

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

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### Commentary

Thromboinflammatory diseases result from the interactions of vascular endothelial cells, inflammatory cells, and platelets with cellular adhesion molecules, plasma proteins, and lipids. Tipping the balance toward a prothrombotic, proinflammatory phenotype results from multicellular activation signals. In this issue of the *JCI*, Li et al. explore the regulation of heterotypic neutrophil-platelet contacts in response to TNF- $\alpha$ -induced venular inflammation with relevance to sickle cell disease (SCD).

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discovery of new disease mechanisms will undoubtedly accelerate the development of therapies in the treatment of cardiac conduction disorders.

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

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**Thromboinflammatory diseases result from the interactions of vascular endothelial cells, inflammatory cells, and platelets with cellular adhesion molecules, plasma proteins, and lipids. Tipping the balance toward a prothrombotic, proinflammatory phenotype results from multicellular activation signals. In this issue of the JCI, Li et al. explore the regulation of heterotypic neutrophil-platelet contacts in response to TNF- $\alpha$ -induced venular inflammation with relevance to sickle cell disease (SCD).**

### Not just misshapen red cells

In a 1910 report of an anemic West Indian man, Herrick first described the “peculiar elongated and sickle-shaped” rbc produced by individuals with sickle cell anemia (1). Over the next century, evaluation of sickle cell anemia-associ-

ated 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  $\beta$ -globin. The pain crises and organ infarctions that manifest in patients were attributed to a mechanical obstruction of blood flow due to the rigid crescent-shaped cells; however, in 1980 Hebbel and others demonstrated that sickle erythrocytes were excessively adherent to vascular endothelial cells (2).

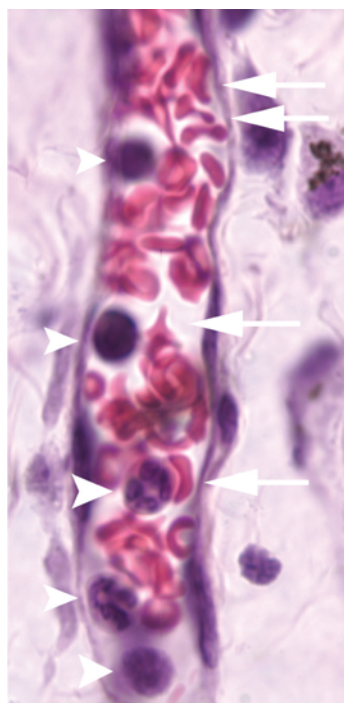
As a result of this seminal observation, researchers in the 1980s and 1990s were able to define the red cell characteristics, endothelial adhesion molecules, and plasma factors responsible for these phenomena. Patients with sickle cell disease (SCD) have marked leukocytosis, thrombocytosis, markers of 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

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**Figure 1**

Histology of a venule in the dorsal skin of transgenic sickle mice after 1 hour of hypoxia and 1 hour of reoxygenation. Dorsal skin samples were taken for histological analysis after S+S-Antilles sickle mice were exposed to 1 hour of hypoxia and 1 hour of reoxygenation. Skin samples were fixed overnight in formalin, cut into 5- $\mu$ m sections, embedded in paraffin, mounted on slides, and stained with hematoxylin and eosin before microscopic examination. The image shows a venule with a suspected vascular obstruction. White arrowheads point to leukocytes that appear to be adherent to the vascular endothelium, and white arrows point to misshapen rbc's inside the venule. Reprinted with permission from *American Journal of Hematology* (24).

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, oxidative stress, and reperfusion/injury physiology (9). Turhan and Frenette demonstrated that leukocytes mediate TNF- $\alpha$ -induced vaso-occlusion, which leads to reduced blood flow and death. Leukocyte-dependent vaso-occlusion was prevented in mice lacking both P- and E-selectins (3). We have shown that treatment with antibodies targeting multiple adhesion molecules, including VCAM-1, ICAM-1, P-selectin, E-selectin,  $\alpha$ 4 $\beta$ 1,  $\alpha$ V $\beta$ 3, vWF, or PECAM-1, inhibits microvascular stasis as assessed with a dorsal skinfold chamber on sickle mice in response to hypoxia-reoxygenation or hemoglobin/heme infusion (11). Histology of a suspected vascular obstruction in the skin of a transgenic sickle mouse following hypoxia-reoxygenation indicates that multiple cell types are present within

the blockage (Figure 1), and several cellular molecules and interactions have been implicated in murine vaso-occlusion (Figure 2).

Human sickle rbc's and platelets interact with monocytes and neutrophils, which can be found within heterotypic aggregates (4, 6). Hemolysis is critical in the vasculopathy of SCD (15), and recent studies demonstrating neutrophil extracellular trap (NET) formation in response to heme further implicate interactions between leukocytes and sickle cells in vaso-occlusion development (16, 17). Leukocytosis in SCD patients is associated with an increased risk of acute chest syndrome, stroke, and mortality, adding credence to the hypothesis that SCD is a disease of inflammation.

### Don't forget the platelet in SCD

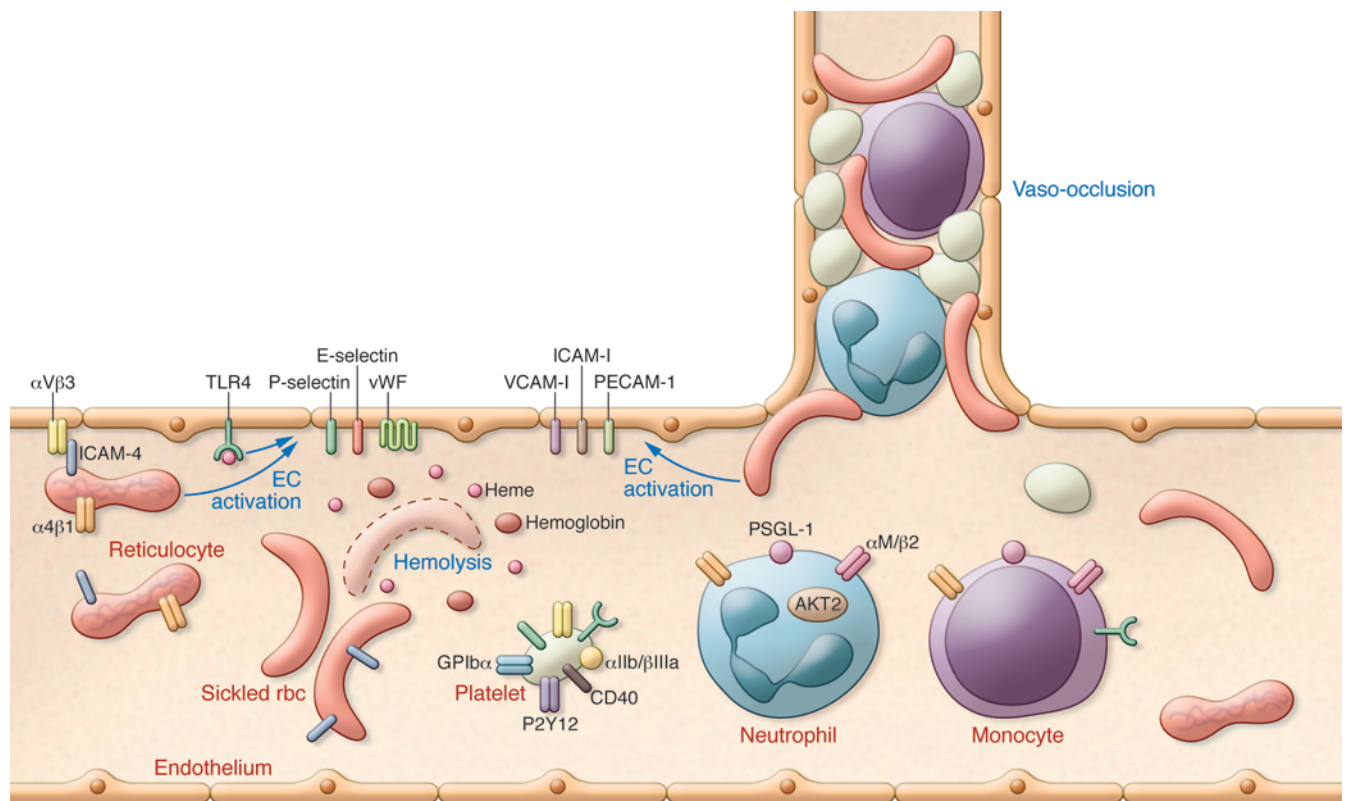
Most patients with SCD have a high platelet count that is related, in part, to inflammation and the asplenic state. Just as activated leukocytes have been investigated in SCD, so have activated platelets (4, 7). Platelets may contribute to vaso-occlusion and stroke in SCD patients due to hemolysis associated with platelet activation via released ADP acting on G protein-coupled purinergic receptor P2Y<sub>12</sub> (18) or heme 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 Ib $\alpha$  (GPIb $\alpha$ ) and integrin  $\alpha$ M/ $\beta$ 2 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 P2Y<sub>12</sub> inhibitor prasugrel to reduce pain crises (18), and the  $\alpha$ IIb/ $\beta$ 3 inhibitor eptifibatid 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).

### 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 AKT2 is critical for neutrophil recruitment and neutrophil-platelet interactions during TNF- $\alpha$ -induced vascular inflammation in live mice. Using in vitro reconstituted systems, Li et al. revealed that both platelet and neutrophil AKT2 are important for heterotypic cell-cell aggregation under shear (21). Basal phosphorylation levels



**Figure 2**

Conceptual model of molecular and cellular interactions that influence the development of vaso-occlusion in SCD mice. Blockade or deletion of factors, including P- and E-selectin, VCAM-1, ICAM-1, and PECAM-1, which are associated with endothelial cell activation, prevents vaso-occlusion in SCD mice. Furthermore, molecules present on platelets, reticulocytes, sickled rbc, neutrophils, and monocytes have been implicated in the development of murine SCD-associated vaso-occlusion. Li and colleagues (21) have determined that neutrophil AKT2 may be required for vaso-occlusion. Despite the identification of a myriad of molecules and cell types that mediate vaso-occlusion, the timing of events and the initiating signals remain to be determined.

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  $\alpha M/\beta 2$  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 antiinflammatories, 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.

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## 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.**

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### 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 liberation 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/insulin-like growth factor pathways, which have assumed central roles in growth and metabolism in higher organisms (3, 4). The importance of these meta-