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

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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. 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. 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. 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. 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. 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, and functions as an initiator of the complement cascade that facilitates the removal of dead cells. 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 pericentral 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/
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

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.
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. Furthermore, it must be noted that the experimental murine models used in this study are still a 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. Simplified in vitro systems can be developed using cocultures of multiple relevant human cells that represent the human in vivo situation accurately. 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. 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.

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