genotypes, and *identical* combinations of GWAS trait loci, express different levels of HbF? Given the identical genetic background, any satisfactory explanation must involve a probabilistic process in differentiation or gene expression influenced by the known genetic traits. Four explanations come to mind. One is the persistence of fetal-like stem or progenitor cells in adults: this seems unlikely because adult F cells are considerably smaller than bona fide fetal cells and are no more likely to express the fetal i-antigen on their surface than A cells.[7] A second explanation is that a small proportion of adult erythroid progenitors exists in a fetal-like transcriptional/epigenetic state. A third explanation is that HbF heterogeneity is caused by small probabilistic changes in gene expression, involving one or more of the known HbF regulators. Finally, the F-cell phenomenon could be explained by alterations in the kinetics of erythroid maturation with premature release of erythroid progenitors that synthesize more HbF than later progenitors.[8] Whichever of these explanations is correct, the amount of HbF and the levels of F cells should be explicable in terms the known genetic factors.

To address this, Khandros et al developed protocols to purify and compare F cells and adult cells (A cells). Surprisingly, only the γ-globin genes, and no other causative genes, were differentially expressed at either the RNA or the protein levels. It will be interesting to determine if there is a critical time point at which there are differences in expression of key regulators of F cells. Alternatively, they may involve undetected posttranslational modifications. The difference seen in γ-globin RNA between F and A cells suggests that differential expression of the fetal genes in such cells is controlled by transcription and/or RNA processing rather than translation. Finally, this phenomenon could involve changes in the kinetics of erythropoiesis, in which case the known regulators of F cells must play an important role in this process. Whatever the answer, this classical Popperian approach to the current theories tells us that there is more to find out.

The F-cell phenomenon is not unique. Patients with α-thalassemia who have a common deletion removing both α genes ($-^{SEA}/\alpha\alpha$) have an excess of β-globin in all erythroid cells, but only 1:1000 of these genetically identical cells have sufficient excess β-globin to form HbH inclusions (HbH cells).[4] Similarly, such individuals produce 1% to 2% of red cells in which embryonic ζ-globin chains are detectable (Z cells).[9] Looking more broadly in erythropoiesis, there are now many traits that determine, for example, the heritability of red cell size,[10] but again genetically identical red cells still show considerable variation. It seems likely that probabilistic variation in any phenotype may occur in genetically identical cells, and, as so often in the past, analysis of globin gene expression in erythropoiesis is leading this interesting area of biomedical research to establish the principles of how such variation occurs and its biological significance.

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THROMBOSIS AND HEMOSTASIS

Comment on Calzavarini et al, page 1969

Regulation of venous thrombosis by platelet protein S

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In this issue of *Blood*, Calzavarini et al solve the long-standing puzzle of the function of protein S (PS) that has been derived from platelets (PS_{plt}) by showing that PS_{plt} limits thrombin formation in large veins.[1]

You discover a leaky pipe and apply putty, hoping that the putty will remain at the leak site and will not swell or drip onto the floor. Our circulatory system faces similar challenges in response to blood vessel injury. Platelets (the putty) rush to the scene and help block the leak. What ensures that platelets remain at the injury site? What controls the size of the surrounding thrombus? Calzavarini and colleagues report that, in large veins, PS_{plt} restrains thrombin generation and promotes confinement of activated platelets and fibrin to the injury site. The authors also show that these PS_{plt} functions are quiescent in large arteries. These findings are critical for our understanding of the activity of PS_{plt}, and they have implications for therapeutic intervention in patients with bleeding disorders.

PS is an essential vitamin K–dependent plasma anticoagulant. Homozygous deficiency of PS usually causes life-threatening purpura fulminans at birth,[2] and heterozygous

PS deficiency elevates the risk of venous thrombosis.[3] Plasma PS acts primarily as an anticoagulant by enhancing the function of activated protein C (APC),[4] by serving as a cofactor to tissue factor pathway inhibitor α (TFPIα) in inhibition of free factor Xa (FXa) in the initial phase of coagulation,[5] and by inhibiting FIXa to block FX activation.[6]

PS_{plt}, which is about 2.5% of total PS, is stored in platelet α granules and released by platelet stimulation.[7] Unlike its well-defined plasma counterpart, the function of PS_{plt} in thrombosis and hemostasis has been largely unknown. Calzavarini et al closed this knowledge gap with an elegant mouse model in which they ablated PS_{plt} expression specifically in the megakaryocyte precursors of platelets. The investigators found that PS_{plt} was essential for regulating thrombosis in veins, at last giving much-needed clarity to an unchartered area.

One of the most exciting findings by Calzavarini et al is that PS_{plt} regulates venous thrombosis but not arterial thrombosis. The difference seems to be based on shear rate. Earlier, Begent and Born[8] assessed the effect of venous shear rate on thrombus formation. They discovered that thrombus growth in a large vein (diameter of 40-60 μm) reached a maximum at a low blood flow of about 400 μm/s, a point at which the number of platelets transported to an injured site remained in balance with the number that adhered to the vessel wall. At a low shear rate, activated platelets secrete PS_{plt}, which then acts as a cofactor for APC and TFPI, thereby controlling FXa and thrombin generation within the thrombus. Conversely, arterial thrombosis occurs at a high shear rate when platelet rich thrombi are formed around ruptured atherosclerotic plaques and damaged endothelium; platelets are crucial in the development of arterial thrombosis at these sites.[9] The reason that PS_{plt} does not contribute to control of arterial thrombosis is because at a high shear rate, the platelets accumulate quickly at the injury site; thus, most of the platelets are not activated to release PS_{plt}.

Another remarkable finding from Calzavarini et al is that mice lacking PS_{plt} had significant reductions in bleeding time and blood loss, presumably because of unrestrained thrombus growth. This observation raises the intriguing possibility, suggested by Calzavarini et al, that targeting PS_{plt} for inactivation might constitute a valuable therapeutic avenue for patients with bleeding disorders.

In summary, by selectively inhibiting the expression of PS_{plt} in a transgenic mouse model, Calzavarini et al explained, for the first time, the function of PS_{plt} in blood coagulation and thrombosis. Unraveling the importance of platelet PS in vivo is breakthrough work in the fields of thrombosis and hemostasis.

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TRANSFUSION MEDICINE

Comment on Escamilla-Rivera et al, page 1983

Opsonization, inflammation, and RBC alloimmunization

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In this issue of *Blood*, Escamilla-Rivera and colleagues show that inflammation can sidestep immunoprophylaxis, with breakthrough red blood cell (RBC) alloimmunization, in a murine model mirroring antenatal administration of Rh immune globulin (RhIg).[1] Successful immunoprophylaxis was associated with early phagocytosis of opsonized RBCs, especially by splenic $CD8a^+$ dendritic cells, which was not observed in the presence of inflammation.

Historically, ~15% of RhD-negative women would develop anti-D during the course of pregnancy, with devastating consequences for subsequent pregnancies. With the introduction of RhIg prophylaxis in the 1960s, the incidence of RhD alloimmunization and hemolytic disease of the fetus and newborn decreased markedly and is considered one of the triumphs of modern medicine. The most recent data show that antenatal and postnatal administration of 300 μg of RhIg can prevent RhD alloimmunization in 98% to 99% of cases.[2] RhIg failures or breakthrough RhD alloimmunization occurs in 0.3% to 1% of women and has been attributed to transplacental hemorrhage prior to RhIg administration, inappropriate administration of low-dose (50 μg) RhIg, or insufficient RhIg to cover a large fetal bleed.[2,3]

In this issue of *Blood*, Escamilla-Rivera and colleagues explore an entirely novel mechanism for breakthrough alloimmunization, building on the known association between RBC alloimmunization and inflammation.[4,5] The authors used a well-described transgenic mouse model expressing the human KEL glycoprotein. To mimic RhIg prophylaxis, wild-type mice were initially transfused with