NF-κB signaling and that this suppression was dependent on the kinase activity of MST1.

Activation of the NF-κB pathway has been found in other models of secondary myelofibrosis,[6] and targeting this pathway has some efficacy in preventing the progression of disease.[7] Therefore, to translate these findings, the authors used an IRAK1/4 inhibitor in the JAK2V617F, Stk3/4 haploinsufficient mouse model of myelofibrosis. This proinflammatory signaling that characterized the myelofibrotic phenotype was inhibited by the IRAK1/4 inhibitor in vivo, providing a logical path to clinical translation that may benefit patients with advanced MPN and increased inflammatory signaling (see figure).

Altogether, Stoner et al provide a compelling argument for a functional role of MST1 loss in the pathogenesis of myeloid malignancies, leading to dysregulated inflammatory signaling and progression to advanced disease. A number of questions still remain, such as identifying the best way of dampening down inflammatory signaling in these diseases and why del(20q) leads to a relatively favorable prognosis compared with other cytogenetic abnormalities. This work builds on a recurrent theme that identifies pathological activation of inflammation and immune pathways in myeloid blood cancers and provides hope that these findings may be leveraged to design new treatments for our patients with these diseases.

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THROMBOSIS AND HEMOSTASIS

Comment on Shao et al, page 1745

Low FV beneficial in FVFVIII deficiency?

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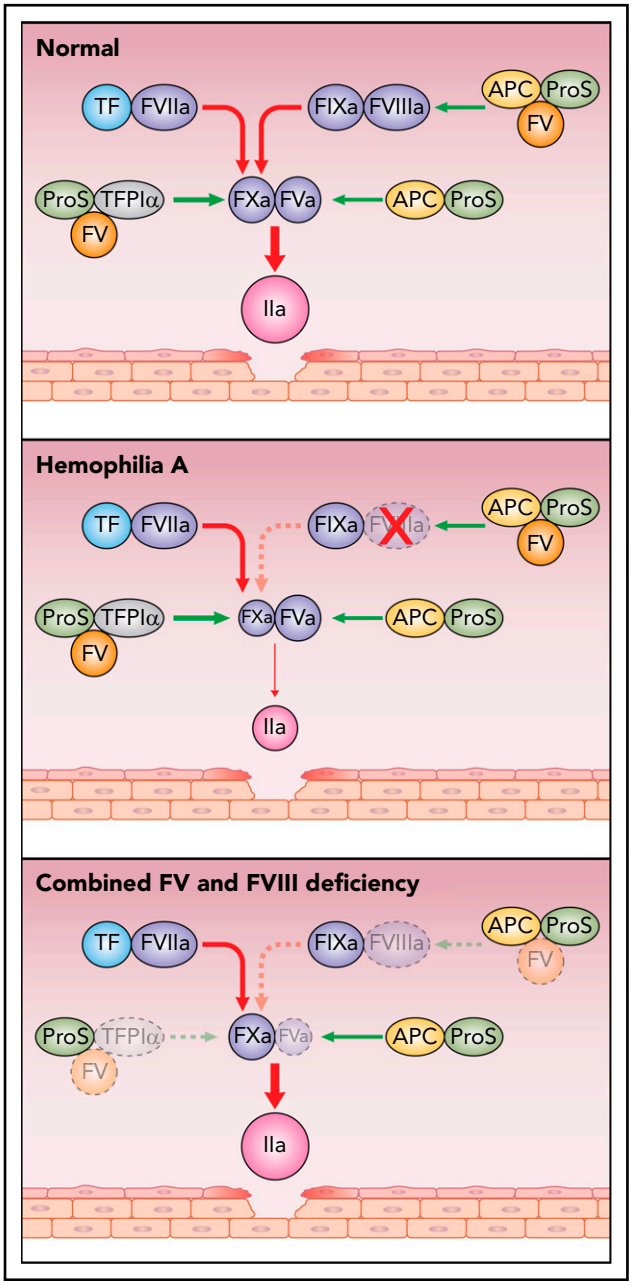
In this issue of *Blood*, Shao and colleagues demonstrate that the low factor V (FV) levels in patients with combined deficiency of FV and FVIII (F5F8D) may in fact be beneficial for the patients and ameliorate their bleeding tendency.[1] This report adds to the growing list of observations that FV not only is procoagulant but also that it works as an anticoagulant. The paper brings new understanding of the basic pathology of F5F8D and also lays the foundation for a change in the treatment strategy of the disease.

Blood coagulation is initiated by vascular damage, which exposes tissue factor (TF) to the circulating coagulation proteins.[2,3] This initiates a series of proteolytic reactions that result in the formation of thrombin and a fibrin clot. Factor VIIa (FVIIa) bound to TF activates factors IX and X (FIX and FX), which team up with their respective activated cofactor, factor VIIIa and Va (FVIIIa and FVa) (see figure). FXaFVa activates prothrombin to generate the initial thrombin. Feedback from this reaction activates the system by converting the procofactors FVIII and FV to their active forms, and the system can now proceed at maximum speed.[4] The role of FIXaFVIIIa is to provide amplification by generating more FXa. The cofactors FVa and FVIIIa are crucial as the enzymes FIXa and FXa have low intrinsic activities. This is illustrated by the severe bleeding phenotype affecting individuals with hemophilia A, due to defective or absent FVIII. In contrast, it has been puzzling that patients with FV deficiency generally have a mild bleeding phenotype. Patients with combined FVIII and FV deficiency also have a mild bleeding phenotype, and the report by Shao and colleagues provides a possible explanation by demonstrating that the low FV levels are associated with efficient thrombin generation. Reconstitution of FV to normal levels results in anticoagulation and decreased thrombin generation. This illustrates the importance of the anticoagulant functions of FV.

To date, 2 anticoagulant properties of FV have been identified (see figure). The discovery of activated protein C (APC) resistance, caused by the FVLeiden mutation, as a major risk factor for venous thrombosis led to the identification of FV as an APC cofactor functioning in synergy with the anticoagulant protein S in the degradation of FVIII in the FIXaFVIIIa complex.[4,5] Loss of the APC cofactor activity due to the FVLeiden mutation is one of the mechanisms that generates a hypercoagulable state. The second anticoagulant function of FV is related to its interaction with full-length tissue factor pathway inhibitor (TFPIα).[5] TFPIα, which regulates both the FVIIaTF complex and FXa, circulates bound to FV. As a consequence, individuals with FV deficiency have low TFPIα and therefore defective anticoagulation, which may be the explanation for the mild bleeding phenotype in FV deficiency.[6] FV serves not only as a carrier of TFPIα in circulation but also as a synergistic TFPIα cofactor together with protein S in the inhibition of FXa.[7,8] A minor splice isoform of FV, denoted FV-Short, which is particularly efficient as



Simplified scheme of pro- and anticoagulant reactions in health, in hemophilia A, and in combined FV and FVIII deficiency. Damage to the vessel wall results in activation of coagulation via FVIIaTF. FVIIaTF and FIXaFVIIIa activate FX; FXa then binds to FVa and activates prothrombin to thrombin. The anticoagulant functions of FV include being a carrier to TFPIα and a synergistic cofactor with protein S (ProS) to TFPIα as well as to APC. In health, the pro- and anticoagulant mechanisms are in balance. In contrast, in hemophilia A, the balance is shifted to anticoagulation with little thrombin generated. In F5F8D, because the anticoagulant functions of FV, TFPIα, and protein S are deficient, the low levels of FV and FVIII are sufficient to efficiently generate thrombin. Red arrows, procoagulant reactions; green arrows, anticoagulant reactions.

a TFPIα carrier and synergistic TFPIα cofactor with protein S, was discovered during elucidation of the pathogenic mechanism of the East Texas bleeding disorder.[9]

The low plasma levels of FV and FVIII in F5F8D result from defective secretion of FV and FVIII due to mutations in either *LMAN1* (lectin mannose binding-1) or *MCFD2* (multiple coagulation factor deficiency-2). The block in secretion is not complete, and therefore, the FV and FVIII plasma levels usually are 10% to 20% of normal. The associated bleeding phenotype is mild to moderate, and bleeding episodes have been treated on demand to increase FV and FVIII levels. Shao and colleagues have used an in vitro thrombin generation assay to investigate platelet-poor plasma from 6 patients with F5F8D. They made the surprising observation that thrombin generation in F5F8D was considerably higher than in that of a normal control. Addition of FVIII increased the thrombin generation further, whereas reconstitution of FV decreased thrombin generation. Because FV is known to carry TFPIα in plasma, and because FV deficiency is associated with low TFPIα, the authors measured TFPIα in the patients and found that the TFPIα plasma concentrations were equally low as those in severe FV deficiency. Subsequent reconstitution of TFPIα to the F5F8D patient plasma resulted in decreased thrombin generation. Platelet-rich plasma demonstrated a different picture with low thrombin generation in F5F8D patients. Addition of FV or TFPIα minimally affected thrombin generation, whereas FVIII addition greatly improved thrombin generation. Thus, in F5F8D patients, the thrombin generation patterns were very different in the presence or absence of platelets, which raises questions about the in vivo relevance in F5F8D patients of these findings. However, an important conclusion that can be drawn from both platelet-rich and platelet-poor plasma is that increase in FVIII results in increased thrombin generation. Increased FVIII levels can be achieved by infusion of 1-Deamino-8-DArgininVasoPressin (DDAVP), which induces secretion of FVIII and von Willebrand factor from endothelial cells but does not affect FV. DDAVP has been used in several cases of F5F8D, and the observation of Shao and colleagues provide a molecular basis for the rationale to use this treatment in F5F8D patients.

FV is a Janus-faced protein with both pro- and anticoagulant properties. The low FV levels in F5F8D may contribute to the mild phenotype. Based on the observation made by Shao and colleagues, it is tempting to speculate that selective inhibition of the anticoagulant properties of FV could be beneficial for hemophilia patients, but this may be difficult to achieve. However, several reports have demonstrated improved in vivo hemostasis in hemophilia after inhibition of different anticoagulant proteins (eg, antithrombin, TFPIα protein C, and protein S).[10]

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TRANSPLANTATION

Comment on Laberko et al, page 1755

Universal donor strategy for primary immunodeficiency

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Arguably, the most difficult transplants are those of patients with primary immune deficiencies with persistent viral infection and no matched donor. In this issue of *Blood*, Laberko et al report an approach to this problem using T-cell receptor (TCR)αβ–depleted donors.[1]

For many years, the outcomes of transplant for patients with primary immune deficiencies have been improving, although outcomes remain worse for patients with no matched family or unrelated donor.[2] With increasingly sophisticated methods of graft engineering, T-lymphocyte–depleted graft outcomes have been improving, particularly for patients with primary immunodeficiency,[3] so that today, survival outcomes for patients receiving T-lymphocyte–depleted grafts are similar to those receiving replete HLA-matched grafts.[4,5] Although overall survival may be similar, there are other risks associated with fully matched, and with T-lymphocyte–depleted transplants. The risk of significant acute graft-versus-host disease (aGvHD) remains for patients receiving matched stem cell sources,[6] an unwanted consequence with no survival advantage in patients with primary immune deficiencies. The risk of viral reactivation continues to complicate outcomes following T-lymphocyte–depleted transplants.[5] Laberko and colleagues, in the biggest reported primary immune deficiency series to date transplanted using the TCRαβ/CD19–depleted grafts, describe outcomes on 2 groups of patients: those receiving matched unrelated donor and those receiving related parental haploidentical stem cells. Ninety-eight patients with combined or other non-T-lymphocyte–related immune deficiencies are reported. Two significant patient groups are omitted from the report: those patients with severe combined immune deficiency and those with Nijmegen breakage syndrome, because of unique problems of transplant pertaining to these groups; the results of these transplants will be important to consider in the future. In an attempt to avoid sequelae of significant aGvHD, the largest group of patients received T-lymphocyte–depleted peripheral blood stem cells from matched or slightly mismatched unrelated donors, whereas the rest received haploidentical parental grafts. Outcomes between the 2 groups were identical in terms of overall survival, which was excellent at 86%, primary graft failure, aGvHD, immune recovery kinetics, and importantly, incidence of viral reactivation, particularly in those with combined immune deficiency, which was high with significant clinical consequences in both groups, and the coequal cause of death with bacterial infection.

Several lessons can be synthesized from this study. First, it is confirmed that survival outcomes of T-lymphocyte–depleted transplants for primary immune deficiency patients are equivalent to outcomes using other donors. Second, as the authors state, the outcomes are equivalent in all examined parameters for T-lymphocyte–depleted unrelated donor or haploidentical related donor transplants. In the pediatric setting, given the ready availability of medically fit parental donors, these are to be preferred, because they are more likely to harbor viral-specific T lymphocytes active against viruses carried by the patient; the cost of sourcing and manipulating the graft is less than for unrelated donors, and there are more quickly available. However, given that the results are equivalent, for older patients, or other situations where medically fit related donors may not be available, transplant physicians may be confident that the use of T-lymphocyte–depleted mismatched unrelated donors, although more costly, leads to extremely good outcomes. The use of haploidentical donors is increasing in the field of hematopoietic stem cell transplantation.[7] This report, with others,[5] highlights the ongoing risk of significant or fatal viral reactivation, even when parental donors are used, despite the infusion of NK cells and TCRγδ T lymphocytes within the graft. Despite the excellent overall survival results, further strategies to reduce the risk of viral reactivation are required. The use of donor or third-party viral-specific T lymphocytes,[8] CD4/CD45RA$^+$–depleted donor lymphocyte infusions,[9] or the infusion of icaspase 9 genetically modified "suicide" T lymphocytes[10] have all been reported. Randomized controlled trials are now required to determine which, if any, of these strategies will improve outcomes of T-lymphocyte–depleted transplants still further, reducing