Comment on De Meyer et al, page 5217, and on Gandhi et al, page 5224

Size does matter: VWF in MI

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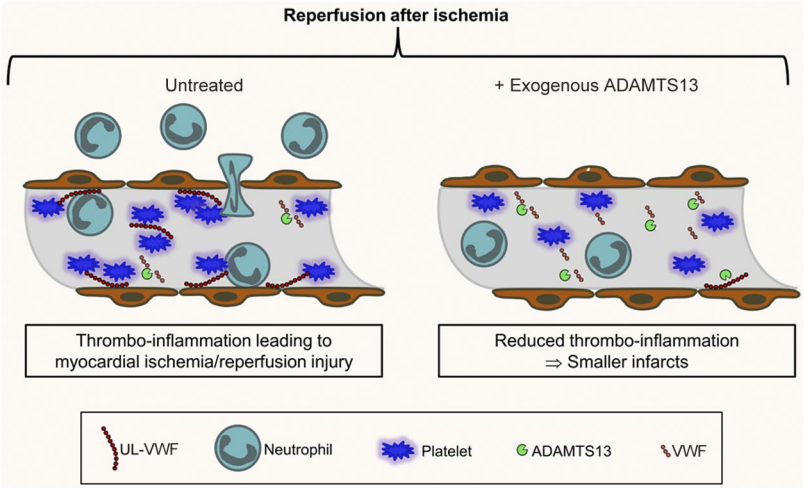
In this issue of *Blood*, 2 independent studies analyzed the relevance of the von Willebrand factor (VWF)–ADAMTS13 axis in a murine model of myocardial ischemia/reperfusion injury. It is demonstrated that genetic ablation, neutralization or increased cleavage of VWF is protective in this experimental setting.[1,2] This strongly supports the concept that interference with the ADAMTS13-VWF axis may be a powerful approach to treat acute thrombo-inflammatory disease states.



ADAMTS13-mediated modulation of VWF activity limits thrombo-inflammatory ischemia/reperfusion injury in the heart. In the ischemic heart, ultra-large (UL)–VWF released from activated or damaged endothelial cells and platelets promotes neutrophil and platelet recruitment that contribute to tissue damage. UL-VWF is cleaved by ADAMTS13 to smaller VWF multimers that circulate in the plasma and are less potent in recruiting neutrophils and platelets. Infusion of exogenous ADAMTS13 reduces UL-VWF levels and infarct size. Similar results were obtained in VWF-deficient mice or mice treated with anti-VWF antibodies. The exact mechanism of how VWF, platelets, and leukocytes promote these processes to induce tissue damage remains to be determined.

Von Willebrand factor (VWF) is a large multimeric protein that is stored as ultra-large von Willebrand factor (UL-VWF) in Weibel-Palade bodies of endothelial cells and in platelet α-granules from where it is released in response to injury or inflammation. Under high shear stress, VWF immobilized on exposed subendothelial collagen or on the surface of activated endothelial cells exposes its binding sites for the platelet receptor glycoprotein (GP) Ib. GPIb-VWF interactions mediate platelet deceleration, thereby allowing other receptors to interact with their ligands to induce cellular activation, firm adhesion, and thrombus formation. This process is crucial to limit posttraumatic blood loss, but also causes myocardial infarction and ischemic stroke, the 2 leading causes of death and severe disability worldwide.

UL-VWF is the most thrombogenic VWF variant and its activity is tightly controlled by the plasma metalloprotease ADAMTS13 that rapidly cleaves it into smaller and less thrombogenic VWF multimers, which circulate in the plasma. This process is essential to prevent excessive intravascular thrombus formation as revealed in patients who suffer from thrombotic thrombocytopenic purpura (TTP), a severe thrombotic disease state caused by acquired ADAMTS13-inhibiting autoantibodies or genetic defects resulting in reduced ADAMTS13 activity.[3] Likewise, increased levels of VWF or decreased levels of ADAMTS13 are associated with an increased risk of ischemic stroke and myocardial infarction.[4] Animal studies have shown that the balance of the VWF-ADAMTS13 axis is a critical determinant of cerebral ischemia/reperfusion injury because absence of VWF[5] or increased levels of ADAMTS13[6] are beneficial, while ADAMTS13 deficiency has detrimental effects on infarct size and neurologic outcome after focal cerebral ischemia.[6,7] Unexpectedly, however, this pathology appears to occur independently of platelet aggregation as anti-GPIIb/IIIa antibodies were not protective in this setting in mice and humans.[8] These data in combination with the observation that cerebral ischemia also elicits a strong inflammatory response with an essential but not fully understood critical involvement of T cells[9,10] led to the concept of thrombo-inflammation as a principal pathomechanism of ischemic stroke.[8] A crucial role of VWF within this concept is further supported by a recent study of Petri et al demonstrating that VWF (and also GPIb) is crucial for efficient leukocyte extravasation during inflammation.[11]

The 2 studies published in this issue of *Blood* now expand the importance of the VWF-ADAMTS13 axis to acute myocardial infarction. In a murine model of cardiac ischemia/reperfusion injury they show that ADAMTS13 deficiency resulted in increased infarct sizes while absence or antibody-blockade of VWF[2] as well as the pre-treatment with recombinant human (rh) ADAMTS13[1] reduced infarct sizes, and consequently, cardiomyocyte apoptosis. Interestingly, this protective effect was associated with reduced neutrophil infiltration into the ischemic myocardium, indicating that VWF activity also determines leukocyte recruitment in cardiac ischemia/reperfusion injury (see figure).[1,2] This suggests that ischemia/reperfusion injury in the brain and in the heart might both be thrombo-inflammatory processes, implying that the pathomechanisms of the 2 leading causes of death might be more similar than previously thought. In the light of the results of De Meyer et al who infused rhADAMTS13 into mice[1] one might speculate that only the UL-VWF (but not the smaller, cleaved VWF multimers) contribute to the pathology of ischemia/reperfusion injury. However, further studies will be required to confirm this hypothesis. Of note VWF neutralizing antibodies[2] as well as rhADAMTS13[1] were infused in parallel with the onset of ischemia that may not exactly reflect the situation in patients with acute myocardial infarction. Therefore, it will be important to test the efficacy of rhADAMTS13 treatment at later

time points during the reperfusion phase. Assuming that these studies will also support the therapeutic use of rhADAMTS13, or similar strategies, modulation of VWF activity appears to be an attractive therapeutic approach to treat myocardial infarction as well as acute ischemic stroke. We hope that this will encourage preclinical studies to translate this promising approach from animals to patients.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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Comment on Arning et al, page 5231

Pediatric stroke and ADAMTS genes

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In a study of 270 children with ischemic stroke and their parents, Arning and colleagues report associations with genetic variants in several genes of the ADAMTS family.[1]

Pediatric stroke is a rare but devastating disease, with substantial short-term mortality that in survivors often leads to lifelong debilitating intellectual and motoric defects.[2] The etiology of pediatric stroke is complex.[3] There is a moderate genetic component, and several genetic abnormalities, particularly in the coagulation system, have been associated with its occurrence. These include common variants such as prothrombin 20210A and factor V Leiden, well-established risk factors for venous thrombosis. It is self-evident that these variants can only contribute to a minor extent to the occurrence of pediatric stroke, given the wide discrepancy between the incidence of pediatric stroke (~ 1 in 20 000) and the prevalence of these variants (~ 1 in 20). Moreover, even for venous thrombosis, where prothrombotic abnormalities usually stand out more than for arterial disease, most carriers of these variants never develop thrombosis. The association with arterial disease for these and other prothrombotic variants is weak, in adults as well as in children, with a nearly negligible risk-enhancing effect.[4] So, there must be more. Because pediatric stroke has also been associated with arterial malformations, sickle cell disease, and infectious diseases, a complex multicausal etiology is likely.

Searching for the cause of a rare disorders with a complex etiology is a daunting enterprise due to the large numbers of patients required to disentangle the various causes. In this issue of *Blood*, Arning and colleagues from several centers in Germany report on a search for genetic causes of pediatric stroke. In a nationwide survey, 270 children with stroke (mean age at the event < 6 years) were included in the study, and analyzed together with their parents. The authors applied the technique of a large number of single nucleotide polymorphisms (SNPs) scattered over the genome, but rather than a genome-wide association study (GWAS) in which patients are contrasted to unrelated healthy reference individuals, they used the family structure in a so-called transmission disequilibrium test (TDT).

The TDT is a clever use of information of relatedness between subjects, in the absence of extended pedigrees allowing true linkage analysis. Because it requires DNA from both parents, it is particularly useful for the genetic analysis of pediatric disorders. First proposed in 1993,[5] the test directly compares the transmission of the candidate allele from a heterozygous parent to the affected child with the expected tranmission probability of each allele (of 50%), and by definition takes household effects into account. Because TDT belongs to the family of linkage analysis and not association analysis, it is not affected by population stratification. Therefore, the results of this analysis are more persuasive than those of previous studies in unrelated individuals.

They succesfully genotyped over 300 000 single nucleotide variants, as well as a large number of variants prevously identified as potential risk factors in adult ischemic stroke. Of the latter, none provided a clear signal, but several variants stood out from the larger group. Four variants were suggestive based on the statistical threshold used, of which 2 were intragenic, in ADAMTS2 and ADAMTS12, with odds ratios close to 2, and minor allele frequencies of 20% to 50%. Moderate associations, below the preset threshold for the discovery, were found for ADAMTS13 and ADAMTS17.

These proteins belong to a class of enzymes called a disintegrin and metalloproteinase with thrombospondin motif (ADAMTS), that contain 18 proteins, ADAMTS1 to ADAMTS19. These proteins share a similar structure, with a metalloproteinase domain, a disintegrin-like domain, and a thrombospondin-type motif. However, they do not have similar function, nor are they located on the same chromosome. Deficiencies of the 2 most well-known proteins in the family even lead to diametrically opposed symptoms: a deficiency of ADAMTS2, which is involved in collagen assembly, leads to Ehlers-Danlos syndrome with hyperelastic skin, hyperflexible joints, and fragile vessel