


eGastroenterology Macrophages clear out necrotic liver lesions: a new magic trick revealed

Robim M Rodrigues , Joost Boeckmans , Tamara Vanhaecke 

To cite: Rodrigues RM, Boeckmans J, Vanhaecke T. Macrophages clear out necrotic liver lesions: a new magic trick revealed. *eGastroenterology* 2023;1:e100024. doi:10.1136/egastro-2023-100024

► Prepublication history for this paper is available online. To view these files, please visit the journal online (<http://dx.doi.org/10.1136/egastro-2023-100024>).

Received 01 September 2023

Accepted 08 September 2023

Liver regeneration is a process that ensures the restoration of liver size and weight on loss of hepatic cells due to acute liver injury, chronic liver disease or partial hepatectomy.¹ The regenerative activity of parenchymal and non-parenchymal cells is essential for the maintenance of the hepatic function and body homeostasis and the mechanisms that drive liver regeneration have been extensively investigated.^{1,2} However, little is known about how dead tissue and necrotic lesions resulting from liver injury are removed during the hepatic healing process. Yet, the natural resolution of these necrotic lesions is essential to prevent further worsening of the hepatic function. In their latest research paper in the *Journal of Clinical Investigation*, Feng *et al* have demonstrated that the repair and removal of necrotic lesions is a well-orchestrated interplay of recruited immune cells, hepatocytes and stellate cells.³ They found that monocyte-derived macrophages (MoMFs) infiltrate the liver and encapsulate the necrotic areas, triggering multiple events leading to their resolution. Although the pro-inflammatory function of macrophages exacerbates liver injury, these cells also play an important role in tissue repair.^{4,5} Using an elegant multiplex immunofluorescent staining technique combined with single cell sequencing, different populations of MoMFs were identified surrounding necrotic areas in the liver of a concanavalin A-induced mouse model of acute hepatitis (figure 1). The repairing role of MoMFs relies on dynamic changes of these cells following a time-dependent sequence of events, starting from early recruitment to the affected areas, which is then followed by specific interactions with different cell types around the necrotic lesions.

The recruitment of MoMFs is mediated by the hepatocytes adjacent to the necrotic zones. These hepatocytes are prompted by danger-associated molecule patterns secreted by the necrotic tissue to express high levels of monocyte chemoattractant protein-1. This potent

chemokine interacts with the C-C chemokine receptor type 2 expressed by monocytes.⁶ The researchers found that infiltrated MoMFs activate the JAG1-NOTCH2 axis, inducing anti-apoptotic, non-proliferative SOX9⁺ hepatocytes. These death-resistant cells form a capsule around the necrotic foci, thereby protecting healthy adjacent cells against further injury, which is then maintained until the necrotic lesion disappears. Furthermore, Feng *et al* discovered a population of MoMFs expressing complement 1q (C1q) that is induced by hypoxia that is associated with necrotic lesions. C1q is known to bind to apoptotic cells, facilitating their phagocytosis by macrophages,⁷ and functions as an initiator of the complement cascade that facilitates the removal of dead cells.⁸ Moreover, C1q⁺-MoMFs express genes coding for lysosomal proteinases, such as cathepsins and legumain that degrade proteins and extracellular matrix. C1q⁺-MoMFs play, therefore, a unique role in the initial clearing of necrotic debris.

At a later stage, a manifest population of MoMFs expressing platelet-derived growth factor subunit B (PDGFB) was also identified. Feng *et al* proved that *Pdgfb*⁺MoMFs are responsible for the activation of hepatic stellate cells (HSCs) with which they are colocalised in the perinecrotic areas. These alpha-smooth muscle actin-positive HSCs (α -SMA⁺HSCs) showed evident contraction clues, which were induced by active peptides cleaved by metalloendopeptidases secreted by MoMFs. As a result, contractile α -SMA⁺HSCs surrounding the necrotic areas squeeze the necrotic nodules promoting their clearance until they are eliminated.

The authors also briefly investigated whether the same interactions between MoMFs, hepatocytes and HSCs occur during the resolution of necrosis in other models of liver injury. They concluded that the necrotic lesions in the *Klebsiella pneumoniae* infection model and in the ischaemia/



© Author(s) (or their employer(s)) 2023. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

In Vitro Toxicology and Dermato-cosmetology (IVTD), Vrije Universiteit Brussel, Brussel, Belgium

Correspondence to

Dr Robim M Rodrigues;
robim.marcelino.rodrigues@vub.be

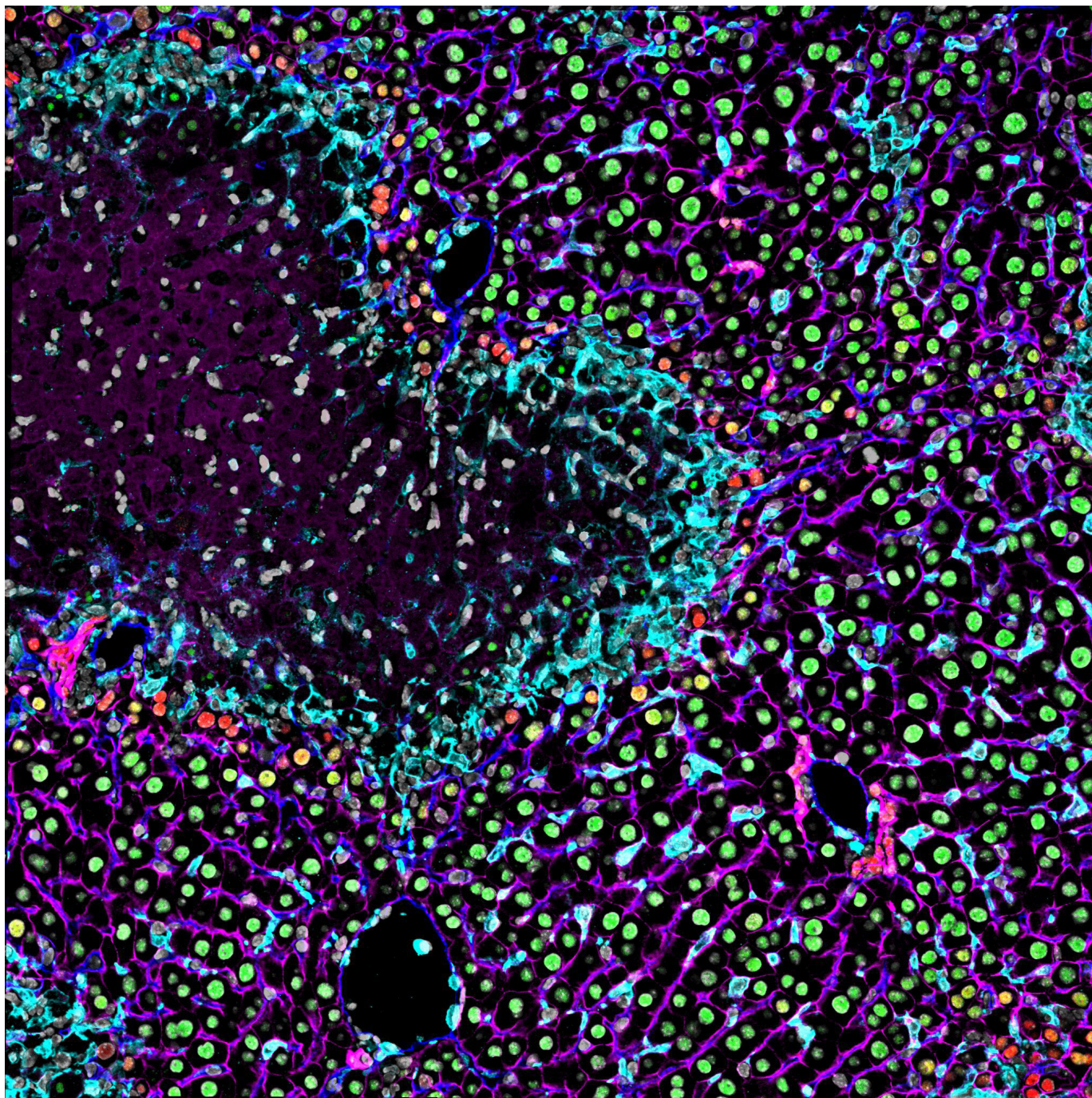


Figure 1 Multiplex immunofluorescence staining of liver tissue 48 hours after concanavalin A-induced liver injury. Monocyte-derived macrophages (MoMFs) (cyan) encapsulate the necrotic areas and promote the expression of SOX9 (red) in hepatocytes (HNF4α; green) adjacent to MoMFs. Endothelial cells (CD31; blue) and the membrane of hepatocytes (β-catenin; magenta) are also shown.

reperfusion model are resolved by similar mechanisms. Yet, no evident aggregation of MoMFs, SOX9⁺hepatocytes and αSMA⁺HSCs was detected surrounding the necrotic lesions induced by carbon tetrachloride or acetaminophen (APAP), suggesting that a different mechanism is in place in those hepatotoxin/drug-induced liver injury models.

The findings of this study could represent an important fundamental for novel macrophage-based immunotherapeutic approaches targeting hepatic necrosis. Autologous

macrophage therapy for liver fibrosis is already being investigated.^{9 10} For example, injected primary human bone marrow-derived macrophages were previously shown to be scavengers of APAP-induced necrotic tissue.⁴ However, the well-orchestrated functions and dynamic phenotypes of circulating macrophages during the resolution of necrosis can now also be explored. This new information should be included in the design and fine tuning of future (pre-)clinical studies. Since necrosis is often observed in various liver diseases with different

aetiologies, macrophage-based therapies have a broad applicability domain.

Nonetheless, it is yet to be investigated if and how the size of the necrotic lesions plays a role in the efficacy of MoMFs-mediated elimination process. The degree of hepatic necrosis depends on the extent of hepatocyte injury as well as the underlying cause, and severe necrosis can even lead to irreversible liver damage.¹¹ Furthermore, it must be noted that the experimental murine models used in this study are still far from the human situation. The extrapolation of these results to human liver injuries, particularly those involving a strong immunological component, remains to be investigated. To this end, the use of human-based in vitro systems might be an interesting alternative approach. For example, human spheroid three-dimensional cell cultures form characteristic natural necrotic cores.¹² Simplified in vitro systems can be developed using co-cultures of multiple relevant human cells that represent the human in vivo situation accurately.¹³ Such in vitro models could be a tool for bridging the gap between animal experimentation and studies including patients suffering from diverse hepatic injuries. Furthermore, necrosis is not a process reserved to the liver alone but is commonly found, after a toxic insult or injury, in other organs as well. Macrophages have also been shown to play a role in the repair of necrotic lesions in other organs.^{14–16} Whether the resolution of necrotic lesions in these cases also occurs by similar MoMFs-mediated mechanisms, is still to be investigated.

Overall, this study smartly reveals the mechanisms behind the ‘disappearing magic trick’ of hepatic necrotic lesions during liver regeneration. It opens new research lines that will have a fundamental impact on therapeutic developments for the resolution of necrosis, and potentially also their prevention.

Acknowledgements The authors thank Dr Dechun Feng for providing an immunofluorescent staining image of necrotic liver lesion repair.

Contributors RMR was responsible for conceiving, writing and revising the final manuscript. JB and TV contributed to the manuscript writing and revision.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests RMR is an *eGastroenterology* Editorial Board Member.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval Not applicable.

Provenance and peer review Commissioned; internally peer reviewed.

Data availability statement No data are available.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

ORCID iDs

Robim M Rodrigues <http://orcid.org/0000-0003-2927-6791>

Joost Boeckmans <http://orcid.org/0000-0001-8399-5872>

Tamara Vanhaecke <http://orcid.org/0000-0002-6685-7299>

REFERENCES

- 1 Michalopoulos GK, Bhushan B. Liver regeneration: biological and pathological mechanisms and implications. *Nat Rev Gastroenterol Hepatol* 2021;18:40–55.
- 2 Ait Ahmed Y, Fu Y, Rodrigues RM, et al. Kupffer cell restoration after partial hepatectomy is mainly driven by local cell proliferation in IL-6-dependent autocrine and paracrine manners. *Cell Mol Immunol* 2021;18:2165–76.
- 3 Feng D, Xiang X, Guan Y, et al. Monocyte-derived macrophages orchestrate multiple cell-type interactions to repair necrotic liver lesions in disease models. *J Clin Invest* 2023;133:e166954.
- 4 Starkey Lewis P, Campana L, Aleksieva N, et al. Alternatively activated macrophages promote resolution of necrosis following acute liver injury. *J Hepatol* 2020;73:349–60.
- 5 Mossanen JC, Krenkel O, Ergen C, et al. Chemokine (C-C motif) receptor 2-positive monocytes aggravate the early phase of acetaminophen-induced acute liver injury. *Hepatology* 2016;64:1667–82.
- 6 Marra F, Tacke F. Roles for chemokines in liver disease. *Gastroenterology* 2014;147:577–94.
- 7 Galvan MD, Greenlee-Wacker MC, Bohson SS. C1Q and phagocytosis: the perfect complement to a good meal. *J Leukoc Biol* 2012;92:489–97.
- 8 Martin M, Blom AM. Complement in removal of the dead - balancing inflammation. *Immunol Rev* 2016;274:218–32.
- 9 Starkey Lewis PJ, Moroni F, Forbes SJ. Macrophages as a cell-based therapy for liver disease. *Semin Liver Dis* 2019;39:442–51.
- 10 Moroni F, Dwyer BJ, Graham C, et al. Safety profile of autologous macrophage therapy for liver cirrhosis. *Nat Med* 2019;25:1560–5.
- 11 Dezső K, Nagy P, Paku S. Human liver regeneration following massive hepatic necrosis: two distinct patterns. *J Gastroenterol Hepatol* 2020;35:124–34.
- 12 Cox CR, Lynch S, Goldring C, et al. Current perspective: 3D spheroid models utilizing human-based cells for investigating metabolism-dependent drug-induced liver injury. *Front Med Technol* 2020;2:611913.
- 13 Xu Q. Human three-dimensional hepatic models: cell type variety and corresponding applications. *Front Bioeng Biotechnol* 2021;9:730008.
- 14 Nishida M, Okumura Y, Fujimoto S-I, et al. Adoptive transfer of macrophages ameliorates renal fibrosis in mice. *Biochem Biophys Res Commun* 2005;332:11–6.
- 15 Zhang J, Muri J, Fitzgerald G, et al. Endothelial lactate controls muscle regeneration from ischemia by inducing M2-like macrophage polarization. *Cell Metabolism* 2020;31:1136–1153.
- 16 Rayees S, Rochford I, Joshi JC, et al. Macrophage TLR4 and PAR2 signaling: role in regulating vascular inflammatory injury and repair. *Front Immunol* 2020;11:2091.