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## A specific test for polycythemia vera?

Distinguishing between polycythemia vera (PV) and other polycythemic disorders can be very challenging. Although the diagnosis of PV may be straightforward if patients have the classic criteria as defined by the Polycythemia Vera Study Group, often patients present with an incomplete phenotype. Thus, a simple, readily available laboratory test to establish a diagnosis of PV would be highly desirable. In this issue, Klippel and colleagues (page 3569) report the utility of polycythemia rubra vera-1 (PRV-1) mRNA quantification in granulocytes for discrimination of PV from other polycythemic disorders. The authors also report that PRV-1 may be overexpressed in the neutrophils of some patients with thrombocytopenia and idiopathic myelofibrosis; it remains to be established if some patients presenting with a thrombocytopenia phenotype may in fact be early PV, as reported by Shih et al,<sup>1</sup> and if those with PRV-1-positive idiopathic myelofibrosis have the spent phase of PV. These investigators have previously reported increased PRV-1 mRNA in PV granulocytes but not in their progenitors. The function of PRV-1 in normal hematopoiesis is unclear, as the amount of this protein does not differ between normal and PV cells.

However, quantification of PRV-1 mRNA may be a useful and specific diagnostic marker of PV. In PV, the EEC assay (endogenous erythroid colonies grown in *in vitro* cultures without erythropoietin) is specific in experienced hands, but it is not easy to standardize; it is labor intensive and requires expensive reagents. Similarly, assays of the clonality of circulating myeloid cells can be performed only in females, and not every female is informative for the X-chromosome-inactivation-based clonality studies. Other newly described PV abnormalities, such as platelet c-Mpl expression,

are difficult to perform and available only in specialized laboratories. In contrast, the PRV-1 test is conceptually simple, has minimal inter- and intra-assay variation, and any competent laboratory equipped with the increasingly widely available real-time polymerase chain reaction (PCR) instrument should be able to perform it. However, occasional patients with congenital polycythemia<sup>2</sup> and familial thrombocytosis<sup>3</sup> were reported to have elevated PRV-1 levels, raising questions about its specificity. Thus, the usefulness and specificity of the PRV-1 test for PV diagnosis remain to be proved in prospective studies. However, due to its simplicity, the PRV-1 assay is attractive and it eventually may become the preferred PV test for all practicing hematologists.

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## Mystery of thiamine-responsive megaloblastic anemia unlocked

Megaloblastic changes in the bone marrow are morphologically quite distinctive, and the several causes of this condition, including specific nutrient deficiencies, metabolic errors, and certain drugs, are well described. The underlying biochemical mechanisms responsible for these conspicuous changes are, however, not very well defined and remain somewhat speculative and controversial. There are basically 2 current theories, both rooted in the concept that nucleotide synthesis is impaired and

that in folate and cobalamin (vitamin B<sub>12</sub>) deficiency, at least, there is a critical lack of thymidine formation from deoxyuridine (dU) leading to catastrophic collapse of orderly DNA synthesis and repair.

In one theory, lack of deoxythymidine triphosphate (dTTP) retards the elongation of newly formed replicating segments of DNA, resulting in fatally fractured pieces that trigger premature apoptosis.<sup>1</sup> In the other theory, build-up of deoxyuridine triphosphate (dUTP) resulting from failure of conversion of dU to thymidine causes an inordinate accumulation of dUTP, which can then substitute for missing dTTP in the machinery of DNA polymerase activity. Misincorporation of dUTP results in excision of the faulty segment followed by misrepair while the famine for dTTP persists, and thus ensues a futile cycle of excision-misrepair.<sup>2</sup> This, too, results in apoptosis, the final common pathway of ineffective hematopoiesis in megaloblastic anemia.<sup>3</sup>

Among the more obscure causes of megaloblastic anemia is the acronymic curiosity thiamine-responsive megaloblastic anemia (TRMA), subject of an article by Boros and colleagues (page 3556). The use of mass spectrometry in conjunction with stable isotope-labeling techniques has made it possible to unlock doors along previously inaccessible hallways of gene function analysis in the metabolomic maze. The door to TRMA was thus opened by Boros et al, who have pioneered the use of stable isotope-based dynamic metabolic profiling (SIDMAP) as a key to better understanding of changes in substrate flow as a basis for drug mechanisms and disease. Teaming up with the Boston group who first identified the loss of function mutation in the high-affinity, low-capacity thiamine transporter in TRMA, the authors have pinpointed the cause of disruption of nucleic acid synthesis that leads ultimately to premature apoptosis in this intriguing genetic disorder.

Through tracking the stable <sup>13</sup>C-labeled glucose in fibroblasts from patients with TRMA, these authors concluded that the underlying lesion in this condition resides in

the pentose cycle, specifically the transketolase enzyme, which requires thiamine pyrophosphate as a cofactor. Through a consideration of the several interconnected pathways of glycolysis, the tricarboxylic acid cycle, and ribose synthesis, the authors defined substrate flux in TRMA and normal wild-type fibroblasts grown in both low- and high-thiamine medium. They concluded that defective high-affinity thiamine transport in TRMA leads to a critical reduction in de novo generation of ribose with consequent cell-cycle arrest that triggers precocious apoptosis. Their results clearly demonstrate a selective and time-dependent loss of ribose synthesis in TRMA patients that is most marked under thiamine-deprived culture conditions and is partially restored by thiamine supplementation, explaining the clinical responsiveness of TRMA patients to high doses of thiamine.

Use of the powerful tools provided by SIDMAP and related techniques that use even more sensitive accelerator mass spectrometry with ultra-low-dose labeling techniques provides the promise to address, perhaps in vivo, similar unanswered questions involving the molecular basis for disease. Applying these methods to the study of the more common conditions that cause megaloblastic anemia, but that are still shrouded in mystery, could ultimately shed similar light on their mechanism.

—Ralph Green

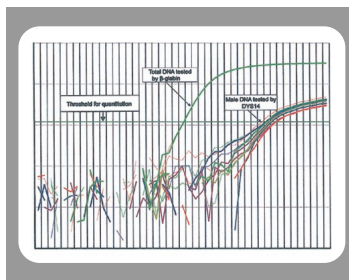
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## Fetal microchimerism—what our children leave behind

Fetal microchimerism (FMc) describes the persistence of low numbers of fetal cells in the

mother after a pregnancy. A number of recent studies suggests FMc may play a role in the etiology of some autoimmune diseases.<sup>1</sup> Remarkably, FMc has been demonstrated to persist for up to 38 years after pregnancy and has been found in multiple lymphocyte subsets and in early lymphoid precursors.<sup>2</sup> In a single patient, FMc was demonstrated in CD34<sup>+</sup> cells, suggesting that FMc may result from the engraftment of a long-term repopulating or stem cell.<sup>3</sup> In this issue of *Blood*, Adams and colleagues (page 3845) have taken the next step and evaluated female hematopoietic cell (HC)



transplant donors for the presence of Y chromosome-specific DNA. Strikingly, Y chromosome DNA could be detected in more than one third of peripheral blood stem cell (PBSC) collections and nearly one half of the CD34<sup>+</sup> selected cell fractions from these female donors. Since the use of multiparous female donors is associated with a higher propensity of graft-versus-host disease (GVHD),<sup>4</sup> the authors speculate that major histocompatibility complex (MHC)-mismatched fetal cells transferred during HC transplantation (HCT) could participate in the induction of GVHD. Unfortunately, a number of factors are lacking from this dataset to completely address such questions. For instance, parity information is not available for most donors, and the assay used detects only male DNA; thus, the actual incidence of FMc in hematopoietic stem cell fractions may be significantly higher than is estimated by this analysis. Likewise, it is not known whether female donors with demonstrable FMc induced a greater incidence of GVHD than other donors in this series.

Despite this, and like all good science, this work raises more questions than it answers. For instance, virtually nothing is known about the circumstances that allow

FMc to occur. Other important questions include what role do such cells play in the pathogenesis of either acute or chronic GVHD. If the hypothesis by Adams et al is correct, then it may be possible to detect the presence of transferred fetal cells in the host after HCT (possibly in GVHD target tissues). To date there have been no reports of chimerism analysis after HCT demonstrating a party other than the donor or recipient, but the studies by Adams et al may prompt such reports.

Not only have fetal cells been detected in the mother, but also maternal cells in the fetus.<sup>5</sup> Such microchimerism might not be all bad for patients requiring HCT, since it might aid, or even enhance, donor selection. This is because a potential sibling donor (with maternal microchimerism) may be tolerant of noninherited maternal antigens, allowing for less rigorous typing of maternal alleles. Similarly, mothers who have had multiple pregnancies (and hence FMc from more than one child) may be tolerant of paternal antigens. Such parents may be ideal donors for their children. In fact, Shimazaki et al have reported a technique of donor selection based on microchimerism analysis to perform haploidentical, 2 to 3 antigen mismatched, non-T-cell-depleted HCT.<sup>6</sup> In this series of 5 patients the incidence of severe (grades 3-4) GVHD was remarkably low (1 of 5 patients). Thus, it is hopeful that the findings by Adams et al may be useful in the understanding of FMc and how (or if) it relates to the pathogenesis of GVHD and whether such information may assist in the selection of HC transplant donors.

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