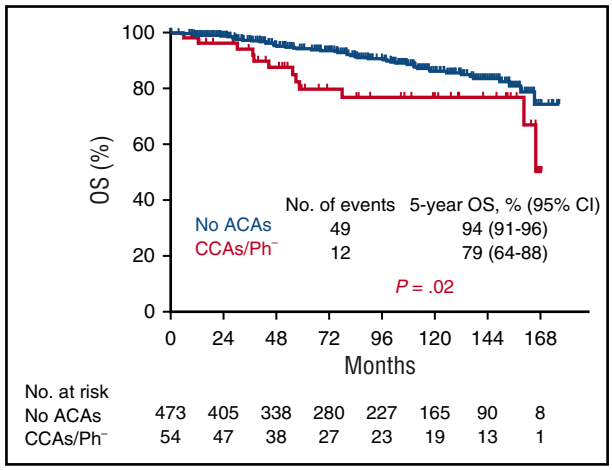

Overall survival (OS) of 54 patients with CML with CCAs/Ph$^-$ compared with 473 patients with no additional chromosomal aberrations (ACAs). Median observation time was 7.6 years. See supplemental Figure 1D in the article by Issa et al that begins on page 2084.

The major reason for decreased survival in patients with CCAs/Ph$^-$ seems to be transformation of CML resulting from genetic instability and the appearance of ACAs, similar to observations in Ph$^+$ cells.[7,8] The occurrence of ACAs in Ph$^+$ and Ph$^-$ cells in the same patient, as observed by others as well,[9,10] would support this idea. Clonal ACAs in CML constitute a negative prognostic sign whether they occur in Ph$^+$ or Ph$^-$ cells.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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● ● ● PHAGOCYTES, GRANULOCYTES, AND MYELOPOIESIS

Comment on Morikis et al, page 2101, and Bai et al, page 2092

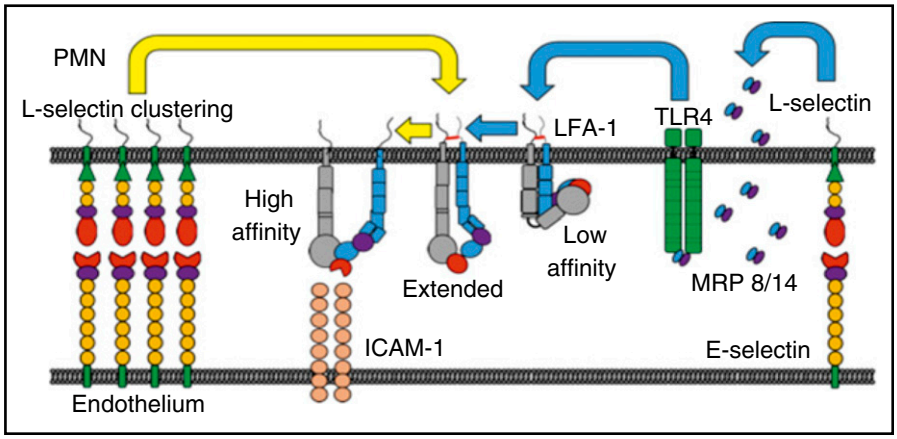
'Tis one thing to adhere, another to migrate

Christopher J. Kuckleburg IPD ANALYTICS

In this issue of *Blood*, we review 2 studies investigating neutrophil integrin activation. Morikis et al describe the molecular dynamics of mechanotransduction, which underlie E-selectin–mediated neutrophil rolling and adhesion. Bai et al investigate CD177-induced integrin activation in regulating adhesion and transmigration.[1,2]

Neutrophil recruitment can be broadly conceptualized as 3 sequential steps: rolling, adhesion, and transmigration. The interaction between endothelial cell selectins (E-selectin) and glycosylated neutrophil ligands (L-selectin, P-selectin glycoprotein ligand-1 [PSGL-1], sialylated glycosphingolipids) promotes neutrophil rolling along the vascular endothelium through catch-bonds and slip-bonds. Adhesion is established through the interaction of neutrophil integrins LFA-1 (CD11a/CD18a) and Mac-1 (CD11b/CD18) with their endothelial cell counterreceptor, ICAM-1. Neutrophil transmigration then occurs at interendothelial junctions requiring associations between LFA-1 and Mac-1 with ICAM-1, ICAM-2, and JAM family members, and the contributions of CD99 and PECAM-1.[3] The spatial-temporal regulation of integrin activation plays a critical role in all steps of neutrophil recruitment.

In contrast to mouse neutrophils, human neutrophils can transition LFA-1 to the high-affinity state via rolling over E-selectin, even in the absence of G protein–coupled receptor signaling.[4,5] Human neutrophils incorporate *N*-glycans with the tetrasaccharide carbohydrate sialyl Lewis^x (sLe^x) onto L-selectin. This difference allows human neutrophils to bind and cluster E-selectin, leading to the release of cytosolic calcium, activation of Src-family kinases, and β2-integrin activation. Although previous studies



E-selectin cross-linking activates the release of myeloidrelated protein (MRP) 8/14 and extension of β2 integrin to a high-affinity state. PMN, polymorphonuclear leukocyte; TLR, Toll-like receptor. See Figure 6A in the article by Morikis et al that begins on page 2101.

have demonstrated that blocking antibodies to E-selectin can prevent human neutrophil arrest under shear conditions, it is still unclear how E-selectin mediates outside-in signaling to promote a shift in β2 integrins from the intermediate to high-affinity conformation.[6]

Using Rivipansel, an sLe^x tetrasaccharide structure mimic that disrupts L-selectin interactions, Morikis and colleagues explored the role of E-selectin in mediating outside-in signaling of neutrophil β2 integrin. The authors found that Rivipansel significantly inhibited neutrophil arrest and migration across interleukin 1β (IL-1β)–stimulated endothelial cells. Because Rivipansel is recognized by all 3 selectins, the authors used blocking antibodies to known E-selectin ligands (CD44, PSGL-1, L-selectin) to determine the relative contributions of each to integrin activation in neutrophil rolling and following E-selectin cross-linking. All blocking antibodies inhibited E-selectin binding; however, only anti-L-selectin antibodies inhibited high-affinity CD18 activation in response to E-selectin cross-linking. Under shear flow conditions, blocking L-selectin interactions also inhibited neutrophil arrest on E-selectin, results similar to those with anti-β2-integrin blocking antibodies. In contrast, PSGL-1 blocking antibodies were unable to disrupt neutrophil arrest.

E-selectin catch-bond formation allows for an increasing force to convert short-lived tethers into strong, longer-lived binding interactions (see figure). To investigate the bond mechanics between sLe^x and E-selectin, the authors observed neutrophil capture under variable shear stress conditions. Both Rivipansel and the PSGL-1 glycopeptide mimetic, GSnP-6, increased neutrophil mean rolling velocity; however, only Rivipansel inhibited neutrophil arrest. It is known that both L-selectin and PSGL can form catch-bonds when tether force and wall shear stress are increased. However, the authors report that tether duration and efficiency were significantly decreased when L-selectin interactions were blocked, as compared with blocking PSGL-1. In addition, the authors observed L-selectin clustering and increased focal adhesion on neutrophils under shear conditions, which were blocked when Rivipansel was used to disrupt L-selectin interactions. Such clustering has previously been associated with Src kinase activity. Indeed, the authors found that Rivipansel inhibited activation of the Src kinase, Lck, and thereby high-affinity β2-integrin expression. Interestingly, the ligation of E-selectin also resulted in the concomitant release of MRP8, which appears to contribute to a shift in β2 integrin to an intermediate-affinity state.

Morikis et al reveal additional insight into the evolutionary differences between mouse and human neutrophils, and demonstrate that efficient high-affinity β2-intergrin expression associated with E-selectin mechanotransduction may require a second signal, stressing the importance of temporal regulation in neutrophil integrin activation. Understanding the contribution of these signaling pathways in neutrophil recruitment will require future investigations. This study also describes the therapeutic utility of Rivipansel for treating vascular occlusive disease and invites the possibly of its use in other conditions to regulate neutrophil recruitment.

Bai and colleagues investigate the next step in neutrophil recruitment: the transition from cell adhesion to migration. For most individuals, CD177 is expressed on a subset of neutrophils (45%-65%), although a small population of individuals (3%-5%) do not express CD177 on any circulating neutrophils ($CD177^{null}$). CD177 is the counterreceptor for PECAM-1 and has been shown to play a role in neutrophil transmigration.[7] CD177 has also been reported to anchor the neutrophil serine protease proteinase 3 to the neutrophil surface.[8] CD177 has been shown to physically associate with β2 integrins, particularly Mac-1 (CD11b/CD18), but the role of this interaction is unclear.[9] Although several studies have demonstrated a migratory advantage for $CD177^{pos}$ neutrophils, other studies have shown that this enrichment does not occur in vivo.[10] Therefore, the role of CD177 in neutrophil recruitment is an area of continued investigation.

Using a PECAM-1–independent transwell system, Bai and colleagues report that CD177 ligation (using a cross-linking CD177 antibody) disrupted $CD177^{pos}$ neutrophil migration in response to IL-8 or fMLP. Ligation of CD177 was found to enhance $CD177^{pos}$ neutrophil adherence and spreading but, paradoxically, ligation of CD177 inhibited neutrophil translocation. The authors found that ligation of CD177 enhanced the expression of CD11a, CD11bA (the active confirmation of CD11b), CD66, and activated Src and extracellular signal-regulated kinase (ERK) signaling. Strikingly, the ERK inhibitor U0126 was found to partially restore neutrophil migration of $CD177^{pos}$ neutrophils following ligation.

Previous studies have reported that CD177 associates closely with β2 integrins.[9] Using fluorescence lifetime imaging microscopy, the authors confirmed close spatial interactions between CD11b and CD177 and report that ligation inhibited integrin internalization. This suggests that CD177 may be able to arrest cell migration not only through integrin activation, but also by inhibiting integrin internalization and recycling.

The ligation of CD177 is most likely to occur when neutrophils are transmigrating, contacting PECAM-1 at endothelial cell junctions during transmigration, a location where arresting neutrophil transmigration

would be undesirable. Therefore, if CD177 inhibits neutrophil migration, then we would expect other factors to compensate for this by promoting integrin recycling or uncoupling trailing integrin associations. Whether this includes neutrophil serine proteases or other soluble factors will be an intriguing area for future investigations.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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● ● ● RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Colucci et al, page 2111

Releasing the FKBP12 brake on hepcidin

Nermi L. Parrow and Robert E. Fleming SAINT LOUIS UNIVERSITY SCHOOL OF MEDICINE

In this issue of *Blood*, Colucci et al add to the understanding of hepcidin regulation by demonstrating a role for the immunophilin FKBP12 in dampening signaling via the bone morphogenetic protein (BMP) type I receptor ALK2.[1]

Hepatocellular expression of the iron-regulatory hormone hepcidin is influenced by several stimuli, including circulating iron levels, erythropoietic activity, and inflammation. The response to iron is mediated by BMP/SMAD signaling; moreover, a complete response to inflammation requires an intact BMP/SMAD pathway. Recent studies using genome-wide RNA interference knockdown technology identified the nutrient-sensing mammalian target of rapamycin (mTOR) pathway as a modifier of hepcidin expression.[2] Rapamycin was found to increase hepcidin expression in cell culture. Hepcidin-mediated iron restriction might explain the microcytic anemia seen in association with pharmaceutical use of rapamycin.[3] Colucci et al dissect the mechanism and demonstrate that rapamycin and the related drug, tacrolimus, upregulate hepcidin by sequestering the FK506 (tacrolimus) binding protein, FKBP12, to upregulate BMP/SMAD signaling. They provide evidence that this immunophilin otherwise serves as a "brake" on hepcidin signaling by its interaction with BMP type I receptor ALK2 and demonstrate increased basal hepcidin expression in cell culture systems by drug-mediated sequestration of FKBP12 or by mutation of ALK2 protein residues required for FKBP12-ALK2 interaction (see figure).

The consequences of mutations in ALK2 that interfere with FKBP12 binding on BMP/SMAD signaling have been previously investigated, because such mutations are responsible for the rare bone disorder fibrodysplasia ossificans progressiva (FOP). Empirical observations and molecular modeling suggest that causative ALK2 mutations result in a gain of function characterized by increased BMP/SMAD signaling (even in the absence of ligand binding and BMP type II receptor phosphorylation). ALK2 also shows enhanced responsiveness to canonical BMP ligands, with 1 study reporting hyperresponsiveness to BMP2 but an unaltered response to BMP6 in a hepatic cell line.[4] Importantly, these mutations render the ALK2 receptor responsive to the transforming growth factor β signaling superfamily cytokine activin A. Activin responsiveness is required to recapitulate the disease phenotype in a mouse model of FOP, including the heterotopic ossification that characterizes the condition.[4]

Colucci et al likewise found that the consequences of abrogating FKBP12-ALK2 interactions on hepcidin expression are much more pronounced when examined in the presence of activin A. The authors suggest that during inflammation, ALK2 is not bound to FKBP12 and is thus rendered sensitive to noncanonical ligands such as activin A. This observation is particularly important, given evidence that ALK2 appears to contribute to the upregulation of hepcidin under inflammatory conditions. This model has implications for iron homeostasis during inflammation, as was suggested by a recent study reporting that the drug momelotinib decreases hepcidin production in a rat model of anemia of chronic disease by inhibiting ALK2.[5] Studies using follistatin (an activin A and B inhibitor) in murine models of acute and chronic inflammation have suggested a role for one or both of these cytokines in hepcidin regulation.[6] Subsequent studies have not supported a role for activin B.[7]

Although these studies suggest participation of ALK2 and activin A in the upregulation of hepcidin during inflammation, some lines of evidence suggest that neither is essential. For example, interleukin-6 administration induces hepcidin expression and decreases serum iron in mice with hepatocyte-specific deficiency of ALK2.[8] Mice with hepatocellular inactivation of activin B do not demonstrate upregulation of SMAD5 yet have complete induction of hepcidin