Transforming the major autoantibody site on ADAMTS13: spacer domain variants retaining von Willebrand factor cleavage activity

Marie Scully

Department of Haematology and National Institute for Health Research Cardiometabolic Programme, UCLH/UCL BRC, London, UK

E-mail: MARIE SCULLY - m.scully@ucl.ac.uk

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Immune thrombotic thrombocytopenic purpura (iTTP) is an acute, rare life-threatening condition associated with antibodies to ADAMTS13, resulting in severe enzyme deficiency, failure of von Willebrand factor (VWF) cleavage, excess platelet-VWF binding and microthrombi formation, resulting in multi-organ damage. iTTP is an immune-mediated condition and antibodies to ADAMTS13 are polyclonal.[1,2] Despite this, and as replicated by a number of groups, nearly 100% of patients demonstrate a specific target region of antibody binding to the spacer domain in the N terminal region of the metalloprotease, ADAMTS13.[3-7] Antibodies can be detected in other ADAMTS 13 domains, typically the TSP 2-8 regions or CUB domains, but to a lesser degree than spacer domain antibodies. Furthermore, spacer antibodies are more likely to inhibit ADAMT13 enzyme activity, as opposed to antibodies in the C terminal region of ADAMTS13, which result in increased ADAMTS13 clearance.[8]

It has been suggested that a possible therapeutic strategy for iTTP would be an ADAMTS13 variant that would prevent antibody binding within the pathogenic region of the spacer domain. As iTTP is a polyclonal antibody response, more than one epitope may need to be considered.

In the current issue of *Haematologica*, Graça and colleagues present a detailed description of the influence of the epitope (R568/F592/R660/Y661/Y665 [RFRYY]),[9] within the spacer domain, the predominant site for autoantibody binding. The capacity for ADAMTS13 to cleave VWF with full-length mutants was reduced in varying amounts. The impact of the variants on autoantibody binding was assessed and compared to that of wild-type ADAMTS13. The comprehensive narrative concludes that non-conservative and alanine modifications of residues RFRYY, within exosite 3 of the spacer domain, were superior in preventing autoantibody binding. Importantly, selected ADAMTS13 variants maintained VWF cleavage mediated by the metalloprotease.

The spacer domain, comprising 130 amino acids, is between a cysteine-rich region and the TSP-2 repeat domain. The region is important and has two major effects. First, the binding to VWF, which is critical in ADAMTS13-mediated cleavage. The importance of spacer domain-mediated cleavage is verified by the resulting proteolytic activity involving the MDTCS compared to MDTC variants. Second, exosite 3, within the spacer domain, contains a cluster of hydrophobic residues. This specific exosite is critical in binding to the A2 region of VWF, but it is also the major epitope for ADAMTS13 autoantibodies.

Residues making up exosite 3 in the spacer domain of ADAMTS13 include Arg660, Tyr661, Tyr665,[7] Arg568 and Phe592.[10] Conservative substitution variants involve replacement of an amino acid within these residues but achieving comparable biochemical properties. A further effect of manipulation of this region is a gain-of-function ADAMTS13 variant. The effect of the gain-of-function variant, previously described, is resistance to ADAMTS13 autoantibodies and increased ADAMTS13 activity.[11] Other variants have been developed, such as alanine modifications, which resulted in reduced ADAMTS13 autoantibody binding but also diminished ADAMTS13 cleavage of VWF.

In their study published in this issue of *Haematologica*, Graça and colleagues generated 42 ADAMTS13 variants, introduced into a full-length ADAMTS13 spacer exosite 3 region. The variants included the gain-of-function fragment, truncated wild-type MDTCS and MDTSC 5x Ala variants as well as conservative, semi-conservative and non-conservative substitutions with asparagine and alanine amino acids. Initially, samples from patients were examined for autoantibody binding against these developed variants, which were compared to wild-type ADAMTS13. Concurrently, each variant was analyzed for its ability to cleave VWF.

Results re-confirmed the predominant binding of ADAMTS13 autoantibodies to the exosite 3 region of the spacer, but antibody binding to the CUB and TSP 2-8 domains was also detected. The ability of the selection of variant ADAMTS13 to resist ADAMTS13 antibody was explored.

The conservative mutants had comparable autoantibody binding, but semi-conservative variants had reduced antibody binding compared to the wild-type spacer domain of ADAMTS13. Variants containing alanine and, to a lesser extent, asparagine were the most successful at preventing ADAMTS13 antibody binding. The gain-of-function mutation only achieved a small reduction in autoantibody binding. However, the 5x Ala full-length mutant (AAAAA) had the best effect in preventing autoantibody binding. Therefore, replacing the spacer epitope with asparagine or alanine amino acids had the greatest influence in averting ADAMTS13 antibodies from binding. The variants were compared to wild-type ADAMTS 13, assessing their ability to cleave VWF. In all the cases, cleavage of VWF was reduced in comparison to the wild-type protein. However, the greatest cleavage was noted in those with conservative and some of the semi-conservative mutants. Single alanine and asparagine changes were associated with the highest ADAMTS13 cleavage activity (Figure 1). VWF cleavage was further confirmed using recombinant VWF in multimeric gels. In general, mutations associated with the lowest ADAMTS13 cleavage activity demonstrated greater antibody resistance.

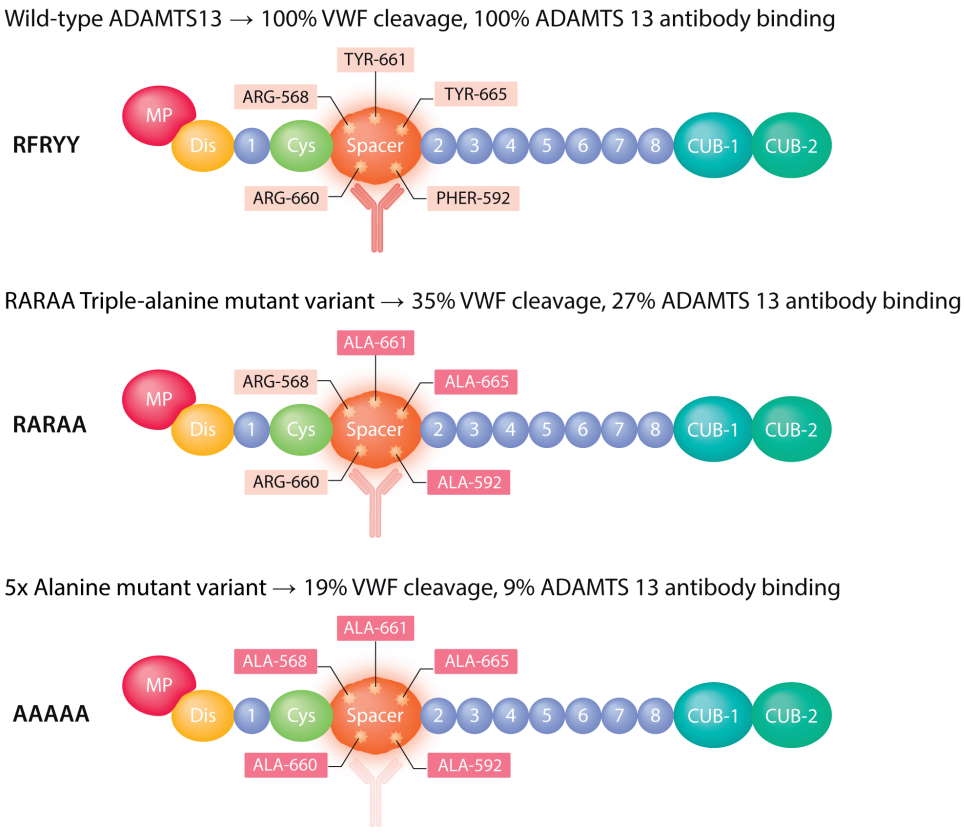

Figure 1. Effect of variants in the exosite 3 region of the spacer domain of ADAMTS13 and their impact on ADAMTS13 autoantibody binding and von Willebrand factor cleavage. The effect of wild-type (WT) ADAMTS13, with 100% ADAMTS13 autoantibody binding involving the amino acid configuration RFRRY, compared to the most productive variants (RARAA and AAAAA), which can reduce ADAMTS13 autoantibody binding but still retain von Willebrand factor (VWF) cleavage activity.

The three aromatic residues rather than the two arginine residues of exosite 3 in the spacer domain appear to have greater importance in ADAMTS 13 antibody binding. The greatest influence was noted with cumulative mutations of the aromatic residues, demonstrating the maximum effect in preventing ADAMTS13 autoantibody binding, which is achieved by re-presenting epitope loops, lowering the surface charge and reducing surface size.[9]

Current therapy for TTP aims to replace ADAMTS13, *via* plasma exchange and immunosuppression to remove autoantibodies to ADAMTS13. The main therapeutic modalities used are steroids and rituximab. Their role in reducing IgG antibody levels has been well described in both the treatment of acute TTP[12] and as prophylaxis,[13,14] usually resulting in normalization of ADAMTS13 activity. The importance of the work by Graca *et al.* is the detailed demonstration and confirmation that development of ADAMTS13 variants could be used to overcome the antibody response in iTTP, preventing autoantibodies to ADAMTS13 from binding to exosite 3 of the spacer domain but ensuring residual ADAMTS13 cleavage activity. There may be a role for these variants in future care of patients, conquering the immunological consequence of ADAMTS13 antibodies.

References

1. Furlan M, Lammle B. Aetiology and pathogenesis of thrombotic thrombocytopenic purpura and haemolytic uraemic syndrome: the role of von Willebrand factor-cleaving protease. Best Pract Res Clin Haematol. 2001;14(2):437-454.
2. Tsai HM, Lian EC. Antibodies to von Willebrand factor-cleaving protease in acute thrombotic thrombocytopenic purpura. N Engl J Med. 1998;339(22):1585-1594.
3. Klaus C, Plaimauer B, Studt JD, et al. Epitope mapping of ADAMTS13 autoantibodies in acquired thrombotic thrombocytopenic purpura. Blood. 2004;103(12):4514-4519.
4. Luken BM, Kaijen PH, Turenhout EA, et al. Multiple B-cell clones producing antibodies directed to the spacer and disintegrin/thrombospondin type-1 repeat 1 (TSP1) of ADAMTS13 in a patient with acquired thrombotic thrombocytopenic purpura. J Thromb Haemost. 2006;4(11):2355-2364.
5. Zheng XL, Wu HM, Shang D, et al. Multiple domains of ADAMTS13 are targeted by autoantibodies against ADAMTS13 in patients with acquired idiopathic thrombotic thrombocytopenic purpura. Haematologica. 2010;95(9):1555-1562.
6. Yamaguchi Y, Moriki T, Igari A, et al. Epitope analysis of autoantibodies to ADAMTS13 in patients with acquired thrombotic thrombocytopenic purpura. Thromb Res. 2011;128(2):169-173.
7. Pos W, Crawley JT, Fijnheer R, Voorberg J, Lane DA, Luken BM. An autoantibody epitope comprising residues R660, Y661, and Y665 in the ADAMTS13 spacer domain identifies a binding site for the A2 domain of VWF. Blood. 2010;115(8):1640-1649.
8. Thomas MR, de Groot R, Scully MA, Crawley JT. Pathogenicity of anti-ADAMTS13 autoantibodies in acquired thrombotic thrombocytopenic purpura. EBioMedicine. 2015;2(8):940-950.
9. Graça NGA, Ercig B, Velásquez Pereira LC, et al. Modifying ADAMTS13 to modulate binding of pathogenic autoantibodies of patients with acquired thrombotic thrombocytopenic purpura. Haematologica. 2020;105(11):2619-2630.

10. Pos W, Sorvillo N, Fijnheer R, et al. Residues Arg568 and Phe592 contribute to an antigenic surface for anti-ADAMTS13 antibodies in the spacer domain. Haematologica. 2011;96(11):1670-1677.
11. Jian C, Xiao J, Gong L, et al. Gain-of-function ADAMTS13 variants that are resistant to autoantibodies against ADAMTS13 in patients with acquired thrombotic thrombocytopenic purpura. Blood. 2012;119(16):3836-3843.
12. Scully M, McDonald V, Cavenagh J, et al. A phase 2 study of the safety and efficacy of rituximab with plasma exchange in acute acquired thrombotic thrombocytopenic purpura. Blood. 2011;118(7):1746-1753.
13. Hie M, Gay J, Galicier L, et al. Preemptive rituximab infusions after remission efficiently prevent relapses in acquired thrombotic thrombocytopenic purpura. Blood. 2014;124(2):204-210.
14. Westwood JP, Thomas M, Alwan F, et al. Rituximab prophylaxis to prevent thrombotic thrombocytopenic purpura relapse: outcome and evaluation of dosing regimens. Blood Adv. 2017;1(15):1159-1166.

A new drug for an old concept: aptamer to von Willebrand factor for prevention of arterial and microvascular thrombosis

Agnès Veyradier[1,2]

[1]Hematology department, French National Reference Centre for Thrombotic Microangiopathies and von Willebrand disease, Hospital Lariboisière, AP-HP.Nord and [2]EA3518 Saint-Louis Research Institute, Paris University, Paris, France.

E-mail: AGNÈS VEYRADIER - agnes.veyradier@aphp.fr

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Von Willebrand factor (VWF) is a large and complex multimeric glycoprotein essential for initiation of hemostasis after vascular injury. VWF is the mediator of platelet adhesion to the subendothelial collagen matrix and of platelet aggregation, especially at high shear rates of blood flow present in the microcirculation and stenotic arteries.[1] Platelet adhesion involves specific sequences of the A1 domain of VWF (VWF-A1) and the platelet receptor glycoprotein Ib (GPIb).[1] The adhesive properties of VWF are proportional to both the size of its multimers and their shear-induced unfolding, which respectively determine the number of available VWF-A1 and their swift from a cryptic to an exposed status able to bind platelet GPIb.[2] Physiologically, in order to prevent the spontaneous binding of VWF to platelets, VWF multimeric distribution is regulated by a specific-cleaving protease, ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin type 1 repeats, member 13).[3] A defect in VWF (related to genetic mutations of *VWF*) causes a bleeding disorder named von Willebrand disease (VWD) while an excess of ultralarge multimers of VWF (UL VWF) (due to a severe deficiency in ADAMTS13 mostly mediated by specific auto-antibodies) causes a thrombotic microangiopathy called thrombotic thrombocytopenic purpura (TTP).[4] In addition, the interaction of VWF-A1 with platelet GPIb also contributes to arterial thrombosis present in atherosclerotic cardiovascular disease (ACD).[5] Consequently, inhibiting the binding of VWF to GPIb by specifically targeting VWF-A1, is a rational approach to decrease both arterial and microvascular thrombosis by preventing the formation of further VWF- and platelet-rich thrombi[2] in both acute ACD[5] and acute TTP[3], respectively.

In the 1990-2000s, two classes of anti-VWF-A1 therapeutic agents were developed for this purpose. On one hand, a humanized single-variable domain immunoglobulin (Nanobody®)[6,7] has recently been approved and commercialized as caplacizumab (Cablivi ™) by Ablynx, a Sanofi company (Sanofi-Aventis, Paris, France) for the treatment of acute acquired TTP in adults, on the basis of positive results in phase II and III trials.[8,9] On the other hand, several aptamers, consisting of single-stranded DNA or RNA oligonucleotides with a specific and stable three-dimensional shape able to recognize their target with high affinity and specificity, were developed and tested in animal models.[10-12] However, only the historical anti-VWF-A1 aptamer, ARC1779, developed by Archemix (Cambridge, MA, USA), was investigated in ACD[13-16] and TTP[17-21] including limited phase II studies.[16,19-21]

In this issue of *Haematologica*, Sakai K and colleagues[22] present the *in vitro* characterization of a recently developed novel aptamer to VWF-A1, TAGX-0004,[23] using an elegant and extensive structural and functional investigation in both static and dynamic conditions (platelet aggregation, shear stress-induced platelet thrombus formation, study of binding to both wild-type VWF-A1 and 16 alanine-scanning VWF-A1 mutants using an electrophoresis mobility shift assay and surface plasmon resonance, and graphic analysis of three-dimensional (3D) structure models of VWF-A1). The authors show that, *in vitro*, TAGX-0004 is able to inhibit the binding of VWF-A1 to platelet GPIb better than the historical aptamer ARC1779 and as well as the Nanobody® caplacizumab. TAGX-0004 is thus presented as a new potential therapeutic option not only in acute TTP but also in various VWF-mediated thrombotic disorders such as acute coronary syndrome (ACS) and cerebral infarction.

In terms of the biochemical properties, the comparison of TAGX-0004 with ARC1779 is solid because those aptamers were used as both monovalent entities with no polyethylene glycol (PEG). The significantly higher affinity of TAGX-0004 for VWF-A1 compared to ARC1779 is likely related to the presence of Ds, an artificial hydrophobic base, able to directly interact with a specific residue (F1366) within VWF-A1. Also, interestingly, the amino acid residues of VWF-A1 identified as binding sites for TAGX-0004 and ARC1779 by the current study did not totally overlap and some slight differences with the originally mapping of VWF-A1 binding sites for ARC1172/ARC1179 performed by Huang and colleagues[24] were also observed. Regarding the similar *in vitro* affinity for VWF-A1 measured for both TAGX-0004 and caplacizumab, the authors mention that direct comparison of an affinity of monovalent entity with