MINI-REVIEW

Mechanisms of alcoholic liver injury

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SW French. Mechanisms of alcoholic liver injury. Can J Gastroenterol 2000;14(4):327-332. There have been numerous recent advances in the understanding of the mechanisms of alcoholic liver disease pathogenesis. Endotoxin-induced Kupffer cell activation plays a role in cytokine-mediated inflammatory changes in the liver, and this can be blocked by a diet high in saturated fat, by a diet containing lactobacillus, which does not produce endotoxin, by neomycin antibiotic sterilization of the gut, by eliminating Kupffer cells, or by removing tumour necrosis factor-alpha with antibody or by using tumour necrosis factor-alpha knockout mice. The fatty liver component is mainly the result of the nicotinamide adenine dinucleotide/reduced nicotinamide adenine dinucleotide redox shift to the reduced state by ethanol oxidation generation of reduced nicotinamide adenine dinucleotide, although this too can be blocked by a diet high in saturated fat. Hepatocytic enlargement occurs due to ethanol-induced inhibition of the ubiquitin-proteasome pathway of cytoplasmic protein degradation and the retention of oxidized proteins in hepatocytes. The liver is scarred by stellate cells that have been activated by inflammatory cytokines and growth factors produced by activated Kupffer cells, and by bile ductule metaplasia. Mallory bodies and balloon cell degeneration develop through the ethanol-induced oxidative stress-protein kinase activation pathway, inhibition of phosphatase activity and inhibition of the ubiquitin-proteasome pathway.

Key Words: Cytokines; Ethanol; Growth factors; Kupffer cells; Stellate cells

Progress in understanding how ethanol causes liver damage has been made possible through the use of rodent models of alcoholic liver disease (ALD). Isolation of liver cellular constituents in culture and the use of mouse knock-out models have further focused investigations of the mechanisms involved. The roles of inflammatory cytokines and chemokines, as well as of growth factors have been further defined (Figure 1). Progress in understanding the role of metabolic changes and consequences of oxidant stress has revealed new concepts regarding liver injury and subsequent fibrosis and cirrhosis in animal models. The mechanisms of hepatocellular swelling, cytokeratin aggregation (ie, Mallory bodies [MBs]) and apoptosis are better understood. This progress is the subject of the present review.

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The focal sinusoidal inflammatory response to Kupffer cell activation in
Figure 1) Schematic representation of the four types of cells involved in
the focal sinusoidal inflammatory response to Kupffer cell activation in
alcoholic liver disease. Cell to cell communications result from various 
secretory products and cell receptors. Cytokines, proteases, free radicals, 
eicosanoids and endotoxin are involved in paracrine and autocrine stimu-
lated responses (arrows). IL Interleukin; LPS Lipopolysaccharide; LTB4 Leukotriene B4; NO Nitric oxide; PGE Prostaglandin E; PMN 
Polymorphonuclear leukocytes; TBX T-box; TGFβ Transforming growth factor-beta; TNF Tumour necrosis factor

The role of the Kupffer cell as the primary target of ethanol 
that endotoxin levels were elevated in the blood of rats fed 
levels and the severity of the liver pathology (ALD) that induced fatty change and liver cell necrosis. 
showed subsequently, using the same model, that replacing gut bacteria with lactobacillus, which do not 
endotoxia, prevented the development of ALD pathology. This has been confirmed by reducing LPS in the 
by feeding the rats neomycin to sterilize the gut (4). The significance of this observation became apparent when it 
with the use of the same rat model, that the liver pathology caused by ethanol feeding was largely pre-
vented by eliminating the Kupffer cells with gadolinium 
Kupffer cells isolated from rats with experimental ALD showed increased TNFα mRNA expression. Kupffer cells isolated from the rat model 
showed increased gene expression of TNFα, interleukin (IL) 
-6 and transforming growth factor-beta (TGFβ) (9). In fact, 
immunohistochemistry experiments performed in the Kupffer cell 
overproduction of TNFα is an important step in the development of ALD.

As is LPS (6,7), and probably accounts for the anorexia that 
these patients experience. Nanji et al (8) reported that livers 
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Of course, Kupffer cell activation by LPS and ethanol 
leads to increased secretion of cytokines IL-1, -6 and -8, 
and the chemokines chemokine induced neutrophil chemotactant, macrophage inflammatory protein 
(MIP), monocyte chemotactant protein-1 (MCP-1), 
platelet-activating factor and insulin-like growth factor 1 (IGF-1), through the activation of nuclear factor kappa B (NFkβ) transcription (1). Oxidative stress activates NFkβ, leading to the induction of inducible nitric oxide synthase 
and nitric oxide generation (1). Through the CD14, recep-
tor LPS activates phospholipase C and protein kinase C (PKC) in the Kupffer cell (12), increases cytosolic calcium and increases reduced nicotinamide adenine dinucleotide 
phosphate oxidase generation of superoxide (1). The LPS- 
CD14 response is augmented by the liver cell release of 
LPS-binding protein, an acute phase response protein stimulated by IL-6 produced by the Kupffer cell. 
Cyclo-oxygenase-2 is induced in the Kupffer cells, which leads to an increase in thromboxane A2, a potent agonist for 
vasoconstriction, thrombosis, ischemic necrosis and inflam-
I (13). This is augmented by activation of 
phospholipase A2 generation of arachidonic acid (13). PKC is also activated by oxidative stress, which activates tyrosine 
kinase and the mitogen-activated protein (MAP) kinase cascade. Both the increase in cytosolic calcium and the 
MAP kinase cascade increase phospholipase A2, which probably accounts for the activation and sensitization of the 
Kupffer cell to augment further injury caused by further challenges with ethanol. Kupffer cells undergo an acute desensit-
antion after challenge followed by increased sensitivity to rechallenge a few hours later (14).

Kupffer cell-mediated injury is amplified by cytokine-
mediated induction of cell adhesion molecules (integrins, ie, 
intercellular adhesion molecule, vascular cell adhesion 
molecule, IL-8 and leukotriene B4.) Monocytes, lympho-
cytes and neutrophils are thus immobilized within the sinus-
oid. These inflammatory foci generate superoxide and proteases. 
Myeloperoxidase generates hypochloric acid, leading to focal damage and hepatocellular necrosis.

Active Kupffer cells also release proteases such as uroki-
nase, which may affect neighbouring hepatocytes because urokinase activates hepatocyte growth factor (HGF) to 
stimulate hepatocellular regeneration.

There are three mitigating circumstances where ethanol 
ingestion may not activate Kupffer cells. For instance, a high 
fat diet is required if ethanol is to induce ALD pathology by 
activating Kupffer cells (15), ie, NFkβ activation, TNFα 
and MIP-1 mRNA expression. Another modifier is the in-
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Liver cell proteins become damaged through peroxidation by the CYP 2E1-generated free radical formation of peroxyls and alkoxys, which leads to the accumulation of oxidized proteins in hepatocytes (29). Concomitantly, ethanol feeding leads to a decrease in ubiquitin in hepatocytes (30). Ubiquitin is necessary to prepare the cytosolic proteins for degradation by the proteasome pathway. Compounding this problem in the removal of cytosolic proteins, ethanol feeding leads to inhibition of the proteolytic enzymes in the proteasome (31). Consequently, proteins accumulate in hepatocytes (31), which probably accounts for the ‘ballooning’ of hepatocytes seen in ALD. Oxidized proteins, in increased amounts, inhibit protein degradation by the proteasomes (32).

Oxidation of mitochondrial DNA is also induced by chronic alcohol ingestion (33). Mitochondrial DNA deletions occur in the liver of alcoholics associated with steatosis (34). Both errors in cytosine loci where wrong nucleotides are incorporated during replication of DNA cause point mutations (34). In addition DNA, deletions are observed in the liver mitochondria of alcoholics (35). These changes are reversible when alcoholic patients stop drinking (36).

The cell origin of collagen in the liver, including the source of scarring and cirrhosis, is the stellate cell, formerly known as the Ito cell or lipocyte. Recent focus on the role of this cell in ALD has revealed that it is activated by fibronectin and cytokines IL-1, TGFβ and IL-6. Fibronectin is derived from sinusoidal endothelial cells (37,38). It is increased in the space of Disse early in the course of ethanol feeding (39), followed by an increase in collagen IV (40), followed by capillarization of the sinusoid by laying down basement membrane by the endothelial cells (40). Although the focal activation of stellate cells that is due to focal necrosis occurs early in the course of experimental ALD (one to two months of alcohol feeding), diffuse activation of stellate cells occurs late in the course of ethanol feeding (five to six months of feeding ethanol) (41). Extensive centrilobular perisinusoidal fibrosis due to diffuse stellate cell activation requires polyunsaturated fatty acids in the diet (42-44) and a high fat diet (14).

Activation of stellate cells, either focally or diffusely, probably results from paracrine stimulation by Kupffer cells, endothelium and hepatocytes, which release TGFβ IL-1, TGFα, platelet-derived growth factor (PDGF), TNFα and IGF-1 (37,45,46). Acetaldehyde (15) and interferon (45) inhibit stellate cell activation. This may explain why activation of stellate cells in vivo takes so long to be induced by ethanol. Activated stellate cells become responsive or hypertensive in tissue culture to cytokines and growth factors through paracrine and autocrine mechanisms. These factors include IGF-1, TGFα, TGFβ, colony stimulating factor-1, HGF, IL-6, PDGF, epidermal growth factor (EGF), MCP-1, fibronectin and endothelin-1 (37,46). The activated stellate expresses a complex phenotype that includes the following newly acquired functions: proliferation, migration, contraction, collagen and fibronectin synthesis, white blood cell chemotaxis and collagenase secretion. Focal in-
Figure 2) Schematic representation of pericellular fibrosis and bile duct metaplasia induced fibrosis. Cytokines, proteases, growth factors and inhibitors are involved. aFGF Acidic fibroblast growth factor; BD Bile duct; BM Basement membrane; C Collagen; E Endothelium; H Hepatocyte; HGF Hepatocyte growth factor; K Kupffer cell; PDGF Platelet-derived growth factor; S Stellate Cell; TGF Transforming growth factor; TIMP Tissue inhibitors of matrix metalloproteinases

jjury, leading to local scarring, is quickly resolved unless the stimulus is sustained. It is not known which factors perpetuate the scarring process that leads to cirrhosis. Scars that do not lead to septal formation can be completely removed by collagenase over time. Thus, permanent scarring is the result of the balance between collagen synthesis and degradation over time. Collagenase inhibitors (tissue inhibitors of matrix metalloproteinases) may be important in maintaining this balance (47).

Activation of stellate cells leads to the deposit of a great variety of extracellular matrix proteins (37) secreted by the stellate cells. Among these is hyaluronic acid (37), which is elevated in the blood during active liver fibrosis. Hyaluronic acid is normally cleared by hepatic endothelial cells, but in hepatic fibrosis in ALD the endothelial cells fail to clear it (48). The CD44 receptor isoform for hyaluronic acid is expressed by stellate cells in liver injury, and this may be a factor in the migration of stellate cells to the site of injury in the localized fibrosis in ALD (49).

One important property of the activated stellate cell is its enhanced contraction in response to endothelin-1 from endothelial cells (50). Thus, activated stellate cell contraction may explain the damage to hepatocytes that occurs in the centrilobular zone because of the liver hypoxia that develops in rats fed ethanol chronically (51).

One important mechanism by which stellate cell proliferation plays a role in the pathogenesis of alcoholic cirrhosis involves the liver cell bile ductular metaplasia phenomenon (52). In this phenomenon, liver cells undergo a phenotypic switch to form ductules in the limiting plate of hepatocytes at the periportal zone during the development of cirrhosis (18). The stellate cells located next to the switched hepatocytes proliferate to provide stroma for the newly formed ductules in a process that recapitulates bile duct formation in the fetal liver (53). This is a self-perpetuating interaction that leads to progressive fibrosis and cirrhosis due to growth factors released by the ductules (acidic fibroblast growth factor), which stimulates stellate proliferation, and HGF and stem cell factor released by the stellate cells, which stimulates bile ductule proliferation (54, 55). Endothelin-1 (56), TGFβ (57), EGF and IL-6 (58) may also participate in this stimulation-proliferation process of perportal fibrosis (Figure 2).

The role of MB formation in ALD has recently been better defined. The formation of MBs in drug-primed mice fed ethanol intragastrically for seven days provides a model to determine their pathogenesis (59) because of the short interval between starting ethanol ingestion and MB formation. In this model, CYP 2E1 was induced and cytokeratin 8 was increased, whereas the mRNA for CYP 2E1 tended to decrease and cytokeratin-8 mRNA was significantly decreased. The discrepancy between the protein levels and the mRNA expression indicates an inhibition of proteolysis by the proteasome as the mechanism for cytokeratin-8 and CYP 2E1 accumulation. This fits the hypothesis that MBs result from the accumulation of cytokeratins that are conformationally altered so as to resist proteolysis, which is inhibited by ethanol ingestion (60). Evidence that the MBs are composed of cytokeratins that have undergone profound alterations in their conformation was obtained by infrared spectroscopy of the amide I spectrum of isolated mouse and human MBs (61).

Hyperphosphorylation of MBs (60) likely accounts for their conformational change. Secondary ubiquitination (62) of MBs may add to their resistance to proteolysis. The question of what is the mechanism of hyperphosphorylated MBs remains. Studies on MB phosphorylation indicate that PKC is the main kinase involved, probably triggered by oxidative stress. Ethanol induces hyperphosphorylation of cytokeratins in hepatocytes within 15 mins in primary tissue culture of hepatocytes through a PKC mechanism (63). Okadaic acid increases phosphorylation of liver cytokeratins in vivo within 15 mins by inhibiting serine-threonine phosphates 1 and 2A (60). Hyperphosphorylation was indicated using an antibody to phosphothreonine. Aggregates of cytokeratin resulted, and these stained positive for cytokeratin and ubiquitin and phosphothreonine in the same manner as do MBs (60). The aggregates were present in 'empty' hepatocytes that failed to stain for cytokeratins except where the aggregates were found. The 'empty' cells correspond to the balloon cells containing MBs seen in ALD in humans (64). These acute changes induced by okadaic acid occurred at the same time that NFκB was activated (60), which is a further indication that oxidative stress was involved (65). The hyperphosphorylation of serine residues (66) as well as the involvement of PKC has been corroborated, although other kinases may also be involved (67).
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REFERENCES
