

Transgenic overexpression of interleukin-8 in mouse liver protects against galactosamine/endotoxin toxicity

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Background/Aims: CXC chemokines function as survival factors for several types of cells. In this study, we investigated whether CXC chemokines promote survival of liver cells following an apoptotic stimulus *in vivo*.

Methods: Apoptosis was induced in mouse liver by treatment with galactosamine and endotoxin (Gal/ET). The influence of CXC chemokines was investigated by comparing Gal/ET responses in wild-type (WT) mice to those in mice with a transgene encoding the CXC chemokine interleukin-8 (IL-8 TG).

Results: IL-8 TG mice displayed less apoptosis and better survival after Gal/ET treatment than did WT mice (60% fewer TUNEL-positive cells at 6 h; 36% better survival at 24 h). Gal/ET toxicity was also preventable in WT mice by pre-treatment with IL-8. Notably, IL-8 was not protective against hepatic apoptosis due to anti-Fas or concanavalin A. In Gal/ET-treated mice, IL-8 promoted liver cell survival by interfering with the mitochondrial pathway of apoptosis. Survival was not attributable to activation of NF- κ B or up-regulation of anti-apoptotic proteins, but coincided instead with activation of Akt and phosphorylation of the pro-apoptotic protein Bad.

Conclusions: IL-8 protects liver cells from Gal/ET-mediated apoptosis by signaling through phosphatidylinositol-3 kinase (PI-3K). This is in keeping with the reported mechanism of chemokine-related survival in other tissues.

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Keywords: Chemokine; Apoptosis; Survival; Caspase; Akt; Inflammation

1. Introduction

The chemokines comprise a family of more than 50 low-molecular-weight cytokines with conserved cysteine residues near their N-terminus [1]. They were initially

characterized as leukocyte chemoattractants, but are now recognized to have a broad range of activities and a wide variety of cellular targets. Chemokines play important roles in embryogenesis, lymphocyte development and trafficking, angiogenesis, wound repair, and tumor metastasis [1–3]. In these processes they promote not only cell migration, but also growth [4–7] and survival [8–10] and changes in phenotype [11,12].

Interleukin-8 (IL-8, CXC ligand-8) is a member of the CXC chemokine subfamily. Like other chemokines it has many functions, including leukocyte chemotaxis, cell proliferation and cell survival [1,7,13–16]. Attention has focused recently on the ability of IL-8 to block apoptosis; this effect was originally demonstrated with hematopoietic cells [8,9,17], but has since been observed with cells from other organ systems such as neurons [18–20] and vascular endothelia [21]. The survival function of IL-8 has been linked to its ability to activate intracellular

Received 27 February 2005; received in revised form 8 June 2005; accepted 8 June 2005; available online 18 July 2005

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Abbreviations: Akt, protein kinase B; Con A, concanavalin A; FADD, Fas-associated death domain; Gal/ET, galactosamine and endotoxin; IAP, inhibitor of apoptosis protein; IKK, inhibitory subunit of NF- κ B kinase; IL-8, Interleukin-8; NF- κ B, nuclear factor- κ B; PI-3K, phosphatidylinositol-3 kinase; TG, transgenic; TNFR1, TNF receptor 1; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling; WT, wild-type; XIAP, X-linked IAP.

phosphatidylinositol 3-kinase (PI-3K) [18,22]. PI-3K phosphorylates protein kinase B (Akt), which in turn can block apoptosis by several means [23]. An important target of Akt is the inhibitory subunit of NF- κ B kinase (I- κ B kinase; IKK). By phosphorylating IKK, Akt can activate NF- κ B, causing the induction of survival proteins from the Bcl-2 family [24–26] as well as the inhibitor of apoptosis protein (IAP) family [27–31].

IL-8 is up-regulated in a variety of liver diseases [32–34], but it is unknown whether this chemokine has a survival function in liver. In experimental animals, acute administration of IL-8 has been reported to protect the liver against toxic injury [35,36]; this salutary effect, however, has not been conclusively linked to inhibition of apoptosis. The objective of this study was to determine whether IL-8 suppresses hepatic apoptosis caused by galactosamine and endotoxin (Gal/ET). When mice with chronically high blood levels of IL-8 (IL-8 TG) were treated with Gal/ET, they developed significantly less apoptosis than WT mice. Hepatic apoptosis was inhibited at the level of mitochondria, downstream of caspase-8. Survival in IL-8 TG mice coincided with increased Akt activity in the liver; however, it was not associated with increased activation of NF- κ B or induction of anti-apoptotic proteins. Instead, IL-8 TG mice displayed increased phosphorylation of Bad, which could account for the reduced activity of the intrinsic pathway of apoptosis after Gal/ET treatment.

2. Materials and methods

2.1. IL-8 transgenic mice

BDF-1 mice with a liver-specific transgene encoding human IL-8 were obtained from Amgen, Inc. (Thousand Oaks, CA). These mice have circulating neutrophilia and impaired chemotaxis to intraperitoneal thioglycollate [37], but organ histology and function are normal [37,38]. IL-8 TG mice were mated with WT BDF-1 mice (Jackson Laboratories, Bar Harbor, ME). Offspring were screened by quantitation of IL-8 in whole blood (R&D Systems, Minneapolis, MN). IL-8 levels in transgenic mice averaged 74.9 ng/ml, compared to 0.13 ng/ml in WT mice.

Animals received humane care based on guidelines set by the American Veterinary Medical Association. All protocols were approved by the Committee on Animal Research at the University of California, San Francisco.

2.2. Gal/ET treatment

Adult male mice were injected intraperitoneally with Gal/ET (700 μ g/kg galactosamine and 100 μ g/kg *E. coli* endotoxin; Sigma Chemical Company, St Louis, MO). Controls received an equivalent volume of sterile saline. In some experiments, WT mice were pre-treated with recombinant human IL-8 (R&D Systems; 2.5 μ g IV) or saline immediately before Gal/ET injection. Mice were killed at specified intervals after toxin administration. In survival studies, Gal/ET-treated mice were monitored until they became moribund and then euthanized.

2.3. Treatment with anti-Fas or concanavalin A

For comparison to Gal/ET, mice were injected intraperitoneally with 1 mg/kg anti-Fas (Jo2; BD Biosciences, San Diego, CA) and killed at 2.5 h. A separate group of mice was injected intravenously with 20 mg/kg

concanavalin A (Con A) (Sigma) and killed at 24 h. Immediately prior to Con A, mice were injected intravenously with either recombinant IL-8 (2.5 μ g/mouse) or saline.

2.4. Serum TNF

TNF was measured in mouse serum by ELISA (R&D Systems).

2.5. Histochemistry and immunohistochemistry

Formalin-fixed liver sections were stained with hematoxylin and eosin and examined for apoptosis by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) (Promega Corporation, Madison, WI). Neutrophils were identified in acetone-fixed frozen sections by immunohistochemistry, using a goat anti-rat IgG (BD Biosciences) and avidin–biotin–peroxidase complex (Vector Laboratories, Burlingame, CA). Neutrophils were quantitated by direct counting of stained cells in five random high-power (20 \times) fields.

2.6. Quantitation of caspase activity

Livers were homogenized in 25 mM HEPES, 5 mM EDTA, 5 mM MgCl₂, 5 mM DTT containing protease inhibitors. Caspase-3 activity was quantitated fluorometrically (CaspACE®, Promega). Results were expressed as pmol of substrate cleaved per 100 μ g protein per hour.

2.7. Expression of apoptosis-related genes

Livers were homogenized in TRI reagent (Molecular Research Center, Cincinnati, OH) for extraction of total RNA. mRNA encoding apoptosis-related genes was quantitated by RNase protection [39], using 20 μ g RNA and Riboquant® multiprobe sets labeled with α -³²P-UTP (Amersham Biosciences, Piscataway, NJ).

2.8. Western blotting

Antibodies were obtained from several sources (Cell Signaling Technology, R&D Systems, Stressgen, Santa Cruz Biotechnology, ProSci and Abcam). Anti-phospho-Bid was donated by Dr Solange Desagher (Montpellier, France). Livers were homogenized in modified radio-immunoprecipitation (RIPA) buffer containing protease inhibitors (Halt®, Pierce Biotechnology, Rockford, IL). Cytosol was prepared as described by Li et al. [40]. Aliquots of liver homogenate or cytosol were separated through SDS-polyacrylamide, transferred to nitrocellulose or PVDF filters (Bio-Rad Laboratories), blocked and incubated with primary antibodies as recommended by the manufacturers. Filters were then washed and incubated with peroxidase-conjugated anti-IgG (1/10,000). Immuno-reactive proteins were detected by chemiluminescence (SuperSignal® West Dura, Pierce).

2.9. Measurement of NF- κ B activation

NF- κ B DNA binding activity was measured in liver extracts (10 μ g protein) using a spectrophotometric assay (TransAM™ NF- κ B p65; Active Motif, Carlsbad, CA). Results were expressed in absorbance units (\times 100).

2.10. Statistics

All quantitative results were expressed as mean \pm SE. Differences between means were compared by analysis of variance, except for survival data, which were analyzed using the Poisson distribution. *P* values <0.05 were considered significant.

3. Results

3.1. Transgenic overexpression of IL-8 protects mice from Gal/ET-induced liver injury

WT mice treated with Gal/ET for 6 h exhibited signs of severe liver injury (Fig. 1). Histology revealed widespread nuclear fragmentation; in 83% of livers, intraparenchymal hemorrhage was evident. In contrast, IL-8 TG mice displayed more modest nuclear fragmentation and only 28% hemorrhage. Consistent with their milder degree of liver injury, IL-8 TG mice survived longer than WT mice after Gal/ET (50% vs. 14% at 24 h, $P < 0.001$; Fig. 2).

3.2. IL-8 reduces Gal/ET-mediated hepatic apoptosis without altering TNF induction

To determine whether IL-8 affects hepatocyte apoptosis in Gal/ET-treated mice, we performed TUNEL staining of liver sections and measured caspase-3 activity in liver homogenates. IL-8 TG livers had 60% fewer TUNEL-positive cells than WT livers, as well as 40% lower caspase-3 activity (Figs. 1 and 3). To confirm that the reduced apoptosis in IL-8 TG mice was due specifically to IL-8, we treated WT mice with recombinant IL-8 (2.5 μ g IV) immediately prior to Gal/ET. Acute IL-8 infusion also reduced caspase-3 activation in

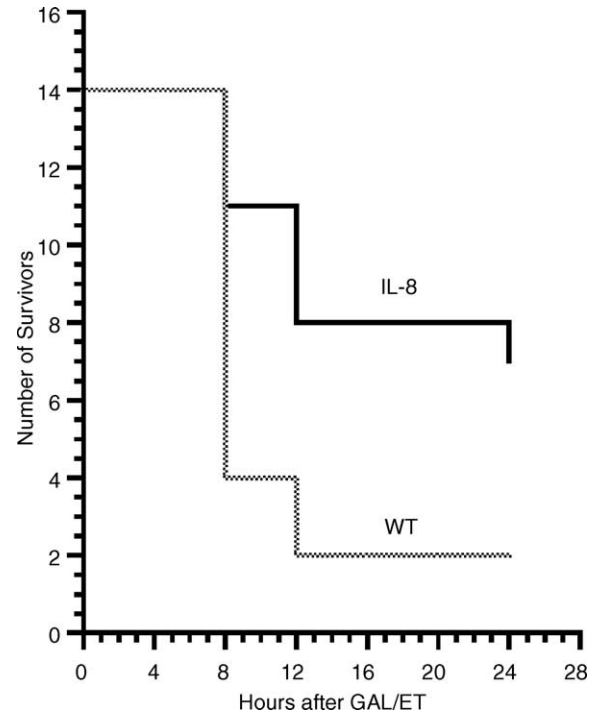


Fig. 2. Survival of WT and IL-8 TG mice after Gal/ET treatment. WT and IL-8 TG mice were treated with Gal/ET and monitored for 24 h or until they reached a moribund state. Graph depicts survival over the 24-h interval (14% WT vs. 50% IL-8 TG, $P < 0.001$).

response to Gal/ET, by 65% ($P < 0.05$, $n = 8$). Because of the central role of TNF in Gal/ET-mediated apoptosis, we investigated whether IL-8 interfered with TNF induction. This was not the case, as serum TNF levels were comparable in both groups of mice 1.5 h after Gal/ET (2111 \pm 980 pg/mL in IL-8 TG vs. 2686 \pm 896 pg/mL in WT, $P > 0.05$, $n = 5$).

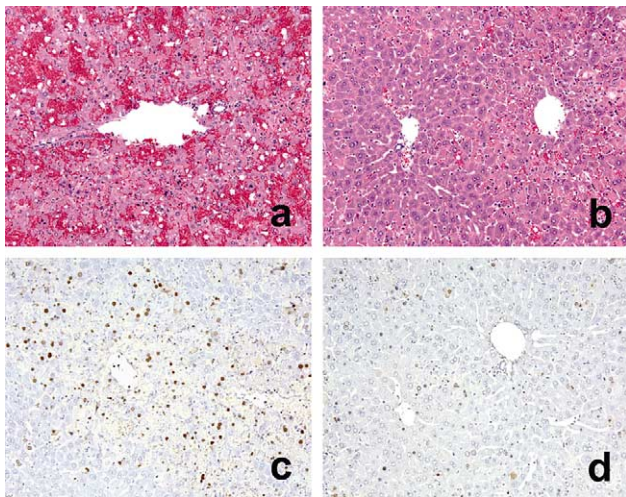


Fig. 1. Liver histology in Gal/ET-treated WT and IL-8 TG mice. Photomicrographs depict livers from WT and IL-8 TG mice 6 h after administration of Gal/ET. Panel a, WT H&E; panel b, IL-8 TG H&E; panel c, WT TUNEL; panel d, IL-8 TG TUNEL. In WT mice, Gal/ET caused widespread nuclear condensation and fragmentation and diffuse intraparenchymal hemorrhage (a). TUNEL-positive nuclei were prominent throughout the liver lobule (c). In IL-8 TG mice, nuclear condensation was mild, and there was no evidence of intraparenchymal hemorrhage (b). TUNEL-positive nuclei were present in IL-8 TG livers but were sparse in comparison to WT livers. Original magnification, 10 \times .

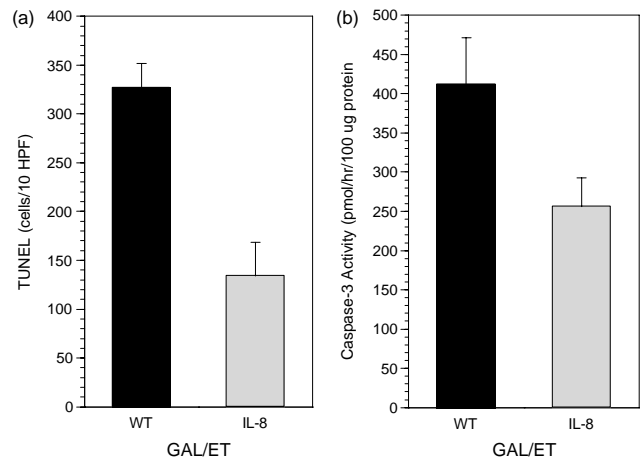


Fig. 3. Quantitative assessment of hepatic apoptosis in WT and IL-8 TG mice after Gal/ET. Panel a depicts the number of TUNEL-positive cells counted in the livers of WT and IL-8 TG mice 6 h after Gal/ET ($P = 0.0002$). Panel b depicts caspase-3 activity in WT and IL-8 TG livers 6 h after Gal/ET ($P = 0.03$). Values represent mean \pm SE for $n = 18$.

3.3. IL-8 does not influence hepatic inflammation in response to Gal/ET

Neutrophil recruitment follows apoptosis in Gal/ET-treated livers and amplifies tissue damage [41]. Because IL-8 can suppress neutrophil chemotaxis [37], IL-8 TG mice might resist Gal/ET toxicity through inhibition of hepatic inflammation. To determine whether IL-8 suppresses Gal/ET-mediated inflammation, we counted hepatic neutrophils in WT and IL-8 TG mice before and after Gal/ET treatment. IL-8 TG mice had more neutrophils than WT mice at baseline, which is consistent with prior reports documenting neutrophils within the sinusoids [37]. After Gal/ET, however, the number of neutrophils in WT and IL-8 TG livers increased identically over baseline (10.1 ± 2.4 cells/high-power field in WT vs. 11.5 ± 0.7 cells/high-power field in IL-8 TG; $n=18$). This excludes immunosuppression as an explanation for IL-8-mediated protection against Gal/ET hepatotoxicity.

3.4. IL-8 does not alter basal expression of apoptotic genes or proteins in the liver

Our initial findings indicated that the principal effect of IL-8 was to limit hepatic apoptosis after Gal/ET treatment. To address the mechanism of the effect, we investigated whether transgenic IL-8 alters the basal hepatic expression of molecules related to apoptosis. We found no difference between WT and IL-8 TG mice in the expression of mRNA encoding procaspases or members of the Bcl-2 family (Fig. 4). Similarly, we identified no disparity between WT

and IL-8 TG mice in the hepatic expression of several pro- and anti-apoptotic proteins (Fig. 5).

3.5. IL-8 interferes with the mitochondrial pathway of apoptosis in the liver

Despite their similar complement of apoptotic machinery, WT and IL-8 TG mice showed distinct differences in apoptosis progression in response to Gal/ET. Specifically, IL-8 TG mice showed little or no cleavage of caspase-9 after Gal/ET treatment, as well as reduced cytoplasmic release of Smac and cytochrome *c* (Fig. 6). Bid cleavage was also less prominent in IL-8 TG than WT mice after Gal/ET treatment; together these data indicate that IL-8 blocks Gal/ET-mediated apoptosis at or above the level of mitochondria. IL-8 did not suppress caspase-8 cleavage in response to Gal/ET, nor did it influence the expression of FADD or Bcl-xL (Fig. 6). This suggests that IL-8 prevents the amplification, rather than the initiation, of the intrinsic pathway of apoptosis. Caspase-3 can cleave Bid [42], creating a feed-forward loop through mitochondria that perpetuates apoptosis even in the absence of ongoing signals from death receptors [43]. Such amplification can be arrested by IAPs [44]. However, neither X-linked IAP (XIAP) nor the related proteins cIAP-1 and cIAP-2 was up-regulated in the livers of IL-8 TG mice after Gal/ET treatment (Fig. 6).

3.6. IL-8 increases Akt activity in the liver and promotes phosphorylation of Bad after Gal/ET

Since the anti-apoptotic effect of IL-8 in transgenic mice could not be linked to a change in expression of any

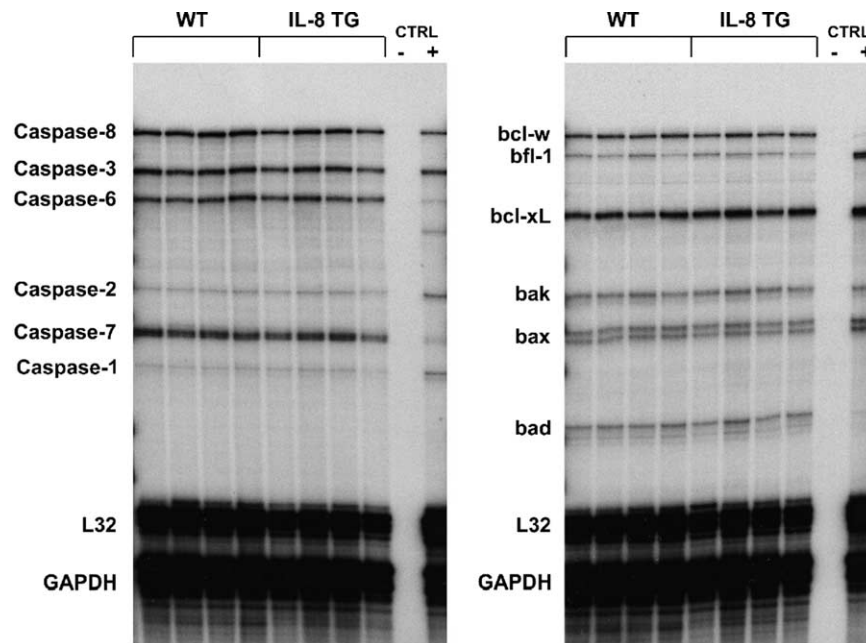


Fig. 4. Basal hepatic expression of genes relevant to apoptosis in WT and IL-8 TG mice. Autoradiograms illustrate the hepatic expression of genes encoding caspases (left panel) and Bcl-2 family members (right panel) in untreated WT and IL-8 TG mice. There was no difference between the two groups in any of the mRNAs examined.

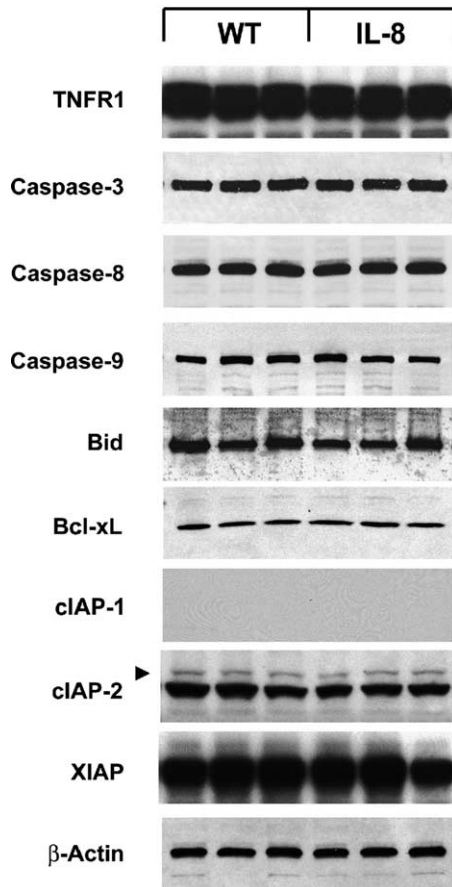


Fig. 5. Basal hepatic expression of proteins relevant to apoptosis in WT and IL-8 TG mice. Autoradiograms depict hepatic expression of proteins encoding TNF receptor 1 (TNFR1), procaspases-3, -8, and -9, Bid, Bcl-xL, and the IAPs cIAP-1, cIAP-2 and XIAP in untreated mice. β -actin is included as a control for protein content. cIAP-1 was undetectable in untreated livers; for the remainder of the proteins, expression was similar in WT and IL-8 TG mice.

component of the extrinsic or intrinsic cell death pathway, we investigated whether IL-8 influences survival signaling in the liver. IL-8 TG mice displayed greater hepatic phosphorylation of Akt than WT mice in response to Gal/ET (Fig. 7), which is in keeping with prior reports that IL-8 activates PI-3K [18,22]. IL-8 TG mice also exhibited increased phosphorylation of Bad (Fig. 7); this protein is a downstream target of Akt, whose pro-apoptotic activity is inhibited by phosphorylation [45]. IKK is also an important target of Akt, and thus we examined whether IL-8 TG mice exhibit exaggerated activation of NF- κ B after Gal/ET treatment. NF- κ B DNA binding was unaffected by IL-8 after Gal/ET, nor was there any evidence of differential induction of NF- κ B-responsive proteins such as Bcl-xL, A1/Bfl-1 (Fig. 6) or iNOS (not shown). We also considered the possibility that IL-8 inhibits apoptosis by phosphorylating Bid, a process that is catalyzed by casein kinase II [46]. Efforts to identify phosphorylated Bid in liver homogenates were unsuccessful (not shown) (Table 1).

3.7. Hepatic apoptosis caused by anti-Fas or Con A is not ameliorated by IL-8

To establish whether the survival effect of IL-8 extends to other models of hepatic apoptosis, we examined caspase-3 activity in the livers of WT and IL-8 TG mice after treatment with anti-Fas or Con A. These agents, like Gal/ET, activate death receptors [47,48]. Unlike Gal/ET, anti-Fas and Con A activated caspase-3 to the same extent in IL-8 TG as WT mice (Table 2). IL-8 overexpression offered no protection from apoptosis, nor did it improve survival (not shown). Even acute infusion of IL-8, which has been reported to ameliorate the biochemical and histologic parameters of Con A toxicity [36], had no effect on Con A-mediated activation of caspase-3. Based on these findings, the cytoprotective effect of IL-8 appears specific for Gal/ET.

4. Discussion

In this study we demonstrate that the CXC chemokine IL-8 substantially limits the hepatotoxicity of Gal/ET in vivo. Whether overexpressed chronically or administered acutely, IL-8 suppresses hepatic apoptosis, reduces liver hemorrhage and lengthens survival in mice treated with Gal/ET. An anti-apoptotic effect of IL-8 in the liver is noteworthy because in most forms of clinical and experimental liver disease, this chemokine has been viewed primarily as an inflammatory mediator [49]. The current findings emphasize that the role of IL-8 in liver goes beyond its function as a leukocyte chemoattractant and activator.

In Gal/ET-treated mice, IL-8 exerted its hepatoprotective effect by interfering with the intrinsic pathway of apoptosis. The chemokine reduced the release of Smac and cytochrome c from mitochondria and limited cleavage of caspase-9; it also suppressed Bid cleavage, which implied interruption of either the initiation or amplification of the mitochondrial pathway of cell death. Notably, IL-8 did not alter hepatic expression of apoptotic proteins either before or after Gal/ET treatment, nor did it prevent Gal/ET-mediated induction of TNF or death receptor signaling through the point of caspase-8 cleavage. IL-8 did, however, enhance Akt phosphorylation in the liver in response to Gal/ET. Phosphorylation of Akt is consistent with a chemokine effect, as CXC chemokines are known activators of PI-3K [18,22].

The anti-apoptotic properties of CXC chemokines are closely linked to their ability to phosphorylate Akt [18,22]. Akt promotes survival by means: it phosphorylates IKK, which activates NF- κ B [50]; it also phosphorylates several components of the cell death machinery (Bad, caspase-9, XIAP), which results in slowing or termination of apoptosis. Additional targets of Akt include forkhead transcription factors, the cAMP response element binding protein (CREB) and glycogen synthase kinase-3 β [23,51]. The precise

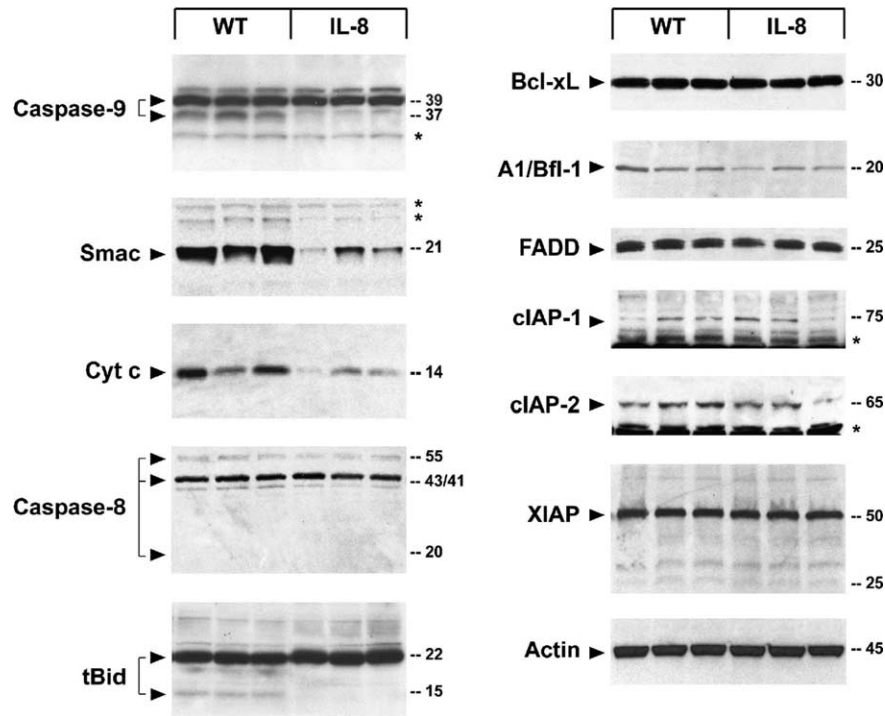


Fig. 6. Expression and cleavage of apoptotic proteins in WT and IL-8 TG livers 6 h after Gal/ET. Western blots illustrate proteins relevant to apoptosis in the livers of WT and IL-8 TG mice. Immunoblots for Smac and cytochrome *c* were performed with purified liver cytosol (see Section 2); all others were performed with whole liver homogenate (see Section 2). 6 h after Gal/ET, IL-8 TG mice showed less of the 37-kDa cleavage product of pro-caspase-9 than IL-8 TG mice. They also exhibited a lesser degree of Smac and cytochrome *c* release into the cytosol, and reduced generation of truncated Bid (15 kDa). By contrast, WT and IL-8 TG mice demonstrated similar degrees of procaspase-8 cleavage (from 55 kDa to 43/41 kDa) in response to Gal/ET. The 20-kDa cleavage product of caspase-8 was not visualized. Additional immunoblots show that hepatic expression of Bcl-xL, A1/Bfl-1 and FADD were comparable in WT and IL-8 TG mice after Gal/ET. Members of the IAP family (cIAP-1, cIAP-2 and XIAP) were also unaffected by IL-8. Survivin was undetectable in either WT or IL-8 TG liver after Gal/ET (not shown). β -actin is shown as a control for protein content. *signifies a non-specific band of immunoreactivity.

means by which Akt facilitates survival varies among different cell types and cell death situations [23]. In cultured hepatocytes, Akt has been reported to prevent TNF-mediated apoptosis by activating NF- κ B [52]. Our experiments, by contrast, show that Akt phosphorylation and survival occurred independently of NF- κ B in an *in vivo* model of TNF-mediated apoptosis. In Gal/ET-treated mice, Akt appears to facilitate survival by phosphorylating and inactivating the pro-apoptotic protein Bad [45]. This is consistent with another recent study performed *in vivo* in which Akt prevented liver cell death from ischemia-reperfusion by phosphorylating Bad rather than activating NF- κ B [53]. Although the signal for phosphorylated Bad in whole liver was weak, this does not diminish the potential importance of this protein to liver cell survival in Gal/ET-treated mice. Indeed, phosphorylated Bad is not easily detected in the liver [52–54], perhaps due to its rapid sequestration with 14-3-3 [55].

We designed our experiments to measure the effect of IL-8 on Gal/ET-mediated hepatic apoptosis while avoiding any potential confounding effect of the chemokine on hepatic inflammation. Neutrophilic inflammation becomes prominent 6–7 h after Gal/ET treatment [56]; thus, we ended our experiments at 6 h, when apoptosis was active but

inflammation minimal. Over the first 6 h after Gal/ET treatment, there were no differences between WT and IL-8 TG mice in the ability of neutrophils to migrate to the liver. This corroborates a recent report by Remick and colleagues [38] demonstrating that neutrophils from IL-8 TG mice maintain normal responsiveness to a variety of

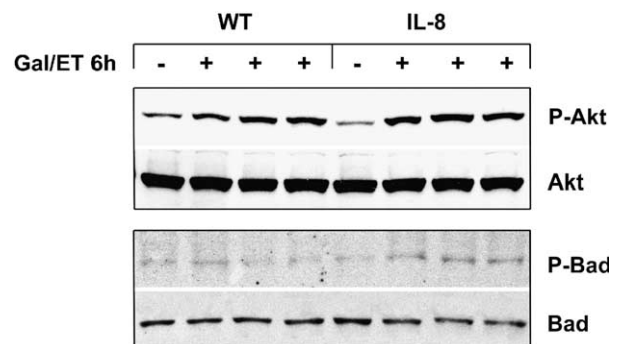


Fig. 7. Phosphorylation of Akt and Bad in the livers of WT and IL-8 TG mice in response to Gal/ET. Autoradiogram illustrates Akt and Bad and their phosphorylated forms in mouse liver either before or 6 h after Gal/ET treatment. After Gal/ET, phospho-Akt (P-Akt) and phospho-Bad (P-Bad) are both more abundant in IL-8 TG than WT mice. Densitometry indicated that the P-Akt/Akt ratio in Gal/ET-treated IL-8 TG mice was 28% higher than that in WT mice ($P < 0.05$).

Table 1
NF-κB activation in the liver in response to Gal/ET treatment

	NF-κB activation (units) ^a	
	WT (n)	IL-8 TG (n)
Untreated	1.6 ± 0.8 (4)	0.6 ± 0.2 (4)
Saline 6 h	1.1 ± 0.3 (3)	1.4 ± 0.2 (3)
Gal/ET 1.5 h	303.6 ± 5.2 (5)	303.7 ± 8.6 (6)
Gal/ET 6 h	230.0 ± 37.6 (3)	270.0 ± 14.4 (3)

^a NF-κB DNA binding activity was measured as described in Section 2. Values represent mean ± SE. There was no significant difference in NF-κB activation between the two groups of mice, regardless of treatment.

Table 2
Caspase-3 Activity in liver following treatment with Con A or Anti-Fas

	Caspase-3 Activity (pmol /100 μg protein/h)		
	No treatment (n)	Anti-Fas (n)	Con A (n)
WT	65.6 (2)	437.5 ± 104.7 (11)	261.7 ± 29.4 (10)
WT + rIL-8	–	–	275.8 ± 49.8 (6)
IL-8 TG	73.6 (2)	846.4 ± 190.8 (11)	305.4 ± 29.0 (9)

WT and IL-8 TG mice were treated with anti-Fas or Con A as described in Section 2. Some WT mice were injected intravenously with 2.5 μg recombinant IL-8 immediately prior to Con A (WT+rIL-8). Values represent caspase-3 activity (mean ± SE) measured in whole liver homogenates at 2.5 h (anti-Fas) or 24 h (Con A). There were no significant differences in caspase-3 activity among treatment groups for either anti-Fas or Con A.

inflammatory stimuli *in vivo*. Because the early neutrophilic response to Gal/ET was not altered by IL-8, our findings confirm that the beneficial effect of IL-8 in Gal/ET-treated mice is due to a specific effect on apoptosis rather than inflammation.

Noteworthy in our study was that IL-8 did not protect liver cells from all forms of death receptor-mediated apoptosis. In particular, IL-8 was ineffective against apoptosis precipitated by anti-Fas. This suggests either that the survival mechanism induced by IL-8 is specific to Gal/ET, or that it is non-specific but activated too slowly to avert Fas-induced apoptosis. Similar discrepancies have been noted previously; for example, Nagaki and colleagues [57] utilized TNF preconditioning to inhibit hepatic apoptosis, and found that preconditioning rescued mice from death due to Gal/TNF but not anti-Fas. IL-8 also failed to protect mice from Con A-mediated apoptosis in our study. This is consistent with evidence that Con A-induced cell death, although dependent upon TNF, is not mediated through FADD/caspase-8 as it is for Gal/ET [58]. Osawa and colleagues reported that IL-8 reduces hepatic injury from Con A [36]. These authors, however, did not address whether Con A had any specific effect on hepatic apoptosis *in vivo*. Our results indicate that IL-8 does not reduce caspase-3 activation in response to Con A. Thus, if IL-8 ameliorates liver injury in the Con A model, it must be acting through a mechanism independent of caspase-3.

Hogaboam and colleagues [35] were the first to note that

IL-8 attenuates toxic liver injury in mice. They discovered that IL-8 inhibits the toxicity of acetaminophen, a compound that causes necrosis rather than apoptosis in the liver [59]. The benefit of IL-8 in acetaminophen-treated mice was attributed to its ability to stimulate hepatocyte mitogenesis and enhance liver regeneration [35,60]. When taken together with the current findings, as well as data that IL-8 protects against Con A toxicity, it appears that this CXC chemokine has the potential to limit liver injury by multiple mechanisms.

One issue not addressed in our study is the means by which IL-8 interacts with liver cells to promote cytoprotection. IL-8 binds to mouse cells via CXC chemokine receptor 2 (CXCR2); this receptor is reportedly inducible in hepatocytes in the setting of acute liver injury *in vivo* [61]. Following Gal/ET treatment, we have been unable to demonstrate induction of CXCR2 on hepatocytes (Ng R et al, unpublished observations). Thus, in the Gal/ET model of injury, IL-8 may be acting through another binding protein or exerting its anti-apoptotic effect indirectly by inducing another compound that interacts with hepatocytes.

In summary, the above experiments demonstrate that the CXC chemokine IL-8 has specific anti-apoptotic activity toward liver cells in the Gal/ET model of acute liver injury. IL-8 mediates survival by interfering with mitochondrial events in the apoptotic cascade. Resistance to Gal/ET toxicity in IL-8 TG mice does not involve activation of NF-κB or up-regulation of anti-apoptotic proteins; it coincides instead with enhanced hepatic phosphorylation of Akt and Bad. This survival pathway may be of particular importance in the setting of liver injury *in vivo*.

Acknowledgements

This work was supported by NIH grants DK61510, AA07810, AA00215, DK26743 and a gift from Mr and Mrs Robert H. Shepard.

The authors thank Dr Scott Simonet and the Amgen Corporation for providing IL-8 transgenic mice, and Dr Solange Desagher for donating phospho-Bid antibody.

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