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PHAGOCYTES, GRANULOCYTES, AND MYELOPOIESIS

Comment on Frey et al, page 1932

Rare disease + lots of sequencing = mechanism?

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In the age of DNA next-generation sequencing, we have come to take for granted the availability of a prodigious amount of data that seemingly sheds light upon disease. However, the reams of sequences that are often produced exceed our ability to understand the biological relevance and the disparate disease states associated with those data. Researchers often make descriptive observations that incorporate hematologic data, but we are sometimes left wanting for the mechanisms involved. In this issue of *Blood*, Frey et al have elucidated the mechanism of the vacuolar protein sorting 45 homolog (VPS45)–Rab GTPase axis, which impacts the biology of embryogenesis and causes congenital neutropenia and myelofibrosis.[1]

Over the years, the Frey group has annotated numerous mutant genes associated with neutropenia and has followed the science wherever it leads. Herein, Frey et al have provided an elegant study that explains the epistasis seen among the partners of the complex, as observed by microscopy as well as biochemically in the protein level regulation of the composite partners and as suggested by previously reported observations on an isolated family pedigree.[2]

Furthermore, their study supports the notion of how defects on each end of the developmental axis can be manifested because of a genetic mutation. Paradoxically, neutropenia that results from defects in trafficking of the granulocyte colony-stimulating factor receptor is empirically associated with differentiation defects, yet *VPS45* and *Rab* gene mutations that result in severe defects with embryonic lethality in the mouse model have the likelihood for hypomorphic expression needed for reported clinical cases.

It is perhaps not so surprising that defects in VPS45-Rab result in a wide-ranging phenotype, given the observed defects in endocytic trafficking. Fascinatingly, it is not only the effect of differentiation that seems to give rise to neutrophil effects. Inherent defects in the neutrophils themselves with respect to phagosome processing suggest a wider-ranging defect in innate immunity.[3] Given the implications for endosomes across the immune system,[4] the phenotype of VPS45-Rab protein dysfunction in adaptive immunity needs to be investigated.

With the occurrence of severe neurocognitive defects seen in VPS45-Rab protein dysfunction with hematologic abnormalities, the effects of dependent endosomal function in neurologic pathways also need to be determined.[5] Again, the empirical basis for such work is evident, because axonal transport of critical proteins is necessary for early growth and development. Ironically, the most obvious phenotypic and cursory findings for VPS45-Rab protein dysfunction provide a window for understanding how pervasive this pathway is for nonhematopoietic and hematopoietic functions alike. Equally so is the unlikely insight such work opens to common diseases of the elderly such as Parkinson disease and Alzheimer disease.[6] VPS45-Rab complexes figure prominently in their pathogeneses, and endosomes are discretely noted microscopically in diseased neurons.

Although a defect in a basic process that leads to early embryonic death has been empirically observed, it is not clear why that defect can have such specificity in its effects in, for example, granulopoiesis. Why do patients with these defects not exhibit a low platelet count or anemia rather than simply neutropenia? If embryonic lethality occurs, then why is late differentiation also affected? The association with myelofibrosis has long been observed, yet a connection to leukemia is less than obvious. The more common genetic associations of MPN gene mutations, such as *JAK2*, *MPL*, and *CALR*, make biological sense with respect to signal transduction pathways,[7] but endosome trafficking does not fit neatly into this paradigm. Conversely, cell stress and forced stem cell proliferation are thought to play a role in the increased leukemia predisposition of even generic aplastic anemia,[8] not to mention the inherited bone marrow failure syndromes, such as Fanconi anemia, dyskeratosis congenita, and Diamond-Blackfan anemia.[9] Thus, convergence on the health of

the stem cell may be the true common denominator, no matter what the underlying cause is.

The lessons learned from a rare genetic disorder provide an important link, but there is still an incomplete picture of the pathway for understanding how stress, development, and specificity in hematopoiesis can lead to profound implications for health. Information gained from rare genetic diseases continues to provide unexpected insights and fulfills the age-old promise of the fruits of its investigation.

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RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Turnis et al, page 1945

MCL-1 and BCL-XL: blood brothers

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BCL-2, discovered as a hallmark of follicular lymphoma in 1985, was the first oncogene described to promote cell survival rather than proliferation. As the founding member of the large BCL-2 family that orchestrates intrinsic apoptosis and as the prime target of venetoclax, it remains a popular therapeutic agent in oncology. Yet, in hematology, as reported in this issue of *Blood*, its prosurvival sibling MCL-1 has stolen the crown now, as elegantly demonstrated by Turnis et al.[1]

Turnis et al show that MCL-1 is both sufficient and essential for the production of red blood cells by ensuring the survival of the first hematopoietic cells committed to the erythroid lineage (ie, early burst-forming unit-erythroid [BFU-E] and colony-forming unit-erythroid [CFU-E] cells and proerythroblasts; see figure). Using a mouse model in which both *MCL-1* alleles were specifically deleted in cells expressing the receptor for erythropoietin (EPO), the authors observed increased apoptosis in these early cell stages and, consequently, a partial block in erythroid maturation. MCL-1 expression in early erythropoiesis turned out to be vital for the organism because biallelic *MCL-1* deletion resulted in anemia and embryonic lethality around E13.5. In vitro differentiation experiments using bone marrow cells derived from adult mice phenocopied this effect: acute *MCL-1* deletion resulted in excessive apoptosis of proerythroblasts and early basophilic erythroblasts. In line with the major function of MCL-1 (ie, preventing the activation of the proapoptotic proteins BAX and BAK), concomitant deletion of BAX and BAK fully restored definitive erythropoiesis.

This finding comes as a surprise. Cells committed to the erythroid lineage were believed to require another BCL-2 homolog, BCL-XL, for survival. This assumption was based on results obtained from several independent laboratories: (1) mice lacking BCL-XL died prenatally, and their fetal livers contained a high number of apoptotic erythroid progenitors[2]; (2) in chimeric mice generated by injection of *Bcl-x*$^{-/-}$ embryonic stem cells into wild-type blastocytes, no mature red blood cells were derived from BCL-XL–deficient hematopoietic cells, whereas myeloid and lymphoid cells were not affected[3]; (3) conditional deletion of BCL-XL in erythroid progenitors using transgenic mice that express the Cre recombinase under the control of the mouse mammary tumor virus long terminal repeat (MMTV-LTR-CRE) caused hemolysis and a left-shifted and increased erythropoiesis. Because BCL-XL–deficient erythroblasts were not depleted, the authors concluded that BCL-XL is only required for the survival of reticulocytes and red blood cells[4]; and (4) complementary work pointed to a role of BCL-XL also in immature erythroid cells, especially when enhanced erythrocyte production is required (eg, after bleeding or in high altitude). Under these conditions, the prosurvival function of BCL-XL is antagonized by BIM, a proapoptotic BCL-2 protein of the BH3-only subgroup. High EPO levels as a consequence of low oxygen foster BCL-XL expression while repressing BIM, thereby increasing the viability of those precursors that proliferate most: proerythroblasts and basophilic erythroblasts. Once the pool of erythrocytes is replenished, EPO levels drop, and the BCL-XL/BIM ratio shifts toward BIM, breaching the apoptosis threshold in a fraction of cells.[5] Turnis et al found that EPO also induces MCL-1 expression, albeit only transiently. Thus, EPO-dependent MCL-1 upregulation may contribute to a rapid and efficient increase of red blood cell production during high demand by fostering the survival of early committed progenitors.

The broad implication of this work become evident when considering the expanding list of BH3 mimetics in cancer therapy. Although venetoclax has been approved by the US Food and Drug