surface of platelets of thrombocytopenic VWD2B mice, suggesting that VWF binding to platelets is needed to induce thrombocytopenia. These VWF-platelet complexes are taken up efficiently by macrophages in liver and spleen, thus accelerating their clearance. Interestingly, increase of circulating levels of the conformationally active form of type 2B VWF also promotes VWF binding by macrophages. The presence of increased levels of conformationally active VWF has also been demonstrated in other acquired conditions with thrombocytopenia (eg, thrombotic thrombocytopenic purpura) with increased uptake of platelets by macrophages. This could be a protective mechanism to remove VWF-platelet complexes, preventing the risk of occlusion of microvasculature in VWD2B. Macrophage depletion leads to a two- to threefold increase of platelet counts in thrombocytopenic mice with the V1316M mutation, characterized by the most severe thrombocytopenia. Macrophage depletion has also been demonstrated to determine platelet increase in a mouse model for immune thrombocytopenia, confirming the importance of these cells in the clearance mechanisms of platelet-containing complexes.

Thus, thrombocytopenia in VWD2B appears to be the result of at least a combination of shortened survival of the abnormal platelets and of an accelerated clearance of the abnormal VWF-platelet complexes by macrophages in liver and spleen. VWD2B appears to be not only a disorder of a plasma hemostatic protein but also of platelets, suggesting that in addition to VWF/factor VIII concentrates, transfusion of normal platelets may be justified in VWD2B patients with bleeding and worsening of their thrombocytopenia due to hemostatic stress situations. Because increased clearance of mVWFs appears to be more frequent than expected in VWD, the model developed by Casari and colleagues could be used to investigate whether mVWFs with accelerated clearance have a preferentially increased uptake by macrophages through enhanced binding to special receptors (eg, low-density lipoprotein receptor–related protein 1, LR1P1), thus highlighting the role of specific VWF regions critical in this regard.

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**THROMBOSIS & HEMOSTASIS**

Comment on Lechtenberg et al, page 2777

**Assembling the machinery of coagulation**

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In this issue of *Blood*, Lechtenberg et al determine the structure of a re-engineered prothrombinase complex built from proteins originally identified in the venom of the Australian eastern brown snake (*Pseudonaja textilis*).1

A pivotal step in coagulation is the production of thrombin by the prothrombinase complex. The structure of this key enzyme system has, however, long eluded detailed characterization, hampering the development of therapeutic pro- or anticoagulants. The prothrombinase complex represents the engine room of the coagulation system, producing a cloud of active thrombin around the site of tissue injury.2 At a general level, the interaction of factor Va (a large multidomain protein) with the serine protease factor Xa on the membrane surface results in formation of the prothrombinase complex (see figure). The prothrombinase assembly then binds to and cleaves prothrombin at 2 distinct sites (Arg 320 and Arg 271), culminating in the release of soluble active thrombin into the surrounding milieu and promoting the formation of a clot.2

Prothrombinase has presented an exceptionally challenging target for crystallographers, not least because of the lability of the complex and the absolute requirement of phospholipids for complex assembly. Further, electron microscopy experiments have only yielded low-resolution data to date.3 Here Lechtenberg and colleagues neatly surmount these problems and determine the structure of a re-engineered form of psuetarin C, a preassembled soluble snake venom prothrombinase.1 The actual structure determined is between the 2 *P textilis* components in their preactivated state: factor V in complex with a truncated form of factor X. In an elegant series of experiments, the authors validate the relevance of their system, showing that the relevant forms of psuetarin C that they investigate are capable of cleaving and activating human prothrombin. The resulting structure provides an exceptional series of insights into the
equivalent human system. Broadly, factor X (and by implication Xa) nestles into the interface between 2 key domains of factor V (and again by implication Va): the A2 and A3 domains. An extraordinary feature of the interaction is, however, an extended sequence in the factor V component that reaches out like an embracing arm to encircle the factor X protease domain. Through a series of modeling experiments, the authors then demonstrate the implications of their structure for the human system. Both simple and sophisticated concepts emerge.

At the basic level, the data reveal how factors Va and Xa essentially rise to the same height off the membrane level, thus positioning key surfaces that must interact with one another in the correct locale (see figure). Crucially, the major interactions between the A2 domain of factor V and factor X as seen in the structure are consistent with previous biochemical observations. Most notably, these data explain why cleavage and removal of the A2 domain by activated protein C will inevitably lead to complex dissociation and prothrombinase inactivation.

A key question that remains to be fully understood relates to how prothrombin is captured and cleaved by the prothrombinase complex. Lechtenberg et al, however, are able to interpret their structure to suggest a logical position for the prothrombin component (Figure 3 in Lechtenberg et al). Crucially, the position of the prothrombin protease domain is dictated by the location and bearing of the active site of the factor X moiety. These data suggest that to be placed appropriately, an extended loop sequence (the a1 loop) likely functions to capture the prothrombin target. Electrostatic complementarity further suggests that prothrombin must be orientated such that its Arg 320 site is cleaved first, consistent with biochemical insights. Following cleavage at Arg 320, the partially processed intermediate moiety (termed meizothrombin) undergoes the classical rearrangement from thezymogen to the protease (active) form. It is suggested that this conformational change drives repositioning of meizothrombin with respect to the prothrombinase complex such that the Arg 271 site can be cleaved. The latter event releases the active thrombin protease into the surrounding environment (see figure).

The current structure finally provides satisfying insight into how the prothrombinase complex assembles and functions to cleave prothrombin. Exciting additional questions now arise, however. These include addressing how the human system is controlled through factor Va interaction with phospholipids and, furthermore, understanding the precise molecular details of how the prothrombin substrate interacts in 2 distinct positions with the prothrombinase complex.

Snake venoms have long been exploited as a rich source of factors of utility in investigating the coagulation system. Here, the weapon of the enemy has once again proved central to understanding human biology. Together, the system developed by the authors of the present study provides a compelling and adaptable platform to determine the complete ternary complex between prothrombinase and prothrombin.

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Assembling the machinery of coagulation

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