Comment on Weeterings et al, page 3227

FVIIa: you’ve come a long way, baby!

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In this issue of Blood, Weeterings and colleagues report that binding to the GPIb/IX/V complex plays a role in mediating the platelet surface procoagulant effects of rFVIIa.

In 1997, our first article on the mechanism of action of recombinant activated factor VII (rFVIIa) in hemophiliacs was summarily rejected by Blood because the reviewers felt that the topic was not of interest to a general hematology audience. However, in the ensuing years, FVIIa has become a hot topic, not only in hemophilia but also for its off-label use to manage intractable hemorrhage in a wide variety of clinical settings. Even with all of this interest, the mechanism of its hemostatic effect is still not fully understood.

Plasma-derived FVIIa was first used in 1983 to provide hemostasis in 2 hemophiliacs with inhibitors. In 1996, rFVIIa was approved in the European Union and in 1999 in the United States for use as a “bypassing agent” in patients with hemophilia A or B (FVIII or FIX deficiency) and an antibody inhibitor. The rationale for FVIIa use was that FVIIa would drive activation of factor X (FX) by FVIIa/tissue factor (TF) in the absence of the “intrinsic” FX-activating complex (FIXa/FVIIIa), thus bypassing the need for FVIII or FIX. In light of the “cascade” model of hemostasis as shown in the first figure, this seemed a reasonable hypothesis. However, it soon became clear that the situation was not that simple. Very high levels of FVIIa were required for hemostatic efficacy, higher than needed to saturate TF binding. Thus, a debate ensued over whether the mechanism of FVIIa activity was “TF-dependent” or “TF-independent.”

Bom and Bertina demonstrated that FVIIa can activate FX on a negatively charged phospholipid surface independent of TF, and Rao and Rapaport initially suggested that this might underlie the hemostatic activity of FVIIa in hemophilia. However, several years later, they reported that FVIIa can compete with zymogen FVII for binding to TF and increase procoagulant activity by forming active FVIIa/TF rather than FVII/TF complexes. This competition could explain why unexpectedly high levels of FVIIa might be needed, even if it acted via a TF-dependent mechanism.

During this time, the understanding of hemostasis was evolving. While the FVIIa/TF pathway was viewed as critically important to initiating hemostasis, the “intrinsic” pathway was recognized as operating specifically on the platelet surface to propagate the burst of thrombin generation required for clot formation. Generation of activated factor X (FXa) on TF-bearing cells cannot necessarily make up for a lack of FXa on platelet surfaces. Thus, since the defect in hemophilia is in platelet surface FXa generation, it seemed logical that high-dose FVIIa might act to remedy this defect. Our group demonstrated that FVIIa binds to activated platelets independent of TF and partially restores thrombin generation in an in vitro model of hemophilia (see second figure). We proposed a platelet surface mechanism in which FVIIa binds to phosphatidylserine on the membrane of activated platelets, explaining the localization of FVIIa activity to a site of injury. A report by van’t Veer et al countered this by providing evidence in support of the Rao and Rapaport hypothesis. They demonstrated that plasma levels of unactivated FVII delayed thrombin generation in a model of hemophilia, and FVIIa could overcome the inhibition. However, this competitive effect of FVIIa saturated at around 10 nM while the platelet surface effect was not saturated at 250 nM. The finding that the efficacy of FVIIa in patients with hemophilia was increased by dose escalation to levels of 100 nM or more tended to support the platelet surface mechanism as did the finding that FVIIa variants with increased TF-independent activity had increased efficacy in animal models. While not ruling out a TF-dependent effect, the current evidence suggests that FVIIa efficacy in hemophilia is due in large part to a platelet surface mechanism.

Now Weeterings et al have opened another chapter in the pursuit of the mechanism of action of FVIIa by demonstrating the involvement of the GPIb/IX/V complex in its platelet surface activity. While FVIIa can bind to a negatively charged lipid membrane, the presence of GPIb/IX/V enhances binding and procoagulant activity. It is tempting to speculate that GPIb/IX/V tends to localize FVIIa quite specifically to platelets. However, the details of the mechanism of FVIIa are still not completely clear. It is important to build on this new model since, as the authors note, a better understanding of the mechanism will allow the development of more active
Comment on De Groot et al, page 3303

Tregitopes switch on Tregs

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In this issue of Blood, De Groot and colleagues report the identification and functional characterization of Tregitopes, which are Treg-activating regions in the Fc portion of the IgG molecule. This important finding has the potential to bring understanding about a number of phenomena related to Ig, including tolerance to Ab variable regions, the tolerogenic properties of immunoglobulin–Ag conjugates, the weak immunogenicity of Fc fusion proteins, and the therapeutic and regulatory effects of clinical preparations of IVIg on autoimmune and inflammatory diseases.

Immunoglobulin (Ig) has long been known to have tolerogenic properties. Thus, antigens (Ags) conjugated to Ig elicit tolerance rather than immunity, and intravenous administration of pooled Ig from multiple donors, known as intravenous immunoglobulin (IVIg), is used in clinical practice to treat autoimmune and inflammatory diseases. The reason for these tolerogenic effects of Ig is not understood, but recently IVIg has been shown to enhance human regulatory T cells (Tregs). This, together with the observation that Fc fusion proteins of soluble receptors and other bioactive molecules are either poorly or nonimmunogenic, and antibody (Ab) variable regions (to which central tolerance should not exist) do not elicit robust autoimmune responses, led De Groot et al to postulate that the Ig molecule must contain regions or epitopes that are stimulatory to Tregs (i.e., Tregitopes).

Using computational epitope mapping, the authors looked for consensus 9 amino acid regions in the human Ig molecule that would bind to multiple HLA class II molecules (on the premise that most Tregs are CD4-restricted). They identified 2 such clusters of major histocompatibility complex (MHC) binding motifs in the Fc molecule that could be presented to T cells. Predicted human Tregitope (hTregitope) sequences 167 and 289 were synthesized and were indeed shown to bind to multiple MHC class II molecules. Using a variety of Ags and culture conditions, the authors presented evidence that these Tregitope peptides activate as well as expand Tregs. The authors conclude that both natural Tregs (nTregs) and Ag-specific adaptive Tregs are affected. However, due to limitations of the experimental setup and the complexities of the human system, the distinction between effects on natural versus adaptive Tregs (as in humans, CD4+CD25high cells are a mixture of both) and between the expansion of preexisting FoxP3+ cells versus their de novo conversion from conventional T cells is not always clear.

In the next step, the functional effects of Tregitopes on Ag-induced cytokine production and surface activation markers are documented using depletion experiments and Ag-MHC tetramers. The authors use a pool of immunogenic peptides derived from the complement component C3d (an autologous
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