The Multifaceted Journey of Neutrophils: Chemotaxis, Migration, and Transmigration in Immune Response

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ABSTRACT

Neutrophils are a crucial component of the innate immune system, acting as the first line of defense against infection. Their ability to swiftly migrate to sites of infection or injury through a process called chemotaxis is fundamental to their function. This review aims to provide a comprehensive overview of the mechanisms underlying neutrophil chemotaxis, migration, and transmigration. We will explore the signaling pathways, molecular players, and physiological processes that guide neutrophils from the bloodstream to sites of tissue damage or infection. Furthermore, the review will discuss the implications of these processes in disease states and potential therapeutic interventions. Chemotaxis begins with neutrophils detecting chemotactic signals, such as chemokines (e.g., IL-8) and complement components (e.g., C5a), which bind to G-protein-coupled receptors (GPCRs) on their surface. This interaction triggers intracellular signaling cascades involving key molecules like phosphoinositide 3-kinase (PI3K), Akt, and mitogen-activated protein kinases (MAPKs). These pathways regulate cytoskeletal dynamics, enabling neutrophils to move directionally. Actin polymerization at the leading-edge forms pseudopodia, propelling the cell forward, while myosin II-mediated contraction at the rear facilitates coordinated movement.

Keywords: Neutrophils, Chemotaxis, Migration, ROS, Transmigration, PMNs and chemoattractants.

INTRODUCTION

Neutrophils are the most abundant type of white blood cells, pivotal in the frontline defense against infections. Their ability to swiftly migrate towards the site of infection or inflammation is critical for the effective elimination of pathogens.1 The processes of chemotaxis, migration, and transmigration are central to neutrophil function. This review provides a comprehensive overview of these processes, highlighting key signaling molecules, receptors, and cellular mechanisms involved.2

Neutrophils play a vital role in host defense by deploying antimicrobial mechanisms, including the release of reactive oxygen species (ROS), antimicrobial peptides, and neutrophil extracellular traps (NETs). However, dysregulated neutrophil chemotaxis can lead to chronic inflammatory diseases, such as rheumatoid arthritis, where persistent infiltration causes tissue damage.3

Understanding Neutrophil chemotaxis and transmigration mechanisms provides insights into disease pathogenesis and reveals potential therapeutic targets. Inhibiting chemotactic receptors like CXCR1 and CXCR2 can reduce neutrophil recruitment in inflammatory diseases, alleviating symptoms and preventing tissue damage. Targeting signaling molecules within chemotactic pathways, such as PI3K inhibitors, also offers therapeutic potential. Enhancing chemotaxis in immunocompromised patients could improve infection control and reduce sepsis risk.4

Figure 1: The leukocyte extravasation cascade is orchestrated by a series of adhesive interactions between leukocytes and endothelial cells (ECs). This process involves several distinct steps, each mediated by specific adhesive molecules. In the neutrophil extravasation cascade, neutrophils initially tether and roll along the endothelium, followed by firm adhesion and arrest. After adhering, neutrophils engage in lateral migration or crawling on the endothelial surface to locate a suitable site for transmigration. During this phase, the formation of the ICAM-1 adhesome and the emergence of F-actin–rich filopodia from endothelial cells are crucial for facilitating diapedesis. Diapedesis, the process of crossing the endothelial barrier, can occur either between endothelial cell junctions (paracellular migration) or through the endothelial cell body (transcellular migration). Once neutrophils traverse the perivascular basement membrane, they migrate into the interstitial tissue. Additionally, neutrophils have the capacity to return to the bloodstream via a process known as...
reverse migration. PMN refers to polymorphonuclear neutrophils.

Chemotaxis: Directed Migration towards Chemotactic Gradients

Definition and Importance
Chemotaxis is the directed movement of neutrophils towards higher concentrations of chemotactic agents, such as cytokines, chemokines, and bacterial products. This process is fundamental for the rapid recruitment of neutrophils to sites of infection or injury. Chemotaxis is the directed movement of neutrophils towards chemical gradients of chemoattractants, which include chemokines, complement components, and bacterial products. This process is initiated when these chemoattractants bind to specific G-protein-coupled receptors (GPCRs) on the surface of neutrophils. Key chemoattractant receptors include CXCR1 and CXCR2, which bind to the chemokine IL-8, and the fMLP receptor, which responds to formylated peptides from bacteria.

Upon receptor activation, downstream signaling pathways are triggered, starting with the activation of heterotrimeric G-proteins. These proteins dissociate into Gα and Gβγ subunits, which then activate a series of intracellular signaling cascades. One critical pathway involves phosphoinositide 3-kinases (PI3Ks), which produce phosphatidylinositol (3,4,5)-trisphosphate (PIP3) at the leading edge of the cell. PIP3 serves as a docking site for signaling proteins that regulate actin polymerization, such as Akt and small GTPases like Rac and Cdc42.

Chemotactic Agents
Chemokines: Chemokines are small cytokines that play a crucial role in directing the movement of cells, particularly during immune responses. For neutrophils, key chemokines include IL-8 (CXCL8), GRO-α (CXCL1), and MCP-1 (CCL2). IL-8 is a potent chemoattractant and activator of neutrophils, mediating their migration to sites of infection or injury. GRO-α also attracts neutrophils and aids in inflammatory responses. MCP-1, although primarily known for recruiting monocytes, can also influence neutrophil behavior. These chemokines bind to specific G-protein-coupled receptors on neutrophils, triggering intracellular signaling pathways that facilitate directed cell migration.

Bacterial Products: Formyl peptides, such as N-formylmethionyl-leucyl-phenylalanine (fMLP), are derived from bacterial proteins and serve as potent chemoattractants for Neutrophil. These peptides bind to the formyl peptide receptor (FPR) on the surface of neutrophils, triggering a cascade of intracellular signaling events. This interaction leads to the activation of G-protein-coupled pathways, promoting chemotaxis, degranulation, and the oxidative burst necessary for bacterial clearance. The high affinity of neutrophils for formyl peptides allows for rapid and targeted migration to sites of bacterial infection, enhancing the immune response and facilitating the destruction of pathogens.

Complement System: Anaphylatoxins, including C5a, are potent chemoattractants generated during the activation of the complement system. C5a is produced by the cleavage of complement protein C5 and plays a critical role in the immune response by attracting neutrophils and other immune cells to sites of infection and inflammation. It binds to the C5a receptor (C5aR) on the surface of these cells, triggering intracellular signaling pathways that enhance chemotaxis, degranulation, and the production of reactive oxygen species. This leads to the rapid recruitment and activation of immune cells, contributing to the clearance of pathogens and the amplification of inflammatory responses.

![Figure 2: Neutrophil polarity during migration. The role of the cell cytoskeleton and the proteins that regulate cell polarity is illustrated.](image-url)

Figure 2: Neutrophil polarity during migration. The role of the cell cytoskeleton and the proteins that regulate cell polarity is indicated as forming lamellipodia at leading-edge and uropod at back.

Chemotaxis Signaling Pathways
Chemotaxis is orchestrated through a series of well-coordinated signaling pathways:

1. **Receptor Activation**: Neutrophils express G-protein-coupled receptors (GPCRs) that bind chemotactic agents. For example, the IL-8 receptor (CXCR1/2) binds IL-8, and the formyl peptide receptor (FPR1) binds fMLP.

2. **G-Protein Signaling**: Upon ligand binding, GPCRs activate heterotrimeric G-proteins, leading to the dissociation of Gα and Gβγ subunits. This triggers downstream signaling cascades essential for chemotaxis.

3. **Intracellular Signaling**: Key pathways include:
   - PI3K/Akt Pathway: Phosphoinositide 3-kinase (PI3K) generates PIP3 at the leading edge of the cell,
recruiting Akt, which promotes cell polarization and motility.

- MAPK Pathway: Mitogen-activated protein kinases (ERK, p38) are involved in cytoskeletal reorganization and directional movement.
- Calcium Signaling: Ca2+ fluxes play a crucial role in actin polymerization and pseudopodia formation.11

Actin Cytoskeleton Remodeling

The chemotactic signal induces rapid remodeling of the actin cytoskeleton:

Actin Polymerization: Upon activation by chemoattractants, actin-related proteins such as the Arp2/3 complex and formins facilitate actin nucleation at the cell’s leading edge. The Arp2/3 complex promotes the formation of branched actin networks, creating a protrusive force for pseudopodia extension. Formins, on the other hand, aid in the elongation of unbranched actin filaments, further supporting membrane protrusion and cell movement.13

Actin-Myosin Contraction: At the rear of the cell, myosin II-mediated contraction generates tension, pulling the cell body forward. Myosin II interacts with actin filaments to produce contractile forces, facilitating cytoplasmic flow and retraction of the trailing edge. This coordinated contraction and relaxation cycle ensures efficient translocation of the neutrophil towards the chemotactic signal, enabling rapid and directed migration towards sites of infection or injury.13,14

Migration: Navigation through Tissues

Amoeboid Movement

Neutrophils migrate using amoeboid movement, a mode of locomotion characterized by rapid, dynamic, and flexible morphology. This movement enables neutrophils to navigate through the extracellular matrix (ECM) and tissue barriers with high efficiency. Amoeboid movement involves continuous cycles of protrusion, adhesion, and contraction. The leading-edge forms pseudopodia driven by actin polymerization, while integrin-mediated adhesions anchor the cell to the ECM. Contraction at the cell’s rear, mediated by myosin II, propels the cell forward. This mode of migration is highly adaptive, allowing neutrophils to quickly respond to chemotactic signals and reach sites of infection or injury.15

Integrin-Mediated Adhesion

Integrins: Integrins are transmembrane receptors that play a crucial role in cell-ECM interactions, essential for neutrophil migration. Key Integrins involved in this process include LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18). LFA-1 binds to intercellular adhesion molecule-1 (ICAM-1) and ICAM-2 on endothelial cells, while Mac-1 interacts with ICAM-1 and other ligands such as fibrinogen. These interactions facilitate neutrophil adhesion, spreading, and migration through the endothelium.16

Adhesion Molecules: Integrins on Neutrophil interact with adhesion molecules like ICAM-1 and vascular cell adhesion molecule-1 (VCAM-1) expressed on activated endothelial cells. This binding is critical for firm adhesion, allowing neutrophils to withstand shear forces in the bloodstream. Once firmly adhered, neutrophils can transmigrate through the endothelial barrier in a process called diapedesis. This integrin-mediated adhesion is regulated by chemokines and other signaling molecules, ensuring precise and efficient neutrophil migration to sites of inflammation or infection.16,17

Degradation of ECM in Neutrophil Migration

Neutrophils secrete matrix metalloproteinases (MMPs) to degrade extracellular matrix (ECM) components, facilitating their movement through tissues. MMPs are a family of zinc-dependent endopeptidases that target various ECM proteins, including collagen, elastin, and proteoglycans. Key MMPs produced by neutrophils include MMP-8 (neutrophil collagenase) and MMP-9 (gelatinase B).18 These enzymes break down structural barriers within the ECM, creating pathways for neutrophil migration. The regulated secretion and activation of MMPs are crucial for neutrophil function, enabling them to efficiently traverse the ECM and reach sites of infection or inflammation, thus playing a vital role in immune defense.19

Role of Chemotactic Gradient in Neutrophil Migration

The maintenance of a chemotactic gradient is critical for directed neutrophil migration. Neutrophils exhibit high sensitivity to these gradients, allowing them to detect and respond to even shallow chemotactic cues. This gradient guides neutrophils to the precise location of infection or inflammation. Receptors on neutrophils sense varying concentrations of chemoattractants, leading to polarized cell signaling and actin cytoskeleton rearrangements that drive directional movement towards higher concentrations of these signals.20

Transmigration: Crossing the Endothelial Barrier

Endothelial Adhesion Cascade

Selectin-Mediated Rolling: The initial tethering and rolling of neutrophils on the endothelial surface are mediated by selectins, specifically E-selectin and P-selectin, and their ligands such as P-selectin glycoprotein ligand-1 (PSGL-1). These interactions are transient and allow neutrophils to slow down and roll along the vascular endothelium, a crucial step in the leukocyte adhesion cascade. This rolling is essential for neutrophils to survey the endothelium for signals indicating sites of infection or injury.17,21

Integrin Activation and Firm Adhesion: Chemokines presented on the endothelial surface bind to G-protein-coupled receptors on neutrophils, triggering intracellular signaling pathways that lead to the activation of Integrins, such as LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18). These activated Integrins undergo conformational changes, increasing their affinity for endothelial adhesion
molecules like ICAM-1 and VCAM-1. This transition from rolling to firm adhesion enables neutrophils to firmly attach to the endothelial surface, resisting shear forces from blood flow.21,22

Transendothelial Migration (Diapedesis): Following firm adhesion, neutrophils transmigrate across the endothelial barrier in a process known as diapedesis. This migration can occur via two routes: paracellular, where Neutrophil pass between endothelial cells, and transcellular, where they pass directly through endothelial cells. The paracellular route involves the transient opening of endothelial junctions, regulated by adhesion molecules such as PECAM-1, CD99, and JAMs. The transcellular route requires the formation of membrane-bound channels or pores in endothelial cells. Both routes ensure neutrophils efficiently reach the underlying tissues to respond to infection or injury.21,22

Molecular Mechanisms of Diapedesis

Functional Adhesion Molecules: Functional adhesion molecules (JAMs) such as JAM-A, JAM-C, and PECAM-1 (platelet endothelial cell adhesion molecule-1) are critical for neutrophil passage during diapedesis. These proteins are located at endothelial junctions and mediate interactions between neutrophils and endothelial cells. PECAM-1 is particularly important as it is expressed on both neutrophils and endothelial cells, facilitating homophilic binding that guides neutrophil through endothelial junctions. JAM-A and JAM-C contribute to the regulation of junctional integrity and provide docking sites that support neutrophil transmigration.21

Cytoskeletal Dynamics: The transmigration of neutrophils involves complex rearrangements of the actin and microtubule cytoskeletons in both neutrophils and endothelial cells. In neutrophils, actin polymerization drives the formation of pseudopodia that push the cell forward. Rho GTPases such as Rac1, RhoA, and Cdc42 orchestrate these cytoskeletal changes. In endothelial cells, cytoskeletal rearrangements are necessary to facilitate cell shape changes and junctional opening, allowing neutrophils to traverse the endothelial barrier. This dynamic remodeling is critical for maintaining endothelial barrier function while permitting Neutrophil passage.24

Endothelial Cell Retraction: For neutrophils to pass through intercellular junctions, endothelial cells must transiently retract. This retraction is regulated by signaling pathways that control cytoskeletal tension and junctional integrity. Key molecules involved include small GTPases, kinases, and phosphatases that modulate actomyosin contractility. The coordinated retraction of endothelial cells creates transient gaps through which neutrophils can migrate, ensuring effective immune surveillance and rapid response to inflammatory stimuli.21,24

Role of Endothelial Activation

Endothelial cells play a crucial role in immune responses, particularly in the recruitment and transmigration of neutrophils to sites of inflammation. Upon activation by pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β), endothelial cells upregulate the expression of adhesion molecules and chemokines. This enhanced expression significantly facilitates Neutrophil recruitment and transmigration.25

Implications in Disease and Therapeutic Potential

Acute Inflammatory Response

Neutrophil recruitment is a hallmark of acute inflammation, playing a vital role in the body's defense against infections and injury. Neutrophils are among the first immune cells to arrive at the site of infection, where they perform functions such as phagocytosis, degranulation, and the release of neutrophil extracellular traps (NETs) to eliminate pathogens. However, excessive or dysregulated neutrophil infiltration can be detrimental, leading to significant tissue damage. This is evident in conditions like acute respiratory distress syndrome (ARDS) and myocardial infarction. In ARDS, excessive neutrophil accumulation in the lungs results in severe inflammation, increased vascular permeability, and impaired gas exchange, contributing to respiratory failure. Similarly, during a myocardial infarction, the rapid influx of neutrophils to the damaged heart tissue can exacerbate injury through the release of proteolytic enzymes and reactive oxygen species, leading to further myocardial damage and impaired cardiac function.26

Chronic Inflammation and Autoimmunity

In chronic inflammatory diseases and autoimmune disorders, persistent neutrophil activation and migration play a critical role in ongoing tissue damage and disease progression. For instance, in rheumatoid arthritis (RA), neutrophils infiltrate the synovial fluid and tissue, where they release pro-inflammatory cytokines, proteases, and reactive oxygen species, contributing to joint inflammation and destruction. Similarly, in systemic lupus erythematosus (SLE), the continuous activation and accumulation of neutrophils can lead to the formation of NETs, which contain autoantigens that exacerbate the autoimmune response, promoting further tissue damage. These mechanisms highlight the dual role of neutrophils in both protective and pathological inflammation.26,27

Therapeutic Potential

Understanding the molecular mechanisms underlying neutrophil recruitment and function offers potential therapeutic avenues to mitigate tissue damage while preserving host defense. Targeting specific pathways involved in neutrophil recruitment, activation, and transmigration can help modulate their activity in various inflammatory conditions. For example, inhibiting selectins or Integrins could reduce Neutrophil adhesion and transmigration, potentially alleviating tissue damage in
acute and chronic inflammatory diseases. Additionally, therapies aimed at modulating cytokine and chemokine signaling could help balance the beneficial and harmful effects of neutrophil infiltration. By fine-tuning the inflammatory response, it is possible to develop treatments that minimize collateral tissue damage while maintaining effective immune defense mechanisms.26,28

Cancer: Dual Roles of Tumor-Associated Neutrophils (TANs)

Neutrophils, traditionally viewed as first responders in acute inflammation, play complex roles in the context of cancer. Tumor-associated neutrophils (TANs) can exhibit both pro-tumorigenic and anti-tumorigenic activities, with their behavior highly dependent on the tumor microenvironment and the specific neutrophil phenotypes present. Tumor-associated neutrophils (TANs) can either support tumor growth and metastasis or exhibit anti-tumor activities, depending on the tumor microenvironment and Neutrophil phenotype.29

Pro-Tumorigenic Roles:

In many cancers, TANs are recruited to the tumor microenvironment, where they can support tumor growth and metastasis. Pro-tumorigenic TANs, often referred to as N2 neutrophils, promote tumor progression through various mechanisms:

- **Secretion of Growth Factors:** TANs secrete factors like vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF), which stimulate angiogenesis, providing the tumor with necessary blood supply and nutrients.
- **Matrix Degradation:** TANs release matrix metalloproteinases (MMPs) that degrade the extracellular matrix, facilitating tumor invasion and metastasis.
- **Immunosuppression:** TANs can suppress adaptive immune responses by producing immunosuppressive cytokines such as TGF-β and IL-10, as well as by inducing regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), thereby protecting the tumor from immune surveillance.29,30

Therapeutic Targeting: Modulating Neutrophil Chemotaxis and Migration

Targeting neutrophil chemotaxis and migration pathways presents promising therapeutic strategies for managing immune responses in various inflammatory and autoimmune diseases. By intervening in these pathways, it is possible to reduce harmful neutrophil infiltration while preserving essential immune functions.31

1. **Inhibitors of Chemokine Receptors:** Chemokine receptors, particularly CXCR1 and CXCR2, play crucial roles in neutrophil recruitment to sites of inflammation. By blocking these receptors, it is possible to reduce neutrophil migration and accumulation, thereby mitigating inflammation and tissue damage.

Small molecule inhibitors and monoclonal antibodies targeting CXCR1/2 have shown efficacy in preclinical models of inflammatory diseases such as chronic obstructive pulmonary disease (COPD) and rheumatoid arthritis. These inhibitors can potentially decrease the inflammatory response and improve disease outcomes by preventing excessive Neutrophil recruitment and activation.31,32

2. **Integrin Antagonists:** Integrins are transmembrane receptors that mediate neutrophil adhesion to the endothelium and subsequent transmigration into tissues. Inflammatory diseases often involve excessive neutrophil infiltration, leading to tissue damage. Integrin antagonists, such as those targeting LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18), can disrupt the interaction between neutrophils and endothelial adhesion molecules like ICAM-1 and VCAM-1. By inhibiting integrin-mediated adhesion, these antagonists can prevent neutrophils from adhering to and migrating through the endothelium, thereby reducing inflammation and preserving tissue integrity. Clinical trials are ongoing to evaluate the efficacy of integrin antagonists in conditions like psoriasis and inflammatory bowel disease.33

3. **Modulating Chemotactic Gradients:** The chemotactic gradient of chemokines and other signaling molecules guides neutrophils to sites of inflammation. Therapeutic strategies aimed at modulating these gradients can redirect neutrophil migration and reduce harmful inflammation. Approaches include the use of decoy receptors, which bind to chemokines and prevent them from interacting with their receptors on neutrophils, and inhibitors that degrade chemokines in the tissue microenvironment. Additionally, manipulating the production or release of chemokines can alter the chemotactic gradient, leading to a more controlled and localized immune response. Such strategies have the potential to ameliorate inflammation in diseases like multiple sclerosis and systemic lupus erythematosus.34

✓ Potential Clinical Applications: The development of therapies targeting neutrophil chemotaxis and migration offers hope for treating a wide range of inflammatory and autoimmune conditions. By selectively modulating neutrophil activity, it is possible to achieve therapeutic benefits without compromising the overall immune response. Continued research and clinical trials will be essential to optimize these approaches and ensure their safety and efficacy in patients.33,34

CONCLUSION

Neutrophil chemotaxis, migration, and transmigration are complex, tightly regulated processes essential for effective immune defense. These mechanisms enable neutrophils to rapidly respond to infections and injuries, playing a pivotal role in the innate immune response. Understanding the
molecular and cellular pathways involved in these processes has provided significant insights into their dual roles in health and disease. Dysregulated neutrophil activity can contribute to the pathogenesis of various inflammatory, autoimmune, and neoplastic diseases. Therefore, continued research into these mechanisms is crucial for developing targeted therapies that modulate neutrophil functions. Such advancements hold the potential to improve clinical outcomes in conditions where neutrophils play a central role, including inflammatory and autoimmune diseases, as well as cancer. By fine-tuning neutrophil activity, it may be possible to reduce tissue damage and inflammation while preserving essential immune defenses, offering a balanced approach to disease management.

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