variation of MK distance to the “sinusoids” (lower channel). 1

Another important advantage of the here-described bioreactor is the striking increase in platelet yields by a magnitude of twofold compared with static conditions. The authors speculate that this setup, upon upscaling, could help to satisfy the demand for platelet transfusions. In support of this, Thon and colleagues assessed the quality of their instant platelet product, generated from murine fetal liver– and human-induced pluripotent stem cell–derived MKs, by performing morphologic and functional studies. With this, they show that their platelet product reflects the cytoskeletal organization and functionality of human and mouse platelets. 1 The next logical step will be to test the quality of the product under in vivo conditions, for example, by substituting platelet-depleted mice with in vitro–generated platelets and assessment of platelet lifetime and function in models of not only hemostasis but also thrombosis and thromboinflammation.

Although the “bioreactor-on-a-chip” is not the first attempt to mimic key features of the MK bone marrow microenvironment, 10 it represents a major advance in this field of research as it not only provides high yields of (apparently) functional platelets but also allows high-resolution real-time visualization of the dynamic process of proplatelet formation in vitro. We are convinced that this design will serve as a basis for further optimization and will stimulate new research in the field of MK biology. These studies will certainly advance our understanding of platelet formation and ultimately the molecular causes of platelet formation defects in patients.

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THROMBOSIS & HEMOSTASIS

Comment on Langdown et al, page 1951

Unexplained bleeding: another player to look out for!

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In this issue of Blood, Langdown et al identify a novel mutation in the thrombomodulin gene that is responsible for a bleeding phenotype that has hitherto never been characterized. 1

Bleeding events that are not caused by anticoagulation/antiplatelet therapies are often due to congenital deficiencies of coagulation factors, for example, factor VIII (hemophilia A) or factor IX (hemophilia B) or acquired hemophilia deficiencies due to the presence of antibodies directed against clotting factors. 2,3 These antibodies may arise as a consequence of treatment of congenital hemophilies, termed alloantibodies, but can also occur from the spontaneous effects of autoantibodies 2 in individuals with prior normal coagulation, for example, acquired factor XIII hemophilia due to the development of antibodies against factor XIII. 4

In this article, Langdown et al identify a novel mechanism that can result in a bleeding phenotype due to a premature stop codon in thrombomodulin (p.Cys537Stop). Thrombomodulin binds thrombin and converts thrombin from a procoagulant enzyme to an enzyme with anticoagulant function via the activation of protein C to activated protein C (APC). 5 As a consequence of the p.Cys337Stop mutation, thrombomodulin is truncated within the C-terminal transmembrane helix, leading to shedding of thrombomodulin from the endothelium into the bloodstream, resulting in unusually elevated plasma levels of free thrombomodulin. This yields greater activation of the protein C anticoagulant system generating APC, which serves to attenuate thrombin formation by the inactivation of activated factors V and VIII. 6 Langdown et al identify the novel mutation in a family with 2 members who experienced excessive bleeding events. No abnormalities were detected in the prothrombin time, activated partial thromboplastin time, thrombin time, and fibrinogen assays. In addition coagulation factors II, V, VII, VIII, IX, X, XI, XII, and XIII, von Willebrand factor antigen, and von Willebrand cofactor activity were all within the normal range. Furthermore, there was no evidence of abnormal platelet function. Plasma antithrombin and protein C levels were also within normal ranges. Interestingly, the prothrombin consumption index (a measure of coagulation factor consumption during whole blood clotting) was greater than twofold for members of the family compared with the normal range, suggesting less consumption of coagulation factors compared with controls. Two members of the family (including one with the excessive bleeding phenotype) had normal levels of protein S, whereas 1 member of the family had reduced levels (51% vs >63% for normal reference limits). The APC ratio was normal in the
2 family members who had a bleeding phenotype. Both family members who exhibited excessive bleeding events were found to have reduced thrombin generation, with a trigger of 5 pM tissue factor (TF) and very low levels of thrombin generation with 1 pM TF. Only partial correction of thrombin generation was observed in normal plasma mixing studies, suggesting the presence of a coagulation inhibitor. When thrombin generation was performed with a fivefold physiological excess of protein C with 5 pM TF as a trigger, thrombin generation for all family members was reduced compared with no change in control plasma. An anti-protein C antibody added to the plasma increased the thrombin generation to normal levels in the plasma from the affected subject, whereas in normal control plasma, the antibody did not make any difference. Plasma levels of thrombomodulin in the affected family members were elevated by 100-fold compared with normal plasma. When truncated thrombomodulin was added to normal plasma with thrombin generation determined by a 1 pM TF trigger of coagulation, a dose-dependent inhibition of thrombin generation was observed. These data confirmed the mode of action by which the affected family members were predisposed to an increased risk of bleeding.

This study therefore highlights that there is a “new kid on the block” to look out for when unexplained bleeding events in patients take place. With bleeding events, a systematic diagnosis through conventional tests to identify the cause of the bleeding in the first instance is performed. However, when these conventional tests appear normal, assessment of thrombin generation (using 1 pM TF) and measurement of plasma thrombomodulin levels may aid in the appropriate diagnosis prior to confirmation by genotyping in instances of bleeding where no other explanation can be found. The identification of this novel mutation in thrombomodulin may help to alleviate misdiagnosed bleeding events and enable suitable therapeutic intervention. This study also highlights the benefits of collaborative efforts of both clinical and basic science laboratories to address clinical problems.

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