Review

The Role of Cytokines in the Pathogenesis and Treatment of Alcoholic Liver Disease

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The Role of Cytokines in the Pathogenesis and Treatment of Alcoholic Liver Disease

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Abstract: Alcoholic liver disease (ALD) is a major cause of chronic liver disease. This term covers a broad spectrum of liver lesions, from simple steatosis to alcoholic hepatitis and cirrhosis. The pathogenesis of ALD is multifactorial and not fully elucidated due to complex mechanisms related to direct ethanol toxicity with subsequent hepatic and systemic inflammation. The accumulation of pro-inflammatory cytokines and the reduction of anti-inflammatory cytokines promote the development and progression of ALD. To date, there are no targeted therapies to counteract disease progression and prevent acute liver failure. Corticosteroids reduce mortality by acting on the hepatic-systemic inflammation. On the other hand, several studies analyzed the effect of the inhibition of pro-inflammatory cytokines and the stimulation of anti-inflammatory cytokines as potential therapeutic targets in ALD. This narrative review aims to clarify the role of the cytokines in the pathogenesis and treatment of ALD.

Keywords: alcohol; oxidative stress; inflammation; gut dysbiosis; biological drugs; probiotics

1. Introduction

Alcoholic liver disease (ALD) is one of the leading causes of chronic liver disease worldwide. In this way, alcohol consumption is often a cofactor in patients with hepatitis B and C and patients with non-alcoholic fatty liver disease [1]. According to the European Association for the Study of the Liver, the diagnosis of ALD should be suspected in the presence of liver damage (clinical signs and/or bio-humoral abnormalities) and a regular alcohol consumption of >30 g/day in men or >20 g/day in women [2]. ALD comprises a broad spectrum of liver lesions ranging from simple alcoholic fatty liver (AFL) to alcoholic steatohepatitis (ASH) and alcoholic hepatitis (AH) [3]. AFL diagnosis is established in patients with known alcohol use disorder (AUD) and hepatic steatosis observed on abdominal ultrasound combined with increased liver enzymes and the absence of other causes of liver disease. However, it is a serious problem due to the non-specific symptoms [4,5]. Similar to AFL, mild ASH rarely presents with clinical symptoms and can only be diagnosed by liver biopsy. AH is a clinical condition with a high mortality rate, and it is diagnosed by the presence of jaundice in the preceding eight weeks and elevated transaminase levels. Patients with AH may present signs of severe hepatic decompensation, such as ascites and hepatic encephalopathy [6]. The pathogenesis of ALD is multifactorial due to complex molecular pathways not yet fully elucidated. Certainly, a key role is

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played by the direct hepatotoxicity of ethanol, lipid peroxidation, stress with reactive oxygen species (ROS) production, and activation of the immune response by cytokines [7]. Although there are clear links between the amount and duration of alcohol consumption and the progression of ALD, other genetic and environmental factors are involved in the development and progression of ALD [8]. This narrative review aims to clarify the role of the cytokines in the pathogenesis and treatment of ALD.

2. Epidemiology and Social Impact of ALD

About 2 million deaths each year are linked to chronic liver disease worldwide [9]. Alcohol consumption is estimated at 43% of the global population. AUD prevalence is around 5% (283 million people), with the highest prevalence among men and women in the European region (14.8% and 3.5%, respectively) and in the Americas (11.5% and 5.1%, respectively) [10]. Alcohol is the leading cause of cirrhosis globally, responsible for almost 60% of cases in Europe, North America and Latin America, and about 35% of patients with AUD develop different forms of ALD [11]. The global prevalence of alcohol-associated cirrhosis was estimated at 23.6 million individuals with compensated cirrhosis and 2.46 million for those with decompensated cirrhosis, respectively [12]. In recent years, there has been an increase in the incidence of ALD, especially in the 15-45 age group [13]. This is a serious health problem because higher alcohol consumption in late adolescence increases the risk of developing severe liver disease, with a higher risk of death by cardiovascular diseases and cancer [14,15]. Mortality related to ALD has increased over the last decade more in developed countries, such as Europe, Asia, Latin America and the United States [16,17]. In addition, another increase in ALD deaths was observed during the Coronavirus Disease-19 pandemic due to higher alcohol consumption related to emotive stress and difficult access to treatments [18]. Especially in its final stage, represented by liver cirrhosis, ALD has a high socio-economic impact. It is estimated that in 2016, liver disease-related spending in the United States was $32.5 billion. Two-thirds of these costs are attributable to hospital or emergency room care. In 20 years, healthcare spending on liver disease has increased by 4% per year [19]. Based on ALD-related disability-adjusted life years from 2016, 21.5 million life years were lost due to ALD, with men affected significantly more than women. A significant portion of these lost life years was attributable to premature death rather than disability [20]. Based on the healthcare claims analysis report, of those who survived, more than 50% were hospitalized within one year and almost 75% in the second year, with a total cost of about $145,000 for each patient [21]. Patients with AUD have an increased risk of psychiatric comorbidities, such as anxiety, affective disorders and schizophrenia [22]. Malnutrition is frequent in ALD patients due to reduced caloric intake, abnormal digestion, increased protein catabolism and abnormal lipid metabolism [23,24]. Patients with ALD should receive appropriate nutritional assessment and support. This has a major socio-economic impact, as in many cases hospitalization is required for adequate parenteral or enteral nutrition [25]. In this way, folate deficiency is the result of reduced dietary folate intake, intestinal malabsorption, reduced hepatic absorption and deposition, and increased urinary excretion [26,27]. This event causes the megaloblastic anemia as a main complication. In addition, chronic alcoholics also have thiamine, vitamin B6 and vitamin B12 deficiency [28]. Several studies conducted in animal models have shown how these deficits contribute to the progression of ALD and the development of hepatocellular carcinoma (HCC) and colorectal cancer due to an epigenetic mechanism involving DNA methylation [29,30]. On the other hand, thiamine deficiency is associated with Wernicke encephalopathy (WE), which presents with altered mental status, gait ataxia and ophthalmoplegia. About 80% of patients with non-treated WE develop Korsakoff's syndrome (KS), characterized by memory disturbances associated with confabulation [31]. A study conducted by Wilson et al. showed that treatment with intravenous thiamine for 5 days rather than 2 days increases the healthcare costs of acute care but reduces the expected lifecycle cost should the patient develop KS [32]. On the other hand, a study performed by Thompson et al. defined how healthcare costs for the management of ALD increased among US patients from 2006 to 2013. Total costs were nearly $145,000 per patient, decreasing from $50,000 in the first year to $10,000 per year in the last years. Liver transplanted patients averaged about $300,000 in transplant-related costs and over $1,000,000 in total healthcare costs over five years [33].

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3. ALD Pathogenesis

The pathogenesis of ALD is based on multiple and complex molecular mechanisms that are not yet fully elucidated. These include direct ethanol hepatotoxicity, lipid peroxidation, oxidative stress with consequent ROS production, activation of the immune response and activation of proinflammatory cytokines [34]. Ethanol metabolism comprises three pathways: i) hepatocyte cytoplasmic alcohol dehydrogenase oxidizes ethanol into acetaldehyde, a highly toxic compound that can alter DNA synthesis; ii) the enzyme cytochrome P450 2E1 (CYP2E1), which oxidizes ethanol into acetaldehyde, generate ROS and triggers oxidative stress and inflammation; iii) heme-containing catalase in peroxisomes oxidize ethanol to acetaldehyde and subsequently the enzyme aldehyde dehydrogenase (ADH) oxidize acetaldehyde into acetate, which is released into the circulatory system, and further oxidized into carbon dioxide in various extrahepatic tissues [35–38]. Chronic alcohol use increases CYP2E1 expression, resulting in increased acetaldehyde concentration, decreased ADH activity, reduced acetaldehyde oxidation, and accumulation of acetaldehyde in hepatocytes. This explains the direct hepatotoxicity of ethanol on the liver [39]. In addition, ethanol and acetaldehyde upregulate adiponectin, signal transducer and activator of transcription 3 (STAT3) and reduce zinc levels, inhibiting 5′-AMP-activated protein kinase and peroxisome proliferator-activated receptor α, resulting in lipid peroxidation and ROS production [40]. The increase in ROS damages mitochondrial DNA and proteins, causing a reduction in mitochondrial ATP and glutathione. ROS-induced release of hepatocellular kinase 1 regulates the hepatocellular apoptosis signal through the cleavage of pro-caspase-3 into caspase-active 3 [41]. Chronic alcohol consumption is involved in the accumulation of intestinal endotoxins and increased permeability of the intestinal wall, facilitating the translocation of lipopolysaccharide (LPS) from the gut to the liver. LPS can bind to toll-like receptors (TLRs) and activate the synthesis and release of cytokines and inflammatory factors, such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, and platelet-derived growth factor, further stimulating the accumulation of neutrophils and macrophages and causing liver inflammation and systemic damage in liver Kupffer cells [42]. Additionally, liver damage activates the proliferation of hepatic stellate cells (HSCs), which enhance the secretion of transforming growth factor-β (TGF-β) and collagen synthesis leading to fibrogenesis [43]. In recent years, the link between the changes in the gut microbiota composition and the ALD development and progression has been investigated [44]. Several studies employing preclinical and clinical models have shown that chronic alcohol consumption induces a decrease in Bacteroidetes and Firmicutes and an increase in Enterobacteriaceae and Proteobacteria, leading to gut dysbiosis [45,46]. Gut dysbiosis is associated with increased intestinal permeability linked to altered tight junctions, with translocation of pathogen-associated molecular patterns (PAMPs) in the liver through the portal vein [47]. In addition, alcohol abuse is associated with decreased levels of butyrate-producing genera and increased levels of proinflammatory Enterobacteriaceae. All of this leads to liver damage due to PAMPs translocation process [48]. The different pathogenetic ways involved in ALD development are summarized in Figure 1.

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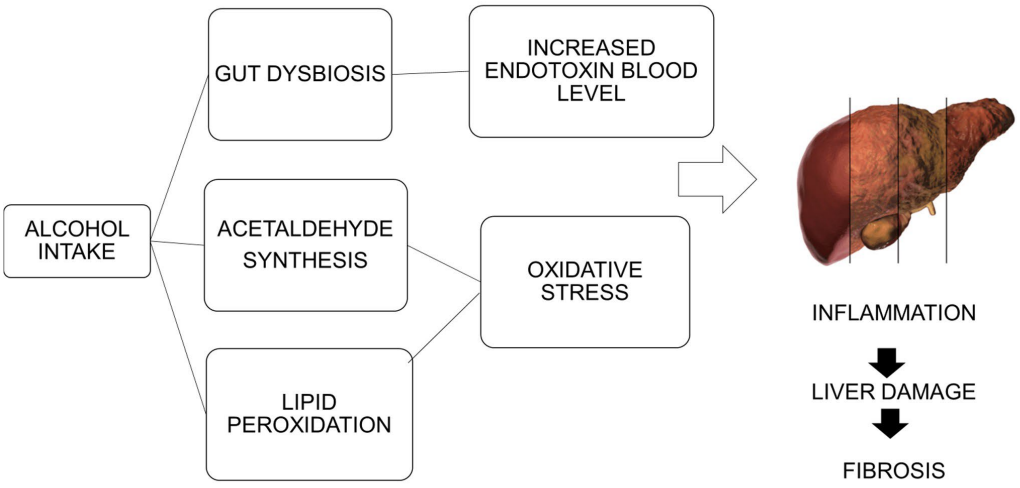

Figure 1. Schematic representation of the different pathogenetic ways involved in ALD development.

4. Role of Pro-Inflammatory Cytokines

4.1. TNF-α

Alcohol-induced damage occurs at several levels, from innate immune cells to hepatocytes. Innate immune cells, including hepatic macrophages (Kupffer cells), play a key role in early alcohol-induced liver damage through the recognition of LPS in the portal circulation. This results in the production of LPS-induced inflammatory cytokines [49]. Among the pro-inflammatory cytokines that promote liver damage, a key role is played by TNF-*α* [50]. Recognition of LPS activates members of the mitogen-activated protein kinase family, including extracellular receptor-activated kinase 1/2 (ERK1/2), p38 and the c-jun-N-terminal kinase, resulting in TNF-*α* production, mediated by oxidative stress [51]. LPS stimulates TLR4 and, at the same time, NADPH oxidase (NOX) interacts with the COOH-terminal region of TLR4, resulting in the generation of ROS in neutrophils and monocytes, which directly activates nuclear factor κB (NF-κB), with increased TNF-*α* concentration [52]. A study conducted by Thakur et al. showed how the use of diphenyliodonium and dilinoleoyl-phosphatidylcholine in Kupffer cells of mice models, reduced ERK1/2 activation, inhibited NOX4, resulting in lower ROS and TNF-*α* production [53]. Convincing evidence about the key role of TNF-*α* in the development of ALD was obtained from transcriptome studies that confirmed its up-regulation in patients with ALD [54]. Furthermore, a higher serum concentration correlates with a worse prognosis and an advanced disease stage [55]. Gonzales-Quintela et al. showed that serum TNF-*α* levels were almost similar in the general population, in teetotalers and alcohol drinkers, while in chronic alcoholics were elevated [56]. Other studies clarified how chronic alcohol stimulates the liver through prolonged activation of NF-κB, resulting in TNF-*α* production. Similar to resident liver macrophages, monocytes from chronic alcoholics also showed increased NF-κB activation compared to the control subjects. Acute alcohol exposure inhibits NF-κB activation, leading to a reduction in TNF-*α* levels [57,58]. A study performed by Mookerjee et al. showed that TNF-*α* is an important mediator of portal and systemic hemodynamic disturbances in ALD. This was the first study in which it was observed that the use of the biological drug infliximab improved cardiovascular hemodynamics, hepatic venous pressure gradient and hepatic and renal blood flow only twenty-four hours after its use [59]. Furthermore, TNF-*α* plays a key role in hepatocyte proliferation and liver regeneration, as reported by in vivo studies [60]. Although TNF-*α* is implicated in alcohol-related liver damage, liver cell death is not the normal outcome when hepatocytes are exposed to this

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cytokine. This suggests that the normal responses of hepatocytes to this cytokine are subverted during liver injury [61].

4.2. IL-8 and CXCL1

Neutrophil infiltration is a key feature of ALD. Unlike other innate immune cells, neutrophils are not liver-resident immune cells [62]. IL-8 plays a key role in the recruitment of neutrophils to liver tissue involving the chemokine CXC motif chemokine ligand 1 (CXCL1). Indeed, serum and liver levels of IL-8 and CXCL1 are directly correlated with disease severity and mortality [63]. A study performed by Patel et al. showed that IL-8 serum levels were significantly higher in patients with severe AH than in those with mild AH and that these levels were better predictors of short-term mortality than conventional prognostic scores [64]. Wieser et al. demonstrated how blockade of IL-8 receptors with short lipopeptides (pepducin) in mice models reduced liver inflammation, weight loss and mortality associated with ALD [65]. Nischalke et al. also showed that the CXCL1 rs4074 single nucleotide polymorphism was associated with increased blood levels of CXCL1 and an increased risk of developing cirrhosis in alcoholics as well as the development of HCC [66]. Mice models treated with a high-fat and ethanol-rich diet significantly upregulated the hepatic expression of several chemokines, including CXCL1, with a reduction of the infiltration and hepatic damage of neutrophils [67]. Roh et al. showed that the production of cytokines, including CXCL1, is mediated by TLR-2 and TLR-9, activated by LPS translocated from the gut and expressed on Kupffer cells. This data showed that in ethanol-treated wild-type mice, there was an increase in the hepatic expression of CXCL1 and the serum level of CXCL1, while TLR2- and TLR9-deficient mice showed significantly lower levels [68].

4.3. IL-1β

As previously reported, chronic exposure to ethanol in ALD sensitizes Kupffer cells to activation by LPS through TLR-4, inducing the production of proinflammatory cytokines, including IL-1β [69]. This is produced as a result of inflammasome activation, mainly of NOD-like receptor protein 3, highly expressed in macrophages and liver monocytes but lower expressed by hepatocytes and stellate cells [70]. NLR family pyrin domain containing 3 (NLRP3) is activated upon binding of PAMPs to TLR. This results in NF-κB activation, culminating in the transcription of pro-IL-1β precursors. Subsequently, procaspase-1 binds to NLRP3, transforming into caspase-1, which is catalytically active for IL-1β processing [71]. A study by Petrasek et al. conducted in murine models found that protein levels of NLRP3 and IL-1β were significantly increased in the liver of chronic alcohol-fed mice compared to the control group. Furthermore, data showed that mice deficient in the NLRP3 and caspase-1 inflammasome components had less steatosis and liver damage than wild-type mice on an alcoholic diet. In addition, IL-1R1 knockout mice were protected against ALD [72]. Cui et al. demonstrated, in Kupffer cells of ethanol-fed mice, an up-regulation of NLRP3 inflammasome components as well as IL-1β [73]. Similarly, a study by Voican et al. showed that increased levels of IL-1β were reported in ALD patients after one week of alcohol withdrawal [74]. In vitro experiments have shown that IL-1β is able to act directly on HSCs, with a subsequent proliferation and trans-differentiation in myofibroblasts, and with an increase in collagen and TGF-β levels [75]. A further function of IL-1β is the activation of invariant natural-killer T lymphocytes (iNKT) [76]. The main role of iNKTs is to determine the hepatic infiltration of neutrophils, a hallmark in the pathogenesis of ALD [77]. iNKT cell-deficient mice were protected from hepatic infiltration of neutrophils and liver damage induced by chronic ethanol binges. In contrast, wild-type mice showed intense hepatic infiltration of neutrophils and marked upregulation of hepatic expression of several inflammation-associated genes. IL-1β also inhibits liver regeneration [78]. Finally, mice treated with an IL-1 receptor antagonist showed a better regeneration of hepatocytes and an increased rate of recovery from liver damage induced by chronic ethanol consumption than untreated mice [79]. Table 1 summarizes the different studies about the involvement of pro-inflammatory cytokines in ALD.

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Table 1. Summary of different studies about the involvement of pro-inflammatory cytokines in ALD.

Cytokine evaluated	Reference	Study design	Outcome
TNF-α	Thakur *et al.*, 2006 [53]	Pre-clinical study	Reduction of ERK1/2 activation and inhibition of NOX4 resulting in lower ROS and TNF-α production after the use of diphenyl-iodonium and dilinoleoyl-phosphatidylcholine in Kupffer cells of mice models
	Ciećko-Michalska *et al.*, 2006 [55]	Case-control study	Higher concentrations of TNF-α in ALD patients were correlated with poor prognosis
	Mandrekar *et al.*, 2006 [58]	Pre-clinical study	Significant reduction in monocyte production of TNF-α in response to LPS or staphylococcal enterotoxin B stimulation eighteen hours after moderate alcohol consumption
	Gonzales-Quintela *et al.*, 2008 [56]	Case-control study	TNF-α levels were almost similar in the general population, in teetotalers and alcohol drinkers, while elevated in chronic alcoholics
	Affò *et al.*, 2013 [54]	Translational study	TNF superfamily receptors are overexpressed in AH humans and mice models
IL-8	Patel *et al.*, 2015 [64]	Case-control study	Serum IL-8 predicts severity and mortality in patients with AH
	Wieser *et al.*, 2017 [65]	Pre-clinical study	Blockade of IL-8 receptors with pepducin reduced liver inflammation, weight loss and mortality

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			associated with ALD in mice models
CXCL1	Nischalke *et al.*, 2013 [66]	Case-control study	CXCL1 rs4074 single nucleotide polymorphism was associated with increased blood levels of CXCL1 and an increased risk of developing liver cirrhosis and HCC
	Chang *et al.*, 2015 [67]	Pre-clinical study	Mice models treated with a high-fat and ethanol-rich diet markedly upregulated the hepatic expression of CXCL1, with a reduction of the infiltration and hepatic damage of neutrophils
	Roh *et al.*, 2015 [68]	Pre-clinical study	Ethanol-treated wild-type mice showed an increased hepatic expression of CXCL1 and serum level of CXCL1, while TLR2- and TLR9-deficient mice showed significantly lower levels
IL-1β	Petrasek *et al.*, 2012 [72]	Pre-clinical study	IL-1β levels were significantly increased in the liver of chronic alcohol-fed mice compared to controls
	Cui *et al.*, 2015 [73]	Pre-clinical study	Up-regulation of NLRP3 inflammasome components and IL-1β in Kupffer cells of ethanol-fed mice
	Voican *et al.*, 2015 [74]	Prospective cohort study	Significantly increased levels of IL-1β in ALD patients after one week of alcohol withdrawal

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	Mathews *et al.*, 2016 [78]	Pre-clinical study	A loss of function of IL-1β protected mice models from hepatic infiltration of neutrophils and liver damage induced by chronic ethanol binges

Legend: TNF-α, tumor necrosis factor-alpha; ERK1/2, extracellular signal-regulated kinase 1/2; NOX4, NADPH oxidase 4; ROS, reactive oxygen species; ALD, alcoholic liver disease; LPS, lipopolysaccharide; AH, alcoholic hepatitis; IL-8, interleukin-8; CXCL1, CXC motif chemokine ligand 1; TLR2, toll-like receptor 2; TLR9, toll-like receptor 9; IL-1β, interleukin-1 beta; NLRP3, NLR family pyrin domain containing 3.

5. Role of Anti-Inflammatory Cytokines

5.1. IL-6 and IL-10

To date, IL-6, IL-22, and IL-10 are the identified cytokines with anti-inflammatory and hepatoprotective roles in ALD. IL-6 and IL-10 regulate the expression of target genes involved in promoting cell proliferation, survival and differentiation through STAT3 [80]. Kupffer cells express high levels of receptors for IL-10. This binding leads to a prolonged activation of STAT3 and inhibits inflammatory responses. Conversely, IL-6 binding to its own receptor leads to transient activation of STAT3, followed by the induction of inflammatory responses. Activation of STAT3 induces the expression of the suppressor of cytokine signaling 3, which in turn inhibits STAT3 activation by IL-6 but does not inhibit IL-10 signaling, leading to an anti-inflammatory effect [81]. The role of IL-6 in ALD is complex and not entirely clear. Some studies have shown how its expression can reduce apoptosis in hepatocytes and lead to mitochondrial DNA repair [82]. In this regard, a study by Zhang et al. in mice models showed that IL-6 can activate enzymes to repair mitochondrial DNA in hepatocytes damaged by chronic alcohol consumption [83]. IL-6 promotes the differentiation of T helper 17 cells and the production of IL-17, contributing to alcohol-related inflammation. However, the activation of STAT3 by IL-6 and IL-10 inhibits the production of other proinflammatory cytokines, reducing liver damage in patients with ALD [84]. Pre-treatment with IL-6 induces hepatoprotection of steatotic liver isotransplants by preventing the apoptosis of sinusoidal endothelial cells, improving hepatic microcirculation and protecting against hepatocyte death [85]. Another study by El-Assal et al. showed that IL-6 knockout mice fed with alcohol exhibited increased liver fat accumulation, lipid peroxidation, mitochondrial DNA damage and hepatocyte sensitization to TNFα-induced apoptosis [86]. IL-10 knockout mice showed a more severe hepatic inflammatory response with higher levels of IL-6 and STAT3 activation than wild-type mice but lower liver steatosis severity and hepatocellular damage after higher alcohol levels or fatty dietary regimen [87]. Byun et al. demonstrated how treatment with polyinosinic:polycytidylic acid in vitro stimulates IL-10 production in HSCs through TLR-3 activation with reduction of alcoholic liver damage [88]. IL-10 plays an important role in the negative regulation of liver regeneration by limiting the inflammatory response and subsequently mitigating hepatic STAT3 activation [89]. A study conducted by Yang et al. on 40 ethnically Taiwanese Han patients with alcoholic liver cirrhosis showed how certain IL-10 promoter polymorphisms allow the development of severe forms of the disease. This result clarifies how a better knowledge of the genetic background of alcoholic liver cirrhosis is essential for its prevention and treatment [90].

5.2. IL-22

IL-22 is a cytokine belonging to the IL-10 family due to the similarity in genetic and protein structures. It is involved in several diseases, such as inflammatory bowel diseases and skin, pancreatic, lung and liver diseases [91]. IL-22 is only produced by hematopoietic cells, while its

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receptor (IL-22R) is expressed in different organs. It's a heterodimer composed of IL-10R2 and IL-22R1: while IL-10R2 is a subunit shared with various IL-10 family receptor complexes, IL-22R1 is only expressed in cells of various organs such as bronchi, liver, pancreas, and gut [92]. IL-22 plays a protective role in alcoholic liver damage through the activation of the STAT3-mediated signaling pathway. This event promotes the overexpression of anti-apoptotic and mitogenic genes, leading to tissue repair and survival of hepatocytes [93]. A recent study performed in mice models with ALD showed that four weeks of treatment with IL-22 reduced autophagy and liver fibrosis in hepatocytes [94]. In a recent prospective cohort study, IL-22 levels were significantly increased in alcohol-associated non-severe hepatitis and alcohol-associated severe hepatitis patients compared to the control group. As reported by the Authors, increased response to proinflammatory cytokines can mitigate liver damage [95]. Another investigation has clarified the role of IL-22 in liver regeneration. It was observed how IL-22 was significantly increased in murine models following liver regeneration and how this signaling blockade caused a reduction in liver regenerative capacity [96]. Table 2 summarizes the different studies on the involvement of anti-inflammatory cytokines in ALD. As previously reported, chronic alcohol consumption causes gut dysbiosis. Indeed, gut microflora imbalance reduces IL-22 production in the small intestine in mice models, which in turn leads to a decrease in antimicrobial C-type lectin regenerating islet-derived 3 gamma (REG3G). The reduction of REG3G causes an increase in bacterial translocation to the liver with consequent steatohepatitis [97].

Table 2. Summary of different studies about the involvement of anti-inflammatory cytokines in ALD.

Cytokine evaluated	Reference	Study design	Outcome
IL-6	Sun *et al.*, 2003 [85]	Pre-clinical study	Pre-treatment with IL-6 induces hepatoprotection of steatotic liver isotransplants by preventing the apoptosis of sinusoidal endothelial cells, improving hepatic microcirculation and protecting against hepatocyte death in mice models
	El-Assal *et al.*, 2004 [86]	Pre-clinical study	IL-6 knockout mice fed with alcohol exhibited increased liver fat accumulation, lipid peroxidation, mitochondrial DNA damage and hepatocyte sensitization to TNFα-induced apoptosis
	Zhang *et al.*, 2010 [83]	Pre-clinical study	IL-6 activates enzymes to repair mitochondrial DNA in hepatocytes damaged by chronic

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			alcohol consumption in mice models
	Miller *et al.*, 2011 [87]	Pre-clinical study	IL-10 knockout mice showed a more severe hepatic inflammatory response with higher levels of IL-6 and STAT3 activation, compared to wild-type mice, but lower liver steatosis severity and hepatocellular damage after higher alcohol levels or fatty dietary regimen
IL-10	Byun *et al.*, 2013 [88]	Pre-clinical study	Treatment with polyinosinic:polycytidylic acid in vitro stimulates IL-10 production in HSCs through TLR-3 activation with reduction of alcoholic liver damage
	Yang *et al.*, 2014 [90]	Case-control study	IL-10 promoter polymorphisms allow the development of severe forms of alcoholic liver cirrhosis
	Meng *et al.*, 2023 [94]	Pre-clinical study	Four-week treatment with IL-22 reduced liver fibrosis in mice models with ALD
IL-22	Sagaram *et al.*, 2023 [95]	Prospective cohort study	IL-22 levels were significantly increased in alcohol-associated non-severe hepatitis and alcohol-associated severe hepatitis patients compared to controls
	Liu *et al.*, 2023 [96]	Pre-clinical study	IL-22 levels were significantly increased in murine models following liver regeneration

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Legend: IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha; IL-10, interleukin-10; STAT3, adiponectin, signal transducer and activator of transcription 3; ALD, alcoholic liver disease; HSCs, hepatic stellate cells; TLR3, toll-like receptor 3; IL-22, interleukin-22.

6. New Therapeutic Approaches

6.1. Biological Drugs

The treatment of ALD is based on AUD therapy and the management of severe hepatitis associated with alcohol abuse [98]. To date, the drugs approved for the AUD are disulfiram, naltrexone, nalmefene, and acamprosate. Disulfiram, naltrexone and acamprosate are used for alcohol withdrawal, while nalmefene acts to reduce alcohol consumption. These drugs act on ethanol metabolism or, at the central nervous system level, by modulating the glutaminergic and opioid pathways [99]. Two other drugs, sodium oxybate and baclofen, are used to treat AUD in some countries, such as Italy and Austria [100]. To date, treatment for severe AH benefits exclusively from the use of corticosteroids. The basic mechanism is the reduction of inflammation by cytokines modulation [101]. Corticosteroids increase survival in the short term but not in the long term and are associated with collateral effects, such as infections [102,103]. Since ALD is characterized by a cytokine storm, a potential treatment may be the inhibition of pro-inflammatory cytokines or the increase of anti-inflammatory cytokines levels. In this way, pentoxifylline, a selective phosphodiesterase inhibitor, has been tested as a therapeutic agent for the treatment of severe AH, acting with a reduction of pro-inflammatory cytokines levels [104]. The STOPAH study comparing the efficacy of pentoxifylline and prednisolone showed that pentoxifylline did not improve survival in AH patients, while prednisolone reduced 28-day mortality [105]. Other agents, such as infliximab and etanercept, which act by blocking TNF-α production, have been studied for the treatment of severe AH. A study conducted in patients with severe AH treated with infliximab and prednisolone showed an increased mortality rate compared to patients treated with prednisolone alone [106]. A further study clarified how a single dose of infliximab could improve the severity and survival rate in severe AH. However, in both studies, the most common side effect was the development of infections. For this reason, further investigations are needed in order to use them as a potential therapeutic target [107,108]. Similarly to infliximab, etanercept has also proved unsuccessful in the treatment of severe AH [109]. Recently, new therapeutic targets have emerged. Already in preclinical models, IL-22 has proven effective in reducing liver damage associated with ALD [110,111]. Phase I clinical trials have demonstrated the safety and efficacy of recombinant human IL-22 Immunoglobulin G (IgG)2-Fc, showing that the latter has good tolerance with good pharmacokinetic and pharmacodynamic properties at the intravenous dose of 45μg/kg [112,113]. Recently, an open-label phase II study was conducted on F-652, a recombinant fusion protein of human IL-22 and IgG2-Fc. Specifically, 18 patients (9 with moderate AH and 9 with severe AH) were enrolled, and three growing doses of F-652 (10, 30, 45 μg/kg) were administered. The treatment proved to be safe, and the patients showed a high rate of improvement as determined by the Lille and Model for End-Stage Liver Disease prognostic scores, with decreased levels of inflammatory biomarkers and increased levels of liver regeneration biomarkers, compared to control subjects. Despite this, the small sample number defines how multi-center, randomized, placebo-controlled studies are needed to confirm the benefits of IL-22Fc therapy in patients with moderate-severe AH [114]. IL-22 has been shown to promote the development of HCC via a STAT3-mediated mechanism [115]. However, studies performed on transgenic mouse models with high levels of IL-22 showed no higher incidence of spontaneous tumor development than in wild-type mice [116]. Another therapeutic target for the treatment of severe AH may be IL-1β inhibition. The ongoing ISAIAH study evaluates the efficacy of the monoclonal antibody canakinumab at a dose of 3 mg/kg intravenously at baseline and after 28 days. The primary endpoint obtained was the histological improvement of AH on liver biopsy after 28 days of treatment compared to baseline, defined as a reduction in lobular inflammation [117]. A study was also conducted on the use of anakinra, an interleukin-1β receptor antagonist. Therapy with anakinra 100 mg by subcutaneous injection for 14 days plus zinc sulfate 220 mg for 90 days was administered and compared to prednisone 40 mg PO daily for 30 days. As reported by the Authors,

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therapy with anakinra plus zinc sulfate improved survival by 90 days in patients with severe AH [118]. However, evaluating if the drug can be eliminated efficiently in patients with impaired kidney function [119] is necessary. Overall, the above-mentioned study provided better knowledge about the effects and metabolism of anakinra in patients with severe AH [118].

6.2. Gut Microbiota Modulation

Gut microbiota modulation can be a safe therapeutic strategy for improving patients' quality of life [120]. In this way, a recent study performed on mice models with alcoholic liver damage showed that the probiotic *Lactobacillus plantarum* reduces the abundance of Gram-negative bacteria, with a reduction of the LPS content in the gut. Moreover, *Lactobacillus plantarum* J26 is able to maintain the integrity of the gut barrier, preventing bacterial translocation to the liver with a consequent reduction of the inflammation linked to alcohol consumption [121]. A further study on murine models compared the protective role of two strains of *Lactobacillus plantarum*, E680 and ZY08, in the ALD. Data reported that the intervention with *Lactobacillus plantarum* ZY08 significantly mitigated alcohol-related hepatic steatosis, liver damage, gut dysbiosis, and relieved plasma LPS levels as well as liver lipid metabolism [122]. A recent study performed in patients with severe AH showed that daily oral administration of *Lactobacillus rhamnosus* GG is associated with a significant reduction in liver damage after one month of treatment [123]. A double-blind, randomized, placebo-controlled, multicenter study is still ongoing with the aim of evaluating the efficacy of bovine colostrum in the treatment of AH. Bovine colostrum has two important components, lactoferrin and IgG: lactoferrin binds to lipid A to neutralize it, and IgG interacts with lymphoid tissue associated with the mucosa and reduces intestinal permeability. Indeed, this compound acts as an immunomodulator, reducing the inflammation typical of ALD [124]. Overall, further studies are required to better define the key role of gut microbiota modulation in the treatment of liver diseases [125,126].

7. Conclusions

In conclusion, pro-inflammatory and anti-inflammatory cytokines play a key role in the pathogenesis and progression of ALD. However, clarifications about the immune pathways involved in this pathology are needed. In this way, new therapeutic approaches, such as biological drugs and probiotics as targets of cytokines and the gut microbiota that modulates them, may be a good therapeutic option. Despite this, new studies are needed to evaluate the use of these promising therapies and their beneficial and adverse effects on ALD.

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Abbreviations

ADH: aldehyde dehydrogenase
AFL: alcoholic fatty liver
AH: alcoholic hepatitis
ALD: alcoholic liver disease
ASH: alcoholic steatohepatitis
AUD: alcohol use disorder
CXCL1: chemokine CXC motif chemokine ligand 1

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CYP2E1: cytochrome P450 2E1
ERK1/2: extracellular receptor-activated kinase 1/2
HCC: hepatocellular carcinoma
HSCs: hepatic stellate cells
IgG: immunoglobulin G
IL: interleukin
iNKT: invariant natural-killer T lymphocytes
KS: Korsakoff's syndrome
LPS: lipopolysaccharide
NF-κB: nuclear factor κB
NLRP3: NLR family pyrin domain containing 3
NOX: NADPH oxidase
PAMPs: pathogen-associated molecular patterns
REG3G: antimicrobial C-type lectin regenerating islet-derived 3 gamma
ROS: reactive oxygen species
STAT3: signal transducer and activator of transcription 3
TGF-β: transforming growth factor-β
TLRs: toll-like receptors
TNF-α: tumor necrosis factor-alpha
WE: Wernicke encephalopathy

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