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DOI 10.1182/blood.2021011475

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## PHAGOCYTES, GRANULOCYTES, AND MYELOPOIESIS

Comment on Xiao et al, page 3533

# Bone and blood: IL-19 to the rescue

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**In this issue of *Blood*, Xiao et al<sup>1</sup> identified interleukin 19 (IL-19) as a potent cytokine capable of promoting expansion and proliferation of neutrophils. Neutropenia is a common consequence of chemotherapy, and there are currently very few and limited treatments available. Surprisingly, osteocytes, the bone cells deeply embedded in the mineralized matrix, are the major source of IL-19, placing these cells, once again, on the list of important regulators of hematopoiesis.<sup>2,3</sup> Osteocytes primary function is to control skeletal homeostasis through 2 secreted proteins: sclerostin,<sup>4,5</sup> a Wnt inhibitor that suppresses bone formation, and Rankl, a cytokine required for osteoclastogenesis.<sup>6</sup> In this article, Xiao et al used a combination of genetically modified mice and in vitro models to provide evidence that osteocytes are also the main source of IL-19, a cytokine that promotes the expansion of neutrophils.**

The article presents 2 major advancements in the field: the identification of IL-19 as a regulator of neutrophils maturation and proliferation and that the osteocytes (and possibly late mature osteoblasts) are the major source of this cytokine.

IL-19 functions as an anti-inflammatory and proangiogenic factor.<sup>7</sup> It belongs to a subfamily that includes IL-20, IL-22, IL-14, and IL-26, and it signals by binding to a receptor complex consisting of an heterodimer of IL-20R $\alpha$  and IL-20R $\beta$  promoting a T helper 2 regulatory T-cell<sup>8</sup> response in a variety of disease contexts.

In this article, the authors first analyzed the role of mechanistic target of rapamycin complex 1 (mTORC1) in osteocytes by generating mice where the expression of this protein was increased, by deleting its inhibitor, tuberous sclerosis complex protein 1 (TSC1), or decreased, by deleting the upstream activator Rheb. mTORC1 regulates both cell proliferation in response to metabolic challenges and myeloid differentiation.<sup>9</sup> When mTORC1

is activated in Dmp1-expressing cells, neutrophils are significantly increased, whereas mTORC1 inhibition induces neutropenia. The in vivo studies are followed by an extensive in vitro characterization of the relative contribution of different cell types, including osteocytes, osteoclasts, endothelial cells, bone marrow stromal cells, lymphocytes, and monocytes. Strikingly, only primary osteocytes recapitulate the in vivo phenotype. Next, the authors identify IL-19 as the factor driving the hematopoietic phenotype. As predicted, IL-19 administration rescues the neutropenia present in the Dmp1-TSC1 knockout (KO) mice, whereas intramedullary administration of IL-19–neutralizing antibody corrects the neutrophilia in the Dmp1-Rheb KO animals. Last, and possibly most importantly, administration of IL-19 protects wild-type mice from neutropenia induced by both chemotherapy and radiation, demonstrating the therapeutic efficacy of this cytokines. It remains unknown whether IL-19 has additional effects on other organs or tissues, but its potential

application in neutropenic states is undoubted. Additional studies will be needed to determine whether factors known to control osteocytes also regulate IL-19 synthesis and secretion and whether this cytokine has additional skeletal and other organ effects. Tissue distribution and downstream signals have only been partially elucidated, and a clearer picture of the function of this cytokine is required. One puzzling finding of this paper is that the phenotype is present only in Dmp1-TSC1 KO animals, and not in mice in which Tsc1 is ablated in osteoprogenitors (Osx-TSC1 KO) or osteoblasts (Ocn-TSC1 KO). This suggests that Tsc1 expression in these cells (osteoprogenitors and osteoblasts) prevents the expansion of neutrophils present in Dmp1-Tsc1 KO animals. One possible explanation is that when Tsc1 is ablated from early osteoprogenitors and osteoblasts, the hematopoietic stem cell niche is altered and can no longer support the expansion of guanosine monophosphate. Further characterization of the hematopoietic phenotype of Osx and Oc-Tsc1 mice will be needed to try to explain this conundrum.

Regardless of these questions, this study is a breakthrough in the development of novel therapeutic interventions to treat neutropenic states.

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

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DOI 10.1182/blood.2021011367

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## RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Mikdar et al, page 3548

# Nucleoside ENTry modulates erythropoiesis

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In this issue of *Blood*, Mikdar et al show that deficiency of ENT1, a nucleoside transporter, leads to defects in erythropoiesis and morphological changes in erythrocytes.<sup>1</sup>

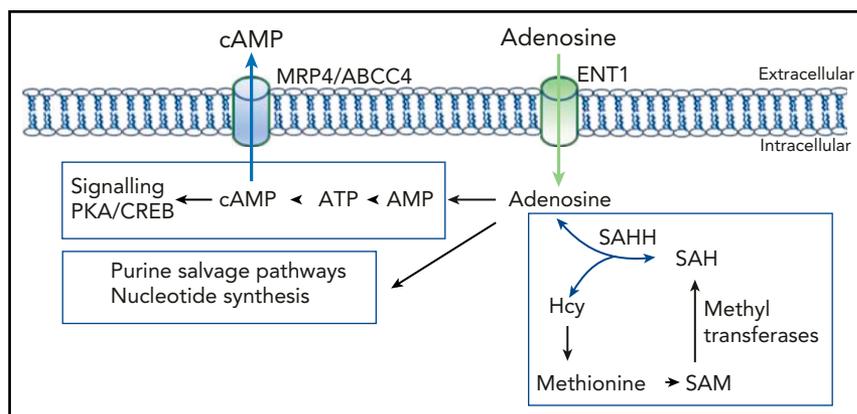
These phenotypes were linked to adenosine transport and could be rescued, in part, by reduced multidrug transporter MRP4/ABCC4 function. The effects of adenosine were first described as a physiological stimulus that regulates cardiac rhythm.<sup>2</sup> Since that study, adenosines have been implicated in the regulation of multiple biological processes. Adenosine transporters can be divided into equilibrative (SLC29) and concentrative (SLC28) transporters. The ENT family of equilibrative transporters uses membrane adenosine concentration gradients as the driving force for influx or efflux. In addition to naturally-occurring nucleosides, nucleotide analogs, such as cytosine arabinoside (ara-C), are substrates. Once intracellular, adenosine metabolism maintains and regulates pivotal

biosynthesis pathways, including synthesis of adenosine triphosphate (ATP), cyclic adenosine monophosphate (cAMP), and nucleotides for DNA/RNA synthesis. In addition, through actions of adenosine kinase and S-adenosylhomocysteine hydrolase (SAHH), adenosine regulates S-adenosyl methionine (SAM)-dependent transmethylation reactions, linking adenosine to epigenetic transcriptional regulation.<sup>3</sup> Importantly, adenosine metabolites also provide substrates for an array of other transporters. For instance, efflux of cAMP is mediated by MRP4/ABCC4. Mikdar et al provide evidence that MRP4/ABCC4 fluxes are linked to ENT1 adenosine influx. Taken together, a picture emerges where adenosine/nucleoside transport and metabolism integrate into a large network of biosynthesis

pathways, thereby regulating a myriad of biological processes (see figure). Thus, it is not surprising that mutations in such transporters lead to pathology. Mutations in mitochondrial-localized ENT3 lead to familial histiocytosis syndrome,<sup>4</sup> whereas ENT1 mutations can result in resistance to chemotherapy (eg, ara-C<sup>5</sup>) or altered binding affinities to specific therapeutics.<sup>6</sup> In addition to this, there is considerable interest in the effects of drugs that target these transporters.

ENT1 is also expressed on erythrocytes and harbors the Augustine blood group system, a high-frequency antigen associated with severe hemolytic transfusion reactions.<sup>7</sup> Augustine-negative subjects do not display any anemia, but they present with altered bone calcification, indicating a role for ENT1 in bone homeostasis.<sup>7</sup> Recently, ENT1 gene regulation during erythropoiesis was shown to be regulated by GATA transcription factors.<sup>8</sup> So, what is the role of ENT1 in erythrocytes and during erythropoiesis? Mikdar et al shed new light on this topic. They examined ENT<sup>-/-</sup> human erythrocytes and found that ENT deficiency leads to macrocytosis and elliptocytosis. Phosphoproteomics uncovered hypophosphorylation of erythrocyte structural proteins that play a role in membrane tethering to the underlying spectrin cytoskeleton (spectrins, protein 4.1, ankyrin, and adducin). Mutations within those proteins lead to elliptocytosis. Surprisingly, no defect in membrane protein tethering to the underlying spectrin cytoskeleton was observed. Although 1 hyperphosphorylated protein, CLNSA1, correlated with macrocytic red cells, the cause of elliptocytosis upon ENT deficiency remains to be elucidated.

Mikdar et al show that ENT1 deficiency reduced human erythrocyte precursor numbers, which resulted in anemia and macrocytosis in Ent1<sup>-/-</sup> mice. These defects could be overcome, in part, by reduced activity of the multidrug transporter MRP4/ABCC4, observed upon inhibition with the broad MRP inhibitor MK-571 or in ENT<sup>-/-</sup> individuals with an additional heterozygous loss-of-function mutation in MRP4. Strikingly, wild-type erythropoiesis was also enhanced in mice treated with ABCC4/MRP4 inhibitors. The results show that erythropoiesis is controlled, in part, by nucleoside transporters and nucleotide exporters; importantly, this pathway may be exploited for therapeutic purposes to treat anemias.



Adenosine levels control multiple biosynthesis pathways. Note that adenosine and homocysteine (Hcy) concentrations coregulate the direction of SAHH. AMP, adenosine monophosphate; CREB, cAMP-response element binding protein; PKA, protein kinase A; SAH, S-adenosyl-L-homocysteine.