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Orchestrating liver repair – a newly discovered function of hepatic iNKT cells

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A recent elegant study entitled “iNKT cells Orchestrate a Switch from Inflammation to Resolution of Sterile Liver Injury” (1) uncovered a novel role of hepatic invariant nature killer T (iNKT) cells in orchestrating the liver repair process after sterile injury. The iNKT cells respond to injury in three phases: the initial repulsion from the injury site, subsequent retention in proximity to the injury, and final re-entry into the injury site. This study demonstrates that iNKT cells are activated by endogenous antigens and cytokines to release IL-4, which plays an essential role in resolving inflammation and promoting tissue repair.

Injury in the absence of infectious microorganisms (termed “sterile injury”) can occur in all tissues in response to a variety of stimuli, such as ischemia-reperfusion, trauma, drug or chemical insult. Similar to infection, sterile tissue injury also results in an inflammatory response with the release of inflammatory mediators and the recruitment of innate immune cells such as neutrophils and monocytes. To better understand the critical role of this inflammatory response in healthy wound healing after sterile injury, a number of studies from the Kubes' laboratory combined the employment of spinning-disk fluorescent confocal intravital microscopy (SD-IVM) and fluorescent reporter mice. These are powerful approaches in revealing the temporal and spatial behavior of multiple cell types simultaneously and visualizing cell-cell interactions in a real-time fashion (1-4). The studies have provided valuable insights into the involvement of innate immune cells and their products in healthy repair after sterile injury of the liver. It is reported that neutrophils are the first to arrive at the site of focal thermal injury of the liver parenchyma (as early as 30 min) and they persist for up to 24h (2). The neutrophils play an important role in removing cellular debris and preventing opportunistic infections before undergoing apoptosis (2, 5). Immediately following neutrophils, mature peritoneal macrophages enter the injury site via a nonvascular route within an hour (Fig. 1). These macrophages dismantle the nuclei of necrotic cells and contribute to revascularization and tissue repair (4). At later time points (8-12h), circulating monocytes are recruited. The cells that initially infiltrate the liver are pro-inflammatory CCR2^{hi}CX3CR1^{low}Ly6C^{hi} monocytes, which form a ring-like structure circumscribing the injured area (3). Over 48h, the pro-inflammatory monocytes transition to

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tissue reparative CCR2^{low}CX3CR1^{hi}Ly6C^{low} monocytes, which then enter the injury site and contribute to tissue repair and remodeling (3). It is clear that the switch of pro-inflammatory monocytes to tissue reparative monocytes occurs locally, but it remains unknown which cells and molecules cause the switch.

The most recent study by Liew *et al* reports that it is the hepatic iNKT cells that orchestrate the monocytes transition from a pro-inflammatory to a pro-reparative phenotype, thereby playing a critical role in healthy repair. iNKT cells are a unique subset of T lymphocytes that express NK cell surface markers as well as a T cell receptor (TCR) containing an invariant chain (V α 14-J α 18 in mice and V α 24-J α 18 in humans). Through this TCR, iNKT cells recognize endogenous and exogenous glycolipid antigens presented by a major histocompatibility complex class I-like molecule, CD1d. In the study by Liew *et al*, iNKT cells were visualized by using a reporter mouse expressing enhanced green fluorescent protein under the control of the CXCR6 promoter (Cxc6^{GFP/+}). Although other cells, such as NK and T cells, also express CXCR6 and are GFP⁺ cells, the authors conducted additional experiments using CD1d tetramers to confirm that the majority of GFP⁺ cells accumulated in the injured site are iNKT cells. The first unexpected and unique behavior of iNKT cells observed was that during the early phase of post-injury (<8h), these cells were repelled from the injury site (Fig. 1). They not only did not enter the injury site but rather made a “U-turn”, perhaps owing to the absence of chemoattractants and their lack of proteases to burrow into injured tissue. This behavior was not affected by blockade of the receptor for class 3 semaphorins or perfusion of the injured site with CXCL9. Depletion of neutrophils, platelets or Kupffer cells did not reduce the number of “U-turns”. The mechanism triggering the repulsion of iNKT cells from the injured site remains a mystery. At 8-24h post-injury, the “U-turns” stopped and iNKT cells were retained abutting the lesion boundary (Fig. 1). This arrest of iNKT cells initially required CD1d-dependent self-antigen presentation by Kupffer cells, liver sinusoidal endothelial cells, and perhaps other CD1d-expressing cells in the liver. Subsequently, the combination of IL-12 and IL-18 played an important role in the retention of iNKT cells. Interestingly, the timing and location of iNKT cell arrest coincided with the transition of the pro-inflammatory monocytes to reparative monocytes. Indeed, the data demonstrated that the arrested iNKT cells produced IL-4, which triggers alternative activation of monocytes/macrophages (6) and has been reported to promote the monocytes switching from a pro-inflammatory to a regulatory phenotype (3, 7). The critical role of iNKT cells in promoting hepatocyte proliferation, monocytes transition, and overall wound repair was further confirmed by using CD1d^{-/-} and J α 18^{-/-} mice, which are deficient in iNKT cells.

Liew *et al* uncovered a fascinating and previously unrecognized function of iNKT cells in orchestrating healthy wound repair. This study filled a knowledge gap in the mechanisms accounting for the switch of monocytes from an inflammatory to a reparative phenotype, which is critical in resolving inflammation and promoting tissue repair. As any pioneering finding, this work raises a number of new questions worthwhile exploring. For example, what are the iNKT-derived mediators that impact the monocyte phenotype transition (in addition to IL-4)? Do monocytes reciprocally affect iNKT cell activation and function? What is the nature of the self-antigen(s) that triggers iNKT cell activation during sterile injury? Why is the self-antigen only induce IL-4, but not IFN- γ , production by iNKT cells?

This unique function of hepatic iNKT cells was discovered in a model of focal and mild liver injury, which may involve different mechanisms of repair (“healthy healing”) compared with other injury models. Will the functional involvement of iNKT cells be different or even opposite during more extensive injury? As an example, a pathological role of iNKT cells has been demonstrated in CCl₄- and methionine-choline-deficient diet-induced liver fibrosis (8). Moreover, is there another cell type that exerts similar functions in other tissues where iNKT cells are not as abundant as in the liver? Finally, few NKT cells are found in human livers, which instead contain mucosal associated invariant T (MAIT) cells, a potential human counterpart of mouse NKT cells. Thus, an important question for future research is how the knowledge gained on mouse NKT cells be extended to human pathophysiological conditions.

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Fig.1. The kinetics and involvement of various innate immune cells during sterile liver injury and repair

Neutrophils are the first to arrive at the injury site (as early as 30 min). Immediately following neutrophils, mature peritoneal macrophages enter the injury site within an hour. During the first 8h after injury, hepatic iNKT cells are repelled from the injury site. Within 8-48h post-injury, iNKT cells are arrested in close proximity to the lesion site, where they are activated by self-antigens and IL-12/IL-18 to produce IL-4. During this time, circulating monocytes are recruited to the same location, and transition from pro-inflammatory $CCR2^{hi}CX3CR1^{low}Ly6C^{hi}$ monocytes to reparative $CCR2^{low}CX3CR1^{hi}Ly6C^{low}$ monocytes. After 48h, the reparative monocytes and iNKT cells enter the injury site and contribute to tissue repair and remodeling.