ADAMTS13 is a blood enzyme that controls the multimer size of the hemostatic protein von Willebrand factor. After synthesis, ultralarge, hyperreactive von Willebrand factor multimers (up to 20,000 kDa) are secreted into the flowing blood and are immediately cleaved by ADAMTS13 into smaller, more quiescent multimers (<10,000 kDa). When ADAMTS13 is deficient, patients suffer from the devastating thrombotic thrombocytopenic purpura (TTP) disorder. In TTP patients, ultralarge von Willebrand factor multimers spontaneously bind platelets, and microthrombi are formed that block arterioles and capillaries. These results in severe organ failure, thrombocytopenia, and hemolytic anemia. TTP can be caused by mutations in the ADAMTS13 gene (congenital TTP) or by the development of autoantibodies against ADAMTS13 (acquired TTP).

ADAMTS13 is a multidomain enzyme consisting of 1,427 amino acids. Plasma ADAMTS13 is heavily glycosylated (20%) and has an apparent molecular weight of 180 to 190 kDa. It is well known that glycosylation plays an important role in many processes such as immune recognition of proteins and protein folding, final structure, secretion, function, and eventual clearance. Only a few studies have investigated the role of glycosylation in ADAMTS13 folding and secretion and in ADAMTS13 function. It has been shown that recombinant ADAMTS13 contains N-linked glycosylation and O-fucosylation sites. Both N-linked glycosylation and O-fucosylation seemed to be crucial for proper folding in the heterologous cells and for efficient secretion of recombinant ADAMTS13. However, when N-linked glycans were removed from recombinant ADAMTS13, the proteolytic activity of ADAMTS13 was not altered.

The glycan profile of recombinant ADAMTS13 might to some extent differ from the glycan structures of plasma ADAMTS13. Hence, to understand the role of glycans in ADAMTS13 biology and pathophysiology, it is crucial to unravel the glycosylation profile of plasma ADAMTS13. Verbij et al used the elegant approach of tandem mass spectrometry with higher-energy collision dissociation and electron transfer dissociation to complete this challenging task. Importantly, they were able to identify or confirm the amino acids that carry ADAMTS13 glycans, and they were also able to unravel the composition of each glycan chain. By using this knowledge, the complete structure of all ADAMTS13 glycosylation chains could be deduced. This work led to 3 categories of glycan structures on plasma ADAMTS13: complex N-linked carbohydrate structures, less complex O-(GalNAc)-linked glycan structures, and simple O-linked fucose and C-linked mannose glycans. Nine of the 10 N-linked glycans are composed of 11 to 13 monosaccharides, including a terminal sialic acid. However, 1 N-linked glycan (8 monosaccharides), situated in the spacer domain, is not sialylated but contains a high mannose structure. The 6 O-(GalNAc)-linked glycans consist of 4 to 7 monosaccharides, again including a terminal sialic acid. Typical for thrombospordin type 1 (TSP) repeats, and with the exception of TSP4, all 7 remaining TSP domains are O-fucosylated with disaccharide structures. Unexpectedly, another O-fucosylation site was identified in the disintegrin domain. Finally, the TSP1, -4, and -7 domains are each C-mannosylated with a single mannose residue.

Knowledge of the glycosylation profile of proteins allows better understanding of the role of glycans in the biology and pathophysiology of proteins. For example, glycans could control the structure of ADAMTS13. The crystal structure of only the disintegrin-like domain of the first TSP repeat/cysteine-rich domain/spacer domain fragment of ADAMTS13 is known. However, it was recently shown that the ADAMTS13 spacer domain interacts with its CUB1-2 domains, suggesting that ADAMTS13 adopts a folded conformation. Thus, it will be interesting to unravel whether the glycans in these domains contribute to the stabilization of this overall folded structure of ADAMTS13. In addition, if glycans stabilize the structure of ADAMTS13, then changes in glycosylation patterns, which could occur spontaneously as a consequence of pathological processes, might lead to different ADAMTS13 conformations. These different conformations might be more prone to proteolysis that renders ADAMTS13 inactive, which could be an explanation of why lower levels of ADAMTS13 activity are detected in certain diseases. In addition, alterations in protein glycosylation may modify or create novel B-cell epitopes. It has been suggested that changes in glycosylation of ADAMTS13 could expose neo-epitopes which could explain the formation of autoantibodies in acquired TTP. Verbij et al also hypothesized that high-mannose glycans identified in plasma...
ADAMTS13 contribute to the binding of ADAMTS13 to the mannose receptor on dendritic cells. Whether glycans in ADAMTS13 indeed contribute to all these processes remains to be determined.

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TRANSPLANTATION

Comment on Shenoy et al, page 2561

Sickle cell disease: the price of cure

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In this issue of Blood, the first prospective trial of unrelated donor bone marrow transplantation (BMT) in children with sickle cell disease (SCD), reported by Shenoy et al, is an important step in extending curative therapy to more children with severe disease.1 Matched related donor BMT for SCD is now well established as a treatment option for children with severe disease.2,3 Overall, the outcome is extremely good, and BMT remains the best immediate prospect for long-term cure for children who are currently experiencing severe sickle-related complications.2,3 Compared with other disease-modifying therapies, such as regular transfusion and hydroxyurea, matched related donor BMT will not only stop ongoing vaso-occlusive crises and arrest progressive organ damage in most cases, but may also lead to regression of damage in some cases.3 Aggregate results from case series and clinical trials show disease-free survival of ~92% and overall survival (OS) of ~95% of children and young adults with SCD with a matched sibling donor. Crucially, <1 in 10 of these families will have to cope with the major adverse outcome of BMT transplant-related mortality (TRM) and severe chronic graft-versus-host disease (GVHD).2,3 Given that SCD causes premature death and disability, particularly in young adults, and has a major impact on the quality of life for patients at all ages and their families,4 these are convincing results.

Can similarly impressive results be achieved for the 80% to 90% of patients with SCD who do not have HLA-matched family donors? A handful of small studies, using haplo-identical, cord blood (CB), or CD34+ peripheral blood cells from matched unrelated donors to treat patients with severe SCD, report an OS of 75% to 100%, although this is at the cost of high rates of graft rejection (38-60%).5 The Blood and Marrow Transplant Clinical Trials Network Sickle Cell Unrelated Donor Transplant (SCURT) study (BMTCTN 0601; #NCT00745420)1 aimed to improve on these results by using a reduced-intensity conditioning (RIC) regimen. The study was conducted between 2008 and 2014 and originally had 2 arms: the BMT arm, the results of which are presented here,4 and a CB transplant arm, which closed early due to a high rejection rate and has been reported separately.5 Twenty-nine children (age, 3-19 years) were treated in the RIC BMT arm of the study using a regimen consisting of alemtuzumab, fludarabine, and melphalan; GVHD prophylaxis was cyclosporine or tacrolimus together with short-course methotrexate and methylprednisolone. In contrast to a similar study in adults,6 no irradiation was used. Nevertheless, the graft rejection rate was low (10%): 27 of 29 patients engrafted, and 1 patient experienced secondary graft rejection. The 1-year and 2-year event-free survival was 76% and 69%, respectively, with an OS of 86% and 79% (see figure). GVHD was the principal transplant-related complication: the day 100 incidence of grade II to IV acute GVHD was 28%, and the 1-year incidence of chronic GVHD was 62% (see figure), which was extensive in 38% of the transplanted children. Overall, 8 of 29 children (27.6%) died, 7 of these as a result of complications of acute or chronic GVHD.

For pediatric hematologists, the results of the SCURT study are disappointing; for some of the families, a personal tragedy. Nevertheless, this is an extremely important study. Not only is this the first prospective trial of unrelated donor transplantation in SCD, but it involved multiple centers and has been steered through with the support of an independent external review committee to reach completion, allowing the results to be shared and lessons to be learned. In particular, as the investigators highlight, this specific RIC protocol was associated with an unacceptably high TRM, mainly due to GVHD. Although the reasons for this are difficult to assess here without full details of each individual case, possible factors may include the timing of the alemtuzumab (early in the protocol) and toxicity in this population of the combination of several agents with gut toxicity. Hence, the approach that enabled a high rate of engraftment in a RIC setting may have been responsible for the high TRM. Advances in the prevention of GVHD associated with
Glycans of plasma ADAMTS13

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