IL-6 pathway in the liver: From physiopathology to therapy

Dirk Schmidt-Arras, Stefan Rose-John*

Summary

Interleukin 6 (IL-6) is a pleiotropic four-helix-bundle cytokine that exerts multiple functions in the body. In the liver, IL-6 is an important inducer of the acute phase response and infection defense. IL-6 is furthermore crucial for hepatocyte homeostasis and is a potent hepatocyte mitogen. It is not only implicated in liver regeneration, but also in metabolic function of the liver. However, persistent activation of the IL-6 signaling pathway is detrimental to the liver and might ultimately result in the development of liver tumors. On target cells IL-6 can bind to the signal transducing subunit gp130 either in complex with the membrane-bound or with the soluble IL-6 receptor to induce intracellular signaling. In this review we describe how these different pathways are involved in the physiology and pathophysiology of the liver. We furthermore discuss how IL-6 pathways can be selectively inhibited and therapeutically exploited for the treatment of liver pathologies.

Introduction

Interleukin-6 and gp130 signal transduction

Interleukin-6 (IL-6) is a four-helical cytokine of 184 amino acids [1]. The protein is synthesized by fibroblasts, monocytes, macrophages, T cells and endothelial cells. IL-6 synthesis and secretion is induced during inflammatory conditions such as upon stimulation of Toll-like receptor (TLR)-4 by lipopolysaccharide or upon stimulation of cells by IL-1 or tumor necrosis factor (TNF)-α [1]. When the IL-6 cDNA was molecularly cloned as B cell stimulating factor 2, it turned out that IL-6 was identical to the 26 kDa protein and to hybridoma growth factor [2].

On target cells, IL-6 binds to the 80 kDa Interleukin-6 receptor (IL-6R), which is not signaling competent. Signaling is initiated upon association of the IL-6/IL-6R complex with a second receptor protein, glycoprotein (gp) 130. Gp130 dimerization leads to the activation of the tyrosine kinase JAK1, which is constitutively bound to the cytoplasmic portion of gp130 [3]. After autophosphorylation, JAK1 phosphorlates five tyrosine residues within the cytoplasmic portion of gp130. This leads to the activation of several intracellular signaling pathways including the MAP kinase and PI3 kinase pathway and the signal transducer and activator of transcription (STAT) 1 and STAT3 pathway. Subsequently, STAT3 is able to upregulate suppressor of cytokine signaling (SOCS) 3, which leads to a downregulation of gp130 signals and thereby represents a negative feed-back loop [3,4].

IL-6 trans-signaling

Importantly, it was shown that IL-6 has only a measurable affinity for the IL-6R but not for gp130. Likewise, the IL-6R has no measurable affinity for gp130 [3]. Only when the IL-6/IL-6R complex is formed, binding to gp130 can be demonstrated [3]. This has important consequences. Whereas gp130 is expressed on all cells of the body, the IL-6R is only expressed on few cell types such as hepatocytes, some leukocytes and some epithelial (e.g. biliary epithelial cells) and non-epithelial cells (e.g. hepatic stellate cells) (Fig. 1A). Therefore, only IL-6R expressing cells can directly respond to the cytokine IL-6 [3].

Interestingly, the IL-6R was shown to be cleaved at the cell surface by the metalloprotease a disintegrin and metalloprotease (ADAM) 17, a process called shedding [5]. The shed soluble IL-6R (sIL-6R) could still bind its ligand IL-6. The complex of IL-6 and sIL-6R was able to associate with gp130 and initiate intracellular signaling [5].

Keywords: Interleukin-6; Interleukin-6-receptor; Trans-signaling; Inflammation; Cancer; Liver regeneration.

Received 16 December 2015; received in revised form 15 January 2016; accepted 3 February 2016

Abbreviations: EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; Fc, constant portion of an IgG antibody; gp130, glycoprotein 130 kDa; IL, interleukin; JAK1, Janus kinase 1; JNK1, Jun amino-terminal kinase 1; LPS, lipopolysaccharide; R, receptor; s, soluble; SOCS3, suppressor of cytokine signaling 3; STAT, signal transducer and activator of transcription; TLR, Toll-like receptor; TNF-α, tumor necrosis factor α.

Institute of Biochemistry, Christian-Albrechts-University Kiel, Olshausenstrasse 40, Kiel, Germany

Key point

On target cells, IL-6 can signal via the IL-6 classic or IL-6 trans-signaling pathway.

* Corresponding author. Address: Institute of Biochemistry, Christian-Albrechts-University Kiel, Olshausenstrasse 40, D-24098 Kiel, Germany. Tel.: +49 431 880 3336; fax: +49 431 880 5007. E-mail address: rosejohn@biochem.uni-kiel.de (S. Rose-John).
IL-6 and the acute phase response

More than 25 years ago, it was found that Interleukin-6 was also identical with hepatocyte stimulating factor 2. Under inflammatory conditions, this factor was known to induce the liver to synthesize a group of proteins called acute phase proteins [19].

In humans the acute phase proteins, which are most induced, include C-reactive protein (CRP), serum amyloid A (SAA), haptoglobin and fibrinogen. Functionally, some acute phase proteins are components of the complement system and of the coagulation cascade. Other acute phase proteins are protease inhibitors, transport proteins or participants in inflammatory responses, such as secreted phospholipase A2 [20,21]. As already mentioned, the major inducer of the hepatic acute phase proteins is the cytokine IL-6, which is secreted by neutrophils, monocytes and macrophages upon TLR stimulation by e.g. lipopolysaccharide [20,21]. Activated myeloid cells in addition release the inflammatory cytokines IL-1 and TNF-α, which can lead to massive production and secretion of IL-6 from other cells such as endothelial cells and fibroblasts thereby functioning as a positive feed-forward loop [22]. Interestingly, in IL-6 knockout mice, the acute phase response is largely inhibited upon injection of turpentine, whereas it is almost normal upon injection of lipopolysaccharide [23]. This might be due to compensation by other IL-6 type cytokines such as Interleukin-11, leukemia inhibitory factor, oncostatin M, ciliary neurotrophic factor, and cardiotrophin 1, which have been demonstrated to induce the synthesis of acute phase proteins in hepatocytes or hepatoma cells. It is therefore believed that members of this cytokine family are actors of liver physiopathology [24,25].

Although not all functions of the acute phase proteins are known, their induced expression is thought to be beneficial for the response of the body to infectious insults and inflammation [21]. Clinically, the extent of the acute phase protein levels such as CRP is used to measure the extent of the inflammatory condition. 80–85% of patients who show CRP levels of more than 100 mg per liter are diagnosed with bacterial infections [26].

The duration of the acute phase response is normally 24–48 h, after which the organism returns to normal liver function. Under severe conditions, such as advanced cancer and the acquired immunodeficiency syndrome, the acute phase can convert to a chronic state of inflammation although the molecular mechanisms of such a chronicity are not completely understood [20,21].

IL-6 and liver regeneration

The most important reaction of the liver to injury is liver regeneration [27,28] and it became clear from experiments with parabiotic animals that soluble extrahepatic factors provide the stimulus for hepatocyte proliferation [29]. Only 2 h after hepatectomy, the level of TNF-α increased followed by a dramatic upregulation of IL-6 levels in the liver vein (Fig. 2A) [30]. After hepatectomy signaling [6]. Strikingly, this could also be demonstrated to occur on cells, which did not express the membrane-bound IL-6R [6]. This process has been named IL-6 trans-signaling (Fig. 1B). Therefore, IL-6 trans-signaling has the potential to largely increase the spectrum of target cells of IL-6 [7].

Hyper-IL-6 is an artificial fusion protein of IL-6 coupled to the sIL-6R via a flexible peptide linker [8] (Fig. 1C). It has been shown to be 100–1000 times more potent than the separate proteins IL-6 and sIL-6R. Hyper-IL-6 has been used to analyze the responsiveness of cells to IL-6 or to the trans-signaling complex IL-6/sIL-6R. It turned out that many cell types including neurons [9], glial cells [10], endothelial cells [11], smooth muscle cells [12], hematopoietic stem cells [13] and embryonic stem cells [14] do not express IL-6R and are therefore dependent on trans-signaling in their response to IL-6.

A fusion protein of the entire extracellular portion of gp130 coupled to the Fc portion of a human IgG1 antibody (sgp130Fc) turned out to be a selective inhibitor of IL-6 trans-signaling [15] (Fig. 1D). IL-6 signaling via the membrane-bound IL-6R was not affected by this protein. This surprising effect could be explained by the fact that neither IL-6 nor IL-6R alone showed a measurable affinity for gp130 [15].

Under normal conditions, IL-6 levels in the blood are extremely low (1–5 pg/ml). Surprisingly, sIL-6R in the blood has been found at concentrations of 40–70 ng/ml. And a soluble form of gp130 (sgp130), which is generated by alternative splicing, is found at concentrations of around 400 ng/ml [16]. We have argued that the sIL-6R and sgp130 constitute a buffer in the blood since IL-6, once secreted will bind to the sIL-6R with an affinity of 1 nM. Thereupon, the complex of IL-6/sIL-6R binds to sgp130 with a hundred times higher affinity (10 pM) and is neutralized. Only when IL-6 levels exceed the sIL-6R concentration, IL-6 can bind to membrane-bound IL-6R on target cells [17,18]. This concept might, however, not be fully valid in case of paracrine activity of IL-6 e.g. in the liver where activated Kupffer cells secrete IL-6 and neighboring hepatocytes respond to a strong local increase in IL-6. 

Key point

While IL-6 classic signaling is crucial for the induction of the acute phase response, IL-6 trans-signaling mediates strong mitogenic signals during liver regeneration.
or liver damage, gut-derived factors like lipopolysaccharide (LPS) activate liver-resident Kupffer cells resulting in a TNF-α-dependent secretion of IL-6 [Fig. 2B] [31].

Consistently, IL-6 knockout mice show impaired liver regeneration [32]. Also under cholestatic conditions, the number of regenerative liver progenitor cells is reduced if IL-6 signaling is blunted [33]. These experimental data pointed to an important role of IL-6 in liver regeneration.

Mice transgenic for the human sIL-6R showed that this protein acts as a serum binding protein for IL-6 and prolongs the half-life of IL-6 [34] and double transgenic mice expressing human IL-6 and human sIL-6R exhibited permanent hepatocyte proliferation [35,36]. We concluded from these results that IL-6 in the presence of sIL-6R was a potent stimulus of liver regeneration [36]. Therefore, the potential of Hyper-IL-6 (Fig. 1C) to accelerate liver regeneration was tested.

When mice were treated with recombinant IL-6 or Hyper-IL-6 after 50% hepatectomy, it turned out that only Hyper-IL-6 significantly accelerated liver weight gain and led to a 36 h earlier peak of mitosis in hepatocytes (Fig. 2C) [37]. Likewise, Hyper-IL-6, but not IL-6, was shown to reverse D-galactosamine mediated liver toxicity and to significantly improve the survival rate of the animals [38]. Interestingly, it was shown that Hyper-IL-6 when genetically delivered via a recombinant adenovirus led to the survival of more than 90% of the mice whereas only 13% of the mock-treated animals survived the D-galactosamine treatment. Treatment with an adenovirus coding for IL-6 resulted only in survival of 21% of the animals [39]. These experiments demonstrated that stimulation of IL-6 trans-signaling via the sIL-6R dramatically accelerated and improved liver regeneration suggesting a physiologic role of the sIL-6R in this process [4,40].

Since hepatocytes express much more gp130 than IL-6R the presence of IL-6 and sIL-6R results in more gp130 activation and therefore to a higher amplitude of the IL-6 signal (Fig. 2D). Furthermore, it was shown that the complex of IL-6 and sIL-6R was internalized much less efficiently than IL-6 leading to longer duration of the IL-6 signal when mediated by trans-signaling [41]. This explains why, in the absence of any liver insult, hepatocytes permanently proliferated in IL-6/sIL-6R double transgenic mice, while hepatocytes did not show any mitogenic response in IL-6 single transgenic mice. The signal induced via the membrane-bound IL-6R on hepatocytes was not sufficient to induce a proliferative response [35,36].

Since all IL-6 type cytokines use the gp130 receptor for signaling it is interesting to ask whether there is potential competition of members of this cytokine family for gp130 (see above). As depicted in Fig. 2D, hepatocytes have been shown to express far more gp130 than IL-6R making it unlikely that gp130 will be the limiting factor in the response to other members of the IL-6 type cytokine family [42].

These experiments demonstrated the potential of exogenous stimulation of IL-6 trans-signaling in the induction of liver regeneration but they did not directly show that IL-6 trans-signaling actually occurred in vivo. Therefore, experiments were conducted with the recombinant sgp130Fc protein, which specifically blocks
IL-6 trans-signaling without affecting signaling via the membrane-bound IL-6R (Fig. 1D). Alternatively, transgenic mice were generated, which overexpress the sgp130Fc protein [43]. In these mice, trans-signaling is abrogated whereas IL-6 signaling via the membrane-bound IL-6R is intact. In these sgp130Fc transgenic mice, the response to D-galactosamine induced liver damage was shown to be compromised. Interestingly, the liver damage-induced glycogen consumption in the liver of the transgenic mice was strongly reduced indicating that glycogen consumption depended on IL-6 trans-signaling [44]. Upon acute CCl₄ damage, blockade of IL-6 trans-signaling led to higher liver damage and to reduced refilling of hepatocyte glycogen stores [45]. In the concanavalin A hepatitis model it was shown that IL-6 classic signaling via the membrane-bound IL-6R rather than IL-6 trans-signaling was responsible for the observed neutrophil accumulation and the induced liver damage [46]. Collectively, these experiments demonstrate that IL-6 trans-signaling in the liver plays an important role in the regenerative response of this organ to injury [47]. Interestingly, IL-6 triggered the activation of YAP and Notch independently of the downstream effector STAT3 in primary hepatocytes and in mouse liver upon partial hepatectomy, suggesting an inflammation induced participation of the YAP and Notch pathways during liver regeneration [48].

**IL-6 in obesity and insulin resistance**

The role of IL-6 signaling on hepatic metabolism, obesity and insulin resistance is discussed controversially. The observation that serum levels of IL-6 correlate with the degree of obesity [49] and the development of type 2 diabetes [50] suggested that IL-6 is causally linked to metabolic disease development. Proteomics analysis of TNF-α-stimulated adipocytes identified the adipokine progranulin (PGRN) as an inducer of IL-6 expression in adipose tissue during obesity [51]. Furthermore, activation of JNK1 in adipose tissue of mice fed a high-fat diet (HFD) resulted in insulin resistance in the liver in an IL-6 dependent manner [52]. Consistently, acute infusions of IL-6 in mice impaired insulin action on the liver and skeletal muscle in hyperinsulinemic-euglycemic clamp analysis [53]. In the liver and skeletal muscle, IL-6 signaling leads to a STAT3-dependent upregulation of SOCS3 (Fig. 3A) which, in turn, impairs insulin-mediated functions.
by muscle cells, leading to an up to 100-fold increase in IL-6 during physical exercise, IL-6 is produced. Interestingly, obesity-associated hyperglycemia. Interestingly, glucose homeostasis in these mice could be restored after TNF-α-inhibition or Kupffer cell-depletion [59]. Consistently, hepatocyte-specific ablation of STAT3 or gp130 also led to increased infiltration of T lymphocytes and exaggerated diet-induced liver inflammation, indicating a protective role of IL-6 on hepatocytes. Interestingly, glycogen consumption and synthesis in hepatoocytes, which accumulate DNA damages due to activated in a TLR- and EGFR-dependent manner [36]. and IL-6 (Fig. 4) [74–76]. IL-6 action on the central nervous system stimulates energy expenditure [57], again underlining a beneficial role of IL-6 in metabolism. Remarkably, we could recently show that most if not all IL-6 signaling in the brain is mediated by trans-signaling [67].

Most of the studies so far used total ablation of IL-6 or tissue-specific inactivation of the IL-6R. However, future studies have to consider that IL-6 classic and IL-6 trans-signaling might differentially regulate metabolism. And indeed, a very recent report demonstrated that blockade of IL-6 trans-signaling impaired the recruitment of inflammatory macrophages to white adipose tissue under a HFD, while it did not alter insulin resistance in that model [68]. Furthermore, glycogen consumption and synthesis in hepatocytes was regulated by IL-6 trans-signaling in two different models of liver damage [44,45].

IL-6 during liver tumorigenesis

IL-6 is crucial for the development of hepatocellular carcinoma

Diabetes, obesity and male gender are associated with an increased risk to develop hepatocellular carcinoma (HCC). Additionally, high serum levels of IL-6 have been reported in several liver pathologies that predispose to the development of HCC, including acute and viral hepatitis [69], alcoholic cirrhosis [70] and primary biliary cirrhosis [71]. In a very recent prospective study, elevated IL-6 serum levels correlated with an increased risk to develop HCC [72]. And in patients suffering from HCC, elevated serum levels of IL-6 and the sIL-6R have been detected [73].

Extensive work on hepatocarcinogenesis has been performed using the murine diethylnitrosamine (DEN)-model of HCC. The cytotoxic effects of DEN are dependent on its metabolic activation by cytochrome P450 2E1 in hepatocytes. Once activated, DEN forms DNA adducts leading to hepatocyte damage. In this early phase of hepatocarcinogenesis, Kupffer cells become activated in a TLR- and EGFR-dependent manner to secrete TNF-α and IL-6 (Fig. 4) [74–76]. IL-6 induces a compensatory proliferation of hepatocytes, which accumulate DNA damages due to DEN. Consistently, IL-6 deficient mice, as well as mice with liver-specific loss of gp130 show a lower incidence of HCC tumors and prolonged survival in the DEN model [75,77]. In mult�drug resistance 2-deficient mice, cholestasis leads to bile acid induced liver injury, inflammation and fibrosis and at a later stage to hepatocyte dysplasia and carcinoma formation [78,79]. Interestingly, in these mice STAT3 or IL-6 ablation aggravated hepatocyte damage through impaired glucose metabolism.
EGFR signaling and enhanced bile acid synthesis leading to enhanced liver fibrosis, predisposing to increased tumor formation [79].

IL-6 expression is a major hallmark of the senescence-associated secretory phenotype. Senescent hepatocytes in chronic liver disease [80,81] or senescent cholangiocytes during primary sclerosing cholangitis [82] therefore also contribute to increased IL-6 expression in the liver under diseased conditions.

Interestingly, the incidence of HCC development is reduced in female mice as estrogen signaling via the estrogen receptor α suppresses expression of IL-6 (Fig. 4) [75], which correlates well with the human situation. Very recently, nuclear receptor coactivator 5 (NCOA5) has been shown to be another transcriptional repressor of IL-6 expression. Predisposition of NCOA5-haploinsufficient mice to insulin resistance, type 2 diabetes and HCC correlated with elevated IL-6 levels [83]. HCC development in the DEN model was aggravated in mice with genetically induced IRF-1 expression [84]. A very recent report suggested, however, that obesity-enhanced HCC formation was independent of the IL-6R on hepatocytes, suggesting that HCC formation might be independent of IL-6 or rely on IL-6 trans-signaling [85]. Indeed, work from our laboratory shows that abrogation of IL-6 trans-signaling decreases tumor burden in the DEN model to a similar degree as in IL-6−/− mice, indicating that IL-6 trans-signaling significantly contributed to tumor formation in this model (personal communication).

In the inflamed liver, IL-6 promotes the polarization of macrophages to a M2 phenotype through induction of the IL-4 receptor α chain [64]. In human HCC, the amount of infiltrating tumor associated macrophages inversely correlates with prognosis in HCC patients and it could be shown that indeed, IL-6 secreted from tumor associated macrophages promoted the expansion of HCC progenitor cells (HcPCs) [86]. Interestingly, at an early stage of hepatocarcinogenesis, HcPCs appeared in ectopic lymphoid structures containing M2 macrophages, B- and T lymphocytes creating a growth-promoting microenvironment [87]. Although HcPCs have a liver progenitor cell phenotype, they originate through dedifferentiation from hepatocytes through dedifferentiation from hepatocytes [88,89]. At a later stage, HcPCs acquire an IL-6 autocrine loop where expression of IL-6 is dependent on LIN28B-mediated degradation of the miRNA Let-7, a negative regulator of IL-6 expression (Fig. 4) [90]. A similar epigenetic switch has been shown to be involved in the generation of breast cancer stem cells [91]. However, the autocrine IL-6 loop alone is not sufficient to drive HCC formation, as HcPCs only formed tumors when transplanted to mice with a fibrotic liver [90]. During the course of HCC development, HcPCs then egress from ectopic lymphoid structures to form HCC nodules in the liver and metastasis to distant organs.

Taken together, while IL-6 secreted from myeloid cells is important during an early phase of HCC development, pre-neoplastic lesions are fed by an autocrine IL-6 loop at a later stage of tumorigenesis, but are still dependent on...
additional signals from the tumor microenvironment to develop full-blown HCC.

Activation of the IL-6/JAK/STAT3 pathway in inflammatory hepatocellular adenoma

Inflammatory hepatocellular adenomas (IHCA) are rare benign tumors of the liver predominantly found in women and frequently associated with oral contraception, obesity and alcohol abuse. They are characterized by constitutive activation of the acute phase genes in hepatocytes and highly polymorphous inflammatory infiltrates in the liver [92].

The discovery of activating mutations in gp130 and downstream signaling molecules, including JAK1, STAT3, Fyn-related kinase (FRK) and G-protein G(s) subunit alpha (GNAS) (Fig. 5) in IHCA highlighted the importance of the IL-6 pathway for the pathogenesis of benign liver tumors. While novel activating STAT3 mutations are found in 12% of IHCA cases [93], small in-frame deletions within the coding region of gp130 are present in 60% of IHCA cases [92]. These deletions vary in length but always cluster within a loop of the extracellular domain D2 which represents an IL-6 contact site. As a consequence stabilizing hydrophobic interactions between domain D2 and D3 are lost and the extracellular domain adopts an active conformation, resulting in constitutive downstream signaling [94]. Persistent activation of IL-6 trans-signaling has already previously been shown to induce hepatocellular adenoma formation in IL-6/sIL-6R double transgenic but not in IL-6 single transgenic mice [95]. These findings suggest that enhanced gp130 activation by IL-6 trans-signaling is needed to induce oncogenic transformation of hepatocytes. Interestingly, the described in-frame deletion mutants of gp130 display a delayed biosynthesis and are therefore predominantly localized within the endoplasmic reticulum, potentially resulting in altered signal transduction [96] contributing to oncogenic transformation (Fig. 5).

Albeit IL-6 has been shown to be critically involved in the development of hepatocellular carcinoma, only 1–2% of HCC cases harbor gp130 deletion mutations [92]. These findings support the notion that hepatocellular adenomas only very rarely progress to HCC and that persistent activation of the IL-6 pathway alone is not sufficient to trigger malignant transformation of hepatocytes [92].

IL-6 directed therapy in liver pathologies

Acceleration of liver regeneration

As described above, different preclinical rodent models of liver regeneration demonstrated that Hyper-IL-6 strongly enhances liver regeneration. Transient infusions of Hyper-IL-6 might therefore
be beneficial to patients after partial liver resection to boost hepatocyte-mediated regeneration. However, these patients very often underwent tumor resection and up to now it is not clear if transient IL-6/Hyper-IL-6 therapy could cause recurrence of tumor growth.

A more elegant way would be to enhance ex vivo expansion of bi-potential liver progenitor cells (LPC) by IL-6 or Hyper-IL-6 prior to its infusion to patients. Indeed IL-6 and even more Hyper-IL-6 were able to enhance proliferation of LPCs in vivo and in vitro and infusion of expanded LPCs were able to regenerate impaired liver function in vivo [33,97].

Neutralization of IL-6 and IL-6R

IL-6 has been previously recognized as growth factor in multiple myeloma and use of a neutralizing anti-IL-6 antibodies (Fig. 6) was effective in multiple myeloma patients [98]. In a very recent clinical phase I/II study, however, monotherapy with the anti-IL6 antibody siltuximab did not show clinical activity against a series of solid tumors [99]. Furthermore, it turned out that anti-IL-6 antibody treatment led to the formation of high molecular weight antibody-IL-6 complexes and thereby prevented IL-6 clearance from the circulation leading to massive systemic IL-6 elevations [100]. This led to the development of tocilizumab, a humanized anti-IL-6R antibody (Fig. 6). Tocilizumab binds to the IL-6 binding site of the IL-6R and thereby prevents IL-6 binding and also high molecular weight antibody-IL-6 complex formation. Tocilizumab blocks IL-6 classic- and trans-signaling. A series of clinical studies have shown its therapeutic benefit in rheumatoid arthritis [101], juvenile idiopathic arthritis [102] and Castleman’s disease [103]. A phase I/II clinical

**Fig. 5. The IL-6/gp130 pathway is constitutively activated in inflammatory hepatocellular adenoma (IHCA).** Activating deletion mutations in gp130 are the most frequently occurring mutations in IHCA. Constitutively active gp130 can induce STAT3 phosphorylation from the endoplasmic reticulum and endosomes, while Ras-activation occurs at the plasma membrane. Activating mutations are also found in STAT3, the Fyn-related kinase (FRK) and G-protein G(α) subunit alpha (GNAS).
trial also showed that tocilizumab might be also beneficial to prevent graft-versus host disease after bone marrow transplantation [104].

Preclinical studies using murine xenograft models showed that anti-IL-6R therapy suppresses tumor angiogenesis and tumor growth in colon cancer and oral squamous cell carcinoma [105,106]. Also, in an HCC xenograft model, tocilizumab reduced HCC growth by blunting IL-6 signaling from tumor-associated macrophages to HCC cells and therefore preventing the formation of cancer stem cells [86]. The fact that expansion of HcPCs is dependent on IL-6 [90], that IL-6 promotes M2 macrophage polarization [64] and that growth of HCC seems to be promoted by M2 macrophages [107] suggest that HCC patients might benefit from an IL-6-directed therapy. Beside its direct effect on tumor growth, anti-IL-6R therapy has been considered for the treatment of tumor cachexia. In preclinical models of cancer cachexia, genetic loss of IL-6 or the use of anti-IL-6 antibodies prevented white adipose tissue browning and therefore counterbalanced cancer cachexia in these models [108]. Consequently, in a study with a single cancer patient, tocilizumab was able to reverse cancer cachexia symptoms [109].

As outlined above, classic IL-6 receptor signaling is believed to control some crucial homeostatic mechanisms such as the hepatic acute phase response [19–21,110]. In contrast, IL-6 trans-signaling seems to play a role in overshooting immunological reactions resulting in autoimmune diseases such as rheumatoid arthritis and inflammatory bowel disease [111].

One therefore has to consider several caveats when using anti-IL-6R antibody therapy. Anti-IL-6R antibodies prevent both, classic IL-6 and IL-6 trans-signaling (Fig. 6). Classic IL-6 signaling however is important for the induction of the acute phase response as a first-line defense to infection and as a prevention of sepsis [112,113]. Classic IL-6 signaling indeed has been shown to be important for the control of bacterial and viral, in particular hepatitis B virus (HBV) infections [110,114]. Also a significant reduction in the number of peripheral neutrophils [115] and an increase in body weight and triglyceride levels has been observed as a side effect of tocilizumab treatment [103]. These parameters therefore have to be tightly monitored under anti-IL-6R treatment.

Given the fact that sgp130Fc transgenic mice do not show a negative metabolic phenotype, while possessing anti-inflammatory properties in various preclinical models [68], sgp130Fc treatment might represent a more advantageous therapy option in diverse disease settings. A recent phase I study has shown that sgp130Fc is well tolerated in humans [116]. Further studies have to reveal if sgp130Fc therapy is an option for the treatment of HCC and other liver pathologies. A phase Ila clinical trial in patients with IL-6 related inflammatory diseases such as inflammatory bowel disease and rheumatoid arthritis is planned for 2016.

**Inhibition of IL-6 downstream signaling**

As described above, IL-6 binding to IL-6R and gp130 results in the activation of cytoplasmic tyrosine kinases of the JAK and Src kinase family and subsequent phosphorylation of STAT3. Inhibition of JAK and Src kinases would therefore blunt intracellular IL-6 signaling. Ruxolitinib and tasocitinib are small molecule inhibitors of JAK kinases (Fig. 6D) and have been clinically approved for the treatment of myeloproliferative
neoplasms and rheumatoid arthritis [117]. Other compounds are currently under development and in clinical trials for hematological malignancies, solid tumors and rheumatoid arthritis [117,118]. Activated STAT3 can also be directly targeted by small molecule inhibitors binding to its SH2 or DNA binding domain (Fig. 6E). These compounds however haven't entered yet clinical trials [119].

There are preclinical data available that both JAK and STAT3 inhibition abrogates the proliferation of HCC cell lines and the growth of orthotopic HCC tumors [119–121]. Consequently, the use of JAK inhibitors for the treatment of IHCA has been suggested [122] and its use for the treatment of HCC might be considered. However, JAK kinase inhibition would not only abrogate both, IL-6 classic and IL-6 trans-signaling, but also interfere with the downstream signaling of several cytokines. One therefore would need to carefully monitor potential side effects.

Conclusions and perspectives

IL-6 is a cytokine with pleiotropic functions in the body. Under physiological conditions it is essential for proper hepatic tissue homeostasis, liver regeneration, infection defense and fine tuning of metabolic functions. Persistent activation of the IL-6 pathway however seems to be detrimental and can even lead to the development of liver cancer.

Although much progress has been made, there are still many open questions concerning the implication of IL-6 in physiology and pathology of the liver. In order to efficiently target only the detrimental effects of IL-6 on the liver, we need to better dissect the effects of IL-6 on different cell types of the liver.

Most of the murine models used so far investigated effects in the complete absence of IL-6. However, in order to better understand the complex nature of IL-6 in the liver and other tissues, a cell type-specific analysis of IL-6 signals is warranted. Deletion of the IL-6R from selected cell types would be informative to analyze the effect of IL-6 classic signaling on different cell types in hepatic pathologies. These experiments would also help to unravel which cells of the liver provide the soluble IL-6R to induce IL-6 trans-signaling. Another elegant way to dissect the different effects of IL-6 signaling in the liver would be the use of mice with an inducible, cell-autonomous and ligand-independent activation of gp130.

Albeit both, classic and trans-signaling pathways lead to activation of the signaling subunit gp130, the effects on intracellular signaling, but also biological effects seem to differ between IL-6 classic and IL-6 trans-signaling. We therefore need a more detailed spatial and temporal cell biological analysis to better explain these effects and to be able to identify cells in complex tissues that underwent IL-6 trans-signaling.

There is now a growing body of evidence that IL-6 trans-signaling is also implicated in liver pathologies. Selective inhibition of IL-6 trans-signaling rather than complete blockade of both IL-6 signaling pathway might therefore be more effective in the treatment of liver pathologies.

Financial support

The work described in this review was supported by grants from the Deutsche Forschungsgemeinschaft Bonn, Germany (SFB 841, projects C1, SFB 877, project A1 and the Cluster of Excellence ‘Inflammation at Interfaces’).

Conflict of interest

S. R-J is an inventor of patents owned by CONARIS Research Institute, which develops the sgp130Fc protein together with Ferring Pharmaceuticals and he has stock ownership in CONARIS. No conflicts of interest, financial or otherwise, is declared by D. S-A.

Authors’ contributions

Both authors contributed equally to this article.

References


Review


