid oxidation, was downregulated in CD-HFD fed WT liver. Notably, loss of *Il15* did not have any impact on alteration of *Acads* and *Acadvl* expression. *Cyp4a10,* the microsomal cytochrome P450 enzyme involved in ω-oxidation, was also decreased in the liver of WT mice receiving CD-HFD and the

expression of *Cyp4a10* was comparable in the liver of CD-HFD fed *Il15* null mice (Figure 3.21).

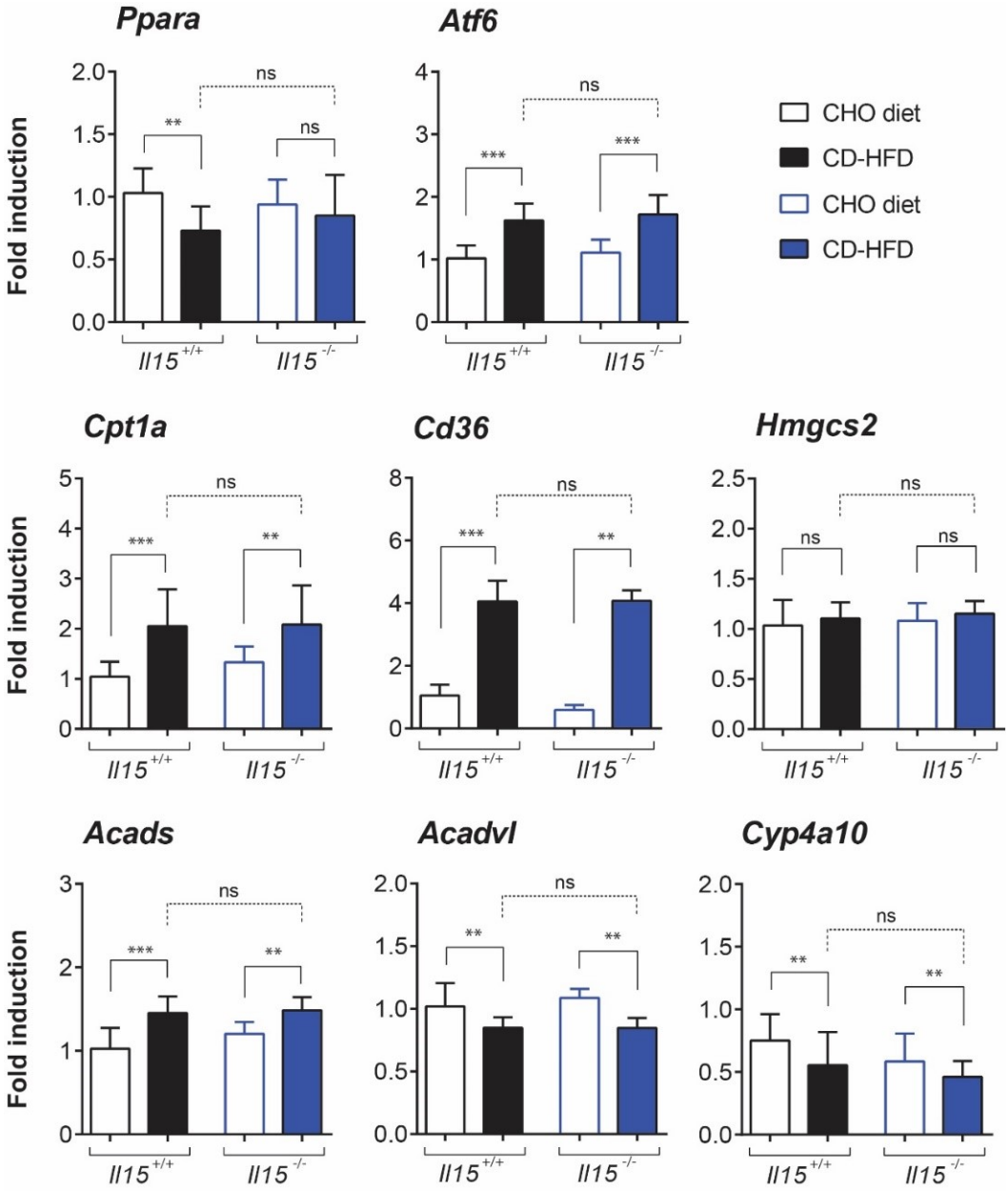

Figure 3.21. Choline-deficient high fat diet for 12 weeks significantly reduced Ppara expression in wild type but not in Il15 deficient mice liver.

*Ppara, Atf6, Cpt1a, Cd36, Hmgcs2, Acads, Acadvl and Cyp4a10 RNA levels were evaluated in liver samples from WT and Il15$^{-/-}$ mice after 12 weeks of dietary manipulation. The data is expressed as relative expression when compared to housekeeping gene (Gapdh). Results are shown as the mean ± SEM of two independent experiments. n = 8 to 11 mice per group. Statistical significance between treated and control groups, followed by Tukey's multiple comparisons test, is given as *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001.*

3.2.8. CD-HFD did not alter Acyl-CoA dehydrogenase family gene expression responsible for long and medium chain fatty acid metabolism

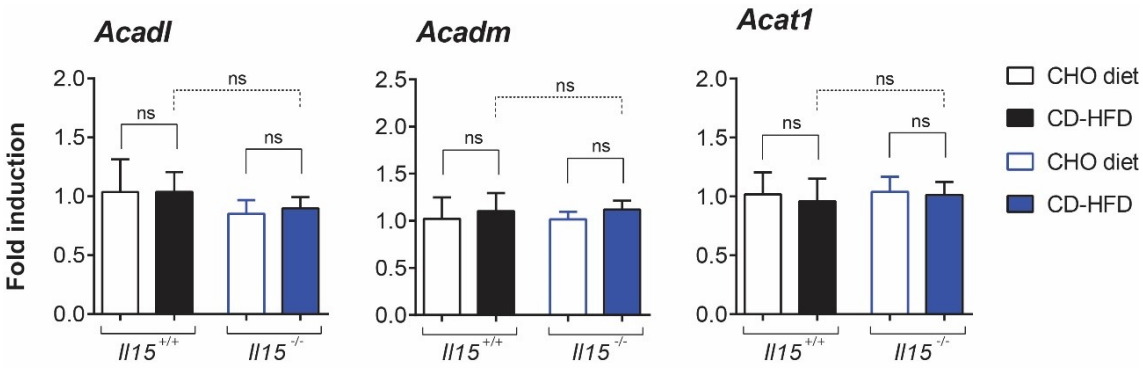

Figure 3.22. Choline-deficient high fat diet did not alter the expression of enzymes responsible for long and medium chain fatty acid metabolism.

*Acadl, Acadm and Acat1 RNA levels were evaluated in liver samples from WT and Il15$^{-/-}$ mice after 12 weeks of dietary manipulation. The data is expressed as relative expression when compared to housekeeping gene (Gapdh). Results are shown as the mean ± SEM of two independent experiments. n = 8 to 11 mice per group. Statistical significance between treated and control groups, followed by Tukey's multiple comparisons test, is given as *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001.*

Another 2 members of ACAD family *i.e. Acadl,* responsible for long chain fatty acid oxidation, and *Acadm,* responsible for medium chain fatty acid oxidation, were not altered by CD-HFD in WT or *Il15*$^{-/-}$ mice liver (Figure 3.22). Excess of cellular cholesterol is stored in the form of cholesteryl ester with the help of acyl-coenzyme A (CoA): cholesterol acyltransferase enzyme. We thus examined the expression of *Acat1* which encodes acyl-coenzyme A (CoA): cholesterol acyltransferase enzyme, and the gene expression of *Acat1* was also not altered in the liver of either WT or *Il15*$^{-/-}$ mice fed with CD-HFD (Figure 3.22).

Minimal regulation of the genes regulating metabolism in the *Il15*$^{-/-}$ mice in the fibrosis model indicate towards the involvement of inflammation pathways rather than direct metabolic alteration in CD-HFD induced hepatic diseases. Reduced expression of *Ppara* in CD-HFD fed WT mice might be mediated through IL-15 induced inflammation rather than direct involvement of IL-15 in the metabolism.

3.3. Choline-deficient high fat diet manipulation for 2 weeks produced significant alteration

To determine whether short-term exposure to CD-HFD was sufficient to produce steatosis/NAFLD like condition, CD-HFD was provided for 2 weeks. Age-matched male WT and *Il15*$^{-/-}$ mice were maintained on regular or CD-HFD and were analyzed as described above.

3.3.1. Choline-deficient high fat diet for 2 weeks is sufficient to upregulate the expression of *Il15* and *Il15ra*

After 2 weeks of CD-HFD, wild type mice showed significant increase in *Il15* and *Il15ra* expression (Figure 3.23), while *Il15* null mice showed significant protection against choline-deficient high fat diet induced elevation in *Il15ra* expressions in liver. Although, *Il15ra* expression was also increased (p<0.05) in *Il15* deficient mice, the expression was significantly low (p<0.01) as compared to choline- deficient high fat diet treated wild type mice. As expected, we did not detect the expression of *Il15* in *Il15* null mice.

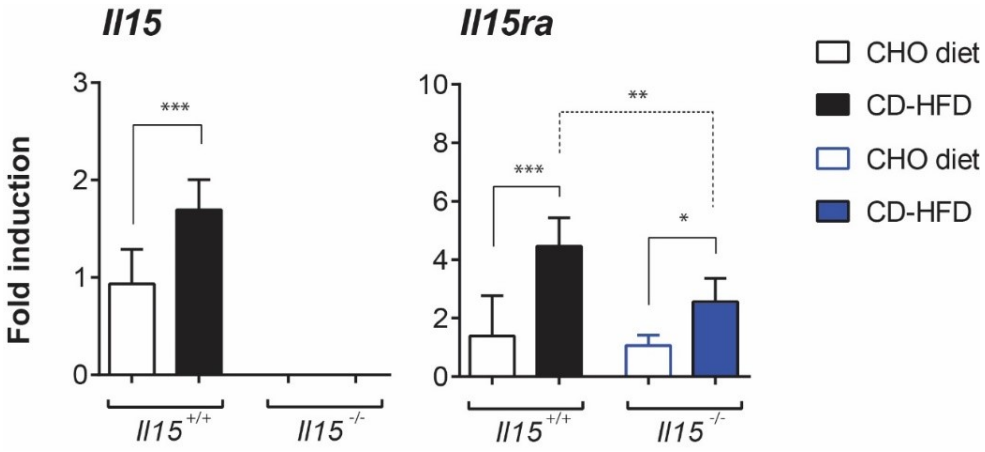

Figure 3.23. Two weeks of CD-HFD manipulation increased Il15 and Il15ra expression.

*Il15 and Il15ra RNA levels were evaluated in liver samples from wild type and Il15 null mice after 2 weeks of dietary manipulation. The data is expressed as relative expression when compared to housekeeping gene (Gapdh). Results are shown as the mean ± SEM of two independent experiments. n = 5 to 7 mice per group. Statistical significance between treated and control groups, followed by Tukey's multiple comparisons test, is given as *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001.*

3.3.2. Choline-deficient high fat diet for 2 weeks induced *Tnfa* and *F4/80* expressions in wild type mice but not in *Il15* deficient mice

The above results indicated involvement of *Il15* and *Il15ra* in the progression of NAFLD and steatosis like condition. As inflammation plays a critical role in the progression of NAFLD to steatosis or NASH followed by fibrosis (Bedossa, 2017), significant increase in the expression of *Tnfa* and *F4/80* in the livers of WT mice maintained on CD-HFD, but not in *Il15* deficient mice, suggests a role for IL-15 in promoting inflammation at early time points (Figure 3.24). Reduced expressions of *Tnfa* and *F4/80* indicated a low degree of inflammation and reduced macrophage infiltration in *Il15* null mice, respectively, and revealed protection against inflammation in the absence of *Il15*.

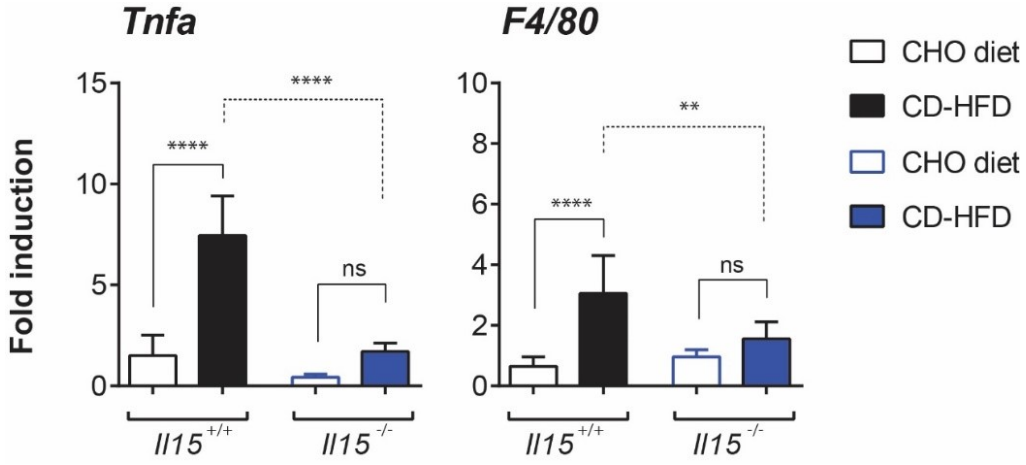

Figure 3.24. CD-HFD diet for 2 weeks increased Tnfa and F4/80 expression in wild type mice but not in Il15 deficient mice.

*Tnfa and F4/80 RNA levels were evaluated in liver samples from wild type and Il15 null mice after 2 weeks of dietary manipulation. The data is expressed as relative expression when compared to housekeeping gene (Gapdh). Results are shown as the mean ± SEM of two independent experiments. n = 5 to 7 mice per group. Statistical significance between treated and control groups, followed by Tukey's multiple comparisons test, is given as *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001.*

3.3.3. *Il15* deficiency reduced fibrosis-associated markers induced by choline-deficient high fat diet for 2 weeks

Although two weeks of choline-deficient high fat diet increased inflammation which was reversible, prolonged administration of choline-deficient high fat diet will leads to inflammation-associated steatosis which develops into liver fibrosis (Itagaki et al., 2013).

Therefore, to check the progression of liver fibrosis, series of genes involved in the progression of fibrosis were assessed.

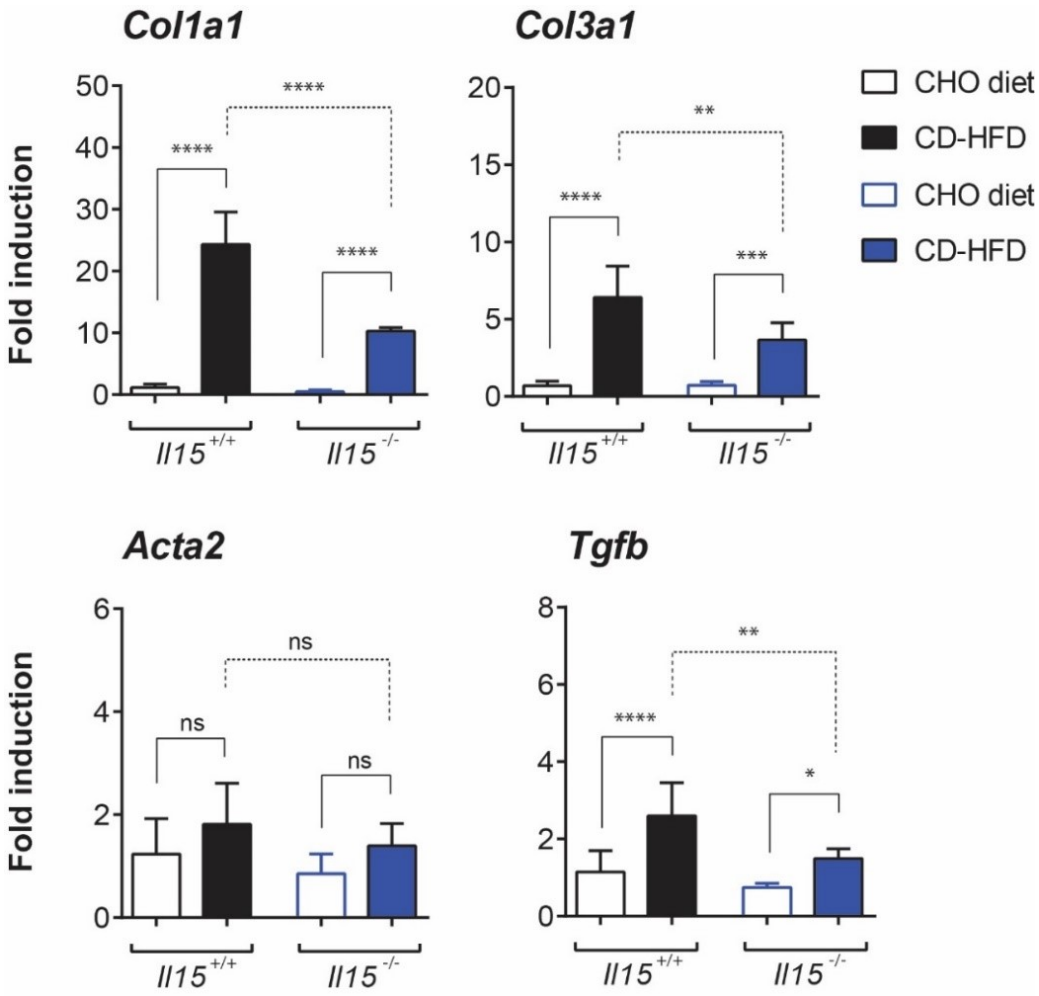

Figure 3.25. Two weeks of CD-HFD diet is sufficient to increase the expression of fibrosis associated genes.

*Col1a1, Col3a1, Acta2 and Tgfb RNA levels were evaluated in liver samples from wild type and Il15 null mice after 2 weeks of dietary manipulation. The data is expressed as relative expression when compared to housekeeping gene (Gapdh). Results are shown as the mean ± SEM of two independent experiments. n = 5 to 7 mice per group. Statistical significance between treated and control groups, followed by Tukey's multiple comparisons test, is given as *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001.*

As depicted in Figure 3.25, *Col1a1, Col3a1* and *Tgfb* expressions were significantly increased (p<0.0001) in the wild type mice after 2 weeks of choline-deficient high fat diet administration but were significantly lower in the *Il15* null mice receiving choline-deficient high fat diet. The expression of *Acta2,* responsible for production of *α-Sma*, was

not significantly altered (Figure 3.25) when compared to the respective control animals receiving normal diet.

3.3.4. Choline-deficient high fat diet for 2 weeks increased chemokine expression in the liver lacking *Il15*

Infiltration of immune cells during hepatic injury is mainly derived from release of chemokines, hence we checked expression of chemokines *Ccl5* and *Cxcl10* receptors. Choline-deficient high fat diet given for 2 weeks showed drastic increase in *Ccl5* and *Cxcl10* expression in wild type mice (Figure 3.26). However, deficiency of *Il15* reduced CD-HFD-induced *CCl5* and *Cxcl10* expression when compared to wild type mice receiving choline-deficient high fat diet, suggesting increased immune cell infiltration in absence of *Il15* due to modulation of chemokines expression.

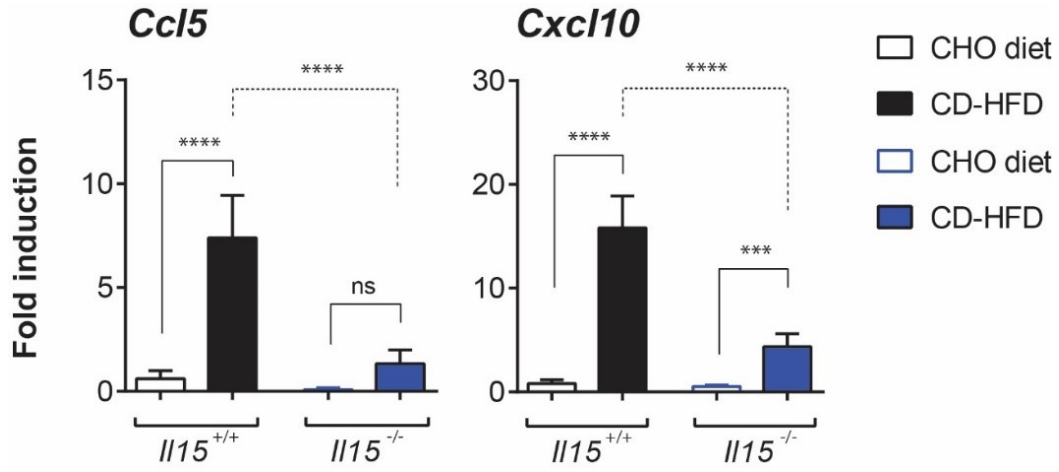

Figure 3.26. CD-HFD diet for 2 weeks induced chemokine expressions but deficiency of Il15 protected against elevation in chemokine expressions.

*Ccl5 and Cxcl10 RNA levels were evaluated in liver samples from wild type and Il15 null mice after 2 weeks of dietary manipulation. The data is expressed as relative expression when compared to housekeeping gene (Gapdh). Results are shown as the mean ± SEM of two independent experiments. n = 5 to 7 mice per group. Statistical significance between treated and control groups, followed by Tukey's multiple comparisons test, is given as *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001.*

3.3.5. Choline-deficient high fat diet for 2 weeks did not alter *Ppara* expression in liver

CD-HFD induces metabolic diseases through metabolic as well as epigenetic alterations. Hence, we studied expression of transcription factor *Ppara*, which is implicated in lipid homeostasis and liver pathogenesis in different contexts. Choline-deficient high fat diet did not alter expression of *Ppara* either in wild type or *Il15* null mice liver (Figure 3.27). This result indicates involvement of inflammation and other pathways rather than metabolic alteration in 2 weeks choline-deficient high fat diet induced hepatic disorders.

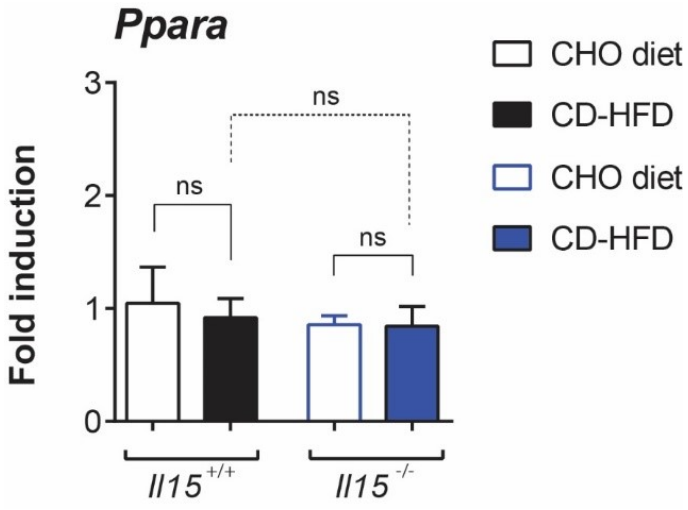

Figure 3.27. Two weeks CD-HFD diet manipulation did not alter Ppara gene expression.

*Ppara RNA level was evaluated in liver samples from wild type and Il15 null mice after 2 weeks of dietary manipulation. The data is expressed as relative expression when compared to housekeeping gene (Gapdh). Results are shown as the mean ± SEM of two independent experiments. n = 5 to 7 mice per group. Statistical significance between treated and control groups, followed by Tukey's multiple comparisons test, is given as *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001.*

3.4. Summary of results

In this work, we investigated the role of IL-15 in the homeostasis, development and pathogenesis of NASH and liver fibrosis. We found that IL-15 plays an inflammatory and profibrogenic role in the pathogenesis of inflammation associated liver fibrosis. Moreover, we observed that loss of IL-15 reduced inflammation and collagen deposition in various experimental animal models. Inflammation induced chemokine expression and immune cell infiltrations were also found to be reduced in the absence of *Il15*. Additionally, proinflammatory and profibrogenic gene expression were also downregulated in the absence of *Il15*. Furthermore, in context of metabolism, we showed that IL-15 mediated inflammation is responsible for the downregulation of *Ppara* gene expression. Overall, our results contribute to a better understanding of the profibrogenic role of IL-15 in the inflammation associated liver fibrosis.

4. Discussion

NAFLD is the most common liver disorder in the western world, with a global estimated prevalence of 20% of cases of liver disorders. The majority of patients have simple steatosis or NAFLD without inflammation or liver damage (Vernon et al., 2011). A subgroup of NAFLD patients develops inflammation associated with ballooning degradation of hepatocytes which leads to development of fibrosis and/or cirrhosis ending with hepatocellular carcinoma (Ong and Younossi, 2007; Vernon et al., 2011; Yoo et al., 2017). NAFLD is part of metabolic syndrome that includes obesity, diabetes and dyslipidemia. Simple NAFLD is a stable disease with or without slow progression over time while inflammation associated NAFLD, also termed as NASH, may lead to advanced fibrosis and cirrhosis which increases mortality due to liver-associated and cardiovascular events (Stal, 2015).

Liver fibrosis is a series of multiple pathological conditions starting from fatty liver to apoptosis and necrosis of hepatocytes which stimulates production and deposition of extracellular matrix and collagen through activation of stellate cells. The early detection of various fibrosis stages and cirrhosis is crucial for diagnosis to start therapeutic interventions. Liver biopsy is considered as a gold standard for the assessment of hepatic inflammation and stages of liver fibrosis, however, due to sampling variation and low patient acceptance due to pain, this invasive technique is not widely acceptable amongst patients (Patel and Shackel, 2014; Stal, 2015). However, recent advanced technique such as serum biomarkers, physical and physiological examination (breath test), made non-invasive techniques more applicable in clinical practices (Patel and Shackel, 2014; Patel et al., 2015; Li et al., 2018). Nevertheless, results of these non-invasive techniques can be influenced by physiological condition of patients due to non-specificity toward liver (Patel and Shackel, 2014; Sharma et al., 2014). Thus, there is a need for the identification of reliable biomarkers for steatosis, steatohepatitis, fibrosis and hepatocellular carcinoma.

Fibrosis results from chronic inflammation, which can be defined as an inflammatory immune response which persists for several months or years with immune cell infiltration, tissue remodeling and repair simultaneously. Even though fibrosis can have distinct etiology, chronic persistent inflammation which induces production of angiogenic factors, proteolytic enzymes, growth factors and profibrogenic cytokines leading to production and deposition of extracellular matrix which eventually alters

normal hepatic architecture due to remodelling, is a hallmark of fibrosis (Tomasek et al., 2002; Friedman, 2004). As inflammation plays a critical role in progression of liver disorders, regulation of inflammatory cascades may prevent the progression of NAFLD to liver fibrosis and cirrhosis.

IL-15 is known to have a pathological role in the development of various autoimmune diseases such as type 1 diabetes, rheumatoid arthritis and inflammatory bowel diseases (McInnes and Liew, 1998; Yokoyama et al., 2009; Bobbala et al., 2012). Previously, we observed that IL-15 is implicated in the pathology of high fat diet induced fatty liver and absence of *Il15* or *Il15ra* retards development of fatty liver (Cepero-Donates et al., 2016a; Cepero-Donates et al., 2016b). Hence, in line with our previous work, here we assessed the role of IL-15, an immune-inflammatory cytokine, in the pathogenesis of liver fibrosis.

It is not known whether human hepatic stellate cells express IL-15 in normal physiological condition as well as in fibrogenesis. IL-15 expression has been observed in liver tissues, hepatocyte and hepatoma cell lines (Golden-Mason et al., 2004; Correia et al., 2009). In this experimental work, we observed constitutive expression of *Il15* as well as *Il15ra* in the mouse liver. Transcriptome microarrays of normal and fibrotic human liver showed a significant increase *IL15* and *IL15Ra* expression in fibrotic liver as compared to healthy liver (Jiao et al., 2016). Increased expression of *IL15* and *IL15Ra* in liver fibrosis supports a role for IL-15 in the progression of the liver pathology.

In line with previous observations, we also observed increased *Il15* and *Il15ra* expression in HFD induced NAFLD as well as CD-HFD or CCl_4 induced liver fibrosis animal models. Previously, Sun and Zemel have also observed increase in *Il15* expression in the skeletal muscles and adipose tissues after high calcium obesogenic diet (Sun and Zemel, 2007). In accordance to this result, we also observed elevated expression of *Il15* in the liver of obese mice receiving high-fat-diet, suggesting involvement of the *Il15* in the pathogenesis of liver ailments.

Inflammation is a common response to any chronic injury leading to formation of scar tissue and ultimately fibrosis. CCl_4 injected wild type mice also showed extensive liver injury, characterized by necrosis, degeneration and ballooning of hepatocytes along with infiltration of mononuclear inflammatory immune cells and widespread fibrosis. CCl_4 administration leads to alteration in various key pathways such as drug metabolism by CYP450 leading to generation of free radicals along with oxidative stress (Scholten et

al., 2015; Dong et al., 2016). However, CCl_4 injected *Il15*$^{-/-}$ mice showed reduced inflammation and fibrosis when compared to WT mice treated with CCl_4. In line with these results, CD-HFD induced fibrotic liver also showed similar results. Recruited proinflammatory macrophages convert to patrolling macrophages and play a critical role in tissue repair and restoration after an injury (Dal-Secco et al., 2015). However, massive infiltration of monocyte derived macrophages is a peculiar feature of acute and chronic liver injury in humans (Zimmermann et al., 2010; Antoniades et al., 2012) and this pro- and anti-fibrotic role of infiltrated macrophages could be attributed to different subsets. In the early phase of liver injury, chemokines recruit Ly6C^{Hi} pro-inflammatory monocytes that promotes fibrosis by direct interaction with stellate cells and through production of TGFβ while Ly6C^{Lo} macrophages demonstrate anti-fibrotic action by accelerating the regression of fibrosis (Karlmark et al., 2009; Baeck et al., 2012; Ramachandran et al., 2012). In line with this data, we also observed increased *F4/80* gene expression as well as CD68+ve macrophages in CCl_4 injected or CD-HFD treated WT mice liver but the expression of *F4/80* and macrophages infiltration were significantly reduced in the absence of IL-15, indicating that IL-15 plays crucial role in the recruitment of macrophages in liver micromilieu during the inflammatory conditions like NAFLD and fibrosis. Earlier, Alleva et al. also showed that IL-15 alters macrophage functions in a cytokine dependent manner (Alleva et al., 1997). In macrophages, IL-15 acts as autocrine regulator of pro- and anti-inflammatory cytokines. High level of IL-15 favors production of pro-inflammatory cytokines such as TNF-α, IL-1 and IL-6 while low concentration favors production of anti-inflammatory cytokine IL-10 via macrophages (Alleva et al., 1997). IL-15 is also known to activate macrophages but underlying mechanism is yet to be elucidated.

Various pro-inflammatory cytokines promote liver fibrosis by promoting secretion of key profibrogenic cytokine TGFβ, leading to activation and proliferation of stellate cells (Borthwick et al., 2013). The expression of *Il6, Il12(p40), Tnfa* and *iNos* genes was significantly increased in WT mice treated with CCl_4 or CD-HFD, whereas expression of these gene was significantly lower in *Il15*$^{-/-}$ mice receiving CCl_4 or CD-HFD, suggesting that IL-15 acts upstream of pro-inflammatory cytokines and is crucial to maintain the production and release of inflammatory cytokines. TNFα has a proven inflammatory role in the development of NASH and progression to fibrosis through upregulating the expression of various fibrogenic molecules such as *Mcp1*, *Tgfb*, *Col1a1*, *Timp1* and

MMPs (Yang and Seki, 2015; Kakino et al., 2018). Increased *Tnfa* expression in fibrotic WT mice is associated with increased inflammation, proliferation and apoptosis of hepatocytes, however the molecular mechanisms of TNFα in liver fibrosis is not well studied. Similarly, increased expression of other pro-inflammatory cytokines such as *Il6* and *Il12(p40),* is also associated with degree of liver damage (Choi et al., 1994; Araujo et al., 2001; Hammerich and Tacke, 2014). The role of IFNγ in the development and homeostasis of the liver is not clear and remains controversial. Previously, Luo et al. observed reduced liver fibrosis in *Ifng*$^{-/-}$ mice due to suppression of inflammatory response of macrophages as well as reduced activation of HSC during the progression of liver fibrosis (Luo et al., 2013). Clinically it is also observed that enhanced level of IFNγ in the serum is directly correlated with liver dysfunction rather than inflammatory disease (Attallah et al., 2016). In line with these observations, we also observed significant elevated expression of *Ifng* in fibrotic WT mice liver. In contrast, IFNγ is known for attenuating liver fibrosis by inhibiting stellate cells proliferation and collagen production, through augmenting NK cell mediated killing of activated HSCs (Czaja et al., 1989; Shi et al., 1997; Shen et al., 2002; Jeong et al., 2008; Gao and Radaeva, 2013). IFNγ treatment improved fibrotic score in patients with chronic HBV infection due to inhibition of profibrotic TGFβ (Weng et al., 2005). It is possible that IFNγ treatment activated NK cells that resulted in the control of HBV infection and had indirect effect on TGFβ expression and fibrosis. However, absence of *Ifng* expression in *Il15*$^{-/-}$ mice liver supports profibrotic role of IFNγ in the development of liver fibrosis, although the reason for impaired *Ifng* expression in IL-15 deficient mice livers remains to be addressed.

During inflammation, activated Kupffer cells, sinusoidal endothelial cells and HSCs produce large amount of NO through iNOS. iNOS is also identified as fibrogenic, due to enhanced expression of *Mmps* and *HIF-1α*, in the development of liver fibrosis (Rockey and Chung, 1996; Mustafa and Olson, 1998; Aram et al., 2008; Anavi et al., 2015; Iwakiri, 2015). Inhibition of *iNOS* or *iNos*$^{-/-}$ mice also showed reduction in experimentally induced liver fibrosis (Aram et al., 2008; Anavi et al., 2015). We also observed a significant increase in the expression of *iNos* gene in WT fibrotic liver that was diminished in the absence of IL-15. Previously, Hou et al. demonstrated increased level of *iNOS* in acetaminophen-induced hepatotoxicity while loss of *Il15* reduced *iNOS* levels (Hou et al., 2012). However, regulation of iNOS through IL-15 signaling is yet to be elucidated.

Inflammatory cytokines increase progression of liver fibrosis by inducing TGFβ, an fibrogenic growth factor, which promotes stellate cell proliferation and drive fibroblast into myofibroblast (Desmouliere et al., 1993; Borthwick et al., 2013). WT mice treated with CCl_4 or CD-HFD showed significant increase in the expression of *Tgfb* gene while *Il15*$^{-/-}$ mice showed reduced expression of *Tgfb* when compared to WT mice, suggesting reduced TGFβ signaling in absence of IL-15. In contrast, Benahmed and colleagues showed that IL-15 inhibits Smad dependent TGFβ signalling in lymphocytes (Benahmed et al., 2007). In line with our hypothesis, CCl_4 or CD-HFD induced collagen and αSMA gene and protein expression was reduced in the absence of IL-15. This indicates that in the absence of IL-15, stellate cells are spared from the effects of inflammation as seen from the reduced expression of *Mmps* and *Timp1* expression.

MMPs are a group of enzymes mainly responsible for the degradation of ECM. However, collective analysis of the role of MMPs in different fibrosis models revealed both properties *i.e.* inhibitory and stimulatory activities in the development of fibrosis (Giannandrea and Parks, 2014; Duarte et al., 2015; Jablonska-Trypuc et al., 2016). Apart from ECM remodeling, MMPs also influence various cellular activities such as survival, proliferation, differentiation, migration and gene expression (Page-McCaw et al., 2007; Giannandrea and Parks, 2014). In the liver, MMPs are expressed by stellate cells and MMPs play crucial role in matrix substrate clearance from the injured environment to maintaining the activated stellate cell phenotype (Guo and Friedman, 2007). Although, MMPs clear various ECM substrates *in vitro*, their *in vivo* replica is not yet clear. The *in vivo* functions of MMPs are influenced by their differential expression at various stages of fibrosis which alter their ability to cleave normal matrix substrate versus fibrotic interstitial fibrillar collagen (Benyon and Arthur, 2001; Gelse et al., 2003; Guo and Friedman, 2007; Giannandrea and Parks, 2014). In the liver, MMP1 and MMP2 are identified as having anti-fibrotic properties while MMP3 and MMP9 promote fibrosis. However, functions of these MMPs depend on progression of fibrosis and are tissue dependent (Giannandrea and Parks, 2014; Duarte et al., 2015). MMP1 and MMP2 contribute to fibrosis resolution via promoting collagen degradation and suppressing collagen expression, respectively (Benyon and Arthur, 2001; Giannandrea and Parks, 2014). MMP9 is also involved in neutrophils and leucocytes infiltration during liver injury (Hanumegowda et al., 2003; Hamada et al., 2008). In line with this, we also observed drastic reduction in CD-HFD induced *Mmp1* and *Mmp9* gene expression in *Il15*

deficient livers when compared CD-HFD fed WT mice. Interestingly, in CCl_4 induced fibrotic mice, expression of *Mmp2* and *Mmp9* is comparable between WT and *Il15*$^{-/-}$ mice. Loss of IL-15 altered expression of *Mmp2* and *Mmp9* in CD-HFD induced liver fibrosis but not in CCl_4 induced fibrosis. TIMP-1 is an endogenous metalloproteinase which contributes in fibrosis via inhibition of different MMPs (Brew et al., 2000). Recently, Thiele and colleagues have identified that TIMP-1 is upregulated, but not essential for the progression of liver fibrosis (Thiele et al., 2017). TIMP-1 expression is upregulated by activated HSCs leading to inhibition of MMPs which contributes to progression of fibrosis (Schuppan et al., 2001; Roderfeld, 2018). CD-HFD induced *Timp1* expression is reduced in the absence of IL-15. However, regulation of MMPs and TIMPs by IL-15 in liver fibrosis is not well studied.

Altered expression of chemokines as well as their receptors play a critical role in the progression and pathogenesis of liver fibrosis. Apart from recruitment of immune cells, chemokines also contribute to immune surveillance and maintain homeostasis of other nonimmune cells, such as fibrogenic response of stellate cells (Marra, 2002; Wasmuth et al., 2010). Activation of liver myofibroblasts leads to production and release of different chemokines *viz*. CCL2, CCL3, CCL5, CCL11, CXCL8, CXCL9 and CXCL10 which further recruits immune cell infiltration in the liver (Holt et al., 2009). Activated stellate cells, Kupffer cells and damaged hepatocytes secrete high level of CCL2 which promotes hepatic infiltration of monocytes leading to fibrogenesis (Karlmark et al., 2009; Seki et al., 2009b; Ehling et al., 2014). Although CCL5 is strongly expressed by hepatic stellate cells, lymphocytes, platelets, macrophages, endothelial cells and hepatocytes also contribute to the secretion of CCL5 (Appay and Rowland-Jones, 2001). CCL5 exerts its action on stellate cells in autocrine as well paracrine manner and controls HSC migration, activation and proliferation (Mafanda et al., 2018). CCL5 is also identified to have detrimental effects during progression of hepatic fibrosis, atherosclerosis, obesity and other inflammatory disorders (Wu et al., 2007; Koenen et al., 2009; Seki et al., 2009a; Mafanda et al., 2018). In line with this, we also observed increased *Ccl2* and *Ccl5* gene expression in CD-HFD as well as CCl_4 injected mice, however the expression of *Ccl2* and *Ccl5* was substantially lower in *Il15* null mice receiving CCl_4 (Figure 3.11 and 3.20).

Notably, CCl_4 or CD-HFD treated wild type mice livers showed increased expression of *Cxcl9* and *Cxcl10*, which are important mediators in tissue scaring and progression of fibrosis by exerting dual effects on intrahepatic immune cells as well as hepatic stellate

cells. CXCR3 associated chemokines such as CXCL9, CXCL10 and CXCL11 have been identified to be involved in inflammation and liver fibrosis due to recruitment of lymphocytes leading to release of interferon and interleukin (Loetscher et al., 1996; Zeremski et al., 2008). Previously, profibrogenic role of CXCL9 and CXCL10 has been proven due to the secretion of IFNγ and IL-9 (Loetscher et al., 1996; Zeremski et al., 2008; Guo et al., 2018; Mazzi, 2019). In contrast to this finding, Wasmuth et al. observed increased liver fibrosis in *Cxcr3*$^{-/-}$ mice indicating an anti-fibrotic role of CXCL9 and CXCR3. (Wasmuth et al., 2009). Reduced expression of *Cxcl9* and *Cxcl10* in *Il15*$^{-/-}$ fibrotic liver could be due to absence of *Ifng* that induces these 2 chemokines. Increased expression of *Ccl5* and *Cxcl10* in CD-HFD induced NASH indicates the secretion of pro-fibrogenic chemokines at the effector phase of the fibrogenic cascade. Apart from CXCR3 associated chemokines, fractalkine receptor CX3CR1, largely expressed by monocyte derived macrophages, monocytes and dendritic cells, was also found to possess antifibrotic role by regulating differentiation and survival of hepatic infiltrated monocytes (Karlmark et al., 2010; Marra and Tacke, 2014). The expression of *Cx3cr1* was found to be abundantly increased in CCl_4 injected WT mice liver with nonsignificant reduction in *Il15*$^{-/-}$ mice as compared to CCl_4 injected WT mice. However, regulation of CX3CR1 via IL-15 remains to be addressed. Reduced fibrosis in *Il15*$^{-/-}$ mice might be attributed to markedly reduced recruitment of immune cells and immune cell-mediated inflammation. However, further studies are needed to unravel the mechanism by which IL15 regulates various chemokines involved in progression of liver fibrosis in different cellular compartment.

As a metabolic hub, the liver maintains whole body energy homeostasis. At the cellular level, mitochondria- the powerhouse of the cell, connects and integrates the hormonal, biochemical and inflammatory pathways to achieve the energetic and biochemical demands of the cell (Mello et al., 2016). In the pathogenesis of obesity and metabolism-associated disorders such as NASH/NAFLD, lipotoxicity, insulin resistance and adipose derived inflammation play a central role. However, central mechanisms responsible for development of fibrosis and hepatic metastasis from NASH are complex and not well understood yet (Ipsen et al., 2018; Rakotoarivelo et al., 2018). In hepatic steatosis, lipid acquisition exceeds its disposal through increased fatty acid uptake and *de-novo* lipogenesis, and reduced fatty acid oxidation (Sozio et al., 2010; Ipsen et al., 2018). PPARα is the main regulatory nuclear receptor that controls lipid metabolism as well as

glycolysis and gluconeogenesis pathways in mice (Rakhshandehroo et al., 2009; Janssen et al., 2015). *Ppara-/-* mice fed with methionine and choline deficient diet showed abundant liver fibrosis when compared with WT mice while treatment with Wy-14,643 (PPARα agonist) showed reduction in fibrosis indicating antifibrotic role of PPARα through reducing fibrotic stimuli such as lipid peroxidation (Ip et al., 2003; Ip et al., 2004). We did not observe reduction in *Ppara* gene expression in WT or *Il15*$^{-/-}$ mice fed with CD-HFD for 2 weeks. This finding is in line with the recent reports indicating negligible alteration in lipid homeostasis with minimal or no inflammation after 2 weeks of HFD feeding (Shang et al., 2017; Andrich et al., 2018; Denver et al., 2018). However, WT mice fed with CD-HFD for 12 weeks showed significant reduction in *Ppara* gene expressions in liver while *Il15*$^{-/-}$ mice did not reveal reduction in *Ppara* expression suggesting IL-15 mediated inflammation plays a critical role in lipid homeostasis alteration through down-regulation of *Ppara* expression. Expression of *Atf6* (transcription factor involved in fatty acid oxidation through binding with *Ppara*), *Cpt1a* (encodes enzyme that transfer long chain fatty acids from acyl-CoA to carnitine to form acyl-carnitine) and *Acads* (responsible for short chain fatty acid oxidation) were significantly upregulated by CD-HFD diet and comparable between WT and *Il15*$^{-/-}$ mice. These observations indicate that IL-15 may not directly regulate lipid homeostasis.

Collectively, our results indicate a pro-fibrotic role for IL-15 in the pathogenesis of liver fibrosis. Thus, IL-15 could be a potential therapeutic target for the management of liver fibrosis.

5. Conclusions

Inflammation associated liver fibrosis is a potentially life-threatening complication associated with high mortality. Several pro-inflammatory and fibrogenic cytokines have been implicated in the pathogenesis of liver fibrosis. The role of IL-15 in the regulation and homeostasis of various immune cells such as NK cells, NKT cells and other lymphocytes has been well characterized but its role in cells such as hepatocytes, stellate cells and Kupffer cells was not well studied. Thus, this study was carried out to investigate the role of IL-15 in hepatocytes and stellate cells.

CD-HFD or CCl_4 treatment increased the expression of *Il15* as well as other pro-inflammatory cytokines such as *Il6, Tnfa* and *Il12*. The livers of WT mice fed CD-HFD also showed reduced *Ppara* gene expression indicating that IL-15 mediated inflammation alters lipid metabolism. In addition to reduced inflammatory mediators, IL-15 deficient livers preserved lipid homeostasis through maintaining *Ppara* regulatory machinery. Liver infiltrated immune cells play a critical role in the activation of stellate cells as well as progression of liver fibrosis. Macrophage infiltration was significantly increased in WT fibrotic liver but not in *Il15*$^{-/-}$ mice liver, and this could be attributed to reduced expression of various chemokine gene (*Ccl2* and *Ccl5*), responsible for macrophage chemotaxis. We also observed reduced ECM deposition such as collagen and α-SMA in *Il15*$^{-/-}$ mice liver indicating reduced stellate cell activation in the absence of IL-15. Interestingly, we observed increased expression of *Ifng* gene in fibrotic livers from WT mice but *Ifng* was not detected in *Il15*$^{-/-}$ mice liver. In a nutshell, we identified pro-fibrogenic role of in the progression of liver fibrosis through upregulation of inflammatory and fibrotic cytokines.

6. Perspectives

The results obtained from the current experimental work indicate a pro-fibrotic role for IL-15 in the pathogenesis of liver fibrosis. While our results indicate that IL-15 regulates inflammation rather than metabolism directly, additional experiments are needed to understand the underlying mechanisms. Recently, Jiao et al. observed that absence of IL-15Rα reduces the ECM deposition and thereby help in reducing fibrosis (Jiao et al., 2016). Study from Jiao and colleagues used *Il15ra*$^{-/-}$ mice to study development of fibrosis rather than *Il15*$^{-/-}$ mice. Additionally, we did not observe *Ifng* gene expression in *Il15*$^{-/-}$ mice liver. Thus, it will be important to know the contribution of IL-15 and IFNγ by analysing the development of fibrosis in the livers of *Ifng*$^{-/-}$ and *Il15*$^{-/-}$*Ifng*$^{-/-}$ mice. Additionally, measurement of various inflammatory and pro-fibrogenic cytokines in these mice plasma might provide detailed mechanisms contributing towards the development of liver fibrosis.

Moreover, we used complete *Il15* knockout mice rather than conditional deletion. Hence, it would be more interesting to prepare primary culture of hepatic stellate cells or hepatocytes from WT or *Il15*$^{-/-}$ mice to assess the role of IL-15 in the production of ECM and proinflammatory cytokines. Additionally, macrophage mediated stellate cell regulation also plays a critical role in progression of liver fibrosis. Therefore, coculture of stellate and macrophage will provide us with additional insights into the molecular mechanisms by which *Il15* deficient macrophages regulate the activation of stellate cells and thereby liver fibrosis.

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8. References:

Abdel-Misih, S.R., Bloomston, M., 2010. Liver anatomy. The Surgical clinics of North America 90, 643-653.

Abdel-Salam, B.K., Ebaid, H., 2008. Upregulation of major histocompatibility complex class II, CD83, CD64, and CD14 on polymorphonuclear neutrophils stimulated with interleukin-15. Journal of microbiology, immunology and infection 41, 462-468.

Adeva-Andany, M.M., Perez-Felpete, N., Fernandez-Fernandez, C., Donapetry-Garcia, C., Pazos-Garcia, C., 2016. Liver glucose metabolism in humans. Bioscience reports 36.

Ahsan, M.R., Islam, K.M., Bulbul, I.J., Musaddik, M.A., Haque, E., 2009. Hepatoprotective activity of methanol extract of some medicinal plants against carbon tetrachloride-induced hepatotoxicity in rats. Eur J Sci Res 37, 302-310.

Alleva, D.G., Kaser, S.B., Monroy, M.A., Fenton, M.J., Beller, D.I., 1997. IL-15 functions as a potent autocrine regulator of macrophage proinflammatory cytokine production: evidence for differential receptor subunit utilization associated with stimulation or inhibition. Journal of immunology 159, 2941-2951.

Alonso-Arias, R., Moro-Garcia, M.A., Vidal-Castineira, J.R., Solano-Jaurrieta, J.J., Suarez-Garcia, F.M., Coto, E., Lopez-Larrea, C., 2011. IL-15 preferentially enhances functional properties and antigen-specific responses of CD4+CD28(null) compared to CD4+CD28+ T cells. Aging cell 10, 844-852.

Amichay, D., Gazzinelli, R.T., Karupiah, G., Moench, T.R., Sher, A., Farber, J.M., 1996. Genes for chemokines MuMig and Crg-2 are induced in protozoan and viral infections in response to IFN-gamma with patterns of tissue expression that suggest nonredundant roles in vivo. Journal of immunology 157, 4511-4520.

Anavi, S., Eisenberg-Bord, M., Hahn-Obercyger, M., Genin, O., Pines, M., Tirosh, O., 2015. The role of iNOS in cholesterol-induced liver fibrosis. Laboratory investigation; a journal of technical methods and pathology 95, 914-924.

Anderson, D.M., Johnson, L., Glaccum, M.B., Copeland, N.G., Gilbert, D.J., Jenkins, N.A., Valentine, V., Kirstein, M.N., Shapiro, D.N., Morris, S.W., et al., 1995. Chromosomal assignment and genomic structure of Il15. Genomics 25, 701-706.

Andrich, D.E., Melbouci, L., Ou, Y., Leduc-Gaudet, J.P., Chabot, F., Lalonde, F., Lira, F.S., Gaylinn, B.D., Gouspillou, G., Danialou, G., Comtois, A.S., St-Pierre, D.H., 2018. Altered Feeding Behaviors and Adiposity Precede Observable Weight Gain in Young

Rats Submitted to a Short-Term High-Fat Diet. Journal of nutrition and metabolism 2018, 1498150.

Anguille, S., Smits, E.L., Cools, N., Goossens, H., Berneman, Z.N., Van Tendeloo, V.F., 2009. Short-term cultured, interleukin-15 differentiated dendritic cells have potent immunostimulatory properties. Journal of translational medicine 7, 109.

Antoniades, C.G., Quaglia, A., Taams, L.S., Mitry, R.R., Hussain, M., Abeles, R., Possamai, L.A., Bruce, M., McPhail, M., Starling, C., Wagner, B., Barnardo, A., Pomplun, S., Auzinger, G., Bernal, W., Heaton, N., Vergani, D., Thursz, M.R., Wendon, J., 2012. Source and characterization of hepatic macrophages in acetaminophen-induced acute liver failure in humans. Hepatology 56, 735-746.

Appay, V., Rowland-Jones, S.L., 2001. RANTES: a versatile and controversial chemokine. Trends in immunology 22, 83-87.

Aram, G., Potter, J.J., Liu, X., Torbenson, M.S., Mezey, E., 2008. Lack of inducible nitric oxide synthase leads to increased hepatic apoptosis and decreased fibrosis in mice after chronic carbon tetrachloride administration. Hepatology 47, 2051-2058.

Araujo, M.I., Bliss, S.K., Suzuki, Y., Alcaraz, A., Denkers, E.Y., Pearce, E.J., 2001. Interleukin-12 promotes pathologic liver changes and death in mice coinfected with Schistosoma mansoni and Toxoplasma gondii. Infection and immunity 69, 1454-1462.

Armitage, R.J., Macduff, B.M., Eisenman, J., Paxton, R., Grabstein, K.H., 1995. IL-15 has stimulatory activity for the induction of B cell proliferation and differentiation. Journal of immunology 154, 483-490.

Asgharpour, A., Cazanave, S.C., Pacana, T., Seneshaw, M., Vincent, R., Banini, B.A., Kumar, D.P., Daita, K., Min, H.K., Mirshahi, F., Bedossa, P., Sun, X., Hoshida, Y., Koduru, S.V., Contaifer, D., Jr., Warncke, U.O., Wijesinghe, D.S., Sanyal, A.J., 2016. A diet-induced animal model of non-alcoholic fatty liver disease and hepatocellular cancer. Journal of hepatology 65, 579-588.

Asselah, T., Bieche, I., Sabbagh, A., Bedossa, P., Moreau, R., Valla, D., Vidaud, M., Marcellin, P., 2009. Gene expression and hepatitis C virus infection. Gut 58, 846-858.

Attallah, A.M., El-Far, M., Zahran, F., Shiha, G.E., Farid, K., Omran, M.M., Abdelrazek, M.A., Attallah, A.A., El-Beh, A.A., El-Hosiny, R.M., El-Waseef, A.M., 2016. Interferon-gamma is associated with hepatic dysfunction in fibrosis, cirrhosis, and hepatocellular carcinoma. Journal of immunoassay & immunochemistry 37, 597-610.

Azimi, N., Brown, K., Bamford, R.N., Tagaya, Y., Siebenlist, U., Waldmann, T.A., 1998. Human T cell lymphotropic virus type I Tax protein trans-activates interleukin 15 gene

transcription through an NF-kappaB site. Proceedings of the National Academy of Sciences of the United States of America 95, 2452-2457.

Badolato, R., Ponzi, A.N., Millesimo, M., Notarangelo, L.D., Musso, T., 1997. Interleukin-15 (IL-15) induces IL-8 and monocyte chemotactic protein 1 production in human monocytes. Blood 90, 2804-2809.

Baeck, C., Wehr, A., Karlmark, K.R., Heymann, F., Vucur, M., Gassler, N., Huss, S., Klussmann, S., Eulberg, D., Luedde, T., Trautwein, C., Tacke, F., 2012. Pharmacological inhibition of the chemokine CCL2 (MCP-1) diminishes liver macrophage infiltration and steatohepatitis in chronic hepatic injury. Gut 61, 416-426.

Bataller, R., Brenner, D.A., 2005. Liver fibrosis. The Journal of clinical investigation 115, 209-218.

Baud, V., Karin, M., 2001. Signal transduction by tumor necrosis factor and its relatives. Trends in cell biology 11, 372-377.

Bedogni, G., Nobili, V., Tiribelli, C., 2014. Epidemiology of fatty liver: an update. World journal of gastroenterology 20, 9050-9054.

Bedossa, P., 2017. Pathology of non-alcoholic fatty liver disease. Liver international : official journal of the International Association for the Study of the Liver 37 Suppl 1, 85-89.

Benahmed, M., Meresse, B., Arnulf, B., Barbe, U., Mention, J.J., Verkarre, V., Allez, M., Cellier, C., Hermine, O., Cerf-Bensussan, N., 2007. Inhibition of TGF-beta signaling by IL-15: a new role for IL-15 in the loss of immune homeostasis in celiac disease. Gastroenterology 132, 994-1008.

Benten, D., Kluwe, J., Wirth, J.W., Thiele, N.D., Follenzi, A., Bhargava, K.K., Palestro, C.J., Koepke, M., Tjandra, R., Volz, T., Lutgehetmann, M., Gupta, S., 2018. A humanized mouse model of liver fibrosis following expansion of transplanted hepatic stellate cells. Laboratory investigation; a journal of technical methods and pathology 98, 525-536.

Benten, D., Kumaran, V., Joseph, B., Schattenberg, J., Popov, Y., Schuppan, D., Gupta, S., 2005. Hepatocyte transplantation activates hepatic stellate cells with beneficial modulation of cell engraftment in the rat. Hepatology 42, 1072-1081.

Benyon, R.C., Arthur, M.J., 2001. Extracellular matrix degradation and the role of hepatic stellate cells. Seminars in liver disease 21, 373-384.

Bertola, A., 2018. Rodent models of fatty liver diseases. Liver Research 2, 3-13.

Bhagavan, N., 2002. Protein and amino acid metabolism. Medical Biochemistry, Harcourt/Academic Press, Burlington, 331-332.

Blaner, W.S., O'Byrne, S.M., Wongsiriroj, N., Kluwe, J., D'Ambrosio, D.M., Jiang, H., Schwabe, R.F., Hillman, E.M., Piantedosi, R., Libien, J., 2009. Hepatic stellate cell lipid droplets: a specialized lipid droplet for retinoid storage. Biochimica et biophysica acta 1791, 467-473.

Bobbala, D., Chen, X.L., Leblanc, C., Mayhue, M., Stankova, J., Tanaka, T., Chen, Y.G., Ilangumaran, S., Ramanathan, S., 2012. Interleukin-15 plays an essential role in the pathogenesis of autoimmune diabetes in the NOD mouse. Diabetologia 55, 3010-3020.

Bonacchi, A., Petrai, I., Defranco, R.M., Lazzeri, E., Annunziato, F., Efsen, E., Cosmi, L., Romagnani, P., Milani, S., Failli, P., Batignani, G., Liotta, F., Laffi, G., Pinzani, M., Gentilini, P., Marra, F., 2003. The chemokine CCL21 modulates lymphocyte recruitment and fibrosis in chronic hepatitis C. Gastroenterology 125, 1060-1076.

Borthwick, L.A., Wynn, T.A., Fisher, A.J., 2013. Cytokine mediated tissue fibrosis. Biochimica et biophysica acta 1832, 1049-1060.

Bouchard, A., Ratthe, C., Girard, D., 2004. Interleukin-15 delays human neutrophil apoptosis by intracellular events and not via extracellular factors: role of Mcl-1 and decreased activity of caspase-3 and caspase-8. Journal of leukocyte biology 75, 893-900.

Brew, K., Dinakarpandian, D., Nagase, H., 2000. Tissue inhibitors of metalloproteinases: evolution, structure and function. Biochimica et biophysica acta 1477, 267-283.

Broux, B., Mizee, M.R., Vanheusden, M., van der Pol, S., van Horssen, J., Van Wijmeersch, B., Somers, V., de Vries, H.E., Stinissen, P., Hellings, N., 2015. IL-15 amplifies the pathogenic properties of CD4+CD28- T cells in multiple sclerosis. Journal of immunology 194, 2099-2109.

Brun, P., Castagliuolo, I., Pinzani, M., Palu, G., Martines, D., 2005. Exposure to bacterial cell wall products triggers an inflammatory phenotype in hepatic stellate cells. American journal of physiology. Gastrointestinal and liver physiology 289, G571-578.

Budagian, V., Bulanova, E., Orinska, Z., Pohl, T., Borden, E.C., Silverman, R., Bulfone-Paus, S., 2004. Reverse signaling through membrane-bound interleukin-15. The Journal of biological chemistry 279, 42192-42201.

Budagian, V., Bulanova, E., Paus, R., Bulfone-Paus, S., 2006. IL-15/IL-15 receptor biology: a guided tour through an expanding universe. Cytokine & growth factor reviews 17, 259-280.

Bulfone-Paus, S., Bulanova, E., Budagian, V., Paus, R., 2006. The interleukin-15/interleukin-15 receptor system as a model for juxtacrine and reverse signaling. BioEssays : news and reviews in molecular, cellular and developmental biology 28, 362-377.

Campbell, I., 2006. Liver: functional anatomy and blood supply. Anaesthesia & Intensive Care Medicine 7, 49-51.

Canbay, A., Bechmann, L., Gerken, G., 2007. Lipid metabolism in the liver. Zeitschrift fur Gastroenterologie 45, 35-41.

Carson, W.E., Ross, M.E., Baiocchi, R.A., Marien, M.J., Boiani, N., Grabstein, K., Caligiuri, M.A., 1995. Endogenous production of interleukin 15 by activated human monocytes is critical for optimal production of interferon-gamma by natural killer cells in vitro. The Journal of clinical investigation 96, 2578-2582.

Castro, R.E., Diehl, A.M., 2018. Towards a definite mouse model of NAFLD. Journal of hepatology 69, 272-274.

Cepero-Donates, Y., Lacraz, G., Ghobadi, F., Rakotoarivelo, V., Orkhis, S., Mayhue, M., Chen, Y.G., Rola-Pleszczynski, M., Menendez, A., Ilangumaran, S., Ramanathan, S., 2016a. Interleukin-15-mediated inflammation promotes non-alcoholic fatty liver disease. Cytokine 82, 102-111.

Cepero-Donates, Y., Rakotoarivelo, V., Mayhue, M., Ma, A., Chen, Y.G., Ramanathan, S., 2016b. Homeostasis of IL-15 dependent lymphocyte subsets in the liver. Cytokine 82, 95-101.

Charlton, M.R., 1996. Protein metabolism and liver disease. Bailliere's clinical endocrinology and metabolism 10, 617-635.

Chen, C.H., Kuo, L.M., Chang, Y., Wu, W., Goldbach, C., Ross, M.A., Stolz, D.B., Chen, L., Fung, J.J., Lu, L., Qian, S., 2006. *In vivo* immune modulatory activity of hepatic stellate cells in mice. Hepatology 44, 1171-1181.

Chiang, D.J., Pritchard, M.T., Nagy, L.E., 2011. Obesity, diabetes mellitus, and liver fibrosis. American journal of physiology. Gastrointestinal and liver physiology 300, G697-702.

Chiang, J., 2014. Liver Physiology: Metabolism and Detoxification, Pathobiology of Human Disease, pp. 1770-1782.

Choi, I., Kang, H.S., Yang, Y., Pyun, K.H., 1994. IL-6 induces hepatic inflammation and collagen synthesis *in vivo*. Clinical and experimental immunology 95, 530-535.

Clapper, J.R., Hendricks, M.D., Gu, G., Wittmer, C., Dolman, C.S., Herich, J., Athanacio, J., Villescaz, C., Ghosh, S.S., Heilig, J.S., Lowe, C., Roth, J.D., 2013. Diet-induced mouse model of fatty liver disease and nonalcoholic steatohepatitis reflecting clinical disease progression and methods of assessment. American Journal of Physiology-Gastrointestinal and Liver Physiology 305, G483-G495.

Correia, M.P., Cardoso, E.M., Pereira, C.F., Neves, R., Uhrberg, M., Arosa, F.A., 2009. Hepatocytes and IL-15: a favorable microenvironment for T cell survival and CD8+ T cell differentiation. Journal of immunology 182, 6149-6159.

Crispe, I.N., 2009. The liver as a lymphoid organ. Annual review of immunology 27, 147-163.

Cui, G., Hara, T., Simmons, S., Wagatsuma, K., Abe, A., Miyachi, H., Kitano, S., Ishii, M., Tani-ichi, S., Ikuta, K., 2014. Characterization of the IL-15 niche in primary and secondary lymphoid organs in vivo. Proceedings of the National Academy of Sciences of the United States of America 111, 1915-1920.

Czaja, M.J., Weiner, F.R., Takahashi, S., Giambrone, M.A., van der Meide, P.H., Schellekens, H., Biempica, L., Zern, M.A., 1989. Gamma-interferon treatment inhibits collagen deposition in murine schistosomiasis. Hepatology 10, 795-800.

Dal-Secco, D., Wang, J., Zeng, Z., Kolaczkowska, E., Wong, C.H., Petri, B., Ransohoff, R.M., Charo, I.F., Jenne, C.N., Kubes, P., 2015. A dynamic spectrum of monocytes arising from the in situ reprogramming of CCR2+ monocytes at a site of sterile injury. The Journal of experimental medicine 212, 447-456.

de Lalla, C., Galli, G., Aldrighetti, L., Romeo, R., Mariani, M., Monno, A., Nuti, S., Colombo, M., Callea, F., Porcelli, S.A., Panina-Bordignon, P., Abrignani, S., Casorati, G., Dellabona, P., 2004. Production of profibrotic cytokines by invariant NKT cells characterizes cirrhosis progression in chronic viral hepatitis. Journal of immunology 173, 1417-1425.

de Lima, V.M., Oliveira, C.P., Alves, V.A., Chammas, M.C., Oliveira, E.P., Stefano, J.T., de Mello, E.S., Cerri, G.G., Carrilho, F.J., Caldwell, S.H., 2008. A rodent model of NASH with cirrhosis, oval cell proliferation and hepatocellular carcinoma. Journal of hepatology 49, 1055-1061.

Delire, B., Starkel, P., Leclercq, I., 2015. Animal Models for Fibrotic Liver Diseases: What We Have, What We Need, and What Is under Development. Journal of clinical and translational hepatology 3, 53-66.

Denver, P., Gault, V.A., McClean, P.L., 2018. Sustained high-fat diet modulates inflammation, insulin signalling and cognition in mice and a modified xenin peptide ameliorates neuropathology in a chronic high-fat model. Diabetes, obesity & metabolism 20, 1166-1175.

Desmouliere, A., Geinoz, A., Gabbiani, F., Gabbiani, G., 1993. Transforming growth factor-beta 1 induces alpha-smooth muscle actin expression in granulation tissue myofibroblasts and in quiescent and growing cultured fibroblasts. The Journal of cell biology 122, 103-111.

Dixon, L.J., Barnes, M., Tang, H., Pritchard, M.T., Nagy, L.E., 2013. Kupffer cells in the liver. Comprehensive Physiology 3, 785-797.

Doherty, T.M., Seder, R.A., Sher, A., 1996. Induction and regulation of IL-15 expression in murine macrophages. Journal of immunology 156, 735-741.

Dong, S., Chen, Q.L., Song, Y.N., Sun, Y., Wei, B., Li, X.Y., Hu, Y.Y., Liu, P., Su, S.B., 2016. Mechanisms of CCl4-induced liver fibrosis with combined transcriptomic and proteomic analysis. The Journal of toxicological sciences 41, 561-572.

Dooms, H., Desmedt, M., Vancaeneghem, S., Rottiers, P., Goossens, V., Fiers, W., Grooten, J., 1998. Quiescence-inducing and antiapoptotic activities of IL-15 enhance secondary CD4+ T cell responsiveness to antigen. Journal of immunology 161, 2141-2150.

Dowman, J.K., Tomlinson, J.W., Newsome, P.N., 2010. Pathogenesis of non-alcoholic fatty liver disease. QJM : monthly journal of the Association of Physicians 103, 71-83.

Duarte, S., Baber, J., Fujii, T., Coito, A.J., 2015. Matrix metalloproteinases in liver injury, repair and fibrosis. Matrix biology : journal of the International Society for Matrix Biology 44-46, 147-156.

Dubois, S., Mariner, J., Waldmann, T.A., Tagaya, Y., 2002. IL-15Ralpha recycles and presents IL-15 In trans to neighboring cells. Immunity 17, 537-547.

Duval, F., Moreno-Cuevas, J.E., Gonzalez-Garza, M.T., Rodriguez-Montalvo, C., Cruz-Vega, D.E., 2014. Protective mechanisms of medicinal plants targeting hepatic stellate cell activation and extracellular matrix deposition in liver fibrosis. Chinese medicine 9, 27.

Ehling, J., Bartneck, M., Wei, X., Gremse, F., Fech, V., Mockel, D., Baeck, C., Hittatiya, K., Eulberg, D., Luedde, T., Kiessling, F., Trautwein, C., Lammers, T., Tacke, F., 2014. CCL2-dependent infiltrating macrophages promote angiogenesis in progressive liver fibrosis. Gut 63, 1960-1971.

El-Zayadi, A.R., 2008. Hepatic steatosis: a benign disease or a silent killer. World journal of gastroenterology 14, 4120-4126.

Farber, J.M., 1997. Mig and IP-10: CXC chemokines that target lymphocytes. Journal of leukocyte biology 61, 246-257.

Fasbender, F., Widera, A., Hengstler, J.G., Watzl, C., 2016. Natural Killer Cells and Liver Fibrosis. Frontiers in immunology 7, 19.

Feau, S., Facchinetti, V., Granucci, F., Citterio, S., Jarrossay, D., Seresini, S., Protti, M.P., Lanzavecchia, A., Ricciardi-Castagnoli, P., 2005. Dendritic cell-derived IL-2 production is regulated by IL-15 in humans and in mice. Blood 105, 697-702.

Fehniger, T.A., Caligiuri, M.A., 2001. Interleukin 15: biology and relevance to human disease. Blood 97, 14-32.

Fimmel, C.J., Brown, K.E., O'Neill, R., Kladney, R.D., 1996. Complement C4 protein expression by rat hepatic stellate cells. Journal of immunology 157, 2601-2609.

Friedman, S.L., 2004. Mechanisms of disease: Mechanisms of hepatic fibrosis and therapeutic implications. Nature clinical practice. Gastroenterology & hepatology 1, 98-105.

Friedman, S.L., 2008. Hepatic stellate cells: protean, multifunctional, and enigmatic cells of the liver. Physiological reviews 88, 125-172.

Gaggero, A., Azzarone, B., Andrei, C., Mishal, Z., Meazza, R., Zappia, E., Rubartelli, A., Ferrini, S., 1999. Differential intracellular trafficking, secretion and endosomal localization of two IL-15 isoforms. European journal of immunology 29, 1265-1274.

Gao, B., Jeong, W.I., Tian, Z., 2008. Liver: An organ with predominant innate immunity. Hepatology 47, 729-736.

Gao, B., Radaeva, S., 2013. Natural killer and natural killer T cells in liver fibrosis. Biochimica et biophysica acta 1832, 1061-1069.

Gao, B., Xu, M.J., Bertola, A., Wang, H., Zhou, Z., Liangpunsakul, S., 2017. Animal Models of Alcoholic Liver Disease: Pathogenesis and Clinical Relevance. Gene expression 17, 173-186.

Gelse, K., Poschl, E., Aigner, T., 2003. Collagens--structure, function, and biosynthesis. Advanced drug delivery reviews 55, 1531-1546.

Ghosh Dastidar, S., Warner, J.B., Warner, D.R., McClain, C.J., Kirpich, I.A., 2018. Rodent Models of Alcoholic Liver Disease: Role of Binge Ethanol Administration. Biomolecules 8.

Giannandrea, M., Parks, W.C., 2014. Diverse functions of matrix metalloproteinases during fibrosis. Disease models & mechanisms 7, 193-203.

Gillgrass, A., Gill, N., Babian, A., Ashkar, A.A., 2014. The absence or overexpression of IL-15 drastically alters breast cancer metastasis via effects on NK cells, CD4 T cells, and macrophages. Journal of immunology 193, 6184-6191.

Giri, J.G., Kumaki, S., Ahdieh, M., Friend, D.J., Loomis, A., Shanebeck, K., DuBose, R., Cosman, D., Park, L.S., Anderson, D.M., 1995. Identification and cloning of a novel IL-15 binding protein that is structurally related to the alpha chain of the IL-2 receptor. The EMBO journal 14, 3654-3663.

Golden-Mason, L., Kelly, A.M., Doherty, D.G., Traynor, O., McEntee, G., Kelly, J., Hegarty, J.E., O'Farrelly, C., 2004. Hepatic interleuklin 15 (IL-15) expression: implications for local NK/NKT cell homeostasis and development. Clinical and experimental immunology 138, 94-101.

Gomez Perdiguero, E., Klapproth, K., Schulz, C., Busch, K., Azzoni, E., Crozet, L., Garner, H., Trouillet, C., de Bruijn, M.F., Geissmann, F., Rodewald, H.R., 2015. Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. Nature 518, 547-551.

Grabstein, K.H., Eisenman, J., Shanebeck, K., Rauch, C., Srinivasan, S., Fung, V., Beers, C., Richardson, J., Schoenborn, M.A., Ahdieh, M., et al., 1994. Cloning of a T cell growth factor that interacts with the beta chain of the interleukin-2 receptor. Science 264, 965-968.

Greenway, C.V., Stark, R.D., 1971. Hepatic vascular bed. Physiological reviews 51, 23-65.

Gressner, A.M., 2001. The up-and-down of hepatic stellate cells in tissue injury: apoptosis restores cellular homeostasis. Gastroenterology 120, 1285-1288.

Gumucio, J.J., 1983. Functional and anatomic heterogeneity in the liver acinus: impact on transport. The American journal of physiology 244, G578-582.

Guo, J., Friedman, S.L., 2007. Hepatic Fibrogenesis. Seminars in liver disease 27, 413-426.

Guo, X., Cen, Y., Wang, J., Jiang, H., 2018. CXCL10-induced IL-9 promotes liver fibrosis via Raf/MEK/ERK signaling pathway. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie 105, 282-289.

Guo, Y., Liang, X., Meng, M., Chen, H., Wei, X., Li, M., Li, J., Huang, R., Wei, J., 2017. Hepatoprotective effects of Yulangsan flavone against carbon tetrachloride (CCl4)-

induced hepatic fibrosis in rats. Phytomedicine : international journal of phytotherapy and phytopharmacology 33, 28-35.

Hamada, T., Fondevila, C., Busuttil, R.W., Coito, A.J., 2008. Metalloproteinase-9 deficiency protects against hepatic ischemia/reperfusion injury. Hepatology 47, 186-198.

Hammerich, L., Tacke, F., 2014. Interleukins in chronic liver disease: lessons learned from experimental mouse models. Clinical and experimental gastroenterology 7, 297-306.

Hansen, H.H., Feigh, M., Veidal, S.S., Rigbolt, K.T., Vrang, N., Fosgerau, K., 2017. Mouse models of nonalcoholic steatohepatitis in preclinical drug development. Drug discovery today 22, 1707-1718.

Hanumegowda, U.M., Copple, B.L., Shibuya, M., Malle, E., Ganey, P.E., Roth, R.A., 2003. Basement membrane and matrix metalloproteinases in monocrotaline-induced liver injury. Toxicological sciences : an official journal of the Society of Toxicology 76, 237-246.

Haslam, D.W., James, W.P., 2005. Obesity. Lancet 366, 1197-1209.

Hebbard, L., George, J., 2011. Animal models of nonalcoholic fatty liver disease. Nature reviews. Gastroenterology & hepatology 8, 35-44.

Heinz, S., Braspenning, J., 2015. Measurement of Blood Coagulation Factor Synthesis in Cultures of Human Hepatocytes. Methods in molecular biology 1250, 309-316.

Holt, A.P., Haughton, E.L., Lalor, P.F., Filer, A., Buckley, C.D., Adams, D.H., 2009. Liver myofibroblasts regulate infiltration and positioning of lymphocytes in human liver. Gastroenterology 136, 705-714.

Horras, C.J., Lamb, C.L., Mitchell, K.A., 2011. Regulation of hepatocyte fate by interferon-gamma. Cytokine & growth factor reviews 22, 35-43.

Hou, H.S., Liao, C.L., Sytwu, H.K., Liao, N.S., Huang, T.Y., Hsieh, T.Y., Chu, H.C., 2012. Deficiency of interleukin-15 enhances susceptibility to acetaminophen-induced liver injury in mice. PloS one 7, e44880.

Ip, E., Farrell, G., Hall, P., Robertson, G., Leclercq, I., 2004. Administration of the potent PPARalpha agonist, Wy-14,643, reverses nutritional fibrosis and steatohepatitis in mice. Hepatology 39, 1286-1296.

Ip, E., Farrell, G.C., Robertson, G., Hall, P., Kirsch, R., Leclercq, I., 2003. Central role of PPARalpha-dependent hepatic lipid turnover in dietary steatohepatitis in mice. Hepatology 38, 123-132.

Ipsen, D.H., Lykkesfeldt, J., Tveden-Nyborg, P., 2018. Molecular mechanisms of hepatic lipid accumulation in non-alcoholic fatty liver disease. Cellular and molecular life sciences : CMLS 75, 3313-3327.

Itagaki, H., Shimizu, K., Morikawa, S., Ogawa, K., Ezaki, T., 2013. Morphological and functional characterization of non-alcoholic fatty liver disease induced by a methionine-choline-deficient diet in C57BL/6 mice. International journal of clinical and experimental pathology 6, 2683-2696.

Iwakiri, Y., 2015. Nitric oxide in liver fibrosis: The role of inducible nitric oxide synthase. Clinical and molecular hepatology 21, 319-325.

Izem, L., Rassart, E., Kamate, L., Falstrault, L., Rhainds, D., Brissette, L., 1998. Effect of reduced low-density lipoprotein receptor level on HepG2 cell cholesterol metabolism. The Biochemical journal 329 (Pt 1), 81-89.

Jablonska-Trypuc, A., Matejczyk, M., Rosochacki, S., 2016. Matrix metalloproteinases (MMPs), the main extracellular matrix (ECM) enzymes in collagen degradation, as a target for anticancer drugs. Journal of enzyme inhibition and medicinal chemistry 31, 177-183.

Janeway, C.A., Jr., 1992. The immune system evolved to discriminate infectious nonself from noninfectious self. Immunology today 13, 11-16.

Janssen, A.W., Betzel, B., Stoopen, G., Berends, F.J., Janssen, I.M., Peijnenburg, A.A., Kersten, S., 2015. The impact of PPARalpha activation on whole genome gene expression in human precision cut liver slices. BMC genomics 16, 760.

Jenkins, S.J., Ruckerl, D., Cook, P.C., Jones, L.H., Finkelman, F.D., van Rooijen, N., MacDonald, A.S., Allen, J.E., 2011. Local macrophage proliferation, rather than recruitment from the blood, is a signature of TH2 inflammation. Science 332, 1284-1288.

Jeong, W.I., Park, O., Gao, B., 2008. Abrogation of the antifibrotic effects of natural killer cells/interferon-gamma contributes to alcohol acceleration of liver fibrosis. Gastroenterology 134, 248-258.

Jiang, J.X., Chen, X., Fukada, H., Serizawa, N., Devaraj, S., Torok, N.J., 2013. Advanced glycation endproducts induce fibrogenic activity in nonalcoholic steatohepatitis by modulating TNF-alpha-converting enzyme activity in mice. Hepatology 58, 1339-1348.

Jiao, J., Ooka, K., Fey, H., Fiel, M.I., Rahmman, A.H., Kojima, K., Hoshida, Y., Chen, X., de Paula, T., Vetter, D., Sastre, D., Lee, K.H., Lee, Y., Bansal, M., Friedman, S.L., Merad, M., Aloman, C., 2016. Interleukin-15 receptor alpha on hepatic stellate cells regulates hepatic fibrogenesis in mice. Journal of hepatology 65, 344-353.

Johnston, J.A., Bacon, C.M., Finbloom, D.S., Rees, R.C., Kaplan, D., Shibuya, K., Ortaldo, J.R., Gupta, S., Chen, Y.Q., Giri, J.D., et al., 1995. Tyrosine phosphorylation and activation of STAT5, STAT3, and Janus kinases by interleukins 2 and 15. Proceedings of the National Academy of Sciences of the United States of America 92, 8705-8709.

Jonakowski, M., Ziolo, J., Kocwin, M., Przemecka, M., Mokros, L., Panek, M., Szemraj, J., Kuna, P., 2017. Role of IL-15 in the modulation of TGF-beta1-mediated inflammation in asthma. Experimental and therapeutic medicine 14, 4533-4540.

Jou, J., Choi, S.S., Diehl, A.M., 2008. Mechanisms of disease progression in nonalcoholic fatty liver disease. Seminars in liver disease 28, 370-379.

Ju, C., Reilly, T.P., Bourdi, M., Radonovich, M.F., Brady, J.N., George, J.W., Pohl, L.R., 2002. Protective role of Kupffer cells in acetaminophen-induced hepatic injury in mice. Chemical research in toxicology 15, 1504-1513.

Kakino, S., Ohki, T., Nakayama, H., Yuan, X., Otabe, S., Hashinaga, T., Wada, N., Kurita, Y., Tanaka, K., Hara, K., Soejima, E., Tajiri, Y., Yamada, K., 2018. Pivotal Role of TNF-alpha in the Development and Progression of Nonalcoholic Fatty Liver Disease in a Murine Model. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme 50, 80-87.

Kandhi, R., Bobbala, D., Yeganeh, M., Mayhue, M., Menendez, A., Ilangumaran, S., 2016. Negative regulation of the hepatic fibrogenic response by suppressor of cytokine signaling 1. Cytokine 82, 58-69.

Karlmark, K.R., Weiskirchen, R., Zimmermann, H.W., Gassler, N., Ginhoux, F., Weber, C., Merad, M., Luedde, T., Trautwein, C., Tacke, F., 2009. Hepatic recruitment of the inflammatory Gr1+ monocyte subset upon liver injury promotes hepatic fibrosis. Hepatology 50, 261-274.

Karlmark, K.R., Zimmermann, H.W., Roderburg, C., Gassler, N., Wasmuth, H.E., Luedde, T., Trautwein, C., Tacke, F., 2010. The fractalkine receptor CX(3)CR1 protects against liver fibrosis by controlling differentiation and survival of infiltrating hepatic monocytes. Hepatology 52, 1769-1782.

Kelly, A.M., Golden-Mason, L., Traynor, O., Geoghegan, J., McEntee, G., Hegarty, J.E., O'Farrelly, C., 2006. Changes in hepatic immunoregulatory cytokines in patients with metastatic colorectal carcinoma: implications for hepatic anti-tumour immunity. Cytokine 35, 171-179.

Kennedy, M.K., Glaccum, M., Brown, S.N., Butz, E.A., Viney, J.L., Embers, M., Matsuki, N., Charrier, K., Sedger, L., Willis, C.R., Brasel, K., Morrissey, P.J., Stocking, K., Schuh, J.C., Joyce, S., Peschon, J.J., 2000. Reversible defects in natural killer and memory CD8 T cell lineages in interleukin 15-deficient mice. The Journal of experimental medicine 191, 771-780.

Kim, Y.O., Popov, Y., Schuppan, D., 2017. Optimized Mouse Models for Liver Fibrosis. Methods in molecular biology 1559, 279-296.

Kobayashi, S., Seki, S., Kawada, N., Morikawa, H., Nakatani, K., Uyama, N., Ikeda, K., Nakajima, Y., Arakawa, T., Kaneda, K., 2003. Apoptosis of T cells in the hepatic fibrotic tissue of the rat: a possible inducing role of hepatic myofibroblast-like cells. Cell and tissue research 311, 353-364.

Koenen, R.R., von Hundelshausen, P., Nesmelova, I.V., Zernecke, A., Liehn, E.A., Sarabi, A., Kramp, B.K., Piccinini, A.M., Paludan, S.R., Kowalska, M.A., Kungl, A.J., Hackeng, T.M., Mayo, K.H., Weber, C., 2009. Disrupting functional interactions between platelet chemokines inhibits atherosclerosis in hyperlipidemic mice. Nature medicine 15, 97-103.

Koyama, Y., Brenner, D.A., 2017. Liver inflammation and fibrosis. The Journal of clinical investigation 127, 55-64.

Krause, H., Jandrig, B., Wernicke, C., Bulfone-Paus, S., Pohl, T., Diamantstein, T., 1996. Genomic structure and chromosomal localization of the human interleukin 15 gene (IL-15). Cytokine 8, 667-674.

Krizhanovsky, V., Yon, M., Dickins, R.A., Hearn, S., Simon, J., Miething, C., Yee, H., Zender, L., Lowe, S.W., 2008. Senescence of activated stellate cells limits liver fibrosis. Cell 134, 657-667.

Kubes, P., Mehal, W.Z., 2012. Sterile inflammation in the liver. Gastroenterology 143, 1158-1172.

Kurys, G., Tagaya, Y., Bamford, R., Hanover, J.A., Waldmann, T.A., 2000. The long signal peptide isoform and its alternative processing direct the intracellular trafficking of interleukin-15. The Journal of biological chemistry 275, 30653-30659.

Lacraz, G., Rakotoarivelo, V., Labbe, S.M., Vernier, M., Noll, C., Mayhue, M., Stankova, J., Schwertani, A., Grenier, G., Carpentier, A., Richard, D., Ferbeyre, G., Fradette, J., Rola-Pleszczynski, M., Menendez, A., Langlois, M.F., Ilangumaran, S., Ramanathan, S., 2016. Deficiency of Interleukin-15 Confers Resistance to Obesity by Diminishing

Inflammation and Enhancing the Thermogenic Function of Adipose Tissues. PloS one 11, e0162995.

Lai, S.M., Sheng, J., Gupta, P., Renia, L., Duan, K., Zolezzi, F., Karjalainen, K., Newell, E.W., Ruedl, C., 2018. Organ-Specific Fate, Recruitment, and Refilling Dynamics of Tissue-Resident Macrophages during Blood-Stage Malaria. Cell reports 25, 3099-3109 e3093.

Lamas-Paz, A., Hao, F., Nelson, L.J., Vazquez, M.T., Canals, S., Gomez Del Moral, M., Martinez-Naves, E., Nevzorova, Y.A., Cubero, F.J., 2018. Alcoholic liver disease: Utility of animal models. World journal of gastroenterology 24, 5063-5075.

Lau, J.K., Zhang, X., Yu, J., 2017. Animal models of non-alcoholic fatty liver disease: current perspectives and recent advances. The Journal of pathology 241, 36-44.

Lau, J.K.C., Zhang, X., Yu, J., 2018. Animal Models of Non-alcoholic Fatty Liver Diseases and Its Associated Liver Cancer. Advances in experimental medicine and biology 1061, 139-147.

Lavin, Y., Winter, D., Blecher-Gonen, R., David, E., Keren-Shaul, H., Merad, M., Jung, S., Amit, I., 2014. Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. Cell 159, 1312-1326.

Lee, H., Jeong, H., Park, S., Yoo, W., Choi, S., Choi, K., Lee, M.G., Lee, M., Cha, D., Kim, Y.S., Han, J., Kim, W., Park, S.H., Oh, J., 2015. Fusion protein of retinol-binding protein and albumin domain III reduces liver fibrosis. EMBO molecular medicine 7, 819-830.

Lee, U.E., Friedman, S.L., 2011. Mechanisms of hepatic fibrogenesis. Best practice & research. Clinical gastroenterology 25, 195-206.

Lemoinne, S., Cadoret, A., El Mourabit, H., Thabut, D., Housset, C., 2013. Origins and functions of liver myofibroblasts. Biochimica et biophysica acta 1832, 948-954.

Leonard, W.J., Lin, J.X., O'Shea, J.J., 2019. The gammac Family of Cytokines: Basic Biology to Therapeutic Ramifications. Immunity 50, 832-850.

Li, C., Li, R., Zhang, W., 2018. Progress in non-invasive detection of liver fibrosis. Cancer biology & medicine 15, 124-136.

Li, Z., Yang, S., Lin, H., Huang, J., Watkins, P.A., Moser, A.B., Desimone, C., Song, X.Y., Diehl, A.M., 2003. Probiotics and antibodies to TNF inhibit inflammatory activity and improve nonalcoholic fatty liver disease. Hepatology 37, 343-350.

Liedtke, C., Luedde, T., Sauerbruch, T., Scholten, D., Streetz, K., Tacke, F., Tolba, R., Trautwein, C., Trebicka, J., Weiskirchen, R., 2013. Experimental liver fibrosis research:

update on animal models, legal issues and translational aspects. Fibrogenesis & tissue repair 6, 19.

Liss, K.H., Finck, B.N., 2017. PPARs and nonalcoholic fatty liver disease. Biochimie 136, 65-74.

Liu, M., Guo, S., Hibbert, J.M., Jain, V., Singh, N., Wilson, N.O., Stiles, J.K., 2011. CXCL10/IP-10 in infectious diseases pathogenesis and potential therapeutic implications. Cytokine & growth factor reviews 22, 121-130.

Liu, Y., Meyer, C., Xu, C., Weng, H., Hellerbrand, C., Dijke, P.t., Dooley, S., 2013. Animal models of chronic liver diseases. American Journal of Physiology-Gastrointestinal and Liver Physiology 304, G449-G468.

Lodolce, J., Burkett, P., Koka, R., Boone, D., Chien, M., Chan, F., Madonia, M., Chai, S., Ma, A., 2002. Interleukin-15 and the regulation of lymphoid homeostasis. Molecular immunology 39, 537-544.

Loetscher, M., Gerber, B., Loetscher, P., Jones, S.A., Piali, L., Clark-Lewis, I., Baggiolini, M., Moser, B., 1996. Chemokine receptor specific for IP10 and mig: structure, function, and expression in activated T-lymphocytes. The Journal of experimental medicine 184, 963-969.

Luo, X.Y., Takahara, T., Kawai, K., Fujino, M., Sugiyama, T., Tsuneyama, K., Tsukada, K., Nakae, S., Zhong, L., Li, X.K., 2013. IFN-gamma deficiency attenuates hepatic inflammation and fibrosis in a steatohepatitis model induced by a methionine- and choline-deficient high-fat diet. American journal of physiology. Gastrointestinal and liver physiology 305, G891-899.

Machado, M.L.V.M., Diehl, A.M., 2016. Animal Models of Nonalcoholic Fatty Liver Disease, in: Chalasani, N., Szabo, G. (Eds.), Alcoholic and Non-Alcoholic Fatty Liver Disease: Bench to Bedside. Springer International Publishing, Cham, pp. 121-145.

Mafanda, E.K., Kandhi, R., Bobbala, D., Khan, M.G.M., Nandi, M., Menendez, A., Ramanathan, S., Ilangumaran, S., 2018. Essential role of suppressor of cytokine signaling 1 (SOCS1) in hepatocytes and macrophages in the regulation of liver fibrosis. Cytokine.

Maher, J.J., 2001. Interactions between hepatic stellate cells and the immune system. Seminars in liver disease 21, 417-426.

Maher, J.J., Lozier, J.S., Scott, M.K., 1998. Rat hepatic stellate cells produce cytokine-induced neutrophil chemoattractant in culture and *in vivo*. The American journal of physiology 275, G847-853.

Mammen, E.F., 1992. Coagulation abnormalities in liver disease. Hematology/oncology clinics of North America 6, 1247-1257.

Mann, D.A., 2014. Epigenetics in liver disease. Hepatology 60, 1418-1425.

Mann, D.A., Smart, D.E., 2002. Transcriptional regulation of hepatic stellate cell activation. Gut 50, 891-896.

Marra, F., 2002. Chemokines in liver inflammation and fibrosis. Frontiers in bioscience : a journal and virtual library 7, d1899-1914.

Marra, F., Pinzani, M., 2002. Role of hepatic stellate cells in the pathogenesis of portal hypertension. Nefrologia : publicacion oficial de la Sociedad Espanola Nefrologia 22 Suppl 5, 34-40.

Marra, F., Tacke, F., 2014. Roles for chemokines in liver disease. Gastroenterology 147, 577-594 e571.

Marzioni, M., Glaser, S.S., Francis, H., Phinizy, J.L., LeSage, G., Alpini, G., 2002. Functional heterogeneity of cholangiocytes. Seminars in liver disease 22, 227-240.

Matsumoto, M., Hada, N., Sakamaki, Y., Uno, A., Shiga, T., Tanaka, C., Ito, T., Katsume, A., Sudoh, M., 2013. An improved mouse model that rapidly develops fibrosis in non-alcoholic steatohepatitis. International journal of experimental pathology 94, 93-103.

Mazzi, V., 2019. Mig chemokine in primary biliary cirrhosis. La Clinica terapeutica 170, e211-e215.

McInnes, I.B., al-Mughales, J., Field, M., Leung, B.P., Huang, F.P., Dixon, R., Sturrock, R.D., Wilkinson, P.C., Liew, F.Y., 1996. The role of interleukin-15 in T-cell migration and activation in rheumatoid arthritis. Nature medicine 2, 175-182.

McInnes, I.B., Leung, B.P., Sturrock, R.D., Field, M., Liew, F.Y., 1997. Interleukin-15 mediates T cell-dependent regulation of tumor necrosis factor-alpha production in rheumatoid arthritis. Nature medicine 3, 189-195.

McInnes, I.B., Liew, F.Y., 1998. Interleukin 15: a proinflammatory role in rheumatoid arthritis synovitis. Immunology today 19, 75-79.

Meazza, R., Verdiani, S., Biassoni, R., Coppolecchia, M., Gaggero, A., Orengo, A.M., Colombo, M.P., Azzarone, B., Ferrini, S., 1996. Identification of a novel interleukin-15 (IL-15) transcript isoform generated by alternative splicing in human small cell lung cancer cell lines. Oncogene 12, 2187-2192.

Mello, T., Materozzi, M., Galli, A., 2016. PPARs and Mitochondrial Metabolism: From NAFLD to HCC. PPAR research 2016, 18.

Mitra, V., Metcalf, J., 2009. Metabolic functions of the liver. Anaesthesia & Intensive Care Medicine 10, 334-335.

Mustafa, S.B., Olson, M.S., 1998. Expression of nitric-oxide synthase in rat Kupffer cells is regulated by cAMP. The Journal of biological chemistry 273, 5073-5080.

Nagy, L.E., 2003. Recent insights into the role of the innate immune system in the development of alcoholic liver disease. Experimental biology and medicine 228, 882-890.

Naito, M., Hasegawa, G., Takahashi, K., 1997. Development, differentiation, and maturation of Kupffer cells. Microscopy research and technique 39, 350-364.

O'Hara, S.P., Tabibian, J.H., Splinter, P.L., LaRusso, N.F., 2013. The dynamic biliary epithelia: molecules, pathways, and disease. Journal of hepatology 58, 575-582.

Ofosu, A., Ramai, D., Reddy, M., 2018. Non-alcoholic fatty liver disease: controlling an emerging epidemic, challenges, and future directions. Annals of gastroenterology 31, 288-295.

Oh, H.S., Cho, W., Tak, S.B., Kim, S., Hong, S.P., Kim, S.O., 2019. Triticum aestivum ethanolic extract improves non-alcoholic fatty liver disease in mice fed a choline-deficient or high-fat diet. Journal of the science of food and agriculture 99, 2602-2609.

Olsen, A.L., Bloomer, S.A., Chan, E.P., Gaca, M.D., Georges, P.C., Sackey, B., Uemura, M., Janmey, P.A., Wells, R.G., 2011. Hepatic stellate cells require a stiff environment for myofibroblastic differentiation. American journal of physiology. Gastrointestinal and liver physiology 301, G110-118.

Olsen, S.K., Ota, N., Kishishita, S., Kukimoto-Niino, M., Murayama, K., Uchiyama, H., Toyama, M., Terada, T., Shirouzu, M., Kanagawa, O., Yokoyama, S., 2007. Crystal Structure of the interleukin-15.interleukin-15 receptor alpha complex: insights into trans and cis presentation. The Journal of biological chemistry 282, 37191-37204.

Ong, J.P., Younossi, Z.M., 2007. Epidemiology and natural history of NAFLD and NASH. Clinics in liver disease 11, 1-16, vii.

Onu, A., Pohl, T., Krause, H., Bulfone-Paus, S., 1997. Regulation of IL-15 secretion via the leader peptide of two IL-15 isoforms. Journal of immunology 158, 255-262.

Oo, Y.H., Shetty, S., Adams, D.H., 2010. The role of chemokines in the recruitment of lymphocytes to the liver. Digestive diseases 28, 31-44.

Otero, Y.F., Stafford, J.M., McGuinness, O.P., 2014. Pathway-selective insulin resistance and metabolic disease: the importance of nutrient flux. The Journal of biological chemistry 289, 20462-20469.

Page-McCaw, A., Ewald, A.J., Werb, Z., 2007. Matrix metalloproteinases and the regulation of tissue remodelling. Nature reviews. Molecular cell biology 8, 221-233.

Paik, Y.H., Schwabe, R.F., Bataller, R., Russo, M.P., Jobin, C., Brenner, D.A., 2003. Toll-like receptor 4 mediates inflammatory signaling by bacterial lipopolysaccharide in human hepatic stellate cells. Hepatology 37, 1043-1055.

Palladini, G., Di Pasqua, L.G., Berardo, C., Siciliano, V., Richelmi, P., Perlini, S., Ferrigno, A., Vairetti, M., 2019. Animal Models of Steatosis (NAFLD) and Steatohepatitis (NASH) Exhibit Hepatic Lobe-Specific Gelatinases Activity and Oxidative Stress. Canadian journal of gastroenterology & hepatology 2019, 5413461.

Papoulas, M., Theocharis, S., 2009. Primary liver tumors: origin and target therapy. Expert Opinion on Therapeutic Targets 13, 957-965.

Patel, K., Bedossa, P., Castera, L., 2015. Diagnosis of liver fibrosis: present and future. Seminars in liver disease 35, 166-183.

Patel, K., Shackel, N.A., 2014. Current status of fibrosis markers. Current opinion in gastroenterology 30, 253-259.

Patidar, M., Yadav, N., Dalai, S.K., 2016. Interleukin 15: A key cytokine for immunotherapy. Cytokine & growth factor reviews 31, 49-59.

Pellicoro, A., Ramachandran, P., Iredale, J.P., Fallowfield, J.A., 2014. Liver fibrosis and repair: immune regulation of wound healing in a solid organ. Nature reviews. Immunology 14, 181-194.

Perera, P.Y., Lichy, J.H., Waldmann, T.A., Perera, L.P., 2012. The role of interleukin-15 in inflammation and immune responses to infection: implications for its therapeutic use. Microbes and infection 14, 247-261.

Pinzani, M., 2015. Pathophysiology of Liver Fibrosis. Digestive diseases 33, 492-497.

Purohit, V., Gao, B., Song, B.J., 2009. Molecular mechanisms of alcoholic fatty liver. Alcoholism, clinical and experimental research 33, 191-205.

Qian, C., An, H., Yu, Y., Liu, S., Cao, X., 2007. TLR agonists induce regulatory dendritic cells to recruit Th1 cells via preferential IP-10 secretion and inhibit Th1 proliferation. Blood 109, 3308-3315.

Rakhshandehroo, M., Hooiveld, G., Muller, M., Kersten, S., 2009. Comparative analysis of gene regulation by the transcription factor PPARalpha between mouse and human. PloS one 4, e6796.

Rakotoarivelo, V., Variya, B., Ilangumaran, S., Langlois, M.F., Ramanathan, S., 2018. Inflammation in human adipose tissues-Shades of gray, rather than white and brown. Cytokine & growth factor reviews 44, 28-37.

Ramachandran, P., Iredale, J.P., 2012. Macrophages: central regulators of hepatic fibrogenesis and fibrosis resolution. Journal of hepatology 56, 1417-1419.

Ramachandran, P., Pellicoro, A., Vernon, M.A., Boulter, L., Aucott, R.L., Ali, A., Hartland, S.N., Snowdon, V.K., Cappon, A., Gordon-Walker, T.T., Williams, M.J., Dunbar, D.R., Manning, J.R., van Rooijen, N., Fallowfield, J.A., Forbes, S.J., Iredale, J.P., 2012. Differential Ly-6C expression identifies the recruited macrophage phenotype, which orchestrates the regression of murine liver fibrosis. Proceedings of the National Academy of Sciences of the United States of America 109, E3186-3195.

Ramanathan, S., Lacraz, G., Cepar Donates, Y., Mayhue, M., France Langois, M., Rola Pleszczynski, M., Ilangumaran, S., 2014. Interleukin-15 in the Regulation of Obesity and Fatty Liver Disease. Journal of Clinical and Experimental Hepatology 4, S32.

Rappaport, A.M., 1980. Hepatic blood flow: morphologic aspects and physiologic regulation. International review of physiology 21, 1-63.

Ratthe, C., Girard, D., 2004. Interleukin-15 enhances human neutrophil phagocytosis by a Syk-dependent mechanism: importance of the IL-15Ralpha chain. Journal of leukocyte biology 76, 162-168.

Raubenheimer, P.J., Nyirenda, M.J., Walker, B.R., 2006. A choline-deficient diet exacerbates fatty liver but attenuates insulin resistance and glucose intolerance in mice fed a high-fat diet. Diabetes 55, 2015-2020.

Regamey, N., Obregon, C., Ferrari-Lacraz, S., van Leer, C., Chanson, M., Nicod, L.P., Geiser, T., 2007. Airway epithelial IL-15 transforms monocytes into dendritic cells. American journal of respiratory cell and molecular biology 37, 75-84.

Reinecker, H.C., MacDermott, R.P., Mirau, S., Dignass, A., Podolsky, D.K., 1996. Intestinal epithelial cells both express and respond to interleukin 15. Gastroenterology 111, 1706-1713.

Reinke, H., Asher, G., 2016. Circadian Clock Control of Liver Metabolic Functions. Gastroenterology 150, 574-580.

Robinson, M.W., Harmon, C., O'Farrelly, C., 2016. Liver immunology and its role in inflammation and homeostasis. Cellular & molecular immunology 13, 267-276.

Rockey, D.C., Chung, J.J., 1996. Regulation of inducible nitric oxide synthase in hepatic sinusoidal endothelial cells. The American journal of physiology 271, G260-267.

Roderfeld, M., 2018. Matrix metalloproteinase functions in hepatic injury and fibrosis. Matrix biology : journal of the International Society for Matrix Biology 68-69, 452-462.

Romero-Gomez, M., 2006. Insulin resistance and hepatitis C. World journal of gastroenterology 12, 7075-7080.

Roskams, T., Yang, S.Q., Koteish, A., Durnez, A., DeVos, R., Huang, X., Achten, R., Verslype, C., Diehl, A.M., 2003. Oxidative stress and oval cell accumulation in mice and humans with alcoholic and nonalcoholic fatty liver disease. The American journal of pathology 163, 1301-1311.

Sabatti, C., Service, S.K., Hartikainen, A.L., Pouta, A., Ripatti, S., Brodsky, J., Jones, C.G., Zaitlen, N.A., Varilo, T., Kaakinen, M., Sovio, U., Ruokonen, A., Laitinen, J., Jakkula, E., Coin, L., Hoggart, C., Collins, A., Turunen, H., Gabriel, S., Elliot, P., McCarthy, M.I., Daly, M.J., Jarvelin, M.R., Freimer, N.B., Peltonen, L., 2009. Genome-wide association analysis of metabolic traits in a birth cohort from a founder population. Nature genetics 41, 35-46.

Safadi, R., Ohta, M., Alvarez, C.E., Fiel, M.I., Bansal, M., Mehal, W.Z., Friedman, S.L., 2004. Immune stimulation of hepatic fibrogenesis by CD8 cells and attenuation by transgenic interleukin-10 from hepatocytes. Gastroenterology 127, 870-882.

Sakhuja, P., 2014. Pathology of alcoholic liver disease, can it be differentiated from nonalcoholic steatohepatitis? World journal of gastroenterology 20, 16474-16479.

Scholten, D., Trebicka, J., Liedtke, C., Weiskirchen, R., 2015. The carbon tetrachloride model in mice. Laboratory animals 49, 4-11.

Schuppan, D., Ruehl, M., Somasundaram, R., Hahn, E.G., 2001. Matrix as a modulator of hepatic fibrogenesis. Seminars in liver disease 21, 351-372.

Schutze, S., Wiegmann, K., Machleidt, T., Kronke, M., 1995. TNF-induced activation of NF-kappa B. Immunobiology 193, 193-203.

Schwabe, R.F., Bataller, R., Brenner, D.A., 2003. Human hepatic stellate cells express CCR5 and RANTES to induce proliferation and migration. American journal of physiology. Gastrointestinal and liver physiology 285, G949-958.

Seigel, L.J., Harper, M.E., Wong-Staal, F., Gallo, R.C., Nash, W.G., O'Brien, S.J., 1984. Gene for T-cell growth factor: location on human chromosome 4q and feline chromosome B1. Science 223, 175-178.

Seki, E., De Minicis, S., Gwak, G.Y., Kluwe, J., Inokuchi, S., Bursill, C.A., Llovet, J.M., Brenner, D.A., Schwabe, R.F., 2009a. CCR1 and CCR5 promote hepatic fibrosis in mice. The Journal of clinical investigation 119, 1858-1870.

Seki, E., de Minicis, S., Inokuchi, S., Taura, K., Miyai, K., van Rooijen, N., Schwabe, R.F., Brenner, D.A., 2009b. CCR2 promotes hepatic fibrosis in mice. Hepatology 50, 185-197.

Seki, E., Schwabe, R.F., 2015. Hepatic inflammation and fibrosis: functional links and key pathways. Hepatology 61, 1066-1079.

Shang, Y., Khafipour, E., Derakhshani, H., Sarna, L.K., Woo, C.W., Siow, Y.L., O, K., 2017. Short Term High Fat Diet Induces Obesity-Enhancing Changes in Mouse Gut Microbiota That are Partially Reversed by Cessation of the High Fat Diet. Lipids 52, 499-511.

Sharma, S., Khalili, K., Nguyen, G.C., 2014. Non-invasive diagnosis of advanced fibrosis and cirrhosis. World journal of gastroenterology 20, 16820-16830.

Shen, H., Zhang, M., Minuk, G.Y., Gong, Y., 2002. Different effects of rat interferon alpha, beta and gamma on rat hepatic stellate cell proliferation and activation. BMC cell biology 3, 9.

Shi, Z., Wakil, A.E., Rockey, D.C., 1997. Strain-specific differences in mouse hepatic wound healing are mediated by divergent T helper cytokine responses. Proceedings of the National Academy of Sciences of the United States of America 94, 10663-10668.

Skov, S., Bonyhadi, M., Odum, N., Ledbetter, J.A., 2000. IL-2 and IL-15 regulate CD154 expression on activated CD4 T cells. Journal of immunology 164, 3500-3505.

Sozio, M.S., Liangpunsakul, S., Crabb, D., 2010. The role of lipid metabolism in the pathogenesis of alcoholic and nonalcoholic hepatic steatosis. Seminars in liver disease 30, 378-390.

Stal, P., 2015. Liver fibrosis in non-alcoholic fatty liver disease - diagnostic challenge with prognostic significance. World journal of gastroenterology 21, 11077-11087.

Starkel, P., Leclercq, I.A., 2011. Animal models for the study of hepatic fibrosis. Best practice & research. Clinical gastroenterology 25, 319-333.

Steel, J.C., Ramlogan, C.A., Yu, P., Sakai, Y., Forni, G., Waldmann, T.A., Morris, J.C., 2010. Interleukin-15 and its receptor augment dendritic cell vaccination against the neu oncogene through the induction of antibodies partially independent of CD4 help. Cancer research 70, 1072-1081.

Stephenson, K., Kennedy, L., Hargrove, L., Demieville, J., Thomson, J., Alpini, G., Francis, H., 2018. Updates on Dietary Models of Nonalcoholic Fatty Liver Disease: Current Studies and Insights. Gene expression 18, 5-17.

Stienstra, R., Saudale, F., Duval, C., Keshtkar, S., Groener, J.E., van Rooijen, N., Staels, B., Kersten, S., Muller, M., 2010. Kupffer cells promote hepatic steatosis via interleukin-1beta-dependent suppression of peroxisome proliferator-activated receptor alpha activity. Hepatology 51, 511-522.

Stonier, S.W., Schluns, K.S., 2010. Trans-presentation: a novel mechanism regulating IL-15 delivery and responses. Immunology letters 127, 85-92.

Strazzabosco, M., Fabris, L., 2012. Development of the bile ducts: essentials for the clinical hepatologist. Journal of hepatology 56, 1159-1170.

Strengell, M., Matikainen, S., Siren, J., Lehtonen, A., Foster, D., Julkunen, I., Sareneva, T., 2003. IL-21 in synergy with IL-15 or IL-18 enhances IFN-gamma production in human NK and T cells. Journal of immunology 170, 5464-5469.

Sudo, K., Yamada, Y., Moriwaki, H., Saito, K., Seishima, M., 2005. Lack of tumor necrosis factor receptor type 1 inhibits liver fibrosis induced by carbon tetrachloride in mice. Cytokine 29, 236-244.

Sun, X., Zemel, M.B., 2007. Calcium and 1,25-dihydroxyvitamin D3 regulation of adipokine expression. Obesity 15, 340-348.

Suraweera, D.B., Weeratunga, A.N., Hu, R.W., Pandol, S.J., Hu, R., 2015. Alcoholic hepatitis: The pivotal role of Kupffer cells. World journal of gastrointestinal pathophysiology 6, 90-98.

Tagaya, Y., Kurys, G., Thies, T.A., Losi, J.M., Azimi, N., Hanover, J.A., Bamford, R.N., Waldmann, T.A., 1997. Generation of secretable and nonsecretable interleukin 15 isoforms through alternate usage of signal peptides. Proceedings of the National Academy of Sciences of the United States of America 94, 14444-14449.

Takeda, J., Kayano, T., Fukomoto, H., Bell, G.I., 1993. Organization of the human GLUT2 (pancreatic beta-cell and hepatocyte) glucose transporter gene. Diabetes 42, 773-777.

Takeshita, T., Asao, H., Ohtani, K., Ishii, N., Kumaki, S., Tanaka, N., Munakata, H., Nakamura, M., Sugamura, K., 1992. Cloning of the gamma chain of the human IL-2 receptor. Science 257, 379-382.

Takeuchi, O., Akira, S., 2010. Pattern recognition receptors and inflammation. Cell 140, 805-820.

Talbott, G., Le, H., Lor, P., Jin, Z., 2018. Reversal of myofibroblast differentiation by phorbol 12-myristate 13-acetate is PKC-independent. The FASEB Journal 32, 687.7.

Tarrats, N., Moles, A., Morales, A., Garcia-Ruiz, C., Fernandez-Checa, J.C., Mari, M., 2011. Critical role of tumor necrosis factor receptor 1, but not 2, in hepatic stellate cell proliferation, extracellular matrix remodeling, and liver fibrogenesis. Hepatology 54, 319-327.

Thiele, N.D., Wirth, J.W., Steins, D., Koop, A.C., Ittrich, H., Lohse, A.W., Kluwe, J., 2017. TIMP-1 is upregulated, but not essential in hepatic fibrogenesis and carcinogenesis in mice. Scientific reports 7, 714.

Thomson, A.W., Knolle, P.A., 2010. Antigen-presenting cell function in the tolerogenic liver environment. Nature reviews. Immunology 10, 753-766.

Tilg, H., Diehl, A.M., 2000. Cytokines in alcoholic and nonalcoholic steatohepatitis. The New England journal of medicine 343, 1467-1476.

Tokunaga, R., Zhang, W., Naseem, M., Puccini, A., Berger, M.D., Soni, S., McSkane, M., Baba, H., Lenz, H.J., 2018. CXCL9, CXCL10, CXCL11/CXCR3 axis for immune activation - A target for novel cancer therapy. Cancer treatment reviews 63, 40-47.

Tomasek, J.J., Gabbiani, G., Hinz, B., Chaponnier, C., Brown, R.A., 2002. Myofibroblasts and mechano-regulation of connective tissue remodelling. Nature reviews. Molecular cell biology 3, 349-363.

Trefts, E., Gannon, M., Wasserman, D.H., 2017. The liver. Current biology : CB 27, R1147-R1151.

Uhlin, M., Masucci, M.G., Levitsky, V., 2005. Regulation of lck degradation and refractory state in CD8+ cytotoxic T lymphocytes. Proceedings of the National Academy of Sciences of the United States of America 102, 9264-9269.

Unanue, E.R., 2007. Ito cells, stellate cells, and myofibroblasts: new actors in antigen presentation. Immunity 26, 9-10.

Van Herck, M.A., Vonghia, L., Francque, S.M., 2017. Animal Models of Nonalcoholic Fatty Liver Disease-A Starter's Guide. Nutrients 9.

van Leeuwen, E.M., Sprent, J., Surh, C.D., 2009. Generation and maintenance of memory CD4(+) T Cells. Current opinion in immunology 21, 167-172.

Verdelho Machado, M., Diehl, A.M., 2018. The hedgehog pathway in nonalcoholic fatty liver disease. Critical reviews in biochemistry and molecular biology 53, 264-278.

Vernon, G., Baranova, A., Younossi, Z.M., 2011. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. Alimentary pharmacology & therapeutics 34, 274-285.

Vernon, H., Kasi, A., 2019. Anatomy, Abdomen and Pelvis, Liver, StatPearls, Treasure Island (FL).

Vinas, O., Bataller, R., Sancho-Bru, P., Gines, P., Berenguer, C., Enrich, C., Nicolas, J.M., Ercilla, G., Gallart, T., Vives, J., Arroyo, V., Rodes, J., 2003. Human hepatic stellate cells show features of antigen-presenting cells and stimulate lymphocyte proliferation. Hepatology 38, 919-929.

Voet, D., Voet, J.G., Pratt, C.W., 2016. Fundamentals of biochemistry: life at the molecular level. John Wiley & Sons.

Waldmann, T.A., Tagaya, Y., 1999. The multifaceted regulation of interleukin-15 expression and the role of this cytokine in NK cell differentiation and host response to intracellular pathogens. Annual review of immunology 17, 19-49.

Wasmuth, H.E., Lammert, F., Zaldivar, M.M., Weiskirchen, R., Hellerbrand, C., Scholten, D., Berres, M.L., Zimmermann, H., Streetz, K.L., Tacke, F., Hillebrandt, S., Schmitz, P., Keppeler, H., Berg, T., Dahl, E., Gassler, N., Friedman, S.L., Trautwein, C., 2009. Antifibrotic effects of CXCL9 and its receptor CXCR3 in livers of mice and humans. Gastroenterology 137, 309-319, 319 e301-303.

Wasmuth, H.E., Tacke, F., Trautwein, C., 2010. Chemokines in liver inflammation and fibrosis. Seminars in liver disease 30, 215-225.

Weng, H.L., Wang, B.E., Jia, J.D., Wu, W.F., Xian, J.Z., Mertens, P.R., Cai, W.M., Dooley, S., 2005. Effect of interferon-gamma on hepatic fibrosis in chronic hepatitis B virus infection: a randomized controlled study. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association 3, 819-828.

Winau, F., Hegasy, G., Weiskirchen, R., Weber, S., Cassan, C., Sieling, P.A., Modlin, R.L., Liblau, R.S., Gressner, A.M., Kaufmann, S.H., 2007. Ito cells are liver-resident antigen-presenting cells for activating T cell responses. Immunity 26, 117-129.

Wong, T., Dang, K., Ladhani, S., Singal, A.K., Wong, R.J., 2019. Prevalence of Alcoholic Fatty Liver Disease Among Adults in the United States, 2001-2016. Jama 321, 1723-1725.

Wu, H., Ghosh, S., Perrard, X.D., Feng, L., Garcia, G.E., Perrard, J.L., Sweeney, J.F., Peterson, L.E., Chan, L., Smith, C.W., Ballantyne, C.M., 2007. T-cell accumulation and regulated on activation, normal T cell expressed and secreted upregulation in adipose tissue in obesity. Circulation 115, 1029-1038.

Wu, T.C., Chen, L.K., Tsai, S.H., Liaw, Y.H., Hwang, B., 2011. Hepatic steatosis: an experimental model for quantification. Archives of gerontology and geriatrics 52, 164-166.

Wu, T.S., Lee, J.M., Lai, Y.G., Hsu, J.C., Tsai, C.Y., Lee, Y.H., Liao, N.S., 2002. Reduced expression of Bcl-2 in CD8+ T cells deficient in the IL-15 receptor alpha-chain. Journal of immunology 168, 705-712.

Xu, J., Tsukamoto, H., 2016. Animal Models of Alcoholic Liver Disease, in: Chalasani, N., Szabo, G. (Eds.), Alcoholic and Non-Alcoholic Fatty Liver Disease: Bench to Bedside. Springer International Publishing, Cham, pp. 103-119.

Yang, Y.M., Seki, E., 2015. TNFalpha in liver fibrosis. Current pathobiology reports 3, 253-261.

Yanguas, S.C., Cogliati, B., Willebrords, J., Maes, M., Colle, I., van den Bossche, B., de Oliveira, C., Andraus, W., Alves, V.A.F., Leclercq, I., Vinken, M., 2016. Experimental models of liver fibrosis. Archives of toxicology 90, 1025-1048.

Yokota, S., Yoshida, O., Dou, L., Spadaro, A.V., Isse, K., Ross, M.A., Stolz, D.B., Kimura, S., Du, Q., Demetris, A.J., Thomson, A.W., Geller, D.A., 2015. IRF-1 promotes liver transplant ischemia/reperfusion injury via hepatocyte IL-15/IL-15Ralpha production. Journal of immunology 194, 6045-6056.

Yokoyama, S., Watanabe, N., Sato, N., Perera, P.Y., Filkoski, L., Tanaka, T., Miyasaka, M., Waldmann, T.A., Hiroi, T., Perera, L.P., 2009. Antibody-mediated blockade of IL-15 reverses the autoimmune intestinal damage in transgenic mice that overexpress IL-15 in enterocytes. Proceedings of the National Academy of Sciences of the United States of America 106, 15849-15854.

Yoo, S., Wang, W., Wang, Q., Fiel, M.I., Lee, E., Hiotis, S.P., Zhu, J., 2017. A pilot systematic genomic comparison of recurrence risks of hepatitis B virus-associated hepatocellular carcinoma with low- and high-degree liver fibrosis. BMC medicine 15, 214.

Younossi, Z., Tacke, F., Arrese, M., Chander Sharma, B., Mostafa, I., Bugianesi, E., Wai-Sun Wong, V., Yilmaz, Y., George, J., Fan, J., Vos, M.B., 2019. Global Perspectives on Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis. Hepatology 69, 2672-2682.

Younossi, Z.M., 2019. Non-alcoholic fatty liver disease - A global public health perspective. Journal of hepatology 70, 531-544.

Yu, M.C., Chen, C.H., Liang, X., Wang, L., Gandhi, C.R., Fung, J.J., Lu, L., Qian, S., 2004. Inhibition of T-cell responses by hepatic stellate cells via B7-H1-mediated T-cell apoptosis in mice. Hepatology 40, 1312-1321.

Zeremski, M., Petrovic, L.M., Chiriboga, L., Brown, Q.B., Yee, H.T., Kinkhabwala, M., Jacobson, I.M., Dimova, R., Markatou, M., Talal, A.H., 2008. Intrahepatic levels of CXCR3-associated chemokines correlate with liver inflammation and fibrosis in chronic hepatitis C. Hepatology 48, 1440-1450.

Zimmermann, H.W., Seidler, S., Nattermann, J., Gassler, N., Hellerbrand, C., Zernecke, A., Tischendorf, J.J., Luedde, T., Weiskirchen, R., Trautwein, C., Tacke, F., 2010. Functional contribution of elevated circulating and hepatic non-classical CD14CD16 monocytes to inflammation and human liver fibrosis. PloS one 5, e11049.

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