inside blood commentary

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Comment on Artz et al, page 529

Dampening neutrophil integrins

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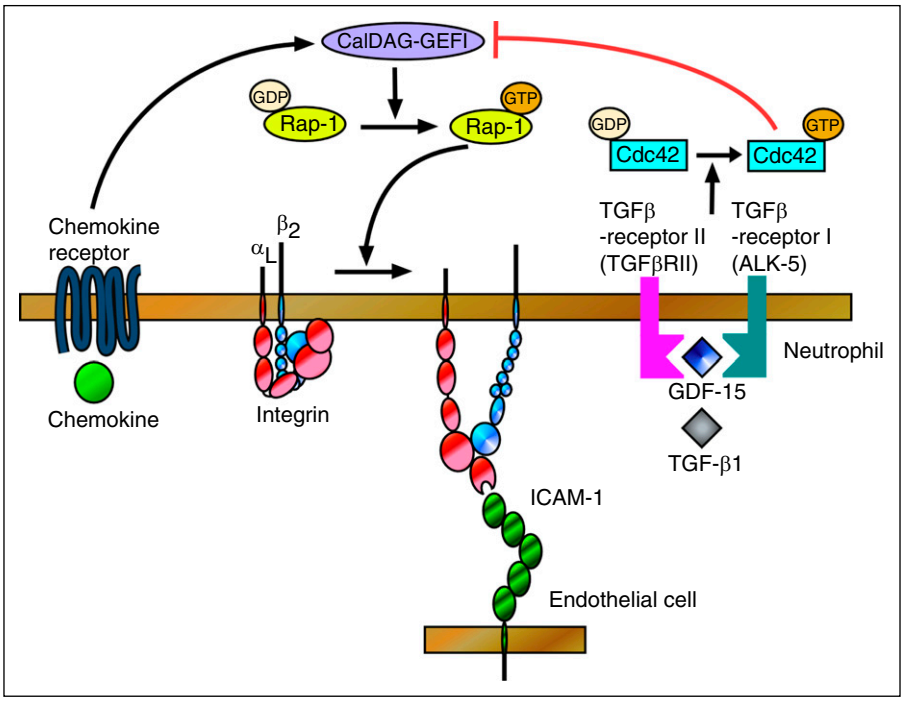
In this issue of *Blood*, Artz et al show that growth differentiation factor 15 (GDF-15), a distant relative of transforming growth factor β (TGF-β), inhibits neutrophil integrin activation through canonical TGF-β receptor heterodimers.[1]

During inflammation, neutrophils roll along venules, arrest, and crawl into perivascular tissues. Interactions of neutrophil ligands with endothelial P- and E-selectin mediate rolling. Interactions of neutrophil integrins with ICAM-1 slow rolling and mediate arrest. Signaling through selectin ligands activates integrin αLβ2 to an extended, intermediate-affinity conformation that slows rolling. Signaling through chemokine receptors activates integrin αLβ2 to an extended, high-affinity conformation that mediates arrest.[2] In both signaling cascades, a key downstream step is activation of the guanosine triphosphatase (GTPase) Ras-related protein 1 (Rap-1), in part through the guanine nucleotide exchange factor (GEF) CalDAG-GEFI (see figure). GTP-bound Rap-1 acts through effectors to recruit talin-1 to the plasma membrane.[3] Binding of talin-1 to the β2 cytoplasmic tail initiates integrin activation.[4] The signaling steps that activate integrins have been intensively investigated. Whether signals can inhibit integrin activation has received less attention.



Signals transmitted through chemokine receptors on rolling neutrophils activate the GTPase Rap-1, in part through CalDAG-GEFI. GTP-bound Rap-1 acts through effectors to convert integrin αLβ2 to an extended, high-affinity conformation, which mediates neutrophil arrest by binding ICAM-1 on endothelial cells. Artz et al demonstrate that TGF-β1 and the distantly related GDF-15 transmit signals through TGF-β–receptor heterodimers, which activate the GTPase Cdc42. GTP-bound Cdc42 inhibits activation of Rap-1, likely by interfering with CalDAG-GEFI. Cdc42, cell division protein 42; GDP, guanosine diphosphate. Illustration by Tadayuki Yago.

Earlier studies revealed that another GTPase, Cdc42, in its active GTP-bound form, decreases chemokine-induced activation of αLβ2 in lymphocytes.[5] GDF-15 was then shown to inhibit chemokine-mediated activation of αLβ2 in neutrophils by activating Cdc42, which blocks activation of Rap-1.[6] Inflammatory mediators induce expression of GDF-15 in many tissues. In GDF-15–deficient mice, more neutrophils arrest and migrate outside venules after inflammatory challenge.[6] Thus, GDF-15 is an anti-inflammatory cytokine that dampens neutrophil integrin activation. Its weak sequence similarity to other TGF-β family members suggested that it signals by distinct mechanisms. But Artz et al demonstrate that GDF-15, and TGF-β1 itself, inhibits chemokine-triggered integrin activation in neutrophils through heterodimers of TGF-β receptor I (TGF-βRI), also known as activin receptor-like kinase 5, and TGF-βRII. Like GDF-15–deficient mice, mice lacking TGF-βRI or TGF-βRII in myeloid cells have enhanced neutrophil arrest and migration after challenge. Both GDF-15 and TGF-β1 use TGF-β receptor heterodimers to activate Cdc42, which is required to inhibit Rap-1 in neutrophils. Strikingly, neither GDF-15 nor TGF-β1 inhibits chemokine-induced integrin activation in CalDAG-GEFI–deficient neutrophils. The

authors did not test whether the lack of CalDAG-GEFI prevents activation of Cdc42, although the latter has other known GEFs. Instead, they suggest that activated Cdc42 somehow prevents CalDAG-GEFI from activating Rap-1. How this might occur is an intriguing topic for future study.

Although Artz et al do not address this possibility, signaling through TGF-β receptors likely also dampens selectin-triggered integrin activation by inhibiting Rap-1. Engaging TGF-β receptors may have other effects. For example, phosphatidylinositol-4-phosphate 5-kinase γ (PIP5KIγ) recruits talin-1 to membranes by direct binding and by generating phosphatidylinositol 4,5 bisphosphate (PIP2).[3] In lymphocytes, constitutively active Cdc42 decreases chemokine-induced generation of PIP2, implying that it negatively regulates PIP5KIγ.[5] Thus, TGF-β–receptor activation of Cdc42 could block talin-1 recruitment by inhibiting both Rap-1 and PIP5KIγ. Artz et al also show that engaging TGF-β receptors on monocytic cells inhibits chemokine activation of α4 integrins, at least in vitro. Therefore, TGF-β receptors may modulate different integrins in different leukocyte subsets.

Artz et al demonstrate that TGF-β1 inhibits neutrophil integrin activation in vitro. Whether it exerts this function in vivo requires further investigation. Most cells express TGF-β1, which exerts pleiotropic effects depending on cellular context. These effects typically regulate gene expression, whereas Artz et al describe a rapid, transcription-independent signaling pathway. To become active, TGF-β1 must dissociate from a latency complex. Activated platelets release large amounts of latent TGF-β1 that can be activated by shear stress under some conditions.[7] Activated platelets bind to neutrophils arrested in venules and transmit signals that regulate integrin αMβ2-dependent crawling.[8] Could platelet-released TGF-β1 contribute to signaling?

Other molecules influence neutrophil integrins. Signaling through neutrophil adenosine A2A receptors hinders selectin- and chemokine-induced β2 integrin activation, as well as β2 integrin outside-in signaling.[9] On the other hand, rolling neutrophils secrete myeloid-related proteins 8 and 14 from the cytosol.[10] The secreted proteins bind in an autocrine manner to toll-like receptor 4 on neutrophils, triggering Rap-1–dependent activation of β2 integrins. The demonstration that TGF-β receptors negatively regulate β2 integrins further emphasizes the complexity of neutrophil responses during inflammation. Determining how diverse signaling pathways cooperate or compete in different contexts may suggest new therapies for inflammatory diseases.

Conflict-of-interest disclosure: The author holds equity in Selexys and Tetherex Pharmaceuticals. ■

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Comment on Guo et al, page 553

IL-4 regulates B-cell receptor signaling in CLL

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In this issue of *Blood*, Guo et al show that interleukin-4 (IL-4) upregulates surface immunoglobulin M (IgM) expression and B-cell receptor (BCR) signaling capacity of chronic lymphocytic leukemia (CLL) cells, providing an explanation of how anergic CLL cells from the peripheral blood become signal competent when they enter the lymph nodes.[1]

The recent approval of the Bruton tyrosine kinase (BTK) inhibitor ibrutinib and the phosphatidylinositol 3-kinase δ (PI3Kδ) inhibitor idelalisib represent major breakthroughs in the treatment of CLL. Although these drugs can inhibit various signaling pathways, they are believed to function primarily by inhibiting signals that the leukemic cells receive through their BCRs.[2] This mechanism of action is supported by numerous lines of evidence supporting a major role for the BCR pathway in the development and progression of CLL, including the expression of BCRs with particular structural and antigen-binding properties, the expression of high levels of BCR-target genes in lymph node-derived CLL cells, and the significant association between clinical course and certain BCR-related features, such as the mutational status of the immunoglobulin heavy chain variable (IGHV) region genes.[3] This evidence, however, is difficult to reconcile with the relatively impaired capacity of CLL cells in the peripheral blood to respond to BCR engagement. Reduced BCR signaling capacity is a general feature of CLL, but is particularly prominent in the CLL subset with IGHV-mutated genes (M-CLL), where the malignant B cells are often completely anergic.[4]

In the article published in this issue of *Blood*, Guo et al investigate the mechanism(s)