This would represent one way of formally testing whether leukocytes were binding directly to platelet–UL-VWF strings in vivo.

An alternative hypothesis to explain elevated recruitment of leukocytes may be that the binding of platelets to UL-VWF strings over the surface of the endothelium serves to further activate the endothelium and induce the expression of cell surface adhesion molecules to which circulating leukocytes bind. This may be a more favorable possibility given the elevated numbers of macrophages in the lesions of Adamts13−/−/ApoE−/− double-deficient mice (compared with ApoE−/− littermates), suggesting that the bound leukocytes are interacting with and transmigrating across the endothelium. Thus, in the absence of ADAMTS13, endothelial-bound UL-VWF strings mediate platelet recruitment to the arterial wall, which as a result deliver certain platelet chemokines and growth factors that provide an additional proinflammatory stimulus to the arterial wall (see figure). Testing such a hypothesis will, however, require careful experimental design.

A further question that arises from the studies of Gandhi et al is the dependency of ADAMTS13 concentration on the proinflammatory effects of UL-VWF strings. The authors compared Adamts13+/+ with Adamts13−/− mice (and not Adamts13+−/− mice) on the ApoE−/− background. Consequently, it remains unclear whether there is a linear relationship between ADAMTS13 concentration and VWF string-dependent leukocyte adhesion, or whether there is a threshold effect in which even very low ADAMTS13 concentrations can protect against the observed recruitment of leukocytes. In ADAMTS13-deficient mice, platelet-decorated UL-VWF strings can be visualized over the activated endothelium by intravital microscopy, whereas such strings seem to appear only transiently in Adamts13+− or Adamts13+−/− mice. A recent study by de Maeyer et al reported platelet–UL-VWF string survival time over the activated endothelium of ~5 seconds in Adamts13+−/−, whereas this was ~13 seconds in Adamts13+− mice. These results might suggest that even a small increase in lifetime of a platelet–UL-VWF string could potentially translate into a proinflammatory stimulus. Whether changes of platelet–UL-VWF string lifetime occur as a function of ADAMTS13 plasma concentration across the normal range in humans will be key to determining whether the proinflammatory effects of VWF strings are pertinent to the pathogenesis of human vascular diseases.

Although there is increasing evidence that ADAMTS13 plasma concentration is inversely correlated with the risk of both myocardial infarction and stroke, these data really measure the risk of an occlusive coronary or cerebral thrombotic event rather than the development of the vascular disease that might precipitate such clinical sequelae. Is there any clinical evidence, therefore, to support the involvement of VWF strings in atherogenesis or vascular disease? In a study by Srámek et al examining atherosclerotic plaque development in normal individuals and in patients with type 3 von Willebrand disease, the authors reported no difference in the plaque score between these groups, suggesting that plaque development occurs independently of VWF strings in humans. However, it must be considered that human atherosclerosis is a far more complex and multifactorial disease than the monogenic ApoE−/− mouse model of atherosclerosis, which makes isolating the contribution of VWF strings to the progression of this disease in humans very difficult. Consequently, further studies will be necessary to better define any involvement of endothelial UL-VWF strings in human disease. This work by Gandhi et al provides an excellent setting to understand how modulation of VWF function by ADAMTS13 over the endothelial surface may influence the proinflammatory conditions in different vascular diseases. This will also provide further clues as to how ADAMTS13 may impact on the vasculature in a manner distinct from its comparatively well-described role in modulating primary hemostasis.

**REFERENCES**


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**Comment on Olson et al, page 2187**

**Killing 2 proteinases with 1 (dual-acting) stone**

**Sriram Krishnaswamy CHILDREN’S HOSPITAL OF PHILADELPHIA**

In this issue of *Blood*, Olson et al establish the biochemical basis for the specificity and selectivity of a dual-action anticoagulant with the designed feature of rapid neutralization, making it extremely attractive for use in anticoagulated patients if surgery is warranted.

The endogenous inhibitory and anticoagulant reactions of antithrombin III, tissue factor pathway inhibitor, and activated protein C highlight the effectiveness of targeting multiple steps in the blood coagulation cascade to limit the clotting response. Olson et al apply...
an analogous concept to inhibit both thrombin function and its formation with a bifunctional inhibitor targeting thrombin and factor Xa, the protease required for prothrombin activation. Although their approach mirrors the multireactant targeting strategy of endogenous regulation, the twist here is that the bifunctional EP217609 molecule inhibits its 2 targets by different mechanisms.

EP217609 contains a fondaparinux-like component fused through a spacer bearing a biotin moiety to a derivative of N-α-(2-naphthylsulfonylglycyl)-4-amidinophenylalanine piperidide (NAPAP). The fondaparinux-like functionality is intended to bind endogenous antithrombin III with high affinity and enhance the suicide inactivation of factor Xa. On the other hand, the NAPAP analog is intended to reversibly bind to the active site of thrombin and inhibit function. The biotin moiety provides a potential handle for the neutralization of the inhibitory properties of EP217609 by abstraction through high-affinity ligation with avidin. Olson et al’s design draws on the established efficacy of heparin pentasaccharide derivatives as anticoagulants without many of the drawbacks of unfractionated heparin but also seeks to resolve the problem of “thrombin rebound” associated with the cessation of heparin and pentasaccharide therapy.

The cleverest of intentions in designing novel inhibitors can be laid to waste by the complexities of coagulation proteinase enzymology. For example, there is no reason to imagine that specificity, selectivity, or other beneficial features of the individual functional groups will be preserved in the fusion construct. There is also the question of whether the NAPAP-like moiety can effectively inhibit thrombin when EP217609 circulates bound to antithrombin III. Despite these qualifications, the approach seemingly works! A prior version of such a fusion–lacking biotin has shown to be a superior anticoagulant in comparison to its analog molecules in animal models of arterial and venous thrombosis and also to prevent “thrombin rebound.” A phase 1 study has reported EP217609 to be well tolerated in healthy subjects with neutralization of its anticoagulant effects after the administration of avidin. The drug is now in phase 2 clinical development as therapeutics. Resolution of the antithrombin conformational change contribution to heparin rate enhancement.

REFERENCES

Related or unrelated donor

John A. Hansen Fred Hutchinson Cancer Research Center

In this issue of Blood, Kanda et al have analyzed data from the Japanese Hematopoietic Cell Transplant Registry to address an important question about the risks associated with hematopoietic cell grafts from HLA 1-antigen mismatched related donors contrasted with 8 of 8 HLA allele matched unrelated donors.™

The study reported by Kanda and colleagues addressed the question of whether a serologically defined 1-antigen HLA-A,B, or DRB mismatched related donor should be preferred to an “8/8” HLA-A,B,C,DR allele MUD. The study population consisted of 327 1-HLA antigen mismatched related donor transplant recipients and 452 8/8-HLA allele matched unrelated donor transplant recipients accessed through the Japanese Transplant Registry Unified Management Program (TRUMP), which includes data from the Japan Society for Hematopoietic Cell Transplantation (JSHCT) and the Japan Marrow Donor Program (JMDP). Patients underwent transplantation for the treatment of acute myeloid leukemia, acute lymphoblastic leukemia, chronic myeloid leukemia, or myelodysplasia between January 2001 and December 2008. Patients received T cell–replete marrow or blood stem cells and 90% to 97% received a conventional cyclosporine– or Tacrolimus–based regimen for GVHD prophylaxis. The related and unrelated cases differed for certain
Killing 2 proteinases with 1 (dual-acting) stone

Sriram Krishnaswamy