

# Megakaryocyte rupture for acute platelet needs

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Circulating platelets were thought to arise solely from the protrusion and fragmentation of megakaryocyte cytoplasm. Now, Nishimura et al. (2015. *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201410052>) show that platelet release from megakaryocytes can be induced by interleukin-1 $\alpha$  (IL-1 $\alpha$ ) via a new rupture mechanism, which yields higher platelet numbers, occurs independently of the key regulator of megakaryopoiesis thrombopoietin, and may occur during situations of acute platelet need.

Platelets, small anucleate cells that circulate in the blood stream, are essential for normal hemostasis but also play major roles in inflammation, immunity, wound healing, tumor metastasis, and the development and maintenance of lymph vessels (Leslie, 2010). Hence, reduced platelet numbers and/or impaired platelet function, as found in the context of numerous pathologies or upon pharmacological intervention, may have a negative impact on a large variety of physiological processes and under certain circumstances can become life threatening (Sachs and Nieswandt, 2007).

Platelets are continuously produced by fragmentation of the cytoplasm of their giant polyploid precursors in the bone marrow, the megakaryocytes. Recent studies using intravital two-photon microscopy of the bone marrow confirmed the formation of long protrusions of megakaryocytes termed proplatelets in vivo, which extend into bone marrow sinusoids where larger cytoplasmic fragments, so-called preplatelets, are shed and further mature within the circulation ultimately giving rise to platelets (Junt et al., 2007; Zhang et al., 2012; Bender et al., 2014). Calculations of platelet consumption and production in humans and mice suggested that platelet production via proplatelet formation is sufficient to maintain platelet count in normal physiology (Kaufman et al., 1965; Junt et al., 2007). However, this mechanism may not be efficient enough to produce sufficient platelet numbers under conditions of increased platelet consumption, such as inflammation/infection, immune thrombocytopenia, or traumatic blood loss. In this issue, Nishimura et al. have now identified an interleukin-1 $\alpha$  (IL-1 $\alpha$ )-induced rupture-type mechanism for platelet production that yields ~20-fold higher numbers of released platelet particles as compared with the classical mechanism of proplatelet formation during the same period of time (Fig. 1). This work provides for the first time an explanation of how megakaryocytes can maintain platelet mass equilibrium and quickly restore platelet numbers

under pathological conditions associated with increased platelet turnover. Even though the platelets released by megakaryocyte rupture were mildly enlarged in size, they were functionally indistinguishable from proplatelet-derived platelets.

The IL-1 $\alpha$  procytokine is expressed in virtually all non-hematopoietic cells, but also in platelets, and is involved in inflammatory processes, modulation of immune responses, and hematopoiesis. IL-1 $\alpha$  is released from damaged endothelial cells and activated platelets, where it triggers the recruitment of immune cells (Rider et al., 2013). As the work from Nishimura et al. (2015) indicates, this cytokine may also stimulate thrombopoiesis and rupture-type platelet release from megakaryocytes to compensate for platelet loss and restore platelet mass equilibrium. This could explain why supplementing cancer patients experiencing chemotherapy-induced thrombocytopenia with IL-1 $\alpha$  accelerated platelet count recovery (Gordon and Hoffman, 1992; Smith et al., 1993). These findings are of particular importance when considering the development of IL-1 $\alpha$  inhibitors to dampen inflammatory processes.

The technical optimization of the temporal and spatial resolution of two-photon intravital microscopy in combination with an elegant series of experiments using a broad variety of knockout mouse models allowed Nishimura et al. (2015) to observe and characterize this alternative mechanism of platelet formation. The mechanism strongly resembles key features of FasL-induced apoptosis, including activation of Caspase-3, disorganization of the cytoskeleton, and membrane blebbing. However, in stark contrast to typical FasL-induced apoptosis, rupture-type platelet formation is relatively quick (within an hour vs. >80 min) and results in the release of a large number of phosphatidylserine-negative particles. These particles carry an increased content of  $\beta$ 1-tubulin, which is reminiscent of disorganized  $\alpha$ - and  $\beta$ -tubulin expression, and has not been described for apoptotic cells (Fig. 1). The increased formation of membrane blebs was accompanied by a reduction in megakaryocyte membrane stiffness that could be reverted by caspase inhibitors. The activation of Caspase-3 represents a central step in rupture-type platelet release, as Caspase-3-deficient megakaryocytes could not use this alternative pathway for platelet production. Future studies will be required to determine how IL-1 $\alpha$  modulates megakaryocyte membrane stiffness and to identify the mechanisms that distinguish rupture-type platelet release from typical FasL-induced apoptosis.

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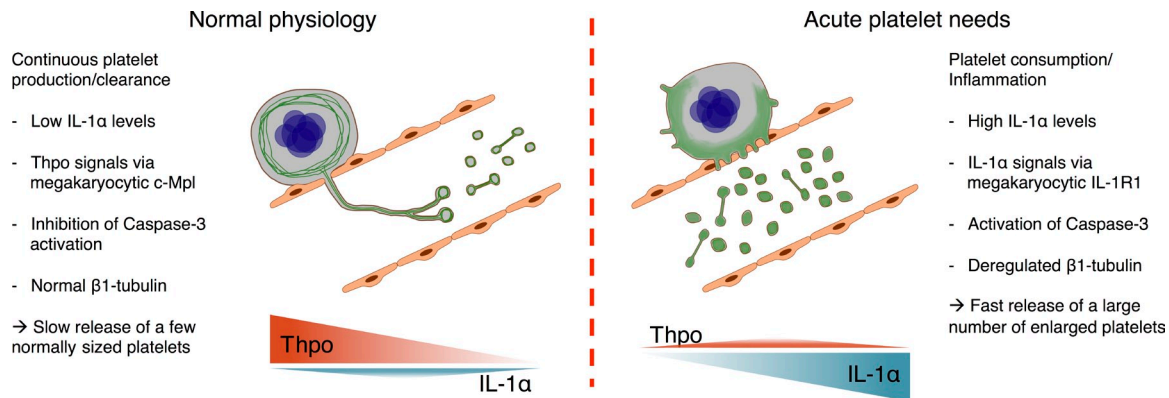


Figure 1. **Platelet production in normal physiology and upon acute platelet needs.** In normal physiology (left), platelets are continuously produced by megakaryocytes via the classical process of proplatelet formation. Under these conditions, thrombopoietin (Thpo) drives megakaryopoiesis by signaling through its receptor c-Mpl, but Thpo is dispensable for proplatelet formation, which is a cell-autonomous process and presumably regulated by the vascular niche. Inhibition of Caspase-3 and a well-organized orchestration of microtubule dynamics (green) are prerequisites for proper proplatelet formation and protrusion into bone marrow sinusoids, where preplatelets are released and further mature within the circulation. Proplatelet formation is a rather slow process with low yields of platelets per period of time but is sufficient to compensate for the continuous loss of aged platelets. Under conditions of increased platelet loss or consumption (right), e.g., as a result of excessive blood loss or in the setting of infection/inflammation, this mechanism might not be sufficient to ensure appropriate platelet supply. Under these conditions, interleukin-1 $\alpha$  (IL-1 $\alpha$ ) levels increase rapidly and trigger rupture-type platelet formation via its receptor IL-1R1 on megakaryocytes. IL-1 $\alpha$  signaling leads to a deregulated expression and organization of  $\beta$ 1-tubulin (green) as well as to the activation of Caspase-3, which in turn leads to a reduction of megakaryocyte membrane stiffness. Together, these processes lead to the formation of multiple membrane blebs that are predominantly released into bone marrow sinusoids to quickly replenish platelet numbers.

Nishimura et al. (2015) find that rupture-type platelet production occurs independently of thrombopoietin (Thpo), the key driver of thrombopoiesis, as rupture-type platelet production constituted the major source of circulating platelets in Thpo-deficient mice. This finding is in line with a study by Ng et al. (2014) showing that megakaryocyte-specific Thpo receptor (c-Mpl)-deficient mice presented a marked thrombocytosis despite the lack of Thpo stimulation during terminal thrombopoiesis. Unfortunately, IL-1 $\alpha$  levels or the presence of rupture-type platelet biogenesis have not yet been assessed in c-Mpl-deficient mice or in patients suffering from congenital amegakaryocytic thrombocytopenia. In addition, it would be of particular interest to assess the contribution of IL-1 $\alpha$ -induced rupture-type platelet release in human patients and also in mouse models reproducing inherited or idiopathic platelet disorders, such as the Wiskott–Aldrich syndrome, Gray-platelet syndrome, or immune thrombocytopenia.

In conclusion, the novel rupture-type platelet release mechanism identified by Nishimura et al. (2015) will help to answer the long-standing question of how circulating platelet numbers are quickly restored under conditions of increased platelet consumption or loss. Furthermore, this finding may lead to the development of new drugs to modulate platelet turnover in humans, but we also need to carefully reconsider previous experimental data on megakaryopoiesis/platelet production to include the possible contribution of IL-1 $\alpha$ -induced platelet release. Overall, the identification of a new mechanism of platelet production has advanced our understanding of platelet production and will certainly stimulate new research in the field of megakaryocyte biology.

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