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BASIC AND TRANSLATIONAL—LIVER

CSF1 Restores Innate Immunity After Liver Injury in Mice and Serum Levels Indicate Outcomes of Patients With Acute Liver Failure

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BACKGROUND & AIMS: Liver regeneration requires functional liver macrophages, which provide an immune barrier that is compromised after liver injury. The numbers of liver macrophages are controlled by macrophage colony-stimulating factor (CSF1). We examined the prognostic significance of the serum level of CSF1 in patients with acute liver injury and studied its effects in mice. **METHODS:** We measured levels of CSF1 in serum samples collected from 55 patients who underwent partial hepatectomy at the Royal Infirmary Edinburgh between December 2012 and October 2013, as well as from 78 patients with acetaminophen-induced acute liver failure admitted to the Royal Infirmary Edinburgh or the University of Kansas Medical Centre. We studied the effects of increased levels of CSF1 in uninjured mice that express wild-type CSF1 receptor or a constitutive or inducible CSF1-receptor reporter, as well as in chemokine receptor 2 (Ccr2)-/- mice; we performed fate-tracing experiments using bone marrow chimeras. We administered CSF1-Fc (fragment, crystallizable) to mice after partial hepatectomy and acetaminophen intoxication, and measured regenerative parameters and innate immunity by clearance of fluorescent microbeads and bacterial particles. **RESULTS:** Serum levels of CSF1 increased in patients undergoing liver surgery in proportion to the extent of liver resected. In patients with acetaminophen-induced acute liver failure, a low serum level of CSF1 was associated with increased mortality. In mice, administration of CSF1-Fc promoted hepatic macrophage accumulation via proliferation of resident macrophages and recruitment of monocytes. CSF1-Fc also promoted transdifferentiation of infiltrating monocytes into cells with a hepatic macrophage phenotype. CSF1-Fc increased innate immunity in mice after partial hepatectomy or acetaminophen-induced injury, with resident hepatic macrophage as the main effector cells. **CONCLUSIONS:** Serum CSF1 appears to be a prognostic marker for patients with acute liver injury. CSF1 might be developed as a therapeutic agent to restore innate immune function after liver injury.

Keywords: Drug-Induced Liver Damage; Clearance; Immune Response; M-CSF.

The liver provides an essential immune barrier against gut-derived pathogens entering the portal circulation.[1] Although surgical removal of liver tissue (partial hepatectomy) results in rapid compensatory up-regulation of metabolic function, the liver's innate immune capacity is markedly impaired.[2,3] Acute toxic liver injury leads to widespread hepatocyte necrosis and compromises barrier function.[4] Changes in gut wall integrity associated with liver failure facilitate the translocation of gut-derived pathogens.[5] Consequently, sepsis is common in patients with liver failure and is strongly associated with high mortality rates.[6,7] Liver transplantation is the only effective therapy for life-threatening liver failure but active sepsis is contraindicated in transplantation.

Hepatic macrophages mediate hepatic innate immune defense and promote hepatocyte proliferation after liver injury.[8,9] Tissue macrophage numbers are controlled during development, and in the steady state, by macrophage colony-stimulating factor (CSF1), which acts through a tyrosine kinase receptor, colony-stimulating factor receptor

Abbreviations used in this paper: **ALF, acute liver failure; ALT, alanine aminotransferase; Ccr2, chemokine receptor 2; CSF1, colony-stimulating factor 1; CSF1R, colony-stimulating factor 1 receptor; Fc, fragment, crystallizable; HMGB1, high-mobility group protein 1; mRNA, messenger RNA; PH, partial hepatectomy.**

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(CSF1R).[10,11] *Csf1*-deficient mice (op/op) have few tissue macrophages and impaired liver regeneration after partial hepatectomy.[12] Hepatic macrophages control circulating CSF1 levels via receptor-mediated endocytosis through CSF1R.[13] In human beings after living donor partial hepatectomy, increased circulating CSF1 is associated with more rapid liver regrowth.[14] In acute toxic liver injury models, monocyte-derived macrophage recruitment is required for necrotic tissue resorption.[15] In human acute liver injury, hepatic macrophages are implicated in tissue repair and low monocyte counts are associated with mortality.[16,17] Based on these findings there is a strong rationale for exploring the potential of macrophage-based therapeutics to improve outcomes after acute liver injury.

Here, we show that high serum CSF1 level is associated with survival in patients with acute liver failure (ALF) and outperforms previous markers of outcome in terms of discriminative ability. We show that CSF1 administration in animal disease models promotes rapid recovery of innate immune function and hence has therapeutic potential in human liver failure.

Materials and Methods

Human Work

Ethical approval was obtained from the South East Scotland Research Ethics Committee for patients undergoing partial hepatectomy (PH) at the Hepatobiliary Unit, Royal Infirmary Edinburgh, between December 2012 and October 2013. Liver failure was defined according to Schindl et al.[7] For the acetaminophen-induced ALF cohort, ethical approval was granted by the local human research ethics committee and informed consent was obtained from all patients, or next of kin, before study entry. This study built on previous analysis of this patient cohort by Antoine et al,[18] representing 78 adult patients admitted to the Royal Infirmary Edinburgh (United Kingdom), or the University of Kansas Medical Center (United States), with acute liver injury. Serial patient samples from a second patient cohort were collected at admission to the hospital (as opposed to admission to the specialist liver center with acute liver failure).[18] Details of serum analyses are provided in the Supplementary Materials and Methods section. Primary hepatocytes were isolated from human liver tissue obtained from liver resection specimens immediately after surgery, with full informed consent and ethical approval from the relevant authorities (National Research Ethics Service reference: 11/NW/0327). See the Supplementary Materials and Methods section for assay details.

Animal Experiments

Animal procedures were approved by the relevant institutional ethics committee (Albert Einstein College of Medicine, United States; University of Edinburgh, United Kingdom; and the University of Glasgow, United Kingdom), and adhered to the Animals (Scientific Procedures) Act of 1986 (United Kingdom) and the National Institutes of Health guide for the Care of Laboratory Animals (United States). Eight- to 12-week-old male mice were used for all experiments. *CCR2*$^{-/-}$, C57Bl/6, and MacGreen mice (Tg[Csf1R-Green fluorescent protein]$_{\text{Hume}}$[19]) were bred and maintained under specific pathogen-free conditions. *Tg*(*Csf1r-Mer2iCre*)$_{jwp}$ were crossed to Rosa floxed stop tomato red and lineage tracing experiments performed as described.[20] Fate tracing bone marrow–derived monocytes was performed using a mouse chimera as previously described.[21] Wild-type C57BL/6 mice were obtained from Charles River (Margate, Kent, UK). Mice were distributed randomly and maintained on A 12-hour light-dark cycle with feed ad libitum. A two-thirds partial hepatectomy was performed as previously described.[22] Acetaminophen intoxication involved intraperitoneal administration of 350 mg/kg acetaminophen (Sigma-Aldrich, St. Louis, MO).[23] The treatment group received 0.75 mcg/g CSF1-Fc, prepared as described previously[24] (control: phosphate-buffered saline), administered subcutaneously immediately after partial hepatectomy or 12 hours after acetaminophen intoxication and subsequently every 24 hours for up to 3 further doses. Reagents and methodology for immunohistochemistry, flow cytometry, quantification of messenger RNA (mRNA), phagocytosis assay, and serum analyses are provided in the Supplementary Materials and Methods section.

Hepatocyte Toxicity and Metabolic Assays

Details of human and mouse hepatocyte toxicity and metabolic assays are provided in the Supplementary Materials and Methods section.

Statistics

Statistical analysis was performed on GraphPad Prism V6.0 (GraphPad Software, Inc, La Jolla, CA), except for logistic regression analyses, which were conducted in *R*.[25] All data are presented as mean ±SEM unless otherwise stated. Two-tailed Student t test and 1-way and 2-way analysis of variance with Bonferroni adjustment were used for analysis of data. Human serum analyses and development of the logistic regression models were completed by a qualified statistician. The level of significance was set at a P value less than .05 for all analyses.

Results

Serum CSF1 Increases According to Extent of Partial Hepatectomy and Is Associated With Survival in Acute Liver Failure

In a cohort of 55 patients undergoing up to 75% PH (cohort details: Supplementary Figure 1*A*), serum CSF1 was increased significantly compared with healthy controls. There was a small reduction on day 1 after surgery followed by a marked increase in CSF1 level by postoperative day 3 (Figure 1*A*). There was no correlation between serum CSF1 level and blood loss (Supplementary Figure 1*B*). The initial decrease in serum CSF1 level may be owing to removal of tumor cells, which secrete CSF1.[26] We hypothesized that the subsequent increase in serum CSF1 level might be produced by proliferating hepatocytes. Because of the risks associated with liver biopsy in human beings, we examined a mouse model of two-thirds PH. CSF1 mRNA was unchanged after PH (Supplementary Figure 1*C*). In the patient cohort the CSF1 increase was related to the extent of resection (Figure 1*B*). Two patients developed postoperative liver failure and both had serum CSF1 levels below the 25th percentile (Figure 1*C*, and the clinical details are shown in Supplementary Figure 1*D*).

We sampled serum from a large patient cohort with established acetaminophen-induced ALF on arrival at the