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Neutrophil Reverse Migration and a Chemokinetic Resolution

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Neutrophil migration to sites of injury or infection is a critical first step in innate immunity. Resolution of neutrophil inflammation is essential for tissue repair but is less well understood. Recent work using intravital imaging in mice to visualize neutrophil dynamics at sterile injuries has advanced our understanding of neutrophil reverse migration and resolution of inflammation in mammals.

Neutrophils act as first responders during an innate immune response and are often the first cells to arrive at a site of infection or injury. While this recruitment is critical for protection against disease, the resolution of neutrophil-mediated inflammation is equally important. Neutrophil retention at a site of inflammation can lead to tissue damage and chronic inflammation. The resolution of neutrophil-mediated inflammation was traditionally thought to occur through neutrophil apoptosis and clearance by macrophages (de Oliveira et al., 2016). This paradigm was first challenged by a study in zebrafish showing neutrophils migrating away from a wound and back into the vasculature (Mathias et al., 2006). This reverse migration, the first to be visualized in an *in vivo* model, was shown to be critical for local inflammation resolution in zebrafish. While this study found an alternative mechanism for neutrophil resolution, it was not commonly accepted that reverse migration was important for neutrophil resolution in mammals, although other studies have shown that, *in vitro*, human neutrophils undergo a type of bidirectional movement termed reverse transendothelial migration or rTEM (Buckley et al., 2006). Neutrophils undergoing rTEM *in vitro* were found to express the markers ICAM1^{hi} and CXCR1^{low}, and an increase in this population was found in the circulation of patients with systemic inflammation (Buckley et al., 2006). Subsequent work in mice showed that neutrophils also exhibit rTEM through a junctional adhesion molecule (JAM-C)-dependent pathway and that this may be associated with distant organ inflammation (Woodfin et al., 2011). More recent work suggested that a LTB4-neutrophil elastase pathway drives this rTEM in mice (Colom et al., 2015). While these studies suggested that a form of reverse transendothelial migration can occur in mammals, it remained unclear whether this was functionally relevant to the resolution of neutrophil-mediated inflammation. The motility mechanisms used by neutrophils to reverse migrate from a site of injury and the fate of reverse-migrated neutrophils also remained unknown.

In a recent issue of *Science*, Wang et al. (2017) used advanced intravital imagining techniques in mice to visualize neutrophil dynamics within a thermal hepatic injury site and in the surrounding tissue. Unlike other inflammatory models such as ischemia-reperfusion injury, this thermal burn model produces a fully resolving sterile injury, allowing researchers to study both neutrophil recruitment and resolution. Using this model, the authors showed that although neutrophils are required for tissue repair during the initial phase of inflammation, after 12 hr, they migrate away from the injury site back into the vasculature. Furthermore, they found that neutrophils leaving the injury site traffic to the lung and eventually the bone marrow, where they undergo apoptosis. Collectively, these findings, for the first time, clearly demonstrated the importance of reverse migration in the resolution of local sterile injury in a mammalian system.

Neutrophil inflammation is known to be important for the clearance of infection and debris from a wound. Consistent with this, Wang et al. (2017) found that neutrophils were critical for clearing the vascular debris following thermal injury and for proper collagen deposition and revascularization in the healing tissue. They observed that even short-term neutrophil depletion led to impaired tissue repair up to 7 days after the thermal injury. This initial phase of neutrophil inflammation was found to be short-lived, with the vast majority of neutrophils leaving the site within 24 hr post injury. Neutrophils reached peak recruitment at 12 hr post injury, but only about 10% remained after 24 hr. A biopsy of the injured area at 12 hr post injury showed low levels of apoptosis, supporting an alternative process than local apoptosis for neutrophil resolution.

Live imaging by Wang et al. (2017) revealed neutrophils leaving the site of injury and entering the vasculature outside the injury barrier (Figure 1). The neutrophils migrated randomly within the injury and directionally at the border. This finding supports studies that suggest neutrophil reverse migration from a wound is, at least in part, a result of chemokine-mediated random motility, or chemokinesis, prior to reverse transmigration (Powell et al., 2017). This is surprising because neutrophils, unlike T cells, are typically viewed as chemotactic cells, whereas T cell migration is often random during immune surveillance (Krummel et al., 2016). Taken together, these studies raise the intriguing possibility that neutrophil random migration may also provide a type of surveillance mechanism where the neutrophils are poised to respond to new cues that mediate further inflammation or, alternatively, resolution.

Many of the key questions about reverse-migrated neutrophils concern their fate following departure from the

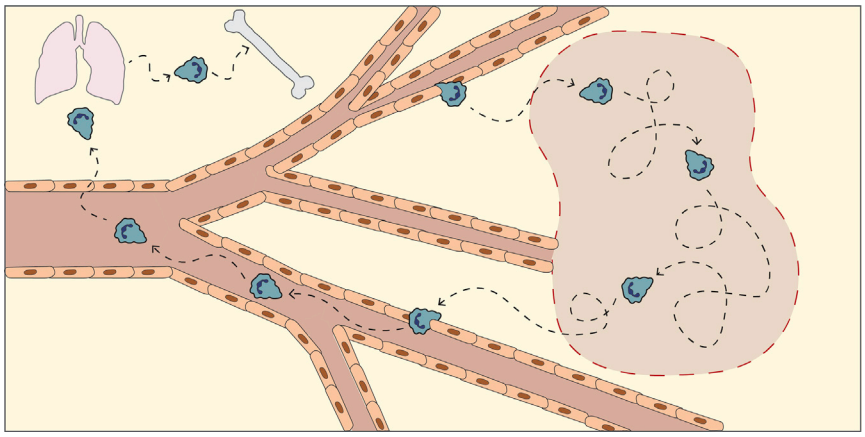

Figure 1. Neutrophil Resolution through Chemokinetic Surveillance and Reverse Migration
Following a thermal hepatic injury in mice, neutrophils (blue) leave the vasculature and migrate to the site of injury (red dotted line) where they clear debris and facilitate wound healing. Neutrophils migrate randomly within the injury site, and upon reaching the border between the injury and normal tissue, they migrate back to the vasculature. A portion of reverse-migrated neutrophils then migrate to the lung en route to the bone marrow where they undergo apoptosis.

primary site of injury. Do reverse-migrated neutrophils have a different, potentially "activated" phenotype following reverse migration? Where do reverse-migrated neutrophils go after leaving a site of injury? Wang et al. used photoactivation of neutrophils specifically expressing photoactivatable-GFP (PA-GFP) within the injury site to track neutrophils and determine their final destination. They found that 24 hr after injury, all the photoactivated neutrophils had left the injury site, and populations of PA-GFP neutrophils could be found in the lung and bone marrow. The increase in neutrophils in the lung was transient with few neutrophils remaining 48 hr after injury. Photoactivated neutrophils were found to have increased expression of CXCR4, and neutrophils in the bone marrow were positive for apoptosis markers. Finally, blockade of CXCR4 led to neutrophil retention in the lung, suggesting that CXCR4 signaling is required for neutrophils to return to the bone marrow. These findings are clinically significant as systematic inflammation following trauma can lead to organ failure. Importantly, it has been shown that patients suffering from acute pancreatitis-associated acute lung injury had reduced expression of JAM-C, a regulator of neutrophil rTEM (Wu et al., 2016). Together these findings indicate that reverse-migrated neutrophils redistribute to the lung following their departure from the primary site of injury. During systematic inflammation, this can potentially lead to tissue damage in the lungs.

The findings presented by Wang et al. (2017) significantly advance our understanding of neutrophil resolution following injury and raise further questions concerning the mechanisms of this reverse migration and how these processes are regulated in a human immune response. Human neutrophils are able to undergo reverse transendothelial migration *in vitro* (Buckley et al., 2006), but what neutrophil intrinsic and extrinsic factors are involved in directing this movement remain largely unknown. Controlling neutrophil resolution is a major clinical challenge because total blockade of neutrophil infiltration leads to reduced healing. Therefore, treatments that allow for a balance between neutrophil recruitment needed for repair and resolution to avoid potential damaging effects of inflammation are needed. Recently, a compound called Tanshinone IIA that promotes neutrophil reverse migration without affecting neutrophil recruitment was identified in a zebrafish screen (Robertson et al., 2014). In light of the new finding that reverse migration is at work in mammals, future work exploring the phenomenon in humans coupled with probing the clinical applications of these compounds to treat chronic inflammation will be an exciting area for future investigation.

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