

*Mechanisms of Disease*FRANKLIN H. EPSTEIN, M.D., *Editor***CYTOKINES IN ALCOHOLIC AND NONALCOHOLIC STEATOHEPATITIS**

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CYTOKINES are pleiotropic regulatory peptides that can be produced by virtually every nucleated cell in the body, including most types of liver cells.^{1,2} The cytokine family consists of several subfamilies: the interleukins, the tumor necrosis factor (TNF) family, interleukin-6 and related cytokines, interferons, chemokines such as interleukin-8, transforming growth factor β , colony-stimulating factors, and others.

In most tissues, including the liver, constitutive production of cytokines is absent or minimal. However, as physiologic and pathologic stimuli activate cells, the production of these autocrine, paracrine, and endocrine effector molecules increases, and they, in turn, orchestrate the tissue's response to the stimulus.^{1,2} There is increasing evidence that several cytokines mediate hepatic inflammation, apoptosis and necrosis of liver cells, cholestasis, and fibrosis,³⁻⁶ but paradoxically, they also mediate the regeneration of liver tissue after injury (Table 1).⁷⁻⁹

Among the various cytokines, the proinflammatory cytokine TNF- α has emerged as a key factor in various aspects of liver disease. Some of the most definitive data on the importance of TNF- α in the pathogenesis of liver disease come from studies of alcoholic and nonalcoholic steatohepatitis in animals. In combination with data from clinical studies, these findings indicate that TNF- α mediates not only the early stages of fatty liver disease but also the transition to more advanced stages of liver damage.

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CYTOKINES AND ALCOHOLIC LIVER DISEASE**Clinical Aspects of Alcoholic Liver Disease**

Long-term consumption of alcohol results in a spectrum of liver abnormalities, ranging from simple fatty liver (steatosis) to steatohepatitis, cirrhosis, and hepatocellular carcinoma.¹⁰ Fatty liver is often undetected, is seldom fatal, and resolves within a few weeks after alcohol consumption has ceased.¹¹ Furthermore, only a minority of consistently heavy drinkers with hepatic steatosis (about 15 to 20 percent) ever have clinically important liver disease,¹¹ suggesting that other host or environmental factors have a role in determining the evolution of alcohol-related liver disease. Risk factors for serious liver damage in habitual alcohol drinkers include certain polymorphisms in alcohol-metabolizing enzymes,¹² obesity,¹¹ exposure to other hepatotoxins (e.g., acetaminophen),¹¹ and infection with hepatitis C.¹³ In many patients, a specific risk factor is never identified. However, numerous anecdotal reports point to an association between episodes of gastrointestinal bleeding or sepsis and an acute, clinical decompensation of alcohol-related liver disease,¹¹ suggesting that endotoxemia potentiates the progression of the disease.¹⁴ The appearance of steatohepatitis is an important rate-limiting step in the development of progressive alcoholic liver disease.¹⁵ One-month mortality rates of 40 to 50 percent have been reported among patients hospitalized with acutely decompensated liver disease due to alcohol-induced steatohepatitis.¹⁶ Furthermore, cirrhosis develops in almost half the survivors within five years.^{11,15}

TNF- α and the Normal Liver

The production of TNF- α is one of the earliest events in many types of liver injury, triggering the production of other cytokines that together recruit inflammatory cells, kill hepatocytes, and initiate a healing response that includes fibrogenesis (Fig. 1). Although the liver's anatomical location and central role in drug and xenobiotic detoxification expose it to factors, such as reactive oxygen species¹⁷ and bacterial endotoxins,¹⁸ that induce the production of TNF- α in other tissues, transcription of the genes for TNF- α and other cytokines is barely detectable in the normal liver. Moreover, the administration of TNF- α to animals¹⁹ or the incubation of hepatocytes with TNF- α in vitro²⁰ promotes the proliferation of hepatocytes rather than their death. The same response occurs after a partial (70 percent) hepatectomy, an insult that increases the production of TNF- α in liver tissue. Indeed, studies of antibody neutralization⁷ and ex-

TABLE 1. KEY PROPERTIES OF CYTOKINES INVOLVED IN LIVER DISEASE.*

Proinflammatory cytokines
Interleukin-1-type cytokines (interleukin-1 α , interleukin-1 β , and TNF- α): prototype proinflammatory cytokines that stimulate the synthesis of acute-phase proteins
Interferon- γ : immunoregulatory cytokine secreted by Th1 that induces TNF- α
Interleukin-12: Th1-directing cytokine that promotes differentiation of T cells into Th1
Interleukin-18: interferon- γ -inducing factor that is proinflammatory at a very early step in the immune response
Antiinflammatory cytokines
Interleukin-1-receptor antagonist: prototype antiinflammatory cytokine that blocks the binding of interleukin-1 to cell-surface receptors
Soluble interleukin-1 receptor type II: binds circulating interleukin-1 α and interleukin-1 β
Soluble TNF receptors p55 (I) and p75 (II): naturally occurring TNF inhibitors that block TNF-regulated inflammatory processes
Glycoprotein 130-signaling cytokines (interleukin-6, interleukin-11, leukemia inhibitory factor, oncostatin M, ciliary neurotrophic factor, and cardiotrophin): proinflammatory and antiinflammatory cytokines that stimulate most acute-phase proteins; interleukin-6 regulates hepatic regeneration and immunoglobulin synthesis
Interleukin-10: prototype antiinflammatory cytokine that regulates B-cell function
Interleukin-4 and interleukin-13: cytokines secreted by Th2 that regulate B-cell function and suppress the synthesis of proinflammatory cytokines
Cytokines involved in immune responses
Interleukin-2, interleukin-4, interleukin-7, interleukin-9, interleukin-12, and interleukin-15
Cytokines secreted by Th1 (interleukin-2 and interferon- γ): proinflammatory cytokines that direct the antiviral response
Cytokines secreted by Th2 (interleukin-4, interleukin-5, and interleukin-10): mediate inflammation, allergic responses, and immunoglobulin synthesis
Cytokines involved in acute liver failure
TNF and TNF receptors p55 and p75
Cell-death receptors (Fas and Fas ligand): critically involved in liver injury and apoptosis
Interleukin-18: mediates TNF- and Fas-related liver failure in animals
Fibrogenic cytokines
Transforming growth factor β : prototype fibrogenic cytokine that is up-regulated by proinflammatory cytokines
Platelet-derived growth factor
Fibroblast growth factor
Antifibrogenic cytokines
Hepatocyte growth factor: antiapoptotic cytokine that promotes liver regeneration
Interferon- α : antiviral, immunomodulatory, and antiinflammatory cytokine

*TNF denotes tumor necrosis factor.

periments with mice that lack type I TNF receptors⁸ indicate that after partial hepatectomy, TNF- α initiates the regeneration of liver tissue by activating type I TNF receptors. Thus, although TNF- α can kill hepatocytes, cell death is not the normal outcome when hepatocytes are exposed to TNF- α . This suggests that normal hepatocyte responses to TNF- α are subverted during liver injury.

TNF- α -Initiated Death Signals and Hepatocyte Viability

Activation of TNF receptors by TNF- α or other members of the TNF superfamily causes an aggregation of receptors, which leads to the recruitment of various adaptor proteins that activate downstream kinases and proteases, including caspases.²¹ Mitochondria are important targets for TNF-initiated death signals. The subsequent release from mitochondria of reactive oxygen species, such as superoxide anion, as well as cytochrome *c* oxidase and other factors that induce apoptosis, plays a pivotal part in TNF-induced cell death (Fig. 2).^{22,23}

The fact that hepatocytes and many other cells can survive the activation of TNF receptors suggests that it is possible to minimize the production of these signals or to neutralize their actions. The entire defensive repertoire has yet to be characterized. However, there is evidence that synthesis of certain protective factors is necessary (Fig. 2), because hepatocytes can be sensitized to TNF-mediated cell death by pretreatment with agents that inhibit RNA or protein synthesis.²⁴ TNF-induced activation of the redox-sensitive transcription factor nuclear factor- κ B is likely to be involved in the protective response, because inhibition of the activity of nuclear factor- κ B promotes the death of hepatocytes exposed to TNF- α .²⁵ Several genes regulated by nuclear factor- κ B encode products that block the activation of caspases or the release of mitochondrial oxidants, including at least three antiapoptotic members of the bcl-2 family,²⁵ manganese superoxide dismutase,²⁶ and inducible nitric oxide synthase.²⁷ Although the experimental data are inconsistent, some findings suggest that the thresh-

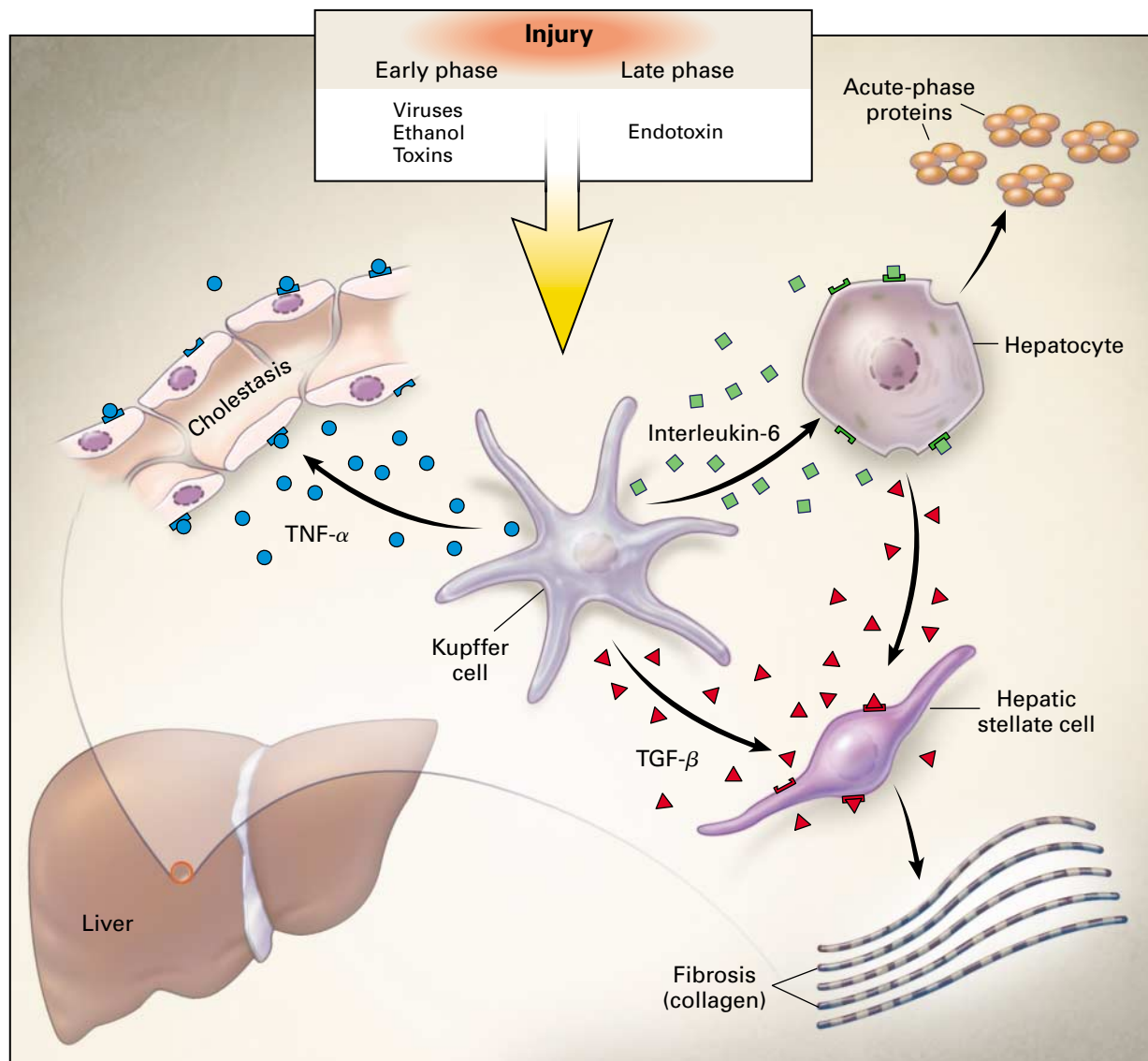


Figure 1. Pathophysiologic Relevance of Cytokines in Chronic Liver Disease.

Most types of cells in the liver, including Kupffer cells, hepatocytes, and stellate cells, either synthesize or respond to cytokines. In the early phase of chronic liver disease, specific agents such as viruses, ethanol, and toxins may stimulate the production of cytokines. In the late phase, endotoxin may be the key agent stimulating cytokine production. Clinical features of chronic liver disease that are mediated by cytokines include cachexia, cholestasis, fibrosis, synthesis of acute-phase proteins, and hypergammaglobulinemia. Whereas proinflammatory cytokines such as tumor necrosis factor α (TNF- α) and interleukin-6 are mainly involved in cholestasis and the synthesis of acute-phase proteins, transforming growth factor β (TGF- β) released by activated Kupffer cells and hepatocytes may be one of the critical cytokines involved in fibrosis. In patients with progressive liver disease, the balance between proinflammatory and antiinflammatory cytokines may be shifted toward the proinflammatory axis, thus the counteracting antiinflammatory cytokines are unable to control inflammation and fibrosis.

old for TNF-induced cell death is reduced in hepatocytes that are deficient in these proteins.²⁸ Thus, liver injury requires at least two “hits”: one that increases the exposure of hepatocytes to TNF- α and another that interferes with the hepatocyte’s normal ability to protect itself from TNF- α -induced cell death (Fig. 3).

The ultimate effect of TNF- α on hepatocytes in vivo is strongly influenced by other cytokines in liver tissue. For example, in mice that are deficient in interleukin-6 increased production of TNF- α induced by partial hepatectomy promotes the death of hepatocytes instead of stimulating their proliferation.⁹ Similarly, interruption of the gene for the antiinflammatory

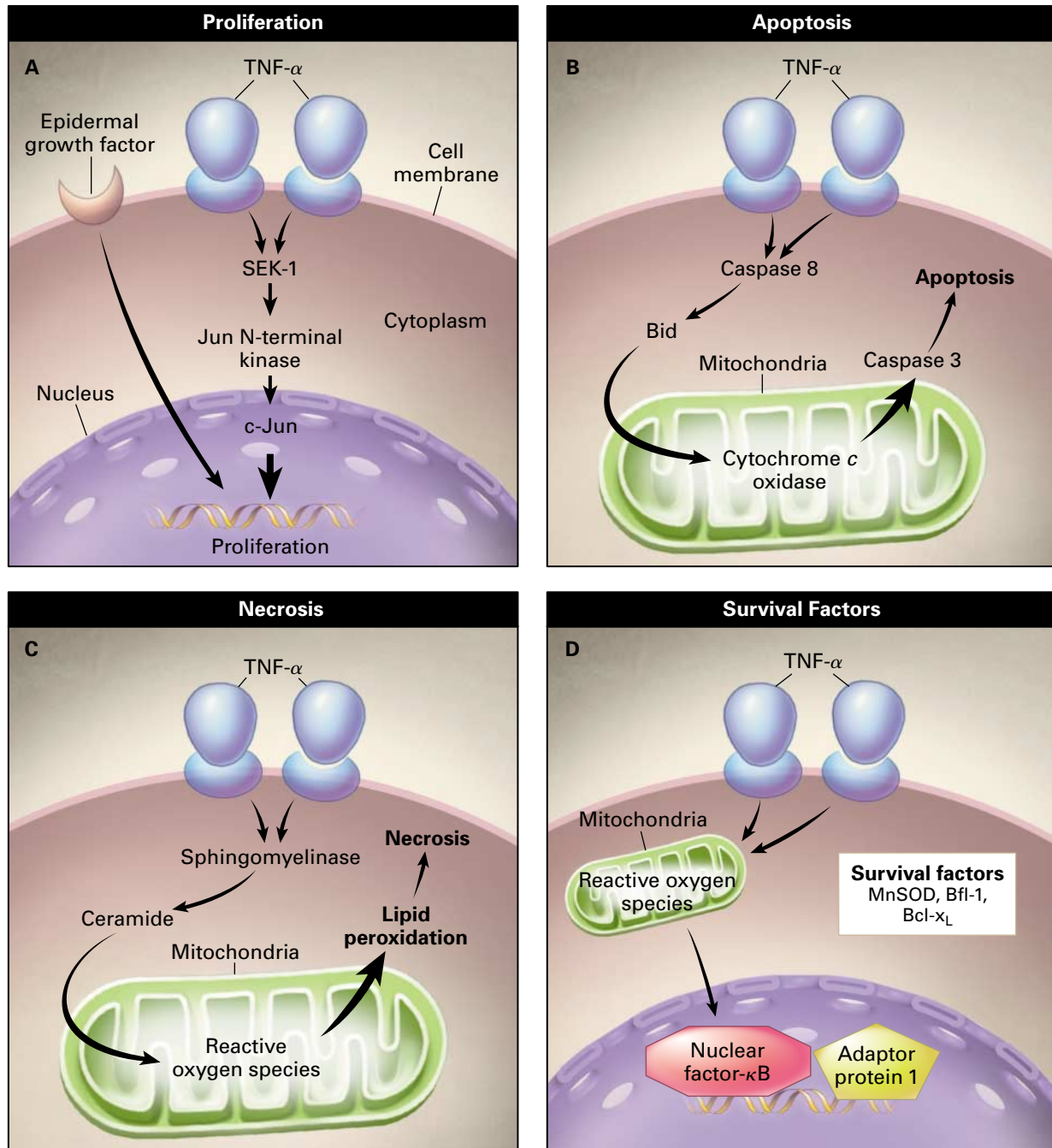


Figure 2. Hepatocyte Responses to Tumor Necrosis Factor α (TNF- α).

The interaction between TNF- α and its receptors on hepatocyte membranes causes the receptors to aggregate, which leads to the recruitment of various adaptor proteins that activate downstream kinases (Panel A), proteases (e.g., caspase 8) (Panel B), sphingomyelinase (Panel C), and transcription factors that regulate cell-survival factors (Panel D).

TNF- α initiates a stress-related protein kinase cascade involving Jun N-terminal kinase 1 (or SEK-1) and Jun N-terminal kinase that culminates in the phosphorylation of the proto-oncogene c-Jun, helping mitogens such as epidermal growth factor to promote proliferation (Panel A). TNF- α activates caspase 8, which cleaves the cytosolic protein Bid, a member of the BH3 subfamily of Bcl-2-related proteins. This truncated form of Bid is redistributed to mitochondrial membranes and permits the release of mitochondrial factors, including cytochrome *c* oxidase, that activate caspase 3 and cause apoptosis (Panel B). The induction of sphingomyelinase by TNF- α increases ceramide, an inhibitor of the activity of the mitochondrial electron-transport chain, leading to increased mitochondrial production of reactive oxygen species, which in turn promote lipid peroxidation and cellular necrosis (Panel C). TNF-related increases in reactive oxygen species also contribute to the activation of oxidant-sensitive transcription factors (e.g., nuclear factor- κ B and adaptor protein 1) that are necessary for the synthesis of cell-survival factors that protect mitochondria (e.g., antiapoptotic proteins such as Bfl-1 and Bcl-x_L and oxidant-detoxifying enzymes such as manganese superoxide dismutase [MnSOD]) (Panel D). Thus, hepatocytes respond to TNF- α by modulating signals that can lead to apoptosis and necrosis, as well as signals that promote cellular survival and proliferation.

ry cytokine interleukin-10 exacerbates TNF-mediated liver injury in mice.²⁹ Conversely, mice that are deficient in interleukin-12,³⁰ interferon- γ ,³¹ or interleukin-18³² are protected against TNF- α -induced liver damage.

Cytokine Studies in Patients with Alcoholic Liver Disease

The translocation of bacterial products from the intestinal lumen to the mesenteric circulation and its lymphatics induces regional and systemic production of TNF- α and other proinflammatory cytokines. Serum concentrations of TNF- α ^{33,34} and several TNF-inducible cytokines, such as interleukin-1,³⁵ interleukin-6,³⁶ and interleukin-8,³⁷ are also increased initially in hospitalized patients with alcoholic steatohepatitis and decline during recovery. This finding, coupled with evidence that long-term ingestion of alcohol increases intestinal permeability³⁸ and that patients with the highest serum cytokine concentrations have the highest rate of in-hospital mortality,^{34,36} indicates that intestinally derived endotoxin and endotoxin-induced cytokines, such as TNF- α , have a role in the pathogenesis of steatohepatitis. Furthermore, serum concentrations of both TNF- α and soluble TNF receptors are correlated with the degree of endotoxemia and the stage of liver disease in patients with alcoholic liver disease.³⁹

TNF- α and Alcoholic Liver Disease in Animals

The possibility that increased production of TNF- α is the consequence, rather than the cause, of alcohol-related liver injury cannot be ruled out. Indeed, the former possibility is suggested by evidence that the serum concentrations of various cytokines are increased in patients with acute or chronic liver diseases.³⁹⁻⁴¹ On the other hand, close temporal associations among Kupffer-cell activation, increased transcription of the genes for TNF- α and related cytokines, hepatic inflammation, and liver-cell death have been reported in rodents with alcohol-induced steatohepatitis.^{42,43} Furthermore, alcohol-associated liver injury is inhibited when the animals are treated with poorly ab-

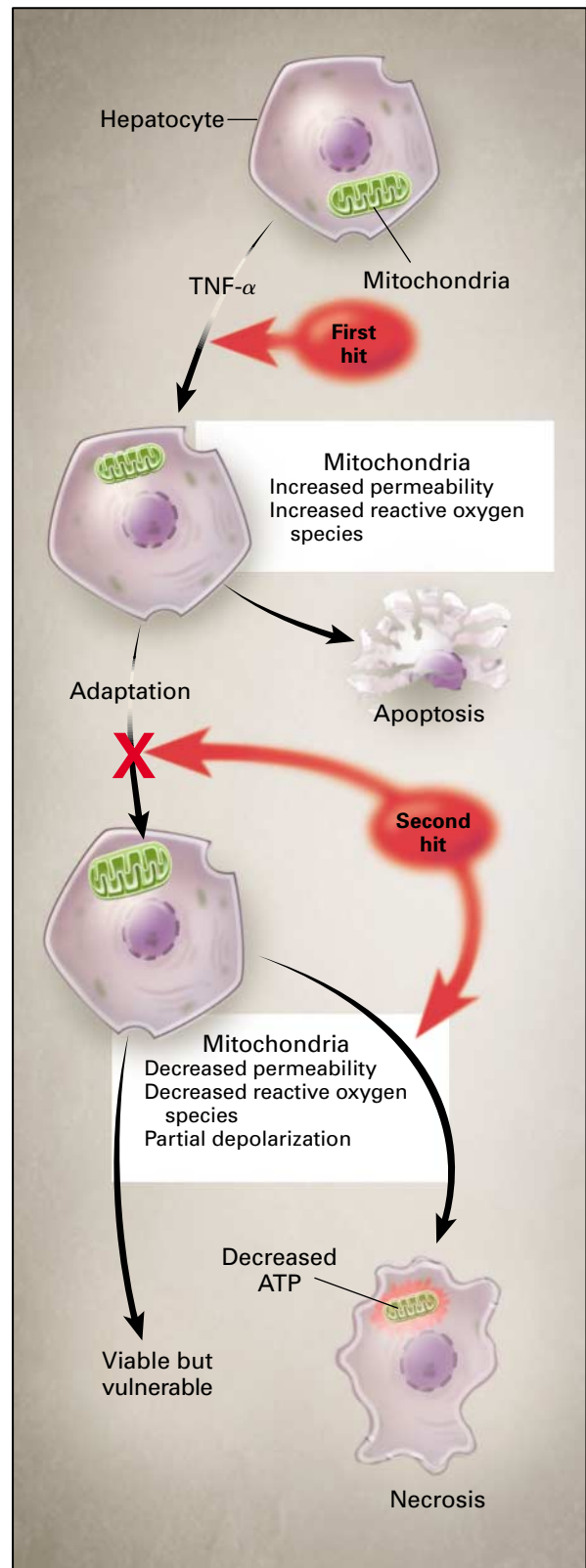


Figure 3. Two-Hit Model of the Progression of Fatty Liver Disease. The earliest stages of fatty liver disease involve increased exposure of hepatocytes to tumor necrosis factor α (TNF- α). TNF- α initiates various intracellular signals that increase mitochondrial permeability and the release of reactive oxygen species. Left unchecked, these responses promote hepatocyte apoptosis. Fortunately, most healthy hepatocytes use some of the potentially lethal signals (e.g., reactive oxygen species) to activate a multifaceted response that permits the hepatocytes to survive. Secondary insults (second hits) that inhibit this adaptation also result in apoptosis. Even when adaptation is successful and hepatocytes remain viable, they become extremely vulnerable to other insults that partially depolarize the mitochondrial inner membrane, dying by necrosis when the mitochondrial membrane potential collapses.

sorbed oral antibiotics⁴⁴ or lactobacillus⁴⁵ in order to decrease endotoxemia or when they are treated with antibodies that neutralize the action of TNF- α .⁴⁶ The recent finding that exposure to alcohol does not induce steatohepatitis in mice in which the gene for type I TNF receptor is disrupted⁴⁷ constitutes the best evidence that TNF- α is a key pathogenic factor in alcohol-related liver injury. Even though TNF- α appears to be necessary for the development of alcohol-related steatohepatitis, however, increased production of this cytokine is not sufficient to cause liver injury. The importance of other factors in the pathogenesis of alcoholic liver disease is supported by evidence that the disease is also inhibited in rats treated with gadolinium chloride to deplete the liver of Kupfer cells. After the administration of gadolinium chloride, hepatic and serum concentrations of TNF- α increase dramatically,⁴⁸ but liver injury does not occur.⁴⁹

Long-Term Alcohol Ingestion and Hepatocyte Vulnerability to TNF- α

Unlike normal hepatocytes, which are resistant to TNF- α -induced apoptosis, hepatocytes from rats with alcohol-induced fatty livers die quickly after in vitro exposure to small amounts of TNF- α .⁵⁰ Several mechanisms have been implicated, many of which involve hepatocyte mitochondria, organelles that play a central part in TNF-induced apoptosis and necrosis.⁵¹ In alcohol-induced fatty livers, the mitochondria have ultrastructural abnormalities, including swelling and disruption of the inner membrane.⁵² These anatomical abnormalities are correlated with alcohol-related inhibition of mitochondrial respiration,⁵² increased generation of mitochondrial oxidants,⁵³ depletion of mitochondrial glutathione,⁵⁴ and multiple mitochondrial DNA deletions similar to those that occur during aging.⁵⁵ Although viable hepatocytes may have these mitochondrial abnormalities, they are likely to potentiate vulnerability to TNF- α -induced cell death. The molecular mechanisms involved in cell death remain uncertain. Long-term exposure to alcohol may promote hepatocyte apoptosis⁵⁶ or necrosis.^{10,57} Perhaps long-term exposure to alcohol potentiates both processes by depleting antioxidants, such as mitochondrial glutathione, that protect hepatocytes from necrosis and apoptosis,⁵⁰ while inhibiting the production of nuclear factor- κ B, an antiapoptotic transcription factor, in response to increased hepatic production of TNF- α .⁵⁸

CYTOKINES AND OBESITY-RELATED FATTY LIVER DISEASES

Similarities between Alcoholic and Nonalcoholic Fatty Liver Diseases

Obesity and alcohol abuse are associated with the same spectrum of liver diseases.⁵⁹ The time course for the progression of disease and the relative risk of cirrhosis are also similar in obesity-related liver dis-

ease and alcoholic liver disease.⁶⁰ The similar pathologic features and natural histories of the two types of fatty liver disease suggest that common pathogenic mechanisms may be involved. Distinct, but mutually enhancing, mechanisms may also be involved, because a fatty liver is more common in patients with obesity and alcohol abuse than in those with either alone. Moreover, obesity is an independent risk factor for cirrhosis in patients who abuse alcohol.¹¹ Thus, elucidation of the mechanisms that cause obesity-related fatty liver disease is likely to clarify the role of pluripotent cytokines, such as TNF- α , in promoting alcohol-induced liver damage.

Liver Disease in Genetically Obese Rats and Mice

Studies of genetically obese ob/ob mice and fa/fa rats have provided information about the pathogenesis of obesity-related fatty liver disease. Both rodent strains have spontaneous mutations that either diminish production of the appetite-suppressing hormone leptin (in ob/ob mice) or inactivate the leptin receptor (in fa/fa rats).^{61,62} Like many obese humans, obese ob/ob mice and fa/fa rats have insulin resistance, hyperglycemia, hyperlipidemia, and fatty livers.⁶³ These rodents also have several immunologic abnormalities, including phagocyte dysfunction, altered transcription of cytokine genes (including constitutive increases in TNF- α), and excessive production of prostanoids by hepatic and peritoneal macrophages.⁶⁴⁻⁶⁶

Just as abnormalities of hepatocyte mitochondria have been found in alcohol-induced fatty livers, ultrastructural and functional alterations of hepatocyte mitochondria have been found in the fatty livers of ob/ob mice.^{67,68} Also, ob/ob mice with fatty livers, like patients and rodents with alcohol-induced fatty livers, have little evidence of associated hepatitis until they are subjected to another insult.⁶⁹ Nevertheless, even in ob/ob mice with early fatty liver disease, hepatocytes synthesize both proapoptotic and antiapoptotic proteins that are not detected in hepatocytes from normal mice,⁶⁹ suggesting that apoptotic stress contributes to obesity-related fatty liver disease.

Hepatic accumulation of fat may be the simplest explanation of obesity-related hepatomegaly, but the administration of drugs that stimulate the proliferation of peroxisomes also increases the ratio of liver weight to body weight in mice.⁷⁰ Hepatomegaly occurs because the rate of hepatocyte apoptosis becomes insufficient to match the rate of proliferation of hepatocytes. Drugs that stimulate peroxisome-proliferator-activated receptor γ , such as thiozolidinediones, stimulate the production of mitochondrial uncoupling protein in adipocytes.⁷¹ Although uncoupling protein is barely detectable in hepatocytes from normal adults,⁷² hepatic synthesis of uncoupling protein 2 increases after the induction of peroxisome-proliferator-activated receptor α in hepatocytes.⁷³ The rate of production and activity of uncoupling protein 2

are also increased in hepatocytes from obese ob/ob mice.⁶⁸ Increased uncoupling protein 2 in mitochondria depolarizes the inner mitochondrial membrane, increasing the activity of the electron-transport chain while limiting the production of superoxide anion and the accumulation of calcium.⁷⁴ Thus, synthesis of uncoupling protein 2 in a fatty liver may help inhibit hepatocyte apoptosis,⁷⁵ and this may explain why the activation of peroxisome-proliferator-activated receptor α increases hepatocyte survival. However, because cells with increased uncoupling-protein activity have partially depolarized mitochondria, they may also be more vulnerable to loss of the mitochondrial inner-membrane potential, with consequent depletion of ATP and necrosis, if exposed to certain secondary insults such as endotoxin or TNF- α .^{64,68,76,77}

Increased production of uncoupling protein 2 has been reported in hepatocytes from some patients with nonalcoholic steatohepatitis or alcoholic hepatitis.⁷⁸ Although it remains uncertain whether uncoupling protein 2 contributes to the pathogenesis of these diseases, both animals and patients with alcohol-induced liver disease or nonalcoholic steatohepatitis have a diminished capacity to replenish liver ATP stores after transient, experimentally induced depletion of ATP.^{68,79} Thus, uncoupling protein 2 may be one component of a general adaptive response that preserves the viability of hepatocytes in fatty livers but also increases the vulnerability of these cells to subsequent insults.

TNF- α AND PROGRESSION FROM STEATOHEPATITIS TO CIRRHOSIS

Transient reconfiguration of the extracellular matrix of the injured liver, which permits the infiltration of inflammatory cells, facilitates the local accumulation of growth factors, and accommodates regenerating hepatocytes, is a critical component of healing after liver injury.⁸⁰ After acute liver injury from various insults — for example, toxins, viral infections, or surgical trauma — the resorption and deposition of the components of the matrix are balanced, and therefore there is no accumulation of fibrous tissue. However, chronic inflammatory conditions, such as alcoholic and nonalcoholic steatohepatitis, alter the composition of the matrix and upset the balance between the synthesis and degradation of the matrix. Consequently, fibrosis occurs, compromising portal venous blood flow, which in turn both compromises hepatic regeneration and promotes the portosystemic shunting of blood that leads to some of the clinical manifestations of advanced liver disease.⁸¹

TNF- α is involved in the pathogenesis of cirrhosis, but additional factors must also be present.⁶ Otherwise, cirrhosis would be a universal consequence of liver injury. Although there is little doubt that cirrhosis is one of the most clinically relevant outcomes of steatohepatitis, a detailed discussion of the mechanisms that regulate the activation of stellate cells,

matrix-gene expression, and matrix remodeling is beyond the scope of this review. TNF- α promotes each of these responses.^{82,83} The remaining challenge is to differentiate the cellular and environmental factors that interact with TNF- α to promote normal matrix remodeling from the factors that promote fibrosis. Although the mechanisms are not well understood, steatohepatitis modifies the response of hepatic stellate cells to injury-related cytokines so that both transcriptional and post-transcriptional mechanisms that promote the deposition of type 1 collagen are induced preferentially.^{82,83} In addition, the microenvironment becomes more conducive to the survival of stellate cells, because the number of stellate cells increases as cirrhosis evolves.⁶ Moreover, even after cirrhosis is well established, the ongoing production of TNF- α and related inflammatory cytokines modulates the expression of enzymes, such as inducible nitric oxide synthase, that regulate the production of vasoactive molecules, which mediate the hemodynamic abnormalities of cirrhosis, such as portosystemic shunting and the hepatorenal syndrome.^{84,85}

INHIBITION OF TNF- α AND TREATMENT OF STEATOHEPATITIS

Current treatments for liver disease related to use of alcohol or obesity focus on reducing alcohol intake and obesity, respectively. Abstinence from alcohol prolongs the survival of patients with alcoholic liver disease, whether or not they have cirrhosis, but usually does not prevent the progression to cirrhosis.^{11,15} Although there is some evidence that abstinence improves the regenerative capacity of the liver in patients with cirrhosis, once cirrhosis has developed, it persists despite the cessation of alcohol use. Similarly, weight loss is an imperfect cure for obesity-related liver disease. Weight reduction may decrease serum aminotransferase concentrations in obese patients,⁸⁶ but rapid or extreme weight loss appears to promote the transition from steatosis to cirrhosis.⁸⁷ Thus, although there is little doubt that obesity is a risk factor for liver disease, weight reduction is not always an effective treatment and may even exacerbate the disease.

On the basis of data from studies of alcohol- and obesity-related liver disease in animals, alternative therapies have been proposed. Treatment with various antioxidants (Table 2) decreases alcohol-induced liver damage in rats^{11,88-91} and improves the fatty liver that develops in rats fed choline- and methionine-deficient diets,⁹² but so far, the benefits have been inconsistent in the small groups of patients with alcoholic liver disease who have been treated with similar agents.⁹³⁻⁹⁷ Few clinical trials of putative antioxidants have been performed in patients with nonalcoholic steatohepatitis.^{97,98} However, evidence that TNF- α mediates insulin resistance in ob/ob mice with fatty livers⁹⁹ and the strong correlation between type 2 di-

TABLE 2. POTENTIALLY BENEFICIAL TREATMENTS IN STUDIES IN ANIMALS OR CLINICAL TRIALS.*

THERAPY	PURPOSE
Antibiotics, lactobacillus	Prevent gut-derived endotoxemia
Anti-TNF antibodies, TNF-receptor antagonists	Inhibit TNF- α activity
Glucocorticoids	Inhibit inflammation
Glutathione prodrugs (S-adenosylmethionine, betaine, choline), vitamin E, silymarin, propylthiouracil	Decrease production of reactive oxygen species
Ursodiol, gemfibrozil	Reduce liver lipids
Leptin, thiozolidinediones	Improve insulin sensitivity

*TNF denotes tumor necrosis factor.

abetes and nonalcoholic steatohepatitis¹⁰⁰ have generated support for trials of insulin-sensitizing drugs. To date, this approach has proved effective only in transgenic mice with steatohepatitis. Mice with adipocyte-targeted overexpression of active sterol regulatory element-binding protein (SREB) 1c are born with lipodystrophy, and severe insulin resistance and steatohepatitis develop in these animals at a young age.¹⁰¹ They have a paucity of white adipose tissue and are therefore deficient in adipose gene products, such as peroxisome-proliferator-activated receptor γ and leptin.¹⁰¹ Treatment of SREB transgenic mice with leptin improves both their insulin resistance and steatohepatitis.¹⁰²

Evidence of the important role that TNF- α has in animals with steatohepatitis suggests that TNF- α is another potential therapeutic target in patients with the disease. Various treatments that decrease the absorption of intestinal endotoxin^{44,45} or inhibit the activity of TNF- α ,^{46,47} an endotoxin-inducible cytokine, prevent alcohol-induced liver disease in animals. Treatment with glucocorticoids has been shown to be effective in carefully selected patients with severe, acute alcohol-induced steatohepatitis (excluding those with active bacterial infections, insulin-requiring diabetes, active gastrointestinal bleeding, or acute pancreatitis).¹⁶ No trials of other antiinflammatory or anticytokine therapies in patients with alcoholic or obesity-related liver disease have been reported.

CONCLUSIONS

Endotoxin-inducible cytokines such as TNF- α , TNF-regulated cytokines, and cytokines that modulate the synthesis and biologic actions of TNF are produced by many cells within the liver and other organs. This extensive cytokine network mediates various aspects of liver injury and repair. Consequently, cytokines ultimately control the pathophysiology and pro-

gression of liver diseases. The importance of cytokines as effector molecules in liver damage has been particularly well demonstrated in patients and animals with alcoholic or nonalcoholic liver diseases ranging from steatosis to cirrhosis. Thus, antagonism of TNF- α and other injury-related cytokines merits evaluation as a treatment for these diseases. However, since the same cytokines are also necessary for the regeneration of tissue after the liver has been injured, cytokine antagonism is not without risk in patients with liver disease, and complete inhibition of TNF- α activity might impair hepatic recovery. The cellular signals that mediate the various actions of TNF- α must be delineated so that new therapeutic agents can be developed to inhibit TNF-initiated cell-death signals preferentially.

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