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Previews

Quo vadis, neutrophil?

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Neutrophil recruitment from blood into tissues is a hallmark of inflammation and anti-microbial host defense. In this issue, De Giovanni et al. describe an unanticipated role for a serotonin metabolite, 5-HIAA, which is produced by activated platelets and mast cells and engages the orphan receptor, GPR35, to recruit neutrophils to inflamed tissues.

Neutrophils, the largest population of bone marrow (BM)-derived immune cells in human blood, act as first responders to acute bacterial and fungal infections and injuries, conditions that elicit their rapid recruitment to affected tissues where neutrophils are indispensable for pathogen clearance and tissue repair. On the flipside, misguided or overactive neutrophil infiltration can also exert detrimental effects that may exacerbate tissue injury. Although these innate immune cells have been studied for over 150 years, there are still gaps in our understanding of how neutrophils navigate from their place of birth in the BM to their end targets in inflamed tissues. Among the key regulators of this voyage are chemoattractants—locally secreted biochemically diverse molecules that usually signal through G-protein coupled receptor (GPCR). In this issue of *Cell*, De Giovanni and colleagues report a novel GPCR-chemoattractant pathway critical for neutrophil recruitment in response to bacterial infections (De Giovanni et al., 2022).

Neutrophil responses to peripheral inflammation depend on the successful completion of four sequential and highly dynamic migration events (Figure 1). Initially, newly generated neutrophils in the BM access the bloodstream by migrating across the endothelial lining of local microvessels. Their circulation half-life measures only ~6 h, because most cells spontaneously leave the circulation and are eliminated within a few days. Extravascular neutrophil numbers rise within minutes after a tissue is subjected to infection or damage. This rapid response requires that blood-borne neutrophils adhere to the luminal surface of microvessels and then emigrate into the affected tissue. This process is initiated when microbial products and/or endogenous pro-inflammatory signals stimulate endothelial cells (ECs) in postcapillary venules to alter their luminal surface properties by displaying adhesion molecules and chemoattractants for blood-borne neutrophils. In addition, blood platelets can be activated, which then adhere to inflamed ECs or accumulate on exposed extracellular matrix in regions where ECs were lost. Consequently, circulating neutrophils that pass through inflamed microvessels encounter a Velcro-like luminal surface composed of activated ECs and patches of adherent platelets. Neutrophils accumulate in these microvessels in a process known as the multi-step adhesion cascade, which requires the sequential engagement of surface adhesion molecules and chemoattractants (Ley et al., 2007). The first step is mediated by members of the selectin family expressed on neutrophils, ECs, and platelets, which bind to sialyl-Lewis^x-like carbohydrates to tether the moving cell and mediate slow rolling along the vessel wall (Lawrence and Springer, 1991; von Andrian et al., 1991). During the rolling phase, the neutrophil is exposed to chemoattractants that may be generated by activated ECs or platelets or originate in the extravascular space and diffuse across the vessel wall. These signals are detected mostly by GPCRs that trigger activation of β2 integrins in neutrophils, which then arrest the rolling cell by binding to their counter-receptors on ECs and platelets. The adherent neutrophil then crawls within the vessel lumen searching for "hotspots" in the vessel wall that permit exit from the vasculature, a process called diapedesis (Nourshargh et al., 2010). Finally, beyond the vessel wall, neutrophils navigate through the extravascular space to find end targets, such as extracellular microbes or tissue debris.

Research spanning several decades has uncovered a plethora of exogenous and endogenous factors that possess chemoattractant properties for neutrophils (Petri and Sanz, 2018). In many pathological settings, several of these agents may arise in parallel, so inhibition of a single chemoattractant pathway *in vivo* may exert only a partial or no discernable effect. In light of this biology, the observations by De Giovanni and colleagues are noteworthy (De Giovanni et al., 2022). The authors investigated the role of GPR35, a GPCR that was not previously implicated in neutrophil migration. GPR35 was expressed at low levels in resting neutrophils but was upregulated upon activation and shown to promote neutrophil recruitment in several settings of tissue inflammation. In addition, GPR35-deficient neutrophils were less efficient in clearing peritoneal bacteria.

Several biomolecules had been proposed previously to act on GPR35, but only with low potency (Kaya et al., 2021). The authors now identify a serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA) as a potent and physiologically relevant GPR35 agonist. The primary cellular sources of 5-HIAA in neutrophil recruitment were activated platelets and extravascular mast cells.

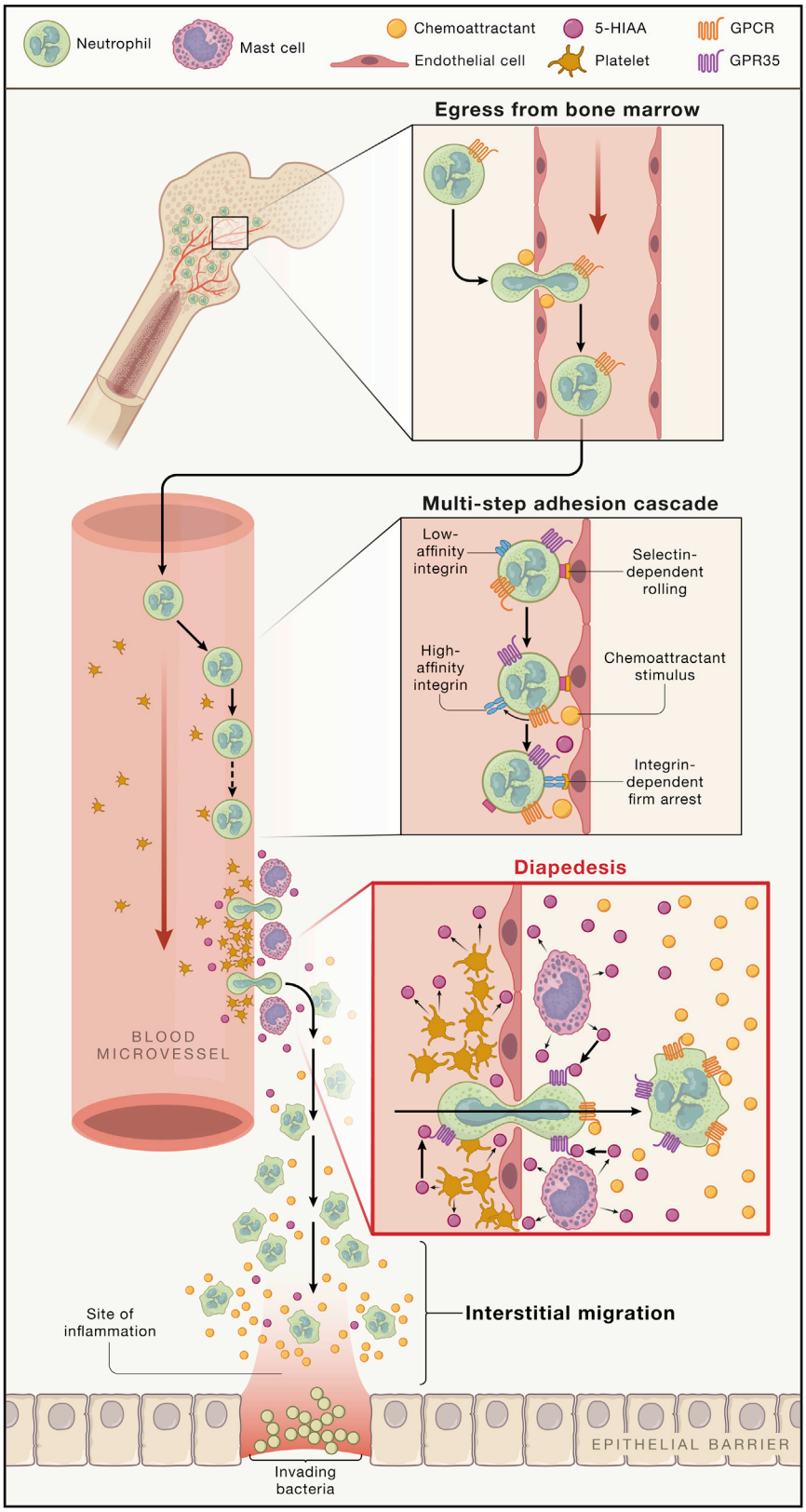

Figure 1. Neutrophils reach their targets in inflamed tissues in four sequential migration events

Neutrophils originate and are stored in large numbers in BM. Egress from BM into the blood circulation, which occurs constitutively and is enhanced by systemic inflammatory signals, makes neutrophils available to every tissue in the body. During inflammation in peripheral tissues, neutrophils engage in a multi-step adhesion cascade mediated by sequential actions of selectins, chemoattractants, and activated integrins, resulting in the accumulation of adherent cells within microvessels. Subsequently, neutrophils diapedese across the vascular wall to access the extravascular space where they engage in interstitial migration to reach their end targets. The diapedesis step occurs preferentially at sites of activated platelet deposition and depends on the serotonin metabolite 5-HIAA produced by intravascular platelets and perivascular mast cells. Activated neutrophils upregulate GPR35 to detect 5-HIAA. At each migration step, neutrophils are guided by the action of a variety of chemoattractants, but the GPR35–5-HIAA axis is particularly critical for neutrophil exit from the vasculature.

Mice deficient in 5-HIAA showed a loss of GPR35-mediated neutrophil recruitment to inflamed tissue, and this phenotype was reflected in animals lacking either platelets or mast cells. Neutrophil recruitment into the inflamed skin of mast-cell-deficient mice could be rescued by local injection of 5-HIAA. *In vitro*, 5-HIAA was sufficient to trigger integrin activation in the multi-step adhesion cascade, and 5-HIAA also induced dose-dependent chemotactic migration of neutrophils across endothelial monolayers. However, *in vivo* experiments did not detect a defect in intravascular neutrophil adhesion upon disruption of the GPR35–5-HIAA pathway, possibly reflecting redundancy of chemoattractant signals that contribute to the multi-step adhesion cascade in living organisms. Rather, the defect in neutrophil recruitment in mutant animals was explained by a critical non-redundant role for GPR35–5-HIAA during the diapedesis step. This pinpoints a unique function of GPR35, which for more than 2 decades had been considered an orphan receptor (Kaya et al., 2021), and also for 5-HIAA, which had not previously been implicated in neutrophil biology.

It is still unclear whether GPR35 agonism can contribute also to mobilization of the BM-resident neutrophil reservoir; however, this effect appears less likely since GPR35 expression on resting

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neutrophils is very low. By contrast, as the receptor is rapidly upregulated on activated neutrophils after extravasation, there could be a potential additional role during interstitial migration. Migrating neutrophils can integrate diverse chemotactic signals from multiple sources with some chemoattractants dominating over competing signals by others (Foxman et al., 1997). Whether 5-HIAA participates in this process and where it ranks in the chemoattractant hierarchy remains to be determined. It will also be important to assess the role of the GRP35–5-HIAA pathway in response to inflammatory triggers other than bacterial infections, especially in settings where neutrophil accumulation may have detrimental effects for the host. Such studies have the potential to ultimately lead to novel therapeutic approaches for a host of inflammatory conditions.

DECLARATION OF INTERESTS

Ulrich H. von Andrian is a founder of Selecta Biosciences and of Monopteros Biotherapeutics and a member of both companies' scientific advisory boards. He is also a member of the scientific advisory boards of Avenge Bio, Beam Therapeutics, Bluesphere Bio, Cygnal, Evelo, Intergalactic, Interon, Mallinckrodt Pharmaceutical, Moderna, Morphic Therapeutics, Rubius, and SQZ.

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Diversifying the menu for crop powdery mildew resistance

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Powdery mildew, a potentially severe crop disease, can be controlled by *mlo* mutations, which suppress fungal proliferation but typically also reduce yield. Li et al. (2022) demonstrate that productivity can be restored by overexpressing a host sugar transporter, thus offering a new option for economically and environmentally benign disease control.

The fungal disease powdery mildew is a threat to many crops in temperate climates. In barley, mutations of *Mildew resistance locus O* (*Mlo*), discovered in 1942, give near-total resistance. This gene is unusual because resistance is conferred by recessive loss-of-function (*mlo*) alleles and because it has been durable, remaining effective against all pathogen races for 8 decades. By contrast, most other major genes controlling disease resistance in plants are dominant and are not durable because they are ineffective against specifically virulent pathogen genotypes.

MLO is a plasma membrane-localized protein with seven transmembrane domains (Büschges et al., 1997). Perhaps remarkably, the mechanism of *mlo*-mediated resistance is still unknown, but the finding that *Mlo* is largely conserved across the plant kingdom has paved the way for inducing resistance by knocking it out in diverse crops including tomato, pea, cucumber, and wheat as well as the model plant *Arabidopsis* (Kusch and Panstruga, 2017). Most artificial *mlo* mutations are associated with reduced yield, but barley breeders can mitigate the yield penalty through reassortment of the genetic background (Kjær et al., 1990). Moreover, although the natural allele *mlo11* has a slightly weaker effect than artificial