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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-κB 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.**

***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

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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-κB kinase; IL-8, Interleukin-8; NF-κB, nuclear factor-κB; 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.

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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-κB kinase (I-κB kinase; IKK). By phosphorylating IKK, Akt can activate NF-κB, 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-κB 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 μg/kg galactosamine and 100 μg/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 μg 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 μg/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×) fields.

2.6. Quantitation of caspase activity

Livers were homogenized in 25 mM HEPES, 5 mM ETDA, 5 mM $MgCl_2$, 5 mM DTT containing protease inhibitors. Caspase-3 activity was quantitated fluorometrically (CaspACE®, Promega). Results were expressed as pmol of substrate cleaved per 100 μg 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 μg RNA and Riboquant® multiprobe sets labeled with α-^{32}P-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 radioimmunoprecipitation (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). Immunoreactive proteins were detected by chemiluminescence (SuperSignal® West Dura, Pierce).

2.9. Measurement of NF-κB activation

NF-κB DNA binding activity was measured in liver extracts (10 μg protein) using a spectrophotometric assay (TransAM™ NF-κB p65; Active Motif, Carlsbad, CA). Results were expressed in absorbance units (×100).

2.10. Statistics

All quantitative results were expressed as mean ± 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.