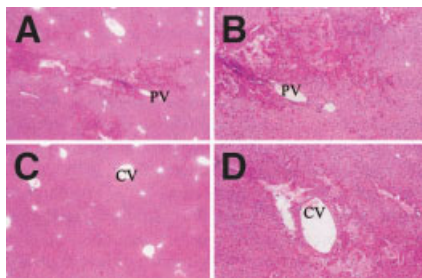


HEPATOLOGY HIGHLIGHTS

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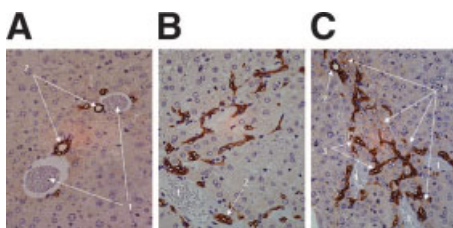
A Complement That Can Kill



The importance of cellular versus humoral immunity in autoimmune hepatitis (AIH) is uncertain. Even with respect to humoral immunity, damage could be caused by antibody-dependent complement versus antibody-dependent

cell-mediated toxicity. Previous studies identified the occurrence of an autoantibody in patients with AIH which recognized a 190-kd molecule on the hepatocyte membrane, and the autoantibody appeared to kill human liver cell lines in a complement-dependent fashion. Yamachi et al. extended this work by cloning a cell line from an AIH patient which produced a monoclonal antibody (MoAb) to a 190-kd molecule. The purified MoAb stained murine hepatocytes in isolation or in liver sections. When the MoAb was injected in mice, after 1 hour the MoAb and C3 complement were “stuck” in the liver but not other tissues. Eight hours after IV MoAb, ALT and AST increased moderately, and areas of central and periportal necrosis were observed (see Fig. A,C) but not in controls given myeloma protein (see Fig. C-D). The absence of cell infiltration along with the colocalization of C3 supports a complement-mediated immunopathogenesis. The MoAb was toxic to huH-7 cells that lack Fas and the MoAb toxicity was not due to contamination by cytokines. This is a very intriguing study but requires much validation. Why is the massive necrosis described by the authors not reflected in marked ALT levels? Was the mode of cell death really necrosis or could it be apoptosis? Also, it would be important to perform *in vivo* dose response and to assess complement levels. Is it possible that endothelial cells are also targets leading to an ischemic injury? What is the identity of the 190 kd protein? Ultimately, this work is intriguing and raises the possibility that the immunopathogenesis of AIH may involve subgroups with distinct or predominant mechanisms with anti-190 kd + complement attack on the liver representing one subgroup. (See HEPATOLOGY 2005;42:149-155.)

Oval Time: For a Quick Start



Oval cells are progenitor cells derived from Canals of Hering at the junction of hepatic cords and small ductules. Kof-

man et al. characterized the morphological response of oval cells in relation to time and dose of acetaminophen (APAP) in a mouse hepatotoxicity model. Oval cells were identified by immunohistochemistry using anti-cytokeratin antibodies (see Fig.). Oval cells increased in number in a biphasic fashion with first phase at 6-8 hours (faster at higher APAP doses) and the second phase peaking at 24 hours. When examined in whole liver sections, the magnitude of the oval cell response was quite modest (increasing from 8 to 9.6 oval cells per portal tract). However, the response was amplified when expressed in relationship to portal vein diameters (oval cells were more abundant in the smallest portal tracts with small veins). This report is a very valuable description of the acute oval cell response to a toxic drug and provides important morphological approaches to more accurately quantify the oval cell response. The authors speculate on several interesting concepts: (1) the oval cells are not killed by the acetaminophen perhaps because they lack *cyp2e1*; another factor may be that they are anatomically far from the paracrine effect of centrilobular toxic metabolite of APAP; (2) the fast response to initial injury is due to the release of stem cell factor (SCF) from hepatocytes which interacts with receptor on oval cell to stimulate proliferation; (3) the second phase of the biphasic oval cell response could represent a wave of maturation of oval cell-derived hepatocytes which are killed by remaining APAP (causing a second peak of release of SCF). All of these are fascinating ideas but require verification. Also, the quantitative contribution of oval cells versus direct hepatocyte proliferation in response to toxin injury needs to be defined in this model. (See HEPATOLOGY 2005;41:1252-1262.)

The CAR Performs But Is It Safe?

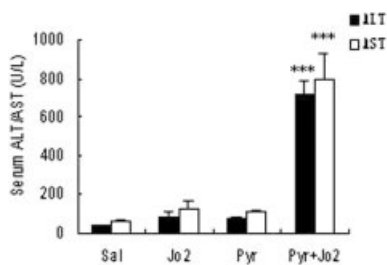
To minimize the retention of bile acids and bilirubin in cholestasis, an adaptive upregulation of the expression of genes of metabolism and export of these compounds is mediated through the actions of transcription factors, CAR, PXR, and FXR. Although this may limit hepatocellular injury, the question addressed by Wagner et al. is whether pharmacological activation of these transcription factors can augment the protective effects of their endogenous activation in mice *in vivo*. The study focused on CAR (phenobarbital and TCPOBOP) and PXR agonists (atorvastatin and PCN). In control mice, only phenobarbital increased *bsep* protein; otherwise, none of these ligands affected mRNA or protein of *ntcp* or *bsep*. On the other hand, *mrp2*, 3, and 4 (mRNA + protein) were induced by CAR agonist and only *mrp3* by PXR agonist. Among the *oatp* family, *oatp2* was selectively increased by both CAR and PXR agonists. Atorvastatin, as reported for the class of drugs, activated PPAR α -induced gene expression as well as *asbt* and *mdr2*. Phase I Cyp hydroxylation genes were induced by both types of agonists but phase II conjugation (*Ugt* and *Sulf*) were more selective CAR target genes. The effect of pretreatment with nuclear receptor agonist in common bile duct ligated (CBDL) mice was assessed. CAR ligands significantly reduced serum bilirubin and bile acid levels and increased urinary bile acid excretion (more pentahydroxylated). PXR agonist mainly decreased serum bile acids. Interestingly, ALT values were increased by both CAR and PXR agonists in CBDL mice. Thus, CAR and, in a more restricted

fashion, PXR ligands coordinately regulate elimination of bile acid and bilirubin phase I and II enzymes and phase III adaptive export pumps to minimize retention in cholestasis. These CBDL experiments were performed after 3 days of pretreatment with agonist. It is not clear whether the agonist responses would be different if given as treatment. The greater ALT increase despite improved measures of cholestasis after *in vivo* CAR and PXR agonist pretreatment in CBDL mice is of considerable interest but unexplained. Although this could be a CBDL rodent model-dependent issue, it raises the question of whether cholestatic cytotoxicity or apoptosis is potentiated by nuclear hormone agonists. Interestingly, this was observed even with the clinically relevant, atorvastatin, which is quite worrisome. (See HEPATOLOGY 2005;42:420-429.)

Antisense Makes Sense

Acyl-CoA:diacylglycerol acyltransferase (DGAT) 1 + 2 catalyze the last step in triglyceride (TG) synthesis. DGAT1 null mice are resistant to obesity, but DGAT2 null mice die shortly after birth. To study the role DGAT2 in lipid metabolism Yu et al. used *in vivo* antisense oligonucleotide (ASO) treatment of mice to knockdown expression. The proprietary (MOE-derivative) ASOs can be given subcutaneously twice weekly. In cultured hepatocytes, ASO decreased DGAT2 (but not DGAT1) mRNA by 75% with corresponding decreased TG synthesis and increased oleate oxidation. Similarly, decreased DGAT2 mRNA and enzyme activity were observed in liver *in vivo* after weeks of ASO treatment. ASO prevented diet-induced fatty liver, TG, diacylglycerol, and free fatty acid (FFA) accumulation, and improved blood lipid levels. This was accompanied by decreased expression of genes involved in TG and cholesterol synthesis and decreased SREBP1 expression in liver. Similarly, in leptin-deficient *ob/ob* mice ASO markedly improved hepatic steatosis. Thus, ASO knockdown of DGAT2 caused decreased total DGAT activity in liver but not in fat, and improved fatty liver but did not decrease adiposity. Interestingly, knockdown of DGAT2 lowered insulin levels but not insulin sensitivity. The lower plasma insulin may be due to decreased FFA levels and would explain why ASO decreased insulin-dependent expression of SREBP1 (which probably exerts the dominant effect in lowering blood and liver lipids). This MOE-ASO treatment strategy seems widely applicable to knocking down specific genes or viruses; it is especially promising because of the rather selective targeting of liver, ease of administration, and stability (administered once or twice per week). (See HEPATOLOGY 2005;42:362-371.)

Induction of Destruction

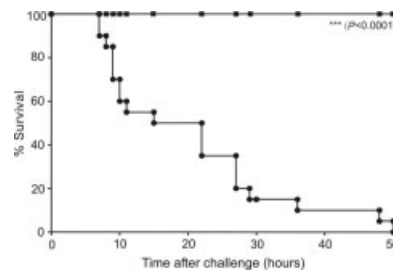


*** $P < 0.001$ for pyrazole/Jo2 vs the other group

The Fas-mediated death pathway may play a role in experimental alcohol liver injury. Since ethanol induces Cyp2E1 and the latter has been suggested to promote liver injury, Wang et al. assessed the possible synergy of these two pathways of

injury. Pyrazole was used as a surrogate for ethanol, since it rapidly induced cyp2e1 with or without Jo2, an agonistic anti-Fas. After a minimally toxic dose of Jo2 was defined, pyrazole pretreatment was found to augment toxicity of Jo2 as reflected in serum ALT (see Fig.) and apoptosis. Interestingly, pyrazole alone increased Fas expression in the liver. Curiously despite >10-fold potentiation of serum ALT, TUNEL-positive apoptosis increased by only $\approx 35\%$ and caspase 3 increased by $\approx 50\%$. Treatment with Cyp2e1 inhibitor partially blocked the potentiated pyrazole effect on Jo2 toxicity and decreased Cyp2e1 activity and expression. Other effects on oxidative and nitrosative stress were observed. Among the numerous questions raised by this interesting work, one important issue that needs to be addressed is whether much of the potentiation of Jo2 toxicity by pyrazole is due to increased Fas expression (seen even with pyrazole alone) and if cyp2e1 inhibitor blocks the increase in Fas expression. Furthermore, it would be important to quantify plasma membrane Fas (since the bulk of Fas is intracellular) as well as to assess the possibility that the discrepancy between the magnitude of change in ALT and apoptosis markers reflects a switch to necrosis perhaps due to oxidative stress-inhibiting caspases. (See HEPATOLOGY 2005;42:400-410.)

Abin Laden In The Liver



When TNF engages its receptor, several signal transduction pathways are activated which either lead to cell death or survival. The latter is mediated mainly by NF- κ B, which allows hepatocytes to resist apoptosis but

at the same time also promotes expression of genes which promote inflammation such as cytokines, chemokines, and adhesion molecules. Wullaert et al. examined the effect of adenoviral expression of ABIN-1 (A20 binding inhibitor of NF- κ B) on these pathways using the TNF/galactosamine model of murine liver injury. ABIN-1 expression blocked the lethality of TNF/galactosamine (see Fig.) while inhibiting histological hemorrhagic injury, apoptosis, and inflammation. Expression of an I κ B α superrepressor did not protect, so the known inhibition of NF- κ B activation by ABIN-1 could not explain its protective effect. This is an elegant paper which clearly demonstrates that overexpression of ABIN-1 protects against TNF-induced apoptosis and NF- κ B activation. However, gene expression is expected to be inhibited in mice treated with galactosamine, so inhibition of NF- κ B activation by ABIN-1 should not have an additional effect. Nevertheless, apoptosis was inhibited. Apparently, TNF binding to its receptor or activation of MAP kinases was not inhibited by ABIN-1. The exact target of ABIN-1 remains to be identified as well as clarification of whether this is an upstream site common to both activation of the apoptosis cascade and NF- κ B. The practical message of this work is that a more effective treatment approach for TNF-induced liver injury is to block both the apoptosis pathway and the proinflammatory NF- κ B pathway and the site of ABIN-1 action would be a key target in future drug development. (See HEPATOLOGY 2005;42:381-389.)