there would be reluctance to advocate long-term primary prophylaxis, this should certainly be offered at times of additional high risk, such as after surgery, immobility, or pregnancy. Clinically, the issue of thrombophilia testing and management is more relevant in the setting of patients who have experienced an event already. If testing has been performed and high-risk thrombophilia has been identified, this should certainly be taken into account when deciding on extended anticoagulation, especially for spontaneous events. The issue of whether all patients with a DVT should be screened for high-risk thrombophilia is unresolved but, for those with a spontaneous event at a young age and a positive family history, this should be considered. Definition of a positive family history is difficult, but the suggestion offered in this paper of more than 20% of relatives affected is not evidence-based and would be dependent on relatives being available for study.

Any decision on whether to offer long-term anticoagulation will depend on the risk of bleeding while on anticoagulants as well as the thrombotic risk. This study reports a very low annual bleeding risk at 0.29% but with wide confidence intervals, because it is based on only 2 events. The authors speculate that this may be because the thrombophilic defect reduces the bleeding risk, and this observation certainly requires confirmation. Alternative explanations are the young age of the cohort, the fact that the patients are cared for by expert centers, and the small number of events.

Conflict-of-interest disclosure: The author declares no competing financial interests.

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ADAMTS13’s tail tale

Karen Vanhoorelbeke, Hendrik B. Feys, and Simon F. De Meyer

In mice, a long form and a short form of the VWF-cleaving protease ADAMTS13 have been identified, the latter lacking the 4 distal carboxyl-terminal domains. While these are not strictly required for regulating normal size distribution of VWF multimers, in this issue of Blood, Banno and colleagues reveal the role of the domains in down-regulating thrombogenesis in vivo.

Since the discovery of ADAMTS13 as a metalloprotease with a multi-domain structure, numerous studies have attempted to shed light on the specific roles of each of the ADAMTS13 domains in digesting large von Willebrand factor (VWF) multimers into smaller, less reactive ones. ADAMTS13 is composed of a signal peptide, propeptide, metalloprotease domain, central TSR (thrombospondin type 1 repeat), Cys-rich region, spacer domain, 7 additional TSRs, and 2 CUB domains. The active site of this enzyme is situated in the metalloprotease domain while the spacer domain plays a crucial role in substrate binding by interacting with a VWF exosite located at the C-terminus of the A2 domain. The exact physiologic significance of the carboxyl-terminal TSRs and the 2 CUB domains still remains unclear, in particular due to the use of different types of in vitro tests, often performed under nonphysiological conditions.

To unravel the in vivo role of the carboxyl-terminal domains of ADAMTS13, Banno and coworkers elegantly take advantage of the presence of 2 kinds of ADAMTS13 genes in laboratory mouse strains. The 129/Sv strain has the ADAMTS13 gene encoding full-length ADAMTS13 while several other strains, including C57BL/6, harbor an Adamts13 gene that expresses a truncated form of the enzyme, lacking the 2 C-terminal TSRs and CUB domains due to the insertion of an intracisternal A-particle retrotransposon. By introgressing the C57BL/6−/−Adams13 gene onto the 129/Sv genetic background, the authors generate congenic mice that had the distal C-terminally truncated ADAMTS13 on a 129/Sv genetic background (Adamts13S^S/S) and use wild-type mice that have full-length ADAMTS13 (Adamts13S^L/L) and ADAMTS13S^S/L mice on the same 129/Sv genetic background for comparison.

The most obvious role of ADAMTS13 is to regulate VWF multimer size. Indeed, ADAMTS13 digests unusually large VWF multimers into smaller less thrombogenic forms, hence preventing the spontaneous intravascular platelet aggregation seen in patients with ADAMTS13 deficiency. Interestingly, Banno et al showed that both Adamts13S^L/L and Adamts13S^S/S mice do not have ultra large VWF multimers in their plasma, implying that the C-terminal domains are not strictly needed for maintaining normal VWF size. Consequently, the 2 C-terminal TSRS and CUB domains are not essential for the removal of ultralarge VWF multimers from the plasma.

Following VWF size regulation, a fascinating role of ADAMTS13 in attenuating thrombus growth has been described, possibly by cleaving VWF multimers that are peripheral to or incorporated in platelet rich thrombi. In this study, Banno et al used the congenic mice to demonstrate that the 2 C-terminal TSRs and CUB domains play a role in the down-regulation of thrombogenesis under high shear conditions. Both in vitro flow chamber experiments at high shear rates and in vivo thrombosis models show that blood from Adamts13S^S/S mice is more thrombogenic. This is evidenced by accelerated thrombus formation and decreased time to occlusion respectively when compared with blood from Adamts13S^L/L mice. Whether this would...
translate into an increased risk of thrombosis in patients having comparable truncated forms of ADAMTS13 remains elusive.

In this article, Banno et al provide the first in vivo insights on the physiological significance of the distal carboxyl-terminal domains of ADAMTS13. The exact mechanism of thrombus size attenuation by ADAMTS13 and, in particular, the specific involvement of the carboxyl-terminal domains still remains to be determined. Does ADAMTS13 digest VWF multimers on the surface of the platelet thrombus or is thrombus size attenuation by ADAMTS13 independent of its VWF-cleaving activity? In this context, it is certainly intriguing that the mechanism of VWF size regulation by ADAMTS13 might be different from that of VWF processing during thrombus growth. Clearly, these new findings provide another impetus in the quest to understand the structure-function relationship of ADAMTS13. Obviously, this is not the end of the tale.

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transplantation

Comment on Kamei et al, page 5041

Scanning for the origins of mHags

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In this issue of Blood, Kamei and colleagues introduce an innovative approach for identifying the genes that encode novel T cell–defined human minor histocompatibility antigens (mHags). In this significant methodologic advance, they demonstrate how the rich human genetic variation data generated for the International Human HapMap Project, together with the available HapMap B-lymphoblastoid cell lines that have undergone extensive genome-wide sequencing, can be used to identify the functional genetic variants responsible for the cellular peptides recognized by selected T–cell clones.

Human minor histocompatibility antigens (mHags) have been recognized as barriers to successful hematopoietic cell transplantation (HCT) from normal donors for more than 30 years.1 Success following HCT is ultimately determined by the ability to achieve sustained engraftment, eradication of abnormal or malignant host cells, and control of graft–versus-host disease (GVHD). Each of these clinical endpoints is influenced by the nature and extent of the genetic disparity between donor and recipient. Graft rejection and GVHD are immune-mediated reactions induced by histocompatibility differences between donor and recipient. GVHD occurs when immune-competent donor T cells are transplanted to an immune-compromised host, and the incompatibility between donor and recipient is sufficient to induce T–cell activation.2 The histocompatibility differences responsible for these T–cell responses are encoded by polymorphic genes located throughout the genome. T–cell recognition of these differences can occur only when the variant peptide in a recipient is foreign to the donor and is appropriately processed and presented at the cell surface by the HLA alleles of the recipient. Polymorphic peptides fulfilling these requirements are called mHags.1,3

Although severe GVHD has an adverse effect on morbidity and mortality, occurrence of GVHD is also associated with lower relapse rates, demonstrating that host reactivity of donort cells can also mediate a significant graft–versus-leukemia (GVL) effect and thereby directly contributes to the curative potential of allogeneic HCT for patients with hematologic malignancy. The GVL effect has become an important model system for exploring new strategies aimed at improving the antitumor potential of T–cell–based immunotherapy. These efforts have largely focused on understanding the mechanisms of GVL and the identification of the molecules that could be the potential targets for T–cell immunotherapy.4,5 Improved techniques for cloning mHag–specific T cells and eluting candidate peptides from major histocompatibility complex molecules in the late 1980s made possible the initial identification of individual mHags. However, the process was difficult, and progress in expanding the library of well-characterized mHags has been slow.

In this issue of Blood, Kamei et al introduce a novel approach for identifying T cell–defined mHag loci using publicly available resources generated by the International HapMap Project and including the B-lymphoblastoid cell lines that were the source of DNA sequenced for the HapMap project and the resulting large dataset of sequence–based genotypes.6-8 These cell lines are publicly available, and once they have been transduced with the appropriate HLA restriction element, they can be tested as targets to determine whether they contain the DNA sequence necessary to encode specific T cell–defined peptides. Mapping of the gene encoding the mHag is accomplished by combining the results of immune–based functional assays with a whole genome association analysis by scanning the known sequence polymorphisms (SNPs) in the vast HapMap database, which currently consists of more than 3 million genetic markers expressed by these reference cell lines. The power and resolution of genetic mapping obtainable with this resource will continue to expand in the future as the numbers of new reference samples sequenced increases, and the racial diversity of the reference panel is broadened. The approach described here by Kamei et al should contribute substantially to the development of a more comprehensive and efficient characterization of mHags. This method may also prove useful for the genetic mapping of other genetic traits.

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