Is the unique bias toward erythroid-megakaryocytic differentiation exhibited by peripheral blood HSPCs altered in hematologic disease? HSC/MPPs from patients with essential thrombocythemia and, surprisingly, β-thalassemia demonstrated increased myeloid production at the expense of the erythroid lineage. The authors suggest that disease-driven changes in the microenvironment and/or hematopoietic dysfunction in the marrow leads to a shift in the differentiation balance of peripheral blood HSC/MPPs.

To further place these studies in a clinical context, extramedullary HSPC composition and function were examined in spleens from 2 patients with hereditary spherocytosis, an example of chronic hematopoietic cell stress. HSC/MPPs from HS spleens displayed increased erythroid transcriptional priming and produced more erythroid colonies in vitro than those from control spleens. HS splenic HSPCs also had a much higher ratio of early erythroid to myeloid progenitors than control. Although there was no change in myeloid expansion of splenic HSPCs, there was increased differentiation along the erythroid line, although not to the level modeled in bone marrow. Taken together, the authors conclude that splenic HSPCs contribute to erythropoiesis in response to anemia in humans.

This study provides an atlas of HSPC transcriptome data for investigators to query and use for comparative studies. It also demonstrates that circulating HSPCs hold promise for use in clinical applications, particularly in disease states because the composition and function of circulating HSPCs may serve as markers for bone marrow dysfunction. They may provide insights into disorders such as cardiovascular disease and stroke, select malignancies, myelofibrosis, and some autoimmune diseases, in which increased numbers of circulating peripheral blood HSPCs have been observed. Could peripheral blood HSPCs provide additional mechanistic insights to our understanding of disease, allowing better diagnosis, monitoring, and treatment? The studies by Mende and colleagues provide a tantalizing "yes" while at the same time providing a beautiful blueprint for future work.

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

REFERENCES

1. Mende N, Bastos HP, Santoro A, et al. Unique molecular and functional features of extramedullary hematopoietic stem and progenitor cell reservoirs in humans. *Blood*. 2022;139(23):3387-3401.
2. Watcham S, Kucinski I, Gottgens B. New insights into hematopoietic differentiation landscapes from single-cell RNA sequencing. *Blood*. 2019;133(13):1415-1426.
3. Martin-Rufino JD, Sankaran VG. Deciphering transcriptional and functional heterogeneity in hematopoiesis with single-cell genomics. *Curr Opin Hematol*. 2021;28(4):269-276.
4. Satpathy AT, Granja JM, Yost KE, et al. Massively parallel single-cell chromatin landscapes of human immune cell development and intratumoral T cell exhaustion. *Nat Biotechnol*. 2019;37(8):925-936.
5. Buenrostro JD, Corces MR, Lareau CA, et al. Integrated single-cell analysis maps the continuous regulatory landscape of human hematopoietic differentiation. *Cell*. 2018;173(6):1535-1548.e16.
6. Takayama N, Murison A, Takayanagi SI, et al. The transition from quiescent to activated states in human hematopoietic stem cells is governed by dynamic 3D genome reorganization. *Cell Stem Cell*. 2021;28(3):488-501.e10.
7. Ranzoni AM, Tangherloni A, Berest I, et al. Integrative single-cell RNA-seq and ATAC-seq analysis of human developmental hematopoiesis. *Cell Stem Cell*. 2021;28(3):472-487.e7.
8. Kaufmann KB, Zeng AGX, Coyaud E, et al. A latent subset of human hematopoietic stem cells resists regenerative stress to preserve stemness. *Nat Immunol*. 2021;22(6):723-734.
9. Mende N, Laurenti E. Hematopoietic stem and progenitor cells outside the bone marrow: where, when, and why. *Exp Hematol*. 2021;104:9-16.

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IMMUNOBIOLOGY AND IMMUNOTHERAPY

Comment on Ma et al, page 3402

Hyperuricemia reduces neutrophil function

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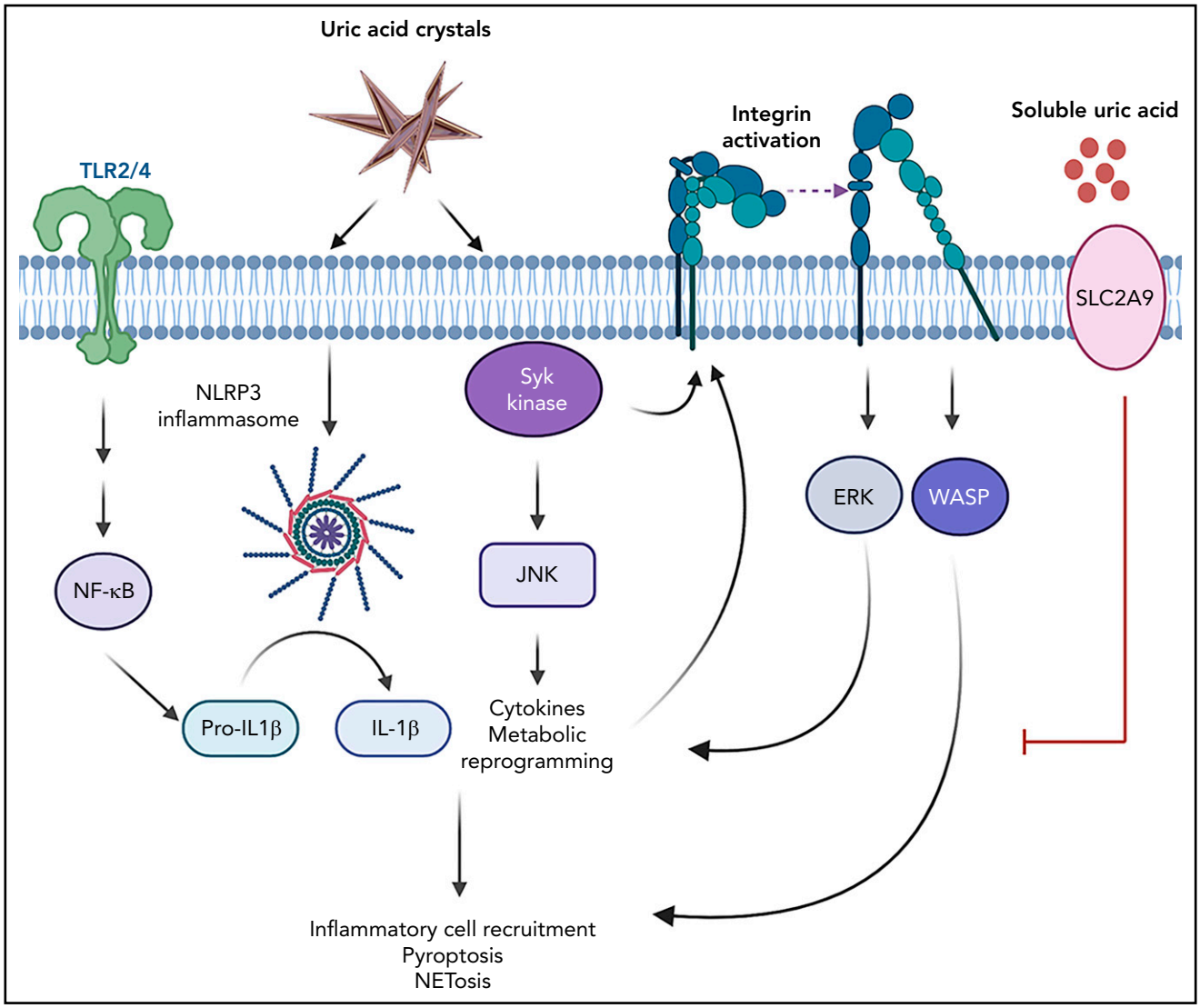
In this issue of *Blood*, Ma et al[1] report that high levels of soluble uric acid (sUA) reduce neutrophil function in patients with chronic renal failure. It is well appreciated that individuals suffering from various forms of chronic renal failure are immunosuppressed.[2] Indeed, infection is the second most common cause of death in patients with kidney disease.

There are many factors that may contribute to the immunodeficiency of renal failure, but the molecular mechanisms that cause these immune defects remain poorly investigated. Clearly, both innate and adaptative immune responses can be affected. In the innate compartment, impairment of both neutrophil and monocyte/macrophage function have been reported. Monocytes from uremic patients manifest reduced cytokine production when stimulated, whereas neutrophils display reduced migratory capacity. In the mononuclear system, one of the molecular mediators of reduced cellular function was found to be high levels of sUA.[3] Hyperuricemia at a level of 7 to 12 mg/dL (found in patients with more severe renal dysfunction) leads to inhibition of monocyte Toll-like receptor (TLR) signaling, which impairs cytokine production and $CD14^+$ monocyte migration in vitro. The authors of the Ma et al article found that UA impairs β2 integrin activation and signaling, which leads to reduced neutrophil migration into inflammatory sites in vivo.

UA is taken up into cells via glucose transporter 9 (Glut9 or SLC2A9). Mice lacking SLC2A9, specifically in hepatocytes, develop moderate hyperuricemia; when the animals are fed an inosine-rich diet, they develop serum UA levels in the range of 7 to 12 mg/dL seen in humans with end-stage renal failure.[4] By using this model, Ma et al found reduced neutrophil recruitment into air pouches (formed on the back of the mouse) in response to 2 different inflammatory stimuli. Most directly, this group used intravital microscopy to directly measure neutrophil rolling and adhesion in the vasculature of the hyperuricemic mice. In response to



The differential effect of soluble vs crystalline UA on innate cells. Figure generated with BioRender software. ERK, extracellular signal-regulated kinase.

inflammatory stimuli, neutrophils in the hyperuricemic mice showed increased rolling velocity, consistent with poor integrin-mediated adhesion, that resulted in reduced transmigration of cells into the inflamed tissues. This effect was partially reversed by treating the animals with rasburicase, which lowers serum urate levels. By using human peripheral blood neutrophils, Ma et al found that exposure to high levels of UA led to poor β2 (CD18) integrin activation and cell surface recycling in vitro (to a number of inflammatory stimuli), causing reduced adhesion and chemotaxis in vitro. This mirrored the reduced in vitro chemotaxis seen in neutrophils isolated from patients with severe renal failure.

Perhaps the most interesting aspect of this biology is the dramatically different effects that soluble versus crystalline UA have on innate cells (see figure). Urate crystals, the causative agent of gout, are extreme activators of innate cells. In primed macrophages that have been exposed to TLR2 or TLR4 agonists and have high levels of pro-interleukin 1 (pro-IL-1), UA crystals lead to direct activation of the NLRP3 inflammasome complex, resulting in extreme production of IL-1β leading to an inflammatory casacade.[5] Monosodium urate crystals also directly activate unprimed (or primed) macrophages, likely through a signaling pathway that involves the Syk and JNK kinases leading to cytokine production and metabolic reprogramming.[6] Similar results have been reported in neutrophils exposed to UA crystals.[7] UA crystals can directly bind to plasma membrane lipids, leading to the aggregation of lipid raft domains in the membrane that in turn activate Syk kinase.[8]

It has long been known that one of the consequences of exposure to UA crystals is integrin (CD18) activation,[9] which enhances the migratory capacity of innate cells. Yet somehow, sUA does just the opposite of all this. A detailed understanding of how sUA is able to oppose β2 integrin activation remains to be provided. Because the effects of sUA on integrin activation are very rapid (experiments are performed with only a 30-minute exposure to UA), the mechanism likely involves some direct biochemical effect. Perhaps UA is competing with purine nucleotides for activation of GTPases involved in changing the integrin conformation.[10] Or perhaps UA could be altering charged interactions between the

signaling molecules involved in integrin activation. Working out these mechanisms will likely require significant biochemical and biophysical studies. Although it is not a major feature of this report, the authors also found that high levels of sUA affect neutrophil phagocytosis. This suggests that UA can have a broader effect on the intracellular actin cytoskeleton of innate cells, limiting membrane dynamics more generally, which may explain the reduced re-cycling of CD18 observed in these studies. Nevertheless, it is clear from this work that some aspects of the immunodeficiency seen in patients with end-stage kidney failure can be explained by the hyperuricemia that causes reduced neutrophil integrin function on top of poor monocyte and macrophage activity. This should increase the urgency for developing inhibitors for SLC2A9 to limit UA uptake into innate immune cells in patients with chronic renal failure.

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REFERENCES

1. Ma Q, Immler R, Pruenster M, et al. Soluble uric acid inhibits β_2 integrin–mediated neutrophil recruitment in innate immunity. *Blood*. 2022;139(23):3402-3417.
2. Steiger S, Rossaint J, Zarbock A, Anders HJ. Secondary immunodeficiency related to kidney disease (SIDKD)-definition, unmet need, and mechanisms. *J Am Soc Nephrol*. 2022;33(2):259-278.
3. Ma Q, Honarpisheh M, Li C, et al. Soluble uric acid is an intrinsic negative regulator of monocyte activation in monosodium urate crystal-induced tissue inflammation. *J Immunol*. 2020;205(3):789-800.
4. Sellmayr M, Hernandez Petzsche MR, Ma Q, et al. Only hyperuricemia with crystalluria, but not asymptomatic hyperuricemia, drives progression of chronic kidney disease. *J Am Soc Nephrol*. 2020;31(12):2773-2792.
5. So AK, Martinon F. Inflammation in gout: mechanisms and therapeutic targets. *Nat Rev Rheumatol*. 2017;13(11):639-647.
6. Cobo I, Cheng A, Murillo-Saich J, et al. Monosodium urate crystals regulate a unique JNK-dependent macrophage metabolic and inflammatory response. *Cell Rep*. 2022;38(10):110489.
7. Tatsiy O, Mayer TZ, de Carvalho Oliveira V, et al. Cytokine production and NET formation by monosodium urate-activated human neutrophils involves early and late events, and requires upstream TAK1 and Syk. *Front Immunol*. 2020;10:2996.
8. Ng G, Sharma K, Ward SM, et al. Receptor-independent, direct membrane binding leads to cell-surface lipid sorting and Syk kinase activation in dendritic cells. *Immunity*. 2008;29(5):807-818.
9. Barabé F, Gilbert C, Liao N, Bourgoin SG, Naccache PH. Crystal-induced neutrophil activation VI. Involvment of FcgammaRIIIB (CD16) and CD11b in response to inflammatory microcrystals. *FASEB J*. 1998; 12(2):209-220.
10. Abram CL, Lowell CA. The ins and outs of leukocyte integrin signaling. *Annu Rev Immunol*. 2009;27(1):339-362.

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LYMPHOID NEOPLASIA

Comment on Alvarez et al, page 3418

Linking epigenome regulation with DNA repair

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In this issue of *Blood*, Alvarez et al[1] present evidence that the epigenetic regulator plant homeodomain finger 6 (PHF6) plays an important role in the prevention of genomic instability in leukemia cells by participating in a wide range of cellular processes that mediate DNA repair and resolution of replication stress.

To protect their genetic information, mammalian cells have evolved a complex network of high-fidelity biological processes to regulate chromatin structures, recognize genomic lesions, and repair damaged DNA. Chromatin regulation involves epigenetic mechanisms such as DNA methylation, histone modifications, and microRNAs, whereas DNA damage response (DDR) relies on a complex network of sensors, transducers, and effectors that coordinate DNA repair with DNA replication. It has recently become clear that epigenetic control and DNA repair cooperate closely. Epigenetic regulators can directly control the expression level and activity of DNA repair proteins, as well as the accessibility of chromatin to the recruited DNA damage repair complexes. It is not surprising, therefore, that important mediators of both DNA repair and epigenetic regulation are frequently altered in cancer cells.[2,3]

PHF6 is an epigenetic regulator frequently inactivated in T-cell acute lymphoblastic leukemia (T-ALL), mixed-phenotype acute leukemia with T-lineage differentiation, and less frequently in acute myeloid leukemia and other myeloid malignancies. Earlier studies have identified PHF6 as a tumor suppressor and have suggested a role for PHF6 in regulating DNA transcription, DNA repair, and hematopoiesis. However, the precise mechanism underpinning its tumor suppressor role remains unclear.[4]

In this important work, Alvarez et al unraveled different PHF6 protein interactions and provided compelling evidence that PHF6 plays a much wider role in suppressing tumorigenesis than previously understood. The authors observed that PHF6 interacts not only with components of the nucleosome remodeling deacetylase (NuRD) complex that associates with condensed chromatin but also with switch/sucrose nonfermentable (SWI/SNF) chromatin remodelers that bind to open chromatin, suggesting a broad role of PHF6 in chromatin remodeling. Moreover, PHF6 was demonstrated to interact with a range of DNA repair factors at sites of damaged DNA, as well as with regulators of cell cycle and DNA synthesis. Through elegant functional studies, the authors confirmed the ability of PHF6 to facilitate resolution of both single-strand and double-strand DNA breaks. They also highlighted a novel function of PHF6 in homologous recombination (HR) repair that involved promoting the nuclear retention of the HR protein BRCA1.

The authors further observed that the role of PHF6 includes the epigenetic regulation of DNA replication, whereby PHF6 protects replication fork integrity by facilitating ataxia telangiectasia and Rad3-related protein (ATR)–dependent activation of the single-strand DNA-binding replication protein A (RPA). Other epigenetic regulators are known to regulate