4. Akin C, Brockow K, D'Ambrosio C, et al. Effects of tyrosine kinase inhibitor STI571 on human mast cells bearing wild-type or mutated c-kit. Exp Hematol. 2003;31:686-692.

5. Opferman JT, Iwasaki H, Ong CC, et al. Obligate role of anti-apoptotic MCL-1 in the survival of hematopoietic stem cells. Science. 2005;307:1101-1104.

RED CELLS

Comment on Hsu et al, page 3088

# The "NO" tipping point

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Pulmonary hypertension in sickle cell anemia, an important risk factor for early mortality, may be caused by intravascular hemolysis leading to decreased NO bioavailability.

Malcolm Gladwell in his bestseller *The Tipping Point: How Little Things Make a Big Difference* points out how little changes can have big effects.[1] For example, when a new hypothesis that proposes a different way of looking at things catches fire, that idea can ripple outward until a "tipping point" is reached, changing the world. Hsu and colleagues in this issue of *Blood* take the idea that intravascular hemolysis reaches a tipping point, leading to global impairment in nitric oxide (NO) bioavailability causing functional pulmonary hypertension in a murine model of sickle cell disease. These studies further amplify the critical physiological effects of plasma hemoglobin and oxidative stress on vascular responsiveness.

Pulmonary hypertension in patients with sickle cell disease is common and portends a poor survival.[2,3] One proposed mechanism for pulmonary hypertension suggests that intravascular hemolysis releases red blood cell (RBC) hemoglobin and arginase into the plasma, scavenging NO and decreasing arginine substrates, and ultimately leading to reduced NO bioavailability. This lack of NO can alter pulmonary vascular endothelial and vasomotor function. Of importance, the end stage of pulmonary hypertension in sickle cell disease is reflected by pathological intimal and smooth muscle changes in the pulmonary vasculature. Many other factors obviously play a critical role in the human disease including chronic hypoxemia, recurrent infections, fibrosis, recurrent thromboembolism, and heart failure.

To test this hypothesis in mice, Hsu et al use the Berkeley mouse expressing exclusively human α- and $\beta^S$-globins. These animals have anemia, sickle RBCs, intravascular and extravascular hemolysis, chronic inflammation, and evidence of excessive oxidative stress. These mice have elevated pulmonary arterial pressures and increased pulmonary vascular resistance. However, unlike the human sickle cell patient with pulmonary hypertension, there was no thickening of the pulmonary arteries, no plexogenic lesion, and no fibrosis noted, but increased intravascular leukocytes were present. These leukocytes may reflect the abnormal rheology in these animals and likely contribute reactive oxygen species promoting oxidative stress within the vessel wall.

Pulmonary hypertension in sickle mice was associated with a global and specific impairment in the pulmonary and vascular responsiveness to both exogenous and endogenous NO. Further proof that hemolysis and oxidative stress were responsible for pulmonary hypertension was shown in normal mice that received a transplant of sickle bone marrow or were induced to hemolyze with alloantibodies. Furthermore, in the sickle mouse, evidence for defects in NO synthase assembly and uncoupling is provided as well as evidence for increased NO consumption. Elevated plasma arginase activity, elevated reactive oxygen species production, and increased tyrosine nitrosylation were identified in the sickle mouse.

This tipping point study adds to the river of evidence that plasma-free hemoglobin, whether derived from hemolysis or hemolyzed RBC transfusion, or given as a therapeutic oxygen carrier, modulates vascular responsiveness.[4] However, care in extrapolating to human sickle cell disease is warranted as pulmonary hypertension takes years, not weeks, to develop in humans and is associated with pathological changes. Also, NO may not be the whole story in pulmonary hypertension in sickle cell patients. Altered vascular prostaglandins, cyclic AMP, and endothelin responses, as well as thromboembolism, probably all conspire. The critical roles of vascular inflammation and oxidative stress, which contribute to altered rheology in sickle cell disease, can also modulate oxidative sensitive NO synthase or tetrahydrobiopterin. Finally, the adaptation to an excess heme load in sickle cell disease and the induction of heme oxygenase-1 may have profound effects on these vascular responses through the release of its products that act as antioxidants (biliverdin/bilirubin, ferritin) or vasodilators (CO).[5]

*The author declares no competing financial interests.*

## REFERENCES

1. Gladwell M. The Tipping Point: How Little Things Make a Big Difference. London, United Kingdom: Little, Brown and Company; 2000.

2. Gladwin MT, Sachdev V, Jison ML, et al. Pulmonary hypertension as a risk factor for death in patients with sickle cell disease. N Engl J Med. 2004;350:886-895.

3. Vichinsky EP. Pulmonary hypertension in sickle cell disease. N Engl J Med. 2004;350:857-859.

4. Rother RP, Bell L, Hillmen P, Gladwin MT. The clinical sequelae of intravascular hemolysis and extracellular plasma hemoglobin: a novel mechanism of human disease. JAMA. 2005;293:1653-1662.

5. Belcher JD, Mahaseth H, Welch TE, Otterbein LE, Hebbel RP, Vercellotti GM. Heme oxygenase-1 is a modulator of inflammation and vaso-occlusion in transgenic sickle mice. J Clin Invest. 2006;116:808-816.

IMMUNOBIOLOGY

Comment on Aksoy et al, page 2887

# Brave new world: birth of immunity

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In this issue of *Blood,* Aksoy and colleagues contribute to the understanding of remarkable immunoregulatory controls at the time of birth by showing that impaired synthesis of interferon-β (IFNβ) and IFN-inducible factors elicited by lipopolysaccharide (LPS) depends on the transcriptional activity of interferon regulatory factor 3 (IRF-3) downstream of Toll-like receptor-4 (TLR4).

The human newborn is profoundly susceptible to intracellular bacterial pathogens that require Th1-dependent host defense pathways for eradication. The molecular basis for the newborn's attenuated response to such infections remains a mystery but is likely a consequence of the immunosuppression required for survival in utero.