Hepatocyte-derived macrophage migration inhibitory factor mediates alcohol-induced liver injury in mice and patients

Graphical abstract

Highlights

- The source of MIF in alcoholic liver disease (ALD) has been investigated.
- In vitro cultured hepatocytes released MIF upon ethanol challenge.
- Chimeric mice not expressing MIF in hepatocytes were protected from ethanol.
- MIF was detected in liver biopsies from patients with alcoholic hepatitis, localized in hepatocytes.
- The study identified hepatocytes as a source of MIF in ALD.

Authors

Veronica Marin, Kyle Poulsen, Gemma Odena, ..., Natalia Rosso, Ramon Bataller, Laura E. Nagy

Correspondence

nagyL3@ccf.org
(L.E. Nagy)

Lay summary

Alcoholic liver disease is a major cause of preventable mortality worldwide, and lacks specific pharmacological therapies. Recent studies have recognized that macrophage migration inhibitor factor (MIF) has a critical role in the inflammatory response to liver damage. However, the cells that produce this protein are still unknown. Our present findings reveal that hepatocytes, the main cell type in the liver, are primarily responsible for MIF production in response to alcohol, which promotes liver injury. Our study suggests that drugs inhibiting MIF production could be beneficial in treating patients with liver disease due to excessive alcohol consumption.

http://dx.doi.org/10.1016/j.jhep.2017.06.014
© 2017 European Association for the Study of the Liver. Published by Elsevier B.V. All rights reserved. J. Hepatol. 2017, 67, 1018–1025
Hepatocyte-derived macrophage migration inhibitory factor mediates alcohol-induced liver injury in mice and patients

Veronica Marin1, Kyle Poulsen2, Gemma Odena3, Megan R. McMullen2, Jose Altamirano3, Pau Sancho-Bru3, Claudio Tiribelli1, Juan Caballeria3, Natalia Rosso1, Ramon Bataller5, Laura E. Nagy2,6,*

1Italian Liver Foundation, AREA science Park, Trieste, Italy; 2Center for Liver Disease Research, Department of Pathobiology, Cleveland Clinic, Spain; 3Institut d’Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain; 4Hospital Clinic of Barcelona, Barcelona, Spain; 5Division of Gastroenterology and Hepatology, Department of Medicine, University of North Carolina at Chapel Hill, NC, USA; 6Department of Molecular Medicine, Case Western Reserve University, USA

Background & Aims: Macrophage migration inhibitory factor (MIF) is a multi-potent cytokine that contributes to the inflammatory response to injury. MIF is expressed by multiple cell types; however, the cellular source and actions of MIF in alcoholic liver disease (ALD) are not well known. Here we tested the hypothesis that non-myeloid cells, specifically hepatocytes, are an important cellular source of MIF in ALD.

Methods: MIF expression was measured in HuH7 and differentiated THP-1 cells in response to ethanol. Ethanol-induced liver injury was assessed in C57BL/6 (WT) and MIf−/− bone marrow chimeras. MIF was measured in peripheral and suprahepatic serum, as well as visualized by immunohistochemistry in liver biopsies, from patients with alcoholic hepatitis (AH).

Results: HuH7 hepatocytes, but not THP-1 macrophages, released MIF in response to ethanol challenge in culture. In chimeric mice expressing MIF in non-myeloid cells (MIf−/− → WT), chronic ethanol feeding increased ALT/AST, hepatic steatosis, and expression of cytokine/chemokine mRNA. In contrast, chimeric mice not expressing MIF in non-myeloid cells (WT → MIf−/−) were protected from ethanol-induced liver injury. Immunohistochemical staining of liver biopsies from patients with AH revealed a predominant localization of MIF to hepatocytes. Interestingly, the concentration of MIF in suprahepatic serum, but not peripheral serum, was positively correlated with clinical indicators of disease severity and with an increased risk of mortality in patients with AH.

Conclusions: Taken together, these data provide evidence that hepatocyte-derived MIF is critical in the pathogenesis of ALD in mice and likely contributes to liver injury in patients with AH.

Keywords: Alcoholic liver disease; Inflammation; MIF; Hepatocytes; Innate immune system; Translational research.

Received 9 January 2017; received in revised form 6 June 2017; accepted 12 June 2017; available online 22 June 2017

* Guest Editor: Didier Samuel.

Corresponding author. Address: Department of Pathobiology, Cleveland Clinic, Lerner Research Institute/NE40, 9500 Euclid Ave., Cleveland, OH 44120, USA. Tel.: +1 216 444 4021; fax: +1 216 636 1493.
E-mail address: nagyl3@ccf.org (L.E. Nagy).

These authors contributed equally to this work.

Lay summary: Alcoholic liver disease is a major cause of preventable mortality worldwide, and lacks specific pharmacological therapies. Recent studies have recognized that macrophage migration inhibitor factor (MIF) has a critical role in the inflammatory response to liver damage. However, the cells that produce this protein are still unknown. Our present findings reveal that hepatocytes, the main cell type in the liver, are primarily responsible for MIF production in response to alcohol, which promotes liver injury. Our study suggests that drugs inhibiting MIF production could be beneficial in treating patients with liver disease due to excessive alcohol consumption.

© 2017 European Association for the Study of the Liver. Published by Elsevier B.V. All rights reserved.

Introduction

Excessive alcohol consumption is the primary cause of liver-related mortality in Western countries.1 Alcoholic hepatitis (AH) is the most severe form of alcoholic liver disease (ALD) and there is an urgent need to develop novel targeted therapies for severe AH.2 Multiple molecular and cellular mechanisms contribute to the development of ALD. Prolonged alcohol abuse leads to an imbalance between intracellular antioxidant defense systems and the production of free radical species, promoting lipid peroxidation.3 Injury to hepatocytes can result in the release of danger-associated molecular patterns (DAMPs) which can activate pathways of sterile inflammation.3 Chronic alcohol consumption also impairs the barrier function of the intestine and promotes bacterial dysbiosis, resulting in increased translocation of pathogen-associated molecular patterns (PAMPS) from the gastrointestinal lumen to the liver through the portal vein.3

Recent studies have identified macrophage migration inhibitory factor (MIF), a pluripotent cytokine/chemokine, as a potential contributor to ethanol-induced liver injury in murine models of ALD.4,5 MIF is constitutively expressed and stored in pre-formed intracellular pools in a wide variety of cell types including immune, endothelial, and epithelial cells. In the liver,
MIF is produced by both hepatocytes and Kupffer cells. MIF signals via the interaction with the CD74 receptor, as well as its coreceptors CXCR2, CXCR4 and CXCR7. These receptors are expressed both on resident hepatic macrophages and peripheral monocytes; therefore, MIF release results in both activation of resident macrophages and recruitment of innate immune cells from the periphery.

MIF concentration is increased in the circulation of patients with ALD, as well as in mice in response to chronic ethanol feeding. Since mice deficient in MIF are protected from ethanol-induced liver injury, it is important to determine the cellular source of MIF in response to ethanol exposure. Making use of cell culture and mouse models, as well as clinical samples from patients with AH, we provide evidence that hepatocyte-derived MIF is important for the progression of ethanol-induced liver injury. Taken together, these data indicate that MIF released from injured hepatocytes likely serves as a DAMP in the progression of ALD, resulting in the recruitment of innate immune cells to the liver and activation of inflammatory pathways. Our results suggest that interfering with the release and/or signaling of MIF may be a viable therapeutic option in the prevention and/or treatment of ALD.

Materials and methods

Cell lines and cell culture

HuH7 is a well-differentiated hepatocyte-derived carcinoma cell line, obtained from Japan Health Science Research Resources Bank (HSRRB, JCRB0403). THP-1 is an acute monocytic leukemia derived cell line (ATCC® TIB-202™). HuH7 and THP-1 cells were cultured as previously described. Mycoplasma contamination has been excluded by testing the cultures periodically using fluorescent staining (Hoechst 33258). Cells were treated with or without 50 nM ethanol or 200 ng/ml lipopolysaccharides ([LPS] from E. coli serotype O127:B8), as detailed in the figure legends.

Generation of bone marrow chimeric mice and ethanol feeding

Female C57BL/6 (wild-type [WT]) mice were purchased from Jackson Laboratories (Bar Harbor, ME). Mif−/− mice on a C57BL/6 background were obtained from Dr. R. Bucala (Yale University, New Haven, CT, USA) and a breeding colony was established at Cleveland Clinic. All procedures using animals were approved by the Cleveland Clinic Institutional Animal Care and Use Committee. Bone marrow chimeras between WT and Mif−/− mice were generated as previously described. Mycoplasma contamination has been excluded by testing the cultures periodically using fluorescent staining (Hoechst 33258). Cells were treated with or without 50 nM ethanol or 200 ng/ml lipopolysaccharides ([LPS] from E. coli serotype O127:B8), as detailed in the figure legends.

Cytokine ELISAs

Cell culture media/Human serum

ELISA was performed in cell culture media and in patient serum following the manufacturer’s instruction (Human MIF Quantikine ELISA Kit, R&D System; Human TNF-α Instant ELISA, eBioscience).

Table 1. Baseline characteristics of patients.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Median (25–75 IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>52 (46–56)</td>
</tr>
<tr>
<td>Male (%)</td>
<td>53 (78)</td>
</tr>
<tr>
<td>Alcohol consumption (g/day)</td>
<td>100 (80–160)</td>
</tr>
<tr>
<td>Laboratory and hemodynamic parameters</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>11.4 (9.9–12.8)</td>
</tr>
<tr>
<td>Leukocyte count × 10^9/L</td>
<td>8.5 (6.3–12.5)</td>
</tr>
<tr>
<td>Platelet count × 10^9/L</td>
<td>112 (77–199)</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>117 (67–156)</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>39 (24–60)</td>
</tr>
<tr>
<td>Serum albumin (g/dl)</td>
<td>2.6 (2.3–3.1)</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.9 (0.7–1.1)</td>
</tr>
<tr>
<td>Serum bilirubin (mg/dl)</td>
<td>6.2 (2.7–19.3)</td>
</tr>
<tr>
<td>International normalized ratio</td>
<td>1.6 (1.4–1.8)</td>
</tr>
<tr>
<td>Alcohol hepatic severity scores at admission</td>
<td></td>
</tr>
<tr>
<td>MELD score</td>
<td>19 (14–25)</td>
</tr>
<tr>
<td>ABIC score</td>
<td>7.83 (6.69–8.66)</td>
</tr>
</tbody>
</table>

IQR, Interquartile range; AST, aspartate aminotransferase; ALT, alanine aminotransferase; MELD, Model for End-Stage Liver Disease; ABIC, Age, serum bilirubin, INR, and serum creatinine.
Staining for H&E was performed in paraffin-embedded mouse liver sections. Terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling (TUNEL) positive staining in liver was analyzed using ApopTag plus In Situ Apoptosis Detection Kit (S7111, Millipore, Billerica, MA). F4/80 (MCA239) (Bio-Rad, Hercules, CA) positive cells were visualized by immunohistochemistry (IHC) in mouse liver and counterstained with DAPI mounting medium (H-1200, Vector Laboratories, Burlingame, CA). IHC for MIF was conducted in liver specimens from patients with AH and subjects with healthy livers using the Bond fully-automated slide staining system (Leica Microsystems). See Supplementary data for additional details.

Statistics

Values are reported as means ± standard error of the mean (SEM). Data were analyzed by analysis of variance (ANOVA) using general linear models procedure (SAS, Cary, NC). If data were not normally distributed, data were log transformed. Multiple comparisons were analyzed using least square means. For human data, correlations between variables were evaluated using Spearman’s rho or Pearson’s r, where appropriate.

For further details regarding the materials used, please refer to the Supplementary data and CTAT table.

Results

**Hepatocytes (HuH7), but not monocyte-derived cells (THP-1), release MIF in response to ethanol challenge**

To understand the cellular sources of MIF in the context of ethanol exposure, HuH7 hepatocytes and differentiated THP-1 monocytes were treated with or without ethanol or LPS. Challenge of HuH7 with ethanol for 4–24 h increased MIF mRNA, while LPS only increased MIF mRNA after 24 h (Fig. 1A). Ethanol induced the release of MIF (but not TNF-α) from HuH7 starting after 8 h (Fig. 1B, C). In contrast, challenge of THP-1 cells with ethanol did not increase MIF mRNA (Fig. 1D) or release of MIF (Fig. 1E). However, LPS modestly increased MIF mRNA at 24 h (Fig. 1D) and stimulated release of MIF and TNFα as early as 4 h (Fig. 1E, F). These data suggested that hepatocytes might be an important source of MIF in the liver in response to ethanol exposure.

**MIF expression in non-myeloid cells contribute to ethanol-induced liver injury in chimeric mice**

Bone marrow transplants were carried out to generate the following chimeric mice: WT → WT (expressing MIF in both myeloid and non-myeloid cells), WT → Mif−/− (expressing MIF only in myeloid cells) and Mif−/− → WT (expressing MIF only in non-myeloid cells). MIF mRNA in livers of WT → Mif−/− mice was substantially lower than in WT → WT and Mif−/− → WT mice (Fig. S2), suggesting that non-myeloid cells are the predominant cell type expressing MIF in the liver.

Chronic ethanol feeding increased ALT and AST levels, as well as hepatic triglycerides, in both WT → WT and Mif−/− → WT mice (Fig. 2A–C). Hepatic steatosis and inflammatory foci were also observed in H&E stained sections of liver from ethanol-fed WT → WT and Mif−/− → WT mice (Fig. 2D). Chronic ethanol feeding to WT → WT and Mif−/− → WT mice also increased TUNEL positive nuclei in the liver (Fig. 2E). In contrast, WT → Mif−/− mice were resistant to chronic ethanol-induced increases in ALT/AST, hepatic steatosis, inflammatory infiltrates and apoptosis (Fig. 2A–E).

Consistent with the histological evidence for increased inflammatory cells after chronic ethanol feeding to WT → WT and Mif−/− → WT mice, expression of mRNA of the chemokines MCP-1, CXCL10, CXCL1 and CXCL2 in liver was also higher in Mif−/− → WT chimeric mice compared to WT → Mif−/− chimeric mice (Fig. 3A). IHC analysis of cells positive for F4/80, a marker of resident macrophages, and Ly6C, a marker of infiltrating monocytes, revealed that while populations of F4/80+ (Fig. 3B) and Ly6C+ (Fig. 3C) cells were maintained at control values in livers of WT → Mif−/− chimeric mice after chronic ethanol feeding, both populations were increased by chronic ethanol in Mif−/− → WT mice (Fig. 3B, C). Ly6C+ cells also clustered into inflammatory foci, as observed on H&E stained liver sections (Fig. 2D).

Taken together, these data from chimeric mice suggest that bone marrow-derived cells do not contribute to MIF release after ethanol feeding in mice. Instead, non-myeloid cells represent the critical cell type generating MIF in response to chronic ethanol feeding. Importantly, the absence of MIF in non-myeloid cells protected mice from chronic ethanol-induced liver injury to a similar extent as in global MIF-deficient mice.

**MIF expression in the liver of patients with alcoholic hepatitis**

We next investigated the expression and localization of MIF in liver biopsies from patients with AH, autoimmune hepatitis (disease control) and non-diseased controls. Increased MIF staining was detected in liver biopsies from patients with AH, as well as autoimmune hepatitis, compared with controls (Fig. 4A). Expression of MIF mRNA was increased 1.4-fold in patients with AH compared to healthy controls (Fig. 4B). However, the cellular localization differed between AH and autoimmune hepatitis.

![Fig. 1. MIF release from HuH7 hepatocytes, but not THP-1 macrophages, in response to ethanol exposure.](image-url)

**A** Expression of MIF mRNA in HuH7 and differentiated THP-1 macrophages was measured by qRT-PCR. MIF and TNF-α accumulation was detected by ELISA in the cell culture medium of (D) HuH7 cells and (E, F) differentiated THP-1 macrophages after challenge with 50 mM ethanol (EtOH) or 200 ng/ml LPS. Values represent mean ± SEM. *p < 0.05 compared to basal, n = 3.

1020 Journal of Hepatology 2017 vol. 67 | 1018–1025
MIF was predominantly localized to hepatocytes and ductular cells in AH patients, while MIF was primarily localized in non-parenchymal cells in autoimmune hepatitis. Hepatocytes and non-parenchymal cells were identified according to their cellular and nuclear morphology. Although the majority of MIF staining was localized in the cytosol of hepatocytes, nuclear staining was also detected in a few cells in sections from patients with AH.

Positive correlation between MIF in suprahepatic serum and biochemical markers of liver disease in patients with alcoholic hepatitis

If hepatocytes are an important source of MIF in response to ethanol, then the concentration of MIF in suprahepatic serum should be correlated with the extent of liver disease in patients with AH. To test this hypothesis, MIF was quantified in peripheral and suprahepatic serum. The concentration of circulating MIF correlated with parameters indicative of disease severity. Peripheral serum levels positively correlated with suprahepatic serum levels. A positive correlation was found between MIF concentration in the suprahepatic, but not peripheral, serum including bilirubin, AST, circulating triglycerides and GGT (Table 2 and Fig. 4C–G). Increased MIF in the suprahepatic serum (Fig. 4H), but not peripheral serum (Fig. 4I), was also associated with an increased risk of mortality.

Discussion

Chronic, heavy alcohol consumption results in injury to hepatocytes, at least in part due to the oxidative stress resulting from ethanol metabolism, as well as the development of inflammation in the liver. However, the complex mechanisms linking hepatocyte injury to inflammation are not completely understood. Release of DAMPs from injured hepatocytes activates the resident Kupffer cells in the liver, leading to production of chemokines and further recruitment of immune cells to the liver. Recent data also suggests that hepatocytes release chemokines in response to...
injury, thus adding to the recruitment of more immune cells.\(^\text{21}\) MIF, a potent chemokine, is released by both immune cells\(^\text{22}\) and hepatocytes\(^\text{11,23}\) in other disease models. Since MIF is a critical mediator of ethanol-induced liver injury, here we sought to identify the predominant cellular source of MIF in response to ethanol exposure. Challenge of HuH7 hepatocytes, but not THP-1 macrophages, with ethanol resulted in the accumulation of MIF in the cell culture media. Importantly, chimeric mice deficient in MIF in non-myeloid cells were protected from chronic ethanol-induced liver injury. Finally, patients with AH had increased expression of MIF in hepatocytes and accumulation in the circulation. The concentration of MIF in the suprahepatic serum correlated with multiple clinically-relevant parameters indicative of disease severity, as well as the risk of death from AH. Taken together, these data suggest that hepatocytes are an important source of MIF in response to chronic ethanol feeding in mice and patients with AH and that the release of MIF from hepatocytes likely provides an important link between hepatocyte injury from ethanol and an exacerbation of inflammation in the liver.

Despite its name, MIF has significant chemotactic activity and is a potent enhancer of macrophage activity, increasing phagocytosis, as well as expression of inflammatory cytokines and inducible nitric oxide synthase.\(^\text{24}\) MIF upregulates the expression of major histocompatibility complex II molecules, co-stimulatory and adhesion molecules, as well as cytokines, in a wide variety of cell types including Kupffer cells, peritoneal macrophages and dendritic cells.\(^\text{25}\) MIF also maintains macrophage viability by suppressing activation-induced macrophage apoptosis by inhibiting p53.\(^\text{26}\) Thus, the ability of MIF to act as a chemokine, as well as to prevent activation-induced apoptosis, increases expression of inflammatory mediators and contributes to MIF’s profound pro-inflammatory effects.

MIF plays an important role in the liver in response to acute and chronic stress,\(^\text{27}\) contributing to T cell mediated injury in murine ethanol-induced liver damage.\(^\text{28}\) In contrast, MIF can also have protective effects at specific phases of liver disease. For example, MIF is required for the recruitment of scar-associated macrophages to the liver; these macrophages are critical for the resolution of fibrosis after an injury.\(^\text{28}\) This recruitment likely...
contributes to the protective function of MIF and its receptor CD74 in response to fibrotic insults. Taken together, these studies suggest a complex interaction between MIF in the progression of liver disease.

Given this complex role, here we first investigated the direct effects of ethanol on HuH7 cells and differentiated THP-1 macrophages, as cellular models of the primary cell types expressing MIF in the liver. When HuH7 cells were challenged with ethanol,
Research Article

MIF mRNA increased by 4 h and MIF accumulated in the cell culture media over 8–24 h, consistent with previous reports that hepatocyte injury in response to CCl₄ increased expression of MIF. In contrast, MIF was not released in differentiated THP-1 macrophages, nor did MIF mRNA increase, in response to ethanol. The differences in the response of these two cell types to ethanol may be related to the ability of HuH7 cells to metabolize ethanol; ethanol metabolism likely contributed to injury of the hepatocytes and release of MIF.21

Based on these cell culture studies, we proposed that if hepatocytes were the primary source of MIF in response to ethanol, then chimeric mice deficient in MIF in non-myeloid cells should be protected from ethanol-induced liver injury. Indeed, when bone marrow chimeric mice expressing WT or Mif⁻/⁻ bone marrow in a WT background were exposed to chronic ethanol feeding, liver injury developed, characterized by increased ALT/AST in the circulation and hepatic steatosis. In contrast, bone marrow chimeric mice expressing WT bone marrow on a Mif⁻/⁻ background were protected from ethanol-induced liver injury. While these studies cannot exclude a potential contribution of MIF in other hepatic cell types, such as endothelial cells22 or hepatic stellate cells,23 they are consistent with a likely role of hepatocytes as an important cellular source of MIF in the liver.

Because of its potent chemokine activity, the absence of MIF should prevent the recruitment of immune cells to the liver in response to ethanol. Indeed, the number of both F4/80⁺ and Ly6C⁺ cells in the liver were increased by ethanol in chimeric mice on a WT background, but not in mice on a Mif⁻/⁻ background. This finding is consistent with published reports that indicate a role of MIF in macrophage/monocyte replenishment and recruitment during ethanol exposure.24,25 Specifically, Barnes et al.24 found that the number of hepatic F4/80⁺ cells is reduced in ethanol-fed Mif⁻/⁻ mice compared to WT mice, suggesting that MIF was critical for the maintenance of the hepatic macrophage population during ethanol exposure. Similarly, the recruitment of Ly6C⁺ monocytes was also reduced in MIF-deficient mice after ethanol feeding and challenge with LP,25 as well as after challenge with CCl₄.26

Although MIF has direct chemokine activity, it can also indirectly influence immune cell recruitment via modulation in the expression of other chemokines. For example, MIF upregulates MCP-1 expression in an autocrine mechanism in mouse liver or hepatocytes following acute CCl₄ exposure.23 MCP-1 contributes to progression of ethanol-induced liver injury, particularly related to its ability to act both as a chemokine and a steatoxin.20 Here we find that in WT → Mif⁻/⁻ expression of mRNA for some chemokines, including MCP-1, CXCL1, CXCL2 and CXCL10 after ethanol feeding was lower than in Mif⁻/⁻ → WT (Fig. 3). Interestingly, CX chemokines, mainly derived from hepatocytes, are known to correlate with mortality in AH.21 Taken together, these data indicate that MIF released from non-myeloid cells in response to ethanol-induced liver injury acted as a signal regulating the expression of a network of chemokines known to be important for the progression of ethanol-induced liver injury.

Our cell culture and mouse studies suggest that MIF is an important danger signal released from hepatocytes in response to ethanol-induced injury. Here we also report that MIF expression, both at the level of mRNA and protein, was increased in livers of patients with AH. IHC revealed a predominant expression of MIF in hepatocytes in AH patients compared to both healthy liver controls and patients with autoimmune hepatitis. Kumagi et al.11 reports that MIF concentrations in the circulation were increased in relation to disease severity in patients with ALD. If this increase in circulating MIF was derived from injured hepatocytes and/or other liver cell types, then MIF concentration in suprarepatic serum should be correlated with the extent of hepatocyte injury in patients with AH. This hypothesis is supported by our finding that suprarepatic, but not peripheral, MIF correlated with bilirubin, AST, GGT and circulating triglycerides, as well as with short-term mortality in AH patients. It will also be important in future experiments to determine if there are post-translational modifications to MIF in patients with AH, such as the redox-dependent conformational isomers reported in other inflammatory diseases.25

It is interesting to note that MIF has protective effects on hepatocytes in the face of high fat diet-induced injury.26,27 Clearly, our data suggest that this potential hepatoprotective effect of MIF is not sufficient to protect hepatocytes in the context of ethanol exposure. Instead, it is likely that the pro-inflammatory, chemoattractive properties of MIF are predominant in AH. These differential effects of MIF are likely related to its interactions with multiple receptors. The cytokine activity of MIF, as well as its protective effects on hepatocytes, is largely due to binding to the CD74/CD44 receptor complex.9,25 In contrast, its chemokine activity is exerted through the formation of different homo- and heterodimers of the CXCR2, CXCR4 and CXCR7 receptors, possibly also in coordination with CD74.10,36,37 These differences in MIF function will be important to keep in mind during the development of potential therapeutic interventions to inhibit MIF action.

In conclusion, our present findings reveal that MIF was released from non-myeloid cells during the progression of ethanol-induced liver injury. Our data are consistent with a predominant role for hepatocyte injury in driving the release of MIF. It is interesting to note that MIF release from injured hepatocytes functions in a similar manner to classic DAMPs, signaling sterile inflammation and the recruitment of inflammatory cells to the site of injury. Strategies to specifically dampen the chemotactic, rather than the potential hepatoprotective effects of MIF are likely to be a relevant therapeutic approach in treating AH.

Financial support

This work was supported in part by NIH grants U01AA020821 (LEN), U01AA021890 (LEN), F32AA024955 (KP) and U01AA021908 (RB); by the Italian Liver Foundation (NR, CT) and grants from Instituto de Salud Carlos III (FIS PI14/00320) and Miguel Servet (CP11/00071) co-financed by FondoEuropeo de Desarrollo Regional (FEDER), Unión Europea, ‘Unamanera de hacer Europa’ (PS-B).

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript. Please refer to the accompanying ICMJE disclosure forms for further details.

Authors’ contributions

Study concept and design: LE Nagy, R Bataller, N Rosso.
Acquisition of data; analysis and interpretation of data: V Marin, K Poul sen, G Odena, MR McMullen, J. Altamirano, P Sancho-Bru, CTiribelli, J. Caballeria, N Rosso, LE Nagy, R Bataller. Drafting of the manuscript: V Marin, K Poul sen, G Odena, MR McMullen, P Sancho-Bru, CTiribelli, N Rosso, R Bataller and LE Nagy.

Critical revision of the manuscript for important intellectual content: V Marin, K Poul sen, G Odena, MR McMullen, P Sancho-Bru, CTiribelli, N Rosso, R Bataller and LE Nagy.


Technical support: MR McMullen.

Acknowledgements

JA wishes to express his gratitude to the Mexican National Council of Science and Technology (CONACyT, Mexico City, Mexico) for partially supporting his predoctoral stay at IDIBAPS.

Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.jhep.2017.06.014.

References

[33] Cupples BL, Bai S, Burgoon LD, Moon J-O. Hypoxia-inducible factor-1α regulates expression of genes in hypoxic hepatic stellate cells important for collagen deposition and angiogenesis. Liver Int 2011;31:230–244.

JOURNAL OF HEPATOLOGY