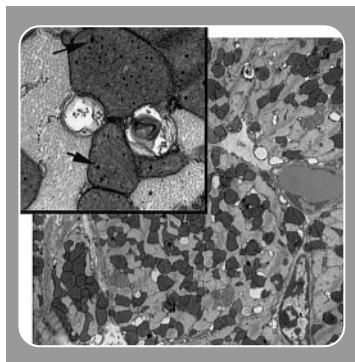


mutations that result in genetic iron overload. Using a murine model of hemochromatosis, the *Hfe* knockout mouse, they show that acute DOX treatment of homozygous knockout mice results in increased iron in the serum and its accumulation in tissues. Besides the expected accumulation of iron in the liver, they also found excessive deposition in the heart.

To further simulate the distribution of hemochromatosis mutations in the general population, they studied the effect of chronic administration of DOX on both heterozygous and homozygous knockout mice. Both groups of mice displayed increased mortality and excessive iron accumulation when compared with wild-type mice. Sig-



nificantly, Miranda et al show that the iron accumulation results in extensive mitochondrial damage in heart tissue.

Recently, Swain et al¹ reported that DOX-related cardiotoxicity and induced congestive heart failure occur at a greater frequency than was previously reported, and that patients of advanced age may be at a greater risk. As iron accumulation increases with age, this observation has profound implications when considered along with the results presented by Miranda et al. These studies warrant a closer look at the relationship between DOX cardiotoxicity and the prevalence of *HFE* mutations and other genetic changes that affect iron homeostasis.

—V. Nathan Subramaniam

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1. Swain SM, Whaley FS, Ewer MS. Congestive heart failure in patients treated with doxorubicin: a retrospective analysis of three trials. *Cancer*. 2003;97:2869-2879.

... And so on to viscosity

Much as Lewis F. Richardson¹ embraced Jonathan Swift's metaphorical use of smaller and smaller fleas to represent the infinite in his modeling of rheologic disturbance—

*Big whorls have little whorls,
Which feed on their velocity,*

*And little whorls have lesser whorls,
And so on to viscosity.*

—we now must accede to the infinite complexity of factors that impart viscosity to sickle cell blood.

Considering that Richardson found an infinite potential for turbulence in the blowing wind and flowing river water, both Newtonian fluids lacking particulate constituents, what was to be expected of sickle cell blood? A seminal opinion on the matter, Ham and Castle's² celebrated description of a "Vicious cycle of erythrostatics," focused on the deleterious effect of the eye-catching sickle erythrocyte on the viscosity of sickle cell blood. This valuation engendered exhaustive studies of sickling and hemoglobin S polymerization, as well as doctrinal interpretations of disease. The elegance of these formulations notwithstanding, detailed understandings of polymerization could neither predict the occurrence nor identify the triggers of acute painful vasoocclusion.³

Perhaps as a result of dogged research, or maybe imaginative insight, or perchance just idle curiosity, important polymerization-independent processes emerged and defined different targets for investigation. As a result, modern views interpret traditional polymerization-based understandings as part of a broad matrix of pathophysiology. However, even as the molecular details of platelet activation, coagulation induction, sickle cell adhesion, endothelial cell agonism, heterocellular interactions, and vasomotor dysregulation are established, the question of causality remains.

The agonists of several of the vasoocclusive pathophysiology include inflammatory cytokines and procoagulant enzymes. Certainly the vascular injury and cell activation

caused by circulating sickle cells might account for inflammation, but what of the thrombin? Proposed mechanisms for thrombin generation include the procoagulant effect of phosphatidyl serine (PS) on the exterior of sickled cells, the expression of tissue factor (TF) by activation and interaction of endothelial cells and monocytes, and the procoagulant activity of hypoxic endothelial cells.³

In this issue, Shet and colleagues (page 2678) have extended our knowledge of these complex mechanisms. They found an increase in erythroid-derived microparticles and markers of thrombophilia during acute painful episodes, but the absence of TF on erythroid microparticles indicates that these are not the triggers of coagulation. Possible causality derives from the generation of both procoagulant PS-positive sickle cells⁴ and erythroid microparticles by the same process, sickling. However, the surprisingly constant rate of sickling would suggest that bursts of PS-positive sickle cells are unlikely to trigger vasoocclusion, and the data in this paper do not establish whether increased erythroid microparticles precede or follow vasoocclusion. Shet et al also found that during pain crises the levels of TF bearing monocyte-derived microparticles increases and correlates with indicators of thrombophilia. But what is the cause of the monocyte activation? These findings by Shet and his colleagues have taken us deeper into the realm of infinite complexity and may have led us closer to the proximate cause of these important perturbations. As is often the case with infinitely complex mechanisms, the real answer may be hidden among the internal deterministic parameters of chaos.⁵

—Stephen H. Embury

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1. Gleick J. *Chaos: Making a New Science*. New York, NY: Penguin Books; 1988:119.
2. Ham TH, Castle WB. Relationship of increased hypotonic fragility of erythrostatics to the mechanisms of hemolysis in certain anemias. *Trans Assoc Am Physicians*. 1940;55:127-132.
3. Embury SH. The not-so-simple process of sickle cell vasoocclusion. *Microcirculation*. In press.
4. Setty BN, Rao AK, Stuart MJ. Thrombophilia in

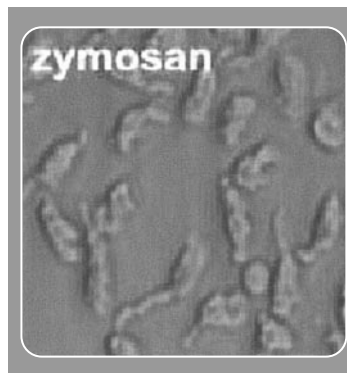
sickle cell disease: the red cell connection. *Blood*. 2001;98:3228-3233.

5. Embury SH, Hebbel RP, Mohandas N, Steinberg MH. Pathogenesis of vasocclusion. In: Embury SH, Hebbel RP, Mohandas N, Steinberg MH, eds. *Sickle Cell Disease: Basic Principles and Clinical Practice*. New York, NY: Raven Press; 1994: 311-326.

Human neutrophils and TLRs: 2, 4, and plenty more

And who could be truly surprised? Toll-like receptors (TLRs) are orthologs of combined developmental polarization and host defense proteins identified in the fruit fly, *Drosophila melanogaster*, and respond to molecular motifs that are distinct and unique features of bacterial, fungal, and virus pathogens (reviewed in Heine and Lien¹ and references within). Neutrophils are the primary and pivotal cells providing innate host defense against these same invading bacterial and fungal pathogens—how could neutrophils have evolved without a full set of these crucial pattern recognition receptors? In this issue, Hayashi and colleagues (page 2660) provide us with a thorough and thoroughly satisfying response to this question, demonstrating that human peripheral blood

neutrophils express not only TLRs 2 and 4, as previously recognized, but also TLRs 1, 5, 6, 7, 8, 9, and 10, indeed all of the known TLRs save for the elusive double-stranded RNA receptor, TLR3. Moreover, the authors have shown that agonist-mediated TLR activation is intimately associated with all that neutrophils do best: phagocytosis, selectin-shedding, generation of superoxide, and production of the cytokines and chemokines, most notably interleukin-8. As a result of this study, interactions among TLRs and TLR ligands now add significant new complexities to the study of neutrophil activation *in vitro*. Equally important, we can now interpret the findings of *in vivo* experiments involving specific TLR deficiencies,



both naturally occurring and genetically engineered, with the added dimension of neutrophil function in mind.

The following questions are among those arising from this work: How do real-life, multivalent pathogens interact with the 9 known TLRs expressed by human neutrophils? Do neutrophils respond to TLR ligands *in situ*, or are they processed and presented to the neutrophils via mechanisms that have yet to be explored? Do the neutrophil TLRs interact with one another to form multicomponent networks similar to those that have been postulated for macrophages and dendritic cells?² Are signals transduced as part of individualized or completely interchangeable pathways?

While the answers to these questions may take some time to emerge, we can consider human neutrophils as completely equipped to participate in full.

—**Helene F. Rosenberg**
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and Infectious Diseases

1. Heine H, Lien E. Toll-like receptors and their function in innate and adaptive immunity. *Int Arch Allergy Immunol*. 2003;30:180-192.
2. Underhill DM. Toll-like receptors: networking for success. *Eur J Immunol*. 2003;33:1767-1775.