Comment on Fahs et al, page 3706, and Everett et al, page 3697

In search of the source of factor VIII

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The cellular site of synthesis of factor VIII in the circulation has long been disputed, but 2 papers in this issue of *Blood* by Fahs et al[1] and Everett et al[2] finally identify the cell type that makes factor VIII in the liver and, by implication, in the rest of the body.

To understand why this quest was so prolonged, one needs to go back to the technologies of the 1970s and 1980s. Factor VIII is a cofactor for conversion of factor X to factor Xa by activated factor IX and is the rarest of all the clotting factors in blood. Not surprisingly, it was the last but one of the classical blood coagulation factors to yield to the advance of molecular biology (in 1984, 2 years before tissue factor and von Willebrand factor). To purify enough protein for biochemical characterization was beyond the limits of technology available to researchers up to 1980. Then, progressively new techniques enabled even the rarest of proteins to be isolated. Thus, factor VIII was purified and sequenced and its gene cloned.[3] Earlier efforts to localize the organs making factor VIII showed that the liver of a normal dog transplanted into a dog with hemophilia corrected the bleeding tendency by elevating the dog's blood factor VIII level up to 50% of normal.[4] In the reverse experiment, a normal dog with a hemophilic liver still maintained a factor VIII level of 50%. Later, we found that a hepatocyte-rich cell fraction from human donor liver contained factor VIII messenger RNA and factor VIII antigen,[5] but there remained lingering doubt about the actual cell type involved. Studies in rodents showed that liver endothelium contained factor VIII antigen,[6] and the original cross transplantation work demonstrated that cells other than hepatocytes must be able to make half the factor VIII circulating in blood.

Recently, it has been shown convincingly that factor VIII activity and antigen are confined to cells sorted individually from liver that carry markers restricted to endothelium.[7] The papers published in this issue[1,2] complete the story using sophisticated cellular biology methods not even dreamt of in the 1980s. In one study using a Cre/*lox*-dependent conditional knockout model, hepatocyte-specific factor VIII knockout mice are indistinguishable from controls, whereas efficient endothelial knockout models display a severe hemophilic phenotype with no detectable plasma FVIII activity. In the other study, *Lman1* conditional knockout mice were generated to characterize the FVIII secretion profiles of endothelial cells and hepatocytes. Because *Lman1* is essential for export of factor VIII from cellular sites of synthesis, it was again shown that only if endothelial cells have no Lmann1 does the factor VIII level fall and hence that they are the primary biosynthetic source of murine FVIII. Conversely, when hepatocytes made no Lmann1, the plasma FVIII pool was unaffected. Taken together, these studies conclusively demonstrate that hepatocytes have no role in factor VIII biosynthesis, a role that is fulfilled by endothelial cells in the liver and elsewhere.

This result must raise the question of why hepatocytes synthesize every other circulating factor necessary for clot formation yet specifically omit one crucial cofactor in the enzymatic cascade leading to thrombin formation and conversion of fibrinogen to fibrin. Several explanations come to mind. Perhaps it is dangerous to have a complete cascade in one cell, with risk of intracellular fibrin formation and adverse consequences for hepatocytes. Against this is the observation that expression of factor VIII at high levels in the hepatocytes of mice receiving gene transfer had no such effect.[8] Alternatively, it may be that having factor VIII made throughout the circulation in cells that also make the carrier molecule von Willebrand factor is efficient and also allows for localized targeted release that occurs when those cells are damaged or in response to certain physiological stimuli such as norepinephrine and exercise. Whatever the evolutionary explanation may be, it is satisfying to know at last the true source of factor VIII.

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Comment on Spoerl et al, page 3832

JAK inhibitors: a home run for GVHD patients?

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In this issue of *Blood*, Spoerl and colleagues demonstrate a promising strategy to control graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (HSCT) by using a Janus kinase (JAK) inhibitor in both mice and humans.[1]

GVHD is mediated by cytotoxic T-cell effectors and dysregulated inflammatory cytokines and remains a major cause of morbidity and mortality after allogeneic HSCT. Corticosteroids are the mainstay of GVHD therapy but no standard salvage therapy has been established for corticosteroid refractory GVHD. Spoerl and colleagues report that JAK1/2 inhibitor ruxolitinib is effective to control GVHD.[1]

JAK inhibitors block JAK–signal transducer and activator of transcriptional factor (STAT) signaling and are approved for the treatment of myelofibrosis.[2,3] JAK inhibitors relieve symptoms related to an excess of proinflammatory cytokines in patients with myelofibrosis, regardless of JAK2 mutational status. In light of the inflammatory nature of GVHD, would JAK inhibitors regulate GVHD? JAK-STAT pathways are also expressed in T cells and therefore also play an important role in regulation of the immune system. T cells are activated in a 3-step process by receiving 3 sequential signals from antigen-presenting cells (APCs). Signal 1 is mediated through T-cell receptor binding to major histocompatibility complex molecules. Signal 2 is the costimulatory signal. Signal 3 is the polarizing signal from APCs, such as cytokines that determine the fate of T-cell differentiation into effector cells. In T cells, JAK1/2 relays the signaling function of many inflammatory cytokines with relevance for GVHD, including interferon-γ (IFN-γ), interleukin-2 (IL-2), IL-6, IL-12, and IL-23 (see figure).

Spoerl and colleagues show that inhibition of JAK1/2 signaling by ruxolitinib reduces GVHD in a mouse model by inhibiting donor T-cell expansion and inflammatory cytokine production, and favoring regulatory T-cell (Treg) differentiation.[1] Based on these observations in mice, the authors then evaluated whether their findings can be translated into the clinic. Six patients with steroid refractory GVHD that had been heavily pretreated were treated with ruxolitinib. All patients are reported to have responded to ruxolitinib with improved GVHD grades and corticosteroid-sparing effects. Despite the small number of patients in this single-center study, the quick and profound responses to ruxolitinib are very impressive.

This study will pave the way to developing a novel therapeutic strategy of GVHD. However, there are many questions remaining to be addressed. First, it has been shown that ruxolitinib also potently impairs APC functions.[4] It remains unclear whether reduction of GVHD is chiefly mediated by T-cell suppression, Treg generation, or APC inhibition. Furthermore, mechanisms of GVHD suppression in patients are unclear; clinical study focuses on treatment of steroid refractory GVHD, whereas the mouse study evaluates prevention of GVHD without steroids. Second, accumulating evidence indicates that ruxolitinib exerts substantial immunosuppressive activity in patients with myelofibrosis, with increases in hepatitis B virus activation and opportunistic infections.[5] JAK-STAT pathways are also essential for cytokine-mediated hematopoiesis; thrombocytopenia and anemia are the major adverse effects of ruxolitinib in myelofibrosis. Thus, use of ruxolitinib after allogeneic HSCT can be a double-edged sword. In this study, the lower ruxolitinib dose of 10 to 20 mg per day was used compared with a standard dose of 30 to 40 mg per day used in myelofibrosis trials.[2,3] Further studies to determine optimal dose and duration of the therapy are warranted to allow better GVHD control, while avoiding profound immunosuppression and cytopenias in allogeneic HSCT patients who have a fragile hematopoiesis and immune system. Finally, ruxolitinib blocks multiple cytokine signaling and affects multiple processes of immunity. However, compared with ruxolitinib, corticosteroids appear to exert much broader effects on multiple cell lineages, while calcineurin inhibitors pinpoint a target downstream of IL-2 signaling in T cells. Better understanding of the difference in mechanisms of action of these 3 classes of immunosuppressants should lead to optimal utilization of JAK inhibitors in the prophylaxis and treatment of GVHD. JAK1/2 selective inhibition spares the IL-2–JAK3–STAT5 signal that is critical for proliferation and survival of Tregs (see figure), but this beneficial effect may be abrogated when combined with other immunosuppressants.[6] Thus, this study suggests that JAK inhibitors may have activity in GVHD and future studies will have to address the efficacy of this approach, when used alone and in combination with other classes of immunosuppressants.

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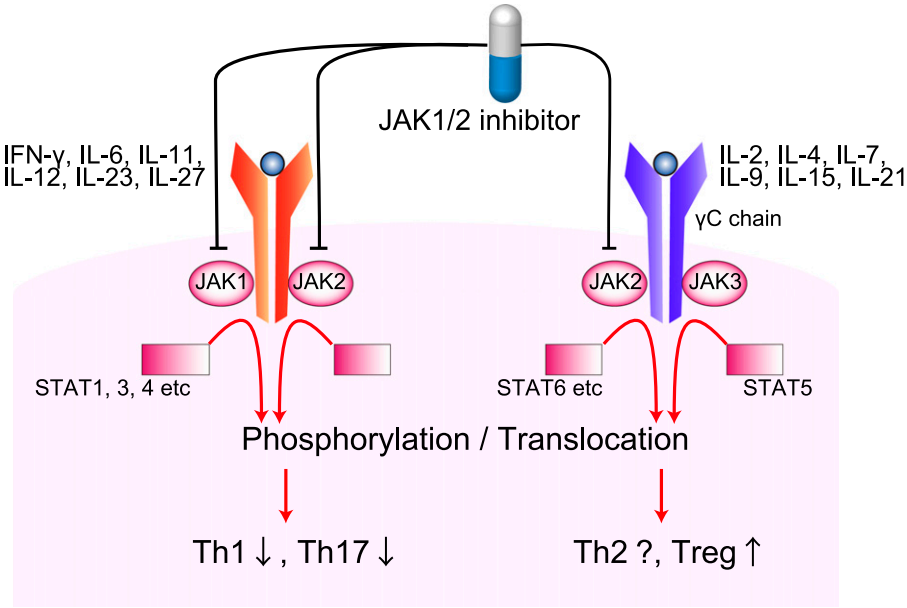

Proinflammatory cytokines, such as IFN-γ, IL-6, and IL-12, ignite serial phosphorylation of JAK1/2, cytokine-receptor chains, and STAT1/3/4. Phosphorylated STATs form heterodimers or homodimers translocate to nucleus, leading to the transcriptions of Th1- or Th17-related genes. On the other hand, γC subunits of IL-2, IL-4, IL-7, IL-15, and IL-21 receptors are associated with JAK3 and STAT5 signaling. JAK1/2 selective inhibition spared the IL-2–JAK3–STAT5 signal and therefore may spare Tregs. Th, T helper.

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