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**DNA damage signals inhibit neutrophil function**

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In this issue of Blood, Harbort et al identify a novel role for DNA damage responses in the regulation of cytokine production and cell death of activated neutrophils.1 They show that reactive oxygen species (ROS) generated by stimulated neutrophils trigger DNA damage signaling, which suppresses proinflammatory functions (see figure). Their work reveals new insights into the control of innate immunity and inflammation.

Damage to DNA is caused by genotoxic agents, such as irradiation and chemotherapies, and during normal biologic processes, including DNA replication, transcription, telomere maintenance, and antigen-receptor assembly in lymphocytes.2 Regardless of the mechanism of injury, DNA breaks activate conserved signaling pathways that are initiated by the DNA damage sensors ATM and ATR (AT and RAD3-related).2 These serine/threonine kinases phosphorylate hundreds of proteins to coordinate DNA repair, cell-cycle checkpoints, and cell-death pathways.2,3 DNA damage responses also regulate cell-type–specific programs, including cell survival and differentiation.4-6 In their study, Harbort et al show that DNA damage signaling is activated by ROS in neutrophils and represses proinflammatory functions.

In response to invading pathogens, neutrophils secrete proinflammatory cytokines, which recruit additional neutrophils and other immune cells.7 Stimulation of neutrophils also triggers a burst of ROS that contribute directly to microbial destruction and function as signaling molecules.7 Defects in the generation of reactive species, such as those found in CGD, result in recurrent infections, poor clearance of pathogens, and hyperinflammation.7

Harbort et al show that the generation of ROS in neutrophils is required for suppressing cytokine production and limiting neutrophil lifespan (see figure). This repression of neutrophil function depends on ROS-mediated activation of DNA damage-response signaling. ATM and ATR function synergistically to inhibit neutrophil function. Loss of ROS or of DNA damage signaling increases cytokine production and prolongs survival of activated neutrophils. Thus, ROS and ATM are necessary to inactivate neutrophil responses and limit inflammation.

ROS are known to cause oxidative damage to DNA but can also directly oxidate and activate ATM without DNA damage intermediates.8 The histone H2AX is phosphorylated (γH2AX) at sites of DNA damage and Harbort et al find that γH2AX is generated in neutrophils following the oxidative burst but is absent in ROS scavenged cells.2 These findings suggest that ROS directly damages DNA in activated neutrophils and that activation of ATM occurs in response to this injury. However, H2AX is phosphorylated by ATM and thus, the decreased generation of γH2AX may be a consequence of reduced ATM activity in the absence of ROS.2 Further work is necessary to determine if DNA is directly damaged by ROS and to define the precise mechanism of ATM activation in stimulated neutrophils.

![Model of the ROS- and ataxia-telangiectasia mutated (ATM)--mediated repression of neutrophil inflammatory responses.](image)

Model of the ROS- and ataxia-telangiectasia mutated (ATM)--mediated repression of neutrophil inflammatory responses. Following stimulation, neutrophils generate a burst of ROS that activates ATM-dependent DNA damage signaling to suppress cytokine production and induce apoptosis. Deficiency in ROS production or in ATM disables this inhibitory circuit, resulting in overproduction of cytokines, enhanced survival of activated neutrophils, and hyperinflammation. The MRN complex is composed of Mre11/Rad50/Nbs1 proteins. "P" represents phosphorylation of ATM, which is the active form of the kinase. AT, ataxia-telangiectasia; CGD, chronic granulomatous disease. See supplemental Figure 12 in the article by Harbort et al, available on the Blood Web site.
Recently, it has been shown that macrophages deficient in DNA damage responses have increased cytokine production. 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 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. 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. 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.

REFERENCES


DOI 10.1182/blood-2015-11-678672
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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. Ne
crotic platelets are a subpopulation of activated platelets that are formed in response to a strong in vitro stimulus. A jambalaya of descriptive names and acronyms has been ascribed to platelets with similar characteristics, among these procoagulant, balloon(ing), coated, SCGP (sustained collagen-induced platelets), and CaT (collagen and thrombin) platelets. Distinguishing in vitro characteristics and functions of necrotic platelets includes high levels of platelet phosphatidyserine 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. 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. 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.
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