FinA and GPIbα regulate platelet size. (A) Increased surface expression of GPIbα is a marker of megakaryocyte maturation and differentiation, whereas FinA expression levels increase as early as in ES cells. Ablation of FinA expression in FlnA<sup>−/−</sup> PF4-Cre megakaryocytes results in the premature production of enlarged platelets with reduced GPIbα expression. Kanaji et al. show that FinA and FinB regulate ES cell maturation and differentiation. (B) Balanced expression levels of FinA and GPIbα in megakaryocytes regulate the production of normal-sized platelets. FinA-null and GPIbα-null platelets, as well as platelets over-expressing GPIbα, are enlarged. Conversely, FinA subcellular localization is altered in GPIbα-null platelets and in HEK293 cells overexpressing GPIbα.

whether GPIbα conversely affects FinA distribution in megakaryocytes and platelets. Interestingly, overexpression of FinA, like lack of FinA 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 FinA 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 FinA 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 FinA and FinB in ES cell maturation and differentiation, and (2) the concept of balanced expression levels of FinA 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 FinA and β1 and β3 integrins similarly regulates megakaryocyte function to produce “fully balanced” platelets.

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

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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.

THROMBOSIS & HEMOSTASIS

Comment on López-Sagaseta et al, page 2914

EPCR encryption induces cellular APC resistance
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 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 phosphatidyglycerol (PCh) but also PCh metabolites, such as lysoPCh and free fatty acids, can occupy the hydrophobic groove of EPCR, 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 best 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 PLA2s 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.
transplantation 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 lesions and a variety of inflammatory conditions. In addition, the link between plasma levels of sPLA₂ and cardiovascular disease seems consistent with a potential role of sPLA₂ in indu-
ing EPCR lipid modifications and possible contributions thereof to cardiovascular disease.⁸,¹⁰ Should such links become more tangible then evaluation of sPLA₂ inhibitors as a therapeutic strategy to boost the endogenous protein C anticoagulant and cytoprotective pathways might become worthwhile.¹⁰

As is often the case with new discoveries that push the frontiers of our knowledge, they raise more questions than they answer. Certainly, the tantalizing observations by López-Sagasta and colleagues raise many new questions, but most importantly, they stimulate the conceptualization of new basic research with the potential of translation into novel thera-
peutic approaches to combat thrombosis and vascular diseases.

Conflict-of-interest disclosure. The authors declare no competing financial interests.

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Comment on Styczynski et al, page 2935

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 outcomes of 453 pediatric BM and PBSC donors.³

The work is long overdue. In the midst of our focus on improving outcomes of our 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 severe 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 tremendously in the way they practice, with consequences for the donor. This gives us an opportunity 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).

Another approach to the problem would be storing autologous units from children when exceeding 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 donors 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 recipient? The highest-risk donors were 4-year-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 others⁴ to be safe in children and it allows multiple collections to be performed until adequate numbers of cells are collected; however, theoretical concerns regarding G-CSF administration to children linger (in spite of many reassuring articles). Younger children would need apheresis catheter placement with its attendant risks, and the smallest (< 20 kg) may be exposed to PRBCs to prime an apheresis machine, defeating the purpose. In addition, 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 anticipated. This takes preparation, however, and
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