A Neutrophil-Centric View of Chemotaxis

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Abstract

Neutrophils are key players of the innate immune system, that are involved in coordinating the initiation, propagation and resolution of inflammation. Accurate neutrophil migration (chemotaxis) to sites of inflammation in response to gradients of chemoattractants is pivotal to these roles. Binding of chemoattractants to dedicated G protein coupled receptors initiates downstream signalling events that promote neutrophil polarisation, a prerequisite for directional migration. We provide a brief summary of some of the recent insights into signalling events and feedback loops that serve to initiate and maintain neutrophil polarisation. This is followed by a discussion of recent developments in the understanding of *in vivo* neutrophil chemotaxis, a process that is frequently referred to as 'recruitment' or 'trafficking'. Here, we summarise neutrophil mobilisation from and homing to the bone marrow, and briefly discuss the role of glucosaminoglycan-immobilised chemoattractants and their corresponding receptors in the regulation of neutrophil extravasation and neutrophil swarming. We furthermore touch on some of the most recent insights into the roles of atypical chemokine receptors in neutrophil recruitment, and discuss neutrophil reverse (transendothelial) migration together with potential function(s) in the dissemination and/or resolution of inflammation.

List of abbreviations

ACKR – atypical chemokine receptors

COPD – chronic obstructive pulmonary disease

DAG – diacylglycerol

fMLF – N-formylmethionine-leucyl-phenylalanine

GAG – glycosaminoglycans

GAP – GTPase activating protein

G-CSF – Granulocyte colony stimulating factor

GEF – guanine nucleotides exchange factor

GPCR – G protein coupled receptor

GTPase – Guanosine trisphosphatase

IP3 – inositol trisphosphate

LTB4 – leukotriene B4

MTOC – microtubular organising centre

PI3K – phosphoinositide 3-kinase

PIP3 – phosphatidylinositol-(3,4,5)-trisphosphate

PLC – phospholipase C

PTEN – phosphatase and tensin homologue

RasGRP – Ras guanyl releasing protein

SHIP – SH2-containing inositol phosphatase

Introduction

Chemotaxis is defined as directed cell migration in response to a gradient of a chemical stimulus, with migration occurring towards a chemoattractant, or away from a chemorepellent. Chemotaxis is critical during embryonic development, where it promotes morphogenetic movements in response to growth factor receptor-mediated gradient sensing by directional coordinated, collective cell migration. Examples of collective developmental chemotaxis include the migration of neural crest cells and the angiogenic sprouting of blood vessels towards growth factors [1]. In contrast, single cell chemotaxis provides a tightly controlled mechanism throughout life by which immune cells are recruited, usually in response to G protein coupled receptor (GPCR) stimulation by chemoattractants.

Chemotaxis has fascinated scientists for decades. Single cells are more amenable to *in vitro* analysis than embryos. Neutrophils, the most abundant circulating leukocytes in man, are short-lived immune cells of the granulocyte lineage. Neutrophils can produce reactive oxygen species and degranulate, releasing cytotoxic products. Combined with their ability to phagocytose and kill ingested microorganisms or to release chromatin-rich extracellular traps, neutrophils provide a first line of defense against bacterial and fungal infections. They are also key effectors in the inflammatory response (for general reviews [2, 3]). Neutrophils are highly motile and migrate as single cells with exquisite speed and directionality in response to chemotattractant stimulation. Chemotaxis *in vivo* is essential for many of the neutrophil's functions throughout its lifetime.

Experimental models of neutrophil chemotaxis

The highly motile primary neutrophil is very short-lived and not amenable to culture, transfection or transduction. Although they chemotax very well, freshly purified human

93 neutrophils are therefore not frequently used for chemotaxis experiments. Alternative models
94 that are not always representative of all facets of neutrophil functions are usually used
95 instead. Freshly isolated (often bone marrow-derived) neutrophils from mice that carry
96 genetic alterations of interest are frequently the model of choice. Mice, or indeed zebrafish,
97 offer in addition the opportunity to investigate neutrophil trafficking *in vivo.* Alternative more
98 tractable alternatives to primary neutrophils for the study of single cell chemotaxis *in vivo*
99 include cultured cells that can be differentiated to become neutrophil-like (e.g. HL-60), and
100 the social amoeba *Dictyostelium discoideum*. Over time, primary neutrophils obtained from
101 knock-out mouse models, transfected cell lines, and genetically modified *D. discoideum*
102 strains in combination with *in vitro* chemotaxis chambers have helped to decipher many
103 facets of the molecular regulation of chemotaxis [4]. Aided by increasingly powerful
104 intravital microscopy, in recent years such *in vitro* studies and relatively straightforward *in*
105 *vivo* recruitment assays have been supplemented with *in situ* observations of neutrophil
106 recruitment. Some of the recent advances in the understanding of the molecular regulation of
107 neutrophil chemotaxis *in vitro* and insights into the molecular control of neutrophil
108 trafficking *in vivo* are discussed here.
109
110 **Chemotaxis as a specialised form of cell migration**
111 In chemotaxis, receptor-mediated chemoattractant gradient sensing promotes cell polarisation
112 and thereby directional cell migration. General features of cell migration have been reviewed
113 in depth elsewhere [5, 6]. *In vitro* cell migration occurs by different modes, depending on
114 whether the cells are on a two-dimensional substrate or within a three-dimensional matrix. In
115 the first instance, cells adopt a flattened shape, form integrin-based adhesions to the
116 substratum, use actin-mediated propulsion led by a lamellipodium at their front, and are
117 characterised by a trailing end [7]. In the latter case, rather than relying on integrin-based

adhesions [8], neutrophils migrate in a frequently non-proteolytic, amoeboid fashion and depend on actin-mediated protrusions and myosin II-mediated contractions to propel themselves through a three-dimensional matrix [9]. *In vivo*, integrin-dependent steps involve the breaching of barriers such as the vessel wall, whereas interstitial migration is thought to be integrin-independent. 'Amoeboid' neutrophil migration in the interstitium involves the selection of a path of least resistance, with neutrophils probing for gaps that permit passage of their multilobular nuclei. Interestingly, leukocytes undergoing amoeboid chemotaxis exhibit a typical microtubular organising centre (MTOC) position behind or, in the case of the neutrophil, in between nuclear lobes. Amoeboid cell migration contrasts with the much slower polarised 'mesenchymal' cell migration (that is exemplified by fibroblasts), which is characterised by MTOC and Golgi apparatus polarisation in front of the nucleus [10-12]. An elegant recent study that employed chemotactic mazes with channels of different sizes demonstrated that the MTOC is a good indicator of the directional choice (or dominant pole) of chemotaxing leukocytes [13]. Resting neutrophils are comparatively devoid of microtubules, with chemoattractant stimulation causing microtubular polymerisation. Interestingly, neutrophil chemotaxis on two dimensional matrices or elastase-depending invasion, but not transendothelial migration or crawling on immobilised chemoattractants was shown to depend upon polymerisation of microtubules [14].

Chemoattractant sensing by G protein coupled receptors

Chemoattractants bind G protein coupled cell surface receptors (GPCRs) which usually signal through $G\alpha_{i/0}$ containing heterotrimeric G proteins (reviewed in [15]). Although there is some promiscuity, many chemoattractants have dedicated receptors. Several classes of chemoattractants are known to act on neutrophils. They comprise lipids [e.g. leukotriene B4 (LTB4)], formylated peptides of bacterial or mitochondrial origin [e.g. N-formylmethionine-

leucyl-phenylalanine (fMLF) which is frequently used *in vitro*], protein fragments (e.g. C5a and C3a complement fragments) and classical chemokines, which are classed according to their conserved cysteine residues into CC and CXC groupings. Table 1 provides a summary of some major neutrophil chemoattractants together with their receptors. Many chemokines can bind to extracellular glycosaminoglycans (GAGs) expressed by endothelial cells (or outside of the vasculature to the extracellular matrix). This serves to essentially immobilise the gradient, which is important, for example in the context of blood flow [16, 17]. The directional cell movement on immobilised chemoattractants is sometimes referred to as 'haptotaxis'. Experiments involving the simultaneous application of several chemoattractants *in vitro* established chemoattractants to exist in a hierarchy, with 'end-target' attractants (e.g. fMLP or C5a) overruling intermediary chemoattractants (e.g. LTB4). Unsurprisingly, the signalling pathways employed by intermediary and end-target chemoattractants are non-identical [18, 19].

Molecular events regulating neutrophil polarisation.

Chemoattractant-sensing GPCRs are distributed uniformly on the neutrophil's surface. Both directional and indeed uniform chemokine receptor stimulation of neutrophils in a dish causes them to polarise, that is to say, adopt the morphology of the migrating cell described above, prior to actually migrating in a directional, or random fashion by chemotaxis or chemokinesis, respectively (Fig 1 for a simplified view of a polarised neutrophil).

On a molecular level, chemoattractant binding induces the G protein coupled chemoattractant receptor to undergo a conformational change that allows it to activate heterotrimeric G proteins, exchanging GDP for GTP on the Gα subunit. This in turn induces the release of the Gβγ subunits, so that both Gα-GTP and Gβγ can activate downstream effectors, including

168 phospholipase C (PLC) β via Gα and Gβγ as well as agonist-activated phosphoinositide 3-
169 kinase (PI3K) by Gβγ subunits [20, 21]. Four agonist-activated PI3Ks are expressed in the
170 neutrophil, PI3Kα, PI3Kβ, PI3Kδ and PI3Kγ [22]. Of these, PI3Kγ is activated directly by
171 Gβγ in concert with Ras-GTP [23], with Ras being activated downstream of PLCβ by
172 RasGRP4 [24]. Both PLCs and agonist activated PI3Ks are well known regulators of
173 phosphoinositides, lipid components of cellular membranes, with PLCs catalysing plasma
174 membrane phosphatidylinositol(4,5)bisphosphate [PI(4,5)P2] hydrolysis to generate inositol
175 trisphosphate (IP3) and diacylglycerol (DAG), whereas PI3Ks phosphorylate PI(4,5)P2 in the
176 3' position, generating the lipid second messenger phosphatidylinositol(3,4,5)trisphosphate
177 (PIP3). Several PI3K isoforms are thought to be involved in chemotaxis, likely at least in part
178 because the individual PI3K isoforms cross-talk extensively [25]. The PI3K pathway
179 provides the mechanistic backdrop to the well-documented PIP3 polarisation to the leading
180 edge of polarised neutrophils, neutrophil-like cells and dictyostelium [26-28]. Many PI3K
181 effectors are regulators of small GTPases, in particular guanine nucleotide exchange factors
182 (GEFs) and GTPase activating proteins (GAPs). These PIP3-responsive regulators of small
183 GTPases in polarised neutrophils together promote actin-dependent protrusion, for example
184 by activating Rac1/2 and Arf6 and inactivating RhoA at the cell's pseudopod (reviewed in
185 [29, 30]). Research by many groups into the function of PI3K/PIP3, and into individual PI3K
186 isoforms in chemotaxis resulted in somewhat contradictory reports. Taken together, this large
187 body of work suggests that individual PI3K isoforms, in particular PI3Kγ/δ, regulate
188 chemokinesis and/or chemotaxis in an assay-, substratum- and in the case of human
189 neutrophils likely also priming-dependent fashion [31-38]. *D. discoideum* cells were shown
190 to be able to chemotax poorly even in the absence of any PI3K isoform [39]. Human
191 neutrophils from chronic obstructive pulmonary disease (COPD) patients and the elderly
192 were characterised by excessive PI3K activity and poor chemotactic directionality, and could

be rescued with low concentrations of inhibitors of the leukocyte-specific PI3Kγ/δ that partially inhibited these enzymes [40, 41]. This suggests that a 'goldilocks principle' applies in chemotaxis, whereby too much PI3K activity may be just as disruptive as too little.

Neutrophil polarisation involves players including the above discussed PI3Ks and their effectors, as well as PIP3 phosphatases. Amongst the numerous phosphoinositide phosphatases that are expressed by leukocytes, the 3' phosphatase PTEN and the 5' phosphatase SHIP are best understood; both were shown to regulate chemotaxis [18, 26, 42]. PTEN-mediated regulation was found to be rather context-dependent, with it being suggested to control chemotaxis in the presence of two opposing gradients, and in distinguishing between end-point and intermediate point chemoattractants [18]. In contrast, SHIP-deficient neutrophils were extremely spread and failed to polarise or chemotax effectively [26, 42]. Further important contributions are likely regulated by feed-back loops. For instance, Rho GTPases, actin polymerisation and PIP3 polarisation at the cell's front act in one such feed-back loop [43-46]. Likely driven by PIP3-dependent Rac GEFs such as P-Rex1/2 and DOCK2 [29], Rac activation has been shown to maintain neutrophil polarity through Hem-1, which assists in polarising the neutrophil by facilitating actin polymerisation and excluding myosin activity at the front of the cells, whilst also promoting Rac activity at the front in a positive feedback loop [47]. The RhoA and Arf6 GAP ARAP3 is being recruited to the plasma membrane in a PIP3 dependent fashion, regulating persistent PIP3 polarisation and chemotactic directionality [48].

In the presence of uniform chemoattractant, neutrophils polarise randomly. Membrane tension is one factor that has been shown to be involved in the regulation of such neutrophil polarisation. Leading edge protrusions generate strong membrane tensions, thus inhibiting the

formation of secondary protrusions elsewhere in the neutrophil, and maintaining persistent polarisation [49]. In neutrophils that make contact with the substratum, a further regulatory input stems from altered membrane curvature. This is thought to be sufficient to break the symmetry of the non-polarised neutrophil, establishing cytoskeletal back polarisation in the adhering neutrophil in a PI4P, SRGAP2 and PIP5K1C90-dependent fashion [50].

In vivo neutrophil chemotaxis (trafficking)

Neutrophils migrate to new locations at least twice, and potentially more during their short lives. All neutrophil trafficking events have their regulation by chemoattractant-mediated chemotaxis in common. The remaining part of this minireview summarises some of the recent insights into *in vivo* neutrophil chemotaxis in a range of situations (see Fig 2 for a schematic diagram).

Neutrophil chemotaxis during mobilisation and homing

Neutrophil differentiation from progenitors occurs in the bone marrow, with 10^7 and 10^{11} neutrophils released into the circulation each day in mouse and human, respectively. The regulation of neutrophil release into the circulation has been elucidated with the help of genetically modified mice. Immature and mature neutrophils are retained in the bone marrow by CXCR4 chemokine receptor expression that is responsive to CXCL12 produced by bone marrow stromal cells. The major mobilising cytokine G-CSF causes downregulation of CXCR4 on neutrophils and of CXCL12 in the bone marrow [51], as well as upregulation of CXCR2. With the CXCR2 agonists CXCL1 and CXCL2 constitutively expressed by bone marrow endothelium, these changes drive neutrophil mobilisation from the bone marrow to the circulation [52].

Circulating neutrophils under homeostatic conditions are short-lived, persisting in the circulation for only one day before becoming senescent. Senescent neutrophils upregulate CXCR4, which increases their sensitivity to CXCL12 that is expressed in the bone marrow. In this way, senescent neutrophils are recruited or 'home' back to the bone marrow, where they undergo apoptosis for clearance by stromal macrophages [53, 54]. Interestingly, both neutrophil release into the circulation and clearance of senescent neutrophils occur in a circadian rhythm, providing immunity while protecting the host [55, 56].

Neutrophil recruitment to inflammatory sites – extravasation

As the first circulating immune cells to be recruited to sites of inflammation, neutrophils present a first line of cellular defense against infections. The initial step of neutrophil recruitment from the circulation into the inflamed tissue is best understood in the inflamed cremaster muscle, a site that is particularly amenable to intravital microscopy. Initially, circulating, non-adhesive neutrophils form L-selectin and β2 integrin-mediated interactions on the luminal face of the wall of post-capillary venules [reviewed in [57]]. This is induced by cytokine production (e.g. TNF) by resident macrophages, which in turn causes upregulation of adhesion molecules (P- and E-selectins and integrin ligands including ICAM1) as well as chemokines by the endothelium. Selectin-mediated interactions cause neutrophil rolling along the endothelium, allowing neutrophil interactions with chemokines to take place. Additional chemokine stimulation drives integrin activation and in turn integrin-mediated neutrophil adhesion to the endothelial surface. Immobilisation of the chemokines to the luminal face of the endothelium occurs due to CXCL1/CXCL2/CXCL8 binding to GAGs, carbohydrate moieties that are expressed on the endothelial cell surface. In some circumstances, GAGs not only bind, but transcytose chemokines [16, 17, 58]. GAG chemokine immobilisation efficiency is chemokine-dependent, with chemokine

immobilisation avoiding chemokine diffusion despite the blood flow in the vessel. In this way, GAG-dependent chemokine presentation ensures that rolling, but not circulating neutrophils are activated while at the same time directing neutrophils to extravasate at specific sites [59].

Neutrophil swarming

Neutrophil-mediated amplification of a chemotactic gradient by neutrophil-mediated release of 'intermediate-target' chemoattractants (such as the lipid mediator LTB4, which was to be found stored in exosomes) can be induced by 'end target' chemoattractants such as C5a, bacterial formylated peptides, and cell death, which leads to the release of formylated mitochondrial proteins [60, 61]. This autocrine-paracrine chemoattractant signal amplification loop causes directional collective neutrophil recruitment ('swarming') in response to the activation of a leading neutrophil (Fig 2). By generating LTB4, the leading neutrophil instigates BLT1-mediated activation of the following neutrophils, which in turn generate more LTB4 [62, 63]. Interestingly, microlesions, such as those caused by the death of individual parenchymal cells have recently been shown to be shielded by resident macrophages. This neatly avoids a neutrophil swarming response to the released formylated peptide and concomitant bystander host injury caused by neutrophil-derived inflammation [64].

Neutrophil recruitment to inflamed sites by series of chemoattractants

In recent years it has come to be recognised that neutrophil recruitment to sites of inflammation *in vivo* is regulated by a hierarchical series of chemoattractants. This principle has been shown to hold true in several in models of sterile inflammation and injury. It is illustrated for example by neutrophil recruitment to the inflamed joint of mice in the K/BxN

serum transfer model of rheumatoid arthritis. A series of elegant studies performed over a number of years that combined mouse genetics and lately multiphoton intravital signalling elucidated the sequential action of neutrophil chemoattractants in this disease model. Hence, the deposition of immune complexes on the surface of the joint triggers the alternative complement pathway, precipitating C5a generation and subsequent C5a deposition on the luminal surface of the joint vasculature, where it is immobilised in a GAG-mediated fashion. C5a binding to its receptor C5aR1 promotes β2 integrin activation, causing neutrophils to arrest, spread and crawl on the joint endothelium. C5a also causes neutrophil-driven amplification of the chemotactic gradient by releasing LTB4, and in turn promoting BLT1-mediated extravasation into the joint tissue by autocrine-paracrine positive feedback loop. Here, immune complex-mediated FcγR stimulation causes neutrophils to release IL-1β. This in turn induces the generation of endothelial cell- and synovial fibroblast-derived CCR1 and CXCR2 chemokine receptors ligands. CCR1 promotes neutrophil crawling on the joint endothelium with neutrophil-generated CXCL2 orchestrating CXCR2-dependent amplification of neutrophil recruitment to the joint [65-68].

Optimisation of neutrophil directional migration by atypical chemokine receptors

In addition to G protein coupled chemokine receptors with signalling function, leukocytes and stromal cells also express atypical chemokine receptors (ACKRs; table 1; Fig 2), which do not signal through heterotrimeric G proteins. ACKRs are also known as scavenger or decoy receptors, since some internalise and degrade chemokines, essentially functioning as sinks to limit excessive inflammation [69, 70]. For example, ACKR2 was shown to limit inflammation by reducing neutrophil directional migration to inflammatory chemokines by competing for CCR1 ligands in a neutrophil autonomous fashion [71].

Neutrophil non-autonomous mechanisms also employ decoy receptors to finely tune neutrophil migration. Unlike other atypical chemokine receptors, ACKR1 optimises leukocyte extravasation by internalising and transcytosing chemokines [72, 73]. Some of the latest studies in this area have coupled high resolution intravital imaging with genetics to demonstrate how atypical chemokine receptors optimise neutrophil recruitment to inflamed sites. Two atypical chemokine receptors were shown to jointly regulate neutrophil recruitment to the inflamed joint in K/BxN serum transfer arthritis. Hence, C5aR2, an atypical C5aR expressed by endothelial cells transports tissue-derived C5a across the endothelium to be exposed on the luminal side, in this way aiding with arresting C5aR1-expressing neutrophils. At the same time, endothelial ACKR1 was shown to transport synovial tissue-derived CXCR2 ligands across the joint endothelium, facilitating neutrophil adhesion and extravasation [74].

A separate study identified how the two CXCR2 ligands, CXCL1 and CXCL2 sequentially direct neutrophil extravasation in the inflamed cremaster muscle. In this instance endothelial and pericyte GAG-immobilised CXCL1 promoted neutrophil adhesion and crawling, whereas CXCL2 controlled transendothelial migration. Fascinatingly, the source of CXCL1 was endothelial cells and pericytes, whereas CXCL2 was generated and released by neutrophils in another example of a paracrine amplification loop of directional neutrophil migration. Neutrophil-derived CXCL2 was subsequently immobilised by ACKR1 expressed by pericytes at venular cell-cell junctions, supporting the correct directionality of neutrophil transendothelial migration [75].

Reverse Migration

To avoid excessive inflammation, neutrophils were long thought to undergo apoptosis, followed by being cleared ('efferocytosed') by resident pro-resolution macrophages at sites of inflammation [76]. Recent observations have, however, suggested that this may not be the only possible fate of the neutrophil in sterile inflammation. Rather than undergoing apoptosis and dying, neutrophils were found to migrate away from a sterile wound in zebrafish larvae, including, on occasion, entering the vasculature [77]. Zebrafish neutrophils express two chemokine receptors, CXCR1 and CXCR2, of which CXCR1 regulates recruitment to the sterile wound, and CXCR2 promotes CXCL8-induced reverse migration, which interestingly occurred by chemokinesis rather than chemotaxis [78], a view shared by a separate study [79]. Interestingly, reverse migration may promote wound healing, since wounds in zebrafish that are genetically deficient in CXCR2/CXCL8 displayed heightened inflammation [78]. This view is supported by other observations made in the zebrafish, where retaining zebrafish neutrophils at the wound site and reducing neutrophil apoptosis by inducing HIF1α was also pro-inflammatory [80]. In a similar vein, tashinone IIA, an active compound from a Chinese medicinal herb, that promoted neutrophil reverse migration was isolated in a zebrafish screen aimed at identifying compounds that would aid the resolution of inflammation [81]. Interestingly, unlike their mammalian counterparts, neutrophils in zebrafish larvae are generally tissue resident [82]. Therefore, the term reverse migration refers merely to the direction of migration in the zebrafish, whilst it generally includes the breaching of the vessel wall in the luminal direction (i.e. reverse transendothelial migration) in mammals. Reverse migration has been suggested to occur, too, in humans. This view is controversial, however, with circulating neutrophils that comprise a 'reverse migration signature' ($CD54^{hi}$ $CXCR1^{low}$) being 4-8x more abundant in patients with systemic inflammation than in healthy individuals [83]. Yet, there is evidence to support a potential role of neutrophil reverse migration in the dissemination of inflammation from mouse models, where reverse migrated mouse

neutrophils observed after ischemia reperfusion injury augmented instances of inflammation in the lung [84]. A subsequent study identified following ischemia reperfusion injury that neutrophil-derived LTB4 and elastase were responsible for loss of junctional JAM-C, permitting neutrophil reverse (transendothelial) migration, with reverse migrated neutrophils again travelling to the lung to spread inflammation [85]. In a separate study the neutrophilic response to a small localised burn in the liver was observed by intravital microscopy. This induced neutrophil recruitment to the injury site, where neutrophils aided tissue repair, phagocytosing dead tissue. Rather than undergoing apoptosis for phagocytosis at the injury site, neutrophils once more left the wound, employing proteases to re-enter the vasculature by reverse transendothelial migration. They entered the lung, and upregulated CXCR4 prior to homing to the bone marrow for non-inflammatory clearance [86]. Clearly neutrophil reverse migration is a very interesting area which remains to be further investigated and fully understood. Does reverse migration only occur in response to sterile injury and, conversely, is apoptosis at the site of inflammation followed by efferocytosis more typical of neutrophils at sites of infection? Could reverse migration be involved in inducing lung injury under certain but not all instances? It will be exciting to follow new developments in this area in the future.

Conclusion

This minireview has highlighted key points of neutrophil chemotaxis, focussing on some molecular events that were shown *in vitro* to regulate neutrophil polarisation and summarising some exciting developments in neutrophil trafficking *in vivo*. The mind-boggling complexity of the regulation of neutrophil chemotaxis is fascinating to the basic scientist and provides evidence of the physiological importance of the process that is being regulated. Meticulous regulation of neutrophil chemotaxis is required to balance neutrophilic

392 inflammation, to ensure adequate host defense while avoiding excessive host damage. As
393 evidenced by rare genetic diseases such as leukocyte adhesion deficiencies, in which β2
394 integrins are absent or their signalling dysfunctional, interfering with leukocyte recruitment
395 leaves the body open to recurrent serious bacterial infections. Conversely, certain chronic
396 inflammatory diseases (e.g. rheumatoid arthritis or chronic obstructive pulmonary disease)
397 are characterised by excessive neutrophilic inflammation. Therapeutically targeting
398 neutrophil chemotaxis to alleviate such conditions may be feasible, but could result in
399 reduced host immunity as a trade-off.
400

Summary Points

- Chemotaxis is defined as directional cell migration towards a source of chemoattractant, whilst chemokinesis is chemoattractant-induced cell migration in the absence of a gradient.
- Chemotaxis bears all the hallmarks of random migration, but in addition is characterised by chemoattractant-induced polarisation, and directionality towards a source of chemoattractant
- Chemoattractants include lipids, peptides, protein fragments and chemotactic cytokines (chemokines). They are classed into intermediary and end-point chemoattractants, and operate in a hierarchical fashion. Chemoattractants signal through G protein coupled receptors. Atypical chemoattractant receptors bind chemoattractant without inducing intracellular signalling.
- Being amongst the fastest chemotaxing cells in the human body, neutrophils provide a first line of defense against infections.
- Leukocyte recruitment to a site of inflammation is directed by chemoattractants and therefore corresponds to chemotaxis *in vivo*. This area has been revolutionised by genetic approaches in combination with intravital imaging. Many of the latest insights are concerned with the integration of different chemoattractants by the migrating cell, paracrine amplification loops ('swarming') and reverse migration (ie away from the source of chemoattractant).

	Receptor	*Chemoattractant*	*Alternative name*	*Function*
Chemokine Receptors	CXCR2	CXCL1	Gro-α (human) KC (mouse)*	Neutrophil recruitment & activation
	CXCR2	CXCL2	Gro-β (human) MIP2 (mouse)*	Neutrophil trafficking
	CXCR1	CXCL8	Interleukin 8 (IL-8)*	Neutrophil recruitment to sites of inflammation
	CXCR4	CXCL12	Stromal cell derived factor 1 (SDF1)	Bone marrow homing
Chemoattractant Receptors	BLT1	LTB4 (leukotriene B4)		Neutrophil recruitment and swarming
	FPR1 (also known as fMLPR) FPR2	Bacterial and mitochondrial formylated peptides, e.g. fMLF		Neutrophil recruitment
	C5aR	C5a		Neutrophil recruitment (eg in autoantibody induced disease)
	C3aR	C3a		Inhibitor of neutrophil mobilisation
Atypical Chemokine receptors	ACKR1 (formerly Duffy antigen receptor)			Chemokine transcytosis; Haematopoiesis and neutrophil blood counts [87]
	ACKR2 (formerly D6)	Inflammatory CC chemokines		Decoy / scavenger receptor

Michael and Vermeren, Table 1.

Figure and Table Legends.

Table 1. Common neutrophil chemoattractants and their receptors. In addition to chemotactic cytokines (chemokines), which bind to chemokine receptors that signal or atypical chemokine receptors that do not signal, neutrophils express a series of chemoattractant receptors, which bind to lipids, peptide, protein fragments or chemokines. In additional to atypical chemokine receptors (ACKRs), there are also atypical chemoattractant receptors, e.g. C5aR2, see main text. * Note, CXCL8/IL-8 and its receptor CXCR1 are lost in the mouse, where CXCL1/KC and CXCL2/MIP2 and their receptor CXCR2 appear to act as functional homologues.

Figure 1. Molecular signalling events in neutrophil polarisation allowing movement towards the chemotactic gradient. Binding of a chemoattractant to the G-protein coupled chemoattractant receptor induces intracellular signalling to regulate neutrophil polarisation. Polarised neutrophils are characterised by accumulation of PIP3 to the leading edge, where effectors such as Rho GEFs and GAPs promote actin polymerisation. Polarisation is maintained by feedback loops, for example inhibiting RhoA at the pseudopod. The bulky nucleus is used as a 'mechanical gauge' that together with the MTOC facilitates the cell's progress through pores in the interstitium. Trailing end retraction is facilitated by microtubule depolymerisation, activating RhoA and triggering actomyosin contractility in addition to feedback loops involving RhoA, Rac and PTEN.

Figure 2. Neutrophils are controlled by chemotaxis. Clockwise, from top left: *Mobilisation and Homing*. CXCR2 signalling leads to the mobilisation of neutrophils from the bone marrow into the bloodstream, whereas upregulation of CXCR4 in senescent neutrophils promotes chemokine-driven homing back to the bone marrow. *Recruitment.*

Resident macrophages at inflammatory sites release pro-inflammatory mediators that promote selectin-mediated interactions between neutrophils and the endothelium. Neutrophils tether and roll along the endothelium, where GAG-immobilised chemokines guide the neutrophils through G protein coupled receptor signalling, regulating integrin-mediated neutrophil extravasation. *Atypical chemokine receptors* have been shown to aid neutrophil recruitment to sites of inflammation. *Swarming.* Certain end-target chemoattractants cause the release of LTB4 containing exosomes. An autocrine-paracrine feedback amplification loop promotes directional migration of many neutrophils in a 'swarm'. *Chemoattractant hierarchies*. *In vivo* the neutrophil encounters multiple chemoattractants, the response to which must be tightly regulated. For example, neutrophils choose 'end-target' chemoattractants over intermediate chemoattractants. *Reverse migration*. Neutrophil reverse (transendothelial) migration has been observed in many contexts and, perhaps depending on circumstances, may or may not have pro-inflammatory consequences. See text for further discussion.

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MM and SV wrote the paper and drafted the figures.

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Competing Interests

The authors declare that there are no competing interests associated with this manuscript.

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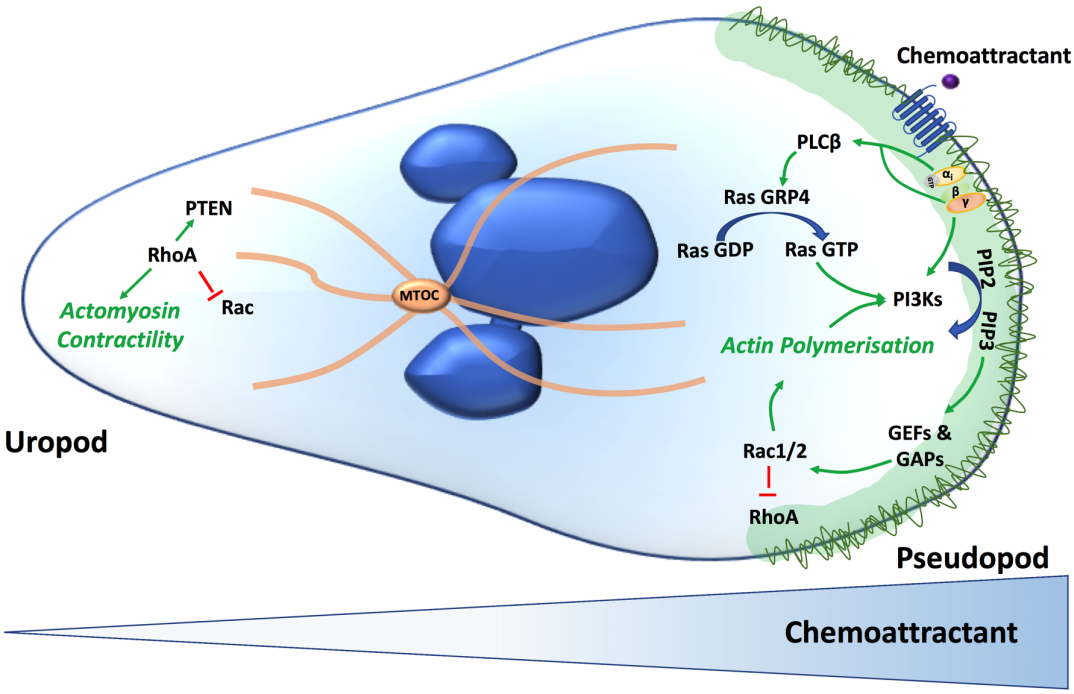
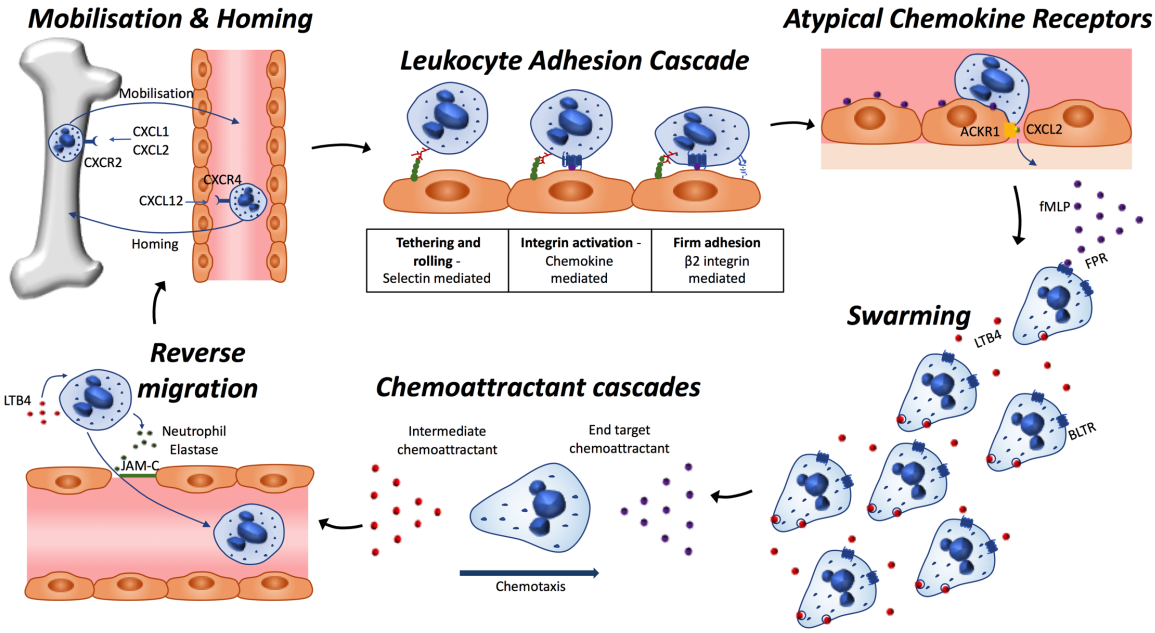


Michael & Vermeren Fig 1



Michael and Vermeren Fig 2