

Dimerized P-selectin, which is expressed on the membrane of activated platelets and the endothelium, binds PSGL-1 on leukocytes to mediate adhesion and signaling, leading to inflammation. These pathways lead to proteolytic shedding of P-selectin, releasing sP-selectin, a monomer. Circulating monomeric sP-selectin binds PSGL-1 less well and does not induce leukocyte signaling, leading to inflammation.

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

REFERENCES

1. Panicker SR, Mehta-D'souza P, Zhang N, Klopocki AG, Shao B, McEver RP. Circulating soluble P-selectin must dimerize to promote inflammation and coagulation in mice. *Blood*. 2017;130(2):181-191.
2. Ushiyama S, Laue TM, Moore KL, Erickson HP, McEver RP. Structural and functional characterization of monomeric soluble P-selectin and comparison with membrane P-selectin. *J Biol Chem*. 1993;268(20):15229-15237.
3. Blann AD, Nadar SK, Lip GY. The adhesion molecule P-selectin and cardiovascular disease. *Eur Heart J*. 2003;24(24):2166-2179.
4. Sakamaki F, Kyotani S, Nagaya N, et al. Increased plasma P-selectin and decreased thrombomodulin in pulmonary arterial hypertension were improved by continuous prostacyclin therapy. *Circulation*. 2000;102(22):2720-2725.
5. Ramacciotti E, Blackburn S, Hawley AE, et al. Evaluation of soluble P-selectin as a marker for the diagnosis of deep venous thrombosis. *Clin Appl Thromb Hemost*. 2011;17(4):425-431.
6. Pabinger I, Ay C. Biomarkers and venous thromboembolism. *Arterioscler Thromb Vasc Biol*. 2009;29(3):332-336.
7. André P, Hartwell D, Hrachovinová I, Saffaripour S, Wagner DD. Pro-coagulant state resulting from high levels of soluble P-selectin in blood. *Proc Natl Acad Sci USA*. 2000;97(25):13835-13840.

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● ● ● PLATELETS AND THROMBOPOIESIS

Comment on Sim et al, page 192

Factor V marks platelet-primed megakaryocytes

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In this issue of *Blood*, Sim et al identify novel distinct human megakaryocyte populations primed for platelet release and establish a new approach to isolate such populations to generate either in vitro or in vivo functional human platelets.¹

Platelets are circulating anucleate cells that play a central role in hemostasis, thrombosis, and inflammation. Platelets are the final products of a complex maturation process that involves the differentiation of multipotent hematopoietic stem cells in mature megakaryocytes. This maturation process is

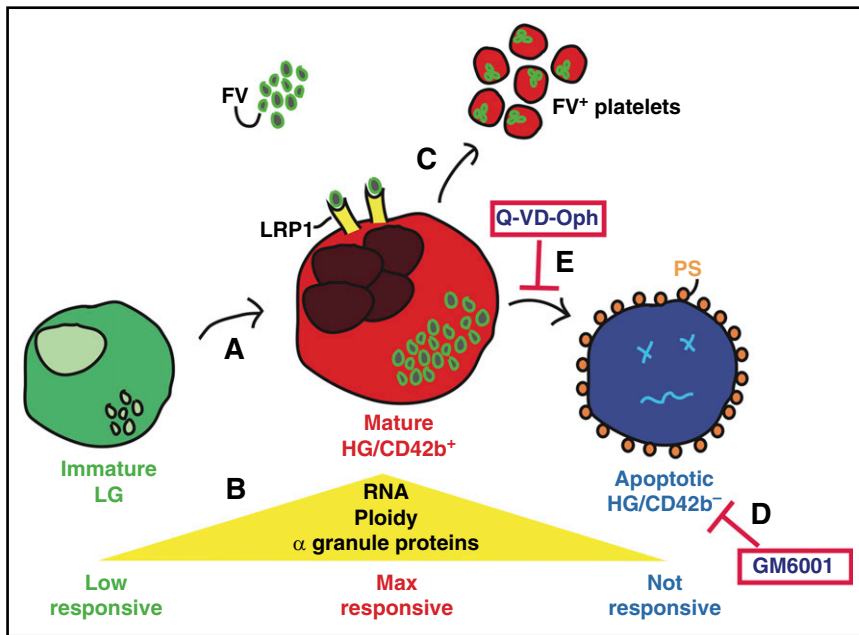
characterized by increase in cell size, ploidy, granular components, megakaryocyte-specific surface receptors, and the formation of an invaginated membrane system.²

Platelet transfusions are commonly used clinically to treat or prevent hemorrhage in people with either thrombocytopenia or

platelet function defects. Platelet transfusions are employed extensively and increasingly, although they have some associated risks, such as allergic reactions and bacterial and viral infections.³ At present, the only source of platelets for transfusion is donation from volunteers.

One of the main difficulties associated with platelet transfusions is the availability of adequate hospital platelet inventories. To overcome this limitation, efforts have been made to find alternative, efficient, non-donor-dependent systems to generate platelets, exploiting in vitro culture systems to differentiate megakaryocytes from hematological stem cells or induced pluripotent stem cells.

There are 2 principal strategies used to generate platelets ex vivo. One method relies on the collection of platelets from megakaryocytes or in a bioreactor system. This method



Schematic showing the newly identified differentiation stages in human megakaryopoiesis. (A) Immature low granular (LG) megakaryocytes (MKs) have low ploidy and few α granules. (B) These LG MKs mature in high granular (HG)/CD42b⁺ MKs characterized by increased ploidy, RNA, and α granule content. (C) Mature HG/CD42b⁺ MKs internalize factor V (FV) into their α granules and release FV-labeled platelets. The FV-labeled platelets are similar in size or larger than human donor platelets. (D) Pharmacological inhibition of CD42b shedding from MKs does not prevent apoptosis or improve MK functionality. (E) However, pharmacological inhibition of apoptosis increases the number of CD42b⁺/FV⁺ MKs that release functional platelets. The figure has been adapted from Figure 7 in the article by Sim et al that begins on page 192.

allows production of large amounts of platelets, but these show weak responses to agonist stimulation and a short half-life when infused in immunodeficient mice.⁴ In a second method, in vitro-differentiated megakaryocytes are infused and undergo platelet release in vivo based on the theory that megakaryocytes shed platelets in the lung.⁵ Two pools of human platelets are detected in the circulation upon infusion of in vitro-grown megakaryocytes. One pool appears a few hours postinfusion and shows comparable size, yield, half-life, and function to donor-derived platelets. A second pool consisting of platelet-like particles is detected immediately upon infusion and differs in size, yield, half-life, and function compared with donor-derived platelets.⁶ Although in vitro culture systems can be exploited to generate megakaryocytes able to release functional platelets in vivo, these systems also generate damaged cells releasing platelet-like

particles. Furthermore, 1 limitation of the in vitro-differentiation system is that only 10% to 30% of ex vivo-differentiated megakaryocytes show the ability to generate platelets; thus, the yield of in vivo releasable platelets remains too low to be used clinically to substitute for donor-derived platelets.⁷ One of the big challenges to overcome is the identification and definition of the maturation stage of the megakaryocyte that is ready to release platelets.

The interesting article by Sim et al identifies specific markers useful in selecting in vitro undamaged human megakaryocyte populations that, when infused in mice, are able to release fully functional platelets. These megakaryocyte populations are characterized by high granularity and CD42 expression. Furthermore, the authors show that these megakaryocyte populations preferentially internalize factor V; endocytosed factor V can

be used as a marker to selectively enrich the population of megakaryocytes primed for platelet release. Because culture conditions over time can damage cells inducing apoptosis and shedding of surface receptors, Sim et al show that pharmacological treatment of differentiating cultures with apoptosis inhibitors can be a useful tool to increase the percentage of fully mature megakaryocytes, thus resulting in increased platelet yield in vivo (see figure).

One caveat of this approach is that the use of an endocytosed protein, such as factor V, as a marker of differentiation is cumbersome to employ on an industrial scale for platelet production for transfusions. Future studies are required to identify new surface markers more easily applicable in isolating such megakaryocyte populations to efficiently generate either in vitro or in vivo functional platelets.

This study sheds new light on the mechanisms of human megakaryopoiesis and thrombopoiesis and provides a new method that could potentially improve platelet production for clinical use.

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REFERENCES

1. Sim X, Jarocho D, Hayes V, et al. Identifying and enriching platelet-producing human stem cell-derived megakaryocytes using factor V uptake. *Blood*. 2017; 130(2):192-204.
2. Deutsch VR, Tomer A. Megakaryocyte development and platelet production. *Br J Haematol*. 2006;134(5):453-466.
3. Wang B, Zheng J. Platelet generation in vivo and in vitro. *Springerplus*. 2016;5(1):787-797.
4. Hu Z, Yang YG. Full reconstitution of human platelets in humanized mice after macrophage depletion. *Blood*. 2012;120(8):1713-1716.
5. Weyrich AS, Zimmerman GA. Platelets in lung biology. *Annu Rev Physiol*. 2013;75:569-591.
6. Wang Y, Hayes V, Jarocho D, et al. Comparative analysis of human ex vivo-generated platelets vs megakaryocyte-generated platelets in mice: a cautionary tale. *Blood*. 2015;125(23):3627-3636.
7. Thon JN, Montalvo A, Patel-Hett S, et al. Cytoskeletal mechanics of proplatelet maturation and platelet release. *J Cell Biol*. 2010;191(4):861-874.

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