Not simply misshapen red cells: multimolecular and cellular events in sickle vaso-occlusion

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Commentary

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Not simply misshapen red cells: multimolecular and cellular events in sickle vaso-occlusion

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Not just misshapen red cells

In a 1910 report of an anemic West Indian man, Herrick first described the “peculiar elongated and sickle-shaped” rbcs produced by individuals with sickle cell anemia (1). Over the next century, evaluation of sickle cell anemia–associated molecular and cellular pathobiology revealed that the polymerization of hemoglobin S and cellular shape change upon deoxygenation were due to a single nucleotide mutation (A to T) in the gene encoding β-globin. The pain crises and organ infarctions that manifest in patients with sickle cell disease (SCD) have marked leukocytosis, thrombocytosis, markers of inflammation, oxidative stress, and a procoagulant phenotype. Other cells have been implicated in sickle-associated vaso-occlusive events, including neutrophils (3, 4), monocytes (5, 6), platelets (4, 7), invariant NKT lymphocytes (8), and the endothelium itself (2, 9–11).

Of mice and men: murine SCD models tell a hot story

Transgenic mouse models of SCD have redefined the pathophysiology of vaso-occlusion. SCD models have been developed...
with varying degrees of severity, exhibiting hemolytic anemia, leukocytosis, markers of inflammation, and activated coagulation. Furthermore, these animal models mimic the pathology of human disease in response to stimuli, including hypoxia-reoxygenation, cytokines, LPS, and hemoglobin/heme (12–14). Kaul and Hebbel showed that pathological stimuli, such as hypoxia-reoxygenation, resulted in decreased blood flow, enhanced leukocyte rolling, adhesion, emigration, and enhanced oxidant production, strongly supporting the concept that SCD is a disease of inflammation.

Most patients with SCD have a high platelet count that is related, in part, to inflammation and the asplenic state. Just as activated leukocytes have been investigated in SCD, so have activated platelets (4, 7). Platelets may contribute to vaso-occlusion and stroke in SCD patients due to hemolysis associated with platelet activation via released ADP acting on G protein-coupled purinergic receptor P2Y12 (18) or heparin interactions with TLR4 (11). Activated platelets can promote sickle red cell adhesion, form platelet-red cell, platelet-neutrophil, and platelet-monocyte aggregates, activate coagulation through CD40 expression, and interact with activated vWF. Platelet aggregates with neutrophils and monocytes result from interactions between platelet P-selectin and P-selectin glycoprotein ligand-1 (PSGL-1) on leukocytes and interactions between platelet glycoprotein Ibα (GPIbα) and integrin αM/β2 on monocytes. High levels of platelet-monocyte and platelet-neutrophil aggregates have been observed in SCD patients. Recent trials have examined the ability of the P2Y12 inhibitor prasugrel to reduce pain crises (18), and the cILb/IIa inhibitor efibizumab is currently being examined to relieve SCD-associated symptoms (19). Additionally, efficacy of a glycomimetic pan-selectin inhibitor (GMI-1070) is now being tested in SCD patients (20).

Don’t forget the platelet in SCD

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How are multicell interactions conducted in this orchestra?

The complex orchestration that leads to vaso-occlusion in SCD requires one undeniable factor: the presence of hemoglobin S. Recently, several models have been proposed for the events leading to vaso-occlusion; however, the pathogenesis of SCD-associated vaso-occlusion remains unclear. If only Raquel Welch and Steven Boyd were available for a fantastic voyage into the vasculature aboard their miniaturized submarine Proteus to record the cellular and molecular events in the hypoxic environment of the post-capillary venules. Is the deoxygenated red cell interacting with the endothelium the first trigger? Or is free hemoglobin/heme acting on the endothelium to blame? Are stiff, misshapen red cells blocking flow? Which proteins, lipids, carbohydrates, and receptors are initially engaged, and how does the crosstalk between cell types proceed? How do neutrophils, monocytes, and platelets aggregate to occlude blood flow? Why is occlusion temporary in most cases, and what signals promote dissolution of the blockade? What factors determine irreversible blockage with subsequent infarction? Why does interference with so many different molecules on cells interfere with vaso-occlusion in mouse studies?

In this issue of the JCI, Li et al. (21) focus on the role of AKT, a Ser/Thr kinase that phosphorylates numerous substrates that are important in cell activation, survival, and proliferation. Isoforms of AKT have specific roles in platelets, macrophages, neutrophils, and endothelial cells. Fluorescence intravital microscopic studies revealed that hematopoietic cell AKT-II is critical for neutrophil recruitment and neutrophil-platelet interactions during TNF-α-induced vascular inflammation in live mice. Using in vitro reconstituted systems, Li et al. revealed that both platelet and neutrophil AKT-2 are important for heterotypic cell-cell aggregation under shear (21). Basal phosphorylation levels
of AKT isoforms were markedly increased in neutrophils and platelets isolated from SCD patients. Inhibition of AKT2 reduced heterotypic aggregation of patients’ neutrophils and platelets in vitro and diminished neutrophil adhesion and neutrophil-platelet aggregation in SCD mice, thereby improving blood flow rates. These results provide important evidence that neutrophil-associated AKT2 regulates αM/β2 integrin (CD18/CD11b) function and promotes neutrophil recruitment and neutrophil-platelet interactions in response to thromboinflammatory conditions such as SCD.

This elegant study by Li and colleagues does not, however, answer the question of who conducts the orchestra of vaso-occlusion, but it provides important insights into the players. Furthermore, the study does not reconcile previous investigations that have demonstrated the importance of P-selectin in vaso-occlusion. Is AKT2 the bad sibling? Caution should be used before targeting AKT2 for SCD therapy. Patients with SCD are prone to infection, and inhibition of AKT could worsen this by inactivating host-defending neutrophils. Mice lacking Akt2 present with hyperinsulinemia and diabetes-like syndrome, because AKT2 is highly expressed in insulin-responsive tissues (22). Previously, the observed phosphorylation of nuclear AKT in livers of sickle mice overexpressing heme oxygenase-1 (HO-1) or after treatment with inhaled CO suggested an antiinflammatory role for AKT (23). The observed AKT phosphorylation induced by HO-1 and CO could possibly be due to selective activation of AKT isoforms AKT1 or AKT3.

Ultimately, the primacy of hemoglobin S, hemolysis, oxidative stress, and subsequent inflammation in SCD pathogenesis rules any approaches to limiting SCD-associated damage. Strategies aimed at minimizing hemoglobin S through gene therapy or stem cell transplantation, and minimizing hemoglobin S polymerization, by increasing hemoglobin F, are the best hopes for minimizing SCD patient morbidity and mortality. Approaches geared toward dealing with hemolysis, either by scavenging hemoglobin/heme or detoxifying heme through HO-1, may limit the inflammation. Other strategies for ameliorating SCD, including antiinflammatory, vasoprotectants, and antithrombotics, will be useful; however, these approaches could compromise host defenses. Herrick never could have imagined over 100 years ago the complexity of the pathobiology of the peculiar-shaped cells that he observed. Hopefully, the use of nanotechnology to provide a “miniature submarine” to explore the vasculature of SCD individuals will be on the horizon to provide us with a better understanding of vaso-occlusion.

Acknowledgments
This work was supported by an NIH grant from the NHLBI (HL115467-01, to G.M. Vercellotti and J.D. Belcher).
Insulin, osteoblasts, and energy metabolism: why bone counts calories

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Recent studies have demonstrated that insulin stimulates bone cells to produce and activate osteocalcin, an endocrine hormone that increases the efficiency of glucose metabolism through its actions on the pancreas and other peripheral tissues. In this issue of the *JCI*, Wei and colleagues directly explore the contribution of insulin signaling in osteoblasts to the disturbances in whole-body glucose metabolism associated with a high-fat diet. In mice fed a high-fat diet, increased uptake of saturated fatty acids by the osteoblast accelerates the ubiquitination and degradation of the insulin receptor. In this setting, impairments in osteoblast insulin signaling reduce serum levels of undercarboxylated osteocalcin, which in turn exacerbate insulin resistance in muscle and white adipose tissue. These findings underscore the importance of insulin-responsive skeletal cells as components of a newly appreciated endocrine network critical for regulating global energy homeostasis.

Conflict of interest: The authors have declared that no conflict of interest exists.

Citation for this article: *J Clin Invest*. 2014;124(4):1465–1467. doi:10.1172/JCI75554.

Bone as a metabolic organ: lessons from evolution

The evolution of a large appendicular skeleton powered by robust skeletal muscles in early tetrapods was a successful strategy for ambulation on land. Additionally, the new skeleton served as a repository for calcium, a scarce commodity in the terrestrial habitat, and the emergence of the parathyroid gland at this juncture provided the means to rapidly access bone calcium through osteoclast-mediated resorption from skeletal stores (1). However, the upgraded musculoskeletal system also increased overall fuel requirements and altered global energy balance, prompting the evolution of other endocrine networks to coordinate energy expenditure (2). Prominent among these emerging networks were the leptin and insulin/skeletal-like growth factor pathways, which have assumed central roles in growth and metabolism in higher organisms (3, 4). The importance of these meta-