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Neutrophil migration in infection and wound repair: going forward in reverse

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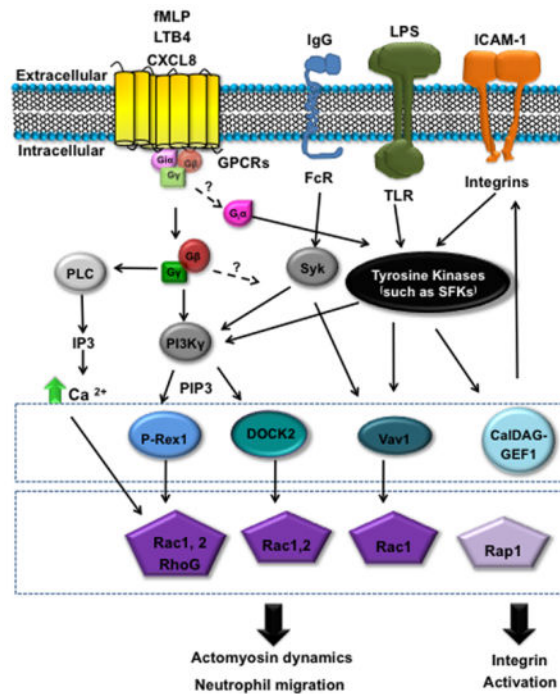
Abstract

Neutrophil migration and its role during inflammation has been the focus of increased interest in the past decade. Advances in live imaging and the use of new model systems have helped to uncover the behaviour of neutrophils in injured and infected tissues. Although neutrophils were considered to be short-lived effector cells that undergo apoptosis in damaged tissues, recent evidence suggests that neutrophil behaviour is more complex and, in some settings, neutrophils might leave sites of tissue injury and migrate back into the vasculature. The role of reverse migration and its contribution to resolution of inflammation remains unclear. Here, we discuss the different cues within tissues that mediate neutrophil forward and reverse migration in response to injury or infection, and the implications of these mechanisms to human disease.

TOC blurb

Neutrophils follow a multitude of signals to reach sites of injury or infection. Understanding how this occurs and what the fate of these neutrophils is provides insight into how immune responses are controlled and chronic inflammation avoided. Here, the authors describe the movement of neutrophils during inflammation.

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Neutrophils are the most abundant leukocytes in the blood and they lead the first wave of host defense to infection or tissue damage. Neutrophils are powerful effector cells that destroy infectious threats through phagocytosis, degranulation, reactive oxygen species (ROS) and neutrophil extracellular traps¹⁻³. Neutrophil loss by disease or therapy-induced side effects has devastating consequences that are characterized by recurrent severe infections. However, excess infiltration and activation of neutrophils at a site of tissue damage can cause chronic inflammation, limit injury repair and lead to loss of organ function^{1,4}. Neutrophils mediate tissue damage through the release of cytokines, proteases and other factors contained in their cytoplasmic granules and also by regulating the activity of the adaptive immune response, including both T and B cell activation. Therefore, the migration and activation of neutrophils must be finely controlled. Recent advances in *in vivo* imaging techniques and new *in vitro* systems incorporating microfluidics and three-dimensional models have enabled researchers to directly visualize and quantify neutrophil behaviour (Box 1). These developments have spawned more complex studies regarding the role of neutrophils in the context of tissue homeostasis and disease, including wound healing, chronic inflammation, infection and cancer. As the role of neutrophils as key modulators of the immune response becomes clearer, it is becoming more important to understand neutrophil migration in the context of both acute and chronic inflammation and how migration is altered in disease.

Box 1

Imaging neutrophil migration

Substantial progress has been made in understanding the mechanisms that regulate neutrophil migration using live imaging. Most of this work has involved the use of

primary human or mouse neutrophils¹⁴⁰ or human-derived neutrophil-like cell lines (HL-60¹⁴¹ or PLB-985¹⁴²). Microfluidic devices are one of the most powerful recent tools used to study the movement of neutrophils in stable chemokine gradients^{94,143–145}. Recent studies using microfluidics have highlighted mechanisms that differentially regulate neutrophil migration in two-dimensional compared with three-dimensional environments¹⁴⁶. These *in vitro* studies are complemented by more recent work imaging neutrophil migration within the tissue microenvironment using zebrafish and mice. Intravital imaging in mouse models has been developed to directly visualize and measure neutrophil migration and function in specific organs after acute tissue damage. These models mainly focus on peripheral tissues in mice that are amenable to imaging, including the cornea¹⁴⁷, ear dermis^{7,68} and cremaster muscle^{110,148–150}. Other methods create “windows” through which immune cell infiltration can be visualized in the brain¹⁵¹ and lungs¹⁵². Neutrophil recruitment to the liver has been studied for many years by externalizing it to allow for high-resolution intravital imaging^{33,153,154}. Nevertheless, these models are invasive, often requiring animal sacrifice shortly after the experiment and are generally not amenable to long-term studies. In addition, these models do not provide high temporal and spatial resolution, making it difficult to analyse behaviours of early recruited neutrophils.

Zebrafish have been used increasingly for the study of innate immune cell function, including neutrophil migration^{155–157}. Transparent larvae allow *in vivo* imaging of neutrophil migration in the context of a whole living organism on the scale of days. Zebrafish neutrophils have the same morphology, behaviour and function as mammalian neutrophils^{158,159} and zebrafish have been used successfully to model human neutrophil disorders^{155,156}. With fluorescent tags, specific cells and subcellular structures can be visualized, and the use of tracking software enables researchers to easily measure multiple parameters of neutrophil motility, including speed and directionality, early after tissue damage. In fact, neutrophil reverse migration was first visualized in zebrafish¹⁰¹ and zebrafish are currently being used to elucidate the mechanisms governing reverse migration¹⁰⁸. Zebrafish larvae are ideal for screening chemical compounds — with such screens a fungal-derived compound that inhibits neutrophil chemotaxis¹⁶⁰ and a compound that promotes neutrophil inflammation resolution¹⁰⁷ were recently discovered.

This Review highlights the signals that regulate neutrophil migration in acute tissue damage, on the basis of three main phases: an early neutrophil recruitment phase induced by short-term signals, an amplification phase of neutrophil infiltration in response to more persistent signals and the resolution of inflammation, which may include neutrophil reverse migration. We also consider the differences and similarities between ‘sterile’ wound-induced and infection-induced neutrophil infiltration. Finally, the potential therapeutic relevance of modulating either neutrophil forward or reverse migration is discussed.

Neutrophil forward migration

Acute tissue damage generates a wide variety of signals produced by complex networks that establish chemoattractant gradients throughout tissues. As ‘leader’ cells in host defense responses, neutrophils must sense, prioritize and integrate all of these chemotactic cues into

a migration response towards the damage^{5,6}. In this section, we focus on the different signals that mediate neutrophil forward migration to a site of damage. This migration is thought to occur in phases (Figure 1): early neutrophil recruitment (sometimes referred to as “scouting”) followed by amplification of the response that is mediated by both tissue-resident and early-recruited cells and results in robust neutrophil infiltration from the bloodstream^{7,8}. The process of neutrophil extravasation from the vasculature into the tissue has recently been reviewed in detail^{9,10}. Here, we highlight some of the signals in these phases, including damage-associated molecular patterns (DAMPs), hydrogen peroxide (H₂O₂), lipid mediators and chemokines.

Neutrophils express more than 30 different receptors that can sense pro-inflammatory mediators and modulate neutrophil migration, function and behaviour including G protein-coupled receptors (GPCRs), Fc receptors, adhesion receptors, cytokine receptors and pattern recognition receptors (PRRs)¹¹. Many of these receptors can directly modulate neutrophil polarization and migration (Box 2). In addition to activating migration, the signalling pathways downstream of these receptors can also affect neutrophil transcriptional activity, phagocytosis, apoptosis, degranulation and ROS production¹². These cues often converge on common intracellular signalling pathways through phosphoinositide 3-kinase- γ (PI3K γ), phospholipase C- β 2 (PLC β 2) or PLC β 3 and/or extracellular signal-regulated kinase (ERK) signalling. These signalling pathways are discussed in more detail in Box 2 and have recently been reviewed^{6,11,13}. Importantly, the pool of exogenous signals that neutrophils respond to after a wound is complex and it is likely that no single cue is absolutely required for migration.

Box 2

Neutrophil signalling in motility

There is an extensive literature on downstream signalling mechanisms that regulate neutrophil directed migration to specific chemokines and other signals^{6,11,161–164} and only general principles are discussed here. Directed migration is mediated by chemoattractant signalling through G protein-coupled receptors (GPCRs) and other receptors that induce the asymmetric localization and activation of key signalling molecules, including phosphoinositide 3-kinase (PI3K), phospholipase C (PLC) and members of the RHO GTPase family, such as RAC¹⁶⁵. The asymmetric distribution of the PI3K product phosphatidylinositol (3,4,5)-trisphosphate (PtdIns(3,4,5)P₃), at the front of polarized neutrophils is a hallmark of gradient sensing induced by chemoattractants^{165–167}. The localized recruitment of PtdIns(3,4,5)P₃ mediates the recruitment of specific adaptor proteins and guanine nucleotide-exchange factors, such as DOCK2 and P-REX1 respectively, that lead to the activation of RAC signalling and polarized actin polymerization during directed migration^{168,169}. Although many attractant cues signal through PI3K and RAC, there is evidence that signalling hierarchies exist in which some chemoattractants are more dependent on p38 mitogen-activated protein kinase or PI3K signalling *in vitro*¹⁷⁰. Specificity can be provided by the interaction of chemokine receptors with different ligands or the binding of receptors to specific downstream binding partners as has been described with binding of CXC-

chemokine receptor 2 (CXCR2) to vasodilator-stimulated phosphoprotein (VASP)³⁸. In addition, different GPCR kinases have been reported to bind to CXCR1 and CXCR2, providing specificity in the regulation of receptor internalization and therefore an additional layer of regulation¹⁷¹.

Less is known about the key signals that mediate neutrophil migration *in vivo*. Zebrafish are an important tool for the analysis of signalling *in vivo* and recent studies have demonstrated that both RAC signalling¹⁷² and calcium flux¹⁷³ at the leading edge are sufficient to guide cell movement in live animals. However, despite recent progress, a challenge for future investigation remains in determining how neutrophils prioritize competing cues *in vivo* through the modulation of specific downstream intracellular signalling pathways that mediate responses to tissue damage.

Detection of early signals by neutrophils

The first signals that are responsible for early neutrophil recruitment released from damaged and necrotic cells after tissue injury are likely to be DAMPs¹⁴. Neutrophils recognize DAMPs by specific PRRs, including Toll-like receptors (TLRs) and NOD-like receptors (NLRs). The wide variety of DAMP molecules includes DNA, proteins (such as high-mobility group box 1 (HMGB1)), N-formyl peptides, extracellular matrix components, ATP and uric acid^{15–17}). The exact signals that govern this early stage in mice are largely unknown but are probably sensed by GPCRs as treatment of neutrophils with pertussis toxin, which inhibits GPCR-G α_i signalling, limits this early chemotaxis towards the damage site⁷. The signals promoting the early recruitment of neutrophils have been investigated in more detail in larval zebrafish, as this model is amenable to live imaging of early neutrophil recruitment after tissue damage (Box 1).

In larval zebrafish, one of the major early signals known to be directly sensed by neutrophils after wounding and to promote early forward migration is H₂O₂. After a tail wound in zebrafish up to a 200 μ m radial gradient of H₂O₂ is formed, beginning only three minutes after wounding, with levels peaking at 20 minutes post-wounding¹⁸, and this H₂O₂ gradient is also observed in wounded *Drosophila* embryos¹⁹. This early H₂O₂ gradient is primarily produced by the enzyme dual oxidase 1 (DUOX1) in epithelial cells, and depletion of this enzyme in either zebrafish or *Drosophila* inhibits neutrophil migration to a wound^{18,19}. Importantly, H₂O₂ also promotes chemotaxis of human²⁰ and mouse²¹ neutrophils *in vitro*. SRC family kinases (SFKs) were found to directly sense this H₂O₂ gradient and are required in zebrafish for early neutrophil recruitment to wounds and in human neutrophils for chemotaxis to H₂O₂ *in vitro*²⁰. Upon H₂O₂ exposure, a specific cysteine residue in the zebrafish SFK LYN is oxidized, promoting the autophosphorylation and activation of LYN and this activates multiple downstream pathways including ERK signalling²⁰. The exact mechanism by which LYN directs the migration of neutrophils towards the source of H₂O₂ is unknown but it is likely that the signalling converges on the activation of PI3K, RAC and ERK to mediate directed migration (Box 2). This H₂O₂-LYN signalling seems to be important only for early neutrophil recruitment in zebrafish²⁰. Moreover, neutrophils from mice deficient in three SFKs transplanted into a wild-type animal were still able to infiltrate to a site of inflammation, although mice deficient in these SFKs are protected from

autoantibody-induced arthritis because SFK were necessary for the inflammatory microenvironment²².

The many DAMPs that are released from damaged and necrotic cells also serve to directly modulate neutrophils. For example, ATP can be sensed by two different classes of receptors, P2Ys (which are GPCRs) or P2Xs (ligand-gated ion channels)²³. Zebrafish treated with apyrase or inhibitors of P2Y and P2X signalling had fewer neutrophils recruited to a wound²⁴. Although ATP can directly induce monocyte and macrophage migration *in vitro* through the activation of P2Y₂ receptors²⁵, it is unclear if ATP directly activates chemotaxis of neutrophils. It has been shown that neutrophil-released ATP can act as an autocrine signal at the leading edge of the cell to amplify chemotactic signals through P2Y₂ receptors and increase the efficiency of chemotaxis²⁶. The autocrine effects of ATP and its metabolite adenosine at the cell front and rear of neutrophils, through the P2Y₂ receptor and A2a receptor, respectively, promote efficient chemotaxis through differential effects on mechanistic target of rapamycin (mTOR) and mitochondrial activity²⁷. There is also evidence that the presence of ATP can enhance the migration of neutrophils towards N-formyl peptides or CXC-chemokine ligand 8 (CXCL8) by activating RHOA^{28,29}.

Early neutrophil chemotaxis and activation is also triggered by N-formyl peptides, such as fMet-Leu-Phe (fMLP). Although these peptides can be derived from bacterial proteins, they are also released from mitochondria after tissue damage and activate human neutrophils by binding to the GPCRs FPR1, FPR2 and FPR3^{30–32}. *In vitro*, FPR1-specific antibodies block neutrophil migration to disrupted mitochondrial products³¹ or necrotic cells³³. FPR1 blockade or deficiency also prevented the guidance of recruited neutrophils to necrotic cells in a localized hepatic injury in mice,³³ highlighting the importance of this signal as a short-range cue for proper neutrophil localization to a site of injury.

Other early signals produced by tissue and tissue-resident cells

The initial signals can be short-lived; studies in zebrafish suggest that the peak of H₂O₂ production is around 30 minutes after injury and the signal is completely cleared one hour later by the myeloperoxidase activity of wound-associated neutrophils³⁴. For more sustained neutrophil recruitment, long-term and long-range signals are also activated, largely composed of chemokines and lipid mediators.

The CXCL8 family comprises some of the main chemokines that are involved in neutrophil migration, including CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7 and CXCL8. These chemokines are sensed by neutrophils through the GPCRs CXCR1 and CXCR2^{35,36}, and activate downstream signalling to vasodilator-stimulated phosphoprotein (VASP), PI3K and SFKs to mediate neutrophil directed migration^{37,38} (Box 2). These chemokines can be produced by both immune cells (including neutrophils, macrophages and T cells) and non-leukocytes (including epithelial and endothelial cells) in response to both injury and infection. Human CXCL8 has been known to be a neutrophil chemoattractant for over 25 years³⁹ and the role of CXCL8-family–CXCR signalling in neutrophil migration has been confirmed *in vivo* in both mice and zebrafish^{40–44}. To attract more distant neutrophils, CXCL8 family chemokines, among other chemokines, can bind to glycosaminoglycans on cell walls and in the extracellular matrix to create chemokine gradients along the tissues and

structures through which neutrophils migrate^{33,42,45}. When HEK293T cells expressing fluorescently tagged CXCL8 were transplanted into zebrafish larvae, the CXCL8 accumulated locally around the transplanted cells but then spread outwards into the vasculature, forming immobilized gradients⁴². Such intravascular extracellular matrix-bound chemokine gradients have also been observed in mouse models of acute hepatic injury, reaching as far away as 650 μm ³³, and when CXCL8 was injected intradermally in rabbits or *ex vivo* in human skin it appeared bound to the lumen of venular endothelial cells⁴⁶.

Lipid mediators are also strong inducers of neutrophil chemotaxis. These mediators are derived from arachidonic acid. After arachidonic acid release by activated cytosolic phospholipase A2 (cPLA2), arachidonic acid is metabolized through multiple enzymatic pathways into a wide variety of molecules each with specific functions. The lipid mediator that is known to have the largest effect on neutrophil migration is leukotriene B4 (LTB4), which is produced from arachidonic acid by 5-lipoxygenase and sensed by the GPCR BLT1 to induce neutrophil polarization and migration (Box 2). Although some LTB4 is produced relatively early after wounding to recruit neutrophils, it also functions in the amplification stage of neutrophil recruitment and is discussed in detail in the next section. Other lipid mediators can also induce neutrophil chemotaxis, including 5-oxo-ETE and 5-KETE⁴⁷.

In most cases, the production of these chemokines and lipid mediators is activated by DAMPs that are released after cell damage. Pre-made stores of chemokines can be rapidly released via exocytosis from endothelial cells that are activated by DAMPs^{48,49}. DAMPs also activate core enzymatic and transcriptional pathways, such as nuclear factor- κB (NF- κB) and AP-1, that result in the production of chemokines and lipid mediators. Recently, in two different mouse models, DAMP-mediated activation of the NLRP3 inflammasome by ATP was linked to persistent neutrophil recruitment^{33,50}. Tissue injury also results in the release of intracellular ion stores, creating alterations in neighbouring cell osmolarity. *In vivo* in zebrafish larvae, cells surrounding tissue wounds can sense changes in osmolarity through the activation of cPLA2 and release of arachidonic acid, which is metabolized into 5-KETE⁵¹. Knockdown of expression of cPLA2 or OXE-R (the GPCR for 5-KETE) inhibited leukocyte recruitment to a wound by decreasing both neutrophil velocity and directionality⁵¹.

Amplification of neutrophil recruitment

Neutrophil recruitment is exponentially amplified in a phase sometimes termed “neutrophil swarming”. To promote this phase, more signals must arise from the early recruited cells — including neutrophils and macrophages — to recruit more neutrophils both directly and indirectly through the further activation of tissue and tissue-resident cells^{8,52}. This is the next step in the cascade of signals that promote neutrophil forward migration and involves many of the same signals that are involved in earlier phases, including LTB4 and CXCL8 family chemokines^{7,52}.

LTB4 can amplify neutrophil responses to primary chemoattractants in multiple ways. *In vitro*, in response to fMLP, human neutrophils generate local microgradients of LTB4 at their leading edge, acting as an autocrine signal and stabilizing neutrophil polarization⁵³. Longer-range gradients of LTB4 are also created to recruit new neutrophils⁵³. In a mouse

model of laser-induced dermal tissue injury, LTB₄ receptor-deficient neutrophils close to a wound were able to undergo chemotaxis to the wound but more distant neutrophils required the LTB₄ receptor to be efficiently recruited, suggesting that LTB₄ is specifically required for persistent and long-term neutrophil infiltration⁵⁴. This defect in recruitment is similar to that observed when all GPCR-G_{αi2} signalling is inhibited⁵⁴, providing evidence that LTB₄ is a major signal in this phase. In addition to the requirement for sensing LTB₄ through its receptor, neutrophils are also a source of LTB₄ and neutrophils genetically lacking 5-lipoxygenase also could not swarm to the wound⁵⁴. LTB₄ production is also important for neutrophil recruitment in mouse models of inflammatory arthritis^{55,56}.

The amplification step is also likely to be initiated through the recognition of DAMPs by the early-arriving cells. In addition to acting as direct neutrophil chemoattractants, N-formyl peptides also activate ERK1, ERK2 and p38 mitogen-activated protein kinase signalling pathways⁵⁷ and stimulate LTB₄ production by neutrophils⁵³. Cyclic ADP ribose (cADPR) (a metabolite of NAD⁺) has also been shown to have a role in this amplification stage in a mouse model of laser-induced dermal tissue injury⁷. Treatment of neutrophils with a competitive inhibitor of cADPR signalling did not affect early neutrophil recruitment but prevented the amplification phase of the neutrophil response⁷. In a mouse model, neutrophil swarming correlated with cell death of some of the initially recruited cells and this probably produces more DAMPs⁵⁴. The exact signals that these dying cells release to induce swarming is unclear because neutrophils deficient in single GPCRs including *Fpr1*, *Fpr2*, *Cxcr2* and *P2rx7* could migrate short distances normally when injected into the damaged tissue in this model⁵⁴, and it is likely that a combination of signals are released.

Other secondary signals are also produced by these early-arriving neutrophils. In a model of inflammatory arthritis, LTB₄-activated neutrophils produce chemokines, such as CXCL2, and the cytokine interleukin-1β (IL-1β), which activates tissue endothelial cells, fibroblast-like cells and macrophages to produce more ligands for CXCR1, CXCR2 and CCR1, further amplifying neutrophil recruitment⁵⁸. Activated neutrophils also express matrix metalloproteinases (MMPs), which can cleave CXCL8 family chemokines to increase their chemotactic effect^{59,60}. Collagen in the extracellular matrix can also be cleaved by neutrophil MMP activity to create collagen-derived chemotactic peptides⁶¹.

In addition to early recruited neutrophils, recruited monocytes and macrophages amplify the neutrophil response. Pro-inflammatory monocytes and macrophages can be recruited by initial DAMP signals but neutrophils also recruit these cells through production of macrophage chemokines and through further activation of surrounding non-immune tissue cells, setting off a feed-forward loop of neutrophil recruitment⁶².

Additional levels of signalling in infection

The tight control of neutrophil recruitment after infection is arguably even more important than after tissue injury. While inflammation must be maintained until the infection is cleared, neutrophils can also provide niches within which pathogens can replicate⁶³. Neutrophil swarms have been observed after infection with multiple types of pathogen, including bacteria⁶⁴ and protozoa⁶⁵. Neutrophil infiltration in response to early recruited neutrophil cell death⁵⁴ is likely to be even more pronounced in infections, as many

pathogens can cause necrosis of host cells⁶⁶. Neutrophil recruitment can also be altered by pathogens that carry virulence factors that directly interfere with or promote neutrophil recruitment^{67–69}. Probably one of the most important differences in infections is the presence of PAMPs (pathogen-associated molecular patterns) in addition to tissue DAMPs, creating an extra level of danger signals (reviewed in REF.⁷⁰). Another major variable in infection is the duration of damage, especially if the pathogen is not cleared. These factors lead to a much wider variety of immune cells participating in and modulating neutrophil recruitment including macrophages^{68,71}, dendritic cells⁷², mast cells⁷³ and T cells⁷⁴.

As in a sterile tissue injury, tissue and tissue-resident cells can sense the presence of infection-induced damage and transmit the signal to neutrophils through the production of chemokines. Perivascular macrophages produce CXCL1 and CXCL2 to promote neutrophil extravasation at sites of bacterial infection in mouse ear skin⁶⁸, whereas dendritic cells produce CXCL1 and CXCL2 after a *Leishmania* infection⁷². Mast cells can indirectly recruit neutrophils to a site of infection through the production of tumour necrosis factor (TNF)⁷⁵ or a tryptase⁷⁶, both of which promote chemokine production from tissue cells. Mast cells also directly recruit neutrophils through the production of leukotrienes such as LTB4 and LTC4⁷⁷.

The same amplification of neutrophil recruitment through feed-forward signalling and macrophage-neutrophil-tissue cell crosstalk is also observed after infection. In a mouse model of uropathogenic *Escherichia coli* infection, tissue-resident macrophages (LY6C^{low}) first sense the infection and produce a variety of chemokines, of which CXCL1 is the most important, to recruit both neutrophils and non-resident inflammatory macrophages (LY6C^{hi})⁷¹. However, these inflammatory macrophages then produce TNF to further activate the tissue-resident macrophages to release preformed stores of CXCL2 that further recruit neutrophils to the infection site⁷¹. In *Staphylococcus aureus* infection, early-arriving immune cells phagocytose the bacteria, leading to inflammasome activation by the bacterial cell wall component peptidoglycan and IL-1 β secretion^{78,79}. IL-1 β then activates non-immune tissue cells to produce more pro-inflammatory cytokines and neutrophil chemokines⁸⁰.

The marked involvement of the adaptive immune system has a key role in the persistent neutrophil inflammation that can be induced by infection. In several models, T cells and T cell-produced cytokines are important for neutrophil recruitment to infection, including *Streptococcus pneumoniae*⁸¹ and *Helicobacter pylori*⁸² infections. Many factors contribute to the crosstalk between T cells and neutrophils in the context of infection⁸³, but the involvement of the cytokine IL-17 seems to be particularly important^{84,85}. In a cutaneous model of *S. aureus* infection, resistance to infection depends on the presence of neutrophils⁸⁶, and both $\alpha\beta$ T cells and IL-17-producing T cells have been shown to be important for the neutrophil response⁷⁴. IL-17 stimulates both tissue cells and leukocytes to produce a variety of cytokines, including CXCL8 family chemokines and granulocyte-colony stimulating factor (G-CSF), which promotes the production and maturation of neutrophils from the bone marrow^{87,88}. After infection with *S. aureus*, mice with lowered IL-17 levels due to $\gamma\delta$ T cell deficiency had lowered levels of CXCL1 and CXCL2 production and consequently impaired neutrophil recruitment⁸⁹. In this case, decreased

neutrophil recruitment resulted in poor infection outcome which could be rescued by administration of recombinant IL-17⁸⁹.

Distinguishing an infection from a wound

It is crucial for the host to distinguish whether or not a tissue injury has an associated infection, as this determines both the strength and length of the appropriate response. Responses to infection, such as an *S. aureus* skin infection, can create especially large neutrophil swarms⁹⁰. Exactly how the host integrates information on the persistence of damage and diversity of signals after wounding or infection into differing responses is not clear and this question is relevant for the development of anti-inflammatory therapies that prevent chronic inflammation but retain a sufficient response to infection. Although many DAMPs and PAMPs signal through similar receptors and converge on similar signalling pathways, there is evidence that cells can interpret and respond to these signals differently⁹¹.

A few studies have directly compared the requirements for neutrophil recruitment to a wound versus an infection. In zebrafish larvae, H₂O₂ was found to be required for the early neutrophil response to a wound but not to *P. aeruginosa* or *S. iniae* infections⁹². Moreover, IL-1 β and MYD88-mediated signalling were required for neutrophil responses to a wound but not to *E. coli* infection⁹³. There is also evidence that neutrophils migrate differently to host-produced versus pathogen-produced signals. *In vitro*, human neutrophils retrotaxed after responding to a gradient of LTB₄, whereas the neutrophils were “trapped” after they arrived at a source of the fungal cell wall component zymosan⁹⁴.

One mechanism for the distinction between a wound and an infection involves CD24 and its ability to specifically trap DAMPs (including HMGB1, HSP70, and HSP90) and prevent their binding to receptors^{95,96}. CD24 interacts with Siglec G, which associates with SH2 domain-containing protein tyrosine phosphatase 1 (SHP1), a negative regulator of NF- κ B activation⁹⁷. In this manner, CD24 decreases NF- κ B-mediated activation of tissue-resident cells such as DCs, and thereby reduces the amplification stage of neutrophil recruitment⁹⁷.

It has been proposed that neutrophils have phenotypic heterogeneity and functional plasticity (Box 3); several markers of different neutrophil phenotypes have been identified, although disease associations and functional characteristics of these phenotypes need to be established⁹⁸. Differing signals at a wound or infection probably define the phenotype of the responding neutrophils (either by altering their phenotype after they arrive at the site or by specifically recruiting certain subsets that pre-exist in the circulation). A future challenge will be to determine the distinct neutrophil subsets that exist in different types of damage including ‘sterile’ versus infected tissue and to probe the complex signalling networks that integrate diverse inputs into a specific response.

Box 3

Neutrophil heterogeneity and plasticity

During the past decade, evidence has been accumulating for the existence of neutrophil subsets with phenotypic heterogeneity and functional plasticity in different models^{106,174,175}. Perhaps the most well-characterized example is in the tumour

microenvironment, where neutrophils develop polarity that is similar to the M1/M2 polarization described for macrophages. Tumour-associated neutrophils can be designated also as N1 (anti-tumorigenic and pro-inflammatory) and N2 (pro-tumorigenic and immunosuppressive)¹⁷⁶. Tumour-associated N2 neutrophils are characterized by high expression of CXC-chemokine receptor 4 (CXCR4), vascular endothelial growth factor and matrix metalloproteinase 9 and are induced in the presence of high levels of transforming growth factor- β (TGF β). By contrast, N1 neutrophils express pro-inflammatory cytokines and chemokines, are able to kill cancer cells and are induced by inhibition of TGF β signalling. Analogous neutrophil subsets may exist in damaged or infected tissues, although these phenotypes have not been well characterized. Subsets of neutrophils have also been defined by surface receptor expression and density (low-density neutrophils versus high- or normal-density neutrophils). For example, CXCR4 expression is increased in aged or senescent neutrophils¹⁷⁷ and is associated with neutrophil trafficking to the bone marrow. Other changes in the expression of surface proteins on neutrophils have been described, including CD63 expression in the airways of patients with cystic fibrosis¹⁷⁸, intercellular adhesion molecule 1 expression associated with systemic inflammation and reverse migration¹²⁰ or CD177 expression associated with autoimmune diseases^{174,179}. Low-density neutrophils have been associated mostly with autoimmune disorders¹⁸⁰, sepsis¹⁸¹, HIV infection^{182,183} and cancer¹⁷⁶. In addition to heterogeneity, neutrophil phenotypes can be plastic. For example, neutrophils under specific inflammatory settings have been reported to differentiate into other myeloid cells types⁸³, including dendritic cells (DCs), and purified bone marrow-derived neutrophils can differentiate into a hybrid population characterized by the expression of DC and neutrophil markers after *in vitro* treatment with granulocyte/macrophage colony-stimulating factor, tumour necrosis factor and IL-4, and this has been verified both *in vitro* and *in vivo*¹⁸⁴. It is likely that the environment (pro- or anti-inflammatory as well as the tissue or organ microenvironment) helps to dictate neutrophil differentiation and modulate the specialization and function of these cells.

Reverse migration of neutrophils

In a successful response to an acute injury, it is crucial to prevent tissue damage by promoting the local resolution of inflammation through the removal of neutrophils from the site of injury⁶². This clearance of neutrophils can occur through apoptosis or necrosis and subsequent phagocytosis by macrophages⁹⁹. However, some early evidence suggested that neutrophils at inflamed sites do not necessarily undergo apoptosis. Hughes *et al.* used a rat model of glomerular capillary injury to track the fate of radiolabelled neutrophils and found that 70% of neutrophils that entered inflamed glomerular capillaries were able to return to the main circulation and did not undergo apoptosis at the site of inflammation¹⁰⁰. More recent studies have demonstrated that neutrophils can leave sites of tissue damage in a process termed neutrophil reverse migration which describes the interstitial migration of neutrophils away from inflamed sites. Neutrophils have also been reported to re-enter the vasculature in a distinct process referred to as neutrophil reverse transendothelial migration (rTEM).

Reverse neutrophil migration within tissues away from the site of injury was first directly visualized *in vivo* in zebrafish larvae, in which it was demonstrated that not all recruited neutrophils die at the site of injury and most leave the site¹⁰¹. In subsequent studies using zebrafish, it was shown that neutrophils that leave a wound can reverse transmigrate into the vasculature (rTEM) and traffic to distal sites post-injury^{102,103}. Moreover, Buckley and colleagues described the ability of human neutrophils to reverse transmigrate through an endothelial monolayer *in vitro*, identifying markers characteristic of these reverse-transmigrated neutrophils (ICAM1^{hi}CXCR1^{low}) and found this neutrophil phenotype in the peripheral blood of patients with systemic inflammation¹⁰⁴. Since these early studies, neutrophil reverse migration and/or rTEM has been visualized in multiple models including zebrafish^{105–108}, mice^{109,110} and *in vitro* human neutrophils^{94,111} (Figure 2). *In vitro* studies using microfluidics demonstrated that more than 90% of human neutrophils can reverse their direction away from a chemoattractant and migrate away continually for distances greater than 1,000 μm ¹¹¹. Together, these studies have suggested that reverse migration is a possible mechanism to locally resolve inflammation and a potential novel target for drug therapy in diseases characterized by excessive neutrophil infiltration. However, a caveat is that neutrophil reverse migration may lead to activated neutrophils being redistributed to other locations in the body, contributing to inflammation elsewhere. It is also important to note that many aspects of neutrophil reverse migration remain controversial including: the exact mechanism(s), the fate of reverse-migrated neutrophils and the occurrence of reverse migration in human disease.

Potential mechanisms of reverse migration

The cues that mediate neutrophil reverse migration from sites of tissue damage remain largely unknown. Reverse migration can be confused with the terms fugetaxis or retrotaxis¹¹², mechanisms that may have only a partial role in neutrophil reverse migration *in vivo*. Several mechanisms have been proposed to explain the reverse migration of neutrophils from inflamed tissues, including a competition between chemoattractant sources at the wound and vasculature. A neutrophil may prioritize competing cues through the downregulation of specific chemokine receptors and/or receptor desensitization. It has also been postulated that new chemorepellent cues may be released at the wound to mediate neutrophil reverse movement. Finally, other factors may influence the potential to leave damaged tissues, including decreased levels of wound chemoattractants and/or neutrophil-intrinsic transcriptional changes¹¹³. Neutrophils constantly sense a variety of stimuli and must process and prioritize all of these inputs to determine their behaviour and it is likely that a process as complex as reverse migration occurs through a combination of these mechanisms.

Relatively few studies have shown a direct link between neutrophil reverse migration and specific pathways (Figure 2). One pathway that has been specifically implicated in neutrophil migration out of wounded tissue in larval zebrafish is the hypoxia-inducible factor 1 α (HIF1 α) pathway¹⁰⁵. In addition to reducing neutrophil apoptosis, HIF1 α activation in zebrafish neutrophils increases the retention of neutrophils at the site of tissue injury¹⁰⁵. It is known that *in vitro* chemoattractants such as CXCL8 can act as chemorepellents in higher concentrations¹¹⁴ and therefore neutrophil behaviour might

change as the cell migrates up a chemokine gradient. Data indicating that reverse-migrated human neutrophils have lowered cell-surface expression of CXCR1¹⁰⁴ provides some evidence for the idea that neutrophils might stop responding to chemoattractants at the wound because of receptor internalization or downregulation. Other tissue-resident cells are also implicated in the control of neutrophil reverse migration. In zebrafish, direct contact of neutrophils with macrophages at a wound induces neutrophils to migrate away, and autocrine redox-SFK signalling in macrophages is required for the resolution of neutrophil inflammation¹⁰⁸. This is an especially intriguing observation in the context of patients with chronic granulomatous disease (CGD) and deficient ROS signalling who develop chronic neutrophil inflammation in tissues¹¹⁵.

The relative levels of different lipid mediators can also promote the resolution of inflammation and may be involved in neutrophil reverse migration. As discussed, LTB4 is a neutrophil chemoattractant, but after lipid-mediator class switching pro-inflammatory lipid mediator production pathways are altered to instead produce pro-resolution mediators such as lipoxin A4 (LXA4)^{116,117}. Other pro-resolution lipid mediators include resolvins and protectins and stop the influx of new neutrophils¹¹⁸. They are also implicated in promoting the resolution of existing neutrophil inflammation: LXA4 can enhance the reverse migration of human neutrophils *in vitro* in a microfluidic device¹¹¹.

Besides reverse migrating through tissue away from an injury, neutrophils may also re-enter the circulation through rTEM. Neutrophils that were recruited to a tissue wound can be observed back in the circulation in zebrafish^{102,103}. In mice, in which there are greater challenges to imaging than in zebrafish, the Nourshargh laboratory has used “disturbed” neutrophil TEM after ischemia-reperfusion injury as a model for rTEM^{110,119}. In this model, junctional adhesion molecule C (JAM-C) at endothelial cell junctions modulates “complete” TEM and a decrease in the levels of JAM-C expression after injury increases the occurrence of rTEM¹¹⁰. Moreover, LTB4 stimulates the production of neutrophil elastase, which cleaves JAM-C, increasing rates of rTEM¹¹⁹, suggesting that LTB4 may be involved in both forward and reverse transmigration to damaged tissue.

What determines whether neutrophils reverse migrate or die at the site of damage? Different neutrophil subsets might be predisposed to reverse migrate from the time they leave the circulation or due to transcriptional changes induced at the injury site^{98,120} (see Box 3). *In vitro*, neutrophils that encounter the fungal cell wall component zymosan have decreased reverse migration^{94,111}, and it is tempting to speculate that neutrophils are less likely to reverse migrate away from sites of infection, at least in part to prevent the dissemination of intracellular pathogens.

It has been proposed that neutrophils do not actively undergo directed reverse migration, but they are simply randomly redistributed away from the wound. By simulating neutrophil behaviour with different mathematical models based on neutrophil migration measurements made in zebrafish, several studies suggested that the migration of neutrophils away from a wound could be best described by a diffusion process rather than a fugetaxis process^{107,121,122}. Therefore reverse migration of neutrophils might be a result of a loss of sensitivity towards recruitment and/or retention signals rather than active repulsion induced

by the release of chemorepellent from the wound or active attraction due to chemoattractants in the blood or vascular endothelial cells. Overall, the mechanisms controlling reverse migration are still not clear. It is likely that multiple processes contribute to the migration of neutrophils away from a site of inflammation and back into the circulation, possibly combined in a reverse migration cascade.

What is the phenotype and fate of reverse-migrated neutrophils?

Do reverse-migrated neutrophils have a different phenotype compared to “naïve” neutrophils? Neutrophils in peripheral tissues are known to be more active transcriptionally and translationally than their counterparts in the circulation¹²³. Neutrophils that have undergone rTEM *in vitro* were found to express specific markers: ICAM1^{hi} and CXCR1^{low}^{104,110}, and these markers have been used to identify neutrophils that had presumably undergone rTEM in peripheral human blood¹⁰⁴ and in secondary sites of inflammation in mice¹¹⁰. In addition, human neutrophils that have undergone rTEM *in vitro* are less susceptible to apoptosis and produce more ROS¹⁰⁴, and mouse neutrophils expressing rTEM markers *in vivo* also produce higher levels of ROS¹¹⁰. By contrast, although zebrafish reverse-migrated neutrophils (not necessarily neutrophils that underwent rTEM) were found to have an activated morphology, they did not have altered ROS production or response to a secondary insult, whether a tissue wound or an infection¹⁰⁶. There is no clear evidence, therefore, that all reverse-migrated neutrophils display a pro- or anti-inflammatory phenotype. These neutrophils may in fact have varying fates or phenotypes according to where and how they are recruited out of the blood into tissues in the first place and what they encounter at the injury site.

Where do neutrophils go after reverse migrating out of the tissue? Zebrafish neutrophils can be found in tissues or the circulation for at least two days after leaving a wound¹⁰². The fate of these neutrophils is important for patients, as systemic inflammation after severe trauma can lead to multiple organ failure¹²⁴. Recently, human patients with acute pancreatitis that developed acute lung injury were found to have a higher level of neutrophils carrying rTEM markers in their circulation¹²⁵. In a mouse model of acute pancreatitis, increasing the level of neutrophils carrying rTEM markers in the circulation and pulmonary vasculature through genetic deletion of JAM-C increased the severity of lung injury and systemic inflammation¹²⁵. Similarly, after ischemia-reperfusion injury in mice, neutrophils with rTEM markers were found re-localized in the lungs¹¹⁰ and experimentally increasing the rates of rTEM resulted in increased neutrophil re-localization and organ damage in the lungs, liver and heart¹¹⁹.

Neutrophils have also been shown to leave inflamed or infected sites, travel through the lymphatics and re-localize to the lymph nodes^{126–128} or bone marrow¹⁰⁹ to affect host defense. These neutrophils can shuttle live pathogens to lymph nodes¹²⁷ and modulate lymphocyte proliferation^{109,126}. Neutrophil migration from inflamed skin to lymph nodes depends on CD11b and CXCR4, and CD11b deficiency in neutrophils decreases T cell proliferation in draining lymph nodes¹²⁶. The transport of antigen from intradermally injected modified vaccinia Ankara virus to the bone marrow by neutrophils can also initiate the expansion of viral-targeting memory CD8⁺ T cells¹⁰⁹. Much more work needs to be

done to further clarify the phenotype and fate of reverse migrated neutrophils and their implications for human disease.

Neutrophil migration: a drug target

The failure to appropriately resolve inflammation can have disastrous effects. Excessive and persistent infiltration of neutrophils into tissues has a role in multiple inflammatory diseases, including rheumatoid arthritis¹²⁹, pulmonary fibrosis¹³⁰ and multiple organ failure¹²⁴, making neutrophil behaviour a potential target for drug therapies. One concern with anti-inflammatory therapies is achieving the resolution of inflammation at a specific site without causing systemic immunosuppression. Another major goal is the identification of molecular targets that distinguish infection from wound-induced inflammation so as to not impair the body's ability to fight invading pathogens. Drugs that induce neutrophil apoptosis or necrosis at an injury site would reduce neutrophil numbers but the clearance of dead cells might increase overall local inflammation. Therefore, both neutrophil forward and reverse migration are attractive targets for anti-inflammatory therapies. We discuss exciting new targets in these pathways below and highlight drugs that are in the clinical trial pipeline in Table 1.

Targeting forward migration

To inhibit neutrophil migration to a site of damage, neutrophil motility machinery, primary signals that recruit neutrophils or the amplification stage of inflammation could be targeted. Colchicine, a microtubule inhibitor, is a known inhibitor of neutrophil chemotaxis and impairs neutrophil recruitment to sites of inflammation *in vivo*. It is used to treat human conditions including gout, chronic pericarditis and familial Mediterranean fever¹³¹. To target neutrophil recruitment signals, the early phase of signalling may be more promising as the amplification phase involves the activation of multiple complex pathways. Conversely, inhibiting specific pathways in the amplification phase could fine-tune the neutrophil response without completely eliminating it. Several clinical trials are currently assessing the effect of CXCR1 and CXCR2 antagonists and inhibitors (Table 1) on inflammatory conditions ranging from cancer to autoimmune diseases (clinicaltrials.gov), and some have reported promising results^{132,133}. However, chemokine receptor antagonist therapy has failed in the past due to differences between animal models and human patients and redundancy of the targets¹³⁴. Other clinical trials are targeting lipid mediator-induced inflammation with LTB4 receptor antagonists. Resolvins are an exciting class of molecules as they can inhibit neutrophil transmigration *in vitro*¹³⁵, and two different resolvins, including chemically synthesized molecules, can significantly decrease the number of neutrophils at a site of damage at least partially through decreased levels of neutrophil chemokines including CXCL1 and LTB4^{135,136}.

Targeting reverse migration

An alternative approach to decrease chronic inflammation is to promote neutrophil reverse migration from local sites of damage. Resolvins and protectins may also resolve inflammation by promoting the removal of leukocytes from injured tissue sites¹³⁷. A drug screen in zebrafish yielded a novel drug known as tanshinone IIA, which is derived from a

Chinese medicinal herb and seems to accelerate apoptosis of neutrophils and promote their reverse migration¹⁰⁷. However, targeting this mechanism raises several concerns, including the fate and activation state of reverse migrated neutrophils. Disseminated neutrophils exhibiting a strong pro-inflammatory or anti-inflammatory phenotype could have deleterious effects such as promoting distal organ damage or systemic immunosuppression, respectively. A better characterization of reverse migrated neutrophils *in vivo* is necessary before further advances in the development of therapies that target this mechanism.

Future perspectives

Remarkable progress has been made in the past decade in understanding the mechanisms that regulate neutrophil migration, including the cells and signals that attract neutrophils to sites of either tissue injury or infection. However, neutrophil reverse migration remains an understudied phenomenon and future work should focus on several key questions to drive the development of new therapies for neutrophil inflammatory disorders. First, the question of whether neutrophil reverse migration is “good” or “bad” for inflammation outcome is still largely unanswered. Although excessive and prolonged neutrophil infiltration can lead to the development of chronic tissue inflammation, the migration of neutrophils back into the circulation might cause systemic inflammation and tissue damage at distal organs. It is likely that the answer to this question depends on the nature, magnitude and location of the inflammation. In the case of infection, premature neutrophil reverse migration could lead to failure to clear the infection and dissemination of intracellular pathogens to other sites. In the case of tissue injury, reverse migration might have positive effects at the local site of inflammation, as the depletion of neutrophils can improve wound healing^{138,139}, but may have negative effects systemically such as multiple organ failure. Another issue is the diversity of neutrophil phenotypes (Box 3). In cancer models, it has been proposed that, similarly to macrophages, neutrophil phenotypes can exist on a spectrum ranging from pro-inflammatory, anti-tumour “N1” neutrophils to anti-inflammatory, pro-tumour “N2” neutrophils. Does a similar plasticity exist at sites of tissue damage or infection? Does the phenotype of the neutrophil have any role in its ability to stay at the injury site or reverse migrate away? In cases of chronic inflammation, is it possible to specifically promote the reverse migration of N1 neutrophils while leaving pro-resolving N2 neutrophils at the wound? Or is it the reverse migration specifically of these N1 neutrophils that can cause systemic inflammation and distal organ damage? Does the outcome of an injury re-programme reverse migrating neutrophils to relay specific messages to different locations in the body? Although many unanswered questions remain, it is clear that neutrophil migration has a crucial role in inflammatory pathology. Fortunately, recent models and technologies have been developed that will enable the further elucidation of forward migration signals and the role of reverse migration in the pathology of inflammatory diseases.

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Glossary

Reactive oxygen species (ROS)

Chemically reactive molecules containing oxygen that, when produced in large amounts, have pro-inflammatory and antimicrobial effects. Physiological levels of ROS have been shown to regulate cellular signalling pathways.

Neutrophil extracellular traps (NETs)

Fibrous networks that are released into the extracellular environment by neutrophils. They are composed mainly of DNA, but also contain chromatin and proteins from neutrophil granules. NETs act as a mesh that traps microorganisms and exposes them to neutrophil-derived effector molecules.

DAMPs

Cues that are derived from stressed, damaged or dead cells. These factors are highly diffusible through tissues and can be either protein-derived or non-protein-derived.

PAMPs

Molecules that are derived from pathogens such as bacteria, fungi or viruses; they are highly diffusible through tissues and can be either protein-derived or non-protein-derived.

Reverse neutrophil migration

The movement of neutrophils away from injured tissues within the interstitium.

Reverse transendothelial migration

The movement of neutrophils back into the vasculature after being in tissues.

Fugetaxis

Also known as retrotaxis. The movement of cells away from a source of chemoattractant *in vitro*.

Biographies

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Key Points

- Complementary models have been developed to study neutrophil migration, including microfluidics and live imaging using mice and zebrafish.
- Neutrophil migration in response to injury or infection occurs in phases: early recruitment, amplification and resolution.
- Early-recruited neutrophils modulate the amplification phase both directly and indirectly, through the activation of tissue and tissue-resident cells, producing sustained signals such as the CXC-chemokine ligand 8 family chemokines.
- Activated neutrophils at a site of inflammation do not necessarily undergo apoptosis but in some circumstances might undergo reverse migration away from the site of damage (reverse neutrophil migration) and/or re-enter the circulation (reverse transendothelial migration (rTEM)).
- Neutrophil forward and reverse migration may be attractive targets for anti-inflammatory therapies.

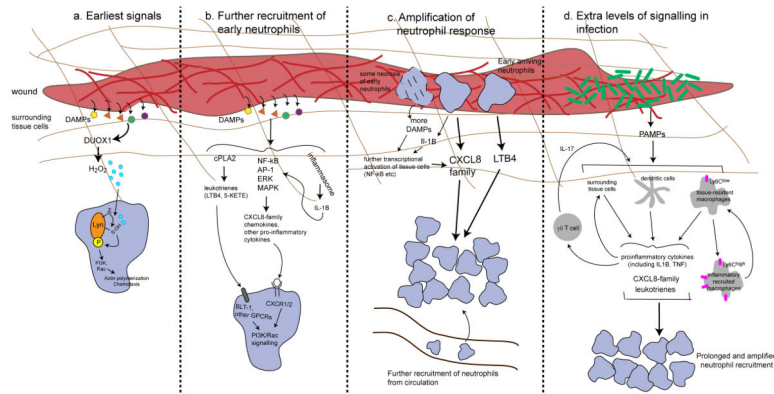


Figure 1. The phases of neutrophil recruitment

The recruitment of neutrophils to a site of damage occurs in several phases. Damage-associated molecular patterns (DAMPs) are released at a tissue injury site and promote the release of hydrogen peroxide (H_2O_2) and direct the recruitment of early-arriving neutrophils through the SRC family kinase LYN (a). DAMPs also induce the production of CXC-chemokine ligand 8 (CXCL8) family chemokines and leukotrienes from surrounding tissue cells to further recruit neutrophils (b). Early-arriving neutrophils are then themselves activated to both directly and indirectly promote further secretion of CXCL8 family chemokines and leukotriene B4 (LTB4) to induce neutrophil recruitment from the circulation and amplification of the response (c). In an infection, extra layers of signalling exist to prolong and amplify neutrophil infiltration, including the release of pathogen-associated molecular patterns (PAMPs) and the involvement of other recruited immune cells, such as macrophages, dendritic cells (DCs) and T cells (d). IL, interleukin; PI3K, phosphoinositide 3-kinase; TNF, tumour necrosis factor.

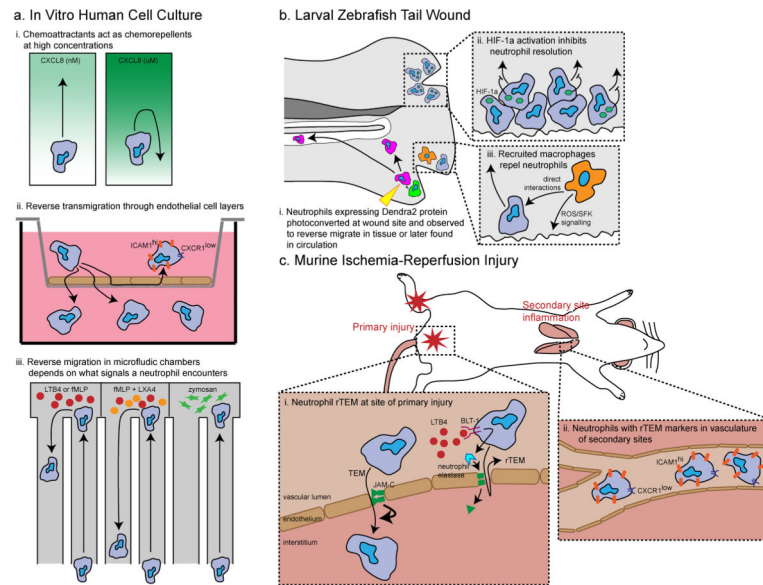


Figure 2. Mechanisms that may be involved in neutrophil reverse migration

Several models for reverse migration have been developed: *in vitro* microfluidic assays or transwell assays (a), larval zebrafish wounding (b), and mouse ischemia-reperfusion injury to model reverse transendothelial migration (rTEM) (c). These studies have reported mechanisms that regulate both neutrophil reverse migration in interstitial tissues and rTEM.

a. Chemoattractants, such as CXC-chemokine ligand 8 (CXCL8), can act as chemorepellents at high concentrations *in vitro*, referred to as fugetaxis (i). Reverse neutrophil transmigration through endothelial cell monolayers has been reported; reverse transmigrated neutrophils have been characterised by high expression of intercellular adhesion molecule 1 (ICAM1) and low expression of CXC-chemokine receptor 1 (CXCR1) (ii). *In vitro* analysis in microfluidics has identified factors that regulate neutrophil forward and reverse migration (iii). The pro-resolving lipid mediator lipoxin A4 (LX4A) induces neutrophil reverse migration whereas zymosan induces neutrophil trapping. **b.** The reverse migration and rTEM of neutrophils has been visualized in larval zebrafish tail wounds using photoconversion (i). The activation of hypoxia-inducible factor 1 α (HIF1 α) in zebrafish neutrophils inhibits neutrophil reverse migration (ii), whereas the migration of macrophages to the wound via reactive oxygen species (ROS)-SRC family kinase (SFK) signalling and their direct interaction with neutrophils promotes neutrophil reverse migration (iii). **c.** A mouse ischemia-reperfusion injury model is used to model neutrophil rTEM or “hesitant” TEM. In this model, junctional adhesion molecule C (JAM-C) on endothelial cells modulates “complete” TEM and leukotriene B4 (LTB4) induces neutrophil elastase expression, which cleaves JAM-C, leading to an increase in rTEM (i, adapted from Colom et al, *Immunity*, 2015). Increased rTEM in this model leads to higher numbers of neutrophils with rTEM markers (ICAM1^{hi} CXCR1^{low}) at secondary sites such as the lungs (ii).

Table 1

Drugs in current clinical trials that specifically target neutrophil migration signals

Drug name	Target	Disease or model	Stage	Reference
SCH 527123	CXCR2	Severe asthma, allergen-induced asthma, COPD, psoriasis and colon cancer	Phase I–II	185
Reparixin	CXCR1 and CXCR2	Ischemia-reperfusion injury; lung, pancreatic islet and kidney transplantation, and breast cancer	Phase II–III	186
DF 2156A	CXCR1 and CXCR2	Active bullous pemphigoid	Phase II	-
AZD-8309	CXCR1 and CXCR2	LPS-induced airway inflammation	Phase I	187
SB-656933	CXCR2	Ulcerative colitis, cystic fibrosis and ozone airway inflammation	Phase I–II	132
GSK1325756	CXCR2	Influenza virus infection and healthy volunteers	Phase II	188
AZD5069	CXCR2	Healthy volunteers and LPS-induced airway inflammation	Phase I	189 190
QBM076	Not known	COPD	Phase II	-

COPD, chronic obstructive pulmonary disease; CXCR, CXC-chemokine receptor; LPS, lipopolysaccharide. More details at clinicaltrials.gov.