tumor cells that ectopically express podoplanin.7

Here, the authors studied CLEC-2 and podoplanin interactions during lung embryonic development. It has previously been observed that global deletion of CLEC-2 or podoplanin is lethal,8,9 and the authors confirmed that these mice have defects in late embryonic lung development (primary septum formation). When CLEC-2 was completely deleted in platelets and megakaryocytes (using Cre-lox plus antibody-mediated deletion of residual CLEC-2), the fatal neonatal defects included lung developmental defects. Previous studies using mice with platelet-specific CLEC-2 deficiency have not reported lung developmental defects,9 but the authors found mild defects that were worse when residual platelet CLEC-2 was completely deleted or if platelets in these mice were depleted by 90%. CLEC-2 signals through the spleen tyrosine kinase to activate platelets, and the authors determined that TGF-β released from activated platelets drives the development of alveolar duct myofibroblasts that are critical to primary septum formation and elastogenesis during late embryogenesis. Curiously, lung developmental defects have not been described in thrombocytopenic mouse models, but it appears that platelet counts must be severely decreased and combined with other defects in platelet activation, such as the absence of CLEC-2 signaling, to produce the lung phenotype.

Why would platelets be important in lung development and regeneration? The strong phenotypes observed in this study may derive from the large vascular surface area where platelets are in continuous circulation and where large numbers of megakaryocytes embolize to produce platelets.10 Additionally, megakaryocytes may live in the lung interstitium, where they could theoretically interact with lymphatic channels to influence lung development. Future studies are needed to determine if the phenotypes observed in the lung are present in other tissues. Investigators in this field should also focus on the mediator(s) released from platelets that have biological influence on the developing lung. Platelet-derived TGF-β is unlikely to be the sole mediator involved because the TGF-β knockout mouse does not have apparent lung developmental defects. Platelets and megakaryocytes are rich sources of other mediators that could influence the tissue matrix during organogenesis and potentially also in disease states characterized by tissue fibrosis.

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

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PLATELETS AND THROMBOPOIESIS

Comment on Lepropre et al, page 1180

Platelet metabolism meets thrombosis
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Metabolic pathways intersect with many processes important in hematolgy, including thrombosis, innate and adaptive immunity, malignant transformation, and stem cell function. These pathways are increasingly recognized as potentially targetable for therapeutic intervention. In this issue of Blood, Lepropre et al identify the energy sensor AMP-activated protein kinase (AMPK) in platelets as a regulator of thrombosis.1

Platelet hyperreactivity is a key factor that promotes arterial thrombosis, and our laboratory and others have described how exogenous factors, including dyslipidemia and hyperglycemia, influence platelet activation. However, the impact of endogenous lipid metabolism on platelet function remains largely unexplored. Lepropre et al demonstrated that platelet AMPK, by phosphorylating acetyl coenzyme A (acetyl-CoA) carboxylase (ACC) and regulating endogenous lipid synthesis, including the arachidonic acid–derived eicosanoid thromboxane A2 (TXA2), thereby contributing to thrombus formation (see figure).

AMPK is a ubiquitously expressed serine/threonine kinase normally activated by low cellular energy state due to its unique ability to sense intracellular AMP levels; as ATP becomes depleted, AMP levels rise and AMPK is activated.2 On activation, it has multiple targets, and the fundamental effect is to suppress metabolic processes that consume energy, such as lipogenesis, while stimulating pathways for energy production, such as fatty acid oxidation and mitochondrial oxidative phosphorylation. The major substrate for AMPK to achieve these effects is ACC, which converts acetyl-CoA to

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Platelet AMPK-ACC pathway regulates lipid contents to control platelet activation and thrombosis

A simplified model showing how the platelet AMPK-ACC pathway integrates signaling through surface receptors for fatty acids and collagen into a metabolic response that promotes thrombosis by enhancing synthesis of thromboxane.

malonyl CoA, a limiting step in synthesis of fatty acids. AMPK, by phosphorylating ACC1/2 on serine residues (Ser79/Ser212), inhibits its activity and thereby puts a brake on fatty acid synthesis (see figure). To explore the in vivo role of the AMPK-ACC pathway in platelets, Lepropre et al used a double knock-in (DKI) mouse model in which the AMPK phosphorylation sites on ACC1/2 were mutated to prevent inactivation. They showed that this resulted in shorter tail bleeding times and enhanced carotid artery thrombosis in a FeCl3-induced injury model. This was associated with increased platelet phospholipid content and enhanced generation of TXA2, a potent platelet agonist, in response to collagen stimulation. The effects of AMPK-ACC inactivation appeared to be specific to collagen as DKI platelets showed normal activation to agonists for CLEC-2 and to G protein–coupled receptors for ADP and TXA2. Surprisingly, DKI platelets also showed normal activation to a small peptide agonist for GPVI, suggesting that there may be a requirement for additional collagen receptors, perhaps integrin α2β1 in AMPK activation.

Platelet hyperreactivity is often observed in patients with diseases associated with thrombotic risk, such as atherosclerosis, hyperlipidemia, diabetes, obesity, cancer, and chronic systemic vasculitis. This may be an important contributing factor to increased risk of myocardial infarction and stroke. Understanding how these conditions influence platelet activation will provide key knowledge for designing novel therapeutic interventions. Previous work focused on signaling through platelet surface receptors known to be components of innate immune system, including toll-like receptors and scavenger receptors. These receptors continuously scan the intravascular environment to detect endogenous and exogenous danger-associated signals. One of these receptors, the type II scavenger receptor CD36, interacts with specific danger signals, including oxidized low-density lipoprotein, to promote platelet activation and thrombosis by generating reactive oxygen species and activating redox sensing/signaling events, clearly implicating cellular metabolism in platelet function. Lepropre et al have now identified another essential metabolic sensor, AMPK, as a regulator of platelet reactivity. This raises the intriguing possibility of “rescuing” the abnormality in platelet metabolism as a therapeutic antithrombotic strategy in certain high-risk conditions. In fact, atherogenic conditions are associated with suppressed AMPK activities in various tissues, and many studies indicate that AMPK activating agents bring potential beneficial effects in other cell types.

An important question not fully addressed is whether platelet bioenergetics are affected by the AMPK-ACC pathway. Lepropre et al detected no obvious alterations in oxygen consumption or glycolysis using the Seahorse metabolic flux assay. However, this should be interpreted with caution as washed platelets used in these assays are a very artificial condition for metabolic measurements. Another interesting question raised here is whether signaling through platelet surface receptors is intrinsically coupled to intracellular metabolic status. For example, CD36 facilitates long-chain fatty acid translocation across the plasma membrane. In myocytes, membrane CD36 binds and suppresses AMPK activity, whereas fatty acid binding releases AMPK for activation, leading to fatty acid oxidation. Because CD36 is highly expressed on the platelet surface, it will be important to test whether CD36, AMPK, and fatty acid metabolism are interrelated to coordinate platelet activation, especially under atherogenic conditions (see figure). Altogether, this interesting paper uncovers a novel metabolic regulatory mechanism in the platelets, which affects thrombosis in vivo, further demonstrating an important link between metabolism and thrombosis.

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

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Comment on Chou et al, page 1198

Rhesus pieces: genotype matching of RBCs

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As Chou et al have elegantly shown in this issue of Blood,1 RH genetic diversity in patients with sickle cell disease (SCD) is prevalent, and matching of donor and recipient red blood cell (RBC) antigens at the molecular level for transfusion is feasible. The Rh blood group system is highly complex, with many antigens defined at the serologic level encoded by the RH and RHCE genes. Genetic exchange between RHD and RHCE is especially common in individuals of African descent, including those with SCD. In fact, ∼90% of studied patients with SCD have at least 1 RH allele that differs from those found in individuals of European descent.2 Because most blood donors in the United States are not of African descent, each RBC transfusion thus exposes recipients with SCD to many “nonself” blood group antigens.

Transfusion therapy can be lifesaving for patients living with SCD, treating disease complications such as acute chest syndrome and preventing recurrent ischemic strokes. However, the formation of RBC alloantibodies is a dreaded transfusion complication. Whereas 3% to 5% of the general transfused patient population will become alloimmunized after transfusion, up to 50% of patients with SCD develop RBC alloantibodies.2 Such alloantibodies can be clinically significant in transfusion and pregnancy settings, leading to difficulties in locating compatible RBCs, transfusion reactions, and hemolytic disease of the fetus and newborn. Whereas some transfusion reactions due to non-ABO antibodies can be mild, others, including delayed hemolytic reactions with bystander hemolysis, can be deadly.

Antigens encoded by RHD and RHCE were identified 3 decades ago to be among the most immunogenic in patients with SCD,3 and serologic matching for D, C/c, and E/e between blood donors and recipients has been adopted as a standard of care.4 Although serologic matching decreases alloantibody formation in general, recent studies have reported unexpectedly high rates of antibodies in the RH family despite such matching.2 These high alloimmunization rates could have been dismissed as merely reflecting patients being transfused at multiple hospitals, including hospitals that do not provide phenotype matched RBCs. However, it is now understood that serologic testing, which involves incubating patient/donor RBCs with antisera and looking for agglutination, fails to detect subtle antigenic variants. As Chou et al describe, 21% of patients that phenotyped as C positive actually possessed a “partial” C antigen only revealed by genotyping (with most demonstrating the hybrid RH(D)(D)lla-CE(4-7)-D). Without genotyping, these patients would have been routinely transfused with C-positive RBCs and may have developed anti-C despite a prophylactic matching strategy (see figure). Furthermore, 30%, 40%, and 36% of described patients had altered D, c, or e antigens, respectively. Although not intuitive, transfusion recipients with conventional D, C/c, or E/e antigens may also be at risk of forming alloantibodies if exposed to donors expressing variant Rh antigens. Such antibodies, which may have historically been categorized as autoantibodies, are in fact alloantibodies.

The take-home messages of the Chou et al article, which involved RH genotyping 587 blood donors of African descent along with 857 patients with SCD, are that RH genetic diversity is similarly widespread among both groups of individuals, and that provision of genotype matched RBCs is logistically feasible with a donor pool composed primarily of individuals of African descent. No prior study has so thoroughly characterized Rh antigens in patients living with SCD, or determined the large-scale feasibility of RBC provision from a blood donor center perspective. It is important to realize that the Rh variant status of patients with SCD will vary based on region of origin, with patients in the northeastern United States having differences in the distribution of Rh antigenic variants and antibodies formed compared with patients in other regions (eg, France).5 It is also worth considering that similar genotyping strategies may benefit other highly transfused patient populations at risk of RBC alloimmunization, such as those with myelodysplastic syndrome6 or thalassemia.

One may ask why RBCs from group O, Rh-negative donors cannot be a priori be selected for transfusion for individuals with SCD. First, such units are typically collected from donors of European descent, and as such, will express non-Rh antigens that are foreign to recipients of African descent, potentially increasing alloimmunization rates to these antigens. Another consideration is that such units are typically reserved for group O, Rh-negative recipients and for women of childbearing age requiring emergent transfusion. Whereas general transfusion patterns of RBCs have decreased over the past few years, the proportion of group O, Rh-negative RBCs that are distributed has increased by 9%.7 Importantly, over one-third of RBCs transfused to D-positive patients with SCD in 1 study were already from D-negative donors,8 with more selective RBC utilization potentially possible with widespread implementation.