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Interstitial leukocyte migration *in vivo*

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Rapid leukocyte motility is essential for immunity and host defense. There has been progress in understanding the molecular signals that regulate leukocyte motility both *in vitro* and *in vivo*. However, a gap remains in understanding how complex signals are prioritized to result in directed migration, which is critical for both adaptive and innate immune function. Here we focus on interstitial migration and how external cues are translated into intracellular signaling pathways that regulate leukocyte polarity, directional sensing and motility in three-dimensional spaces.

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Introduction

The trafficking of leukocytes into peripheral tissues and lymphoid organs is critical for both innate and adaptive immune function and has been extensively reviewed [1–3]. A key step in this process is the interstitial motility of leukocytes within three-dimensional (3D) spaces, which can be either random or directed. Interstitial motility involves cycles of motility and arrest that is orchestrated by a complex hierarchy of external cues that are translated into changes in intracellular signaling. This function is evolutionarily conserved as demonstrated by the chemotactic interstitial migration of *Drosophila* hemocytes (immunosurveillance cells) to sites of tissue damage and infection (reviewed in [4]).

The rapid single-cell migration of leukocytes is classically known as 'amoeboid' migration. The term 'amoeboid' is based on a changing cell morphology, but can encompass different modes of locomotion (reviewed in [5,6]). Pseudopod driven gliding motility is the major mode of locomotion. During this mode of motility, actin polymerization below the leading plasma membrane generates forces for membrane extension. This is coupled with actomyosin contraction in the cell's rear, which detaches the cell and propels the cell body forward. Leukocytes can also display a blebbing type of migration under some conditions where actomyosin contraction generates hydrostatic pressure to form bleb-based protrusions.

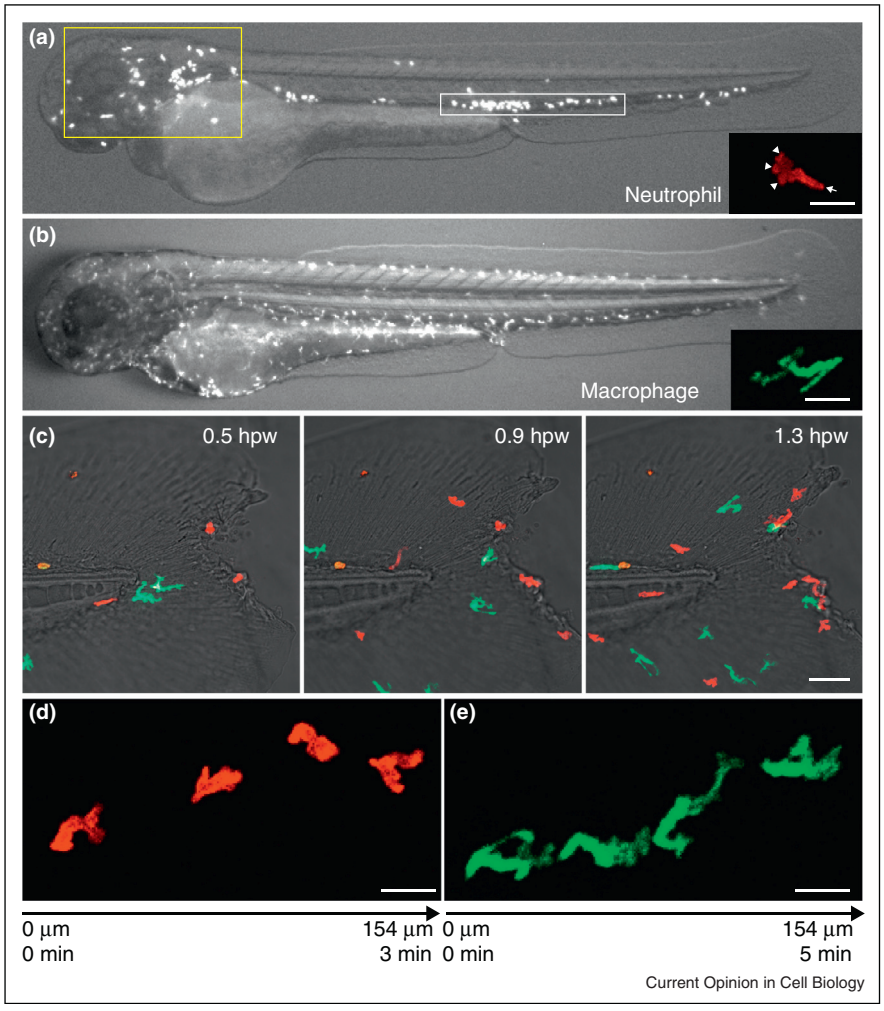
Migration in 3D

Efficient leukocyte migration through interstitial tissues is a key component of the normal function of the cells of the innate and adaptive immune system. The patrolling function of leukocytes requires cells to maneuver rapidly through complex matrix environments at speeds of up to 20–30 μm per minute. The type of protrusions formed by different leukocytes can be quite distinct, as demonstrated by neutrophils and macrophages migrating *in vivo* (Figure 1a and b). Neutrophils project small pseudopodia that often bifurcate, while macrophages frequently extend long, thin filopodia-like protrusions in many directions as they maneuver through the interstitium toward damaged tissues within zebrafish embryos (Figure 1c–e). T cells, on the other hand, often migrate randomly in their surveillance of lymph nodes in mice, with extension of small pseudopodia at the leading edge [7]. The mechanisms that regulate the generation and maintenance of amoeboid pseudopodia and biased selection are under intense investigation both *in vitro* and *in vivo* (reviewed in [8,9]).

Leukocyte amoeboid motility, in general, occurs in the absence of strong adhesive interactions with surrounding cells or tissues (reviewed in [10]), although leukocyte arrest often requires integrin-mediated adhesion, in particular during transendothelial migration (reviewed in [11]). Integrin-mediated adhesion is essential for migration on two-dimensional surfaces. However, integrins can be dispensable for leukocyte migration in interstitial tissues [12•] or in 3D collagen lattices [13]. The idea that movement within confined spaces can be integrin-independent was paradigm shifting and highlights the key role for adhesion in limiting leukocyte migration speed and mediating leukocyte arrest rather than an essential role in motility under many *in vivo* conditions.

Interstitial environments contain both soluble and tissue-bound cues that help guide leukocytes to migrate or mediate cell arrest. Here, we review the key steps in interstitial leukocyte migration including sensing of environmental cues and the intracellular signaling mechanisms that orchestrate leukocyte motility and cell arrest (Figure 2a and b).

Figure 1



Neutrophil and macrophage distribution, morphology and response to wounding in zebrafish embryo at 3 days post fertilization. **(a)** Neutrophils distribute mainly in the mesenchymal tissue in the head region (yellow box) and in the caudal hematopoietic tissue (white box). (Inset in (a)) A typical migrating neutrophil has multiple pseudopodia (arrow heads) and a uropod (arrow). Scale bar = 20 μm. **(b)** Macrophages distribute in the whole embryo with no clear tissue localization. (Inset in (b)) A typical migrating macrophage shows multiple spindly protrusions. Scale bar = 20 μm. **(c)** Time series image of neutrophils (red; lifeact-Ruby) and macrophages (green; dendra) migrating to tail fin wound. Neutrophils have arrived at the wound at 0.5 hours post wounding (hpw) and macrophages arrived at later time points. Scale bar = 50 μm. Time series image of a neutrophil **(d)** or a macrophage **(e)** migrating from left to right toward the wound. Scale bar = 20 μm.

Cell signaling underlying leukocyte motility

Chemoattractants transmit signals through heterotrimeric G-protein-coupled receptors (GPCRs) [14], which activate a plethora of effectors [15,16]. One of the key effectors is the class Ib phosphatydylinositol-3-kinase (PI(3)K) (Figure 2c). Although the role of PI(3)K during *in vitro* chemotaxis is controversial, PI(3)Kγ is required for cell polarity and motility *in vivo* [17•,18,19]. PI(3)K promotes Rac-mediated actin polymerization at the leading edge and generates F-actin anteroposterior polarity (dynamic F-actin at the leading edge and stable F-actin at the rear; Figure 2c and f). Inhibition of PI(3)K results in impaired F-actin polarity and neutrophil recruitment to both wounds and infection in zebrafish [17•,20]. The importance of PI(3)K signaling is further illustrated by studies showing that the modulation of PI(3)K signaling can result in an inflammation phenotype. The PI(3)K products PI(3,4,5)P3-PI(3,4)P2 are localized to the leading edge of zebrafish neutrophils *in vivo* [17•,21]. PI(3,4,5)P3 can be hydrolyzed to PI(3,4)P2 by SH2-domain-containing inositol 5-phosphatases (SHIP). SHIP limits myeloid cell motility through the modulation of PI(3)K signaling *in vitro* [22] and *in vivo* in zebrafish [21]. In addition, SHIP knockout mice show increased myeloid infiltration into vital organs [23,24]. Taken together, the modulation of