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

## REFERENCES


1. Hegde S, Kaushal N, Ravindra KC, et al. delta12-prostaglandin J3, an omega-3 fatty acid-derived metabolite, selectively ablates leukemia stem cells in mice. *Blood*. 2011; 118(26):6909-6919.
2. Mozaffarian D, Lemaitre RN, King IB, et al. Circulating long-chain omega-3 fatty acids and incidence of congestive heart failure in older adults: the cardiovascular health study: a cohort study. *Ann Intern Med*. 2011;155(3): 160-170.
3. Roynette CE, Calder PC, Dupertuis YM, Pichard C. n-3 polyunsaturated fatty acids and colon cancer prevention. *Clin Nutr*. 2004;23(2):139-151.
4. Hassane DC, Guzman ML, Corbett C, et al. Discovery of agents that eradicate leukemia stem cells using an in silico screen of public gene expression data. *Blood*. 2008;111(12): 5654-5662.
5. Hu Y, Swerdlow S, Duffy TM, Weinmann R, Lee FY, Li S. Targeting multiple kinase pathways in leukemic progenitors and stem cells is essential for improved treatment of Ph+ leukemia in mice. *Proc Natl Acad Sci U S A*. 2006; 103(45):16870-16875.
6. Persons DA, Paulson RF, Loyd MR, et al. Fv2 encodes a truncated form of the Stk receptor tyrosine kinase. *Nat Genet*. 1999;23(2):159-165.
7. Moreau-Gachelin F, Tavitian A, Tambourin P. Spi-1 is a putative oncogene in virally induced murine erythroleukemias. *Nature*. 1988;331(6153):277-280.


● ● ● PHAGOCYTES & GRANULOCYTES

Comment on Lilla et al, page 6930

# Deleting Mcl-1 in mast cells: getting 2 birds with 1 stone

**Booki Min** CLEVELAND CLINIC FOUNDATION

In this issue of *Blood*, Lilla and colleagues report that Mcl-1 (myeloid cell leukemia sequence 1), an intracellular antiapoptotic factor in a multiple hematopoietic lineage cells, plays a crucial role in regulating survival of both mast cells and basophils.[1]

Mcl-1 belongs to the bcl-2 family proteins and has been considered a master regulator of cell survival. Earlier studies that used Mcl-1-floxed mice that express Cre recombinase under different cell type–specific promoters demonstrated that Mcl-1 plays an essential role in maintaining survival of multiple cell types including lymphocytes, neutrophils, hepatocytes, and neurons.[2-4] As both mast cells and basophils are also known to express Mcl-1, the authors directly investigated whether Mcl-1 exerts antiapoptotic functions in these cells by selectively deleting Mcl-1 by expressing Cre recombinase under the promoter of carboxypeptidase A3 (CPA3; referred to as *Cpa3-Cre;Mcl-1$^{fl/fl}$* mice hereafter), a gene highly expressed in mast cells and basophils. First, they confirmed CPA3-induced Cre recombinase expression in various cell types. As expected, high levels of Cre expression was observed in peritoneal mast cells. Conversely, the level of CPA3-induced Cre expression in basophils was substantially lower than that of mast cells. Moreover, the level of Cre expression in other granulocytes such as eosinophils and neutrophils was not different from that in basophils. When *Cpa3-Cre; Mcl-1$^{fl/fl}$* mice were analyzed, mast cell numbers were dramatically reduced in all tested tissues. The authors also found that the numbers of basophils in these mice were substantially diminished, while other granulocytes including eosinophils and neutrophils, despite the similar CPA3-induced Cre expression, were not affected by the lack of Mcl-1. Therefore, a selective defect in the survival of both mast cells and basophils is achieved in the *Cpa3-Cre;Mcl-1$^{fl/fl}$* mice.

Using the *Cpa3-Cre;Mcl-1$^{fl/fl}$* mice, the authors then tested if Mcl-1 deficiency in mast cells and basophils results in impaired biologic function of these cells in vivo. Consistent with reduced numbers, all tested inflammatory responses, mast cell–dependent passive cutaneous anaphylaxis, basophil-dependent chronic allergic inflammation, and IgE-dependent passive systemic anaphylaxis were severely impaired in *Cpa3-Cre;Mcl-1$^{fl/fl}$* mice, demonstrating that Mcl-1 is indeed indispensable for the development, survival, and biologic functions of both mast cells and basophils.

Particularly interesting from the current study is that the survival of mast cells and basophils in *Cpa3-Cre;Mcl-1$^{fl/fl}$* mice appears to be differentially regulated; for example, survival defect of mast cells is much greater than that of basophils. Although this could be attributed to the different levels of Cre expression in these cells, the finding that Mcl-1 deficiency fails to affect the homeostasis of other granulocytes that express similar CPA3-induced Cre strongly suggests that an additional survival factor might be involved. Measuring expression of Mcl-1 as well as other antiapoptotic molecules in different cell types of *Cpa3-Cre;Mcl-1$^{fl/fl}$* mice and whether the level of expression changes during immune responses will thus be of great importance. Likewise, *LysM-Cre;Mcl-1$^{fl/fl}$* mice in which Mcl-1 is selectively deleted in myeloid precursors result in severe neutropenia, although macrophage development normally occurs.[2]

Elucidating a mechanism(s) underlying cell type–specific Mci-1–dependent (and independent) survival remains to be an area of future investigation. Specifically, a factor that induces Mcl-1 needs to be identified as Mcl-1 may be induced by different mechanism depending on the cell types. For example, IL-3 up-regulates Mcl-1 expression in basophils,[5] although the lack of IL-3 has no obvious defect in maintaining basal level production of mast cells and basophils,[6] strongly suggesting that Mcl-1–dependent survival of mast cells and basophils reported in the current study may be IL-3–independent. Alternatively, IL-5 and stem cell factor may be involved in up-regulating Mcl-1 as demonstrated in erythroleukemic cell line.[7] Mcl-1 expression may also be modulated depending on the maturation status.[8] In support of this possibility, molecular pathways leading to Mcl-1 expression were reported to be diverse.[5,7] Equally important is how Mcl-1 exerts antiapoptotic functions. Mcl-1 deficiency-induced apoptosis can be reversed by codeletion of proapoptotic molecules such as BIM, BAX, BAK, and PUMA,[2] suggesting that a balance between antiapoptotic and proapoptotic pathways is needed. Mcl-1 may regulate expression of apoptosis mediators. Mcl-1 was also shown to be a direct target of caspases.[9] In this context, investigating mechanisms underlying Mcl-1–mediated cell survival in different cell types will be important.

In sum, mast cells and basophils share similarity in phenotypes as well as in developmental pathways from which they arise, although recent studies have identified some nonredundant roles of each subset in immunity. Importantly, animal models developed thus far showed no common defects in either

subset. Mast cell–deficient $Kit^{W/Wv}$ or $Kit^{W\text{-}sh/W\text{-}sh}$ mice carry mutations of the c-Kit gene, which obviously do not affect basophil biology. On the other hand, basophil-deficient Mcpt8-DTR or basoph8 mice carry a transgenic receptor that mediates toxin-mediated cell death and mast cells in these mice remain intact. In mice deficient in IL-3, both mast cell and basophil generation are severely impaired only after parasite infection.[6] Therefore, *Cpa3-Cre; Mcl-1$^{fl/fl}$* mice will prove a useful animal model from which both redundant and nonredundant functions of mast cells and basophils in vivo can be unveiled.

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

## REFERENCES


1. Lilla JN, Chen CC, Mukai K, et al. Reduced mast cell and basophil numbers and function in Cpa3-Cre; Mcl-1fl/fl mice. *Blood*. 2011;118(26):6930-6938.

2. Steimer DA, Boyd K, Takeuchi O, Fisher JK, Zambetti GP, Opferman JT. Selective roles for antiapoptotic MCL-1 during granulocyte development and macrophage effector function. *Blood*. 2009;113(12):2805-2815.

3. Opferman JT, Iwasaki H, Ong CC, et al. Obligate role of anti-apoptotic MCL-1 in the survival of hematopoietic stem cells. *Science*. 2005;307(5712):1101-1104.

4. Opferman JT, Letai A, Beard C, Sorcinelli MD, Ong CC, Korsmeyer SJ. Development and maintenance of B and T lymphocytes requires antiapoptotic MCL-1. *Nature*. 2003;426(6967):671-676.

5. Didichenko SA, Spiegl N, Brunner T, Dahinden CA. IL-3 induces a Pim1-dependent antiapoptotic pathway in primary human basophils. *Blood*. 2008;112(10):3949-3958.

6. Lantz CS, Boesiger J, Song CH, et al. Role for interleukin-3 in mast-cell and basophil development and in immunity to parasites. *Nature*. 1998;392(6671):90-93.

7. Huang HM, Huang CJ, Yen JJ. Mcl-1 is a common target of stem cell factor and interleukin-5 for apoptosis prevention activity via MEK/MAPK and PI-3K/Akt pathways. *Blood*. 2000;96(5):1764-1771.

8. Druilhe A, Arock M, Le Goff L, Pretolani M. Human eosinophils express bcl-2 family proteins: modulation of Mcl-1 expression by IFN-gamma. *Am J Respir Cell Mol Biol*. 1998;18(3):315-322.

9. Wardle DJ, Burgon J, Sabroe I, Bingle CD, Whyte MK, Renshaw SA. Effective caspase inhibition blocks neutrophil apoptosis and reveals Mcl-1 as both a regulator and a target of neutrophil caspase activation. *PLoS One*. 2011;6(1):e15768.


● ● ● THROMBOSIS & HEMOSTASIS

Comment on Choi et al, page 6963

# A cofactor for factor XI activation

**David Gailani** VANDERBILT UNIVERSITY

During formation of a blood clot, the key enzyme thrombin is formed through the coordinated activities of a group of plasma proteases (factors VIIa, IXa, Xa, and XIa). Central to this process are protein "cofactors" (tissue factor, and factors Va and VIIIa) that facilitate the activation and/or activity of the plasma proteases on membranes of platelets and tissues. In this issue of *Blood*, Choi and colleagues present intriguing results showing that a nonprotein, inorganic polyphosphate (PolyP), can enhance thrombin generation by serving as a cofactor for formation of factor XIa.[1]

Factor XI, the precursor of factor XIa, differs structurally in several important respects from the vitamin K–dependent (VKD) coagulation protease zymogens (the thrombin precursor prothrombin and factors VII, IX, and X).[2] For example, factor XI lacks the Gla-domain through which the VKD proteins bind to phospholipid membranes. In addition, factor XIa does not appear to require a cofactor to mediate its primary hemostatic function, activation of factor IX. These properties reflect the different natural histories of factor XIa and the VKD proteases.

The VKD proteases and their cofactors form the core of an ancient hemostatic mechanism that is common to all vertebrate organisms. Factor XI, in contrast, is a relative newcomer, appearing during mammalian evolution as the result of a duplication of the gene for plasma prekallikrein.[3] The protease zymogens prekallikrein and factor XII, along with high molecular weight kininogen, comprise the plasma kallikrein-kinin system (KKS). The KKS participates in a number of homeostatic and host-defense functions, including the innate immune response to invading microorganisms.[4,5] KKS components assemble and are activated on the surface of microorganisms, generating antimicrobial peptides and contributing to complement activation.[5] The capacity of the KKS to bind to surfaces is also important for initiating blood coagulation in vitro in the activated partial thromboplastin time assay (aPTT) used in clinical practice. In the aPTT, anionic substances such as purified earths trigger reciprocal activation of factor XII and prekallikrein in a process called contact activation. Activated factor XII (factor XIIa) then propagates clotting by activating factor XI. Given its close ties to the KKS, then, it is not surprising that factor XI activation by factor XIIa is enhanced in vitro by a variety of polyanions, including the bacterial product dextran sulfate and glycosaminoglycans such as heparin. The importance of factor XIIa-mediated factor XI activation to hemostasis has been questioned, justifiably, because of the absence of a bleeding disorder in persons lacking factor XII. Other proteases including various forms of thrombin activate factor XI and may be more physiologically relevant activators.[2] However, regardless of the activating protease, factor XI activation proceeds slowly in the absence of a polyanion, strongly suggesting that a cofactor (perhaps with features of a polyanion) is required to promote the reaction.

Choi et al have now convincingly demonstrated that PolyP secreted from activated platelets is a potent enhancer of factor XI activation by the α and β forms of thrombin.[1] Previously, PolyP has been shown to influence blood coagulation by (1) induction of factor XII activation (contact activation), (2) acceleration of factor V activation by factor Xa, and (3) enhancing fibrin fibril thickness.[1,6,7] PolyP is a linear polymer of inorganic phosphate groups linked by high-energy phosphoanhydride bonds. A minimum PolyP chain length is required to support factor XI activation by thrombin, suggesting a template mechanism in which factor XI and thrombin bind to the polymer in proximity to each other. PolyP also supports factor XI autoactivation, consistent with previous descriptions of the effects of polyanions such as dextran sulfate and heparin on factor XI in solution.

In 1972, Walsh observed that collagen-stimulated platelets possessed a procoagulant activity that required factor XI but not factor XII, and proposed that this activity could explain why factor XII deficiency is not associated with a bleeding disorder.[8] Subsequent work from several groups showed that activated platelets support factor XI activation by factor XII–dependent and –independent mechanisms. These findings are entirely consistent with the properties of PolyP presented by Choi et al, and with their observation that activated platelets and platelet releasates support factor XI activation in a manner that is blocked by a PolyP specific binding protein.[1] Taken as a whole, this work