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● ● ● PLATELETS & THROMBOPOIESIS

Comment on Debrincat et al, page 5850

Death regulates platelet birth and life

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In this issue of *Blood*, Debrincat et al show that Mcl-1 and Bcl-xL coordinately regulate megakaryocyte survival and ultimately affect platelet life-span.[1]

In recent years it has become apparent that platelet survival depends on the interplay between proteins of the Bcl-2 family, which are critical regulators of the "intrinsic" apoptosis pathway. The Bcl-2 family contains both prosurvival and proapoptotic members, and the balance between these competing systems regulates the apoptotic switch. The prosurvival family consists of Bcl-2, Bcl-x_L, Mcl-1, A1, and Bcl-w. Prosurvival proteins maintain cellular viability by restraining proapoptotic Bak and Bax, which represent the effector arm of the intrinsic pathway. Once activated, Bak and Bax damage mitochondria, triggering a cascade of events that ultimately leads to activation of caspase-9, the so-called "initiator" caspase that triggers the apoptotic cascade.[2]

Bcl-x_L, a member of the Bcl-2 family of prosurvival proteins, is clearly essential for platelet survival, both in vitro and in vivo (see figure). Its role is to control the activity of Bak, and to a lesser extent Bax.[3] Mice lacking Bcl-x_L exhibit a shortened platelet life span of only 24 hours, compared with 5 days in their wild-type counterparts[3] More recently, mice specifically lacking Bcl-x_L in the megakaryocyte lineage have been generated.[4] These animals had platelet counts reduced to less than 5% of those of wild-type mice, due in part to a platelet life span reduced to only 5 hours. The study has also shown that mature megakaryocytes depend on the function of Bcl-x_L to efficiently produce platelets, although Bcl-x_L loss alone does not impair the growth or maturation of megakaryocytes, indicating that apoptotic factors may be redundant.[4]

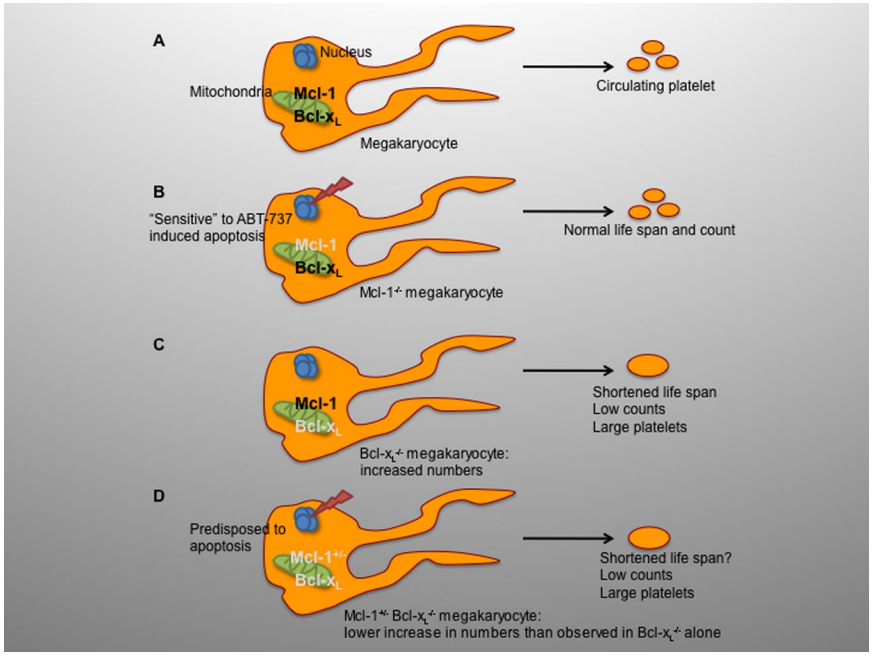
Debrincat et al add Mcl-1 to the repertoire of prosurvival Bcl-2 family members affecting platelet formation and survival. They propose that the combination of both Bcl-x_L and Mcl-1 is essential for the viability of the megakaryocyte lineage. Using an elegant system in which Mcl-1, alone or in combination with Bcl-XL, is specifically deleted in mouse megakaryocytes, they convincingly demonstrate that Mcl-1 is dispensable for normal megakaryocytopoiesis and platelet life span, even when stressed to produce new platelets after platelet ablation.[1] However, the role of Mcl-1 in platelet life span remains unclear, as circulating platelets do not contain Mcl-1. It is likely that Mcl-1 affects the sensitivity of megakaryoctes to undergo apoptosis, as the BH3 mimetic compound ABT-737, which binds to and inhibits the prosurvival proteins, Bcl-2, Bcl-x_L and Bcl-w, was toxic to megakaryocytes when applied in mice deficient in Mcl-1.

Further, mice with megakaryocyte-specific deletion of Bcl-x_L and Mcl-1 (Bcl-$x^{Pf4\Delta/Pf4\Delta}$ Mcl-$1^{Pf4\Delta/Pf4\Delta}$ mice) die in preweaning stages and Bcl-$x^{Pf4\Delta/Pf4\Delta}$ Mcl-$1^{+/Pf4\Delta}$ survivors have low platelet counts (< 5% of wild-type levels) with increased size and fewer megakaryocyte numbers than those of mice with the single Bcl-xL deletion. The low megakaryocyte numbers may result from developing megakaryocytes being prone to apoptosis. Interestingly, megakaryocyte-specific deletion of Bcl-x_L and Mcl-1 produces hemorrhage and lethality. Specifically, Bcl-$xL^{Pf4\Delta/Pf4\Delta}$ Mcl-$1^{Pf4\Delta/Pf4\Delta}$ embryos possess aberrant connections between the blood and lymphatic vascular networks, resulting in blood-filled lymphatic vessels. They also exhibit focal hemorrhages, consistent with a failure of hemostasis. However, the embryonic liver morphology appears normal, with no apparent signs of bleeding. It is intriguing that other organs but not the liver have impaired vessel-lymphatic networks. Are platelets not necessary for proper establishment of the liver morphology? What other signals keep the liver vessel and lymphatic systems intact? It would be of particular interest to investigate whether the double Bcl-$xL^{Pf4\Delta/Pf4\Delta}$ Mcl-$1^{Pf4\Delta/Pf4\Delta}$ deficient embryos are able to establish an early bone marrow function.

The work by Debrincat et al opens new investigative avenues and raises interesting questions. For example, why are mature megakaryocytes in particular dependent on Bcl-x_L and Mcl-1? Why do mature platelets lack Mcl-1, but express Bcl-x_L? How does the megakaryocyte "sort and package" its apoptotic machinery while producing platelets? Why does only a certain subset of megakaryocytes have an increased sensitivity toward apoptotic events?

The same group has previously shown that Bak-deficient mice exhibit an almost doubling of platelet life span in vivo.[3] The extended life span of $Bak^{-/-}$ $Bax^{-/-}$ platelets in vivo raises interesting questions relevant to platelet circulation. Why do $Bak^{-/-}$ $Bax^{-/-}$ platelets not circulate beyond 10 days? By what means are $Bak^{-/-}$ $Bax^{-/-}$ platelets removed from the circulation? The observations indicate that other clearance mechanisms regulate platelet life span.

Glycan modifications on platelet surface proteins are becoming increasingly recognized



Schematic drawing of pro- and antisurvival proteins in thrombopoiesis. (A) In mature megakaryocytes platelet production is coordinately regulated by the balanced expression of the prosurvival members Bcl-x_L and Mcl-1. (B) Loss of Mcl-1 does not affect platelet production and platelet lifetime. However, when challenged by the proapoptotic ABT-737 substance, Mcl-1 deficient megakaryocytes are more "sensitive" to cell death. (C) Megakaryocyte-specific loss of Bcl-x_L leads to severely reduced platelet life span, low platelet counts, and increased platelet volume. In addition, mice with megakaryocyte-specific loss of Bcl-x_L have higher megakaryocyte numbers in the bone marrow as a compensatory mechanism to boost platelet production. (D) Megakaryocyte-specific loss of Bcl-x_L and 1 allele of Mcl-1 results in low platelet counts and enlarged platelets, similar to the phenotype observed in Bcl-$x_L^{Pf4\Delta/Pf4\Delta}$ mice. However, Bcl-$x_L^{Pf4\Delta/Pf4\Delta}$ Mcl-$1^{+/Pf4\Delta}$ megakaryocytes are prone to premature cell death leading to megakaryocyte numbers lower than those found in Bcl-$x_L^{Pf4\Delta/Pf4\Delta}$ mice. Megakaryocyte-specific loss of Bcl-x_L and Mcl-1 leads to hemorrhage and mortality (not shown).

in mediating platelet clearance.[5] Loss of sialic acid (desialylation) exposes the next underlying sugar, galactose, and leads to removal of platelets. Recently, the hepatic asialoglycoprotein receptor 1/2 (Ashwell-Morell receptor) has been shown to remove platelets that are desialylated as a result of sepsis or long-term refrigeration.[6,7] Mice lacking the ST3Gal-IV sialyltransferase gene are thrombocytopenic due to increased clearance by Ashwell-Morell receptors of platelets with elevated terminal galactose residues.[6,8] Interestingly, mice lacking Ashwell-Morell receptors have elevated platelet counts,[9] and transfused wild-type platelets circulate longer in mice deficient for the Ashwell-Morell receptor.[7] The spleen does not appear to regulate platelet life span in mice.[4] Moreover, the primary clearance site for platelets in dogs after administration of ABT-737 is the liver.[10] Together, these data indicate that the liver clears aged/senescent platelets. Thus, platelets lacking proapoptotic proteins, such as $Bak^{-/-}$ $Bax^{-/-}$ platelets, may exhibit an altered glycosylation, which may be recognized by hepatic lectins leading to platelet clearance.

In conclusion, the study by Debrincat et al further elucidates the role of apoptotic proteins in platelet production and survival. They show that the combination of Bcl-x_L and Mcl-1 is essential for the viability of the megakaryocyte lineage, as deletion of both Bcl-x_L and Mcl-1 severely impairs megakaryocytopoiesis and leads to ectopic bleeding. However, deletion of Mcl-1 alone does not affect platelet life span or megakaryocyte function. Further investigations will continue to shed light on parameters dictating platelet birth and life.

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

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● ● ● TRANSPLANTATION

Comment on Markey et al, page 5918

GVHD-associated immunodeficiency: soil or seed?

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In this issue of *Blood*, Markey and colleagues demonstrate that immunodeficiency associated with graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (SCT) is caused by functional impairment of dendritic cells.[1]

Graft-versus-host disease and infection are the major complications of allogeneic SCT, and their close relationship has been recognized. One of the primary characteristics of GVHD is a profound and long-lasting immunodeficiency that allows development of severe opportunistic infections, while the role of systemic immunosuppressive drugs used for prevention and treatment of GVHD is well appreciated. It has been assumed that GVHD-mediated immunodeficiency is primarily attributed to defects within the T-cell