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Reviews

The role of cytokines in the pathophysiology of chronic liver diseases

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Summary. Many of the biological activities of cytokines are similar to clinical manifestations and abnormalities of laboratory parameters observed in chronic liver diseases (CLD). Evidence of impaired cytokine synthesis in CLD comes from studies of serum or plasma levels, supernatants of peripheral blood mononuclear cells stimulated with various agents and from studying cytokine expression locally in the liver. Circulating levels of several cytokine-regulated molecules such as neopterin, soluble IL-2 receptor, adhesion molecules, and metabolites of the nitric oxide pathway are elevated in patients with CLD. Thus inhibition of cytokine synthesis or modulation of their activity could provide not only important information about their pathophysiologic relevance but also have a profound impact on disease progression in CLD. These studies will also show whether prolonged anti-cytokine treatment with interleukin-1- or tumor necrosis factor-inhibitors interferes with host defense mechanism.

Key words. Cytokines – Liver diseases

Introduction

Cytokines are regulatory peptides that can be produced by virtually every nucleated cell type in the body, such as lymphocytes, monocytes/macrophages, epithelial cells, fibroblasts and many others [15, 51, 61]. They usually are simple polypeptides or glycoproteins with a molecular weight of less than 30 kDa and are often described as hormone-like substances, although there are several features distinguishing between polypeptide hormones and cytokines. Characteristic features of cytokines are: (1) their overlapping spectrum of actions, (2) their transient action radius and (3) their multiple cell origin. Constitutive production of cytokines is absent or low and their production is regulated by several inducing agents at both the transcriptional and translational level. Cytokines act via binding to highly specific cell surface receptors [15, 51, 61].

This group of potent multifunctional peptides is rapidly growing and includes: interleukins (ILs) 1-13, IL-6-related cytokines, like oncostatin M, ciliary neurotrophic factor, granulocyte colony-stimulating factor, and leukemia inhibitory factor (LIF), IL-8 and related molecules, tumor necrosis factor-α (TNF) and TNF-β, colony-stimulating growth factors, stem cell factor, erythropoietin, interferons (IFNs), platelet-derived growth factor (PDGF), transforming growth factors (TGFs) and various other growth factors.

An important definition criterium for cytokines is their critical role as mediators of inflammatory responses. Inflammation plays a critical role in most diseases leading to chronic liver disease (CLD) and end-stage liver cirrhosis [45]. The prototype of these proinflammatory cytokines are IL-1 and TNF. IL-6 is not a proinflammatory cytokine, but shares with IL-1 and TNF the ability to stimulate T and B cells, to augment cell proliferation and to initiate or suppress gene expression for several proteins. This review will primarily focus on the role of IL-1, IL-6 and TNF in the pathophysiology of CLD. The following aspects will be addressed: (1) biological effects of cytokines and their relation to CLD, (2) evidence for impaired synthesis of cytokines and cytokine-dependent molecules in CLD, (3) cell sources and stimuli for cytokine synthesis and (4) possible therapeutic anti-cytokine strategies in CLD.

Biological effects of cytokines and their relation to CLD

Many of the biological actions of cytokines are known clinical manifestations and abnormalities of laboratory parameters observed in CLD [45]. A comparison of the biological properties of IL-1, IL-6 and TNF, as well as clinical symptoms and laboratory abnormalities seen in patients with CLD, is shown in Table 1. With respect to many biological properties, IL-1, IL-6 and TNF show synergistic behavior.

Cachexia

The cachexia syndrome is characterized by anorexia, weight-loss, anemia and net losses of whole body lipid and protein, and is very often found in patients with end-stage CLD [45]. TNF, which was initially termed "cachectin", has been widely implicated as one of the putative mediators of cachexia [61]. Several investigators so far have proven that injections of sublethal doses of TNF cause weight loss, anorexia and anemia [58, 61]. The most convincing proof of its role in the development of cachexia comes from a study of genetically engineered Chinese hamster ovary cells continuously secreting TNF, which were implanted into nude mice [40]. Over 6–8 weeks the mice became anorectic and experienced severe weight loss, whereas control animals did not become anorectic. Interestingly, another study showed that animals did not develop tolerance to be anorexigenic effects of TNF [59]. However, TNF seems not to be the only mediator of cachexia. Sherry et al. [47] showed that passive immunization against TNF leads only to a partial abrogation of anorexia and weight loss, suggesting that other mediators may also be involved in catabolic regulation.

IL-1 seems to be involved either directly or as an inducer of TNF in cachexia development. In contrast to TNF, there arises rapid tolerance to the anorectic property of IL-1 [15]. Recently Gershenwald et al. [23] reported that antibodies to IL-1 receptor type I block the weight loss associated with inflammation, further supporting a role for IL-1 in cachexia. Other studies also demonstrate a role for IFN-γ, LIF and IL-6 in the pathophysiology of cachexia [7, 33, 50]. Therefore, the synergism of various cytokines rather than one single cytokine may be responsible for the development of cachexia in general and cachexia observed in patients with CLD.

Fibrosis and cirrhosis

The liver reacts to chronic inflammation and injury by the development of fibrosis and cirrhosis, caused by an increase in synthesis and deposition of various extracellular matrix proteins like collagen, laminin, fibronectin and others [8]. These matrix proteins are synthesized by several cell types, such as Ito cells (liver fat-storing cells), fibroblasts and hepatocytes. The mechanisms underlying the increased induction of collagen synthesis and subsequent fibrosis are not well understood. TGF-β and PDGF are thought to be key cytokines involved in the pathogenesis of fibrogenesis [3, 30, 41]. Inflammatory cytokines, however, may also be involved in this process [15, 51, 61].

Peterson and Isbrucker [41] recently showed that antibodies to PDGF, but not to IL-1β, reduced the fibroproliferative activity of monocyte-conditioned medium obtained from patients with liver disease. IL-1β, however, increases fibroblast proliferation and directly increases the transcription of collagen type I, II and IV [15]. An explanation for the failure of antibodies to IL-1β to suppress fibroproliferative activity of the monocyte-conditioned medium in the aforementioned study could be the presence of other inflammatory cytokines such as TNF in the monocyte-conditioned medium.

TNF has been shown to be able to upregulate PDGF, and therefore inhibition of both IL-1 and TNF together may be necessary to reduce fibroproliferative activity [20]. In accordance with the assumption of an important role for IL-1β in fibrosis development, a specific IL-1 antagonist, namely IL-1 receptor antagonist, (IL-1Ra), has been demonstrated to reduce collagen deposition in experimental hepatic fibrosis in a rat model [32]. The role of IL-1, however, remains controversial, as another preliminary study shows no effect of the IL-1Ra on fibrosis development in a similar model [21]. In the case of TNF, both stimulatory and inhibitory effects on collagen synthesis have been described [3]. Increased TNF mRNA levels are observed in the livers of rats treated with carbon tetrachloride, a toxin known to induce liver damage and fibrosis [11]. TNF is a potent mitogen for human diploid fibroblasts and may act synergistically with other growth factors [61]. IL-6 counters the proliferative effects of IL-1 on fibroblasts [15, 51], suggesting that IL-6 is not a "fibrogenic" cytokine. In summary, data suggest that, as inflammation in general precedes fibrosis development, proinflammatory cytokines are involved in the pathophysiology of fibrosis and cirrhosis formation. Induction of PDGF and TGF-β by proinflammatory cytokines could play an important role in the pathway of fibrosis formation.

Table 1. Biological effects of interleukin (IL)-1, IL-6 and tumor necrosis factor (TNF) and their relation to chronic liver disease (CLD)

	IL-1	IL-6	TNF	CLD
Cachexia	+	+	+	+
Fibroblast proliferation	+	−	+	+
Cholestasis	?	?	+	+
Hypergammaglobulinemia/ B cell Ig synthesis	+	+	+	+
Acute-phase proteins	+	+	+	+
Induction of NO	+	−	+	+
Induction of adhesion molecules	+	−	+	+
Macrophage activation	+	+	+	+
Induction of IL-1, TNF	+	−	+	+
Induction of IL-6	+	−	+	+

Ig, Immunoglobulin; NO, nitric oxide

Cholestasis

Endotoxin administration has been found to induce cholestasis in vivo [24]. In addition, it has been recently reported that TNF administration to humans leads to cholestasis [24]. High-dose IL-2 therapy, which is associated with the appearance of high circulating TNF levels, also results in cholestasis in some patients [19]. Experimental biliary obstruction in mice leads to prolonged increased levels of the cytokines TNF and IL-6 [6]. Recently nitric oxide (NO) has been shown to inhibit canalicular contraction suggesting a possible role for NO in cholestasis [18].

Therefore cytokines are associated with cholestasis either as a causative agent or are releaesed into the circulation subsequent to experimental obstructive jaundice.

Hypergammaglobinemia

Polyclonal immunoglobulin (Ig) stimulation is a common feature of CLD [60]. The role of IL-1 and TNF in hypergammaglobinemia may be mainly indirect, in inducing IL-6. IL-1 acts as a helper or cofactor during the activation process of B cells, particularly with IL-4. Other studies show that IL-1 synergizes with various B cell growth and differentiation factors, such as IL-2, IL-5 or IL-6, leading to enhanced proliferation and antibody synthesis [15]. IL-6 is the most important cytokine with regard to B cell stimulation and Ig synthesis [26]. IL-6 acts on B cells at the mRNA level and induces biosynthesis of secretory-type IgG [26]. IL-6 acts on B cells activated with pokeweed mitogen to induce IgM, IgG and IgA production, but not on resting B cells. Furthermore, anti-IL-6 antibodies inhibit pokeweed mitogen-induced Ig production, suggesting that IL-6 is essential for pokeweed mitogen-induced Ig production [26]. IL-6 action on B cells is therefore dependent on other co-stimulatory factors, such as IL-2 [26]. There exist disease-specific Ig patterns in CLD, and therefore in addition to IL-6 several other cytokines such as IL-2, IL-4 and IL-5 may be important in the dysregulation of Ig synthesis observed in CLD.

Acute-phase proteins

The acute-phase response is a systemic reaction of the organism to inflammation or tissue injury. The regulation of these proteins is well understood, although the list of involved cytokines is quickly growing. The cytokines IL-1, IL-2, IL-6, IL-11, TNF, TGF-β, LIF and oncostatin M have so far been shown to induce various acute-phase reactants [15, 26, 51, 61]. IL-6 seems to be the most important cytokine of the acute-phase response, since it is involved in the synthesis of all known acute-phase proteins [3, 26]. Fibrinogen, for example, is not induced by IL-1 and TNF, but by IL-6 in vitro [3, 26]. Whereas the regulation of acute-phase protein synthesis by cytokines is better understood, the functions of these acute-phase reactants are still unclear. However, there is increasing evidence that acute-phase proteins have anti-inflammatory potential and may therefore be important mediators in the termination of inflammatory events. Data obtained in our laboratory suggest that C-reactive protein (CRP) is a major inducer of IL-1Ra but only a weak inducer of IL-1β, suggesting that CRP acts preferentially as an anti-inflammatory mediator (H. Tilg et al., in preparation).

Intermediary metabolism of the liver

This topic has been recently reviewed [3]. Cytokines regulate the intermediary metabolism of amino acids, proteins, carbohydrates, lipids and minerals. Patients with CLD often present with disturbances of intermediary metabolism similar to those mediated by cytokines [45].

Evidence for impaired synthesis of cytokines and cytokine-regulated molecules in CLD

Activated macrophages are well documented in CLD [45, 57]. Proinflammatory cytokines can either activate macrophages or are released by activated macrophages [15, 26, 51, 61]. Macrophage-released cytokines like IL-1 or TNF can activate other macrophages, resulting in a chronic condition of activity and a cascade of other inflammatory or fibrogenic substances (e.g., IL-8 and related molecules, PDGF, TGF-β) [15, 51, 61].

Cytokines

Evidence of impaired cytokine synthesis in CLD comes from studies of serum or plasma levels, supernatants of peripheral blood mononuclear cells (PBMC) stimulated with various agents and from studying cytokine expression locally in the liver. Most published literature of serum or plasma levels of cytokines in liver diseases concentrates on acute liver diseases [3]. There are few reports on circulating cytokines in CLD. Khoruts et al. [28] reported elevated plasma levels of TNF, IL-1α and IL-6 in more than 50% of patients with stable alcoholic liver cirrhosis [28]. Similar results were obtained by Deviere et al. [13]. Sheron et al. [46] demonstrated increased circulating TNF levels in patients with chronic hepatitis B infection [46]. We recently showed that serum levels of IL-1β, IL-6, TNF and IFN-γ are slightly elevated in most patients with CLD [53]. Patients in the cirrhotic stage of their disease exhibited significantly higher levels than non-cirrhotic patients. Enhanced endogenous cytokine production in our study was independent of underlying disease and cytokine levels did not correlate with liver function parameters [53].

Many studies show altered spontaneous and endotoxin-induced cytokine production in PBMC obtained from patients with various CLD [53]. In most studies spontaneous and endotoxin-induced cytokine synthesis in PBMC is increased compared with healthy volunteers [13, 14, 22, 34, 46, 65, 66], although several reports show decreased cytokine synthesis [2]. However, when studying cytokine production in PBMC one has to consider that disease activity may play an important role. The best example in favor of this assumption comes from a study with patients in septic shock, a disease which is well known for cytokine overproduction [15, 51, 61]. In this study, endotoxin-induced IL-1α, IL-1β, IL-6 and TNF production by monocytes was remarkedly reduced relative to that of normal controls [36]. We recently observed a similar phenomenon, when studying IL-8 production in PBMC obtained from patients undergoing high-dose IL-2 therapy [54]. PBMC isolated on day 1 before the start of therapy produced significantly more IL-8 than PBMC

isolated on day 4 of therapy. Therefore the contradictory results with regard to cytokine production by PBMC from patients with various liver diseases are not surprising and one may find both increased and decreased cytokine production by PBMC in the same patient depending on the activity of the underlying disease.

Enhanced expression of cytokines in the livers of patients with CLD has also been demonstrated by immunohistochemical studies. Yoshioka et al. [67] reported focal production of TNF in liver tissue sections from patients with chronic hepatitis or hepatitis B-related cirrhosis. Volpes et al. [62] recently showed enhanced hepatic expression of both p55 and p75 TNF receptors in chronic inflammatory liver diseases. However, much more information about the local expression of cytokines in CLD is needed.

Cytokine-regulated molecules

Elevated levels of several cytokine-regulated molecules have been demonstrated in various CLD. Neopterin is a degradation product of the pteridine pathway and is mainly regulated by IFN-γ, although in vivo other cytokines can induce its synthesis [63]. We and others have shown that urinary and serum neopterin levels are elevated in CLD, irrespective of the etiology of underlying liver disease [12]. As shown for circulating cytokines, serum neopterin levels in cirrhotics are usually higher than in non-cirrhotics A. Wilmer et al., in preparation. Soluble IL-2 receptor (sIL-2R) is shed by activated leukocytes. Müller et al. [35] reported increased serum sIL-2R levels in patients with CLD, again independent of etiology. Serum levels of CRP, the prototype acute-phase reactant, are also elevated in many patients with CLD [53]. In contrast, albumin synthesis is decreased in many of these patients [53]. Albumin synthesis can be downregulated by various cytokines [15, 51, 61]. A negative correlation between circulating proinflammatory cytokines and albumin levels in patients with CLD was shown [53]. The leukocyte adhesion molecule intracellular adhesion molecule-1 (ICAM-1) is induced by proinflammatory cytokines and can be detected on bile ducts in patients with primary biliary cirrhosis and primary sclerosing cholangitis [1]. Very high levels of circulating ICAM-1 were found in patients with primary biliary cirrhosis and primary sclerosing cholangitis and levels were elevated in other patients with CLD [1]. These data together strongly support the hypothesis that in patients with CLD, irrespective of etiology of the underlying disease, cytokines and their products are continuously synthesized and released.

Nitric oxide

NO, initially described as an endothelial-derived relaxing factor, has recently been recognized as a mediator of macrophage function [37]. A variety of cell types have been shown to produce NO from *L*-arginine, by either a constitutive and/or an inducible enzyme [37]. This enzyme is called NO synthase (NOS), and three different NOS have been cloned so far (cerebellar, endothelial/constitutive and macrophage/inducible). Endotoxin and proinflammatory cytokines induce endothelial and macrophage NOS [37]. Most studies with the inducible NOS have been done in animals, and little is known about the existence of inducible NOS enzyme activity in specific human cell types, despite its clear role in the elimination of tumor cells and intra- and extracellular pathogens, as well as in the induction of sustained hypotension [37]. Recently Nussler et al. [39] reported that freshly isolated human hepatocytes induce NO biosynthesis after stimulation with IL-1, TNF, IFN-γ and endotoxin. These data are the first published evidence of the existence of inducible NO biosynthesis in a human cell type. Hunt and Goldin [27] recently showed that monocytes from patients with alcoholic liver disease have increased nitrite production compared with healthy controls. Nitrite and nitrate are the stable endproducts of the NO pathway [37]. Therefore there is evidence that not only NOS inducers like endotoxin and cytokines circulate in patients with CLD but also that NO synthesis is increased in these patients [56].

The possible role of NO as a mediator of the hyperdynamic circulation of cirrhosis, a condition characterized by excessive splanchnic vasodilation and hyporesponsiveness to vasoconstrictors, has been recently reviewed [49, 64]. There is evidence that NO plays a role in the hyperdynamic circulation of cirrhosis and this suggests that endotoxin and circulating proinflammatory cytokines may be the trigger for increased endothelial or macrophage NO synthesis [49, 64]. NO may also be involved in the liver tissue injury seen in cirrhosis, in reduced hepatocyte protein synthesis and endotoxin- or drug-induced hepatotoxicity [49]. Whether these NO effects are mediated by the inducible or constitutive enzyme is not yet known. If the endotoxemia and cytokinemia found in patients with CLD leads to induction of NO synthesis, blocking of either cytokine production or NO production by specific antagonists could have a major impact on complications such as cirrhotic ascites or hepatorenal syndrome and various other NO-mediated effects.

Cell sources and stimuli of cytokine synthesis

Almost all cell types are involved in cytokine synthesis [15, 51, 61]. This is especially true for macrophages; Kupffer cells in the liver represent the major macrophage pool of the body. Kupffer cells, but also hepatocytes and Ito cells, have been shown to synthesize various cytokines in response to endotoxin and other stimuli [57]. However, it is rather unclear how much the liver itself contributes to the cytokine production found in CLD. Other peripheral tissues, as well as circulating leukocytes, may also be major contributors. The liver is the main site of clearance for circulating cytokines in the body [3]. Therefore not only increased synthesis but also lack of hepatic cytokine clearance may be a reason for cytokine imbalance in these patients [3]. The precise stimuli causing cytokine release

in CLD have not been defined. Endotoxin seems likely to be an important stimulus, since patients with CLD show elevated endotoxin levels and endotoxin is an important inducer of cytokines in vitro [15, 51, 61]. The contribution of various hepatitis viruses, antigens, autoantigens, immune complexes, metabolic products or Igs needs to be investigated.

Possible therapeutic anti-cytokine strategies in CLD

If cytokines are indeed important mediators in the pathophysiology of CLD, then inhibition of their synthesis or activity should provide therapeutic benefit in patients with CLD. Not surprisingly, and again reflecting the importance of IL-1 and TNF in human disease, the most successful anti-cytokine strategies so far have been developed for IL-1 and TNF.

IL-1Ra is a natural product of monocytes which has been found to occupy and block IL-1 receptors and thereby downregulate IL-1 activity [4, 16]. This inhibitor is a member of the IL-1 family, based upon its amino acid sequence homology and similarities in gene structure to IL-1α and IL-1β [4, 16]. The IL-1Ra is an absolutely unique substance since it is the only natural competitive antagonist of another cytokine/hormone/neurotransmitter ever described. IL-1Ra reduces IL-1-induced effects in many in vitro and animal models of inflammation [17]. Although there is as yet only limited data regarding clinical efficacy in humans, preliminary results in a prospective study show a decrease in sepsis-associated mortality from 44% to 16% [17]. So far no data are available on the use of IL-1Ra in animal models of acute liver failure. As mentioned above, IL-1Ra has been used in two animal models of experimental fibrosis to investigate the importance of IL-1 and preliminary data show controversial results [21, 32]. For IL-1 there also exist other possible blocking mechanisms. IL-1β requires proteolytic cleavage to generate the active form of the molecule [15]. A cysteine protease that specifically cleaves pro-IL-1β at ALA117 has been described and cloned [9, 52]. Selective inhibitors of this enzyme are already available for in vitro studies and will be of great interest for in vivo study.

Natural TNF inhibitors have been initially described in the urine of febrile patients with leukemia [44]. These proteins were found to bind to TNF and to neutralize its biological activity. Both inhibitors were subsequently purified, sequenced, and their cDNAs cloned [29, 31, 43]. The cDNA sequences revealed that the proteins represent the extracellular portions of membrane-associated receptors generated by proteolytic cleavage [38, 42]. These inhibitors have therefore been termed soluble TNF receptors (TNFsR p55 and p75). In animal studies of septic shock, the administration of TNFsRp55 increased survival in otherwise lethal endotoxemia [5]. Therapeutic trials in humans are therefore eagerly awaited; so far no studies have been performed in animal models of acute or CLD with these inhibitors. Those studies, like studies with IL-1Ra, will undoubtedly reveal how important the contributions of IL-1 and TNF are in the pathophysiology of liver diseases.

We recently observed increased circulating levels of IL-1Ra and TNFsRp55 in patients with CLD [55]. Elevations were only slight for IL-1Ra and more pronounced for TNFsRp55 and, as described for cytokines in these patients [53], independent of underlying disease. Therefore we conclude that circulating elevated cytokine antagonists found in CLD may reflect ongoing disease activity and are probably not able to counteract endogenous IL-1 and TNF.

There are many other anti-cytokine strategies, including pharmacological substances like pentoxifylline ore pentamidine which also decrease cytokine synthesis [25]. Those substances are widely available and will be studied in the future in various models of liver disease. New anti-cytokine strategies, such as selective inhibitors of transcription factors, will be developed in the next few years, providing us with a whole array of options for interfering with cytokine pathways.

Conclusion

Cytokines are important mediators in the pathophysiology of liver diseases, thus inhibition of their synthesis or modulation of their activity could provide not only important information about their pathophysiological relevance but also have a profound impact on disease progression in CLD. These studies will also show whether prolonged anti-cytokine treatment interferes with host defense mechanisms. Although it is clear that many cytokines are involved in disease processes, interference with a single cytokine may be sufficient to inhibit tissue pathology. In various models of septic shock inhibition of one cytokine can significantly inhibit lethality. There is also increasing evidence that inhibition of the final pathway of many cytokines, namely NO with specific antagonists like N-amino-*L*-arginine, increases mortality in animal studies with endotoxin [10]. Recently it has also been shown that endogenously synthesized NO prevents endotoxin-induced glomerular thrombosis, proving that some NO is necessary to prevent platelet aggregation [48]. Therefore the best approach in most experimental animal and human studies of various liver diseases may be in blocking a single or two cytokines rather than blocking a late effector molecule like NO.

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