Protein-tyrosine Phosphatase 1B Deficiency Protects against Fas-induced Hepatic Failure*

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Genetic disruption of protein-tyrosine phosphatase 1B (PTP1B) in mice leads to increased insulin sensitivity and resistance to weight gain. Although PTP1B has been implicated as a regulator of multiple signals, its function in other physiological responses in vivo is poorly understood. Here we demonstrate that PTP1B-null mice are resistant to Fas-induced liver damage and lethality, as evidenced by reduced hepatic apoptosis in PTP1B-null versus wild type mice and reduced levels of circulating liver enzymes. Activation of pro-apoptotic caspases-8,-9,-3, and -6 was attenuated in livers from PTP1B-null mice following Fas receptor stimulation, although components of the death-inducing signaling complex were intact. Activation of anti-apoptotic regulators, such as the hepatocyte growth factor/Met receptor tyrosine kinase, as well as Raf, ERK1/2, FLIP, and the NF-κB pathway, was elevated in response to Fas activation in livers from PTP1B-null mice. Using PTP1B-deficient primary hepatocytes, we show that resistance to Fas-mediated apotosis is cell autonomous and that signals involving the Met, ERK1/2, and NF-κB pathways are required for cytoprotection. This study identifies a previously unknown physiological role for PTP1B in Fas-mediated liver damage and points to PTP1B as a potential therapeutic target against hepatotoxic agents.

The nonreceptor tyrosine phosphatase PTP1B is the most widely studied prototype for the PTP superfamily and was first purified from human placenta (1). PTP1B is a ubiquitously expressed 50-kDa protein possessing a N-terminal catalytic domain followed by tandem proline-rich motifs (2, 3). At its C terminus, PTP1B contains a small hydrophobic endoplasmic reticulum-targeting sequence (4). PTP1B has been implicated in the modulation of multiple cytokine and growth factor-activated signaling pathways, as demonstrated by its ability to dephosphorylate growth factor receptors, including the insulin, IGF-1 (5), EGF (6), platelet-derived growth factor (7) receptors, as well as nonreceptor tyrosine kinases, Src (9), p210Bcr-Abl (10), Jak2, and Tyk2 (11).

The role of PTP1B in regulating the activity of the insulin receptor has been extensively studied. PTP1B-null mice show hyperphosphorylation of the insulin receptor in liver and muscle tissue upon stimulation with insulin (12, 13). Moreover, these mice are resistant to weight gain caused by a high fat diet through the ability of PTP1B to regulate the leptin receptor, via dephosphorylation and subsequent termination of signaling from the downstream kinase Jak2 (14, 15). PTP1B has also been shown to interact with a number of SH3 domain-containing proteins through its proline-rich motifs, including p130Cas (3), p62Dok (16), β-catenin (17), Grb2, and Crk (3), which are thought to target this phosphatase to distinct cellular protein complexes. Although PTP1B has been implicated as a regulator of diabetes and obesity, its function in other physiological responses regulated in vivo by tyrosine kinase signaling is poorly understood. For example, multiple cytokine and growth factor signals, such as TNF-α, EGF, HGF, IGF-1, and insulin, as well as interleukin 6, play important roles as anti-apoptotic and growth-promoting signals in response to surgical or chemical liver damage (18–21). Several of these are targets for PTP1B (6, 12, 13, 22). Deregulation of the apoptotic program is pathophysiologically involved in liver disease (23). Acute hepatic failure induced by hepatic toxins such as Fas ligand (FasL), or the agonistic Fas antibody (Jo-2) is characterized by uncontrolled apoptosis of hepatocytes mediated by the death receptor Fas (FasR) (24). Moreover, the Fas receptor is present on the plasma membrane of normal hepatocytes and is overexpressed in some chronic hepatic diseases such as that induced by the hepatitis B (25) or hepatitis C viruses (24, 26). Binding of FasL to its cognate receptor causes hepatic apoptosis via caspase activation. This occurs through FasR-mediated assembly of the death-inducing signaling complex (DISC) (27). The DISC complex consists of the Fas receptor, caspase-8, caspase-10, and FADD. Autoproteolytic cleavage of caspase-8 is an early detectable event in Fas-induced apoptosis, leading to caspase-3 activation either in a mitochondria-independent manner or via a mitochondria-dependent pathway that proceeds via Bid and caspase-9 (28, 29).

To determine whether PTP1B plays a role in regulating signals involved in liver damage and survival, we determined the sensitivity of PTP1B-null and wild type (WT) mouse hepatocytes to FasR activation, using two complementary experimental systems: whole liver in vivo and in vitro primary cultures. Protection from cell death and fulminant hepatic failure was evident by reduced blood levels of liver enzymes as

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well as reduced apoptosis in liver sections of PTP1B-null versus wild type mice after injection with agonistic Fas antibody (Jo-2). This identifies a previously unknown function for PTP1B in modulating hepatic apoptosis in response to Fas-induced liver damage.

**EXPERIMENTAL PROCEDURES**

**Animals and Antibodies**

WT and PTP1B-null mice are hybrids of 129Sv and BALB/c backgrounds (12). Female mice (4–6 weeks) were used for experiments. The antibodies were obtained as follows: monoclonal anti-Jo-2, anti-c-Raf, anti-phosphotyrosine (PY20), and polyclonal anti-EGFR (BD Biosciences, San Diego, CA); polyclonal anti-FLIP (Axxora LLC, San Diego, CA), polyclonal anti-PTP1B antibody (Upstate Biotechnology, Inc., Lake Placid, NY); mouse monoclonal anti-Met, polyclonal anti-Fas, anti-IkBa, anti-insulin receptor, and anti-IGF1 receptor (Santa Cruz Biotechnology, Santa Cruz, CA); polyclonal anti-caspase-9 and anti-caspase-8 (Stressgen, Victoria, Canada); polyclonal anti-caspase-3, anti-ERK1/2 antibody, and anti-phospho-ERK1/2 pTpY202/204 antibody (Cell Signaling Technology, Beverly, MA); rabbit polyclonal anti-c-Raf pYpY340/341 antibody and polyclonal pIR/IGF1RpYpYpY1158/1162/1163 antibody (Biosource, Camarillo, CA).

**Injection of Jo-2 and Histology**

Wild type and PTP1B-null mice were injected intraperitoneally with 0.3 μg/g body weight of Jo-2 in 200 μl of phosphate-buffered saline. After 6 h, the mice were sacrificed, and the liver tissue was recovered by dissection. The livers were fixed in 10% buffered formalin and embedded in paraffin. 4-μm sections were cut and stained with hematoxylin and eosin. All of the mouse manipulations were carried out in accordance with McGill University animal care guidelines.

**Serum ALT and AST Activity**

Wild type and PTP1B-null mice were injected intraperitoneally as above. After 4 or 6 h, the mice were anesthetized and exanguinated by cardiac puncture, and serum was collected. The liver damage was quantitated by measuring serum ALT and serum AST levels using ALT IFCC and AST IFCC modified reagent without pyridoxal phosphate (Roche Applied Science). The data were acquired using a Hitachi 911 analyzer.

**Immunoblotting**

Livers were homogenized using a Mixer Mill MM301 (Glen Mills Inc., Clifton, NJ) in 1% Triton lysis buffer (50 mM HEPES, pH 7.4, 150 mM NaCl, 2 mM EGTA, pH 8.0, 1.5 mM MgCl2, 10% glycerol, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride, 1 mM Na2VO4). The homogenates were centrifuged at 13,000 rpm to remove debris. The protein extracts were resolved on 10% SDS-polyacrylamide gels, transferred onto nitrocellulose membranes (Hybond; Amersham Biosciences), and probed with antibodies as described, followed by appropriate horseradish peroxidase-conjugated secondary antibodies. All of the immunoblots were visualized by enhanced chemiluminescence (Amersham Biosciences).

**Hepatocyte Preparation, Culture, and Flow Cytometry**

The hepatocytes were isolated according to a modified version of the two-step collagenase method originally developed for rats (30, 31). The mouse livers were perfused with a 25 mM Ca2+-free HEPES buffer, pH 7.5, containing insulin (0.5 μg/ml) and EGTA (0.5 mM), followed by Dulbecco's modified Eagle's medium/Ham's F-12 modified medium containing collagenase (0.2 Wünsch units/ml) and Ca2+ (5 mmol/liter). 0.5 × 10⁶ cells/cm² viable hepatocytes were plated on attachment media (Dulbecco's modified Eagle's medium/Ham's F-12 medium with selenium-free (5 μg/μl), insulin (5 mg/liter), transferrin (5 mg/liter), and gentamycin (50 μg/ml)) on fibronectin-coated dishes. Post-attachment, dexamethasone (10⁻⁷ M) and EGF (20 ng/ml) were added to the culture medium. For cell surface Fas receptor staining, hepatocyte cell suspensions were incubated with mouse Fc block (CD16/CD32) (BD Biosciences) and Fas-specific anti-phycocerythrin antibody (BD Pharmingen, San Diego, CA). CD62L hamster IgG2A (PharmMingen, San Diego, CA) conjugated to phycocerythrin was used as an isotype control. The data were acquired using a FACS Scan flow cytometer (Becton Dickinson, San Jose, CA) and analyzed with CellQuest software (Becton Dickinson, San Jose, CA).

**Thymocyte Preparation and Viability Assay**

Thymocytes were removed aseptically from WT and PTP1B-null mice, and single cell suspensions were prepared by gently teasing through a 70-μm nylon mesh. Thymocytes (0.15 × 10⁶ cells/well) were cultured in 96-well flat-bottomed plates in complete RPMI medium (with glutamine, β-mercaptoethanol, and gentamycin) supplemented with 10% fetal bovine serum and treated with Jo-2 (0.0625–1 μg/ml) at 37 °C and 5% CO₂ in a final volume of 100 μl for 6 h. Cell viability was measured using the colorimetric WST-1 assay (Roche Applied Science) as per the manufacturer’s instructions.

**Assessment of Apoptosis**

**TUNEL Assays**—The livers were fixed in 10% buffered formalin, paraffin-embedded, sectioned (4 μm), stained to visualize apoptosis using the Apoptag fluorescein in situ apoptosis detection kit (Serologicals Corporation, Norcross, GA). Tissue sections were stained with anti-digoxigenin fluorescein conjugate antibody and counterstained with 4′,6′-diamino-2-phenylindole (0.5 mg/ml).

**Annexin V Analysis**—The hepatocytes were plated at a density of 0.5 × 10⁶ cells/cm² on fibronectin-coated dishes and preincubated with HGF (20 ng/ml) for 6 h and/or UO126 (20 μM) (BD Transduction Laboratories, Mississauga, Canada), NF-κB inhibitor peptide (SNS50) (50 μg/ml) and inactive control peptide (SNS50M) (50 μg/ml) (Calbiochem, La Jolla, CA), Genistein (50 μM) (Sigma-Aldrich), or PHA-665752 (0.1 μM) (Pfizer Pharmaceuticals) for 1 h prior to treatment with Jo-2 antibody (0.5 μg/ml), FasL (10 ng/ml) (Sigma-Aldrich), or TNF-α (10 ng/ml) for 4 h. TNF-α treatment was carried out in the absence or presence of actinomycin D (10 μg/ml). Annexin V staining (Roche Applied Science) was performed following the manufacturer’s instructions. Annexin V-positive cells were visualized by fluorescence microscopy. Apoptosis observed in untreated hepatocytes was taken as base line and subtracted from the treatment group.

**RESULTS**

**PTP1B-null Mice Are Protected against Fas-induced Liver Failure**—Multiple cytokine and growth factor signals that play important roles as anti-apoptotic and growth-promoting signals in response to surgical or chemical liver damage (18–21) are known targets for PTP1B (6, 12, 13). We therefore investigated the role of PTP1B in protection against liver damage. To induce acute liver failure, PTP1B-null and WT mice were intraperitoneally injected with a lethal dose of the FasR-specific antibody Jo-2 (0.3 μg/g of body weight). The majority of PTP1B-null mice showed no overt signs of clinical compromise, loss of appetite, or decreased activity after injection with Jo-2 and were resistant to the lethal effect of the Fas-specific antibody (75%) (Table 1). In contrast, 84% of their WT counterparts presented with signs of clinical compromise, including tachypnea, shallow breathing, and prostration indicate-
tive of liver failure and were sacrificed (Table 1). The livers from these WT mice turned dark red in color following injection of Jo-2, which is indicative of liver hemorrhage, whereas livers from PTP1B-null mice did not show this phenotype (Fig. 1A). Histological analysis of livers from the Jo-2-injected WT mice that showed distress revealed parenchymal necrosis, hemorrhage, and hepatocyte apoptosis, whereas livers from the majority of PTP1B-null mice showed no significant histologic pathological features (Fig. 1B). As a measure of apoptosis, DNA fragmentation, a hallmark of apoptotic cells, was quantified by the incorporation of anti-digoxigenin-fluorescein in liver sections (TUNEL staining). Liver sections from Jo-2-injected WT mice showed a significant number of positive TUNEL staining cells. In contrast, few positive-staining apoptotic cells were observed in sections from either saline-injected controls or PTP1B-null mice injected with Jo-2 (Fig. 1, C and D). However, livers from the PTP1B-null mice that showed distress in response to Jo-2 (25%) (Table 1) showed parenchymal necrosis and hepatocyte apoptosis to similar levels as WT animals (supplemental Fig. S1). Blood samples were collected from WT and PTP1B-null mice at different time points (4 and 6 h) following Jo-2 injection and assayed for levels of two circulating liver enzymes, ALT and AST, elevated levels of which are indicative of liver damage. Levels of ALT and AST were significantly elevated in WT mice 6 h post-Jo-2 treatment (Fig. 1E). In contrast, ALT and AST levels were near base line in PTP1B-null mice in response to Jo-2 6 h post-injection (Fig. 1E) and remained at these levels even when assessed at 24 h (data not shown). Taken together, our data demonstrate that PTP1B-null mice are resistant to Fas-mediated liver damage.

**TABLE 1**

| Fas-specific antibody injection | Alive | Sacrificed* |
|--------------------------------|-------|-------------|
| PTP1B-WT                       | 7/43 (16%) | 36/43 (84%) |
| PTP1B-KO                       | 24/32 (75%) | 8/32 (25%) |

* The mice were sacrificed within 6 h upon showing signs of distress.

**FIGURE 1.** PTP1B deficiency protects against Fas-induced liver apoptosis. Mice were intraperitoneally injected with 0.3 μg/g body weight of the Fas-specific antibody, Jo-2. The current model of Fas engagement suggests that the Fas receptor, upon binding of FasL, recruits FADD, followed by caspase-8. Formation of this DISC complex activates caspase-8, thus initiating the caspase cascade leading to the activation of downstream caspase-9, caspase-7, caspase-6, and caspase-3 (27). To determine the step in the apoptotic signaling cascade that is blocked because of a loss of PTP1B, we studied the presence of activated caspases in liver lysates from WT and PTP1B-null mice post-Jo-2 treatment. Consistent with our histological data (Fig. 1, B–D), cleavage of caspases-8, -9, -3, and -6, normally activated following FasR oligomerization (32), was detected in liver extracts from WT mice 6 h post-Jo-2 treatment but was significantly
Loss of PTP1B Protects against Fas-mediated Liver Failure

Induction of Signals in Livers from Fas-treated PTP1B-null Mice—In liver development and T cells, resistance to apoptosis induced by death receptors is tightly regulated by genes activated by the transcription factor NF-κB (33–36). An investigation of proteins involved in the NF-κB pathway in liver extracts revealed that levels of IκBα, the inhibitor of NF-κB signaling, were decreased in both Jo-2-treated PTP1B as well as WT mice (Fig. 3). However, the decrease in IκBα levels was significantly greater in PTP1B-null mice when compared with WT (Fig. 3), indicating greater activation of this pathway in PTP1B-null mice. Consistent with this, levels of FLIPI, a downstream transcriptional target of NF-κB (37, 38) that can act as an inhibitor of caspase-8 activation by interacting with FADD and caspase-8 in the DISC complex (39), were elevated in PTP1B-null mice following Jo-2 treatment when compared with WT mice (Fig. 3). In addition, MAPK enzymes (ERK1/2), which have been shown to promote survival in response to Fas (40, 41), were activated in PTP1B-null mice in response to Jo-2 but not in their WT counterparts, as assayed using anti-phospho-ERK antibodies (Fig. 3). An upstream activator of ERK1/2, Raf kinase, was also activated, as determined using a phosphospecific antisemur in Jo-2-treated PTP1B-null mice (Fig. 3).

Hepatocytes from PTP1B-null Mice Are Protected against FasL and TNF-α-mediated Damage, but Thymocytes Are Not Resistant to Fas-induced Apoptosis—To establish whether protection from apoptosis is cell autonomous, the sensitivity of primary hepatocytes isolated from PTP1B-null and WT mice to both Jo-2 and the physiological FasL was assessed quantitatively by counting apoptotic cells after staining for Annexin V, an early indicator of apoptosis. In support of our in vivo data, primary hepatocytes prepared from PTP1B-null mice were more resistant to Jo-2- or FasL-induced apoptosis, when compared with hepatocytes prepared from WT mice. The number of apoptotic hepatocytes from WT mice was 25 and 30% in response to Jo-2 and FasL, respectively, whereas PTP1B-null hepatocytes did not show an increase in apoptosis in response to these treatments (Fig. 4, A and B). In agreement with results obtained by Musallam et al. (71), hepatocytes from WT BALB/c mice are sensitive to Jo-2 in the absence of actinomycin D. To test whether the resistance of hepatocytes from PTP1B-null mice was specific to FasL-induced apoptosis, hepatocytes from these mice were treated with TNF-α in the presence and absence of actinomycin D. As previously shown (42), hepatocytes from WT animals are sensitive to TNF-α-induced apoptosis in the presence of actinomycin D (Fig. 4A). In contrast, hepatocytes isolated from PTP1B-null mice are resistant to apoptosis in response to TNF-α (with actinomycin D) compared with WT mice (28% apoptotic cells) (Fig. 4, A and B).

To examine whether the resistance to apoptosis observed in PTP1B-null mice was tissue- or cell type-specific, we examined the sensitivity of thymocytes isolated from PTP1B-null and WT mice to Jo-2-induced apoptosis. In contrast to PTP1B-null hepatocytes, PTP1B-null thymocytes are not protected from Fas-induced apoptosis in vitro, whereas thymocytes derived from WT or PTP1B-null mice undergo apoptosis to a similar extent (Fig. 4C). In support of this, thymuses from both PTP1B-null and WT mice were invovled following Jo-2 treatment,
indicating occurrence of apoptosis (data not shown). Our data show that the resistance to Fas-mediated apoptosis observed in PTP1B-null mice is cell type-specific.

**Protection against Fas-induced Apoptosis Requires Activation of Both the ERK and NF-κB Signaling Pathways**—We have shown that ERK1/2 and NF-κB signaling pathways are activated in PTP1B-null mice in response to Fas engagement (Fig. 3). To examine whether ERK1/2 or NF-κB activity is required for the observed resistance of PTP1B-null mice to Jo-2-induced liver apoptosis in *vivo*, hepatocytes were treated with a pharmacological MEK-1/2 inhibitor (UO126) (43) or a peptide inhibitor of NF-κB (SN50) (44) prior to Jo-2 exposure. Pretreatment with UO126 or SN50, but not vehicle or control peptide (SN50M), increased apoptosis of hepatocytes prepared from PTP1B-null mice in response to Jo-2 to a level similar to that of WT hepatocytes (Fig. 5, A and C). Moreover, pretreatment of primary hepatocytes derived from WT mice with activators of the MEK-ERK1/2 pathway, such as the HGF, protected WT hepatocytes from Jo-2-induced cell death (Fig. 5B).

Notably, the protection mediated by growth factor pretreatment was abrogated in the presence of UO126 (Fig. 5, A and B). Together, these data indicate a critical role for MEK-ERK signals, as well as NF-κB-dependent signals, in the protection of PTP1B-null hepatocytes from Jo-2-induced apoptosis.

**Receptor Tyrosine Kinase Phosphorylation Increases in Response to Fas-induced Apoptosis in PTP1B-null Mice**—Modulation of receptor tyrosine kinases including the insulin, EGF, platelet-derived growth factor, IGF-1, and erythropoietin receptors by PTP1B is thought to proceed via the ability of PTP1B to dephosphorylate these substrates (5–8). To examine whether the resistance of PTP1B-null mice to Fas-induced apoptosis is dependent on tyrosine kinase activity, primary hepatocytes from PTP1B-null and WT mice were pretreated with Genestein, a general protein-tyrosine kinase inhibitor (45), prior to Jo-2-induced apoptosis (Fig. 6A). Following Genestein pretreatment, hepatocytes from PTP1B-null mice undergo apoptosis to a similar level as hepatocytes from WT mice, demonstrating that a tyrosine kinase-de-
The role of PTP1B in the regulation of insulin signaling and as a regulator of diabetes and obesity has been studied extensively (12, 13, 51). However, its function in other physiological responses regulated in vitro by tyrosine kinase signaling is poorly understood. Many signaling pathways regulated by tyrosine kinases have been shown to play a role during liver regeneration and in the protection of hepatocytes from apoptosis (19–21, 52). To address this, we investigated the role of PTP1B in response to liver damage induced by Fas. Our data indicate that the absence of PTP1B markedly protects against Fas-induced massive liver apoptosis and fulminant hepatic failure in vivo (Table 1 and Fig. 1). Interestingly, the 75% of PTP1B-null mice that showed no signs of distress in response to Jo-2 (Table 1) showed no overt signs of liver damage as indicated by no elevation in circulating AST and ALT liver enzyme levels (Fig. 1E) or liver apoptotic index, as assessed through quantitation of TUNEL-positive nuclei (Fig. 1, C and D). Together these results identify PTP1B as an important modulator of the Fas-mediated apoptotic response. These data are also consistent with a recent report that injection of adenovirus expressing PTP1B into PTP1B-null mice consistently caused higher elevation of circulating transaminase liver enzymes than adenovirus alone, indicating that elevated levels of PTP1B may potentiate adenovirus-induced hepatic damage (53).

Binding of the FasL or cross-linking of Fas with agonistic antibodies, such as Jo-2, promotes the recruitment and subsequent activation by proteolytic cleavage of procaspase-8 (54). In hepatocytes the formation of the DISC and activation of caspase-8 are followed by a cascade of
events leading to activation of caspases-9 and -3 and apoptosis (27). In livers from PTP1B-null mice, protection from apoptosis occurred at the level of the initiator caspase-8; cleavage of caspase-8, as well as downstream caspases-3 and -9, was abrogated in PTP1B-null but not WT animals (Fig. 2A). No difference was seen in the protein levels of different components of the DISC complex in saline-injected WT versus PTP1B-null mice. The level of Fas receptor present on the cell surface (Fig. 2), as well as FADD and caspase-8 (Fig. 2A), were similar between WT and PTP1B-null animals (Fig. 2B), indicating that components of DISC in this pathway are intact. Primary hepatocytes prepared from PTP1B-null but not WT mice were protected against apoptosis when treated with either Jo-2 or the Fas ligand (Fig. 4), indicating that resistance to apoptosis is cell autonomous.

In livers from PTP1B-null mice, protection against Fas-mediated apoptosis correlated with an elevation and/or activation of numerous anti-apoptotic signaling proteins that regulate the caspase and apoptotic cascade. These include FLIP, ERK1/2, and NF-κB (Fig. 3) (33–36, 55, 56). NF-κB activation, as measured by IkBa degradation, was observed in both WT and PTP1B-null mice, but activation was greater in PTP1B-null mice in response to Jo-2 (Fig. 3). These data are in agreement with previous experiments (57) where mice lacking TNFα receptors were resistant to Fas-mediated liver damage 5–8 h post-Jo-2 injection and showed greater NF-κB activation in comparison with WT. FLIP has been characterized as a specific inhibitor of death receptor-mediated apoptosis, possibly through its ability to compete with caspase-8 for recruitment to the DISC (39), or alternatively FLIP can also promote the activation of anti-apoptotic NF-κB and ERK signaling pathways (58, 59). Consistent with this, in intact livers of PTP1B-null mice, elevated FLIP levels correlate with ERK1/2 activation, IkBa degradation, and protection from Fas-mediated apoptosis (Fig. 3). In support of this, inhibition of ERK1/2 activation by a MEK1/2 inhibitor (UO126) or inhibition of NF-κB by a peptide inhibitor (SN50) sensitizes primary hepatocytes derived from PTP1B-null mice from Fas-mediated apoptosis (Fig. 5, A and C), suggesting a critical role for both ERK1/2 and NF-κB in this cytoprotection. Similarly, a decrease in FLIP levels correlated with decreased ERK1/2 activation and increased sensitivity to Fas-mediated apoptosis in keratin 8-null hepatocytes (41). Although the exact mechanism is yet unknown, ERK1/2-mediated protection against Fas can occur via inhibition of caspase-8 activation (56, 60, 61).

The resistance of PTP1B-null hepatocytes to Fas-mediated apoptosis requires tyrosine kinase activity, because PTP1B-null hepatocytes are rendered susceptible to Fas-mediated apoptosis by pretreatment with Genestein, a general inhibitor of tyrosine kinases (Fig. 6A). Although multiple tyrosine kinases are expressed in liver, (20, 46 – 48) and some known substrates of PTP1B (IR and IGF-1R) show elevated levels of phosphoryrosine following Fas activation (Fig. 6B), several lines of evidence support a role for the HGF/Met receptor in the hepatoprotection of PTP1B-null mice (19, 52). The Met receptor was the predominant phosphoprotein elevated in Fas-treated PTP1B-null mice when compared with control mice (Fig. 7, A and B). In addition, the Met receptor is a substrate for PTP1B in vitro, and HGF is a potent hepatoprotective agent against Fas-mediated liver damage both in vivo (52, 62) and in primary hepatocytes in vitro (49). Hepatocytes from liver-specific Met knock-out mice are hypersensitive to Fas-induced apoptosis (19), and Met is essential for efficient liver regeneration (18, 19). Notably, ERK1/2 activation, which is important for efficient liver regeneration, requires the activation of the Met receptor in vitro (18), whereas in primary hepatocytes in vitro, Akt signals appeared predominant over ERK1/2 (49). We failed to observe an elevation in phospho-Akt in livers from Jo-2-treated PTP1B-null mice. This may reflect a robust negative regulatory pathway for Akt activation in intact livers. Nevertheless, pretreatment of primary hepatocytes from WT mice with HGF protects these cells from Fas-mediated apoptosis (Fig. 5B), and this protection is diminished by inhibiting ERK1/2 with UO126 (Fig. 5B), supporting a requirement for Met for ERK1/2 activation during liver regeneration (18).

Hence, the anti-apoptotic effect observed in primary hepatocytes derived from PTP1B-null mice is dependent on the activation of physiological modulators of liver regeneration, such as the Met receptor and anti-apoptotic pathways involving NF-κB and ERK1/2 (Figs. 3 and 5). Although the Fas receptor is highly expressed in thymocytes and hepatocytes (63) and PTP1B is expressed in both cell types, resistance to Fas-induced apoptosis is limited to hepatocytes (Fig. 4). Thymocytes from PTP1B-null mice did not differ in their response to Jo-2 when compared with thymocytes from WT mice (Fig. 4C). The observed tissue selectivity may reflect differences in the expression of targets for PTP1B, such as the Met receptor, which is highly expressed in hepatocytes, but is only weakly expressed in thymocytes from adult mice (64).

Similarly, the sensitivity of 25% of PTP1B-null mice to Jo-2-induced liver damage may reflect differences in the levels of expression of targets for PTP1B, resulting in differences in the threshold of anti-apoptotic pathways activated, such as ERK1 and ERK2 (data not shown).

Fibroblasts from PTP1B-null mice are also resistant to ER-mediated stress (65). In this process, decreased levels of phosphorylated INK and p38 MAPK correlated with protection (65). We failed to observe any difference in the levels of INK or p38 in livers of PTP1B-null mice compared with WT mice (data not shown). This may reflect the time at which livers were harvested for analysis, i.e. 6 h post-Jo-2 injection, by which time changes in INK or p38 MAPK activity may not be detected. Alternatively, this may reflect differences between PTP1B substrates in primary hepatocytes and immortalized fibroblasts.

Fas-mediated apoptosis is an efficient process through which damaged hepatocytes are excluded from the liver (66). The present results regarding the response of primary hepatocytes to Fas and TNF-α show that PTP1B-null hepatocytes are resistant to both apoptosis-inducing signals. Because the TNF-α receptor can induce apoptosis in the presence of actinomycin D via activation of caspase-8 in a manner similar to Fas (67), this suggests a common role for PTP1B as a positive modulator of both of these signals in primary hepatocytes. A similar inhibition of caspase induction and death receptor-induced liver damage was recently demonstrated for the compound suramin (68). Notably, suramin is an inhibitor of PTP1B (69) at concentrations shown to provide protection to death receptor-mediated liver damage (68). Together these data suggest a potential role for PTP1B in suramin-regulated resistance to death receptor-induced apoptosis.

Interestingly, a recent report has identified a role for a PTP1B-related phosphatase, T cell protein-tyrosine phosphatase, in the negative regulation of ERK1/2 activity downstream from the TNF receptor, through dephosphorylation and inactivation of the Src kinase (70). In contrast, PTP1B has been proposed to activate Src in fibroblasts through dephosphorylation of a negative regulatory site on Src (pY527) (9). Consistent with this, we observed elevated Src pY527 phosphorylation in livers from Fas-treated PTP1B-null mice when compared with WT mice (data not shown). Thus our data do not implicate Src in the mechanism of ERK1/2 activation in Fas-treated PTP1B-null mice.

PTP1B-null mice live and reproduce normally (12, 13). When these animals are subjected to the stress of a high fat diet, they show protection against diabetes and obesity through increased phosphorylation of signaling components associated with the insulin, leptin, and growth
hormone receptors (12–15, 46). In response to Fas activation, a known stress inducer, we have shown, in PTP1B-null mice but not WT mice, elevated tyrosine phosphorylation and downstream signaling of receptor tyrosine kinases involved in liver injury, namely the Met receptor tyrosine kinase. Notably, only subtle differences in these signals were observed in untreated animals, consistent with previous studies (12–15). Hence, PTP1B acts as a potential rheostat to integrate cellular responses to extracellular stress. Our observation that the majority of PTP1B-null mice are protected against Fas-mediated liver damage suggests that pharmacological manipulation of PTP1B activity may constitute a viable therapeutic modality for treatment against hepatotoxins and liver damage.

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