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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.

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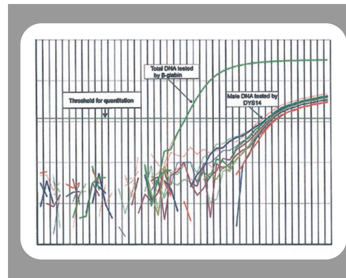
Fetal microchimerism—what our children leave behind

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.[1] 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.[2] In a single patient, FMc was demonstrated in CD34$^+$ cells, suggesting that FMc may result from the engraftment of a long-term repopulating or stem cell.[3] 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$^+$ 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),[4] 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 factions 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.[5] 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.[6] 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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Superactive analogs of factor VIIa: superglue for bleeding patients?

Recombinant factor VIIa was introduced in clinical medicine 20 years ago and has been shown to be a highly effective prohemostatic agent. Primarily, recombinant factor VIIa was used in patients with congenital or acquired hemophilia and inhibiting antibodies toward factor VIII or IX, for which it has been licensed in most countries. In recent years, the potential of recombinant factor VIIa to act as a prohemostatic agent in other categories of patients with severe coagulation defects, or in patients with a pre-existing normal coagulation system but who experience excessive or life-threatening bleeding, has been explored. Although this application of recombinant factor VIIa is still in its initial phase and most of the experience is based on uncontrolled series of observations, this agent is indeed a promising candidate for (adjunctive) treatment for serious bleeding in many types of patients.

In this issue of *Blood,* Tranholm and colleagues (page 3615) describe the properties of a series of analogs of factor VIIa, developed by genetic engineering using native factor VIIa as a template. These mutant compounds have a much higher enzymatic activity toward factor X, but similar binding affinity to tissue factor, compared with native factor VIIa. When tested in a tail-bleeding model in mice with acquired (antibody-induced) hemophilia A, the authors show that the factor VIIa analogs have a dose-dependent and potent ability to shorten the bleeding time and to reduce blood loss. The potency of the factor VIIa analogs was estimated to be 3- to 4-fold higher than that of native recombinant factor VIIa.

These interesting results can be helpful in understanding the mechanism of action of recombinant factor VIIa in bleeding patients. Recombinant factor VIIa is thought to act locally at the site of tissue injury and vascular wall disruption, by binding to exposed tissue factor (or replacing zymogen factor VII from tissue factor) and generating small amounts of thrombin, sufficient to activate platelets. The activated platelet surface can then form a template on which recombinant factor VIIa can bind and directly or indirectly mediate further activation of coagulation, resulting in the generation of much more thrombin. The relative contribution of factor VIIa binding to tissue factor or to platelets is unclear. The present study underlines the importance of the tissue factor–independent effect of factor VIIa and demonstrates that the tissue factor–binding properties of factor VIIa may be less important in further improving the prohemostatic effect.

It is tempting to speculate whether these superactive analogs of factor VIIa could result in more potent prohemostatic drugs. Although recombinant factor VIIa is effective in preventing or arresting bleeding in most patients with complex hemostatic disorders, there is a substantial 10% to 20% of cases with unsatisfactory hemostasis. The factor VIIa analogs might provide a more potent treatment option for these patients. Also, patients with excessive and life-threatening bleeding, for example in trauma or upon major surgery, could benefit from highly potent prohemostatic agents. Obviously, the superior efficacy of superactive factor VIIa analogs in these situations needs to be tested in clinical trials before any such claim can be substantiated. Also, the safety of these compounds may be a concern. In view of its potent prohemostatic effect, recombinant factor VIIa is surprisingly safe with an estimated incidence of thrombotic complications in 1% to 2% of patients, including complications that may not be related to the administration of the agent. In the current study by Tranholm et al, there was no sign of fibrin deposition after administration of the factor VIIa analogs at histologic analysis of the kidneys (although a more complete pathologic analysis, involving other organs, would have strengthened this observation). Nevertheless, a precise safety profile of analogs of recombinant factor VIIa will be required as well to assess their potential place in clinical medicine. Awaiting further evidence, superactive analogs of factor VIIa seem to be promising agents for prevention and treatment of excessive bleeding in the future.

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P2X1: definitely not an ADP receptor

The story of platelets and purine nucleotides is one of the longest and most exciting in the biology and pathology of hemostasis. It began in the 1960s with the discovery of the role of adenosine diphosphate (ADP) as an important platelet agonist; like a good novel this story follows large avenues, but also dead ends, and turnabouts. Among the large avenues was the use of cloning technology, production, and analysis of knock-out and/or transgenic mice, along with the careful clinical, biologic, and molecular genetic analysis of patients suffering from ADP-dependent platelet defects, which led to the identification of the 3 purine receptors $P2X_1$, $P2Y_1$, and $P2Y_{12}$ in platelets. The combination of all 3 receptors appears to correspond to the formerly termed P2T receptor. $P2Y_1$ and $P2Y_{12}$ are both G-protein–coupled receptors (GPCRs) specific for ADP. Acting in concert, $P2Y_1$ mediates Ca^{2+} mobilization through a $G\alpha q$ pathway, while $P2Y_{12}$ elicits a $G\alpha i$-dependent pathway, both acting concurrently to activate integrin $\alpha IIb\beta 3$ and initiate platelet aggregation.[1] One specific feature of $P2X_1$ is that it is not a GPCR, but a

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