Purinergic signaling and immune cell chemotaxis. Focus on “The UDP-sugar-sensing P2Y_14 receptor promotes Rho-mediated signaling and chemotaxis in human neutrophils”

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Purinergic receptors enable cells to detect and respond to extracellular nucleotides and adenosine. Presently, eight G protein-coupled P2Y receptors, four adenosine receptors, and seven ionotropic P2X receptors have been cloned and characterized (11). In addition, multiple ectonucleotidases and pathways for nucleotide release have been identified in mammalian cells where they function collaboratively within signaling complexes to regulate a variety of physiologic process (2). In immune cells, these complexes facilitate recognition of nucleotides released from damaged or stressed cells, which in turn triggers autocrine purinergic feedback pathways that promote recruitment and inflammatory responses at the site of infection (3, 6). Interactions between paracrine and autocrine purinergic signaling mechanisms allows immune cells to modify their response to danger signals and find-me cues released by surrounding tissues. Purinoceptor stimulation also activates inflammasomes, resulting in secretion of cytokines that contribute to the overall inflammatory response (6).

Overview of neutrophil recruitment and chemotaxis. Neutrophils are polymorphonuclear leukocytes that are stored in bone marrow then rapidly released into the circulation in response to infection. They can be recruited from the bloodstream into affected tissues by various chemoattractant molecules produced by microorganisms or by host cells within infected and inflamed tissues. Neutrophil extravasation initially involves adhesion to the endothelium, a process that is mediated by P-selectin ligand-1 (PSGL-1) located at the tips of neutrophil microvilli. PSGL-1 binds to P-selectin to produce tethering and rolling of neutrophils along the endothelium of the microcirculation (6). Exposure to chemoattractant molecules under these conditions results in expression of integrins on the cell surface that strengthens neutrophil adherence to endothelial cells. Transmigration then occurs through paracellular or transcellular pathways without damaging endothelial integrity. Once neutrophils enter the extravascular space they migrate towards the source of chemoattractant mediators from the site of inflammation or tissue damage. The widely accepted local excitation, global inhibition model for gradient sensing and chemotaxis suggests that a combination of leading edge positive feedback loops and longer range negative feedback mechanisms translate and amplify weak extracellular chemoattractant gradients into well-defined internal signals that direct pseudopod protrusion at the leading edge while suppressing signals that would yield protrusions along the lateral and trailing margins of polarized cells (6).

Autocrine purinergic signaling and modulation of the chemotactic response. Activation of chemoattractant receptors expressed by neutrophils evokes the polarized release and accumulation of ATP at the cell surface nearest to the chemoattractant source (3, 7). P2Y_2 receptors adjacent to the site of release become activated and amplify intracellular signals initially generated by chemoreceptor stimulation. As neutrophils undergo polarization, proteins involved in purinergic signaling such as pannexin 1 (channels through which ATP is released), the ectonucleotidase CD39 and A3 adenosine receptors redistribute to the leading edge of the cell. Additional ATP release associated with lamellipodia protrusion is hydrolyzed by CD39 to produce adenosine, which binds to A3 receptors to strengthen purinergic feedback pathways necessary for migration towards the chemoattractant source (Fig. 1). Negative, longer range autocrine purinoceptor feedback mediated by A2A adenosine receptors blocks cell activation at sites away from the leading edge of the cell and supports cell migration by promoting retraction at the trailing edge (7). Thus autocrine purinergic feedback loops represent a specific example of local...
excitation, global inhibition that modulates the chemotactic response of neutrophils initiated by chemooattractants emanating from a site of infection or inflammation.

**UDP-glucose and P2Y14 receptor-mediated neutrophil motility.** The present study by Sesma et al., published in this issue of the *American Journal of Physiology-Cell Physiology* (10), investigated the role of P2Y14 receptors (P2Y14-R) in neutrophil chemotaxis. P2Y14-R is a Gs-coupled receptor that is activated by UDP and UDP-sugars, but not by ATP, ADP, or other naturally occurring nucleotide di- or triphosphates. Earlier studies showed that UDP-glucose is released with mucins from the airway epithelium by exocytosis under conditions of inflammation and unlike ATP or UTP, extracellular UDP-glucose is stable and not readily degraded by ectonucleotidases (10). UDP-glucose was also found to stimulate alveolar and airway epithelial cells to secrete proinflammatory chemokines capable of inducing neutrophil recruitment (8). Results from Sesma et al. (10) demonstrate that UDP-glucose functions as a stable chemooattractant for neutrophils in response to tissue damage or inflammation. In addition, chemotaxis was partially inhibited when neutrophils and differentiated HL60 cells were treated with apyrase, suggesting that ATP release was associated with UDP-glucose stimulation (10).

Neutrophil exposure to UDP-glucose induced RhoA activation, rearrangement of the cytoskeleton and stimulated cell migration. Treatment with Rho kinase inhibitors decreased migration, which is consistent with previously published data showing that Rho kinase activation is associated with cell polarization along the axis of movement and myosin-dependent retraction of the trailing edge of the cell. The proposed mechanism of RhoA activation evoked by UDP-glucose binding to P2Y14-R appears to involve pertussis toxin-sensitive Gi activation and subsequent Gβγ-induced increases in phosphoinoside 3-kinase (PI-3-kinase) activity. The resulting stimulation of phosphatidylinositol(3,4,5)trisphosphate production facilitates Rho-G protein exchange factor recruitment to the plasma membrane and enhanced RhoGTP formation, similar to fMLP receptor signaling in other immune cells (1).

**Implications.** An issue that was not addressed by Sesma et al. (10) involves the cellular localization of P2Y14-R and whether its distribution changes in response to the presence of a gradient for UDP-sugars. This would have implications for gradient amplification since chemooattractant receptors have been reported to redistribute to the leading edge of the cell during polarization and migration. P2Y14-R redistribution may coincide with activation and recruitment of PI-3 kinase and the movement of lipid rafts to the leading edge of the cell. Asymmetric redistribution of lipid rafts has been shown to occur following stimulation with chemooattractants and electric fields. Interestingly, treatment of DMSO-differentiated HL60 cells with pertussis toxin inhibits raft redistribution in response to stimulation with fMLP, suggesting that rafts function as signal amplification platforms during chemotaxis (5). Based on the results of Sesma et al. (10), the post-receptor signaling events associated with P2Y14-R could facilitate trafficking of receptor-containing rafts to the leading edge to enhance UDP-sugar detection.

Although a specific role for P2Y14-R in autocrine modulation of neutrophil chemotaxis was not specifically identified by Sesma et al. (10), previous studies have shown that UDP functions as a P2Y14-R agonist (4). Therefore, it is conceivable that UTP release from neutrophils and subsequent CD39-mediated UDP accumulation at the leading edge during lamelipodia protrusion could activate P2Y14-R (and potentially P2Y6-R) to provide local amplification of intracellular signals originally produced in response to a chemooattractant gradient. Moreover, in addition to the previously described role for Gs-coupled P2Y2 receptors in chemooattractant signal amplification, Gi activation following UDP binding to P2Y14-R would presumably broaden the array of post-receptor signaling pathways that would be activated by autocrine ATP and UTP release. Ultimately, it may be that P2Y14-R functions as both a primary chemooattractant detection mechanism for UDP sugars and as a component of autocrine purinergic signaling responsible for modulation of the neutrophil chemotactic response.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author.

**AUTHOR CONTRIBUTIONS**

S.M.O. prepared the figure; drafted the manuscript; edited and revised the manuscript; and approved the final version of the manuscript.

**REFERENCES**