differentiating PEMs from both MEPs and common myeloid progenitors (CMPs) to achieve speedy regeneration of erythroid cells.\textsuperscript{5} Confirmation of the human model awaits the further analysis of PEM cells in vivo in humans and demonstration of the importance of these cells in the human erythropoietic stress response. Due to the difficulties of conducting such studies in humans, studies in primate model systems that are very similar to humans could be valuable. Minor differences in the human and murine models will require further clarification. In the human model, the PEM population is derived directly from the MEP, whereas the murine model suggests that PEMs may be derived from both MEPs and CMPs. In addition to the PEM population, the authors also described a population of macrophages associated with differentiating erythroblasts that was expanded in a CID-independent manner. Further experiments will be required to characterize the mechanism of macrophage expansion and the relationship of these cells to the differentiating erythroblasts.

The ability of PEM cells to directly and rapidly differentiate into erythrocytes suggests that the erythroid stress differentiation pathway might be involved in the generation of erythrocytes containing increased levels of fetal hemoglobin (HbF) (F cells). In human and nonhuman primate models, HbF and F cells are normally present at low levels but increase during erythropoietic stress.\textsuperscript{5,6} Because more primitive cells are programmed to express higher levels of HbF than more differentiated cells, increased HbF expression can result from commitment to differentiation at more primitive stages of the erythroid differentiation pathway.\textsuperscript{7} Interestingly, Belay et al observed, as predicted, a higher ratio of $\gamma$-globin in PEMs than in erythroid cells. Further understanding of the mechanisms controlling HbF and F cell numbers could impact the treatment of sickle cell disease and $\beta$-thalassemia, where increased HbF and F cell numbers are beneficial.

One of the potentially promising applications of the current findings is to exploit the expansion of erythroid cells from bipotent PEM progenitors free of exogenous cytokines in culture using the CGS-CID system as described by Belay et al.\textsuperscript{1} Currently, scaling up to produce 1 unit of packed cultured red blood cells (RBCs) from human peripheral blood CD34\textsuperscript{+} cells is feasible but is limited by excessive production costs, which are largely due to the requirement for exogenous cytokines.\textsuperscript{8} Therefore, exogenous cytokine–free ex vivo–cultured human RBC production would enable a significant reduction in costs. Certainly, much more work will need to be done to demonstrate that the purified PEM-derived RBCs not only are functional in vivo and behave similar to native RBCs but also are free of contaminating myeloid progenitors. Of note, as proof of principle, cultured human RBCs have already been generated and transfused in a single human subject.

In conclusion, Belay et al have identified a human bipotent erythroid-megakaryocytic precursor cell with the ability to rapidly differentiate into both erythrocytes and megakaryocytes. The qualities of this human bipotent cell, analogous to those of murine PEM cells arising during stress erythropoiesis, strongly suggest that the erythroid differentiation pathway is likely governed in a similar manner in response to stress erythropoiesis in both mice and humans. The identification of this new erythroid-biased bipotent population may reveal a cost-effective means of generating cultured human RBCs for possible blood transfusions in the future.

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Comment on Feng et al, page 1034

VWF and complement

Bernhard Lämmlle UNIVERSITY MEDICAL CENTER, MAINZ

In this issue of Blood, Feng and coworkers present data suggesting a role for von Willebrand factor (VWF) in the proteolytic inactivation of complement C3b by factor I (CFI).\textsuperscript{1} Whereas smaller VWF multimers, especially dimers, tetramers, and hexamers, enhance C3b inactivation by CFI, large and unusually large VWF multimers are devoid of this cofactor activity and, therefore, they enhance complement activation by the alternative pathway C3 convertase, C3bBb.

When a severe deficiency of VWF-cleaving protease, either caused by autoantibodies inhibiting its activity or by a constitutional defect without circulating inhibitors, was first reported in a series of patients with sporadic or familial thrombotic thrombocytopenic purpura (TTP), respectively, a differential diagnosis between TTP and atypical hemolytic uremic syndrome (HUS) seemed to become possible.\textsuperscript{2} In contrast to patients clinically diagnosed with TTP, those with a diagnosis of atypical HUS showed normal or only mildly decreased activity of the VWF-cleaving
protease,7 which nowadays is known as ADAMTS13 (a disintegrin and metalloprotease with thrombospondin type 1 domains, known as ADAMTS13) which plays an important role in the regulation of von Willebrand factor (VWF). This enzyme helps to control the formation of platelet clumps by cleaving VWF into smaller fragments.

The extent of complement activation upon admission was even found to be associated with fatal cases showing more extensive complement activation. It is less clear, however, whether complement activation in acute episodes of acquired or hereditary ADAMTS13-deficient TTP is truly a driving pathogenetic factor, or rather an epiphenomenon following ischemic organ damage by widespread VWF-platelet clumping leading to microvascular thrombosis.

Nevertheless, it is clear that the defense systems, complement and hemostasis, are intrinsically linked at many levels. For instance, the endothelial transmembrane protein, thrombomodulin (THBD), functions as a regulator of the complement to platelet clumping in the microcirculation by enhancing C3b degradation by CFI, which suggested that the cofactor function was indeed attributable to VWF and not to a copurified contaminant. Feng and colleagues conclude that normal plasma VWF multimers contribute to inhibit complement activation by enhancing C3b degradation, whereas larger VWF species, including the unusually large VWF multimers found in patients lacking ADAMTS13 activity, do not have CFI cofactor function that would enhance complement activation.1

The in vivo significance of these findings, however, is unclear at present. As nicely shown by the authors with their different VWF fractions obtained by sizing chromatography of normal plasma cryoprecipitate, normal plasma obviously contains not only VWF fractions rich in dimers, tetramers, and hexamers contributing to complement regulation, but also fractions with larger multimers devoid of complement regulatory function, which rather favor complement activation. The net result of VWF in normal plasma and the in vivo role of VWF in the activation or regulation of the complement system clearly needs further study. The article by Feng et al stimulates our interest in the highly complex interactions of complement and hemostasis, both in normal physiology and in disease states such as the thrombotic microangiopathies.

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Comment on Bruneau et al, page 1038

DGKE disruption ditches complement and drives p38 signaling

K. Vinod Vijayan  BAYLOR COLLEGE OF MEDICINE; MICHAEL E. DEBAKEY VETERANS AFFAIRS MEDICAL CENTER

In this issue of Blood, Bruneau et al provide evidence that disruption of diacylglycerol kinase ε isofrom (DGKE) does not upregulate complement activation, but rather induces endothelial damage via the activation of p38 mitogen–activated protein kinase (MAPK). These in vitro findings may support a new pathophysiologic mechanism for atypical hemolytic-uremic syndrome (aHUS) in a subset of patients with DGKE gene mutations.

aHUS is a thrombotic microangiopathy characterized by thrombocytopenia, anemia, and microthrombi found predominantly in the kidney. Recurrent clinical course is common, and end-stage renal disease develops in more than half of the affected individuals. Mutation in one or more of the genes that encode proteins of the alternative complement pathway, such as factor H, factor I, membrane cofactor protein (MCP), complement factor H–related proteins CFHR1 and CFHR3, thrombomodulin, and factors B and C3, is a hallmark of this disease. Mechanistically, these mutations render excessive complement activation, which damages glomerular endothelial cells and likely supports microthrombi via local tissue factor exposure, thrombin generation, and platelet adhesion/aggregation. Indeed, a monoclonal antibody to complement C5 (eculizumab) has proven efficacious in aHUS patients with complement defect.

The strong complement-aHUS link was challenged when 2 independent reports in 2013 revealed recessive loss-of-function mutations in the DGKE gene in a subset of patients with aHUS and membranoproliferative glomerulonephritis, respectively. DGKE encodes for an enzyme DGKε that is distinct from the complement pathway. In fact, DGKε is a lipid kinase that can phosphorylate specifically diacylglycerol (DAG) with an arachidonoyl group at the sn-2 position of the glycerol backbone and generate phosphatidic acid (PA) (see figure). DAG is generated predominantly by PLC–mediated hydrolysis of PIP2 downstream of G protein–coupled receptors and integrins. Thus, loss-of-function mutations in DGKE are predicted to alter the intracellular levels of arachidonic acid containing DAG and PA, with potential changes in cellular signaling downstream of these bioactive lipids. How disruption of DGKε might contribute to the pathophysiology of aHUS is currently unknown.

The article by Bruneau et al1 is timely and begins to address this important and significant question. The authors show that disruption of DGKE by siRNA from ECs of 2 different vascular beds (human umbilical vein ECs and human microvascular ECs) can enhance expression of ICAM-1, E-selectin (E-Sel), and tissue factor (TF), with a concomitant increase in platelet adhesion (see figure). DGKε-depleted ECs revealed an increase in p38 MAPK signaling in phosphoproteomic studies. More importantly, p38 inhibitor blocked the increased ICAM-1 and E-Sel expression in DGKε-depleted ECs. Thus, loss of DGKε can trigger endothelial activation and display a prothrombotic phenotype. Intriguingly, the authors also show that disruption of DGKE induces...
VWF and complement
Bernhard Lämmle

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