with endothelial cell and platelet β1 integrins during thrombus formation in vivo, thereby regulating integrin activity.5

Integrins are integral membrane proteins that mediate cell-cell adhesion and cell-matrix interactions. These molecules are essential for hematopoiesis, vascular development, immune and inflammatory responses, and hemostasis. Integrins are signaling receptors that can signal information bidirectionally across the plasma membrane. In circulating blood cells, integrins are normally in an active (resting) state with low affinity for their ligands. Upon activation, they can quickly change their conformation to an activated, high-affinity state, a process often referred to as inside-out signaling (see figure).6

In the present study, Hahm et al demonstrate a new mechanism by which the adhesiveness of the β2-integrin Mac-1 can be regulated under inflammatory conditions. The authors show that the specific deletion of PDI in myeloid cells abolishes neutrophil recruitment. By using blocking antibodies and knockout mice, they convincingly demonstrate that eliminating or blocking PDI reduces neutrophil adhesion and crawling by regulating Mac-1 adhesiveness. Although Mac-1 is also involved in neutrophil transmigration,7 the authors have not investigated this important step in this study. In addition to this, the in vivo data in the present study show that exogenous PDI can also bind to Mac-1. However, as exogenous PDI still binds to αMβ2 integrin-deficient neutrophils, it is likely that the regulatory role of PDI in integrin function is not limited to Mac-1.

Integrins contain several highly conserved cysteine residues. Some of the cysteines are disulfide-bonded and some exist as free thiols. Cleavage of disulfide bonds appears to be involved in the activation of integrins.3,9 The reducing agent dithiothreitol activates integrins on platelets and neutrophils by reduction of disulfide bonds within the integrin’s cysteine-rich repeat.3,9 The study by Hahm et al demonstrates that PDI on the cell surface of neutrophils interacts with activated Mac-1 within lipid rafts via electrostatic interactions and catalyzes thiol exchange on the αM subunit of Mac-1 (see figure), thereby regulating Mac-1 adhesiveness during neutrophil activation. Integrin adhesiveness is regulated by modulating integrin affinity and avidity.10 Blocking of PDI in human neutrophils did not reduce the binding of the reporter antibody CBRM 1/5, which only detects the activated form of Mac-1, but abolished Mac-1 clustering,1 suggesting that PDI does not affect inside-out-mediated affinity regulation of Mac-1, but rather the stabilization of the integrin in the activated conformation. The results of this study stand in contrast to another study showing that modification of disulfide bonds in the I domain of the αM subunit alters the affinity of ligand binding.9 These data suggest that the modification of some cysteine residues regulates integrin affinity, whereas modification of other cysteine residues modulates integrin avidity. Future studies are necessary to examine whether PDI has a binding specificity to a specific integrin subunit and determine which cysteine residue on Mac-1 is modified by PDI.

In summary, these findings put forward a novel and important role for PDI in regulating neutrophil recruitment during inflammation. An important aspect of these findings is that PDI interacts with activated Mac-1 in lipid rafts and modifies thiol exchange on Mac-1, thereby regulating Mac-1 adhesiveness and neutrophil recruitment. Further studies are anticipated that will investigate the specific mechanisms underlying the regulation of β2-integrin activation on leukocytes, which remain unknown.

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Comment on Bueno et al, page 3867

The ongoing conundrum of MLL-AF4 driven leukemogenesis

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In this issue of Blood, Bueno and colleagues explore the developmental impact, as well as the transforming capacity, of the mixed-lineage leukemia (MLL)-AF4 fusion protein in combination with activation of FMS-like tyrosine receptor 3 (FLT3) in human embryonic stem cells (hESCs).1

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MLL-AF4+ pro-B acute lymphoblastic leukemia (ALL) in infants represents an aggressive, high-risk type of childhood leukemia arising from prenatally acquired preleukemic t(4;11) chromosomal translocations. However, despite various reported attempts, accurately mimicking MLL-AF4–driven leukemogenesis in mice remains a difficult task. Enforced expression of the MLL-AF4 fusion protein in cord blood–derived human hematopoietic stem cells (HSCs) transplanted into immunodeficient mice either results in the development of malignancies that deviate from the pro-B ALL phenotype as observed in humans, or does not lead to neoplasia at all.2 Obviously, these discrepancies raise important questions: (I) Does MLL-AF4+ pro-B ALL in infants arise in CD34+ HSCs? (II) Are MLL-AF4 fusion proteins driving leukemogenesis on their own, or are cooperative genetic lesions required? (III) Do MLL-AF4 fusion proteins exert sufficient transforming capacity? For instance, Bursen et al recently showed that not MLL-AF4, but enforced expression of its reciprocal fusion protein AF4-MLL in murine HSCs or progenitor cells, induced ALL in mice without the requirement of MLL-AF4.3 In contrast, Tamai et al showed that enforced expression of MLL-AF4 in murine HSCs is sufficient to induce ALL, but demonstrated that the process of transformation is significantly accelerated by cooperative K-Ras mutations.4 Nonetheless, these experiments remain to be repeated in HSCs of human origin to appreciate the relevance of AF4–MLL and/or RAS activation in the development of MLL-AF4+ pro-B ALL. Moreover, the reciprocal AF4–MLL fusion transcript is present in the majority of, but not all, patients with MLL-AF4+ ALL,3 and RAS mutations are found in ~25% of the cases.5 Hence, distinct mechanisms of transformation, as well as the involvement of yet unknown genetic events, cannot be ruled out.

Meanwhile, Dr Pablo Menendez and coworkers have been elegantly addressing the question of the cell of origin from which MLL-AF4+ pro-B ALL may arise.6 On the basis of their earlier observations that bone marrow–derived mesenchymal stem cells from patients with MLL-AF4+ pro-B ALL harbor and express the MLL-AF4 fusion gene,6 Menendez et al reasoned that this type of leukemia may well arise in hematopoietic mesodermal or hemangioblastic precursors sprouting from differentiating hESCs. To test this hypothesis, this research group recently created a cellular system to study early hematopoietic development in MLL-AF4–expressing hESCs. Interestingly, introducing MLL-AF4 expression in hESCs enhanced the specification of hemogenic precursors, but impaired further hematopoietic commitment in favor of an endothelial cell fate. Alas, MLL-AF4 expression alone appeared not sufficient to induce leukemia in hESC-derived hematopoietic cells.5 In the present study, Bueno et al explored the impact of FLT3 activation on the hematopoietic fate of MLL-AF4–expressing hESCs. Patients with MLL-AF4+ pro-B ALL frequently display constitutive FLT3 activation, usually as a result of high-level FLT3 expression, or sporadically from activating mutations within the tyrosine kinase domain.5 Activated FLT3 positively affects several signal transduction pathways, all of which favor cell survival and proliferation, and supposedly provides (pre-) leukemic cells with a growth advantage and possibly with enhanced transforming capacity. Hence, FLT3 activation may well be an additional genetic event required for MLL-AF4–driven leukemogenesis. Interestingly, Bueno et al5 show that in MLL-AF4–expressing hESCs, activated FLT3 is capable of abolishing hematopoietic differentiation, indeed suggesting a role for FLT3 activation in the development of MLL-AF4+ pro-B ALL. However, FLT3 activation did not seem sufficient to cooperate with MLL-AF4 in transforming hESC-derived hematopoietic cells. Nonetheless, the presented MLL-AF4–expressing hESC model represents an intriguing experimental system that hopefully soon will also be used to explore the impact of other potential secondary oncogenic lesions. In the meantime, it remains important to keep searching for alternative target cells that may resemble the actual cell of origin, as well as additional (epi)genetic hits that potentiate MLL-AF4–driven leukemogenesis (eg, use of whole-genome sequencing approaches).

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