NKTL. When investigating coimmunoprecipitates, they observed an association of both proteins. Pharmacologic inhibition of the JAK/STAT5 signaling pathway led to an increased H3K27 trimethylation, suggesting an indirect inhibition of the methyltransferase activity of EZH2 by JAK3. The same effect was observed when JAK3 expression was inhibited by small interfering RNAs, whereas overexpression of JAK3 led to the contrary effect and a decrease in H3K27 trimethylation. Using an anti-phosphotyrosine antibody on immunoprecipitated EZH2, the protein was found to be phosphorylated. This finding was substantiated by the observation that treatment of cells with a JAK inhibitor decreased EZH2 phosphorylation. To identify the precise tyrosine phosphorylation site, the authors bioinformatically identified 3 potential residues (Y23, Y244, and Y523) and generated mutants wherein they substituted tyrosine by alanine. Only the EZH2 Y244A mutant showed significantly reduced cell growth. The Y244A residue was verified as the putative phosphorylation site, using a specific polyclonal antiserum. Treatment of NKTL cells with a JAK3 inhibitor reduced the level of phospho-EZH2 Y244.

Next, the authors investigated the effect of JAK3-mediated phosphorylation of EZH2 on the expression of genes that are downregulated by the histone methyltransferase activity of EZH2. They performed gene expression microarray studies and derived a JAK-STAT activation gene signature and an PRC2-repressed target-gene signature and found a strong positive correlation between the 2 expression signatures.

Using specific EZH2 mutants, the authors identified a set of 93 potential target genes of phosphorylated EZH2 and validated these findings by chromatin immunoprecipitation–quantitative polymerase chain reaction analysis of selected genes. Immunoprecipitation revealed that phosphorylated EZH2 was no longer associated with the PRC2 components SUZ12 and EED (see figure) but instead formed a complex with RNA polymerase II, indicating that the downstream effects of Y244-phosphorylated EZH2 are not dependent on the context of the PRC2.

The application of JAK3 kinase inhibitor PF956980 dramatically decreased NKTL cell growth in cell culture whereas inhibitors of the methyltransferase activity had little or no effect.

In summary, the work of Yan et al reveals interesting new aspects of the molecular biology of EZH2, which was previously known as a methyltransferase and mainly a gene silencer in the context of the PRC2 complex. Yan et al show that EZH2 can also act as transcriptional activator when phosphorylated by JAK3 and that this function is independent and largely exclusive of its methyltransferase activity and the PRC2 complex. Several questions remain to be answered, especially concerning the downstream targets of phosphorylated EZH2. The clinically interesting aspect is of course the perspective that certain patient groups, exhibiting overexpression of (unmutated) EZH2, might benefit from the treatment with JAK inhibitors. There even is the potential of developing specific inhibitors of Y244-phosphorylated EZH2.

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

REFERENCES

1. Yan J, Li B, Lin B, et al. EZH2 phosphorylation by JAK3 mediates a switch to noncanonical function in natural killer/T-cell lymphoma. *Blood.* 2016;128(7):948-958.
2. Kim KH, Roberts CW. Targeting EZH2 in cancer. *Nat Med.* 2016;22(2):128-134.
3. Herviou L, Cavalli G, Cartron G, Klein B, Moreaux J. EZH2 in normal hematopoiesis and hematological malignancies. *Oncotarget.* 2016;7(3):2284-2296.
4. Kim E, Kim M, Woo DH, et al. Phosphorylation of EZH2 activates STAT3 signaling via STAT3 methylation and promotes tumorigenicity of glioblastoma stem-like cells. *Cancer Cell.* 2013;23(6):839-852.
5. Ernst T, Chase AJ, Score J, et al. Inactivating mutations of the histone methyltransferase gene EZH2 in myeloid disorders. *Nat Genet.* 2010;42(8):722-726.
6. Ntziachristos P, Tsirigos A, Van Vlierberghe P, et al. Genetic inactivation of the polycomb repressive complex 2 in T cell acute lymphoblastic leukemia. *Nat Med.* 2012; 18(2):298-301.
7. Morin RD, Johnson NA, Severson TM, et al. Somatic mutations altering EZH2 (Tyr641) in follicular and diffuse large B-cell lymphomas of germinal-center origin. *Nat Genet.* 2010;42(2):181-185.
8. McCabe MT, Graves AP, Ganji G, et al. Mutation of A677 in histone methyltransferase EZH2 in human B-cell lymphoma promotes hypertrimethylation of histone H3 on lysine 27 (H3K27). *Proc Natl Acad Sci USA.* 2012;109(8): 2989-2994.
9. Souroullas GP, Jeck WR, Parker JS, et al. An oncogenic Ezh2 mutation induces tumors through global redistribution of histone 3 lysine 27 trimethylation. *Nat Med.* 2016;22(6):632-640.
10. Morera L, Lübbert M, Jung M. Targeting histone methyltransferases and demethylases in clinical trials for cancer therapy. *Clin Epigenetics.* 2016;8:57.

DOI 10.1182/blood-2016-06-723916

● ● ● MYELOID NEOPLASIA

Comment on Leiva-Juárez et al, page 982

A sniff to chase ill humors away

John R. Wingard UNIVERSITY OF FLORIDA COLLEGE OF MEDICINE

Pneumonia is a major cause of death in acute leukemia patients. In this issue of *Blood*, Leiva-Juárez et al demonstrate a novel way to prevent pneumonia in acute leukemia by inhalation of a synergistic combination of Toll-like receptor 2/6 (TLR 2/6) and TLR9 agonists (Pam2-ODN) to induce protection by bolstering mucosal defenses against challenges by relevant bacterial and mold pathogens.[1] This protection occurred in the setting of neutropenia, despite chemotherapy without exacerbating lung toxicity, in the presence of uncontrolled leukemia, and was associated with rapid pathogen killing mediated by lung epithelial cells. This study extends earlier work by the Evans group studying inducible resistance by lung epithelial cells in in vitro and in animal models.

TLRs are a family of important pattern recognition cell receptors (PRRs) expressed on the membranes of leukocytes and epithelial cells that have roles in the regulation of innate and adaptive immune responses.[2] They engage microbes on entering the host by recognition of pathogen-associated molecular patterns. Once engaged, they trigger downstream intermediary signals that lead to elaboration of inflammatory cytokines and other transcription events. Polymorphisms of TLR genes have been recognized to be associated with susceptibility to infection in a variety of medical conditions including acute leukemia[3] and after allogeneic hematopoietic cell transplant (HCT).[4-6]

There is a precedent for targeting PRRs therapeutically to bolster anti-infectious protection. Pentraxin 3 (PTX3) is a soluble PRR shown to be important in protection against *Aspergillus* pneumonia after HCT. In 1 study, a particular genetic variant of PTX3 that was associated with PTX functional deficiency was associated with a doubling of the risk for invasive aspergillosis (IA) after allogeneic HCT.[7] In a murine HCT model, administration of PTX3 provided protection against the development of IA after an intranasal challenge.[8]

These observations remind us that, although the flotilla of hematopoietic cells and circulating molecules that we normally think of providing protection are very important and often determinant in controlling infection, those are not the only host components of innate immunity. The anatomic barriers of skin, mucosa, and lung epithelia make up the first line of defense. These, like the hematopoietic elements, have a repertoire of functions, some of which are inducible and responsive to various signals that can be manipulated to augment protection.

Certainly, we have learned the importance of avoiding breeches in the integument by avoiding venous and urinary catheters and that recognition has led to practice changes to enhance safety. We are beginning to pay attention to the importance maintaining integrity of the gut mucosa, with its associated intestinal immune system, and its interaction with gut microbiota to shape inflammatory responses. This study emphasizes the lung as another opportunity to augment protection to a key portal of entry for microbes.

Our standard approaches to infection prevention have focused on antibiotics to reduce the hordes of microbes threatening at our door, use of myeloid growth factors to shorten neutropenia, and the occasional use of granulocyte transfusions to replace deficiencies of phagocytes. Multiple studies of antibiotics and antifungal drugs have shown their benefits as prophylaxis, with survival advantages in some instances, particularly in acute leukemia. Yet, we now recognize the problems of emergent antibiotic resistance. We are also beginning to see the unintended deleterious consequences of alteration in the gut microbiota that reduces colonization resistance and is associated with increases in systemic infections, graft-versus-host disease, and lower survival after HCT.[9,10] Myeloid growth factors also have a protective role in shortening neutropenia, and granulocyte transfusions may have a role in patients with refractory infections, but they too do not abrogate the risk of infection. If successful, targeting TLRs will not replace these but would be complementary.

There are unanswered questions. Delivery by inhalation is highly desirable but it offers challenges. The delivery device must get the drug to the site of need. Producing the right particle size to reach distal sites in the tracheobronchial tree is crucial. Will this approach be successful with all of the multiple polymorphisms of the TLR molecules or just certain variants? Is there a risk that with certain variants found to be associated with greater risk of infection, there might be an increase in susceptibility? If used after allogeneic HCT, how will this affect risk of graft-versus-host disease?

Studies such as this also suggest additional opportunities. As noted, there are multiple PRRs other than the TLR family. Those too could be targets to exploit. Clearly, more work needs to be done, but this is a good start.

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

REFERENCES

1. Leiva-Juárez MM, Ware HH, Kulkarni VV, Zweidler-McKay PA, Tuvim MJ, Evans SE. Inducible epithelial resistance protects mice against leukemia-associated pneumonia. *Blood*. 2016;128(7):982-992.
2. Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. *Cell*. 2006;124(4):783-801.
3. Schnetzke U, Spies-Weisshart B, Yomade O, et al. Polymorphisms of Toll-like receptors (TLR2 and TLR4) are associated with the risk of infectious complications in acute myeloid leukemia. *Genes Immun*. 2015;16(1):83-88.
4. Bochud PY, Chien JW, Marr KA, et al. Toll-like receptor 4 polymorphisms and aspergillosis in stem-cell transplantation. *N Engl J Med*. 2008;359(17):1766-1777.
5. Carvalho A, Cunha C, Carotti A, et al. Polymorphisms in Toll-like receptor genes and susceptibility to infections in allogeneic stem cell transplantation. *Exp Hematol*. 2009; 37(9):1022-1029.
6. de Boer MG, Jolink H, Halkes CJ, et al. Influence of polymorphisms in innate immunity genes on susceptibility to invasive aspergillosis after stem cell transplantation. *PLoS One*. 2011;6(4):e18403.
7. Cunha C, Aversa F, Lacerda JF, et al. Genetic PTX3 deficiency and aspergillosis in stem-cell transplantation. *N Engl J Med*. 2014;370(5):421-432.
8. Gaziano R, Bozza S, Bellocchio S, et al. Anti-Aspergillus fumigatus efficacy of pentraxin 3 alone and in combination with antifungals. *Antimicrob Agents Chemother*. 2004;48(11):4414-4421.
9. Taur Y, Xavier JB, Lipuma L, et al. Intestinal domination and the risk of bacteremia in patients undergoing allogeneic hematopoietic stem cell transplantation. *Clin Infect Dis*. 2012;55(7):905-914.
10. Holler E, Butzhammer P, Schmid K, et al. Metagenomic analysis of the stool microbiome in patients receiving allogeneic stem cell transplantation: loss of diversity is associated with use of systemic antibiotics and more pronounced in gastrointestinal graft-versus-host disease. *Biol Blood Marrow Transplant*. 2014;20(5):640-645.

DOI 10.1182/blood-2016-06-723932

● ● ● PLATELETS AND THROMBOPOIESIS

Comment on Izzi et al, page 1003

Pairing megakaryopoiesis methylation with PEAR1

Andrew D. Johnson NATIONAL HEART, LUNG, AND BLOOD INSTITUTE

In this issue of *Blood*, Izzi et al pair a platelet function–associated single-nucleotide polymorphism (SNP) with allele-specific methylation at a cytosine guanine dinucleotide (CpG) island and regulation of platelet endothelial aggregation receptor 1 (*PEAR1*) RNA expression in megakaryopoiesis, describing one of the first epigenetic-SNP links to platelet biology.[1]

PEAR1 encodes a transmembrane tyrosine kinase receptor expressed in platelets and endothelial cells and plays a role in platelet cell activation.[2] Discovered in 2005, PEAR1 was found to be phosphorylated following platelet aggregation in a proteomic screen.[2] Subsequently, we and others found association of common genetic variants in or near *PEAR1* with platelet aggregation in response to agonists including adenosine diphosphate and epinephrine,[3,4] collagen,[4] and thrombin.[5] The genetic study with greatest statistical power and

2016 128: 889-890
doi:10.1182/blood-2016-06-723932

A sniff to chase ill humors away

John R. Wingard

Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.