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Galectin-1 attenuates hepatic ischemia reperfusion injury in mice☆

Yufu Ye[a,b,1], Wei Wang[a,1], Weichen Zhang[a], Yifan Peng[a], Yuanxing Liu[a], Songfeng Yu[a], Qi Chen[c], Lei Geng[a], Lin Zhou[a], Haiyang Xie[a], Mingchun Lai[a], Jun Yu[a,*], Shusen Zheng[a,b,*]

[a] Key Laboratory of Combined Multi-organ Transplantation, Ministry of Public Health, Department of Hepatobiliary and Pancreatic Surgery, First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China
[b] Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, Hangzhou, China
[c] Ningbo First Hospital (Ningbo Hospital of Zhejiang University), Ningbo, China

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ABSTRACT

Background: Hepatic ischemia reperfusion injury (IRI) is a primary cause of organ dysfunction occurring during liver resection surgery and transplantation. Galectin-1, an endogenous lectin expressed on lymphoid organs, plays an important role in governing innate and adaptive immunity. This study was designed to determine the therapeutic role of galectin-1 and underlying mechanism in hepatic IRI.

Methods: Male C57BL/6 mice were subjected to 90 min of partial hepatic ischemia followed by reperfusion with or without treatment with recombinant galectin-1 (rGal-1) or neutralizing anti-IL-10 antibody. Mice were sacrificed at 6 and 24 h following reperfusion. Liver damage related enzymes were determined and cytokines/chemokines were measured by qPCR and ELISA.

Results: Administration of rGal-1 significantly attenuated hepatic IRI, including a remarkable reduction in serum ALT/AST levels and an improved liver histology score compared to controls. rGal-1 treatment reduced TUNEL positive apoptotic hepatocytes, attenuated proinflammatory cytokines (TNF-α, IL-6, IL-1β, IL-12, IFN-γ, IL-17) and chemokines (CXCL-1, CXCL-10) levels, but upregulated IL-10 expression, compared with controls. In addition, rGal-1 increased the production of IL-10 in hepatic macrophages *in vivo* and *in vitro*. Blockade of IL-10 using neutralizing anti-IL-10 antibody reversed the protection of galectin-1 in hepatic IRI in mice.

Conclusion: These data suggest that galectin-1 may attenuate hepatic IRI via an IL-10-dependent mechanism, which is a promising therapeutic target.

1. Introduction

Hepatic ischemia reperfusion injury (IRI) during liver resection and transplantation is a major cause of liver dysfunction. IRI causes early graft failure and increases the risk of acute and chronic rejection [1]. Innate immunity plays an important role in the modulation of IRI in liver. In response to certain stimuli or stress, Kupffer cells (KCs) have been well documented in the pathogenesis of liver IRI by producing proinflammatory cytokines (TNF-α and IL-6), chemokines and reactive oxygen species (ROS) [2]. However, some other studies raised conflicting results showing that KCs are also protective against liver IRI with via the up-regulation of IL-10 [3]. In addition, chemokines including CXCL10 plays an important role in mediating hepatic inflammatory response to IRI, whose deficiency protects liver against IRI via an IL-10-dependent manner [4]. Moreover, adaptive immunity also gets involved in the regulation of liver IRI. $CD4^+$ T lymphocytes, particularly Th1 and Th17 subsets, have been reported as key mediators of liver inflammation via the production of proinflammatory cytokines including IFN-γ and IL-17 in a murine model of liver transplantation [5,6]. Although the modulation of hepatic IRI have been linked to liver immunity, including activating of KCs, recruiting neutrophils and promoting inflammatory T cell differentiation, there is no effective therapy to completely prevent or eliminate ischemia reperfusion-triggered liver damage currently.

Galectin-1 (Gal-1), a member of galectin family, is expressed in a variety of cells, such as thymic epithelial cells, endothelial cells,

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* Corresponding authors at: Division of Hepatobiliary and Pancreatic Surgery, Department of Surgery, First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China.
E-mail addresses: dryujun@zju.edu.cn (J. Yu), shusenzheng@zju.edu.cn (S. Zheng).

[1] Y.Y. and W.W. contributed equally to this work.

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dendritic cells, macrophages, fibroblasts, and bone marrow stromal cells [7]. Gal-1 has been implicated in various biological activities, including cell adhesion, apoptosis, growth, metastasis and the regulation of immune homeostasis [8]. Moreover, Gal-1 also plays a critical role in directing innate and adaptive immune responses. We have reported that Gal-1 selectively blunted Th1/Th17 responses, dampened the secretion of pro-inflammatory cytokines/chemokines and promoted the apoptosis of activated lymphocytes [9]. In consistent with our findings, recombinant galectin-1 (rGal-1) has been verified as a therapeutic target in other models of inflammatory disease, including arthritis [10], hepatitis [6], type-1 diabetes [11], and liver transplant rejection [9]. However, how Gal-1 governs inflammatory responses in liver IRI is not fully understood. In the current study, we demonstrated that administration of Gal-1 ameliorated liver injury in a murine model of ischemia reperfusion injury. Mechanically, we revealed that Gal-1 modulated inflammatory liver immune profile via an IL-10-dependent manner, suggesting that Gal-1 is a promising therapeutic target in liver IRI.

2. Materials and Methods

2.1. Animals

Specified pathogen-free 8- to 10-week-old female C57BL/6 mice were purchased from the Shanghai Experimental Center, Chinese Academy of Science. Mice were maintained under specific pathogen-free conditions within the animal facility of The First Affiliated Hospital, School of Medicine, Zhejiang University, provided with water and food ad libitum, and housed under 12-h light/dark cycles. All animal experiments were approved by the Animal Care Committee of Zhejiang University in accordance with the Principles of Laboratory Animal Care.

2.2. Hepatic IRI model

A mouse model of warm partial IRI was performed as described [12]. Briefly, mice were anesthetized, injected with heparin (100 U/kg, intraperitoneally), and the arterial and portal venous blood supply to the cephalad lobes was interrupted by an atraumatic clip. After 90 min of local ischemia, the clip was removed. Sham controls underwent the same procedure, but without vascular occlusion. Recombinant galectin-1 (Peprotech, USA) was injected i.p. (0.25 mg at day −1 and 0.5 mg at day 0) 30 min before surgery. IL-10-neutralizing antibody (BD Biosciences) was administered (0.5 mg at day −1 and 0.5 mg at day 0) with or without rGal-1. Saline was injected at equivalent doses and schedules as controls. Animals were sacrificed after 6 or 24 h of reperfusion.

2.3. Hepatocellular damage assay

Transaminase (ALT and AST) levels were measured using an Automatic Chemical Analyzer 7600-100 (Hitachi, Ltd, Tokyo, Japan) in the clinical chemistry laboratory at The Fist Affiliated Hospital, School of Medicine, Zhejiang University.

2.4. Liver histology evaluation

Formalin-fixed paraffin-embedded liver tissues were cut into 4-μm thick sections placed on polylysine-coated slides and stained with hematoxylin and eosin for histology analysis. All liver specimens were evaluated by a single pathologist who was unaware of the experimental scheme. Liver sections were graded for IRI using the system devised by Suzuki et al. [13].

2.5. Quantitative real-time polymerase chain reaction

RNA isolation and mRNA levels were determined by real time quantitative PCR (RT-qPCR) as described in our previous study [14], using an ABI PRISM 7500 real-time PCR System and SYBR Green PCR master mix (Applied Biosystems, Foster City, CA, USA). Target gene expressions were calculated by their ratios to the housekeeping gene hypoxanthine-guanine phosphoribosyl transferase.

2.6. Terminal transferase-mediated dUTP nick end-labeling staining

Paraffin-embedded sections were prepared and stained for apoptotic cells by the terminal transferase-mediated dUTP nick end-labeling (TUNEL) method using a commercially available kit (ApopTag® Peroxidase In Situ Apoptosis Detection Kit S7100; Chemicon International Inc., Billerica, MA, USA), following the manufacturer's instructions. TUNEL-positive cells were counted in 10 high-power fields under light microscopy (×400), as described [12].

2.7. Caspase-3 activity assay

Caspase-3 activity was determined in liver samples using a commercially available Caspase 3 Activity Assay Kit (Beyotime, China) according to the manufacturer's instructions. Optical density measurements at 405 nm were performed using a microplate reader (Bio-TeK).

2.8. ELISA

The concentrations of IL-10 and TNF-α were measured by ELISA assay using a commercially available enzyme-linked immunosorbent assay kit (eBioscience, San Diego, CA) in accordance with the manufacturer's instructions.

2.9. Generation of bone marrow-derived macrophages and isolation of hepatic macrophages

Mouse myeloid DCs were generated from C57BL/6 mouse bone marrow cells as previously described [10]. Briefly, the collected bone marrow extract harvested from femurs and tibias was then cultured in RPMI 1640 (Invitrogen Life Technologies, Carlsbad, CA) containing 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/mL penicillin, 100 lg/mL streptomycin, 1 mM sodium pyruvate, and 1 mM HEPES (Corning, NY), and supplemented with 30 ng/mL macrophage colony stimulating factor (M-CSF, BioLegend, San Diego, CA). After 7 days of culture, adherent macrophages were harvested for further experiments. Non-parenchymal cells from mouse liver were isolated by a 2-step collagenase perfusion, as described in our previous study [15]. Positive selection of F4/80+ cells (hepatic macrophages) from nonparenchymal cells was performed using the QuadroMACS column separation kit (Miltenyi Biotech, Cambridge, MA) [16]. Macrophages were pretreated with rGal-1 (10, or 100 μM) and then were subjected to LPS challenge (100 ng/mL, Sigma-Aldrich).

2.10. Statistical analysis

Statistical analysis was performed using the SPSS 11.6 for Windows (SPSS, Chicago, IL, USA). All values are expressed as the mean ± SD. Differences between experimental groups were analyzed using ANOVA and Tukeýs test. $P < 0.05$ was considered statistically significant. Graphic presentation was performed using the GraphPad Prism® 4.0 package (GraphPad, San Diego CA, USA).

3. Results

3.1. Galectin-1 attenuates hepatic ischemia/reperfusion injury

As galectin-1 selectively blunts Th1 and Th17 responses by inhibiting secretion of pro-inflammatory mediators [9], we postulated that administration of rGal-1 mitigates liver ischemia/reperfusion (I/R)

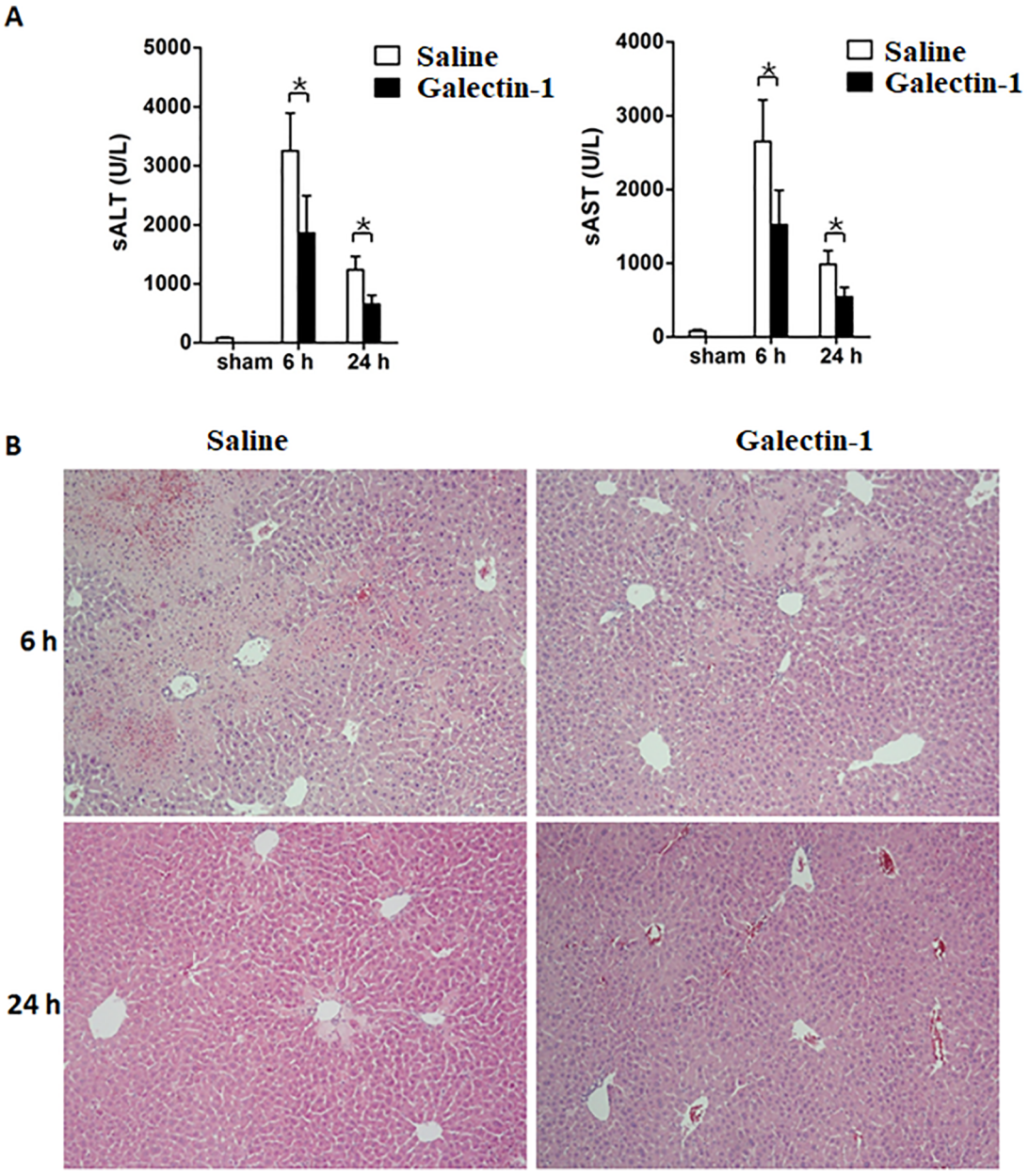


Fig. 1. Liver damage induced by IRI in mice with or without rGal-1 pretreatment. (A) Hepatocellular damage measured by sALT/AST levels at 6 and 24 hours (ALT: 6 h: 3185.6 ± 856.3 vs. 1936.5 ± 862.4; $p < 0.001$; 24 h: 1210.6 ± 286.5 vs. 816.6 ± 176.3; $p < 0.001$; AST: 6 h: 2612.8 ± 678.7 vs. 1516.1 ± 493.8; $p < 0.001$; 24 h: 956.4 ± 196.2 vs. 532.5 ± 35.7; $p < 0.001$). (B) Representative liver histology (H&E staining; magnification ×100) of liver lobes harvested 6 and 24 hours after reperfusion. Data are representative of n = 5.

injury. To test this in vivo, mice were given rGal-1 (500 μg per mouse) or saline 30 min prior to hepatic I/R challenge. We found that 90 min of warm hepatic ischemia followed by 6 or 24 h of reperfusion robustly increased serum ALT and AST levels in saline-treated mice compared to sham-operated controls (Fig. 1a). In parallel with tremendous changes in serum markers, liver histology analysis demonstrated severe sinusoidal congestion, massive extent of necrosis and inflammation following hepatic IRI (Fig. 1b). In consistent with our hypothesis, administration of rGal-1 dampened liver IRI, resulting in a ~40% reduction in serum ALT/AST levels compared to controls (Fig. 1a). In addition, liver histology analysis revealed mild liver damage and minimal inflammatory cell infiltrates in rGal-1-treated mice (Fig. 1b).

3.2. Galectin-1 ameliorates I/R-triggered hepatocyte apoptosis

Since administration of rGal-1 in vivo attenuates I/R-induced liver injury, we hypothesized that rGal-1 governs hepatic damage via regulating hepatocyte death. To further understand how rGal-1 interferes with cell death in hepatic I/R, TUNEL staining and caspase 3 activity assays were performed to determine I/R-triggered apoptosis. In consistent with our hypothesis, rGal-1 treatment markedly reduced the number of TUNEL positive hepatocytes (Fig. 2a and b) and the level of hepatic caspase-3 activity (Fig. 2c) compared to saline-treated controls, suggesting that administration of rGal-1 dampens hepatocyte apoptosis in a murine model of liver I/R.

3.3. Galectin-1 mitigates pro-inflammatory cytokine and chemokine profiles induced by hepatic I/R

To further profile the microenvironment in the murine model of hepatic IRI following rGal-1 treatment, we examined I/R-induced cytokine/chemokine milieu in liver at 6 h of reperfusion following 90 min

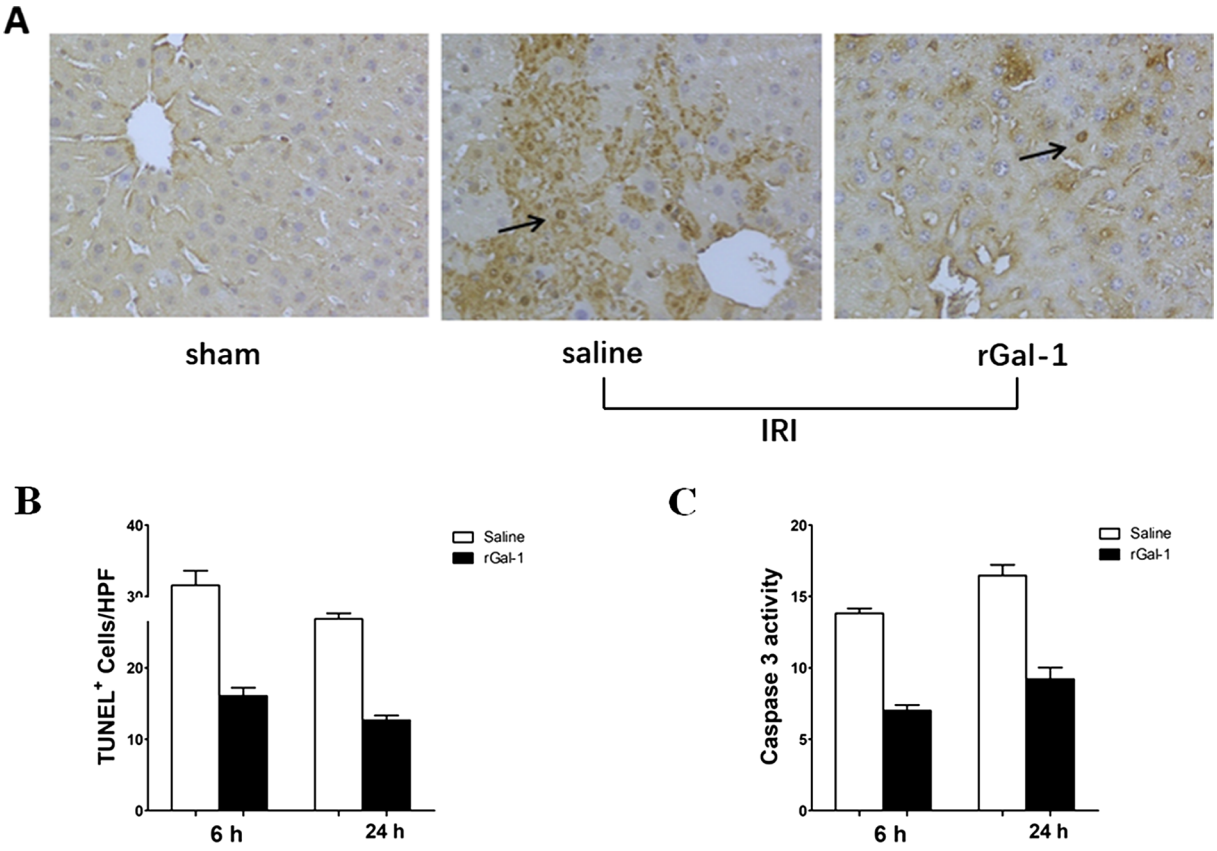


Fig. 2. Galectin-1 ameliorates I/R- triggered hepatocyte apoptosis. (A) Representative TUNEL-stained sections of hepatic necrosis/apoptosis (arrows) in ischemic lobes (magnification ×400). (B) Lower frequency of hepatic TUNEL$^+$ cells in the rGal-1-treated group compared with controls (6 h: 31.6 ± 8.3 vs. 16.4 ± 6.1; $P < 0.05$; 24 h: 26.3 ± 3.7 vs. 12.6 ± 2.9; $P < 0.05$). (C) rGal-1 treatment reduced the level of hepatic caspase-3 activity compared with the saline-treated controls. Data are representative of n = 5. Three sections per mouse were examined.

of warm ischemia. Hepatic mRNA expression of cytokines (TNF-α, IL-6, IL-1β, IL-12, IFN-γ, IL-17 and IL-10) and chemokines (CXCL1, CXCL10) were remarkably increased following I/R. Administration of rGal-1 *in vivo* markedly dampened the upregulation of hepatic pro-inflammatory cytokines and chemokines gene expressions, whereas IL-10 gene expression was significantly increased (Fig. 3A). In parallel with changes in hepatic mRNA profile, serum IL-10 level was increased whereas TNF-α was concomitantly decreased (Fig. 3B). These results indicate that rGal-1 treatment yields an anti-inflammatory microenvironment in the mouse model of warm IRI.

3.4. Galectin-1 augments the production of IL-10 in hepatic macrophages

Since rGal-1 treatment increased hepatic I/R-related production of IL-10, one of the most potent anti-inflammatory cytokines in liver, we postulated that selected toll-like receptors (TLRs) activation promotes IL-10 expression. To establish whether the upregulation of IL-10 are hepatic macrophage dependent, hepatic macrophages were isolated from rGal-1-treated and control mice following I/R. Elevated IL-10 expression was detected in hepatic macrophages from rGal-1-treated mice compared with control mice (Fig. 4A). We then investigated the effect of Gal-1 on IL-10 produced by bone marrow-derived and hepatic macrophages in response to lipopolysaccharide (LPS). Isolated macrophages were pretreated with rGal-1 and subjected to LPS challenge supervene. In parallel with our hypothesis, LPS stimulation resulted in a significant increase in IL-10 level. rGal-1 further increased IL-10 production by both bone marrow-derived and hepatic macrophages in a dose dependent manner, suggesting that Gal-1-induced IL-10 upregulation in KCs is TLR4 dependent in hepatic IRI (Fig. 4B).

3.5. IL-10 neutralization restores hepatic IRI in rGal-1-treated mice

To further explore the role of IL-10 in hepatic IRI protection by Gal-1, IL-10-neutralizing antibody was administered prior to the onset of ischemia, with or without rGal-1. Compared to rGal-1 treatment alone, anti-IL-10 mAb induced a remarkable increase in serum ALT/AST levels (Fig. 5a). Histology analysis revealed that livers harvested from anti-IL-10 mAb-treated mice presented greater extent of edema, sinusoidal congestion, and cell necrosis compared to those following rGal-1 treatment alone (Fig. 5b), which was comparable to sham-controls. Blockade of IL-10 in rGal-1-treated mice aggravates hepatic IRI, suggesting that Gal-1 may attenuate hepatic IRI via an IL-10-dependent manner.

4. Discussion

Galectin-1 has been well documented in recent studies as a negative regulator of both innate and adaptive immune responses [7]. Gal-1 has been reported to be protective in murine models of autoimmune arthritis, diabetes, encephalomyelitis, myocarditis, polymicrobial sepsis and hepatitis [17]. In the current study, we provide the first experimental evidence of a therapeutic role of Gal-1 in attenuating hepatic IRI via an IL-10-dependent manner. We demonstrated that administration of rGal-1 significantly attenuated hepatic IRI, as evidenced by a significant reduction in serum ALT/AST levels, an improved liver histology score and reduced TUNEL positive apoptotic hepatocytes. rGal-1 treatment elicited an altered inflammation response, with reduced gene induction of pro-inflammatory cytokines/chemokines, and concomitant increased anti-inflammatory IL-10 production. Blockade of IL-10 reversed hepatic IRI in rGal-1-treated mice. These data strongly suggest

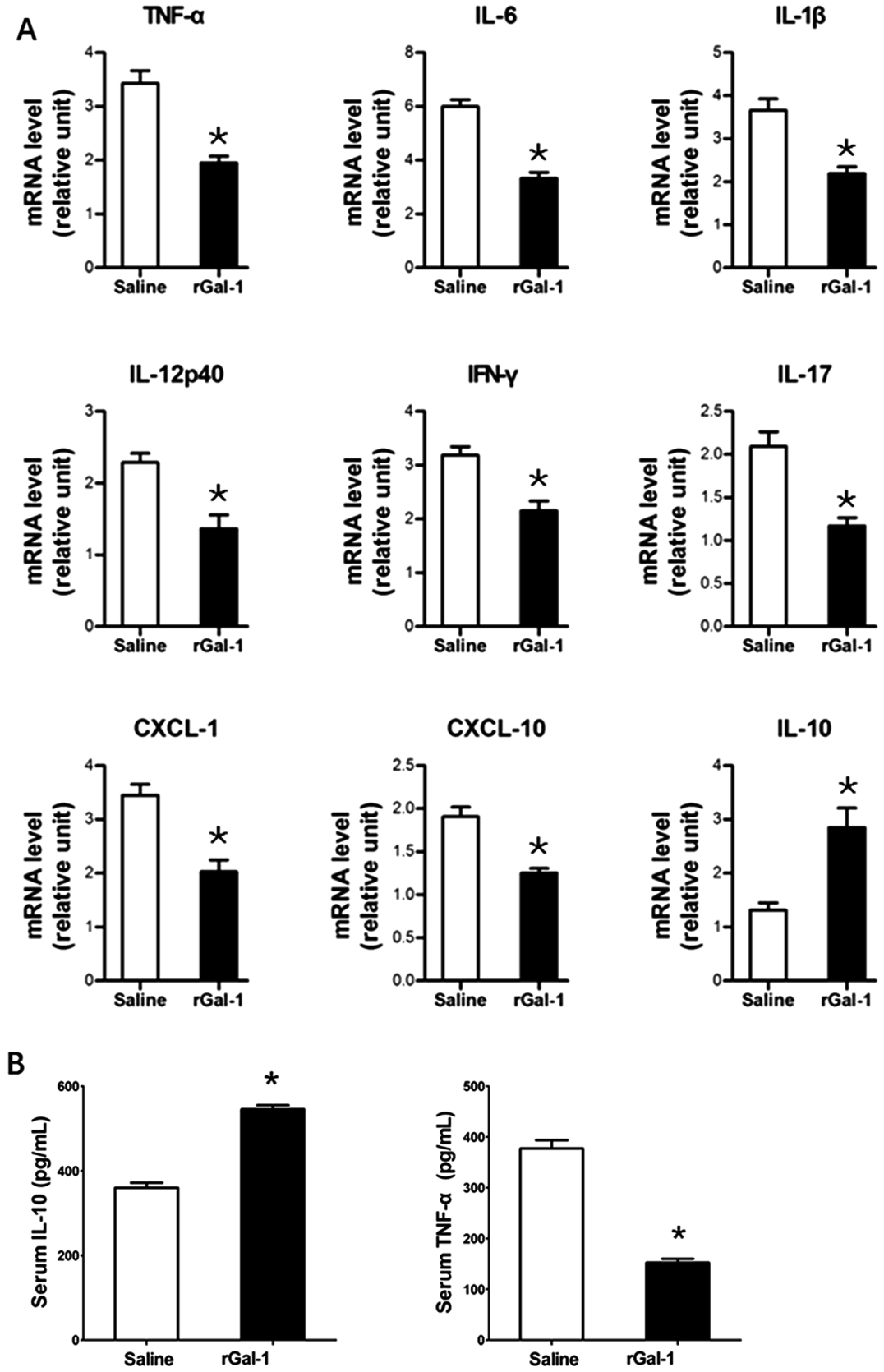


Fig. 3. Effect of rGal-1 administration on intrahepatic mRNA levels of cytokines and chemokines. (A) Quantitative real-time PCR detection of gene expression of cytokines (TNF-α, IL-6, IL-1β, IL-12, IFN-γ, IL-17 and IL-10) and chemokines (CXCL-1, CXCL-2) in IRI livers. Data were normalized to GAPDH gene expression. (B) Serum IL-10 and TNF-α were detected by ELISA. Means and SEM are shown (*$P < 0.05$). Data are representative of n = 5.

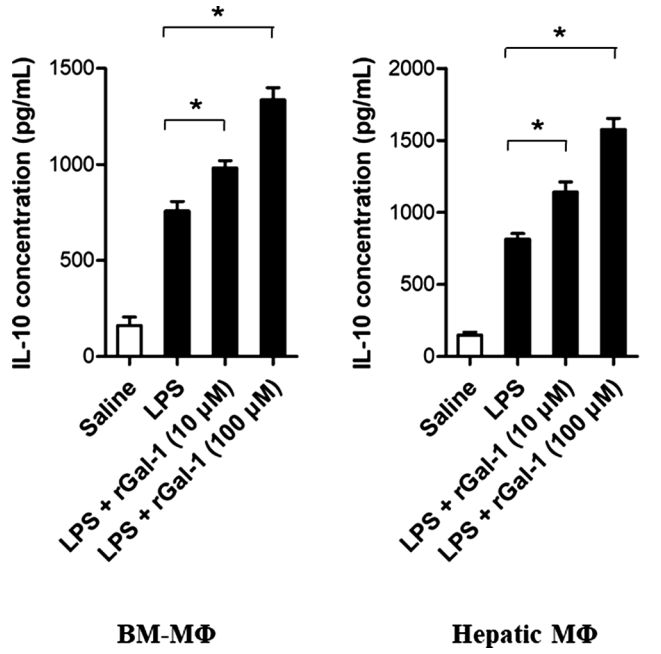
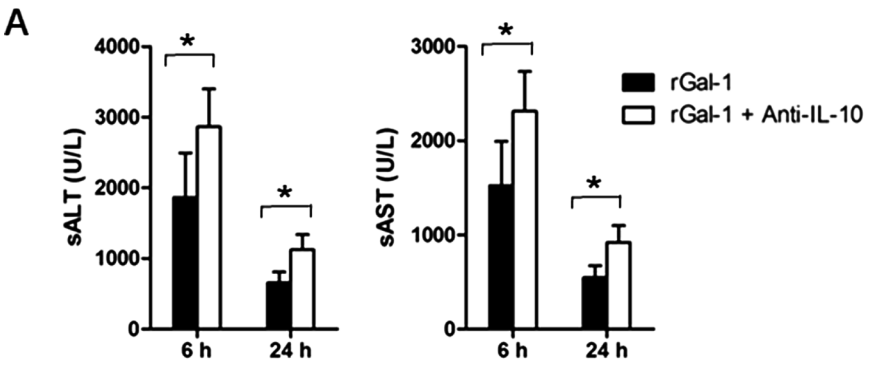


Fig. 4. Effect of rGal-1 on IL-10 production by macrophages. Bone marrow derived (Left) or hepatic (Right) Macrophages (1×10^6 cells/mL) were incubated for 6 hours with 100 ng/mL LPS, with or without galectin-1 pretreatment (10 or 100 μM, 1 h). Supernatants were collected after 6 h, and IL-10 levels were assessed by ELISA. Mean values ± standard errors of the mean (SEM) of five separate experiments performed in triplicate are shown.

that Gal-1 represents a promising therapeutic target for treatment of hepatic IRI.

Hepatic IRI activates KCs, liver sinusoidal endothelial cells (LSECs) and hepatocytes, leading to a release of reactive oxygen species (ROS) and proinflammatory cytokines/chemokines. KCs plays a central role in the physiopathology of hepatic IRI. In the acute phase of IR-triggered inflammation responses after reperfusion, liver injury was linked to T-lymphocyte/KC activation [18]. Activation of KCs results in the release of proinflammatory cytokines and chemokines, which are responsible for neutrophils recruitment and T cell activation [18]. In our study, rGal-1 treatment significantly attenuated expression of proinflammatory cytokines (TNF-α, IL-6, IL-1β, IL-12, IFN-γ, IL-17) and chemokines (CXCL-1, CXCL-10), which have been identified as the main mediators of IR-triggered liver inflammation. Activated KCs increase the oxidative stress by the release of superoxide radicals, such as TNF-α and IL-1, leading to hepatocellular damage [19]. In the subacute phase of IRI, activated neutrophils dominate local damage cascade [18]. CXCL-1 is one of the key chemo-attractants facilitating neutrophil recruitment in hepatic IR-triggered inflammation [20]. IFN-γ enhances the sequestration of neutrophils to liver. rGal-1 treatment inhibited the intrahepatic expression of CXCL-1 and IFN-γ in our hepatic IRI model. These results suggest that galectin-1 may underline its inhibitory effects in acute inflammation via suppressing proinflammatory cytokines/chemokines and limiting neutrophil recruitment.

KCs are considered as the predominant producer of IL-10 in liver [21]. IL-$10^{-/-}$ mice have been shown to have greatly increased susceptibility to hepatic IRI [22]. In our study, administration Gal-1 *in vivo* attenuated hepatic IRI along with an increase in hepatic IL-10 and macrophage-derived IL-10. rGal-1 pretreatment *in vitro* significantly augmented IL-10 production by both bone marrow-derived and hepatic macrophages in response to LPS stimulation. Blockade of IL-10 reversed liver pro-inflammatory phenotype and aggravated the severity of liver damage, suggesting that galectin-1-induced macrophage-derived IL-10 production may be a protective mechanism in hepatic IRI. It has been shown that galectin-1 endows DCs with tolerogenic potential, which can promote IL-10-mediated T cell tolerance and suppress autoimmune neuroinflammation [7]. IL-10 and galectin-3 cooperatively interact to protect cells from IRI [23]. Moreover, galectin-9 and its receptor Tim-3 have also been shown to protect against liver IRI [18,24]. Thus,



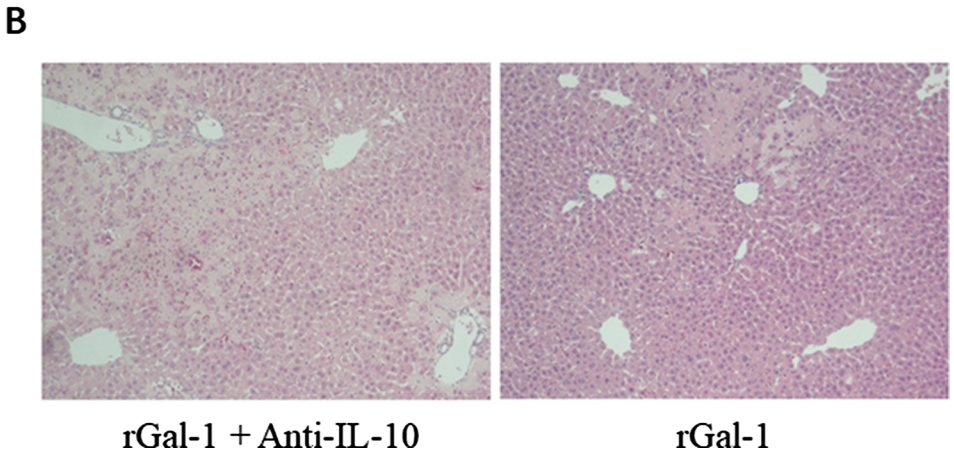


Fig. 5. Blockade of IL-10 in rGal-1-treated mice aggravated hepatic I/R injury. (A) The neutralization of IL-10 aggravated liver injury in rGal-1-pre treated mice, as evidenced by sALT/AST levels (ALT: 6 h: 1936.5 ± 862.4 vs 2912.3 ± 814.2; $p < 0.001$; 24 h: 816.6 ± 176.3 vs 1123.1 ± 246.7; $p < 0.001$; AST: 6 h: 1516.1 ± 493.8 vs 2213.8 ± 564.7; $p < 0.001$; 24 h: 532.5 ± 35.7 vs 951.9 ± 156.4; $p < 0.001$). (B) Representative liver histology (H&E staining; magnification ×100) of liver lobes harvested 6 hours after reperfusion. Data are representative of n = 5.

galectin-glycan interactions may have evolved to regulate antigen-presenting cell homeostasis and influence immune tolerance. However, how Gal-1 modulates IL-10 production in macrophages remains elusive, and further investigations need to illustrate underlying mechanisms.

Although hepatic IRI is an innate immune-dominated tissue inflammation, we and others have demonstrated an important role of activated T cells, particularly of CD4 T cells, in the pathogenesis of IR-triggered liver inflammation. In our previous studies using liver transplantation model, we found that Th1 and Th17 immunity contributes to acute liver allograft rejection [9]. In our current study, we found that hepatic ischemia reperfusion resulted in significant increases in the mRNA expressions of Th1 and Th17-related cytokines (IL-12, IIFN-γ, IL-17), which confirmed the critical role of Th1 and Th17 immunity in mediating hepatic IRI. Moreover, Gal-1 has been linked to tolerance induction and considered as a negative regulator of Th1- and Th17-driven immune responses. Indeed, rGal-1 treatment significantly attenuated gene induction of IL-12, IFN-γ and IL-17. These data suggest that Gal-1 may serve a dual role in the modulation of innate and adaptive immune system. However, it remains unknown how Gal-1 exerts such broad activities with distinctive pro- and anti-inflammatory functions.

In summary, in the current study we provide evidence of a critical role of galectin-1 in attenuating hepatic IRI via an IL-10-dependent mechanism, suggesting a potential approach for therapeutic intervention aimed at limiting liver inflammation in the context of ischemia reperfusion injury.

Author contributions

Y.Y. and W.W. designed and finished experiment in vivo and in vitro. Z.W. and P.Y. provided the mouse hepatic ischemia reperfusion model. L.Y. and Y.S. participated in the immunohistochemistry experiments. G.L. participated in the real-time polymerase chain reaction (PCR) experiments. Z.L. and X.H. participated in the reagents purchase and preparation. L.M. designed experiment in vivo. Y.J. and Z.S. designed experiment in vitro and in vivo.

Declaration of Competing Interest

The authors declare no conflicts of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2019.105997.

References

[1] K. Nakamura, S. Kageyama, B. Ke, T. Fujii, R.A. Sosa, E.F. Reed, N. Datta, A. Zarrinpar, R.W. Busuttil, J.W. Kupiec-Weglinski, Sirtuin 1 attenuates inflammation and hepatocellular damage in liver transplant ischemia/Reperfusion: From mouse to human, Liver Transpl. 23 (2017) 1282–1293.

[2] E.E. Montalvo-Jave, T. Escalante-Tattersfield, J.A. Ortega-Salgado, E. Pina, D.A. Geller, Factors in the pathophysiology of the liver ischemia-reperfusion injury, J. Surg. Res. 147 (2008) 153–159.

[3] J.D. Ellett, C. Atkinson, Z.P. Evans, Z. Amani, E. Balish, M.G. Schmidt, N. van Rooijen, R.G. Schnellmann, K.D. Chavin, Murine Kupffer cells are protective in total hepatic ischemia/reperfusion injury with bowel congestion through IL-10, J. Immunol. 184 (2010) 5849–5858.

[4] Y. Zhai, X.D. Shen, F. Gao, A. Zhao, M.C. Freitas, C. Lassman, A.D. Luster, R.W. Busuttil, J.W. Kupiec-Weglinski, CXCL10 regulates liver innate immune response against ischemia and reperfusion injury, Hepatology 47 (2008) 207–214.

[5] Y. Liu, H. Ji, Y. Zhang, X.D. Shen, F. Gao, T.T. Nguyen, X. Shang, N. Lee, R.W. Busuttil, J.W. Kupiec-Weglinski, Negative CD4 + TIM-3 signaling confers resistance against cold preservation damage in mouse liver transplantation, Am. J. Transplant. 15 (2015) 954–964.

[6] L. Santucci, S. Fiorucci, F. Cammilleri, G. Servillo, B. Federici, A. Morelli, Galectin-1 exerts immunomodulatory and protective effects on concanavalin A-induced hepatitis in mice, Hepatology 31 (2000) 399–406.

[7] J.M. Ilarregui, D.O. Croci, G.A. Bianco, M.A. Toscano, M. Salatino, M.E. Vermeulen, J.R. Geffner, G.A. Rabinovich, Tolerogenic signals delivered by dendritic cells to T cells through a galectin-1-driven immunoregulatory circuit involving interleukin 27 and interleukin 10, Nat. Immunol. 10 (2009) 981–991.

[8] I. Camby, M. Le Mercier, F. Lefranc, R. Kiss, Galectin-1: a small protein with major functions, Glycobiology 16 (2006) 137R–157R.

[9] Y. Ye, S. Yan, G. Jiang, L. Zhou, H. Xie, X. Xie, X. Yu, Y. Ding, J. Tian, Y. Dai, S. Zheng, Galectin-1 prolongs survival of mouse liver allografts from Flt3L-pretreated donors, Am. J. Transplant. 13 (2013) 569–579.

[10] A.J. Iqbal, D. Cooper, A. Vugler, B.R. Gittens, A. Moore, M. Perretti, Endogenous galectin-1 exerts tonic inhibition on experimental arthritis, J. Immunol. 191 (2013) 171–177.

[11] I. Gomez-Tourino, C. Sanchez-Espinel, A. Hernandez-Fernandez, A. Gonzalez-Fernandez, E. Pena-Gonzalez, J. Rodriguez, J.M. Garcia-Lopez, R. Varela-Calvino, Galectin-1 synthesis in type 1 diabetes by different immune cell types: reduced synthesis by monocytes and Th1 cells, Cell. Immunol. 271 (2011) 319–328.

[12] H. Ji, X. Shen, F. Gao, B. Ke, M.C. Freitas, Y. Uchida, R.W. Busuttil, Y. Zhai, J.W. Kupiec-Weglinski, Programmed death-1/B7-H1 negative costimulation protects mouse liver against ischemia and reperfusion injury, Hepatology 52 (2010) 1380–1389.

[13] S. Suzuki, L.H. Toledo-Pereyra, F.J. Rodriguez, D. Cejalvo, Neutrophil infiltration as an important factor in liver ischemia and reperfusion injury. Modulating effects of FK506 and cyclosporine, Transplantation 55 (1993) 1265–1272.

[14] Y. Ye, X. Xie, J. Yu, L. Zhou, H. Xie, G. Jiang, X. Yu, W. Zhang, J. Wu, S. Zheng, Involvement of Th17 and Th1 effector responses in patients with Hepatitis B, J. Clin. Immunol. 30 (2010) 546–555.

[15] X. Xie, Y. Ye, L. Zhou, G. Jiang, H. Xie, X. Feng, Y. He, S. Zheng, Kupffer cells promote acute rejection via induction of Th17 differentiation in rat liver allografts, Transplant. Proc. 42 (2010) 3784–3792.

[16] A. Jiang, H. Okabe, B. Popovic, M.E. Preziosi, T. Pradhan-Sundd, M. Poddar, S. Singh, A. Bell, S.G. England, S. Nagarajan, S.P. Monga, Loss of Wnt secretion by macrophages promotes hepatobiliary injury after administration of 3,5-diethoxycarbonyl-1, 4-dihydrocollidine diet, Am. J. Pathol. 189 (2019) 590–603.

[17] V. Sundblad, L.G. Morosi, J.R. Geffner, G.A. Rabinovich, Galectin-1: a jack-of-all-trades in the resolution of acute and chronic inflammation, J. Immunol. 199 (2017) 3721–3730.

[18] Y. Uchida, B. Ke, M.C. Freitas, H. Yagita, H. Akiba, R.W. Busuttil, N. Najafian, J.W. Kupiec-Weglinski, T-cell immunoglobulin mucin-3 determines severity of liver ischemia/reperfusion injury in mice in a TLR4-dependent manner, Gastroenterology 139 (2010) 2195–2206.

[19] S. Li, M. Hong, H.Y. Tan, N. Wang, Y. Feng, Insights into the role and interdependence of oxidative stress and inflammation in liver diseases, Oxid. Med. Cell Longev. 2016 (2016) 4234061.

[20] A.B. Lentsch, H. Yoshidome, W.G. Cheadle, F.N. Miller, M.J. Edwards, Chemokine involvement in hepatic ischemia/reperfusion injury in mice: roles for macrophage inflammatory protein-2 and KC, Hepatology 27 (1998) 1172–1177.

[21] P. Knolle, J. Schlaak, A. Uhrig, P. Kempf, Meyer zum, K.H. Buschenfelde, G. Gerken, Human Kupffer cells secrete IL-10 in response to lipopolysaccharide (LPS) challenge, J. Hepatol. 22 (1995) 226–229.

[22] S. Dinant, R.L. Vetelainen, S. Florquin, A.K. van Vliet, T.M. van Gulik, IL-10 attenuates hepatic I/R injury and promotes hepatocyte proliferation, J. Surg. Res. 141 (2007) 176–182.

[23] Y.J. Lee, Y.K. Song, Cooperative interaction between interleukin 10 and galectin-3 against liver ischemia-reperfusion injury, Clin. Cancer Res. 8 (2002) 217–220.

[24] H. Hirao, Y. Uchida, K. Kadono, H. Tanaka, T. Niki, A. Yamauchi, K. Hata, T. Watanabe, H. Terajima, S. Uemoto, The protective function of galectin-9 in liver ischemia and reperfusion injury in mice, Liver Transpl. 21 (2015) 969–981.