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Series: Neutrophils in Action

Feature Review

Reverse Migration of Neutrophils: Where, When, How, and Why?

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Neutrophil migration to injured and pathogen-infected tissues is a fundamental component of innate immunity. An array of cellular and molecular events mediate this response to collectively guide neutrophils out of the vasculature and towards the core of the ensuing inflammatory reaction where they exert effector functions. Advances in imaging modalities have revealed that neutrophils can also exhibit motility away from sites of inflammation and injury, although it is unclear under what circumstances this reverse migration is a physiological protective response, and when it has pathophysiological relevance. Here we review different types of neutrophil reverse migration and discuss the current understanding of the associated mechanisms. In this context we propose clarifications to the existing terminology used to describe the many facets of neutrophil reverse migration.

Trends

Neutrophils can exhibit abluminal-to-luminal motility through endothelial cells (rTEM) and reverse interstitial migration (rIM) in models of inflammation. Emerging data suggest that the former can mediate systemic dissemination of a local inflammatory response whereas the latter is a protective physiological event that facilitates inflammation resolution.

In models of inflammation, neutrophil rTEM is enhanced and reduced in EC JAM-C$^{-/-}$ and neutrophil elastase (NE)$^{-/-}$ mice, respectively, demonstrating novel roles of these molecules in regulation of neutrophil trafficking.

Neutrophil rIM is suppressed by genetic activation of the HIF pathway in zebrafish, an intervention that causes a delay in inflammation resolution.

Targeting neutrophil reverse migration represents a novel approach for development of drugs aimed at modulating inflammation.

Introduction

The motility of leukocytes from the bloodstream to interstitial tissues is a fundamental host defence reaction [1]. In the context of neutrophils and innate immunity, this process is largely initiated by pathogen-associated molecular patterns (PAMPs), released from invading microorganisms, or by damage-associated molecular patterns (DAMPs), derived from damaged, dead, or environmentally stressed cells [2,3]. Such danger signals can be detected by sentinel cells including mast cells, macrophages, and dendritic cells, which in turn can release a variety of mediators that promote leukocyte recruitment [1,4]. The mechanisms that regulate leukocyte accumulation into tissues are complex and need to be tightly regulated because defective leukocyte migration leads to immune deficiency disorders while excessive or aberrant leukocyte trafficking can be damaging to the host [3,5,6]. Broadly, this event involves breaching of venular walls and leukocyte crawling within the interstitium to sites of tissue injury or infection (Box 1; the reader is referred to recent comprehensive reviews for mechanistic details [1,7–9]). Once arrived at inflammatory sites, neutrophils can exhibit numerous cellular responses such as release of additional mediators, generation of reactive oxygen species (ROS), phagocytosis, and extrusion of neutrophil extracellular traps (NETs) [3,6], functions that are all ultimately aimed at eliminating the cause of the inflammatory reaction and promoting resolution of inflammation.

The efficient migration of neutrophils to sites of inflammation is governed by the ability of these cells to rapidly detect and respond to attractant molecules [1]. This ensures movement of leukocytes, classically in an amoeboid and polarised manner, towards the foci of the

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Box 1. Neutrophil Migration from the Vascular Lumen to the Interstitial Tissue

The migration of leukocytes out of the vascular lumen and within the interstitial tissue is commonly mediated by coordinated presentation of directional cues to leukocytes on the surface of cells (e.g., ECs and pericytes) and extracellular matrix structures (e.g., heparin sulphate glycosaminoglycans, HS GAGs). Once leukocytes have encountered stimulatory molecules within the lumen of microvessels, a series of adhesive pathways, classically described by the 'leukocyte adhesion cascade', guide them out of the vasculature and into the surrounding tissue [14]. This initiates with leukocyte rolling, mediated by weak and reversible attachment of leukocytes to ECs, followed by further activation of leukocytes leading to leukocyte arrest and crawling on the inner aspect of venular walls, as previously detailed [7,14] (see also Figure 1).

Following luminal interactions, leukocytes need to breach ECs and the second cellular layer of venular walls, the pericyte sheath. Pericytes are mural cells that are typically embedded within the vascular basement membrane (BM). Our understanding of this stage of leukocyte migration is scant, but recent evidence has indicated that neutrophils and monocytes breach venular walls by migrating through gaps between adjacent pericytes and sites within the venular BM exhibiting lower deposition of BM extracellular matrix protein constituents [78,79]. In addition, high-resolution confocal intravital imaging of neutrophil behaviour within mouse cremasteric venular walls identified significant neutrophil sub-EC crawling, as supported by ICAM-1-expressing pericyte processes [29]. Together, it is now apparent that, beyond the vascular lumen, full breaching of the venular wall by leukocytes involves an additional cascade of molecular cues and adhesive mechanisms [1,30]. Of relevance, there appears to be a steep gradient of HS scaffolds between the vascular lumen and the basolateral aspect of endothelial cells [80]. Such a profile could provide a means through which a gradient of chemokines is established across the vessel wall, aiding the passage of leukocytes out of the vasculature in a luminal-to-abluminal manner. Once the leukocytes have fully breached venular walls, they are required to migrate within the interstitial tissue to reach the foci of infection or injury. This phase of leukocyte migration has been the subject of several elegant works involving different modes of advanced confocal intravital microscopy, studies that have begun to shed light on the mechanisms through which efficient leukocyte interstitial motility is achieved [31,32,35,81]. Such investigations have identified multiple patterns of leukocyte migration, and numerous models of cellular and molecular cascades have been proposed [8,9,35,82].

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inflammatory response. As with all motile cells, neutrophils are required to integrate numerous signals to choreograph their movement within complex 3D structures. At sites of inflammation, this is largely regulated by presentation of directional cues in soluble form or, more commonly, in an immobilised fashion, providing a haptotactic gradient. Although the details of how attractant molecules are presented in tissues remain unclear, there is now solid evidence for the existence of functional chemotactic gradients *in vivo* [10]. Additional factors that can modulate movement of neutrophils include shear force (relevant to luminal leukocyte responses), the cellular and molecular composition of the interstitial tissue, and the potential existence of repulsive molecules. The profile and directionality of neutrophil migration out of the vascular lumen and within the interstitial tissue is thus regulated by the resultant processing of multiple signalling factors, both mechanical and biochemical. This commonly leads to a net migratory response of recruited neutrophil populations towards the core of an inflammatory insult, from which it is proposed they are subsequently cleared through apoptosis and uptake by tissue macrophages. Within the past 10 years, investigations of neutrophil behaviour at single cell level have also shown that neutrophils can migrate away from sites of inflammation. We review here the existing evidence for this enigmatic cellular behaviour within inflammation and immunity, describe the different types reported, discuss the proposed mechanisms and, importantly, the potential physiological and pathological roles of this phenomenon. Furthermore, because there is some ambiguity regarding the terms used to describe the various modes of neutrophil reverse migration, we propose some clarity on nomenclature.

Neutrophils Can Show Different Modes of Reverse Migration

Since the first reports of neutrophil reverse migration [11–13], several types of this response have now been identified in different stages and contexts of leukocyte trafficking (Figure 1, Key Figure). These events are loosely referred to as 'neutrophil reverse migration', an expression that requires optimisation and clarity (Box 2 and Figure 1). Of note, this term does not distinguish between neutrophils that exhibit a U-turn and cells that show a true reversal of polarity, or any in-between responses that reflect altered gradient sensing. While acknowledging this limitation, for simplicity