

a model of disseminated AML, the virus was effective at reducing AML burden from blood, bone marrow, liver, and spleen after systemic administration, particularly when combined with the anti-PD-L1 antibody. This translated into a significant survival benefit for virus-treated mice compared with controls—an effect that was again enhanced by the addition of the anti-PD-L1 antibody. Depletion of mouse natural killer cells and CD8⁺ T cells, but not CD4⁺ T cells, negated the survival benefit, suggesting that these immune cells were important for the antileukemia response. When the mice tissues were examined at necropsy, the authors demonstrated robust VSV recovery from the tumors and high PD-L1 expression in saline-treated controls. Those tumors treated with the anti-PD-L1 antibody showed increased infiltration by CD8⁺ cytotoxic T cells and decreased PD-L1 expression. Next, the authors report a critical observation to support the concept of crosspriming. From the treated mice, the authors were able to isolate both VSV-specific and leukemia-specific cytotoxic T cells, with the highest levels found in the combination-treated cohort. Together, these observations provide evidence that the viral infection alone and in combination with the anti-PD-L1 antibody is functioning also as an immunotherapeutic approach. Finally, the authors performed ex vivo analysis of primary patient samples and demonstrated high infectivity with VSV among patients with chronic myelomonocytic leukemia and monocytic AML.

This preclinical study outlines a very novel approach to AML treatment, combining an oncolytic virus with immune checkpoint inhibition, revealing virotherapy as an immunotherapy (see figure). Such a strategy could expand the spectrum of malignancies that may be amenable to immunotherapy outside of those that are most immunogenic. These results need to be confirmed in future studies, but several challenges remain. Systemic virus administration in patients with AML would require achievement of large viral titers and overcoming the host serum and immune factors that could neutralize the virus. Clearance and sequestration by the liver and spleen, although not observed in the mouse model, are important obstacles with systemic administration in humans.³ As evidenced in the ex vivo patient samples, the virus robustly

infected only a subset of cases. Therefore, choosing the right virus and appropriately targeting it to the malignant leukemia cells will remain an ongoing challenge. Because the immune response appears necessary in the antileukemia activity, how this approach fares in previously treated patients who have received chemotherapy and may have a depleted immune repertoire remains to be seen. These and other questions will need to be addressed, but the findings here provide an interesting way to refocus an antileukemia immune response.

Conflict-of-interest disclosure: T.M.K. has received research funding from Bristol-Myers Squibb. ■

REFERENCES

1. Shen W, Patnaik MM, Ruiz A, Russell SJ, Peng K-W. Immunovirotherapy with vesicular stomatitis virus and PD-L1 blockade enhances therapeutic

outcome in murine acute myeloid leukemia. *Blood*. 2016;127(11):1449-1458.

2. Thol F, Schlenk RF, Heuser M, Ganser A. How I treat refractory and early relapsed acute myeloid leukemia. *Blood*. 2015;126(3):319-327.

3. Russell SJ, Peng KW, Bell JC. Oncolytic virotherapy. *Nat Biotechnol*. 2012;30(7):658-670.

4. Andrbacka RH, Kaufman HL, Collichio F, et al. Talimogene laherparepvec improves durable response rate in patients with advanced melanoma. *J Clin Oncol*. 2015;33(25):2780-2788.

5. Naik S, Nace R, Barber GN, Russell SJ. Potent systemic therapy of multiple myeloma utilizing oncolytic vesicular stomatitis virus coding for interferon- β . *Cancer Gene Ther*. 2012;19(7):443-450.

6. Postow MA, Callahan MK, Wolchok JD. Immune checkpoint blockade in cancer therapy. *J Clin Oncol*. 2015;33(17):1974-1982.

7. Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. *Science*. 2015;348(6230):69-74.

DOI 10.1182/blood-2016-01-690891

© 2016 by The American Society of Hematology

● ● ● PLATELETS AND THROMBOPOIESIS

Comment on Huang et al, page 1459

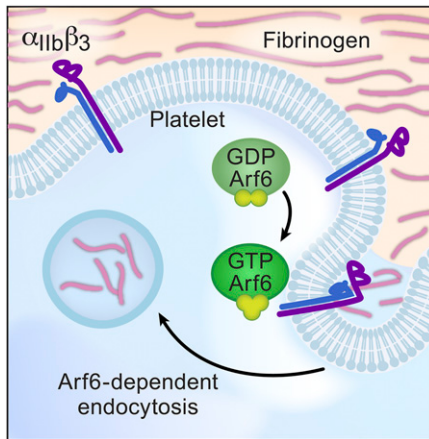
Arf6 arbitrates fibrinogen endocytosis

Matthew T. Rondina and Andrew S. Weyrich UNIVERSITY OF UTAH; THE GEORGE E. WAHLEN DEPARTMENT OF VETERANS AFFAIRS MEDICAL CENTER

In this issue of *Blood*, in a departure from studies of classic platelet function, Huang et al turn their attention to endocytosis and show that adenosine 5'-diphosphate-ribosylation factor 6 (Arf6) plays a key role in fibrinogen engulfment.¹ Although platelets are known to bind, absorb, and load their granules with plasma proteins, this report is one of the first to explore mechanisms that control endocytosis in this anucleate cell. Huang et al demonstrate that Arf6-dependent endocytosis is restricted to fibrinogen, implying that Arf6 also modulates trafficking of $\alpha_{IIb}\beta_3$ integrins in platelets. Consistent with this notion, deletion of Arf6 in platelets enhances spreading on fibrinogen and accelerates clot retraction (see figure). However, activation of surface $\alpha_{IIb}\beta_3$ is unaffected, and Arf6 deficiency does not alter thrombosis in vivo. These incongruous results point toward the complexity of anucleate platelets and the need for more detailed studies to understand intracellular trafficking, recycling, and endocytosis in platelets and their precursors.

Arf6 is a small GTPase that localizes to membranes and endosomal compartments.² In nucleated cells, Arf6 regulates endocytic membrane trafficking and thereby impacts cell motility, cell division, and lipid homeostasis. Arf6 has also been linked to actin remodeling, which may explain why genetic disruption and pharmacologic inhibition of Arf6 in mouse and human platelets, respectively, affects platelet spreading. Like

other small GTPases, Arf6 cycles between an active GTP-bound and inactive GDP-bound conformation. When cycling between these 2 states, Arf6 facilitates ligand internalization at cell surfaces, endosomal recycling, and fusion of endosomal membranes with plasma membranes. Previous studies from Whiteheart's group^{3,4} showed that Arf6 rapidly converts from a GTP- to GDP-bound state in activated human platelets and that



Model of Arf6-mediated fibrinogen endocytosis. Arf6 is a key intracellular trafficking molecule and, in platelets, enhances fibrinogen engulfment into intracellular vesicles. Arf6 also influences platelet spreading and clot retraction through mechanisms that remain incompletely understood. GDP, guanosine diphosphate; GTP, guanosine triphosphate.

a second wave of integrin-dependent outside-in signaling suppresses return of Arf6-GDP to Arf6-GTP. Kanamarlapudi et al⁵ also found that Arf6 regulates P2Y trafficking in platelets. The current work extends both of these observations and provides unequivocal evidence that Arf6 regulates the internalization of fibrinogen in platelets.

By modulating the internalization and trafficking of integrins, Arf6 strongly impacts cell migration, invasion, and shape in nucleated cells. In regard to cell shape, Arf6-deficient platelets spread more readily on fibrinogen compared with controls. The reasons for enhanced spreading are not completely understood and may be due to more rapid recycling of activated $\alpha_{IIb}\beta_3$ to and from the surface of Arf6-deficient platelets. However, $\alpha_{IIb}\beta_3$ surface levels and inside-out activation of the integrin are similar between Arf6 knockout and wild-type platelets, as is downstream activation of Rac1 (Ras-related C3 botulinum toxin substrate 1) and RhoA (Ras homolog gene family, member A). As hypothesized by the authors, future studies are needed to determine whether Arf6 actually controls $\alpha_{IIb}\beta_3$ trafficking in platelets by redirecting this critical integrin and its binding partner (fibrinogen) into “fast-track” endocytic pathways that reduce surface and/or internal residence time. Delineating reasons why Arf6-deficient platelets display accelerated clot retraction but have normal aggregation responses will also be informative.

The study by Huang et al is undoubtedly a prelude to future investigations focused

on platelet endocytosis. As one can imagine, alterations in the endocytotic process may greatly affect the repertoire, level, and/or activity of proteins in platelet granules. This may have important implications in human diseases where alterations in the regulation and release of platelet granule proteins may contribute to thrombotic events or bleeding. For example, higher levels of plasma fibrinogen have been associated with an increased risk of cardiovascular disease.⁶ In other acute infectious settings, such as sepsis, fibrinogen levels may be markedly increased or decreased (especially with disseminated intravascular coagulation), influencing both bleeding and thrombosis.⁷

Last, this report extends previous studies from the same group demonstrating that the Arf6 pathway is present and active in platelets, albeit the results are somewhat discordant between mouse and humans. In 2006, they showed that Arf6 inhibitors effectively blocked spreading of human platelets on collagen, whereas Arf6-deficient mouse platelets exhibit increased spreading on fibrinogen with no effective change when adherent to collagen. In human platelets, the ARF inhibitors also blocked many of the functional responses and signaling steps that were unaffected in the current study. There are many possible explanations for these differential responses, including off-target effects of the Arf6 inhibitors or differential expression of Arf6 signaling components between mouse and human platelets. Although responses may

differ partially between mice and humans, these studies point toward a central role for Arf6 in mediating platelet endocytosis. Perhaps more importantly, the current study highlights the power and limitations of comparing functional responses between mouse and human platelets. This is an important point for investigators to arbitrate as they seek to understand traditional and new functions of platelets and their importance in health and disease.

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

REFERENCES

- Huang Y, Joshi S, Xiang B, et al. Arf6 controls platelet spreading and clot retraction via integrin $\alpha_{IIb}\beta_3$ trafficking. *Blood*. 2016;127(11):1459-1467.
- Schweitzer JK, Sedgwick AE, D'Souza-Schorey C. ARF6-mediated endocytic recycling impacts cell movement, cell division and lipid homeostasis. *Semin Cell Dev Biol*. 2011;22(1):39-47.
- Choi W, Karim ZA, Whiteheart SW. Arf6 plays an early role in platelet activation by collagen and convulxin. *Blood*. 2006;107(8):3145-3152.
- Karim ZA, Choi W, Whiteheart SW. Primary platelet signaling cascades and integrin-mediated signaling control ADP-ribosylation factor (Arf) 6-GTP levels during platelet activation and aggregation. *J Biol Chem*. 2008;283(18):11995-12003.
- Kanamarlapudi V, Owens SE, Saha K, Pope RJ, Mundell SJ. ARF6-dependent regulation of P2Y receptor traffic and function in human platelets. *PLoS One*. 2012;7(8):e43532.
- Kaptoge S, Di Angelantonio E, Pennells L, et al; Emerging Risk Factors Collaboration. C-reactive protein, fibrinogen, and cardiovascular disease prediction. *N Engl J Med*. 2012;367(14):1310-1320.
- Levi M, Opal SM. Coagulation abnormalities in critically ill patients. *Crit Care*. 2006;10(4):222.

DOI 10.1182/blood-2016-01-692145

● ● ● RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Dulmovits et al, page 1481

Down the repressors! Up the fetal hemoglobin!

Christopher H. Lowrey GEISEL SCHOOL OF MEDICINE AT DARTMOUTH

One of the major goals of hemoglobinopathy research has been to devise improved pharmacologic strategies for the induction of fetal hemoglobin (HbF) in people with sickle cell disease and β -thalassemia. In this issue of *Blood*, Dulmovits and colleagues report that pomalidomide, a drug approved by the US Food and Drug Administration (FDA) for treatment of multiple myeloma, induces HbF production by decreasing levels of several key transcriptional repressors of fetal globin gene expression.¹ In addition, they show that pomalidomide induces HbF in differentiating erythroid cells from people with sickle cell disease and in myeloma patients.