Trends in Immunology

CellPress

Review

Feedback Amplification of Neutrophil Function

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As the first line of innate immune defense, neutrophils need to mount a rapid and robust antimicrobial response. Recent studies implicate various positive feedback amplification processes in achieving that goal. Feedback amplification ensures effective migration of neutrophils in shallow chemotactic gradients, multiple waves of neutrophil recruitment to the site of inflammation, and the augmentation of various effector functions of the cells. We review here such positive feedback loops including intracellular and autocrine processes, paracrine effects mediated by lipid (LTB_4), chemokine, and cytokine mediators, and bidirectional interactions with the complement system and with other immune and non-immune cells. These amplification mechanisms are not only involved in antimicrobial immunity but also contribute to neutrophil-mediated tissue damage under pathological conditions.

Trends

Neutrophil recruitment and activation is amplified by positive feedback loops involving neutrophil-derived mediators acting on neutrophils themselves.

Feedback amplification is critical for neutrophil-dependent antimicrobial functions but also for neutrophil-mediated tissue damage.

Feedback amplification can be observed at various levels including intracellular, autocrine, paracrine, and complex processes.

Feedback amplification is involved in various neutrophil functions including cellular polarization, neutrophil recruitment, effector responses, and interactions with other cells.

Neutrophil-derived proinflammatory mediators include LTB_4, chemokines (primarily CXCL molecules), and various cytokines.

Tyrosine kinase signaling pathways play a critical role in feedback amplification of neutrophil function.

Overview of Feedback Amplification of Neutrophil Function

Homeostatic mechanisms mostly rely on negative feedback mechanisms. However, rapid and robust responses to external stimuli often require positive feedback mechanisms. Typical examples of positive feedback include the action potential, ovulation, and blood clotting, all of which result in dramatic changes in biological processes.

Neutrophils form the first line of innate immune defense against bacterial and fungal pathogens [1]. They are equipped with a multitude of cell-surface receptors for the recognition of microbial pathogens and the inflammatory environment [2], and migrate to the site of inflammation through a multistep recruitment process triggered by chemotactic agents including chemokines, lipid chemoattractants (primarily LTB_4), formyl-peptides, and complement fragments [3–5]. At the site of infection/inflammation, neutrophils use an armamentarium of effector functions, including phagocytosis, respiratory burst, degranulation, and neutrophil extracellular trap (NET) formation, to eliminate the invading microorganisms [6,7]. In addition, neutrophils also participate in the organization of the overall immune response [6–9]. Despite being short-lived, terminally differentiated cells with limited transcriptional activity, they can undergo stimulus-induced gene expression changes leading to chemokine and cytokine release into the extracellular space [6,10,11]. Given their robust effector functions, neutrophils also play a major role in tissue damage during infectious and non-infectious inflammatory diseases [6,12].

Several recent reports indicate important roles for self-perpetuating positive feedback amplification loops in promoting neutrophil recruitment and activation. Such feedback loops act at various levels, including amplification within single cells, paracrine interaction between different neutrophils, and more complex feedback loops involving other biochemical and cellular processes (Figure 1). These amplification loops have likely evolved to ensure swift and robust responses against invading microorganisms, but they also contribute to neutrophil-mediated tissue damage during infectious and sterile inflammatory diseases. We review here the various mechanisms of feedback amplification of neutrophil function and their relevance to

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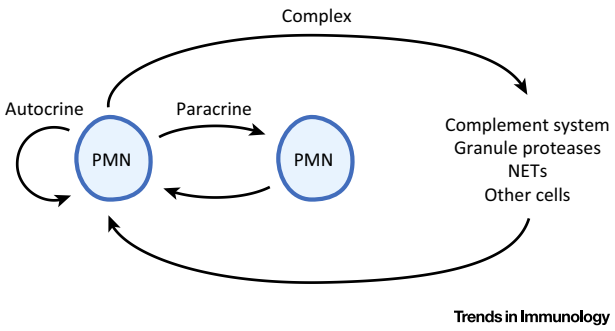

Figure 1. Overview of Neutrophil Amplification Mechanisms. Activated neutrophils can directly enhance their own activity/migration through autocrine and paracrine routes. In addition, they can promote their own functions by modifying the activation state of the complement system, the release of various granule proteases, the formation of neutrophil extracellular traps, or by influencing the activity of other immune/non-immune cells. Abbreviations: NETs, neutrophil extracellular traps; PMN, polymorphonuclear cell (neutrophil).

neutrophil-mediated *in vivo* biological processes. Understanding feedback amplification of neutrophil function may facilitate the development of novel therapeutic strategies in diseases characterized by excessive neutrophil activation.

Feedback Amplification at the Single-Cell Level

Recent reports indicate that the function of single neutrophils is amplified by several intracellular or autocrine feedback amplification pathways.

Intracellular Feedback Loops Promoting Neutrophil Polarization

One of the most prominent biochemical features of neutrophil polarization is the accumulation of phosphatidylinositol (3,4,5) trisphosphate (PIP_3) at the leading edge. The cellular PIP_3 gradient is substantially steeper than that of the extracellular chemoattractant gradient, and exogenous PIP_3 leads to the accumulation of endogenous PIP_3 at the leading edge [13], suggesting the involvement of molecular amplification processes. Chemoattractant receptors trigger initial PIP_3 production at the leading edge through PI3-kinases activated by G-protein $\beta\gamma$ subunits (Figure 2). This initial PIP_3 production leads to activation of Rac small GTPases, which then activate the WAVE2 complex scaffolded by the Hem-1 protein, eventually leading to actin polymerization and activation of further endogenous PI3-kinases at the leading edge [13–15]. In addition, Rac GTPases also activate the NADPH oxidase and promote reactive oxygen species (ROS) production at the leading edge. This results in local inhibition of PTEN, a lipid phosphatase responsible for the degradation of PIP_3, leading to further accumulation of PIP_3 at the leading edge [16]. These mechanisms (Figure 2) contribute to the translation of a shallow extracellular gradient into robust neutrophil polarization.

While the leading edge is characterized by Rac activation and actin polymerization-based protrusions, the trailing edge (uropod) is dominated by Rho activation and myosin-based contraction through the Rho-activated kinase ROCK which phosphorylates and activates the myosin light chain MLC [5]. This differential signaling defines the biochemical basis of 'frontness' and 'backness' in polarized neutrophils (Figure 2). Interestingly, the two domains are mutually exclusive due to mutual inhibition of each other at the biochemical level [17]. Constitutive activation of the uropod components Rho, ROCK, or MLC inhibited the establishment of leading-edge features such as PIP_3 accumulation, Rac activation, or actin polymerization, while inhibition of the Rho/ROCK/myosin pathway had the opposite effect [17]. Furthermore, inhibition of 'frontness' features such as actin polymerization or Gi activation [18], or by knockdown of the leading edge organizer Hem-1 [15], inhibited 'backness' features such as uropod-specific localization of Rho and phosphorylated MLC. The reciprocal inhibition between 'frontness' and 'backness' features (i.e., the inhibition of the inhibitors) provides an additional level of positive feedback amplification of neutrophil polarization and contributes to the