The University of Groningen

Department of Liver Fibrosis

&

Molecular Drug targetting

Investigating the Role of Interleukin 6 In Liver Fibrosis

Winnie kim chindong
S4231651

Supervisor: Dr Poelstra Klaas

Table of Content

Abstract:

Liver fibrosis is a complex pathological process characterized by excessive deposition of extracellular matrix proteins, leading to liver dysfunction and potential progression to cirrhosis. At The University of Groningen, the Department of Liver Fibrosis and molecular drug targeting carried out research on IL-10 and its receptors in liver macrophages. The goal of the research was to find out what part of IL10 binds to what part of the Il10 receptor. Knowing this can help in mitigating liver fibrosis. Part of the experiment carried out in this research was to stimulate the macrophages with a few factors such as LPS, IL10, and parts of IL-10 known as P1 and P2 and investigate the amount of cytokines they produce. For this part of the experiment, the pCR was used with a focus on detecting IL-6 DNA. To delve a little deeper, Interleukin-6 (IL-6) is a multifunctional cytokine and has emerged as a key player in the pathogenesis of liver fibrosis. However, the precise mechanisms by which IL-6 influences liver fibrosis remain incompletely understood. The PCR test result showed various amounts of IL-6 when liver macrophages were stimulated. This thesis aims to comprehensively explore the role of IL-6 in liver fibrosis, focusing on its impact on hepatic stellate cells (HSCs), inflammatory responses, and fibrogenesis. Additionally, the potential therapeutic implications of targeting the IL-6 pathway in the management of liver fibrosis will be investigated.

Introduction

The liver is a crucial organ that performs numerous essential functions within our body. It acts as a remarkable filter, diligently removing toxins and waste products from the bloodstream. In addition, it plays a vital role in digestion by producing bile, which aids in the breakdown of fats. Furthermore, the liver serves as a storage facility for glucose, ensuring a steady supply of energy when needed.

However, the liver is vulnerable to various factors that can cause damage, including excessive alcohol consumption, genetic disorders, and diseases, among others. When such damage occurs, the liver undergoes a reparative process where scar tissue forms in an attempt to heal the injured areas. This scarring of tissue is known as liver fibrosis

Liver fibrosis occurs when there is an excessive buildup of extracellular matrix proteins such as collagen in the liver. These proteins are produced by activated hepatic stellate cells which are normally quiescent in healthy livers. When there is liver damage, these cells become activated and start producing collagen to repair the damaged areas. If the injury persists, the collagen accumulates and forms scar tissue which can potentially impair the liver's functionality if it becomes extensive or widespread. [1]

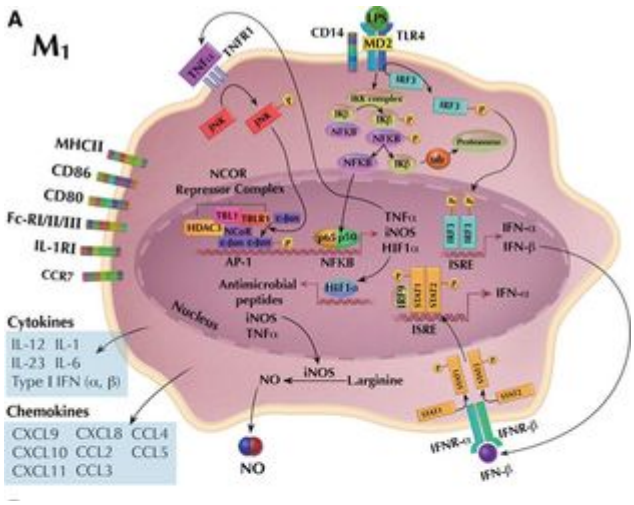
Of particular interest in this study are the roles of interleukin 6 (IL-6) and macrophages in the development and progression of liver fibrosis. It is essential to understand how the production of IL-6, particularly by macrophages, influences the activation of hepatic stellate cells and subsequent collagen synthesis. Investigating the interplay between IL-6, macrophages, and the fibrotic process will provide valuable insights into the mechanisms underlying liver fibrosis and potentially uncover novel therapeutic targets.

Theoretical background

In the liver, Kupffer cells are a specialized group of cells that are needed to activate the stellate cells. These Kupffer cells belong to a group of immune cells known as macrophages. Depending on the stimuli they receive, Kupffer cells can undergo activation and transform into either M1 or M2 macrophages. [2]

M1 activation is mostly through lipopolysaccharide (LPS), a component of bacterial cell walls that triggers an immune response. Other stimulants include pro-inflammatory cytokines such as interleukin 1 (IL-1), tumour necrosis factor-alpha (TNF-alpha), and interferon-gamma (IFN-gamma). Additionally, pathogens, such as bacteria and viruses, as well as tissue damage and immune signals, can also activate macrophages.[3]

Figure 1; macrophage type 1 [4]

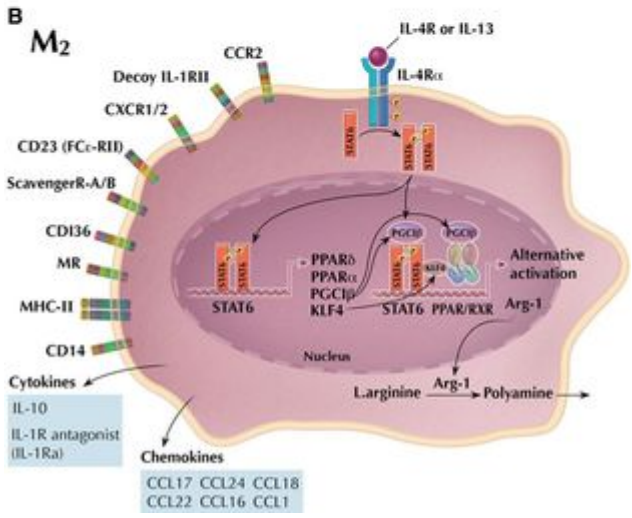


This figure showcases a type one macrophage, which, when stimulated by TNF-alpha/LPS/IFN-gamma, initiates a cascade of reactions, releasing various cytokines such as IL-12, IL-1, IL-23, IL-6, and type 1 interferon-gamma and beta. Additionally, it releases chemokines including CXCL9, CXCL8, CCL4, CXCL10, CCL2, CCL5, CXCL11, and CCL3. These factors are all pro-inflammatory and can contribute to the progression of liver fibrosis when they circulate in the bloodstream and bind to hepatic stellate cells.

When Kupffer cells are transformed into M1 macrophages, they release certain substances called cytokines and chemokines. These molecules attract other immune cells to the injured area in the liver. Consequently, these immune cells produce proinflammatory substances like tumour necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6). The sustained inflammation caused by these substances triggers the activation of hepatic stellate cells (HSCs). HSCs are responsible for producing collagen and other extracellular matrix proteins. As the activated HSCs proliferate and migrate to the site of injury, they produce excessive amounts of collagen, leading to the formation of scar tissue or fibrosis.

On the other hand, when Kupffer cells are transformed into M2 macrophages by stimulating with various factors, including interleukin 4 (IL-4), interleukin 13 (IL-13), interleukin 10 (IL-10), transforming growth factor-beta (TGF-beta), and glucocorticoids. M2 responds to injury or inflammation by producing anti-inflammatory cytokines and growth factors that promote tissue repair and regeneration. These include interleukin-10 (IL-10), TGF-beta and insulin-like growth factor-1 IGF.

Figure 2; Macrophage type 2[5]



This figure showcases a type two macrophage, which, when stimulated by IL-4 or IL-13, initiates a cascade of reactions, releasing various cytokines such as IL-10, IL-1R antagonist, IL-Ra). It also releases chemokines including CCCL17, CCL24, CCL18, CXCL22, CCL16, CCL16, CCL1, and CCL3. These factors are all anti-inflammatory and reduce the progression of liver fibrosis. Looking at Figure 2, The m2 macrophages

has other receptors like CD23, cXR1/2, CCr2, CD14, Mr, MHC-11, etc. These receptors on M2 macrophages reflect are used for antigen presentation, immune cell recruitment, pathogen recognition, and immune modulation. They contribute to the diverse roles that M2 macrophages play in tissue repair, immunoregulation, and resolution of inflammation.

M1 and M2 macrophages can express each other's receptors but this hugely depends on the state of polarization of the initial kupffer cells.

This report was prompted by the findings of a PCR test experiment that involved the stimulation of macrophages with various substances, including LPS (lipopolysaccharide), interleukin 10 (IL-10), and two parts of interleukin 10 called P1 and P2. Different combinations of these substances were tested, and the simulations were conducted at different times. The purpose of the experiment was to understand the effects of these stimulations on the production of interleukin 6 (IL-6).

Figure 3

Graph 1; Relative iL6 production when macrophages are stimulated with lps/il10/p1/p2

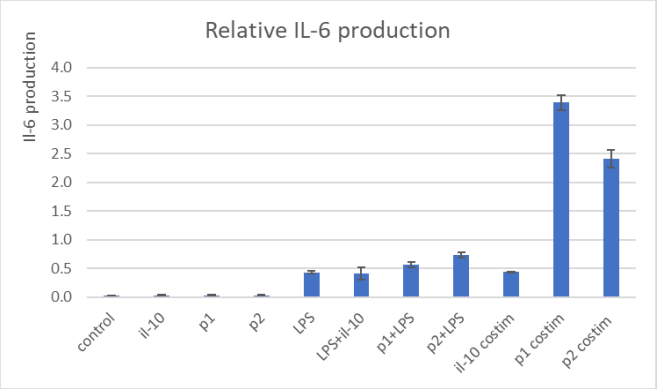


Figure 3 Graph 1 illustrates the production of IL-6 by macrophages in response to various stimulatory factors. The y-axis represents the amount of IL-6 produced, while the x-axis depicts the different factors used to stimulate the macrophages. 11 wells of macrophages in medium were used.
From left to right, the first bar represents the control group, where only macrophages were present in the medium without any additional stimulation. The second bar represents the scenario where IL-10 was added to the macrophages, while the third bar represents the addition of a specific part of IL-10 (p1) to

the macrophages. Similarly, the fourth bar represents the addition of another part of IL-10 (p2) to the macrophages. The fifth bar represents the stimulation of macrophages solely with LPS.

Moving to the next set of bars, the sixth bar depicts the situation where LPS was added to the macrophages and left to incubate for two hours before IL-10 was introduced, and both were allowed to incubate for an additional hour. The same process was repeated for the seventh bar, but p1 was used instead of IL-10, and for the eighth bar, p2 was used.

Finally, the ninth, tenth, and eleventh bars represent the co-stimulation experiments, where LPS and IL-10 or p1 or p2 were added simultaneously to the macrophages. Each of these experimental conditions was incubated for a maximum of 3 hours in a 37-degree Celsius incubator.

The results of this experiment revealed that when macrophages were stimulated at the same time with LPS and P1, the production of IL-6 was significantly higher compared to other combinations. It is important to note that LPS is known to be a causative factor for liver fibrosis. On the other hand, IL-10 is recognized as an inhibitor of pro-inflammatory processes. Both LPS and IL-10, including its subcomponents P1 and P2, play significant roles in liver fibrosis.

Figure 4: LPS, IL10 and IL6 Receptors on macrophages. [6]

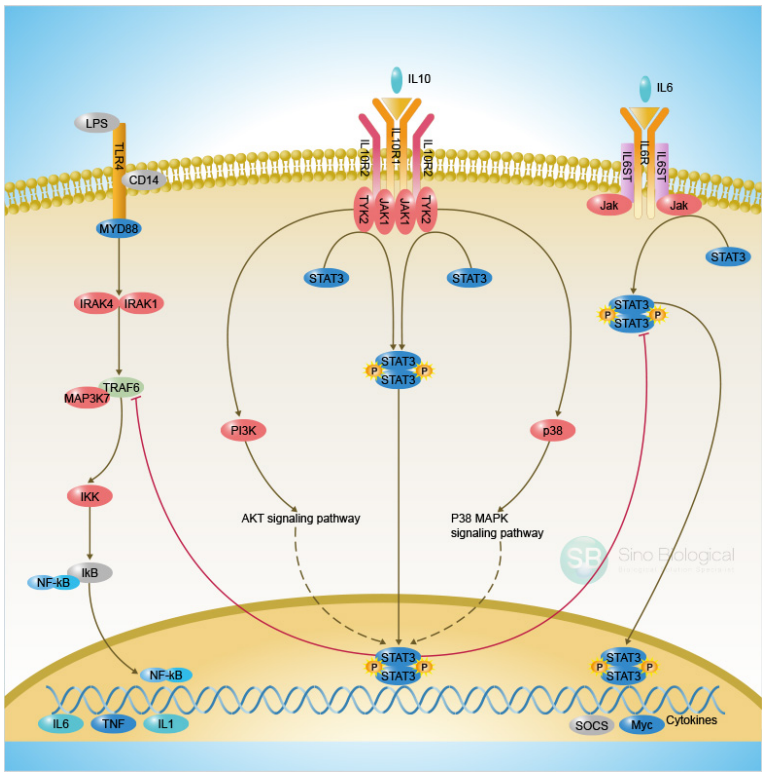


Figure 4 depicts the 3 types of receptors we would like to focus on in this research. The LPs receptor, the IL-10 receptor and the IL-6 receptor. These receptors play a major role in either promoting liver fibrosis or reducing liver fibrosis. Their mechanisms are dealt with in the previous paragraphs below.

IL-10

IL-10 is an anti-inflammatory cytokine produced by various immune cells, including macrophages, T cells, and regulatory T cells. It exerts immunosuppressive effects and plays a critical role in controlling excessive inflammation and maintaining immune homeostasis. IL-10 signalling occurs through the IL-10 receptor, which consists of two subunits: IL-10R1 and IL-10R2. IL-10R1 is responsible for activating the JAK-STAT signalling pathway upon binding to interleukin 10 (IL-10). IL-10R1 interacts with JAK (Janus kinase) family members, particularly JAK1 and TYK2 (Tyrosine kinase 2), leading to their activation. Activated JAKs subsequently phosphorylate the cytoplasmic domain of IL-10R1, creating docking sites for STAT (Signal Transducer and Activator of Transcription) proteins.

On the other hand, IL-10R2, the other subunit of the IL-10 receptor complex, does not directly activate signalling pathways itself. Instead, IL-10R2 functions as a co-receptor, enhancing the binding affinity of IL-10 to IL-10R1 and facilitating downstream signalling through the JAK-STAT pathway.
TYK2, one of the JAK family members, plays a crucial role in mediating IL-10 signalling. It is involved in the activation and phosphorylation of STAT proteins in response to IL-10 binding to IL-10R1. TYK2 kinase activity is necessary to initiate the downstream signalling events regulating gene expression and cellular responses mediated by IL-10.[6]

LPS

LPS, also known as endotoxin, is a component of the outer membrane of Gram-negative bacteria. It is a potent stimulator of the immune system and can trigger strong inflammatory responses. LPS activates macrophages and other immune cells through Toll-like receptor 4 (TLR4), which recognizes and binds to LPS. This binding initiates intracellular signalling pathways, including the MyD88-dependent and TRIF-dependent pathways, leading to the activation of transcription factors such as NF-κB and the production of pro-inflammatory cytokines, including IL-6 and TNF-alpha. LPS-induced inflammation is a

critical component of the host's defence against bacterial infections. However, excessive or uncontrolled LPS-mediated inflammation can contribute to the pathogenesis of sepsis, inflammatory diseases, and tissue damage.[6]

IL-6

IL-6 is mostly known for its pro-inflammatory properties. It is a cytokine produced by various cells, including macrophages, T cells, and fibroblasts. It plays a crucial role in immune responses, inflammation, and tissue homeostasis.This is because Il6 has a pleiotropic effect and is a key mediator of inflammation. However, recent studies show that IL-6 also acts as an anti-inflammatory cytokine at later stages of liver fibrosis in an attempt to dampen the effect.

IL-6 signalling occurs through the IL-6 receptor (IL-6R), which consists of two subunits: the ligand-binding IL-6Rα and the signal-transducing gp130 (IL-6ST). Upon binding of IL-6 to the IL-6R, a signalling cascade is initiated, leading to the activation of various downstream pathways, such as the JAK-STAT pathway. IL-6 has pleiotropic effects and can modulate immune responses, hematopoiesis, acute-phase reactions, and tissue repair[6]. A study by Chen L et al found that IL-6 was involved in the activation of hepatic stellate cells (HSCs), which are responsible for producing collagen and other extracellular matrix proteins in liver fibrosis.

Based on these findings, it is crucial to further investigate the role of IL-6 in liver fibrosis. Understanding the mechanisms underlying IL-6's involvement in this condition could provide valuable insights into the disease process and potentially lead to the development of new therapeutic approaches.
Therefore, the research question arising from this investigation are:

Research questions

- Based on the experiment results, which part of IL-10 (p1 and p2) exhibits a suggestive indication of higher affinity towards IL- 10R1 or IL-10R2 receptors?
- What is the exact role of interleukin 6 in the progression of liver fibrosis?
- What is IL6's potential as a therapeutic?

Discussions

Exploring Affinity Patterns of IL-10 Fragments (p1 and p2) Towards IL-10 Receptors: Insights from Experimental Results;

To address the first question, the analysis will focus on examining the PCR graph and referring to relevant research papers.

Upon analyzing Figure 3 Graph 1, and based on the Figure 3 description above, the following observations were made:
In the first bar where nothing was used to stimulate the macrophages, no IL-6 was produced as expected. When IL-10, p1 and p2 (P1 and p2 are IL-10 with some parts cleaved) were used to stimulate the macrophages, no IL-6 was produced either (Bar 2,3,4). However, when LPS was used to stimulate the macrophages (Bar 5), the amount of IL-6 DNA increased as expected. This is because LPS as seen above stimulates the macrophages to produce pro-inflammatory cytokines which IL-6 is among. Initially, it was expected that IL-6 production would be the highest when only LPS is used to stimulate the macrophages. This expectation was based on Figure 4 which illustrates LPS binding to the TLR4 on macrophages, initiating a cascade of reactions leading to IL-6 production. Figure 3 instead shows a low amount of IL-6 DNA detected (Bar 5) when compared to the other Bars. This low amount could be due to several factors such as the duration of incubation which might have impacted the magnitude of IL-6 DNA production.

For bar 6,7 and 8, LPS was first added to the macrophages and incubated for 2 hours at 36 degrees room temperature before the addition of cytokines. The IL-10, which is bar 6 in Figure 3 demonstrated a reduced synthesis of IL-6 compared to bar 5 with LPS alone. This indicates the IL-10 inhibitory effect. However, when p1 was used (Bar 7), IL-10 synthesis increased compared to Bar 6. Furthermore, p2 (Bar 8) resulted in even greater IL-6 synthesis. This could indicate that p1 potentially binds to IL10R1, which is a receptor involved in initiating the observed cascade of reactions. However, it is important to note that

while the higher amount of IL-6 DNA produced with p2 could suggest its affinity for the IL-10R2 receptor, we cannot conclusively establish this relationship. The increased IL-6 DNA production could also be attributed to other factors influenced by IL-10, and further investigation is required to determine the specific binding affinities of p1 and p2 to their respective IL-10 receptors.

The effects of simultaneously applying LPS and cytokines to the macrophages were further investigated, with the same incubation time of 3 hours. Here as explained in Figure 3, the LPS and IL-10/ p1/p2 were administered to the macrophages at the same time and then incubated for 3 hours. It is highlighted on the graph with the word “costim”. When IL-10 and LPS were simultaneously added (Bar 9), a small amount of IL-6 DNA was detected. In contrast, the combination of p1 and LPS led to a significant increase in IL-6 DNA production (Bar 10). However, in comparison to bar 10, the combination of p2 and LPS (Bar 11) resulted in a smaller amount of IL-6. The affinity of p1 and p2 to the IL-10R1 or IL-10R2 receptor for the costim contradicts the assumptions made when LPS was used to stimulate the macrophages first for two hours before adding the cytokines. However, co-stimulation results could be considered more reliable since the cells were unaffected by prior stimulation, enhancing the reliability and clarity of the results. However concrete conclusion can not be drawn from this experiment and further investigations are needed.

The role of Interleukin 6 in the Progression of liver fibrosis

To investigate the role of IL-6 in the progression of liver fibrosis, it needs to first be understood that the release of IL-6 by macrophages acts as a potent proinflammatory signal and has been implicated in the activation of HSCs. IL-6 can exert its effects on HSCs through multiple mechanisms, contributing to their activation and subsequent fibrogenesis.

Firstly, According to the Journal of clinical investigation [7] IL-6 can directly act on HSCs by binding to its receptor, IL-6R, expressed on HSCs. This IL-6-IL-6R interaction triggers signalling cascades, notably the activation of the Janus kinase (JAK)/signal transducer and activator of the transcription (STAT) pathway as seen above. Once activated, the STAT proteins translocate to the nucleus and initiate the transcription of target genes involved in HSC activation and fibrogenesis. There are several target genes involved in HSC activation and fibrogenesis that are regulated by STAT proteins. One of the most well-studied genes is

alpha-smooth muscle actin (α-SMA), which is a marker of myofibroblast activation. STAT3 has been shown to directly bind to the α-SMA promoter and activate its transcription in HSCs [8]. Another important gene is the tissue inhibitor of metalloproteinases-1 (TIMP-1), which inhibits the activity of matrix metalloproteinases (MMPs) that degrade ECM proteins. STAT3 has also been shown to upregulate TIMP-1 expression in HSCs [9]. Additionally, STAT1 has been shown to induce the expression of transforming growth factor-beta 1 (TGF-β1), which is a potent profibrotic cytokine that stimulates ECM production and inhibits ECM degradation [10].

The mechanism by which these genes cause liver fibrosis involves the dysregulation of ECM turnover. In normal liver tissue, there is a balance between ECM synthesis and degradation. However, in liver fibrosis, there is an excess of ECM synthesis and a decrease in ECM degradation, leading to the accumulation of ECM proteins in the liver. The increased expression of α-SMA and TIMP-1 by activated HSCs contributes to this imbalance by promoting ECM synthesis and inhibiting ECM degradation, respectively. TGF-β1 also plays a key role in this process by stimulating the production of ECM proteins and inhibiting the activity of MMPs [11]. The final product of this process is liver fibrosis, which is characterized by the accumulation of collagen and other ECM proteins in the liver. This can lead to the distortion of liver architecture, impaired liver function, and ultimately cirrhosis and liver failure.

Furthermore, IL-6 can influence HSC activation indirectly through the modulation of immune responses. IL-6 promotes the differentiation of T helper 17 (Th17) cells, which produce pro-inflammatory cytokines such as interleukin 17 (IL-17). Th17-derived IL-17, and according to a study by Zhang et all,[12] IL-17 acts on HSCs through its receptor, IL-17RA, which is expressed on the surface of these cells. IL-17 binding to IL-17RA activates several downstream signalling pathways, including NF-kB and MAPK/ERK, which promote HSC activation and proliferation.

NF-kB is a transcription factor that regulates the expression of genes involved in inflammation, cell survival, and proliferation. Activation of the NF-kB signalling pathway by IL-17 promotes the expression of pro-inflammatory cytokines, such as TNF-alpha and IL-6, which further stimulate HSC activation and proliferation. It is important to note that while TNF alpha and IL-6 are generally associated with pro-inflammatory effects, it has been found to have both pro-fibrotic and anti-fibrotic properties. In the case of liver fibrosis, they can exhibit both pro-fibrotic and anti-fibrotic effects depending on the stage of fibrosis and the cellular environment. During the early stages of liver fibrosis, they promote

inflammation and activate hepatic stellate cells (HSCs), to produce excessive extracellular matrix components. However, as liver fibrosis progresses to advanced stages, it can exert anti-fibrotic effects. This is done by upregulating the expression and activity of matrix metalloproteinases (MMPs), inducing apoptosis in HSC, or modulation of the immune response. Also, In the early stages, IL-6 has a positive feedback loop as it further binds to the HSC IL-6 receptors and initiates the transcription of target genes involved in HSC activation and fibrogenesis.
The MAPK/ERK pathway activates transcription factors such as Elk-1 and c-Fos that bind to the fibronectin promoter and enhance its transcription in HSC.

According to Kimura A et al [13], IL-6 has also been shown to cause an effect on the balance between regulatory T cells (Tregs) and Th17 cells. Tregs are responsible for suppressing immune responses and maintaining self tolerance while TH17 cells promote inflammation and contribute to pathogenesis. Il6 promotes the differentiation and expansion of TH17 cells while inhibiting Treg development and function. The dysregulation of this balance, with a shift towards Th17 dominance, can contribute to sustained HSC activation and fibrosis progression.

As previously discussed, IL-6 directly influences HSCs through the activation of JAK/STAT pathways and the transcriptional regulation of target genes like TGF-β involved in HSC activation and fibrogenesis. In addition to these direct effects, IL-6 can also interact with profibrotic factors, like the TGF-β amplifying the fibrogenic response through several pathways. One of which involves the activation of the Smad signalling pathway. So TGF-β after secreted by HSC binds to its receptor again on the HSC on the cell surface, leading to the phosphorylation and activation of Smad2 and Smad3. These activated Smads then form a complex with Smad4 and translocate to the nucleus, where they regulate gene expression. IL-6 can now enhance this process by promoting the phosphorylation of Smad2 and Smad3 and increasing their nuclear translocation [14]. This crosstalk between IL-6 and TGF-β and the positive feedback loop further potentiates HSC activation and ECM production.

The activation of HSCs by IL-6 and subsequent ECM deposition are critical steps in the development of liver fibrosis. The persistent and dysregulated production of IL-6 during chronic liver injury creates a microenvironment conducive to sustained HSC activation, perpetuating the fibrogenic process. Therefore, targeting IL-6 or its downstream signalling pathways presents a potential therapeutic strategy for mitigating HSC

The Therapeutic Potential of IL-6

In recent years, there has been growing interest in the potential of IL6 as a therapeutic target for liver fibrosis. Despite its pro-fibrotic effects, IL6 has also been shown to have anti-fibrotic effects in the later stages of liver fibrosis just as TNF- alpha. IL6 can induce the expression of matrix metalloproteinases (MMPs) in hepatic stellate cells (HSCs), leading to the degradation of extracellular matrix (ECM) proteins (15). MMPs are a family of enzymes that play a crucial role in ECM remodelling and degradation. The expression and activity of MMPs are tightly regulated by various cytokines and growth factors, including IL6 (16).

IL6 binds to its receptor on the cell surface, which activates a signalling pathway that ultimately leads to the activation of transcription factors such as NF-κB and AP-1. These transcription factors then bind to the promoter regions of MMP genes and activate their transcription.

Once synthesized, MMPs are secreted into the extracellular space where they can cleave various components of the ECM. For example, MMP-1 is able to cleave collagen fibres, while MMP-2 and MMP-9 can degrade basement membrane components such as laminin and type IV collagen. The activity of MMPs is tightly regulated by endogenous inhibitors such as TIMPs (tissue inhibitors of metalloproteinases) and RECK (reversion-inducing cysteine-rich protein with kazal motifs).

IL6 can also induce the expression of adiponectin, which has been shown to have anti-fibrotic effects in liver fibrosis (17). Adiponectin is an adipokine that is predominantly secreted by adipocytes. It has been shown to have pleiotropic effects on various metabolic and inflammatory processes, including insulin sensitivity, lipid metabolism, and inflammation (18). Adiponectin has also been shown to have anti-fibrotic effects in liver fibrosis by inhibiting HSC activation and collagen synthesis (19)

The mechanism of IL6-induced adiponectin expression involves the activation of the JAK/STAT signaling pathway in adipocytes or macrophages. Upon binding to its receptor in adipocytes, IL6 activates the JAK family of kinases, which in turn phosphorylate and activate STAT3. Activated STAT3 then translocates to the nucleus and binds to specific DNA sequences, resulting in the transcriptional activation of target genes, including adiponectin. This is often common in the late stages of liver fibrosis. In macrophages, IL6-induced adiponectin expression is mediated by the JAK/STAT5 pathway. Activation of STAT5 by IL6 leads to the upregulation of adiponectin gene expression.

The synthesis of adiponectin is regulated by several factors, including IL6. Adiponectin is synthesized as a precursor protein called pre-adiponectin, which undergoes post-translational modifications, including cleavage and multimerization, before being secreted into the circulation.

When adiponectin is secreted into the circulation, it binds to its receptors AdipoR1 and AdipoR2, which are expressed on the surface of hepatic stellate cells (HSCs). Adiponectin binding to its receptors leads to the activation of AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor alpha (PPARα) signalling pathways. Activation of AMPK inhibits HSC activation by suppressing transforming growth factor beta 1 (TGF-β1) signalling and reducing the expression of alpha-smooth muscle actin (α-SMA), a marker of activated HSCs. Activation of the PPARα signalling pathway inhibits collagen synthesis by reducing the expression of collagen type I alpha 1 (COL1A1) and tissue inhibitor of metalloproteinase 1 (TIMP1), which are involved in extracellular matrix deposition. Thus reducing liver fibrosis.

Moreover, adiponectin has been shown to inhibit the nuclear factor kappa B (NF-κB) signalling pathway, which is involved in inflammation and fibrosis. Inhibition of the NF-κB signalling pathway reduces the expression of pro-inflammatory cytokines such as tumour necrosis factor-alpha (TNF-α) and interleukin 6 (IL-6), which are known to promote HSC activation and collagen synthesis. This decrease in pro-inflammatory cytokines especially in the early stages of liver fibrosis contributes to the reduction of progression of liver fibrosis. The anti-fibrotic effects of adiponectin in liver fibrosis have been demonstrated in various animal models and clinical studies. Low levels of adiponectin have been associated with the severity of liver fibrosis in patients with non-alcoholic fatty liver disease (NAFLD) and chronic hepatitis C virus (HCV) infection.

As a therapeutic, directing IL-6 to adipose tissue specifically could be a solution since it induces the expression of adiponectin, which in turn can exert protective effects on the liver by suppressing the activation of hepatic stellate cells (HSCs), reducing the expression of pro-inflammatory cytokines as seen above. This in turn reduces collagen deposition and mitigates liver fibrosis. This solution could be archived via the use of nanoparticles coated with antibodies that bind specifically to receptors on adipose tissue cells. These nanoparticles can then be loaded with IL-6 and delivered directly to the adipose tissue. Since IL-6 is considered a key cytokine due to its diverse functionalities in immune

response, another method could be, In the early stages of liver fibrosis, the IL-6 receptor can be blocked. This could be done via the use of monoclonal antibodies.

Some recent discoveries of IL-6 as a therapeutics

IL-6 has also been shown to have anti-fibrotic effects in some contexts. For example, one study found that IL-6 knockout mice had increased liver fibrosis compared to wild-type mice after bile duct ligation-induced liver injury (23). The study suggested that this might be due to impaired clearance of apoptotic cells in the absence of IL-6. In contrast, another study found that blocking IL-6 signalling using an IL-6R antibody reduced liver fibrosis in a mouse model of NASH [24]. The study suggested that this might be due to the inhibition of HSC activation and collagen production. Another study found that treatment with an IL-6 inhibitor reduced liver fibrosis in a rat model of liver injury induced by carbon tetrachloride (CCl4) [25]. The study suggested that this might be due to the inhibition of HSC activation and the promotion of HSC apoptosis.

It is clear that IL-6 plays a complex role in the pathogenesis of liver fibrosis. While it has pro-fibrotic effects, it also has anti-fibrotic effects in some contexts. Its potential as a therapeutic target for liver fibrosis is still being investigated. However, using it to target adipose tissue is much more promising than most of the studies analyzed in this section.

Conclusion

The research conducted to investigate the role of IL-6 in liver fibrosis and its potential as a therapeutic target has revealed the intricate nature of IL-6's involvement in this pathological condition. The research first explored IL-10's interaction with its receptors and indicated a need for further investigation to determine the specific binding affinities of IL-10's parts (p1 and p2) to either IL-10R1 or IL-10R2. Also, the role of Il6 in liver fibrosis suggests that IL-6 plays a multifaceted role, promoting inflammation and activating hepatic stellate cells (HSCs), especially in the early stages, ultimately leading to fibrosis. However, as liver fibrosis progresses and reaches the late stages, the role of IL-6 shifts towards an

anti-inflammatory function. This change in IL-6's activity is thought to be a compensatory mechanism aimed at dampening ongoing inflammation and fibrosis.

Finally, the therapeutic potential of IL-6 in liver fibrosis shows promise, particularly when targeting IL-6 in adipose tissue. However, additional research, including ongoing clinical trials, is necessary to fully evaluate the effectiveness and safety of IL-6-targeted therapies in human subjects. Overall, these findings emphasize the dynamic nature of IL-6's role and highlight the need for further research to unravel its complexities and explore its therapeutic implications for different stages of liver fibrosis.

References

1) Bataller, R. and Brenner, D.A. (2005). Liver fibrosis. *Journal of Clinical Investigation*, [online] 115(2), pp.209–218. doi:https://doi.org/10.1172/jci24282.
2) Dixon, L.J., Barnes, M., Tang, H., Pritchard, M.T. and Nagy, L.E. (2013). Kupffer Cells in the Liver. *Comprehensive Physiology*, 3(2). doi:https://doi.org/10.1002/cphy.c120026.
3) Martinez, F.O. and Gordon, S. (2014). The M1 and M2 paradigm of macrophage activation: time for reassessment. *F1000Prime Reports*, 6. doi:https://doi.org/10.12703/p6-13.
4) Tugal, D., Liao, X. and Jain, M.K. (2013). Transcriptional Control of Macrophage Polarization. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 33(6), pp.1135–1144. doi:https://doi.org/10.1161/atvbaha.113.301453.
5) NA (n.d.). [online] Sinobiological. Available at: https://www.sinobiological.com/pathways/il10-signaling-pathway [Accessed 6 Jun. 2023].
6) Frontiers (2014). *Frontiers | Peer Reviewed Articles - Open Access Journals*. [online] Frontiers. Available at: https://www.frontiersin.org/.
7) Interleukin-6 Directly Modulates Hepatic Transepithelial Sodium Transport and Liver Fibrosis in Vivo published in the Journal of Clinical Investigation in 2015
8) Yang L, et al. Signal transducer and activator of transcription 3 mediates hepatic stellate cell activation and liver fibrosis in vivo. Hepatology. 2009;51(4):1162-71.
9) Yu F, et al. STAT3 activation in hepatic stellate cells via interleukin 6-dependent Jak kinase-independent pathway. Oncogene. 2015;34(47):6013-23.
10) Dooley S, et al. TGF-beta signal transduction in hepatic stellate cells. J Hepatol. 1999;31 Suppl 1:68-75.

11) Bataller R, Brenner DA. Liver fibrosis. J Clin Invest. 2005;115(2):209-18.
12) Zhang, Y., Li, J., Lou, J., Zhou, Y., & Bo, L. (2020). The role of interleukin-17 in liver disease. Advances in clinical and experimental medicine: official organ Wroclaw Medical University, 29(5), 621-626.
13) Kishimoto T. IL-6: regulator of Treg/Th17 balance. Eur J Immunol. 2010 Jul;40(7):1830-5. doi: 10.1002/eji.201040391. PMID: 20583029…..
14) Wynn, T. A. (2008). Cellular and molecular mechanisms of fibrosis. The Journal of pathology, 214(2), 199-210.
15) Zhang CY, Yuan WG, He P, Lei JH, Wang CX. Liver fibrosis and hepatic stellate cells: Etiology, pathological hallmarks and therapeutic targets. World J Gastroenterol. 2016;22(48):10512-10522.
16) Iredale JP. Matrix metalloproteinases in liver disease. Prog Mol Biol Transl Sci. 2017;148:85-106.
17) Kaser S, Moschen A, Cayon A, et al. Adiponectin and its receptors in non-alcoholic steatohepatitis. Gut. 2005;54(1):117-121.
18) Yamauchi T, Kadowaki T. Adiponectin receptor as a key player in healthy longevity and obesity-related diseases. Cell Metab. 2013;17(2):185-196.
19) Wang Y, Lam KS, Xu JY, et al. Adiponectin inhibits cell proliferation by interacting with several growth factors in an oligomerization-dependent manner. J Biol Chem. 2005;280(18):18341-18347.
20) Ding L, et al. Adiponectin protects against liver fibrosis by attenuating hepatic stellate cell activation. J Gastroenterol Hepatol. 2013;28 Suppl 1:165-72.
21) Xu A, et al. The role of adiponectin in renal fibrosis. Front Physiol. 2019;10:451.
22) Wang Y, et al. Adiponectin inhibits hepatic stellate cell activation by targeting the PTEN/Akt pathway. Biochem Biophys Res Commun. 2010;398(4):623-8.
23) Miura K, Kodama Y, Inokuchi S, et al. Toll-like receptor 9 promotes steatohepatitis by induction of interleukin-6 in mice. Gastroenterology. 2010;139(1):323-334.e7.
24) Tanaka N, Matsubara T, Krausz KW, et al. Role of interleukin-6 in hepatic heat shock protein expression and protection against acetaminophen-induced liver disease. Biochem Biophys Res Commun. 2015;464(4):982-987.
25) Zhang Y, Li J, Lou Y, et al. Interleukin-6 inhibition reduces liver fibrosis and mortality in bile duct-ligated mice. Mol Med Rep. 2015;12(1):75-82.