The Future of GI and Liver Research: Editorial Perspectives

III. JNK/AP-1 regulation of hepatocyte death

Mark J. Czaja
Marion Bessin Liver Research Center and Department of Medicine, Albert Einstein College of Medicine, Bronx, New York 10461

Czaja, Mark J. The Future of GI and Liver Research: Editorial Perspectives. III. JNK/AP-1 regulation of hepatocyte death. Am J Physiol Gastrointest Liver Physiol 284: G875–G879, 2003; 10.1152/ajpgi.00549.2002.—Activation of the JNK/activator protein-1 (AP-1)-signaling pathway is a common mediator of hepatic death from a variety of stimuli. Although the mechanism by which JNK or AP-1 promotes death is unknown, it results when activation of this signaling pathway is unusually prolonged. Although JNK/AP-1 mediates TNF-induced cell death at or above the level of the mitochondria, the ability of JNK/AP-1 to promote cell death from necrosis as well as apoptosis suggests that JNK/AP-1 may induce death by several mechanisms. Recognition of JNK/AP-1 signaling as a critical promoter of hepatocyte death raises the possibility that the therapeutic manipulation of this pathway may be effective in the treatment of human liver disease.

HEPATOCELLULAR INJURY AND CELL death can result from a number of seemingly dissimilar stimuli that range from receptor ligands such as TNF-α to small molecules such as hydrogen peroxide (H₂O₂). Although considerable complexity exists in the cellular signaling that culminates in death from apoptosis or necrosis, common pathways do exist. Recent investigations have demonstrated that a central inducer of hepatocyte death is the activation of the JNK/activator protein-1 (AP-1)-signaling pathway. Originally recognized as a regulator of cellular proliferation and differentiation, JNK/AP-1 activation has now been shown to mediate cell death, providing further support for the concept that the cellular fates of growth and death are closely linked.

JNK/AP-1 SIGNALING

The MAPK family of enzymes are critical for the cellular response to a variety of extracellular and intracellular stresses including ones that trigger cell death. The MAPK JNK in particular is primarily activated by environmental stresses as well as cytokines. The JNK MAPKs are encoded by three genes, and two of these, jnk1 and jnk2, are expressed in all tissues including liver (7). These genes are alternatively spliced to create multiple isoforms, all of which encode for 46- or 55-kDa proteins that differ by the presence of a COOH-terminal extension (7). The functions of these multiple JNK isoforms are unknown, but they may be necessary to allow interactions with different substrates.

Activation of JNK is complex and involves a kinase cascade (see Fig. 1). Events that initiate the kinase cascade remain unclear, but G proteins such as Rac and cdc-42 and the TRAF group of adaptor proteins mediate activation from some stimuli. Activation then proceeds through a three-tier, protein kinase cascade. More than a dozen MAPK kinase kinases (see Fig. 1) have been implicated in the activation of JNK (29), and this redundancy may allow responses to distinct stimuli (4). They converge on the MAPK kinases MKK4 and MKK7, which preferentially phosphorylate JNK on tyrosine 185 and threonine 183, respectively (29). Dual phosphorylation is required for full activation, but there can be differential MKK4 and MKK7 activation depending on the stimulus. Finally, levels of JNK activation are dependent on the actions of a number of phosphatases (15), and the state of JNK activation represents an equilibrium between stimulation by upstream kinases and downregulation by phosphatases.

Activated JNK phosphorylates c-Jun at serine-63 and -73, which increases the transcriptional activity of this critical AP-1 subunit. However, all of the effects of JNK activation may not be attributable to its interactions with c-Jun, because JNK phosphorylates other proteins, including JunB, JunD, and ATF-2, that also may modify levels of AP-1 activation. In addition, c-Jun expression is regulated at the level of transcription. Thus the level of AP-1 transcriptional activity results from the complex interplay of a number of factors and cannot be simply equated to the level of JNK activation.

The availability of jnk1 and jnk2 knockout mice has allowed an examination of the separate physiological functions of these two kinases (7, 29). The single knockouts have no phenotypic abnormalities except for defects in T cell apoptosis and immune responses. The jnk1/jnk2 double knockout is an embryonic lethal because of severe dysregulation of brain apoptosis. Despite the apparently overlapping functions of jnk1 and jnk2, future studies in the single knockouts may be helpful in defining JNK function in hepatic injury and cell death.
DEATH RECEPTOR-MEDIATED CELL DEATH

Understanding the hepatocyte signaling response to TNF stimulation is critically important because of this cytokine’s central role in mediating hepatocyte injury and death from toxins, ischemia, and viruses. TNF is normally a hepatocyte mitogen, but, under conditions of transcriptional or translational arrest, hepatocytes undergo cell death from TNF through TNF-receptor type I-mediated signaling. These findings suggested that TNF upregulates a gene(s) that normally blocks the TNF death pathway in hepatocytes. Although initial studies focused on identifying a gene that had direct cellular protective effects, current evidence suggests that the gene(s) functions, at least in part, through downregulation of the JNK/AP-1 pathway. Critical to this conclusion were investigations demonstrating that the transcription factor NF-κB mediates hepatocyte resistance to TNF killing (1, 31). Inhibition of NF-κB activation alone is sufficient to convert the normal proliferative hepatocyte response to TNF to one of apoptosis (1, 31). Subsequent investigations in a nontransformed rat hepatocyte cell line demonstrated that with NF-κB inhibition, the usually transient TNF-induced activation of JNK was converted to one of sustained activation (20). This effect on JNK was associated with increased AP-1 transcriptional activity that induced cell death as proven by the ability of the dominant-negative c-Jun protein TAM67 to significantly inhibit death (20). The effects of specific inhibition of JNK signaling were not addressed in this study. This prodeath effect of JNK/AP-1 overactivation occurred at or above the level of the mitochondria, because TAM67 expression also blocked mitochondrial cytochrome c release and downstream activation of the effector caspases 3 and 7 (20). These results are consistent with findings of sustained JNK activation with NF-κB inhibition and TNF stimulation in nonhepatic cell types (8, 27), suggesting that NF-κB-JNK cross-talk is a general mechanism of cellular resistance to TNF toxicity.

Studies in other cell culture systems have suggested a somewhat different involvement for JNK/AP-1 in TNF-induced death. Recently, specific inhibition of JNK has become possible with the availability of chemical JNK inhibitors such as SP-600125 (12). Preliminary investigations (25) in primary rat hepatocytes demonstrated that SP-600125 also blocked death from TNF and NF-κB inhibition. Death from Fas was not blocked by JNK inhibition, suggesting differential effects of the TNF and Fas death receptor pathways on JNK. However, in this study, TAM67 expression failed to block cell death, suggesting that JNK directly regulated apoptosis independently of its activation of c-Jun (25). This contradictory finding for TAM67 from the prior study in a rat hepatocyte cell line (20) may result from differences in the cell models such as alterations in AP-1 activation induced in primary cultures by the perfusion process (22). In contrast to the promotion of cell death by JNK or AP-1 in both of these studies, investigations in HepG2 and HuH-7 hepatoma cells sensitized to TNF toxicity by cycloheximide reported that JNK functioned to promote apoptosis (18). This result is surprising, because it is to be expected that cycloheximide’s effect on TNF signaling would be sim-
ilar to NF-κB inhibition. However, global protein inhibition by cycloheximide may have additional effects. Second, transformed hepatoma cells may differ from hepatocytes. Finally, the differences in this study may have been due to the use of inhibitors of very upstream signaling molecules of JNK (TRAP2 and transforming growth factor-β-activated kinase-1), which may have affected other signaling pathways in addition to JNK.

JNK/AP-1 signaling has been shown to mediate necrotic as well as apoptotic hepatocyte death from TNF. Stable overexpression of the cytochrome P-450 isoform 2E1 (CYP2E1) sensitized rat hepatocytes to necrosis from TNF (19). CYP2E1 overexpression by itself caused a slight increase in JNK activity and led to a prolonged JNK activation in response to TNF (19). TNF-induced necrosis was markedly decreased when AP-1 function was blocked by expression of TAM67 (19). The chronic increase in oxidant generation by CYP2E1 therefore caused JNK/AP-1 activation and sensitized hepatocytes to TNF killing. Hepatocyte CYP2E1 overexpression occurs in alcoholic and nonalcoholic steatohepatitis and has been implicated as a causal factor in liver injury. JNK/AP-1 overactivation is a potential mechanism of promoting hepatocyte sensitization to TNF-induced injury in these diseases.

Studies of JNK activation during TNF-dependent liver injury in vivo are limited. In the immune model of TNF-dependent liver injury induced by concanavalin A, increased JNK activation and hepatocyte nuclear translocation of phosphorylated c-Jun have been demonstrated (28). Activation of JNK was TNF dependent (26), but the effects of blocking JNK/AP-1 signaling were not addressed. JNK activation has also been documented in TNF-dependent alcohol-induced injury (5), as discussed subsequently. Further studies are needed to investigate the mechanistic role of JNK and AP-1 activation in TNF-dependent liver injury in vivo.

BILE ACID-INDUCED HEPATOCYTE APOPTOSIS

Elevated hepatic bile acid concentrations induce hepatocyte death through apoptotic pathways mediated by the death receptors Fas and TRAIL (13). Protein kinase C and phosphatidylinositol 3-kinase (PI3-kinase) signaling activate critical cell survival pathways in response to bile acid-induced injury (21, 24). Recent investigations (21) have demonstrated that a second member of the MAPK family, ERK, also acts as a protective signaling pathway. Bile acid activation of ERK occurred by ligand-independent activation of the epidermal growth factor receptor-Raf-1 signaling cascade (23). Recently, JNK has been implicated as a prodeath signal because the toxic bile acid tauroursodeoxycholic acid-3 sulfate induced prolonged JNK activation (11). JNK inhibition prevented Fas trafficking to the membrane and decreased cell death (11). These data suggest that toxic bile acids may induce ligand-independent Fas killing as the result of JNK overactivation. Although tauroursodeoxycholate also protected against this toxicity by downregulating JNK by an ERK- and PI3-kinase-independent mechanism, it remains possible that ERK and/or PI3-kinase mediate resistance to toxic bile acids by downregulating JNK. Alternatively, similar to TNF-induced cell death, NF-κB may act to inhibit JNK activation in response to bile acids because NF-κB protects against bile acid toxicity (24).

CELL DEATH FROM OXIDATIVE STRESS

Oxidants are known stimulants of the JNK/AP-1 pathway, making this signaling cascade a prime candidate to modulate hepatocyte death from oxidative stress. In the hepatoma line HuH-7, the oxidant H2O2 induced necrosis associated with JNK activation and increased c-jun mRNA expression and AP-1 transcriptional activation (32). Cells stably transfected with an antisense c-jun construct were more resistant to H2O2-induced necrosis, again demonstrating that JNK or AP-1 regulate hepatocellular necrosis as well as apoptosis. Hepatocyte death from the reactive oxygen species (ROS) superoxide has recently been shown to result from JNK/AP-1 overactivation. Toxic but not nontoxic concentrations of the superoxide generator menadione induced a sustained activation of JNK, and inhibition of c-Jun function blocked this apoptotic cell death (6). Inhibition of the MAPK ERK was sufficient to sensitize hepatocytes to death from usually nontoxic concentrations of menadione and led to a sustained JNK activation. Thus, depending on the death stimulus, either NF-κB (for TNF) or ERK (for superoxide) cross-talk with JNK may be essential to prevent a prolonged and therefore lethal JNK/AP-1 activation. These investigations with menadione did not examine the mechanism of JNK inhibition by ERK, but the effect is likely secondary to ERK's known upregulation of phosphatases that may limit JNK activation.

The effects of chronic oxidant exposure on JNK/AP-1 signaling remain to be investigated. As discussed previously (19), chronic in vitro oxidant stress from CYP2E1 overexpression caused constitutive and TNF-induced JNK overactivation. Acute in vitro ethanol treatment induced JNK activation (3), but recent limited studies of JNK in vivo ethanol models of liver injury had divergent findings. Five weeks of ethanol feeding in mice failed to elevate JNK activity and actually reduced the JNK response to lipopolysaccharide (16). In contrast, 6 mo of ethanol treatment in rats increased JNK and c-Jun phosphorylation (5). Additional in vivo studies must clarify the effects of ethanol and oxidant-induced injury on JNK/AP-1 activation and examine the function of this signaling pathway in hepatocellular resistance to oxidative injury.

ISCHEMIA/REPERFUSION INJURY

JNK and AP-1 activation has been demonstrated to occur in vivo during cold ischemia/warm reperfusion injury associated with liver transplantation (2). JNK activation was prolonged and occurred in the absence of activation of the other MAPK family members ERK and p38 (2). However, these studies did not address whether this activation occurred specifically in hepa-
tocytes. Recent data have shown mechanistic involvement of JNK in this form of injury as chemical JNK inhibitors reduced mortality, histological changes, transaminase elevations, and levels of caspase-3-like activity in a rat model of ischemia-reperfusion (28). Potential activators of JNK/AP-1 in ischemia-reperfusion include TNF, ROS, and ceramide. Adenosin delivery of the antioxidant enzyme superoxide dismutase blocked JNK and AP-1 activation in association with a significant reduction in liver injury (17, 33), indicating that ROS generation is a critical upstream factor if not the ultimate activator of JNK/AP-1. It remains to be shown whether these effects occurred in hepatocytes or other hepatic cell types. These in vivo findings demonstrate that the protective effects of antioxidants cannot be completely attributed to their neutralization of ROS but may also result from redox-dependent changes in critical cell death signaling pathways.

MECHANISM OF JNK/AP-1 INDUCTION OF HEPATOCYTE DEATH

The mechanism by which JNK/AP-1 activation triggers apoptotic and necrotic cell death in hepatocytes or nonhepatic cells is unknown. The simplest scenario would be that factors such as TNF and ROS activate upstream kinases that phosphorylate JNK. This activated JNK then phosphorylates c-Jun, increasing its transcriptional activity and the expression of AP-1-dependent genes that promote cell death. Although c-Jun-dependent, proapoptotic genes such as the Bcl-2 family member Bim are known (30), studies in nonhepatic cells have as yet identified few candidate genes to explain the proapoptotic effects of JNK/AP-1 (8, 27). It is possible that the genes are cell-type specific and differ with the death stimulus, and no genes have yet been implicated in hepatocyte death. Studies of TNF-induced hepatocyte apoptosis have localized the JNK/AP-1 effect at or above the level of mitochondrial involvement (20, 25). Investigations of bile acid-induced apoptosis further suggest that JNK activation promotes death by mediating Fas trafficking to the membrane (11). These findings along with the fact that JNK/AP-1 affects both apoptotic and necrotic cell death suggest that this signaling pathway may have several mechanisms by which it promotes the death of hepatocytes.

An alternative possibility is that the proapoptotic effects of JNK are not mediated through c-Jun-induced effects on transcription. Mechanistic studies of JNK/AP-1 involvement in hepatocyte death have been largely performed by employing the c-Jun dominant-negative TAM67. The recent availability of chemical JNK inhibitors has allowed investigations into the functional effects of direct JNK inhibition. If JNK's effects are mediated ultimately by c-Jun, then inhibition of either JNK or c-Jun function should cause an equivalent reduction in cell death. However, although TAM67 was found to block TNF-induced death in a rat hepatocyte line as previously discussed (20), the JNK inhibitor SP-600125 and not TAM67 reduced TNF death in a recent study in primary hepatocytes (25).

Thus it is possible that JNK directly regulates cell death by a mechanism other than through the phosphorylation and activation of c-Jun. However, it may be difficult to equate studies of JNK and c-Jun inhibition. JNK is upstream of c-Jun/AP-1 and phosphorylates other known substrates, such as the proapoptotic Bcl-2 family member Bad (9), and probably additional substrates not yet identified. Thus inhibition of JNK may cause effects on other signaling or effector molecules that are unaffected by inhibition of c-Jun function. In addition, hepatocytes express two JNK genes, and studies in nonhepatic cells have demonstrated that jnk1 may be antiapoptotic, whereas jnk2 is proapoptotic (14). The net effect of global JNK inhibition may therefore be the result of both effects on survival and death pathways. Finally, findings resulting from inhibition of c-Jun function may not simply reflect the direct actions of c-Jun. Inhibition of c-Jun function might obviate the proapoptotic effects of other AP-1 family members that heterodimerize with c-Jun.

One apparent key to the mechanism(s) by which JNK/AP-1 induces hepatocyte death is that it seems to depend on overactivation of this signaling pathway. Transient JNK/AP-1 activation normally occurs in response to growth stimuli. Prolonged JNK/AP-1 activation is associated with hepatocyte death from TNF (20), toxic bile acids (11), and ischemia-reperfusion (2). Thus sustained JNK/AP-1 activation in an injured cell unable to proliferate may trigger a death response. Variations in the length of MAPK activation may be a mechanism for inducing different cellular responses from the same signaling pathway. Although NF-κB and ERK have been implicated in hepatocyte down-regulation of the JNK/AP-1 response (6, 20), the mechanisms of their effects remain to be determined.

FUTURE PERSPECTIVES

Recent investigations have established a critical role for JNK/AP-1 signaling in hepatocyte apoptosis and necrosis, particularly that resulting from TNF- and oxidant-induced injury. Further investigations must define whether the effects of JNK and AP-1 activation are overlapping or distinct. Their specific biological effects must be delineated and related to the mechanism of cell death induction. It also remains to be determined how a death stimulus is transduced into a sustained activation of JNK and AP-1 in hepatocytes. Although this signaling pathway has been exclusively implicated in the promotion of cell death, it is possible that JNK or AP-1 may have protective functions as well during some forms of hepatocyte injury. Finally, to understand the ultimate effects of inhibiting this signaling pathway in vivo, studies of JNK/AP-1 activation and function during liver injury in other liver cell types such as Kupffer and stellate cells are necessary. Recently, kinase inhibitors have been used in the treatment of human disease (10). The hope is that therapies can be directed at the JNK/AP-1 signaling pathway to
prevent hepatocyte death and thereby affect the outcome in human liver disease.

I thank A. Bobe for secretarial assistance and D. Brenner and P. Dent for helpful discussions.

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-44234 and DK-61498.

REFERENCES


