Recently, it has been shown that macrophages deficient in DNA damage responses have increased cytokine production.[4,5] The findings of Harbort et al complement these other studies and, collectively, they reveal that deficiency of ATM disrupts immune regulation beyond the established deficiencies in B and T lymphocytes. Patients with AT, a multisystem disorder secondary to mutations in ATM, have recurrent infections, autoimmune disease, inflammatory lung disease, and cutaneous granulomas that cannot be attributed solely to defects in adaptive immunity. Indeed, elevated serum levels of interleukin-8 and granuloma formation in AT patients may be direct consequences of increased cytokine production and prolonged survival of ATM-deficient neutrophils.[9,10] By demonstrating that loss of ATM results in defects in innate immunity and dysregulation of inflammatory responses, Harbort et al provide a new understanding of the clinical manifestations and complications of this devastating disease.

Harbort et al show that the hyperinflammatory profile of ATM-deficient neutrophils mirrors the phenotype observed in ROS-deficient neutrophils from CGD patients (see figure). This suggests that the increased inflammation in CGD is, in part, a consequence of the defect in ROS-mediated ATM activation and the associated repression of proinflammatory cytokines. Exogenous induction of DNA damage by exposure to chemotherapy inhibits cytokine production and rescues the hyperinflammatory phenotype of activated ROS-deficient neutrophils (ie, those from CGD patients). It is intriguing to speculate how these findings could be applied to devise new strategies for controlling the inflammatory disease in CGD patients through modulation of DNA damage signaling.

The findings of Harbort et al further support that DNA damage responses have critical cell-type–specific functions beyond their canonical role in DNA repair and cell-cycle checkpoint.[4-6] Characterization of these pathways will establish the important contributions of DNA damage signaling to normal development and disease states.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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● ● ● PLATELETS AND THROMBOPOIESIS

Comment on Hua et al, page 2852

Not dead yet

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In this issue of *Blood*, Hua et al use a novel marker of platelet activity to demonstrate that the necrotic platelet, a highly procoagulant subpopulation of activated platelet, uniquely contributes to fibrin formation and platelet accumulation within the forming thrombus.[1]

Necrotic platelets are a subpopulation of activated platelets that are formed in response to a strong in vitro stimulus.[2] A jambalaya of descriptive names and acronyms has been ascribed to platelets with similar characteristics, among these procoagulant,[3] balloon(ing),[4] coated,[5] SCiP (sustained collagen-induced platelets),[6] and CoaT (collagen and thrombin) platelets.[7] Distinguishing in vitro characteristics and functions of necrotic platelets includes high levels of platelet phosphatidylserine externalization facilitating the activity of the tenase and prothrombinase complex[7]; increased calpain activation and degradation of intracellular proteins, including the cytoplasmic domain of integrin $\alpha_{IIb}\beta_3$[3]; decreased adhesiveness[8]; and cellular rounding and microparticle formation.[4]

Although many features and functions have been described in vitro, the necrotic platelet's role in hemostasis and thrombosis continues to be debated. Depending on the model, either hemostatic or thrombotic functions have been attributed. And in some settings, minimal to no contribution of the necrotic platelet has been suggested. Agonist-initiated platelet phosphatidylserine externalization, a key feature of the necrotic platelet, requires the channel protein TMEM16F, also known as anoctamin-6. TMEM16F deficiency results in a hemorrhagic diathesis, suggesting an important role for the procoagulant function of this subpopulation in hemostasis.[9] Conversely, using a photochemically induced model of thrombosis, impaired necrotic platelet formation, occurring as a result of cyclophilin D deficiency, resulted in accelerated thrombotic occlusion.[5] Finally, recent studies have even questioned the physiologic role of platelets in supporting local thrombin generation. Fibrin formation and prothrombinase activity are minimally associated with platelets in a laser-induced nonocclusive thrombus.[10]

In this study, Hua et al use a novel marker of necrotic platelet formation to investigate the localization and function of the necrotic platelet in vivo.[1] 4-[*N*-(*S*-glutationylacetyl)amino] phenylarsonous acid (GSAO) is an arsenical with a high affinity for intracellular protein thiols. Labeled GSAO had been demonstrated to identify late apoptotic and necrotic-nucleated cells. Using this marker, the authors identify a necrotic-activated platelet subpopulation characterized by GSAO and surface P-selectin positivity. Validating the utility of GSAO as a marker of necrotic platelets, formation of this GSAO-positive subpopulation was regulated by the peptidylprolyl isomerase cyclophilin D, a mitochondrial protein that functions to regulate necrotic cell death in platelets.[5]

GSAO labeling of forming thrombi in murine arterioles demonstrated the spatial association of necrotic platelet formation with fibrin formation in an occlusive thrombus initiated by extramural ferric chloride application. That the necrotic platelet drove both fibrin formation and platelet accumulation in this model was clearly demonstrated by abrogation of GSAO labeling, fibrin formation, and platelet accumulation in mice with cyclophilin D null platelets. Platelet heterogeneity was observed both within the occlusive thrombus, as discrete foci of necrotic platelet formation, and between thrombi, depending on the mechanism of thrombus initiation. Distinct from the occlusive thrombus, few necrotic platelets, if any, were observed in a laser-induced nonocclusive thrombus injury model. This minimal role is consistent with the lack of association of fibrin formation with the platelet mass in this model.

As demonstrated here[1] and by others,[4] necrotic platelet formation can be targeted independent of more traditional platelet activation pathways. This suggests that pathways regulating necrotic platelet formation might provide an alternative target for modulation of thrombotic and other platelet-dependent pathologies. Studies using the GSAO reagent described here in other models of platelet-facilitated thrombotic and inflammatory conditions can be expected to provide novel insights into the mechanism and function of necrotic platelets.

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● ● ● RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Brown et al, page 2863

Transporting down the road to dehydration

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In this issue of *Blood*, Brown and colleagues provide strong evidence that erythrocyte hydration is an important modifier of disease severity in sickle cell disease (SCD).[1]

Erythrocyte dehydration is a critical component of disease pathogenesis in SCD because sickle hemoglobin polymerization is exquisitely dependent on its cellular concentration.[2] Complex interactions between the activity of several water and solute transport systems lead to cellular dehydration, forming dehydrated sickle erythrocytes, known as dense cells. Dense cells are much more prone to hemoglobin S polymerization, sickling, and vaso-occlusion. They also exhibit decreased deformability, increased fragility, and increased adhesion to endothelial cells, leukocytes, and sickle erythrocytes, exacerbating endothelial damage and facilitating vaso-occlusion.

Erythrocyte dehydration is a critical step in the SCD hemolytic pathway, with numbers of circulating dense cells positively correlated with severity of hemolysis in SCD patients.[3] Hemolysis and the hemolytic rate contribute to significant complications of SCD including pulmonary hypertension, stroke, priapism, and leg ulcers.

Three pathways mediating cation loss and cellular dehydration in sickle erythrocytes have been identified: (1) a sickling-induced, deoxy-dependent nonselective pathway called P^{sickle} that mediates an increase in permeability to calcium and other ions at the initiation of the dehydration cascade; (2) a calcium-activated potassium channel, the Gardos channel,[4] which when activated is the primary mediator of potassium and water loss in SCD; and (3) a K-Cl cotransport (KCC) pathway.

KCC cotransport is the primary volume-sensitive cation transport pathway in erythrocytes.[5] KCC activation leads to loss of potassium, chloride, and water, reducing cell volume and increasing cellular hemoglobin