whether GPIbα conversely affects FlmA distribution in megakaryocytes and platelets. Interestingly, overexpression of FlmA, like lack of FlmA expression, results in retention of GPIbα within the ER in transfected HEK293 cells. Conversely, excessive expression of enhanced GFP-fused GPIbα, without GPIbβ and GPIX, traps FlmA in the ER in HEK293 cells, and ES cell–derived megakaryocytes expressing excessive enhanced GFP-fused GPIbα produce platelets that are 2 to 3 times larger than those derived from control cells. Together, the data indicate that the right balance between the expression levels of FlmA and GPIbα is required for optimal trafficking to the plasma membrane and normal platelet size (see figure panel B).

Consistently, platelets isolated from transgenic mice expressing excessive levels of a chimeric protein composed of the transmembrane and cytoplasmic domains of GPIbα fused to the extracellular domain of the IL4 receptor (IL4R-GPIbα) are larger and contain more immature IL4R-GPIbα than control platelets, confirming in vivo that high expression levels of GPIbα, not only its reduced expression, results in the formation of enlarged platelets.

In conclusion, the study by Kanaji et al introduces (1) the role of FlmA and FlnB in ES cell maturation and differentiation, and (2) the concept of balanced expression levels of FlmA and GPIbα in megakaryocytes in the regulation of platelet size. Their paper advances our understanding of the role of these proteins in hematopoietic cells, but also points to exciting new directions for research. It would be particularly interesting to investigate whether balanced expression of FlmA and β1 and β3 integrins similarly regulates megakaryocyte function to produce “fully balanced” platelets.

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THROMBOSIS & HEMOSTASIS
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EPCR encryption induces cellular APC resistance

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In its relatively short history, novel insights on the endothelial protein C receptor (EPCR) have provided time-after-time fertile grounds for research on the interactions between coagulation factors and the vasculature. Yet again EPCR provides an opportunity to explore new frontiers, as López-Sagaseta and colleagues in this issue of Blood discover a novel lipid-based regulatory mechanism for EPCR that suggests its involvement in vascular disease may be more intricate than previously appreciated.1
EPCR was identified as an endothelial receptor for protein C, capable of enhancing the activation of protein C by the thrombin-thrombomodulin complex. Subsequently, direct cytoprotective effects of activated protein C (APC) on cells were found to require EPCR and a homologous EPCR-dependent cellular pathway for factor VII(a) has been suggested. These functions in physiologically and therapeutically relevant pathways, such as the cytoprotective protein C pathway and a novel factor VII(a) cellular pathway, earned EPCR its reputation as a cytoprotective receptor. Now López-Sagaseta and colleagues provide yet another dimension to EPCR as they describe a mechanism by which an endothelial-derived phospholipase can modify or “encrypt” EPCR to lose its ability to bind APC, thereby rendering EPCR unable to mediate APC’s direct cytoprotective activities on cells. These findings conceptualize a novel phenomenon of “cellular APC resistance” similar to the well-known “anti-coagulant APC resistance” associated with an increased risk for venous thrombosis. Although more work needs to be done to establish (patho)physiologic relevance of “cellular APC resistance,” López-Sagaseta et al provide intriguing hints as to its potential involvement in cardiovascular disease.

The EPCR crystal structure revealed the presence of a phospholipid buried in its hydrophobic groove that helps stabilize the interactive surface of EPCR for interactions with the GLA-domain of APC. The current paper demonstrates that not only phosphatidylcholine (PCh) but also PCh metabolites, such as lysoPCh and platelet-activating factor (PAF), can occupy EPCR’s hydrophobic groove, although resulting in an EPCR with reduced affinity for APC. This raises the immediate question of whether other lipids can incorporate in EPCR as well. Insights garnered from EPCR’s homology to CD1 lipid antigen-presenting molecules, especially CD1d, support the idea that they might. Various phospholipids and sphingolipids were found to occupy the hydrophobic groove of CD1d. Not only can CD1d incorporate different lipids, the type of lipid has been implicated to contribute to a biased cytokine response of natural killer T cells, as the lipid head group protruding from the hydrophobic binding groove participates in the CD1d interactive surface with the T cell receptor. Future studies will have to determine whether these observations for CD1d will also be true for EPCR.

As a branch of the large phospholipase family, phospholipases A2 (PLA2s) hydrolyze phospholipids at the sn-2 position producing free fatty acids and lysophospholipids. Most PLA2s are expressed intracellularly but some secreted PLA2s (sPLA2s) circulate in plasma and are well known for their proatherogenic properties and participation in inflammation via the generation of bioactive lipid mediators. Extending their observation that lysoPCh and PAF-loaded EPCR resulted in diminished APC binding, López-Sagaseta et al found that sPLA2 group V (sPLA2-V) can modify the EPCR lipid, as incubation of endothelial cells or purified EPCR with sPLA2-V resulted in diminished APC binding and inhibition of EPCR-dependent antiapoptotic effects of APC on cells. This suggests a model in which EPCR encryption by lipid-editing enzymes results in cellular APC resistance (see figure). This model may have important implications for thrombotic and inflammatory vascular disease because EPCR inactivation in vivo, either genetically or induced by blocking antibodies, aggravates and increases susceptibility to thrombotic and inflammatory disease.

What counter measures can cells employ to restore EPCR’s cytoprotective functions when inflammatory mediators up-regulate sPLA2s to induce cellular APC resistance? Perhaps EPCR shedding induced by inflammatory mediators should be viewed as a protective measure to rid cells of encrypted EPCR. Answers to this and many other questions remain unclear at present. What is clear is that the intriguing observations by López-Sagaseta and colleagues will stimulate innovative thinking on the regulation of EPCR function in vascular disease.

Obviously one should also be cautious in the interpretation of these results. Purified systems and in vitro cell culture do not represent a physiologic environment. Plasma
contains a broad spectrum of lipid-rich lipo-protein particles, abundant lipid carrier pro-
teins, and a host of other factors that all could potentially negate the reported effects on
EPCR. Thus, the implications of EPCR lipid editing, EPCR encryption, and cellular APC
resistance for vascular disease remain to be
determined. Notwithstanding, sPLA2s are
expressed abundantly in atherosclerotic le-
sions and a variety of inflammatory conditions.
In addition, the link between plasma levels of
sPLA2 and cardiovascular disease seems con-
sistent with a potential role of sPLA2s in in-
ducing EPCR lipid modifications and possible
collections thereof to cardiovascular dis-
ease. Should such links become more tangible
then evaluation of sPLA2 inhibitors as a
potential approach to combat thrombosis and
vascular diseases.

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A donor’s a person, no matter how small

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After 30+ years of considering BM donation from sibling children a safe and effective
standard based on 2 single-center studies,1,2 Styczynski and colleagues in this
edition of Blood provide us with a multicenter, prospective study of safety out-
comes of 453 pediatric BM and PBSC donors.3

The work is long overdue. In the midst of
our focus on improving outcomes of their
sicker siblings, we have too-long neglected
what should be an ongoing task of ensuring
that we (1) know in detail current common
and rare risks of these procedures and (2) work
to minimize or eliminate side-effects in these
healthy children as they go through the BM or
peripheral blood stem cell (PBSC) collection
process.

This study confirms our long-held belief
that BM and PBSC collection from minor
donors is generally safe, resulting in temporary
discomfort in a portion of donors (only 1 se-
vire adverse event [nonlife-threatening] was
reported in 453 donations). This distinguishes
BM and PBSC donation from riskier sibling
solid organ donation, counter to a recent
American Academy of Pediatrics policy
statement that calls for BM/PBSC and solid
organ donors to be approached in a similar
fashion.4 But the data presented in the study
points out an area where we have room for
improvement. It is clear that centers vary tre-
mendously in the way they practice, with con-
sequences for the donor. This gives us an op-
portunity to define best practices and raise the
safety bar even further.

The most obvious lesson we learn from this
study is that both being small and being much
smaller than your recipient puts donors at
higher risk for requiring a blood transfusion
and additional apheresis procedures, pain, and
cardiovascular complications after anesthesia
(see figure). Styczynski et al clearly point out
that removing > 20 cc/kg of marrow from donors markedly increases their likelihood of
needing an allogeneic packed red blood cell
(PRBC) transfusion (hazard ratio 4.8, P < .001).

Harvesting a maximum of 20 cc/kg from a
donor is already the standard of care in most
North American centers, and if this approach
is followed, transfusions of allo-PRBCs to do-
nors can largely be avoided. But the study
brings to the fore a dilemma often encountered
in pediatric BMT—what do you do when the
sibling donor is much smaller than the recipi-
ent? The highest-risk donors were < 4 years
old, and most of these donors were smaller
than their recipients. Harvesting a maximum of 20 cc/kg from a donor means that if their
recipient is twice their weight (average
2-year-old donating to a 7-year-old) one will
be giving 10 cc/kg to the recipient, an amount
considered reasonable only if red cell depletion
for ABO mismatch is not required. It is clear
that too few cells to a recipient leads to worse
outcomes, so when larger differences in weight
occur or when red cell depletion is needed,
harvesting more than 20 cc/kg may be needed.

What does one do for such a donor/recipient
weight discrepancy? Clearly, PBSC donation
has been shown by Styczynski and colleagues
and others5 to be safe in children and it allows
multiple collections to be performed until ade-
quate numbers of cells are collected; how-
ever, theoretical concerns regarding G-CSF
administration to children linger (in spite of
many reassuring articles6). Younger children
would need apheresis catheter placement with
its attendant risks, and the smallest (< 20 kg)
may be exposed to PRBCs to prime an aphere-
sis machine, defeating the purpose. In addi-
tion, current literature suggests that the risks
of chronic GVHD in children receiving PBSCs
may outweigh their benefit, although this has
not been studied versus the competing risk of
low cell dose.

Another approach to the problem would be
storing autologous units from children when
exceeding 20 cc/kg from a harvest is antici-
pated. This takes preparation, however, and

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