REVIEW

Innate immune cells orchestrate the repair of sterile injury in the liver and beyond

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There is a close association between inflammation and sterile injury, however not all sterile injuries are the same. While a regulated inflammatory response is crucial for proper healing, a dysregulated or nonterminating response leads to disrepair. While immune cells are thought to contribute to the disrepair, they may also be critical for proper healing and as such, their actions may dictate the end result. In all forms of sterile injury, release of damage-associated molecular patterns from necrotic cells causes robust recruitment of innate immune cells. The subsequent release of toxic mediators from immune cells is thought to be damaging in non-resolving sterile injuries in which the dysregulated immune response leads to chronic inflammatory disease. While similar mediators may be released from immune cells in resolution of acute injury, the spatial localization, timing, and self-termination may all be critical. In this review, we summarize the recent advances in our understanding of the temporal and spatial recruitment of various innate immune cells that beget appropriate healing of acute injuries. Where possible we try to compare this appropriate response to dysregulated sterile injuries in an attempt to identify novel therapeutic targets.

Keywords: iNKT cells · macrophages · monocytes · neutrophils · sterile injury

Introduction

Innate immune cells are the first line of defense against infection [1]. Once deployed, they try to clear or contain the invaders. Innate immune cells including neutrophils, monocytes, and macrophage release highly toxic chemicals such as oxidants and proteases to kill pathogens. These same immune cell-derived molecules, however, can cause bystander tissue injury [2]. As such, these immune cells and their arsenal of toxic molecules have been implicated in causing injury in sites of sterile inflammation. The evidence for this contention is based on the fact that (1) there is a robust recruitment of innate immune cells to an afflicted site [3], (2) these cells become activated and have the capacity to release toxic molecules, and (3) preventing recruitment or inhibiting these toxic molecules reduces inappropriate inflammation. However, the aforementioned results are mostly derived from animal models that try to emulate human diseases that are iatrogenic in nature being caused by toxins, drugs, or bad diets, or that mimic surgical interventions such as ischemia/reperfusion for which a well-evolved immune response is lacking. By contrast, traumatic injury may have a well-evolved immune response and as such, understanding a proper immune response to injury may help elucidate where responses are dysregulated in inappropriate...
or chronic inflammation. In this review, we will first focus on a model of injury and proper repair and then highlight what happens in disrepair.

**Immune cell recruitment**

Sterile injury causes necrosis of host cells and release of intracellular contents. Many of these molecules function as damage-associated molecular patterns (DAMPs), recruiting immune cells such as neutrophils in the same manner as PAMPs from microbes [2, 3]. In fact, in some cases, DAMPs and PAMPs may be closely associated, identical or at the very least use the same innate immune receptors. As such, it remains unclear how immune cells such as neutrophils distinguish between sterile and microbial afflications, however their behavior appears to be vastly different in these two different environments [4, 5]. Aside from neutrophils, there is a growing list of different innate immune cells that get recruited to the site of sterile inflammation and our understanding of their role in different models of sterile injury is still often unclear [4, 6–8]. Moreover, the intercommunication between these cells to ensure coordination of repair also remains unelucidated.

The inflammatory response early in injury is important for proper healing. This is manifested as the clearance of dead cells and debris from the injured tissue by neutrophils and perhaps other immune cells. As such, specific immune cells must be able to detect some of the first signals released from a site of injury. This process of leukocyte recruitment out of the vasculature to the site of infection or injury was documented more than a century ago [1] and is now quite well defined [9]. Briefly, neutrophils, eosinophils and monocytes are recruited to a site of inflammation because of the increased expression of various adhesion molecules and chemokines on the surface of endothelial cells lining the vasculature. While injury to the cremaster muscle, skin, brain, and intestine causes rapid upregulation of selectins that induce neutrophil rolling along the vessel wall bringing neutrophils in close contact with expressed chemoattractants and activating molecules, tissues such as liver and lung may be somewhat different. Since much of the recruitment occurs inside the capillaries of the lung and sinusoidal capillaries of the liver, the rolling step is bypassed and cells tend to adhere. Chemokines such as CXCL1 and CXCL2 that are expressed on the surface of endothelium activate integrins including CD11/CD18 and CD49d for firm adhesion. Neutrophils tend to use the former while eosinophils rely more on the latter with monocytes using both integrins [10–12].

Necrotic cells at the site of injury release DAMPs that are intracellular molecules not normally seen by immune cells. This would include molecules such as ATP, HMGB1, DNA, and formylated peptides produced by mitochondria (ancient bacteria) [3]. Release of these molecules in extracellular environment activates innate immune cells. Due to its short half-life, extracellular ATP can exert its effect only in the vicinity of the cells under stress. Extracellular ATP activates the NLRP3 inflammasome in innate immune cells as well as in epithelial cells by acting on P2X receptors [13]. Inflammasome activation leads to maturation and secretion of IL-1β that leads to production of pro-inflammatory molecules and activation of distant immune cells. Extracellular ATP also activates P2Y receptors with potentially two distinct consequences: (a) clearing apoptotic cells by providing a “find me” signal to phagocytic cells and (b) leading to an allergic reaction [13]. Necrotic cells are also known to release IL-1α into the extracellular environment, which activates innate immune cells and parenchymal cells to release pro-inflammatory molecules [14]. HMGB1 is a DNA chaperone that is passively released by necrotic cells into the extracellular milieu. It can also be secreted into the extracellular environment by cells under tremendous stress. Once in the extracellular milieu HMGB1 acts as a danger signal, activates, and mobilizes immune cells by acting on CXCR4, receptor for advanced glycation end products (RAGE), and toll-like receptors (TLR) [13]. Most of these DAMPs are themselves incapable of functioning as chemoattractants. However, mitochondrial formylated peptides were shown to be important in recruiting neutrophils in models of sterile injury where massive necrotic cell death resulted in the release of large quantity of mitochondrial peptides in the systemic circulation [15, 16]. Mitochondrial formylated peptides activate the formylated peptide receptors (FPFRs) on neutrophils. FPFRs are important in immune cell recruitment in self-resolving as well as dysregulated chronic sterile injuries. In addition to formylated peptides, mitochondrial DNA and RNA can also lead to a profound inflammatory reaction by activating the endosomal TLRs [17]. Mitochondrial nucleic acids are very similar to that of bacteria so endosomal TLRs respond similarly to both activators. It is doubtful that formylated peptides or any single chemoattractant can set up long gradients in sterile injuries and as such, multiple gradients of chemoattractants are usually formed. Indeed, in a model of liver injury, where a small thermally induced wound obliterated all cells in a 200 μm² area, a gradient of chemokines including CXCL1 and CXCL2 were produced by the endothelium and local tissue macrophage (Kupffer cells) [6]. The chemokines were expressed on the blood vessel lumen allowing neutrophils to adhere as much as 600 μm away from the thermal injury and then follow the chemokine gradient to within 100 μm of the wound. Close to the injury border (within 100 μm), endothelial cells lose their glycosaminoglycans (GAGs) that are known to present chemokines and as such, the gradient was not present proximal to the injury. However, platelets bound to these injured sinusoids and provided a platform for neutrophils to migrate on. At this stage formylated peptide receptors were key to recruiting neutrophils into the injury site.

Similar injury in the skin also induces a similar pattern of neutrophil recruitment [6], however, injury to a different tissue modifies what chemoattractants are released. In a very tiny laser induced injury in the skin where very few if any cells were killed, the initial response of neighboring neutrophils was supposedly mediated by DAMPs, however, the swarming of distant neutrophils did not rely on these molecules. Rather, it was dependent on neutrophil relay chemoattractant LTB4 [18]. This class of molecules is thought to be produced by the migrating neutrophils to recruit more neutrophils. What the first neutrophils were
following was not clear but in this type of injury it appears that some of the early responding neutrophils died and “exploded” recruiting the swarm of additional neutrophils. Similar mechanisms of neutrophil recruitment have also been seen in other tissues such as lymph nodes [18]. LTB4 released by early responding neutrophils was important not only for swarming of neutrophils but also for neutrophils to enter into the core of such injuries. Whereas high affinity integrins and G-protein coupled receptors (GPCRs) were required for infiltrating into the core of the injury but not for neutrophil swarming [18]. As such, it is clear that neutrophils arrive early at the site of injury, but their exact role has only recently been elucidated.

\begin{table}
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\textbf{Innate immune cell types} & \textbf{Healing mechanism} \\
\hline
Neutrophils & Phagocytosis of cellular debris [22, 26], angiogenesis [31, 32, 42, 43], phenotypic switching of macrophages [23, 49], regeneration of the injured parenchyma [25, 44, 45], mucosal regeneration [47], synthesis of extracellular matrix [48], and reverse transmigration out of the injury site [26]. \\
Monocytes & Phagocytosis of cellular debris [7], digestion of extracellular matrix [71], differentiation into pro-resolving monocytes and macrophages [7, 75, 91]. \\
iNKT cells & Conversion of inflammatory monocyte into reparative monocytes by secreting IL-4 [8], hepatocyte regeneration [8]. \\
Tissue resident macrophages & Secretion of inflammatory cytokines to recruit other immune cells [6], phagocytosis of dead cells [4, 23]. \\
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Neutrophils initiate healing

By having the most potent and largest abundance of toxic molecules, designed to kill pathogens, the neutrophil has been under most scrutiny as the potential villain in sterile injury [19]. However, numerous reports are now suggesting that the neutrophil may have a beneficial critical role in the reparative phase of injury [20–26]. In fact a recent report has implicated the neutrophil in first removing dead vasculature and creating tunnels for new vasculature and then removing the remaining debris from injured tissue (Table 1) [26]. Since neutrophils can secrete metalloproteases to activate VEGF and can release other proteases to help degrade injured cells and matrix, they are likely critical in healing [27]. Neutrophil-derived heparanase can also potentially release VEGF by degrading heparan sulphate moieties of the extracellular matrix [28]. In the laser-induced skin injury model of Germain and colleagues, the neutrophils appeared to displace the collagen matrix whereas in the focal thermal liver injury model of Phillipson and colleagues identified a subset of neutrophils that selectively gets recruited to newly transplanted pancreatic islets. These neutrophils, which they termed pro-angiogenic neutrophils, express copious amount of MMP9 and CXCR4 and are required for initiating angiogenesis to support the transplants. These pro-angiogenic neutrophils are attracted to the site by VEGF secreted from the hypoxic transplants [37]. Whether this is a circulating subset of neutrophils or neutrophils that adapt to the environment to become pro-angiogenic remains to be fully elucidated.

Neutrophils themselves can also be a source of VEGF [27, 32, 38]. Indeed, in a corneal injury model, neutrophils were shown to directly produce VEGF. Estrogen-dependent endometrial angiogenesis during the estrous cycle in humans is always
associated with VEGF

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Neutrophils have also been reported to promote mucosal regeneration, neutrophil-derived antimicrobial peptides that are commonly known as cathelicidins (cathelin-related antimicrobial peptide (CRAMP) in mice and LL-37 in humans) were shown to be critical for wound revascularization [42]. Cathelicidins deposit on the site of endothelial injury and act on endothelial FPR to induce permeability and neovascularization. To initiate neovascularization, neutrophil-derived cathelicidins recruit endothelial progenitor cells, promote their survival, and induce secretion of growth factors such as EGF and VEGF by the progenitor cells [42, 43].

There is a growing body of evidence that neutrophils can also help in healing parenchymal cells. In response to acid- or LPS-induced lung injury, neutrophils help the regeneration of lung epithelial cells and pneumocytes (Table 1). This appears to be dependent upon neutrophil elastase-mediated cleavage of E-cadherin and activation of β-catenin, MMPs, and FGF1 pathways [44, 45]. All of these pathways have been implicated in efficient regeneration of alveolar epithelium. Neutrophils can also secrete neutrophilic factors such as oncomodulin to promote optic nerve regeneration after ocular injury [25]. In a spinal cord injury model, neutrophil depletion adversely affected astrocyte reactivity as well as diminished preservation of white matter and axons leading to poor functional recovery of hind limb mobility [46]. Neutrophils have also been reported to promote mucosal regeneration [47]. Whether these are direct effects of neutrophils on parenchymal cells or a result of neutrophils allowing for extracellular matrix reconstruction that permits parenchymal cell regrowth remains to be determined. Human neutrophils were reported to synthesize fibronectin extracellular matrix, which permitted infiltrated stromal cell-mediated bone synthesis [48].

In addition to promoting tissue regeneration, neutrophils have been shown to release mediators that convert myeloid cells to an anti-inflammatory or pro-resolving phenotype (Table 1) [49]. For example, neutrophils cause phenotypic switching of macrophage to promote more efficient phagocytosis of dead cells and debris in injured tissue. To this end, in a model of myocardial infarction, neutrophils were shown to release neutrophil gelatinase-associated lipocalin, a factor that polarizes cardiac macrophages to a MER tyrosine kinase (MERTK) high phenotype necessary for very efficient clearing of apoptotic cardiomyocytes. MERTK is a member of the Tyro3, Axl, and Mer receptor tyrosine kinases (TAM RTKs) family, which is crucial for macrophages to phagocytose apoptotic cells and resolve inflammation (recently reviewed in [50]). Inefficient polarization of the macrophage in neutrophil-depleted animals was associated with poor healing of the infarct [23].

Regardless of the exact mechanism by which the neutrophils impose their effects, they must eventually give way to other cells to complete the healing process. As such, in a proper healing response the tissue becomes devoid of neutrophils after a relatively short period of time (24–48 h). The prevailing view regarding neutrophil clearance from wounds is that neutrophils undergo apoptosis within the injury site. Macrophages and monocytes phagocytose neutrophils within the wound to minimize damage that could potentially be induced by the release of damaging molecules from dying neutrophils [51]. While this view is widely held, the evidence is not so compelling. Monocytes and macrophage are rarely found filled with many neutrophils. It may however be that dying neutrophils make apoptotic bodies that are then cleared. However, the timing of these events is not entirely aligned with this view [6, 7, 52]. In a number of models of tissue injury, neutrophils seem to disappear prior to monocyte and macrophage infiltration [26, 53]. In addition, removal of monocytes and/or macrophage did not change the rate of neutrophil disappearance from a site of sterile injury [26]. While there is significant evidence that neutrophils die after phagocytosing bacteria [54–57], it seems this paradigm has simply been extended to sterile injury.

This view of neutrophils dying and being eaten by other cells has recently been challenged by numerous studies. In larval zebrafish, sterile injury to the fin elicited a rapid neutrophil recruitment response. However, many of these immune cells migrated back to the vessels and re-entered the vasculature. This process was termed reverse transmigration [26, 53] and helped heal the injury (Table 1) [58]. In a subsequent study, some of the molecular mechanisms were described including the fact that while CXCR1 was necessary to recruit neutrophils to the site of injury, CXCR2 and its ligand Cxcl8a induced random migration that resulted in reverse transmigration. Studies have been extended to mammalian systems. Woodfin and colleagues reported that neutrophils can enter the subjacent space in inflamed cremaster muscle but then return into the vasculature [59]. In the liver thermal injury model, reverse transmigration was observed after neutrophils excavated the damaged area and helped sculpt tunnels/sleeves for new vasculature. Neutrophils migrated back into the circulation at about 16 h post entry (Fig. 1). This process was impaired in cathepsin C knockout animals. The cathepsin C deficient mice are unable to activate numerous proteases including elastase. This observation was similar to the work of Colom et al., who reported that neutrophil elastase was critical for reverse transmigration as a result of proteolytic cleavage of endothelial cell junctional adhesion molecule-C (JAM-C) [60]. JAM-C is an important regulator of polarized neutrophil migration across the endothelial barrier. Absence or reduction of JAM-C causes disruption of this polarized behavior of neutrophils. This disruption was key to reverse transendothelial migration of neutrophils in a model of ischemia-reperfusion injury of the cremaster muscle where neutrophils came back into the lumen from the abluminal area [59]. However, reverse transmigration of neutrophils that have already chemotaxed into the site of injury back into the circulation may require cleavage of endothelial junctional

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molecules on their way back. So, proteases such as neutrophil elastase likely play an important role in neutrophil reverse transmigration in the model of sterile liver injury [26]. Further mechanistic studies will clarify whether only neutrophil elastase and JAM-C or any other regulators such as changes in chemokine or cytokine gradient and activation of specific signaling molecules are also important for this interesting neutrophil behavior. Inhibition of exit or entry of neutrophils both resulted in impaired healing suggesting that the presence of neutrophils was necessary for healing but their exit was equally important. By 24 h post injury, only a few neutrophils remained in the injury foci.

Since venous blood flows back to the heart and lungs, it is not surprising that both the Nourshargh and Kubes groups found that neutrophils returned to the pulmonary vasculature. Woodfin et al. used increased ICAM-1 on the surface of the emigrated neutrophils as a signature to track the cells to the lungs. Wang et al. used a photo-activation strategy to track neutrophils that had been to the liver parenchyma and had then migrated to the lung. It was possible to observe the behavior of these cells and they were seen to firmly adhere in the pulmonary capillaries for extended periods. While Woodfin et al. suggested that the neutrophils were leaving cremaster inappropriately and entering the lung where they caused injury [59], Wang et al. concluded that the reverse transmigration and sojourn back to the lungs was a natural path for these cells. In fact, this group showed that while in the lung, the neutrophils upregulated CXCR4, the homing receptor for the bone marrow, where they then migrated. They argued that the lung may function as a critical organ de-priming the neutrophils and instructing them to return to their site of origin. While much work is necessary to fully elucidate why the neutrophils go to the lungs, both groups may be correct. Indeed, neutrophils during large traumatic insults may go to tissues to help repair but due to the size of the injury, may then migrate to the lung to de-prime and inadvertently injure the lung a condition known as adult respiratory distress syndrome [61, 62]. Although how the neutrophils are induced to stop in the lung vasculature remains unknown, upregulation of specific adhesion molecules may be one possibility. The other view is that these activated neutrophils are more rigid and as such, get stuck in the lung [63, 64]. While the latter view makes sense since the neutrophils are thought to be larger than the diameter of some pulmonary capillaries, heart capillaries are also narrow, and the neutrophils should have also gotten stuck in the capillaries of the heart for which there is little evidence. Recently, Yipp and colleagues demonstrated that aged neutrophils may interact with B cells in the lung. During this interaction, B cells mark neutrophils to undergo apoptosis and subsequent clearance by macrophages [65]. Clearly there is something unique about the pulmonary circulation that may function as both a filter for and instructing them to return to their site of origin. While much work is necessary to fully elucidate why the neutrophils go to the lungs, both groups may be correct. Indeed, neutrophils during large traumatic insults may go to tissues to help repair but due to the size of the injury, may then migrate to the lung to de-prime and inadvertently injure the lung a condition known as adult respiratory distress syndrome [61, 62]. Although how the neutrophils are induced to stop in the lung vasculature remains unknown, upregulation of specific adhesion molecules may be one possibility. The other view is that these activated neutrophils are more rigid and as such, get stuck in the lung [63, 64]. While the latter view makes sense since the neutrophils are thought to be larger than the diameter of some pulmonary capillaries, heart capillaries are also narrow, and the neutrophils should have also gotten stuck in the capillaries of the heart for which there is little evidence. Recently, Yipp and colleagues demonstrated that aged neutrophils may interact with B cells in the lung. During this interaction, B cells mark neutrophils to undergo apoptosis and subsequent clearance by macrophages [65]. Clearly there is something unique about the pulmonary circulation that may function as both a filter for activated neutrophils and an instructive organ to these same cells.

While neutrophils are highly plastic cells, there is a growing body of evidence that suggests that there are different subsets of neutrophils. Neutrophil subsets have mostly been investigated in the context of cancer. Based on their antitumor or tumor promoting functions, neutrophils were classified as N1 and N2, respectively, much like M1 and M2 tumor-associated macrophages. They are also classified based on their buoyancy, maturity, localization, and so on. Apart from their role in cancer, a subset of proangiogenic neutrophil was also described in humans and mice. Studies on neutrophil subsets have been extended to infection, ischemia-reperfusion, and other inflammatory diseases. However, it is not very clear whether all of these subsets are just differentially polarized forms of neutrophils due to the local environment or transcriptionally regulated subsets (reviewed in [66]). It would be interesting to study whether different subsets of neutrophils get recruited in self-resolving versus non-resolving injuries or whether these injuries differentially polarize neutrophils into different...
Role of monocytes in sterile injury

In mice, monocytes are classified into two distinct subsets: Ly6C\textsuperscript{hi} proinflammatory and Ly6C\textsuperscript{lo} patrolling or reparative monocytes. Ly6C\textsuperscript{hi} monocytes express high levels of C-C motif chemokine receptor 2 and low levels of C-X-C motif chemokine receptor 1 (CCR2\textsuperscript{hi}, CX3CR1\textsuperscript{lo}) whereas Ly6C\textsuperscript{lo} patrolling monocytes express low level of C-C motif chemokine receptor 2 and high level of C-X-C motif chemokine receptor 1 (CCR2\textsuperscript{lo}, CX3CR1\textsuperscript{hi}) [68]. In humans, there appear to be three distinct monocyte populations, namely classical (CD14++/CD16–), intermediate (CD14++, CD16+), and nonclassical (CD14+, CD16–) monocytes [69]. During sterile injury, there is no question that monocytes are recruited; however, their exact role remains somewhat unclear. Numerous studies have reported this monocytic influx into the injury, but only a couple of studies have actually tracked these cells. Interestingly, two groups showed that monocytes are recruited to the periphery of the injury and encircle the damaged area [7, 18]. However, their exact role was unclear. Recent work using lineage tracing studies demonstrated that the inflammatory CCR2\textsuperscript{hi} monocytes slowly morphed into CX3CR1\textsuperscript{lo} monocytes and subsequently to monocyte-derived macrophages [7, 70–72]. Delaying this switch by inhibiting the cytokines that contributed to the metamorphosis of these CX3CR1 cells delayed healing [7]. Orphan nuclear receptor Nuclear Receptor Subfamily 4 Group A Member 1 (Nr4a1) was shown to be responsible for monocyte phenotype transition and survival of the patrolling monocytes [73, 74] and Nr4a1−/− mice, which lacked the capacity for monocyte switching had a delayed healing phenotype [75]. In a recent study, carbon tetrachloride-induced sterile liver injury models, there is some contradiction about the role of these recruited monocytes. Some literature suggested that recruitment of inflammatory monocytes aggravated the early phase of liver injury [76–78] while some other groups observed no difference in liver damage in the presence or absence of monocytes [79–82]. Using a dual reporter where CCR2-RFP and CX3CR1-GFP were present, Dal-Secco at al. showed that inflammatory monocytes were recruited and surrounded the afflicted tissue within 8 h and formed a pronounced ring around the injury by 24-h post injury. Recruited inflammatory monocytes start transitioning into Ly6C\textsuperscript{lo} and CX3CR1-GFP high monocytes at 48- and 72-h post injury (Fig. 1). This transition was mediated by IL-4 and IL-10, since tandem inhibition of IL-4 and IL-10 dramatically reduced the monocyte switch and subsequent healing. Blocking of inflammatory monocyte recruitment in CCR2−/− mice also delayed the clearance of debris from the injury site and healing of the injury (Table 1) [7]. It remains unclear whether CCR2 was only critical for release of monocytes from the bone marrow or also recruitment to the injury site. In the liver, the CCR2−/− mice had a complete reduction in monocyte recruitment [7] whereas in the intestine only 50% reduction was observed ([83], unpublished observations by Honda and Kubes). The authors argued that the microbiota was also important in recruiting monocytes in the gut. It is worth mentioning that in the kidney vasculature, painting the endothelium with a TLR7 ligand resulted in recruitment of patrolling monocytes that appeared to help replace the endothelium [84] and this occurred independent of CCR2 as the CX3CR1 monocytes were already patrolling the resident vasculature.

Role of iNKT cells as the switch from inflammation to resolution

Understanding the switch from inflammation to resolution could be extremely important therapeutically to interrupt an ongoing chronic inflammatory response. It is well appreciated that during infection IFN-γ is produced to help eradicate pathogens whereas resolution is highlighted by cytokines such as IL-4 and IL-10. As such, much attention was paid to identifying cells that could produce ample IL-4 and IL-10. These cells would have the property of being able to detect sterile injury. Invariant natural killer T (iNKT) cells are a unique T-lymphocyte population that has an invariant TCR capable of detecting both bacterial as well as self-glycolipids. In the liver, they constitute 30% all lymphocytes where they constantly patrol the sinusoids in a random fashion [85, 86]. They exhibit remarkable functional plasticity by producing either Th1 or Th2 type cytokines depending on the source of the activation signal [87]. Indeed, they can produce interferon gamma (IFN-γ) when activated by certain bacterial products [85, 88] and IL-4 when stimulated with non-microbial self-glycolipids. The general thinking is that there are subsets of these iNKT cells that respond to various classes of glycolipids.

In a recent study using focal thermal liver injury model, Pei et al. visualized the dynamic behavior of iNKT cells in real time [8]. At early time points (4-h post injury), iNKT cells approached the injury but instantly took a U-turn from the injury border. Subsequently, they became stationary around the injury border and localized at this site from 8 to 48-h post injury. Finally, they migrated inward into the injury site but only after 48 h (Fig. 1). It is not clear whether iNKT cells were kept away overtly from the injury at early time points or simply not retained at the injury. However, by 8 h, Kupffer cells and liver sinusoidal endothelial cells presented self-antigen to iNKT cells via CD1d. This self-antigen presentation activated TCR and functioned as the initial retention signal for iNKT cells. By 24 h, the retention of iNKT cells was no longer CD1d dependent but rather required cytokine dependent signaling (IL-12 and IL-18). TCR activation resulted in a massive amount of IL-4 secretion by iNKT cells around the injury border, which contributed to the switching of monocytes (from
inflammatory to reparative phenotype). The co-localization of the monocytes and iNKT cells at the border of the injury was suggestive of intercellular communication (Fig. 1). IL-4 released by iNKT cells also helped in hepatocyte proliferation in the injury border. While self-antigen presentation to iNKT cells was a well-known phenomenon in thymus [86], clearly, this could also happen in the periphery to contribute to repair of injured tissue. It is worth noting that the repair response and the switching of monocytes was simply delayed in the absence of iNKT cells (Table 1) and other IL-4 producing immune cells also likely contributed. Indeed, removal of iNKT cells only accounted for about half of the IL-4 production, so other cells such as innate lymphoid cells, NK cells, and T cells could also contribute to the healing response. In humans where there are fewer iNKT cells other cells may play a bigger role.

Interestingly, simple activation of iNKT cells with α-galactosyl ceramide a molecule that binds to CD1d and maximally activates iNKT cell TCR results in some liver injury, suggesting these cells may also have the capacity to injure when maximally activated. In a concanavalin A-induced hepatitis model that activates the immune system in a dysregulated manner, iNKT cells inflicted damage as their absence protected the liver from injury. Additionally, liver-derived iNKT cells are well known to mount a strong perforin-mediated cytotoxic insult against many different cancer cells [89]. Clearly, the type of stimulus will dictate whether iNKT cells induce an inflammatory or repair function. As such, these cells could be modulated to affect inflammation and repair with the right glycolipid therapy.

Macrophages and repair

Tissue resident macrophages such as Kupffer cells, microglia, cardiac macrophage, and alveolar macrophage are important for maintaining tissue homeostasis and are regarded as stationary cells or cells with limited mobility within the tissue they reside in [4]. The majority of macrophages that are recruited to the site of injury were shown to be differentiated from recruited monocytes [82]. As such, there are numerous sources of macrophages that may contribute to both inflammation and repair. A huge amount of attention has been given to the role of tissue resident macrophage and their role in healing and/or harming tissue. In the heart, yolk-sac-derived resident macrophages were shown to be critical for healing after myocardial ablation. Recruitment of monocyte-derived inflammatory macrophages impairs the ability of resident cardiac macrophages to repair a healing heart [90]. In contrast, Nahrendorf et al. showed that monocyte-derived macrophages (inflammatory and anti-inflammatory) help in healing injured myocardium [75].

In some injuries, the tissue is obliterated as is the case in burn and under these conditions, new macrophage must be recruited to allow for healing. As we discussed earlier, circulating monocytes can definitely enter tissues to become macrophages. Indeed, publications in liver, heart, and intestine suggest that these monocytes come to the injury site, transition into reparative monocytes and then into macrophages [7, 70, 72]. An alternative view is that inflammatory monocytes get recruited to the injury site in the early phase of injury, which gives rise to inflammatory macrophages. Inflammatory macrophages clear dead cells, digest extracellular matrix, and may worsen [71] or help resolve the ongoing injury [75, 91]. At the late phase of injury, reparative monocytes infiltrate the injury site and differentiate into reparative macrophages, which is crucial for healing [75]. Inflammatory macrophages can also differentiate into reparative macrophage after phagocytosing apoptotic cells within the injury site [71]. This conversion is crucial during the resolution phase of an injury [71]. Tissue resident macrophages have also been shown to play a role in injury and inflammation. In the liver, tissue resident macrophages (Kupffer cells) become activated and release pro-inflammatory cytokines during the early phase of injury and inflammation [6]. This in turn causes recruitment of inflammatory immune cell recruitment in the injury site. Depletion of Kupffer cells reduces neutrophil recruitment (Table 1) [6] in a focal thermal injury model. Neutrophil recruitment in this model of liver injury was later shown to be crucial for timely healing of such injuries [26]. So, Kupffer cells potentially play an important role in healing thermal liver injury by orchestrating neutrophil recruitment. In a Concanavalin A-induced liver injury model, however, Kupffer cell depletion was associated with reduced severity of the liver injury [92]. A healing role of resident cardiac macrophages was also highlighted by Lavine et al. [90] in a genetic model of cardiomyocyte ablation. Therefore, the resident macrophage has the capacity to injure or resolve injury depending on the insult.

Our recent discovery of the recruitment of large peritoneal macrophages to the site of sterile liver injury is the first report of nonvascular recruitment of mature macrophages to the site of injury [4]. These macrophages were the large peritoneal macrophage that were GATA6+ and could enter the injury site within an hour, proliferate locally, and help in healing the injury. They were seen to rip apart dead cells or at least the labeled nuclei but did not appear to take up these particles (Fig. 1). In fact, the released DNA appeared to form a coating over the entire wound. Neutrophils got recruited to the site of injury almost at the same time and appeared to take up many particles made by peritoneal macrophages. Large peritoneal macrophages did not use integrins (β1 and β2) and Gαi-chemokine signaling to get to the site of injury. While ATP is important in allowing cells to home in on injured tissue, it is unlikely that it serves as a chemoattractant based on its very short half-life. It is possible that the peritoneal macrophage patrol on the surface of all the visceral organs and upon injury are already very close to the injury site and may not require chemotactic signals to enter the injury. These macrophages utilize CD44 to interact with its ligand hyaluronan exposed in the damaged matrix. The cells were avidly adherent and over time migrated deeper into the injury site [4]. We would predict that this could occur in all organs and all cavities and initial work in the heart suggests that similar cavity macrophage reside in the pericardium (unpublished observations by Deniset and Kubes).
Conclusions

In self-reparable sterile injury models, many different types of innate immune cells get recruited. They are critical for healing such injuries. If their functions are carefully analyzed, it appears that each of the innate immune cells has designated tasks, and have their own individual programs but must communicate with one another to play coordinated roles in repair. For example, peritoneal macrophages come to the injured liver within an hour and start dismantling dead cells into small cellular debris, which are taken up by neutrophils that swarm into the site at a similar time frame (Fig. 1). Similarly, iNKT cells start accumulating around the injury site at the same time when inflammatory monocytes start coming to the site. These two cell types remain spatially associated around the injury for many hours. During this time, there is inflammatory monocyte transition to reparative phenotype under the influence of iNKT cell derived IL-4 (Fig. 1). If sterile inflammation persists with recurrent stimuli or inert stimuli that cannot be removed, the recruited innate immune cells may ultimately cause injury rather than repair leading to chronic inflammatory diseases. Clearly, the immune cells go to sites of injury to help heal tissue, at least in self-resolving models of injury [20–25], but in non-resolving situations may contribute to injury. As such, therapy will require a much more sophisticated approach of local regulation rather than the blunt approach of preventing total recruitment, which in our opinion will result in less inflammation but poor repair.

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Abbreviations: DAMP: damage-associated molecular pattern · GAG: glycosaminoglycan · GPCR: G-protein coupled receptor · JAM-C: junctional adhesion molecule-C · MERTK: MER tyrosine kinase · Nr4a1: Nuclear Receptor Subfamily 4 Group A Member 1 · TIMP: tissue inhibitor of matrix metalloproteinase · VEGF: vascular endothelial growth factor

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