XPO1–cargo interactions (see figure). Previous studies have shown that the closely related SINE compounds KPT–251, KPT–276, and KPT–330 have strong antileukemic activity and minimal and acceptable adverse effects in acute myelogenous leukemia and CML in blast crisis.6–8 Notably, the clinically relevant XPO1 inhibitor KPT–330 leads to apoptosis and impairment of leukemia clonogenic potential of leukemia but not normal CD34+ progenitors and significantly increased survival of leukemia mice. Mechanistically, KPT–330 altered the subcellular localization of leukemia–regulated factors, including RNA–binding heterogeneous nuclear ribonucleoprotein A1 and the oncogene SET, thereby inducing reactivation of protein phosphatase 2A tumor suppressor and inhibition of BCR–ABL1 in CML blast crisis cells. Because XPO1 is important for leukemia cell survival, KPT–330 may represent an alternative therapy for TKI–refractory Ph+ leukemias.6 Thus, the notion that RAN/XPO1 activity controls oncogene kinase–independent drug resistance in both AML and CML1 further supports the use of the available XPO1 inhibitors in therapeutic protocols for those patients. Notably, the SINE KPT–330 is currently in clinical trials for advanced hematologic malignancies and solid tumors (NCT01607892 and NCT01607905). Furthermore, the work of Khorashad et al10 opens the gateway to characterize microenvironment–generated signals responsible for altered XPO1 expression/activity and, consequently, to develop strategies to efficiently counteract drug resistance in AML as well as in those cases of CML not responding to TKI monotherapy.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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Comment on Bartels et al, page 1782

The end of the line for neutrophils

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In this issue of Blood, Bartels and colleagues demonstrate that acetylation of the transcription factor CCAAT enhancer binding protein ε (C/EBPε) is essential for terminal neutrophil granulocyte differentiation.1

Neutrophils, the most abundant granulocytes, are essential for host innate immune defense. Neutropenia results from damage to the bone marrow or depletion or destruction of neutrophils by drugs, diseases, or congenital disorders that block neutrophil differentiation. Neutropenic individuals are extremely susceptible to bacterial infection, and febrile neutropenia increases the risk of mortality in cancer patients receiving myelosuppressive chemotherapy.2 Prophylactic use of granulocyte colony–stimulating factor (G–CSF) reduces mortality by increasing neutrophil numbers.2 A better understanding of the mechanisms that regulate granulopoiesis and terminal neutrophil differentiation could spur development of new strategies to overcome neutropenia and improve clinical outcomes.

The C/EBP transcription factor family is essential for granulopoiesis and terminal differentiation of neutrophils.3 C/EBPβ is predominately expressed in immature myeloid cells, and lack of expression leads to a block at the myeloblast stage.3 C/EBPβ is expressed from the metamyelocyte stage and on during maturation.3 C/EBPβ–deficient mice display normal granulocyte differentiation and steady–state levels of neutrophils but are unable to produce neutrophils in response to cytokine

C/EBPε
KAC/KDACi
Myeloblast
Ac
Ac
C/EBPε
Grane genes
Ac
Granule
C/EBPε
KAC/KDACi
Neutrophil
Ac
C/EBPε
Ac?
Eosinophil

Acetylation of C/EBPε is essential for terminal neutrophil differentiation and function. C/EBPε is acetylated during granulocytic differentiation toward neutrophils by lysine acetylases (KAC, ε p300) or lysine deacetylase inhibitors (KDACi, eg, nicotinamide inhibiting KDAC SIRT1). Acetylation on K121 and K198 is essential for terminal neutrophil granulocyte differentiation and function. C/EBPε is acetylated during granulocytic differentiation toward neutrophils by lysine acetylases (KAC, ε p300) or lysine deacetylase inhibitors (KDACi, eg, nicotinamide inhibiting KDAC SIRT1). Acetylation on K121 and K198 is essential for terminal neutrophil differentiation of neutrophils and expression of secondary granule proteins. Differential acetylation of C/EBPε could be important for determining differentiation along the neutrophil or eosinophil lineages.

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expression or infection during “emergency” granulopoiesis. C/EBPε is expressed at the promyelocyte to myelocyte stage, and knockout mice lack neutrophils and eosinophils because of a block at this stage. Interestingly, mutations in the CEBPE gene account for some cases of human neutrophil-specific granule disease, a rare congenital disorder, characterized by increased circulating, immature neutrophils, and recurrent pyogenic infections. Also, induction of C/EBPε by G-CSF is important for inducing neutrophil maturation.

Expression of C/EBPε is restricted primarily to the myeloid lineage in 4 protein isoforms of 32, 30, 27, and 14 kDa. C/EBPε32/30 activates transcription, C/EBPε27 represses transcription, and C/EBPε14 acts as a dominant negative. The contribution of these different isoforms toward granulocytic differentiation remains largely unknown as is the importance of posttranslational modifications. Phosphorylation, sumoylation, and acetylation modulate the transcriptional activities of several C/EBP family members. C/EBPε is phosphorylated at several sites, and depending on the amino acid residue that is phosphorylated, granulocyte differentiation can be either blocked or increased. Also, sumoylation of lysine residues in C/EBPε inhibits granulocyte gene expression. Acetylation of C/EBPβ is functionally linked to transcriptional activity, but its impact on granulopoiesis is unknown. Phosphorylation of C/EBPε on threonine 75, which is located in the transactivation domain, leads to increased DNA binding and transcriptional activity, and sumoylation of a lysine residue 121 (K121) within repression domain I enhances transcriptional activation by C/EBPε.

A potential role for acetylation in regulating neutrophil production and differentiation was suggested by these authors as lysine deacetylase inhibitors (KDAC) affect myeloid differentiation. Skowkow et al demonstrated that nicotinamide treatment increases expression of C/EBPε32/30 acetylation of lysine residues on C/EBPε32/30, and expression of the lactoferrin and cathelicidin antimicrobial peptide genes. Nicotinamide treatment augments neutrophil killing and enhances clearance of Staphylococcus aureus both in vitro and in vivo; however, the importance of C/EBPε32/30 acetylation in mediating these outcomes has not been determined in either study. The current study extends these findings and provides the first experimental evidence that acetylation at specific lysines (K121 and K198) is essential for C/EBPε32/30 transcriptional activity and terminal differentiation of neutrophils (see figure). In a series of experiments, they demonstrated acetylation of C/EBPε32/30 in cell culture and CD34+ umbilical cord blood cells after G-CSF-induced neutrophil differentiation. They showed that C/EBPε32/30 acetylation status is regulated by SIRT1 and p300 (a lysine acetylase, KAC) and that nicotinamide, an inhibitor of lysine deacetylase SIRT1, increases levels of acetylated C/EBPε32/30 in CD34+ umbilical cord blood cells differentiated toward neutrophils (see figure). Acetylation was required for transcriptional activity as a “lysine”-dead version of C/EBPε32/30 (all 15 lysines mutated to arginine) lost activity. Mass spectrometry analysis identified 4 acetylated lysines: 2 in repression domain I (K100 and K121), 1 lysine between repression domain II and the basic region (K198), and 1 in the basic region (K202). Mutation analysis revealed that K121 and K198 are required for transcriptional activation and that loss of K121 acetylation decreased DNA binding. A functional role for K121 and K198 acetylation in neutrophil differentiation was demonstrated by retroviral transduction of lysine mutants into CD34+ umbilical cord blood cells differentiated into neutrophils. The lysine dead C/EBPε32/30 dramatically reduced the percentage of mature neutrophils by 10-fold and lactoferrin protein levels by threefold. Adding back K121 and K198 increased the percentage of mature neutrophils and restored levels of lactoferrin protein to nearly that observed for the wild-type C/EBPε32/30. Previously, the authors observed that overexpression of wild type C/EBPε32/30 in hematopoietic progenitor cells promotes eosinophil differentiation at the expense of neutrophils and C/EBPε27 and C/EBPε14 block eosinophil differentiation.

The authors observed this phenomenon with retroviral transduction of the wild-type C/EBPε32/30, but the R121/198K mutant restored neutrophil differentiation, and no increase in eosinophil precursors was observed. One conclusion could be that acetylation at these lysines is selectively important for differentiation along either the neutrophil or eosinophil lineages (see figure). A limitation of the current study is that the conclusions are based on in vitro experiments. Future studies in a mouse model could provide more definitive evidence that acetylation of C/EBPε is essential during granulopoiesis in vivo.

Prior studies with nicotinamide suggest a potentially important function for acetylation of C/EBPε members in boosting neutrophil numbers and function. The current study’s novel findings provide for the first time the important experimental evidence that acetylation of C/EBPε is critical for terminal neutrophil differentiation. These findings establish a foundation for future work toward developing therapies that more effectively and specifically manipulate neutrophil production and function with the goal of treating neutropenia and neutrophil dysfunction.

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Comment on Kapur et al, page 1793

C-reactive protein boosts antibody-mediated platelet destruction

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In this issue of Blood, Kapur et al show that C-reactive protein (CRP) enhances IgG-mediated phagocytosis of platelets and patients with immune thrombocytopenia (ITP) have elevated CRP levels, which predicted slower platelet recoveries and bleeding severity.1

Antibody-mediated platelet destruction in fetal or neonatal alloimmune thrombocytopenia (FNAIT) and ITP occurs primarily through engagement of immunoglobulin (IgG) opsonized platelets with activating Fc receptors (FcRs) on the surface of phagocytes in the spleen and liver, resulting in phagocytosis and thrombocytopenia.2 In FNAIT, for example, the antiplatelet IgG antibodies cross the placenta and target the fetal platelets, which can result in serious complications such as intracerebral hemorrhage. Antibody titer has been shown to be related to fetal platelet counts; however, this correlation is far from perfect, as there are many cases not attributed to antibody titer alone,4 suggesting other factors such as IgG Fc–fucosylation may be involved.5 Even more striking, in ITP, there is no test that reliably predicts bleeding outcomes or severity of bleeding. Thus, a tool for the prediction of both FNAIT and ITP severity is highly warranted.

Kapur et al describe a series of elegant studies in which a serum factor was found to enhance IgG-mediated responses (respiratory burst and phagocytosis) against platelets.1 The involvement of the complement cascade was excluded, and the factor was found to be present in healthy sera and appeared to be acting against platelets in a calcium-dependent manner. As such, CRP was convincingly identified as the serum factor that enhanced IgG-mediated phagocytosis of platelets.

CRP belongs to the pentraxin family, which contains a calcium–dependent ligand binding site and is a major acute-phase protein and a known ligand for FcRs.6 CRP is significantly upregulated during infections (from <0.05 to >500 mg/L), but is also present in healthy sera, albeit at a lower concentrations (~0.8 mg/L in healthy adult volunteers).6 There was an intriguing correlation between CRP concentrations in 14 different healthy sera and antibody–mediated phagocytic activity against platelets.1 In addition, purified CRP was clearly shown to enhance antibody–mediated phagocytic responses against platelets, and CRP depletion and repletion experiments corresponded to IgG-mediated phagocytic activity against platelets.

Several experiments were performed to elucidate the working mechanism of CRP in IgG-mediated targeting of platelets. Platelet activation was shown to be involved, but not via the platelet FcyRIIa. Using pneumococcal cell wall polysaccharide (CWPS), which binds to the phosphorylcholine–ligand binding site on CRP, the authors demonstrated an abrogation of the IgG-enhancing serum effect in a phagocytic assay. Furthermore, using a novel innovative approach of cellular surface plasmon resonance, CRP binding to platelets was further explored. CRP was absorbed on sensor surface spots on a chip and IgG-opsonized platelets were circulated over the chip, measuring the specific interaction of platelets to CRP with high sensitivity. IgG-opsonized platelets demonstrated increased binding to CRP compared with isotype control (nonbound) platelets. Importantly, the binding of platelets to CRP was completely blocked by coinjection of CWPS, suggesting that CRP binds to platelet-membrane phosphorylcholine exposed after platelet activation induced by the antiplatelet antibodies. The nature of this platelet activation was further explored by using both inhibitors and an enhancer of oxidation. Platelet oxidation was found to be associated with increased binding to CRP and, using oxidation inhibitors in the platelet phagocytosis assay, the authors additionally demonstrated a role for the phagocyte reduced NAD phosphate (NADPH) oxidase system.

The aforementioned experiments were conducted with antibodies from maternal FNAIT sera (anti–HPA-1a antibodies), as well as a human monoclonal FNAIT antibody, B2G1. As CRP levels were found to be
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