The transcription factor interferon regulatory factor-1 mediates liver damage during ischemia-reperfusion injury

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The transcription factor interferon regulatory factor-1 mediates liver damage during ischemia-reperfusion injury. ISCHEMIA-REPERFUSION (I/R) injury is a pathophysiological process whereby hypoxic organ damage is accentuated following return of blood flow and oxygen delivery to the compromised tissue. Transient episodes of hepatic ischemia occur during solid organ transplantation, trauma, hypovolemic shock, and elective liver resection when inflow occlusion or total vascular exclusion is used to minimize blood loss. The pathophysiology of liver I/R injury includes both direct cellular damage as the result of the ischemic insult as well as delayed dysfunction and damage resulting from activation of inflammatory pathways. Histopathological changes include cellular swelling, vacuolization, endothelial cell disruption, neutrophil infiltration, and hepatocellular necrosis (9, 29, 32).

The distal interacting elements in the cascade of inflammatory responses resulting in organ damage following hepatic I/R injury have been extensively studied. Activation of Kupffer cells with production of reactive oxygen species (ROS), upregulation of inducible nitric oxide synthase (iNOS) in hepatocytes, activation of c-Jun NH2-terminal kinase (JNK), upregulation of proinflammatory cytokines, and neutrophil accumulation have all been identified as contributing events to the inflammation-associated damage (5, 8, 12, 13, 18, 24). The molecular mechanisms by which the initial cellular injury contributes to propagation of the inflammatory response is poorly understood.

One transcription factor that plays a central role in orchestrating gene expression during inflammation is interferon (IFN) regulatory factor-1 (IFN-IRF-1) (35). IRF-1 is a nuclear transcription factor originally identified as one of the key factors responsible for type I IFN expression (IFN-α and IFN-β) and other IFN-inducible genes (26, 31). We have previously shown that IRF-1 mRNA is upregulated in cultured rat hepatocytes in response to IFN-γ, TNF-α, and IL-1β stimulation (10). It was subsequently shown that cytokine-induced ROS and iNOS are at least in part regulated by IRF-1 (20, 22, 23, 40). However, the role of IRF-1 in regulating cytokine expression and the inflammatory response following hepatic I/R injury has not been previously described. In the current study, we demonstrate that IRF-1 is a necessary and sufficient transcription factor mediating early cytokine production and the inflammatory response secondary to liver I/R injury.

MATERIALS AND METHODS

Animals. Male C57BL/6 IRF-1 knockout (−/−) and IRF-1 wild-type (+/+) mice (8–12 wk old) were purchased from The Jackson Laboratory (Bar Harbor, ME). Male Sprague-Dawley rats weighing 250–300 g were obtained from Charles River (Boston, MA) for hepatocyte isolation experiments. Male Lewis (LEW) rats weighing 200–300 g were purchased from Harlan Sprague Dawley (Indianapolis, IN) for orthotopic liver transplantation experiments. All animals were maintained in a laminar-flow, specific pathogen-free atmosphere at the University of Pittsburgh. Animal protocols were approved by the Animal Care and Use Committee of the University of Pittsburgh, and the experiments were performed in adherence to the National Institutes of Health guidelines for the use of laboratory animals.

Liver ischemia. A nonlethal model of segmental (70%) hepatic warm ischemia was used. The I/R protocol was initiated with the
abdominal wall being clipped of hair and cleansed with Betadine. Under pentobarbital sodium (40 mg/kg ip) and methoxyflurane (inhalation) anesthesia, a midline laparotomy was performed. With the use of an operating microscope, the liver hilum was dissected free of surrounding tissue. All structures in the portal triad (hepatic artery, portal vein, and bile duct) to the left and median liver lobes were occluded with a microvascular clamp (Fine Science Tools, San Francisco, CA) for 60 min, and reperfusion was initiated by removal of the clamp. This method of segmental hepatic ischemia prevents mesenteric venous congestion by permitting portal decompression through the right and caudate lobes. We have previously carried out a time course to determine the optimal ischemia time period for the induction of hepatic injury (21). Less than 60 min of ischemia produced only minimal plasma transaminase elevations, whereas greater than 75 min of ischemia was poorly tolerated with gross evidence of poor reperfusion of the ischemic lobes. A reproducible level of hepatic damage was observed using 60 min of ischemia and thus used for our study.

After application of the clamp, the abdomen was covered with a sterile plastic wrap to minimize evaporative loss. Throughout the ischemic interval, evidence of ischemia was confirmed by visualizing the pale blanching of the ischemic lobes. The clamp was then removed and gross evidence of reperfusion based on immediate color change was assured before closing the abdomen with continuous 4-0 polypropylene suture. Either the absence of ischemic color changes or the lack of response to reperfusion was a criterion for immediate death and exclusion from further analysis. Temperature was monitored by rectal temperature probe and was maintained at 37°C by means of a warming pad and heat lamp. At the end of the observation period following reperfusion, the mice were anesthetized with inhaled methoxyflurane and were killed by exsanguination. Sham animals underwent anesthesia, laparotomy, and exposure of the portal triad without hepatic ischemia. Animals were killed at predetermined time points (3–6 h) after reperfusion for serum and liver samples.

Adenoviral IRF-1 vector construction and delivery. An E1- and E3-deleted recombinant adenovirus encoding murine IRF-1 was constructed as previously described (17). Recombinant adenoviruses were propagated in HEK-293 human embryonic kidney cells and purified by cesium chloride density gradient centrifugation and subsequent dialysis. Titers of viral particles were determined by optical densitometry, and plaque-forming unit (pfu) concentrations were confirmed by formal plaque assay technique. For adenoviral transfection in vivo, recombinant adenovirus was diluted to an appropriate final concentration in normal saline. The total volume of adenovirus-containing solution was injected intravenously, and the animals were killed 48 h later.

Orthotopic liver transplantation. Basic techniques of liver harvesting and orthotopic transplantation without hepatic arterial reconstruction were according to the method previously described (14). Adenoviral IRF-1 (2 × 10⁹ pfu) or AdLacZ (2 × 10⁹ pfu) was injected via the penile vein to prospective donor animals under brief methoxyflurane anesthesia. Four days later, the donor liver was harvested. All liver grafts were kept in a bath of University of Wisconsin (UW) solution at 4°C for 3 h of cold preservation period and orthotopically transplanted into syngeneic LEW recipients. All of the initial transplants were performed by a single surgeon who has been performing rat liver transplantation for more than 5 years.

Hepatocyte isolation. Hepatocytes were isolated from normal rats by an in situ collagenase (type IV, Sigma) perfusion technique, modified as described previously (42). Hepatocytes were separated from the nonparenchymal cells by two cycles of differential centrifugation (50 g for 2 min) and further purified over a 30% Percoll gradient. Hepatocyte purity exceeded 98% as assessed by light microscopy, and viability was typically greater than 95% as determined by trypan blue exclusion assay.

Cell culture. Hepatocytes (5 × 10⁶) were plated on 100-mm gelatin-coated petri dishes in 6 ml of culture media. Media consisted of Williams media E (Gibco Life Technologies, Gaithersburg, MD) with t-arginine (0.5 mmol/l), insulin (10⁻⁸ mol/l), HEPES (15 mmol/l), L-glutamine, penicillin, streptomycin, and 10% low endotoxin calf serum (HyClone Laboratories, Logan, UT). After a 24-h incubation (37°C, 95% air-5% CO₂) the media were changed, and the following cytokines were added for 2–24 h: 100 U/ml IFN-γ or IFN-β (Amgen, Thousand Oaks, CA); 500 U/ml TNF-α (Genzyme, Cambridge, MA); 5 U/ml human recombinant IL-1β (Cistron Biotechnology, Pine Brook, NJ); and 10 μg/ml lipopolysaccharide (Escherichia coli 0111:B4, Sigma).

RNA isolation and Northern blot analysis. Cellular RNA was extracted from hepatocytes with the RNAzol B (Biotecx Laboratories, Houston, TX)-modified method of Chomczynski and Sacchi (4). A
DNA probe for hepatocyte IRF-1 was generated with RT-PCR. Briefly, 5 μg total hepatocyte RNA was used for first-strand cDNA synthesis with oligodeoxythymidine primers and Moloney murine leukemia virus reverse transcriptase. PCR amplification was performed with *Thermus aquaticus* polymerase (Perkin-Elmer, Norwalk, CT) and custom-designed oligonucleotide primers based on the known sequence for rat T-cell IRF-1 (42). The PCR product identity was verified by DNA restriction enzyme analysis and then used as a probe to determine hepatocyte IRF-1 mRNA levels by Northern blot hybridization. Northern blot analysis was performed with 20-μg RNA aliquots as previously described (11). After blots were probed for IRF-1, membranes were stripped with boiling 5 mmol/1M EDTA and 0.1% sodium dodecyl sulfate and rehybridized with a probe to 18S rRNA to control for variations in the amount of RNA per lane.

**Isolation of nuclear and cytoplasmic proteins.** Frozen liver tissues were suspended in buffer containing 10 mM Tris (pH 7.5), 1.5 mM MgCl₂, 10 mM KCl, and 0.1% Triton X-100 and lysed by homogenization. Nuclei were recovered by microcentrifugation at 7,500 rpm for 5 min. The supernatant containing cytoplasmic and membrane protein was collected and stored at −80°C for Western blot analysis. Nuclear proteins were extracted at 4°C by gently resuspending the nuclei pellet in buffer containing 20 mM Tris (pH 7.5), 20% glycerol, 1.5 mM MgCl₂, 420 mM NaCl, 0.2 mM EDTA, and 0.1% Triton X-100 followed by a 1-h incubation at 4°C with occasional vortexing. After microcentrifugation at 13,000 rpm for 15 min at 4°C, the supernatant containing nuclear protein was collected. Protein concentration was quantitated with bicinchoninic acid protein assay reagent (Pierce Chemical, Rockford, IL).

**Liver damage assessment.** To assess hepatic function and cellular injury following liver ischemia, serum alanine aminotransferase (ALT) levels were measured using the Opera clinical chemistry system (Bayer, Tarrytown, NY).

**Histopathology.** Formalin-fixed liver samples were embedded in paraffin and cut into 6-μm-thick sections. Tissues were stained with hematoxylin-eosin, and slides were assessed for inflammation and tissue damage.

**SDS-PAGE and Western blot analysis.** Western blot analysis for IRF-1 and phosphorylated and total kinase forms of p38 and JNK was performed as described (28). Primary polyclonal antibody to IRF-1 (1:1,000; Santa Cruz Biotechnology, Santa Cruz, CA), phosphorylated p38 and JNK (1:1,000; Cell Signaling Technology, Beverly, MA), and primary polyclonal antibody to total p38 and JNK (1:1,000; Santa Cruz Biotechnology) were used for Western blot analysis. Membranes were developed with the Super Signal West Pico chemiluminescent kit (Pierce) and exposed to film.

**SYBR green real-time RT-PCR.** Total RNA was extracted from the liver using the TRIzol reagent (Life Technologies, Grand Island, NY).
according to the manufacturer’s instruction. mRNA for TNF-α, IL-6, iNOS, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was quantified in duplicate by SYBR green two-step, real-time RT-PCR. After the removal of potentially contaminating DNA with DNase I (Life Technologies), 1 μg of total RNA from each sample was used for RT with oligo dT (Life Technologies) and SuperScript II (Life Technologies) to generate first-strand cDNA. The PCR mixture was prepared using SYBR green PCR Master Mix (PE Applied Biosystems, Foster City, CA) using the primers as previously described (27, 30). Thermal cycling conditions were 10 min at 95°C followed by 40 cycles of 95°C for 15 s and 60°C for 1 min on an ABI PRISM 7000 sequence detection system (PE Applied Biosystems). Each gene expression was normalized with GAPDH mRNA content.

Statistical analysis. Results are expressed as means ± SE. Group comparisons were performed using Student’s t-test or ANOVA. Differences were considered significant at P < 0.05.

RESULTS

IRF-1 is involved in liver I/R injury. To determine the role of IRF-1 during hepatic I/R injury, IRF-1 knockout mice and their wild-type counterparts were subjected to warm I/R. Sixty minutes of warm hepatic ischemia followed by 6 h of reperfusion significantly increased serum ALT levels in wild-type mice compared with sham-treated controls. In contrast, IRF-1 knockout mice were partially protected from hepatic injury, exhibiting an ~60% decrease in ALT levels compared with wild-type mice (Fig. 1A). This protection was also evident as early as 3 h after reperfusion in IRF-1 knockout mice (data not shown). Liver histology confirmed the hepatic damage after I/R injury. Severe sinusoidal congestion and hepatocellular necrosis was present in liver tissue from wild-type mice, whereas minimal damage was noted in samples from IRF-1 knockout mice (Fig. 1B). To determine whether the IRF-1-dependent injury was associated with changes in IRF-1 protein levels, Western blot analysis was performed on liver lysates from animals subjected to hepatic I/R (Fig. 2). Following 60 min of warm ischemia, IRF-1 protein expression was upregulated as early as 1 h after reperfusion and then returned to basal levels by 12 h. As expected, IRF-1 protein expression was absent in IRF-1 knockout mice (data not shown). These data implicate IRF-1 as a mediator of liver damage during hepatic I/R injury.

Fig. 5. Recombinant adenoviral gene delivery of IRF-1 induces significant hepatic injury. A: C57BL/6 mice were administered recombinant adenovirus encoding murine IRF-1 [Ad-IRF1; 5 × 10⁹ or 1 × 10¹⁰ plaque-forming units (pfu)] or empty adenoviral vector control (Ad-null; 1 × 10⁹ pfu). Western blot shown is representative of three experiments with similar results. B: serum ALT levels were measured in mice treated 48 h prior with varying doses of Ad-IRF1 (1 × 10⁹, 1 × 10⁸, 3 × 10⁷ pfu) and Ad-null (3 × 10⁹ pfu). IRF-1 expression with viral transfection correlated positively with increased hepatocellular injury in a dose-dependent manner. Data are means ± SE; n = 4 mice/group. C: hematoxylin-eosin-stained liver sections from mice treated with Ad-IRF1 (3 × 10⁹ pfu) or Ad-null (3 × 10⁹ pfu) 48 h prior to death (original magnification ×200). Images are representative liver sections from four mice per group.
IRF-1 is involved in the production of inflammatory mediators. Proinflammatory cytokines, including TNF-α and IL-6, and expression of intracellular adhesion molecule (ICAM)-1 play key roles and can be readily assessed as markers in the pathophysiology of hepatic I/R injury (6, 39). Using real-time RT-PCR, we measured steady-state mRNA levels for these mediators in the liver after I/R. Compared with sham-treated animals, liver I/R in wild-type animals resulted in increased expression of TNF-α, IL-6, and ICAM-1 mRNA 3 h after reperfusion (Fig. 3). IRF-1 knockout animals subjected to I/R exhibited significant increases in hepatic mRNA levels of these inflammatory mediators compared with sham-treated animals; however, IRF-1 knockout mice undergoing I/R had significantly lower production of hepatic cytokines compared with wild-type animals. We previously have shown that iNOS contributes to liver injury due to I/R (21, 36). Hepatic iNOS mRNA levels were increased 3 h after reperfusion in IRF-1 wild-type mice compared with sham animals (Fig. 3). As with TNF-α, IL-6, and ICAM-1 expression, IRF-1 knockout mice exhibited decreased hepatic iNOS expression after I/R.

IRF-1 modulates inflammatory signaling pathways after I/R. Activation of MAPks is a prominent intracellular signaling event during redox stress, and a role for JNK activation in liver I/R injury has been demonstrated (1, 2). To determine whether IRF-1 had a role in MAPk activation, we assessed phosphorylation of JNK and p38 in IRF-1 wild-type and knockout mice. Following I/R, phosphorylation of JNK was increased in liver tissue of wild-type mice but not in IRF-1 knockout mice (Fig. 4). There was no difference in p38 phosphorylation or total cellular levels of JNK and p38 between IRF-1 wild-type and knockout mice.

Recombinant adenoviral gene delivery of IRF-1 induces significant hepatic injury in the absence of I/R. Because IRF-1 knockout mice were protected from hepatic I/R injury (suggesting that IRF-1 contributes to liver damage), we sought to determine whether overexpression of IRF-1 would result in comparable liver injury in normal animals. Administration of recombinant adenovirus encoding murine IRF-1 (AdIRF-1) in vivo resulted in abundant IRF-1 protein expression in the liver of C57BL/6 mice in a dose-dependent manner (Fig. 5A). There was minimal IRF-1 expression in animals given equivalent plaque-forming units of empty adenoviral vector control (Ad-null). The increase in IRF-1 expression following AdIRF-1 transfection correlated positively with increased hepatocellular injury in a dose-dependent manner (Fig. 5B). There was no hepatic injury in mice given empty adenoviral vector control. Liver histology confirmed the hepatic damage after overexpression of IRF-1. Severe hepatocellular necrosis was present in liver tissue from mice treated with AdIRF-1, whereas no damage was noted in samples from mice treated with Ad-null (Fig. 5C).

Recombinant adenoviral gene delivery of IRF-1 activates JNK signaling and increases iNOS expression. Because the IRF-1 knockout studies indicated a role for IRF-1 in activating JNK phosphorylation during hepatic I/R injury, we examined the effect of AdIRF-1 overexpression on MAPk signaling. Transfection of AdIRF-1 resulted in the phosphorylation of JNK (Fig. 6A) without a concomitant change in p38 phosphorylation (data not shown). We also examined the effect of IRF-1 overexpression on hepatic iNOS expression and observed that delivery of 1 × 10^9 pfu AdIRF-1 (but not empty adenoviral vector) increased iNOS protein expression in the liver (Fig. 6B).

Recombinant adenoviral gene delivery of IRF-1 to donor livers worsens orthotopic liver transplantation injury. To elucidate whether hepatic injury after I/R was dependent on IRF-1 expression specific to the liver or overall IRF-1 expression in the immune system, we used a model of orthotopic liver transplantation in rats. We treated the donor animal with adenovirus encoding IRF-1 (2 × 10^9 pfu) and transplanted this liver overexpressing IRF-1 into a syngenic rat. Adenovirus encoding LacZ (2 × 10^9 pfu) was used as a control. This model allows us to differentiate between the role of hepatic IRF-1 (only in the transfected donor liver) and IRF-1 in the immune system (the recipient’s immune system does not have overexpression of IRF-1). Previously, we reported that adenoviral delivery of marker genes (AdLacZ or AdGFP) to the donor animal allows for the majority of uptake to be in the donor liver with strong expression at 4 days after injection (34). The adenoviral-transduced liver graft was then transplanted, and staining of the liver showed strong transgene expression beginning as early as 3 h posttransplant and lasting up to 2 wk (3). Importantly, staining of extrahepatic organs (lung, spleen, heart) showed that there was no escape of virus from the liver. Therefore, we felt this was a suitable model to address the question of hepatic-specific IRF-1 expression. We selected a dose of AdIRF-1 (2 × 10^9 pfu) in the donor animal, which is known to yield reasonable transgene expression with ~30% transfection efficiency (3, 34). After 3 h of cold preservation time, animals transplanted with livers overexpressing IRF-1 (the AdIRF-1 group) exhibited worsened hepatic injury compared with animals receiving donor livers treated with marker...
gene AdLacZ (ALT 523 ± 64 vs. 331 ± 10 IU/l, respectively; n = 4 animals/group, P < 0.05). These results suggest that the enhanced liver damage was due to IRF-1 and not a nonspecific effect of adenovirus. Furthermore, these results support the notion that hepatic IRF-1 alone is sufficient to mediate liver damage after I/R injury.

Inflammatory signals regulate IRF-1 gene expression in cultured hepatocytes. Because both endogenous and exogenous IRF-1 contributed to liver damage, we sought to further characterize the induction of IRF-1 gene expression in the liver. Therefore, we examined the effect of specific inflammatory mediators on IRF-1 mRNA induction in cultured hepatocytes. IFN-γ, IFN-β, TNF-α, IL-1β, IL-6, and LPS were tested for their ability to upregulate IRF-1 gene expression in cultured hepatocytes. In unstimulated hepatocytes, a basal level of IRF-1 mRNA was detected (Fig. 7A). IFN-γ and IFN-β dramatically upregulated IRF-1 mRNA levels, whereas TNF-α and IL-1β induced IRF-1 mRNA to a lesser extent (Fig. 7A). Stimulation with IL-6 or LPS did not significantly increase IRF-1 mRNA levels as measured by Northern blot analysis. Because IFN-γ appeared to be the most potent stimulus for IRF-1 mRNA expression in cultured hepatocytes, a dose response and time course of IFN-γ stimulation was performed. Significant induction of IRF-1 mRNA was observed with as low as 1 U/ml of IFN-γ (Fig. 7B). IRF-1 mRNA was induced as early as 1 h after stimulation and remained upregulated for 48 h (Fig. 7C). To show that induction of IRF-1 gene expression also resulted in enhanced protein levels, Western blot analysis for IRF-1 protein was also performed in cultured hepatocytes stimulated with IFN-γ. In agreement with mRNA levels, significant increases in IRF-1 protein levels were seen by 1 h after IFN-γ stimulation (Fig. 7D).

Hepatic IRF-1 is an immediate-early transcription factor. IRF-1 has been shown to function as an early-immediate nuclear transcription factor in certain cell types (31, 42). To determine whether hepatic IRF-1 gene expression was also regulated in a similar manner, we stimulated rat hepatocytes with IFN-γ or IFN-β along with cycloheximide (5 μg/ml) to inhibit de novo protein synthesis. IFN-γ- or IFN-β-stimulated hepatocellular IRF-1 mRNA was superinduced by addition of cycloheximide (Fig. 8). Cycloheximide alone also superinduced basal IRF-1 mRNA expression, suggesting that IRF-1 gene expression does not require de novo protein synthesis and is regulated by labile nuclear proteins. These findings are consistent with the function of IRF-1 as an early-immediate transcription factor in the liver. As expected, addition of the transcriptional inhibitor actinomycin D completely abrogated IRF-1 mRNA expression (Fig. 8).

DISCUSSION

The damage to the liver caused by ischemia and reperfusion continues to be an important limiting factor in many clinical settings such as liver surgery, transplantation, and low-flow
states. The distal interacting elements in the cascade of inflammatory responses resulting in organ damage following hepatic I/R injury have been well described; however, the mechanisms that orchestrate this response are poorly understood. Because the transcription factor IRF-1 has been shown to be important for gene regulation during inflammation, the purpose of this study was to test the hypothesis that IRF-1 plays a role in hepatic I/R by coordinating the events that lead to tissue injury and organ damage. The major and novel findings of this investigation are 1) IRF-1 is upregulated after hepatic ischemia and reperfusion. 2) IRF-1 exerts a harmful role in hepatic I/R injury by modulating the expression of multiple inflammatory mediators, 3) the mechanism of injury is explained in part by an increase in JNK MAPK signaling cascade, and 4) hepatocellular IRF-1 expression itself is regulated by specific cytokines.

IRF-1 is a transcription factor found to be involved in host defense against pathogens, tumor prevention, and development of the immune system (19, 35). We show that IRF-1 also plays a role in mediating liver injury after I/R. Mice with a null mutation of the IRF-1 gene are protected from hepatic I/R and have diminished expression of hepatic TNF-α, IL-6, and ICAM-1. In addition, IRF-1 knockout mice have decreased expression of iNOS, an IRF-1 inducible gene (15). We previously have shown that iNOS contributes to liver injury due to I/R (21, 36). These results suggest that IRF-1 coordinates the activity of multiple inflammatory mediators involved in the pathophysiology of hepatic I/R injury.

To further elucidate the molecular mechanism of IRF-1-mediated injury, we investigated its effect on MAPK pathways. The MAPK family represents a group of proteins involved in signal transduction of a variety of cellular stimuli. The JNK subgroup of MAPKs, also known as stress-activated protein kinase, is activated in response to environmental stresses (41). JNK phosphorylation occurs in cultured rat hepatocytes following hypoxia alone, with further increase upon reoxygenation (7, 25). JNK activation has also been found in the liver after I/R (1, 2, 36) and hemorrhagic shock (25). Our findings of both a reduction in JNK activation in IRF-1 knockout mice following I/R and abundant JNK activation following recombinant adenoviral expression of IRF-1 in vivo suggest IRF-1 accounts for MAPK activation following I/R. Several studies have shown that JNK blockade with pharmacological JNK inhibitors does inhibit hepatic I/R injury as assessed by histology and serum transaminases in animal models of both warm (37) and cold (38) I/R injury. Our findings are consistent with the important role of JNK in liver I/R injury identified in these reports. The IRF-1 knockout mice exhibited ~60% decrease in hepatocellular necrosis compared with the wild-type mice. These data suggest that while IRF-1-mediated liver damage is a significant component of the hepatic I/R injury, there are also IRF-1-independent mechanisms contributing to the liver injury as the knockout mice were not completely protected. Several groups have shown a role for NF-κB in mediating liver damage, and previously we have shown that in the rat cold I/R injury model, NF-κB activation has a bimodal peak at 1 and 12 h postreperfusion, indicating the complexity of hepatic I/R injury (33).

Because loss of IRF-1 results in protection from liver ischemia, we hypothesized that IRF-1 overexpression would lead to further injury after I/R. Interestingly, we found that transfection of recombinant IRF-1 using an adenoviral vector in vivo results in robust hepatic damage even in the absence of I/R injury. The injury seen with adenoviral IRF-1 treatment was not due to the effects of the adenoviral infection itself, because injection of an empty adenoviral vector control did not cause hepatocellular damage. However, it is unknown whether the mechanism of IRF-1-mediated injury during ischemia and reperfusion is the same as that occurring with IRF-1 overexpression.

Although our study demonstrates the importance of IRF-1 in initiating the inflammatory gene expression that results in organ injury after ischemia and reperfusion, the mechanisms of IRF-1 induction remain unclear. IRF-1 mRNA has been shown to accumulate in response to a variety of IFNs and cytokines (19). Indeed, we find that these mediators are able to upregulate IRF-1 in cultured hepatocytes. Some of these factors, such as IFN-γ, TNF-α, and IL-1, are also expressed after hepatic I/R and have been shown to play key roles in the pathophysiology of I/R injury (6, 16, 39), possibly in part by initiating IRF-1 mRNA expression. It is unknown whether there is a direct connection to the control of IRF-1 signaling with these different cytokine pathways or whether this represents a more global phenomenon of IRF-1 response to hepatocellular cytokine-induced stress.

In conclusion, we showed the transcription factor IRF-1 is upregulated after hepatic ischemia and reperfusion. We also showed that IRF-1 knockout mice are protected from organ injury after I/R and that overexpression of IRF-1 results in liver damage even in the absence of ischemia. Our findings suggest that IRF-1-dependent signaling contributes to tissue damage after I/R. Strategies targeting IRF-1 may be considered in clinical settings of ischemic liver injury to minimize organ damage.

REFERENCES

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