Interferon regulatory factor-2 is protective against hepatic ischemia-reperfusion injury

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Klune JR, Dhupar R, Kimura S, Ueki S, Cardinal J, Nakao A, Nace G, Evankovich J, Murase N, Tsung A, Geller DA. Interferon regulatory factor-2 is protective against hepatic ischemia-reperfusion injury. Am J Physiol Gastrointest Liver Physiol 303: G666–G673, 2012. First published June 28, 2012; doi:10.1152/ajpgi.00050.2012.—Interferon regulatory factor (IRF)-1 is a nuclear transcription factor that induces inflammatory cytokine mediators and contributes to hepatic ischemia-reperfusion (I/R) injury. No strategies to mitigate IRF1-mediated liver damage exist. IRF2 is a structurally similar endogenous protein that competes with IRF1 for DNA binding sites in IRF-responsive target genes and acts as a competitive inhibitor. However, the role of IRF2 in hepatic injury during hypoxic or inflammatory conditions is unknown. We hypothesize that IRF2 overexpression may mitigate IRF1-mediated I/R damage. Endogenous IRF2 is basally expressed in normal livers and is mildly increased by ischemia alone. Overexpression of IRF2 protects against hepatic warm I/R injury. Furthermore, we demonstrate that IRF2 overexpression limits production of IRF1-dependent proinflammatory genes, such as IL-12, IFNβ, and inducible nitric oxide synthase, even in the presence of IRF1 induction. Additionally, isograft liver transplantation with IRF2 heterozygote knockout (IRF2<sup>–/–</sup>) donor grafts that have reduced endogenous IRF2 levels results in worse injury following cold I/R during murine orthotopic liver transplantation. These findings indicate that endogenous intrahepatic IRF2 protein is protective, because the IRF2-deficient liver donor grafts exhibited increased liver damage compared with the wild-type donor grafts. In summary, IRF2 overexpression protects against I/R injury by decreasing IRF1-dependent injury and may represent a novel therapeutic strategy.

LIVER ISCHEMIA-REPERFUSION (I/R) injury occurs in several clinical settings and is known to involve direct cellular ischemic damage, as well as activation of the innate immune system, leading to a complex system of inflammatory pathways (16, 37–41). While much has been learned regarding the initial events during hepatic I/R that lead to activation of the immune system and release of inflammatory mediators, the process is not fully elucidated, and possible strategies to reduce I/R injury are important to explore.

The interferon (IFN) regulatory factors (IRFs) are a family of IFN-inducible transcription factors that are known to be involved in the transcription of genes involved in antiviral defense, immune regulation, and malignancy (reviewed in Ref. 23). IRF1 is upregulated in response to a variety of cytokines and, in turn, activates the transcription of multiple genes and inflammatory mediators, including type I IFNs, inducible nitric oxide synthase (iNOS), and others (5, 12, 19, 21). The involvement of IRF1 in liver I/R injury has been demonstrated in cold (13, 35) and warm (34) I/R injury models. Additionally, the downstream products of IRF1 activation, such as the type I IFNs (39) and iNOS (31, 32), are known to contribute to liver I/R injury.

IRF2 was first described in 1989 as a nuclear factor that is structurally similar to, but functionally distinct from, IRF1 (8). This transcription factor was found to bind the same regulatory sequences as IRF1 but suppressed, rather than activated, transcription of these IFN-inducible genes. However, more recently, IRF2 has been found to have several transcriptional activating roles: it can function as an oncogene in activation of certain cell cycle genes (36), and it can contribute to activation of cytokine production in some infectious and cytokine stimulation models (17, 24, 27). While IRF2 can function as a transcription repressor or activator, depending on the cell type and inflammatory state, its role during hypoxic or acute hepatic inflammation, such as liver I/R injury, is unknown.

With the understanding that IRF1 activation contributes to liver I/R injury and that IRF2 can act as a competitive inhibitor of IRF1 binding to regulatory sequences, we hypothesized that blocking the IRF1-mediated proinflammatory signaling pathways with enhanced IRF2 expression may serve as a novel therapeutic strategy to ameliorate liver I/R injury. In this study, we demonstrate that endogenous intrahepatic IRF2 is protective and that overexpression of IRF2 diminishes induction of IRF1-dependent genes and decreases subsequent liver injury.

MATERIALS AND METHODS

Liver function tests. Hepatic function after warm and cold liver I/R was assessed using a veterinary chemistry analyzer (model 4000 Dri-chem, HESKA, Loveland, CO) to measure serum alanine aminotransferase (ALT) levels.

Hepatocyte isolation. Hepatocytes were isolated from wild-type (WT) mice by an in situ collagenase (type IV, Sigma) perfusion technique, modified as described previously (35). Hepatocytes were separated from the nonparenchymal cells by two cycles of differential centrifugation (50 g for 2 min) and further purified with a 30% Percoll gradient. Hepatocyte purity was >98% as assessed by light microscopy, and viability was typically >95% as determined by Trypan blue exclusion assay.

Cell culture. Hepatocytes (3 × 10<sup>6</sup>) were plated on 60-mm gelatin-coated petri dishes in 3 ml of culture medium, which consisted of Williams medium E (GIBCO Life Technologies, Gaithersburg, MD) with l-arginine (0.5 mmol/l), insulin (10<sup>−6</sup> 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% CO<sub>2</sub>), the medium was changed and the cells were treated as described.

Preparation of adenoviral vectors. An E1- and E3-deleted adenoviral vector carrying the human AdIRF1, AdIRF2, or AdLacZ cDNA...
was constructed as previously described (14). Concentrations of AdIRF1, AdIRF2, and the control adenovirus AdLacZ were determined by plaque-forming assay and expressed as plaque-forming units (pfu). All vectors were diluted with saline to the concentrations described for intravenous injection into the mouse. For cell culture treatment, cells were washed with warmed PBS; then the virus was diluted to the described concentration in serum-free medium (Opti-MEM) and placed on the cell culture for 3 h. After 3 h, the virus was removed, and the cells were allowed to recover for 21 h prior to further treatments.

Isolation of cytoplasmic and nuclear proteins. Frozen liver tissues or cell cultures were suspended in buffer containing 10 mM HEPES (pH 7.9), 1.5 mM MgCl2, 10 mM KCl, and 0.5% Nonidet P-40 and homogenized for 20 s with a Polytron homogenizer (Kinematica, Littau, Switzerland). Nuclei were recovered by microcentrifugation at 7,500 rpm for 5 min. The supernatant containing cytoplasmic protein was collected and stored at −80°C for Western blot analysis. Nuclear proteins were extracted at 4°C by gentle resuspension of the nuclei pellet in buffer containing 20 mM HEPES (pH 7.9), 10% glycerol, 1.5 mM MgCl2, 10 mM KCl, and 0.2 mM EDTA followed by 30 min of 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 and frozen at −80°C. All buffers contained the following additional ingredients: 0.2 mM phenylmethylsulfonylfluoride, 0.5 mM dithiothreitol, 0.1 mM sodium vanadate, and protease inhibitors. Protein concentration was quantified with Bio-Rad Protein Assay (Bio-Rad Laboratories, Hercules, CA).

Western blot analysis. Western blot analysis was performed using nuclear or whole cell lysate from liver specimens (20–50 µg), as previously described (35). For IRF1 and IRF2, 20 µg of nuclear protein were used. Membranes were incubated with primary polyclonal antibody for IRF1 or histone-3 (Santa Cruz Biotechnology, Santa Cruz, CA), IRF2 (Cell Signaling Technology, Beverly, MA), iNOS (Transduction Laboratories, Lexington, KY), or actin (Sigma-Aldrich, St. Louis, MO). After incubation with secondary goat anti-rabbit antibody (Pierce Chemical, Rockford, IL), membranes were developed with the Super Signal detection systems (Pierce Chemical) and exposed to film.

Real-time RT-PCR. The mRNAs for IL-12, IFNβ, and iNOS were quantified in duplicate using SYBR Green two-step, real-time RT-PCR, as previously described (22). Gene expression was normalized with GAPDH mRNA content.

Animals. Male WT (C57BL/6) mice (8–12 wk old) were purchased from Jackson Laboratory. Animal protocols were approved by the University of Pittsburgh Institutional Animal Care and Use Committee and were performed according to the National Institutes of Health guidelines for the use of laboratory animals. IRF2−/− mice were generated by targeted disruption, as previously described (20). IRF2−/− mice, backcrossed to C57BL/6 mice for three to five generations at the time we received them, were the kind gift of Dr. T. Mak (Agen Institute, Toronto, ON, Canada). All IRF2 mice bred in our colony were genotyped.

In vivo gene delivery and warm I/R. For in vivo transfection, mice were intravenously injected (tail vein) with AdIRF2 or AdLacZ at doses of 1.5 × 108 or 7.5 × 108 pfu for 24–48 h prior to assessment of predetermined end points. For warm liver I/R, mice were subjected to 1 h of ischemia and euthanized at predetermined reperfusion time points for tissue sample collection. A nonlethal model of segmental (70%) hepatic warm ischemia was used as previously described (33). Sham animals underwent anesthesia, laparotomy, and exposure of the portal triad without hepatic ischemia. Baseline or untreated animals were given anesthesia and euthanized without exposure of the portal triad.

Orthotopic liver transplantation. The techniques of liver harvesting and murine orthotopic liver transplantation (OLTx) without hepatic artery reconstruction were based on the method described by Qian et al. (25). Briefly, after cholecystectomy and insertion of a polyethylene stent (PE-10, Becton Dickinson, Franklin Lakes, NJ) into the bile duct, donor animals were heparinized, and liver grafts were perfused with 1.0 ml of University of Wisconsin (UW) solution via the portal vein. Excised liver grafts were prepared with cuffs on the infrahepatic vena cava and portal vein and then stored in UW solution for 24 h at 4°C. After removal of native livers in recipient animals, grafts were orthotopically implanted by anastomosis of the suprahepatic vena cava with a running 10-0 nylon suture and connection of the cuffs to the recipient portal vein and inferior vena cava. Anhepatic time averaged 19.8 ± 1.7 min. The bile duct was connected via the ligation over the stent. The abdominal wall was then closed in two layers. Our group recently showed that this murine OLTx technique results in 98% survival (35).

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

RESULTS

IRF2 can be safely overexpressed using adenoviral vector. Recombinant adenovirus encoding murine IRF2 (AdIRF2) was used in initial experiments to demonstrate the safety of nuclear overexpression of IRF2. It is known that transfection with adenoviral vectors can elicit nonspecific inflammation, depending on viral titer levels, particularly in mice. Mice were injected with 1.5 × 108 or 7.5 × 108 pfu of AdLacZ (control) or AdIRF2, as previously described (34). Mice injected with 1.5 × 108 pfu did not experience hepatic damage at 36 h, as measured by serum ALT levels (Fig. 1A). Also, there was no difference in liver injury between the AdLacZ and AdIRF2 groups. In contrast, in mice injected with 7.5 × 108 pfu of AdIRF2 or AdLacZ, ALT levels rose, suggesting nonspecific liver damage from the adenovirus. Therefore, the lower dose of 1.5 × 108 pfu was used for further experiments. To document in vivo gene transfection and transgene expression, mice were injected with a single dose of 1.5 × 108 pfu of AdIRF2 or AdLacZ and euthanized at 24, 36, and 48 h. Nuclear protein extracts from liver tissue were examined for expression of IRF2 protein. Nuclear levels of IRF2 protein were increased in AdIRF2 transected mice compared with AdLacZ mice, confirming successful in vivo gene transfer (Fig. 1B). The low level of hepatic IRF2 nuclear protein detected in the normal (baseline) and AdLacZ-injected mice reflects constitutive expression of endogenous IRF2. These results suggest that IRF2 overexpression can be achieved in mouse livers without causing nonspecific hepatic damage by using a low adenoviral titer.

IRF2 overexpression is protective in hepatic warm I/R injury. We previously showed that IRF1 activation occurs early following warm (34) and cold (13, 35) liver I/R injury and contributes to liver damage. However, the expression pattern of IRF2 following I/R injury has not been described. The time course of nuclear expression of IRF1 and IRF2 proteins was determined using Western blot analysis of nuclear extracts from hepatic parenchyma following warm I/R injury. Nuclear IRF1 expression increased at 1 h following I/R, with maximum expression at 3 h after reperfusion (Fig. 2A). IRF2 expression, in contrast, was induced slightly above sham levels by ischemia alone but did not increase further with reperfusion (Fig. 2A). To determine the effect of IRF2 overexpression on hepatic I/R, mice were pretreated with AdIRF2 vector or AdLacZ and subjected to hepatic warm I/R. Overexpression of IRF2 significantly reduced serum ALT levels 3.7-fold, with a 73% decrease from 3,700 IU/l in the AdLacZ-treated mice to 1,000 IU/l in the
overexpression is protective during hepatic I/R by inhibiting activation of IRF1-driven proinflammatory genes.

*IRF2 overexpression does not alter nuclear IRF1 levels in vivo or in vitro. IRF1 and IRF2 compete for binding to DNA regulatory elements, and the ultimate expression of IFN-responsive genes depends on the balance between these two regulatory transcription factors (9). While IRF1 and IFNβ work in a positive-feedback loop, where each induces transcriptional activation of the other, it is unknown if IRF1 and IRF2 can alter the expression of the other. Additionally, while increased IRF1 expression in response to stimulation with IFNγ is well known, IRF2 expression following the same stimulation is not established (9, 15, 28). To determine whether IRF1 expression is affected by IRF2 overexpression, nuclear protein extracts from hepatic parenchyma from mice injected with AdLacZ or AdIRF2 were subjected to Western blot analysis. As expected, AdIRF2-treated mice had higher levels

AdIRF2-treated mice (Fig. 2B). Hence, overexpression of IRF2 is protective during I/R injury.

*IRF2 overexpression results in decreased cytokine and IRF1 target gene production. IRF1 results in the transcriptional activation of a variety of target genes, including many that have been implicated in hepatic I/R injury, such as iNOS, type I IFNs, and IL-12. IRF2 is known to bind to the same *cis*-acting DNA sequences and competitively inhibit IRF1 protein-DNA binding. Using quantitative RT-PCR in mice injected with AdIRF2 or AdLacZ and subjected to warm I/R, we examined the expression of hepatic IRF1 target genes. Sham-treated mice had low basal levels of hepatic IL-12, which was not significantly affected by IRF2 overexpression (Fig. 3A). Hepatic IL-12 mRNA was markedly increased by I/R, and IRF2 overexpression significantly decreased IL-12 mRNA levels compared with AdLacZ-treated animals. Additional specific IRF1 target genes associated with I/R injury include IFNβ and iNOS. IFNβ and iNOS mRNA were increased after hepatic I/R compared with baseline levels, and AdIRF2-treated mice also demonstrated significantly decreased transcriptional activity of these genes (Fig. 3, B and C). These data suggest that IRF2

*Fig. 1. Interferon regulatory factor (IRF)-2 overexpression does not affect IRF1 levels. C57BL/6 mice (8–12 wk old) underwent adoptive transfer of IRF2 or control vector LacZ by tail vein injection of 1.5 × 10⁸ or 7.5 × 10⁸ plaque-forming units (pfu) of AdIRF2 or AdLacZ, and serum alanine aminotransferase (ALT) levels were determined at 24, 36, and 48 h. A: serum ALT levels at 36 h after treatment with 1.5 × 10⁸ or 7.5 × 10⁸ pfu of AdIRF2 or AdLacZ. Values are means ± SE (n = 3 per treatment). B: Western blots of liver nuclear extracts at 24, 36, or 48 h after injection of 1.5 × 10⁸ pfu of AdIRF2 or AdLacZ show expression of IRF2 and histone in the liver. Blots are representative of 3 per treatment.

*Fig. 2. IRF2 overexpression is protective in warm liver ischemia-reperfusion (I/R) injury. C57BL/6 mice (8–12 wk old) underwent partial warm liver I/R. A: untreated mice were subjected to 1 h of partial ischemia with predetermined euthanasia time points after 0, 1, 3, and 6 h of reperfusion. Liver nuclear extracts were subjected to Western blot analysis for IRF1 and IRF2 to determine a time course of activation. B: mice treated with AdIRF2 or AdLacZ control were subjected to 1 h of partial warm ischemia followed by 6 h of reperfusion, and serum ALT levels were determined. Values are means ± SE (n = 3 per treatment). *P < 0.05.
IFNγ induces hepatocyte IRF1 expression as expected and that overexpression of IRF2 does not alter basal or IFNγ-induced IRF1 expression.

**IRF2 overexpression decreases IRF1-dependent iNOS transcription in vitro.** Stimulation of hepatocytes by IFNγ has previously been shown by mRNA analysis to activate transcription of IRF1 (6) and iNOS (7). IFNγ-dependent iNOS expression is mediated by IRF1 binding in the iNOS promoter (12, 19, 29, 30), and IRF1−/− mice show diminished iNOS induction (12, 26). As expected, in vitro stimulation of hepatocytes with IFNγ resulted in iNOS protein induction after 24 h of stimulation (Fig. 5A). This induction was inhibited in a dose-dependent fashion in response to IRF2 overexpression using AdIRF2 transfection. In contrast, AdLacZ control vector did not alter expression of iNOS in response to IFNγ stimulation. These findings confirm a suppressive role for IRF2 in downregulating IFNγ-induced IRF1-dependent iNOS transcriptional activation in hepatocytes. Next, we sought to show that IRF2 would also inhibit iNOS gene expression stimulated by exogenous IRF1 overexpression. Hepatocytes transfected with AdIRF1 demonstrated increased iNOS expression in a dose-dependent fashion (Fig. 5B). In contrast, AdIRF2 transfection alone did not induce iNOS expression in hepatocytes. Cotransfection with AdIRF1 and AdIRF2 with MOI of 50 resulted in barely detectable iNOS expression, confirming a role for IRF2 in repressing IRF1-mediated transcriptional activation of iNOS. These findings show that IRF2 functions to suppress IRF1-mediated transcriptional activity in hepatocytes. Additionally, we wanted to confirm that this effect was not limited to hepatocytes but also applies in other cell types such as macrophages or Kupffer cells, which are also known to contribute to I/R injury. RAW 264.7 cells did overexpress IRF2 with AdIRF2 transfection compared with control (data not shown), and this IRF2 overexpression inhibited expression of iNOS following stimulation with LPS, IFNγ, or a combination of both. Therefore, we found that IRF2 expression is able to block IRF1 activity in other important cell types as well.

**IRF2 heterozygote liver grafts result in increased injury after hepatic transplantation.** The previous experiments demonstrate that IRF2 overexpression could block IRF1-mediated inflammatory signaling and decrease liver I/R injury; however, they do not address the functional role of endogenous IRF2. Therefore, IRF2+/− mice were used to determine the effect of decreased IRF2 expression on liver I/R injury. Heterozygote mice were used, because IRF2−/− homozygous mice breed poorly and were phenotypically abnormal. To determine the baseline expression of IRF2 in these mice, hepatic nuclear protein extracts from IRF2+/− mice were examined and showed that baseline expression of IRF2 was significantly decreased in IRF2+/− mice compared with WT control mice (Fig. 6A). Additionally, nuclear protein extracts from an IRF2−/− mouse demonstrated no IRF2 protein. In contrast, baseline levels of nuclear IRF1 were not affected in any of the nuclear protein extracts, including the IRF2+/− mice. Therefore, the IRF2 heterozygote mice were used for liver graft donation for OLTx into WT mice. Murine OLTx using IRF2+/− donor grafts showed a twofold increase in liver injury measured by serum ALT compared with WT donor grafts (Fig. 6B). These results confirm a protective role for endogenous hepatic IRF2, because
IRF2 relative deficiency in the IRF2+/− mice resulted in increased liver damage.

**DISCUSSION**

Liver I/R injury occurs in the clinical settings of liver transplantation, hepatic resection surgery, and hemorrhagic trauma. Previously, we showed that the nuclear transcription factor IRF1 contributes to liver damage in warm and cold I/R injury through multiple mechanisms, including expression of proinflammatory cytokine mediators of inflammation, activation of intracellular signaling cascades, and promotion of apoptosis through expression of death receptors and ligands (13, 14, 34, 35, 39). No strategies aimed at decreasing IRF1-mediated liver injury have been defined. IRF2 is a ubiquitous endogenous protein antagonist of IRF1 (8, 9). Therefore, we examined the role of IRF2 in liver I/R injury. The major and novel findings of this study are as follows:

1) endogenous intrahepatic IRF2 is protective against liver I/R injury, as IRF2−/−-deficient mice exhibited more severe liver damage after OLTx;
2) IRF2 overexpression reduces hepatic I/R injury; and
3) IRF2 overexpression significantly decreases induction of IRF1 responsive hepatic inflammatory mediators IL-12, IFNγ, and iNOS.

A major mechanism of action of IRF2 is to competitively inhibit induction of IRF1 inflammatory target genes. While IRF2 was originally described as an IRF1 antagonist (8, 9), it has also been shown to function as an IRF1 agonist or transcriptional activator in response to infection or certain cytokine stimulation. In macrophages, IRF2 contributes to IL-12 and IFNγ expression in response to LPS with or without stimulation with IFNγ (27) and contributes to IRF1 activation and IFNα release in lipoteichoic acid stimulation in a model of *Staphylococcus aureus* infection (17). IRF2 functions to upregulate IL-7 expression in human intestinal epithelial cells in response to IFNγ stimulation (24). While IRF2 has the potential to function as a transcriptional inhibitor or activator in different inflammatory states, its role during the immune response to liver I/R injury has not been addressed.

Since IRF1 contributed to hepatic injury during warm and cold I/R (13, 34, 35), we initially sought to block the actions of IRF1 by overexpressing IRF2. In vivo expression of IRF2 was achieved with 1.5 × 10⁸ pfu of AdIRF2, which did not induce nonspecific inflammation (Fig. 1A). We recognize the limitations of not being able to use adenoviral delivery in patients; however, it provides excellent gene delivery for in vivo hepatic transfection and, therefore, was used to establish proof-of-principle in this study. Strong AdIRF2 expression was observed 24–48 h after injection. Mice that were overexpressing IRF2 were then subjected to warm I/R injury and showed a >70% decrease in serum ALT levels compared with AdLacZ-treated animals. This protection was mediated in part by IRF2 downregulating induction of IRF1-mediated inflammatory cyto-

Fig. 4. IRF2 overexpression does not alter nuclear IRF1 levels in vitro. A: liver nuclear protein extracts were analyzed by Western blot for expression of IRF1, IRF2, and histones to determine relative expression of these proteins after treatment with 1.5 × 10⁸ pfu of AdIRF2 or AdLacZ. B: primary cultured hepatocytes were subjected to gene transfer with 1.5 × 10⁸ pfu of AdIRF2. Cells were incubated for 24 h prior to nuclear protein extraction. Western blot analysis for IRF1 or IRF2 was performed on nuclear extracts. MOI, multiplicity of infection. C: primary cultured hepatocytes underwent gene transfer with AdIRF2. After incubation for gene transfer, cells were treated with IFNγ (100 U/ml). Nuclear protein extracts were analyzed for IRF1 and IRF2 by Western blot.
IL-12, IFN-β, and iNOS in vivo. Moreover, IRF2 inhibited the expression of the IRF1-dependent iNOS gene in vitro in a dose-dependent manner.

Since overexpressed IRF2 was successful in blocking IRF1-mediated inflammatory gene activation and decreasing subsequent liver injury, we next sought to determine the role of endogenous IRF2 during hepatic I/R injury, and we also show that IRF2 can be used to protect against I/R injury.

In addition to the roles described above, it is also clear that IRF1 and IRF2 contribute to immune system development. IRF1−/− and IRF2−/− mice demonstrate defective differentiation of T helper type 1 cells (18). Interestingly, while IRF2−/− mice have reduced numbers of natural killer cells, IRF1−/− mice do not have reduced numbers of these cells, but the natural killer cells that are present have decreased function in response to a variety of stimuli (4, 18). While IRF1 and IRF2 clearly have related functions in immune system development, there are differences in the mechanisms that lead to their immune-deficient state. One limitation of this study is that we focused our in vitro investigations on the role of IRF2 in hepatocytes and only briefly considered the role in immune cells, specifically macrophages or Kupffer cells, which also contribute to I/R injury. In these cell types, IRF2 signaling is complex and varies according to stimulus. In response to Toll-like receptor 2 activation with lipoteichoic acid, IRF2 appears to be upregulated and to lead to activation of IRF1 and secretion of type I IFNs (17). In contrast, in response to LPS, IRF2−/− KO mice had significantly increased TNFα levels and greater numbers of apoptotic Kupffer cells (3). These antiapoptotic effects of IRF2 appear to signal through STAT1/3 and caspase-1 (2). We previously demonstrated the importance of IRF1 in hepatocytes and nonparenchymal cells in contributing to liver damage during I/R (35). Future investigations may be

Fig. 6. IRF2 heterozygote liver grafts result in increased injury after transplantation. A: wild-type (WT), IRF2 heterozygote, and IRF2 knockout mice were euthanized, and liver tissue was sampled. Nuclear protein extracts were obtained, and baseline levels of IRF1 and IRF2 were determined by Western blot analysis. B: serum ALT levels were determined following liver transplants in wild-type (WT-WT, n = 5) and IRF2 heterozygote (IRF2−/+ to WT, n = 3) liver transplants.
warranted to more fully elucidate the role of IRF2 in these other cell types but were outside the limits of this study.

Previous reports demonstrated that IRF2 expression can be induced by IFNβ, IFNγ, TNFα, or LPS in some cell types under specific conditions (1, 9, 10), while other reports indicate constitutive expression (11). The expression and induction patterns are not well described for IRF2 in hepatocytes. In this study, IRF2 is expressed at a low baseline level in untreated or sham liver tissue and induced following liver ischemia, but the expression does not increase further following reperfusion; rather, it is stably induced over this period. It is unknown what leads to the induction of IRF2 during I/R, and this could be due to cytokines, general cellular stress, or oxidative stress, among other possibilities. The factors leading to increased IRF2 will be the subject of further investigation. Furthermore, other pharmacological methods for induction of IRF2 may lead to potential therapeutic options in liver I/R injury.

As already mentioned, a limitation of this study is the use of adenovirus for overexpression of IRF2. While the data clearly indicate a beneficial effect, other viral or nonviral delivery methods will have to be pursued in the clinical setting. Nonetheless, the current data provide confirmation that IRF2 functions as an antagonist to IRF1-mediated signaling pathways in this setting. Importantly, this offers a novel therapeutic strategy for combating hepatic I/R injury by decreasing IRF1-mediated injury through IRF2 overexpression. Further studies are warranted to optimize IRF1 blockade and IRF2 delivery.

REFERENCES


