Pre-miR 29b is fragmented by dicer. The active miR 29b binds DNMT3A, 3B, and Sp1 mRNA either blocking translation or targeting them for degradation. Loss of Sp1 protein in turn down-regulates DNMT1 expression.

(DNMT) depletion. They further demonstrate that replacement of miR 29b oligonucleotides results in both re-expression of the methylated tumor suppressor genes p15INK4b and ESR1 (to an extent comparable to that using the DNMT inhibitor decitabine) and differentiation in AML cell lines.

The putative hypomethylating agents, 5-azaacytidine and decitabine, are the first agents to have had a significant impact on prognosis for patients with high-risk meleodysplastic syndrome and have shown promising efficacy in elderly patients with AML.8 These agents are incorporated into DNA forming adducts with and inactivating DNMTs 1, 3A, and 3B which are responsible for maintenance and de novo methylation, respectively. Both aberrant DNA hypermethylation of tumor suppressor genes and overexpression of DNMT isoforms have been demonstrated in a variety of malignancies including AML. Although controversy remains with regard to the mechanism of action of these drugs, there is considerable circumstantial evidence that tumor suppressor gene re-expression plays an important role in their efficacy.9

The demonstration of a potentially pharmacologically active miRNA provides a unique opportunity for further insight into the activity of 5-azacytidine and decitabine. Debate continues over the mechanism of action of these so-called hypomethylating agents. However, no definitive evidence shows that methylation reversal is responsible for their clinical efficacy. In fact, if these agents exert their effects via hypomethylation of tumor suppressor genes (and resultant gene re-expression), their activity should be mimicked in vitro and in vivo models by expression of exogenous miR 29b. While miRs could conceivably replace or augment the azanucleosides therapeutically, as with all genetically driven therapies, effective delivery to the tissue of interest will be a challenge. Ultimately, even if these agents do not prove to be useful Pharmaceuticals, they should provide an opportunity to finally understand whether demethylation is central to the benefit seen with 5-azacytidine and decitabine.

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PHAGOCYTES & GRANULOCYTES

Comment on Yost et al, page 6419

Babies born without safety NET

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In this issue of Blood, Yost and colleagues demonstrate that neutrophils from newborn infants lack the ability to produce so-called extracellular traps and exhibit impaired extracellular killing of bacteria.

Newborn babies present with various immaturities of their humoral and cellular immune system, particularly during the first days of life. This has important and deleterious consequences. Bacterial infection is a major cause of death and long-term morbidity in preterm newborn infants.1 Therefore, despite the origin of the word (from immatus, existing at the time of birth), the innate immune responses against microbial invasion are apparently impaired in neonates. Defective antibacterial function of neutrophils of neonates has thus been noted for some time, and circumstantial evidence for an immaturity of granulopoiesis—which might explain the frequent development of neutropenia in neonates in response to bacterial sepsis—has also been documented.1 However, the syndrome of neonatal neutrophil deficiency remains incompletely understood.

Neutrophils are essential effector cells of the innate immune system and play a crucial role in the killing of microorganisms. Neutrophils kill pathogens through reactive oxygen species (ROS)—dependent and—independent mechanisms. The former pathway is associated with activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase at the phagosomal membrane. Patients suffering from chronic
When stimulated with growth factors, granulocytes from CGD patients thus failed to produce NETs (nuetrophil extracellular traps) that capture and kill microorganisms. Experimental studies in this issue of Blood, Yost et al now reveal a novel innate immune defect in neonates: impaired NET formation.4

In contrast, NET formation could not be detected when control or LPS-stimulated neonatal neutrophils were examined (middle panels: term; right panels: preterm). The net result of this novel defect was shown to be impaired extracellular killing of bacteria. See the complete figure in the article beginning on page 6419.

Neutrophils from term and preterm neonates thus fail to form NETs upon stimulation ex vivo with inflammatory agonists (see figure). In contrast, NET formation could not be detected when control or LPS-stimulated neonatal neutrophils were examined (middle panels: term; right panels: preterm). The net result of this novel defect was shown to be impaired extracellular killing of bacteria. See the complete figure in the article beginning on page 6419.

Stimulated neutrophils also produce extracellular structures called neutrophil extracellular traps (NETs) that capture and kill microorganisms.2 NETs consist of an extracellular web of fibers composed of DNA, histones, and granule proteins such as elastase. It was suggested that NETs may enhance the efficiency of bacterial killing by providing a high local concentration of antimicrobial molecules. Moreover, these extracellular structures are apparently active even when the neutrophil itself has died,3 thus serving to prolong the tour of duty for these cells. In a series of elegant experiments in this issue of Blood, Yost et al now reveal a novel innate immune deficiency of neonates: impaired NET formation.4

Neutrophils from term and preterm neonates thus fail to form NETs upon stimulation ex vivo with inflammatory agonists (see figure). Importantly, the authors also show that neutrophils isolated from term infants were defective for extracellular killing of bacteria. Previous studies have indicated that NET formation is dependent on the generation of ROS by NADPH oxidase.2 Neutrophils derived from CGD patients thus failed to produce NETs when stimulated with Staphylococcus aureus or PMA, a potent activator of the oxidative burst.3 In contrast, Yost et al report that generation of ROS alone is not sufficient to induce NET formation in neonatal neutrophils. Indeed, they show that the impaired NET formation in neutrophils from newborn babies is not due to a deficiency of NADPH oxidase activation in those cells.4 Thus, the signaling events that govern the formation of NETs remain to be elucidated. However, the discovery that neonatal neutrophils lack the ability to cast NETs suggests a potentially useful model system to further dissect the underlying signaling cascades.

The prevailing view is that neutrophils undergoing apoptosis are recognized and removed by neighboring macrophages before their disintegration, and it is thought that this process also contributes to the resolution of inflammation.3 In this context, the discovery that activated neutrophils may burst and extrude extracellular lattices of chromatin and other intracellular constituents begs this question: How are NETs dismantled after an infection has been cleared? A related question is, of course, whether the prolonged presence of NETs in the extracellular milieu may induce autoimmune responses. It is not inconceivable that inadvertent immune responses to vast amounts of DNA and associated histones may occur.

Finally, an important question is whether NET formation could also contribute to tissue injury during severe infection. A recent report showed that platelets can activate neutrophils to produce NETs, which retain their integrity under flow conditions and are able to ensnare bacteria in the vasculature.6 In fact, these events occurred primarily in the narrowest of vessels, the sinusoidal capillaries where NETs would have the greatest capacity for bacterial trapping. However, this antimicrobial mechanism occurred at the expense of tissue injury to endothelium and liver tissue in a mouse model of endotoxemia.

Finding new strategies to enhance the neonatal immune defense against infection is an important goal. The novel observation that NET formation is reduced in neutrophils obtained from neonates draws our attention to the importance of extracellular mechanisms of antimicrobial defense, and could also inspire new therapeutic approaches.

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

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Comment on Zou et al, page 6428

Escaping the niche

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In this issue of Blood, Zou and colleagues unravel a long-standing mystery—how megakaryocyte precursors make the transition from the collagen–rich osteoblastic bone marrow niche to the collagen–poor vascular niche while expressing 2 receptors that interact with collagen—GpVI and the α5β3 integrin.

PLATELETS & THROMBOPOIESIS

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