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REVIEW ARTICLE

von Willebrand factor sialylation—A critical regulator of biological function

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Abstract

von Willebrand factor (VWF) undergoes complex post-translational modification prior to its secretion into the plasma. Consequently, VWF monomers contain complex N-glycan and O-glycan structures that, together, account for approximately 20% of the final monomeric mass. An increasing body of evidence has confirmed that these carbohydrate determinants play critical roles in regulating multiple aspects of VWF biology. In particular, studies have demonstrated that terminal ABO blood group has an important effect on plasma VWF levels. This effect is interesting, given that only 15% of the N-glycans and 1% of the O-glycans of VWF actually express terminal ABO(H) determinants. In contrast, the vast majority of the N-glycans and O-glycans on human VWF are capped by terminal negatively charged sialic acid residues. Recent data suggest that sialylation significantly regulates VWF functional activity, susceptibility to proteolysis, and clearance, through a number of independent pathways. These findings are of direct clinical relevence, in that quantitative and qualitative variations in VWF sialylation have been described in patients with VWD, as well as in patients with a number of other physiologic and pathologic conditions. Moreover, platelet-derived VWF is significantly hyposialylated as compared with plasma-derived VWF, whereas the recently licensed recombinant VWF therapeutic is hypersialylated. In this review, we examine the evidence supporting the hypothesis that VWF sialylation plays multiple biological roles. In addition, we consider data suggesting that quantitative and qualitative variations in VWF sialylation may play specific roles in the pathogenesis of VWD, and that sialic acid expression on VWF may also differ across a number of other physiologic and pathologic conditions.

KEYWORDS
ADAMTS-13, glycosylation, von Willebrand disease, von Willebrand factor

1 | INTRODUCTION

von Willebrand factor (VWF) is a large multimeric plasma glycoprotein that plays essential roles in maintaining normal hemostasis.[1] First, VWF binds to exposed subendothelial tissues at sites of vascular injury and subsequently facilitates platelet tethering to form a primary platelet plug. Second, VWF also binds to procoagulant factor VIII (FVIII), thereby protecting it from proteolysis and significantly extending its plasma half-life.[2] von Willebrand disease (VWD) is the commonest inherited human bleeding disorder, and is caused by quantitative or qualitative reductions in plasma VWF levels.[3,4] In contrast, elevated plasma levels of the VWF-FVIII complex represent a dose-dependent risk factor for both arterial

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and venous thrombosis.[5] Consequently, understanding the factors that modulate plasma VWF activity is of direct clinical relevance. Human VWF is heavily glycosylated, and a series of studies have highlighted that the sugar determinants expressed on VWF play key roles in regulating multiple aspects of its biology.[6] In this review, we examine recent evidence supporting the hypothesis that VWF sialylation may be of particular importance in this context. In addition, we also consider data suggesting that quantitative and qualitative variations in VWF sialylation may play specific roles in the pathogenesis of VWD, and that sialic acid expression on VWF may also differ across a number of other physiologic and pathologic conditions.

Essentials

- von Willebrand Factor (VWF) is extensively glycosylated with serial studies demonstrating that these carbohydrate determinants play critical roles in regulating multiple aspects of VWF biology.
- Terminal sialic acid residues, expressed on both the N- and O-linked glycans of VWF, regulate VWF functional activity, susceptibility to proteolysis and plasma clearance in vivo.
- Quantitative and qualitative variations in VWF sialylation have been reported in patients with von Willebrand Disease, as well as in a number of other physiological and pathological states.
- Further studies are warranted to define the molecular mechanisms through which N- and O-linked sialylation impacts upon the multiple biological activities of VWF.

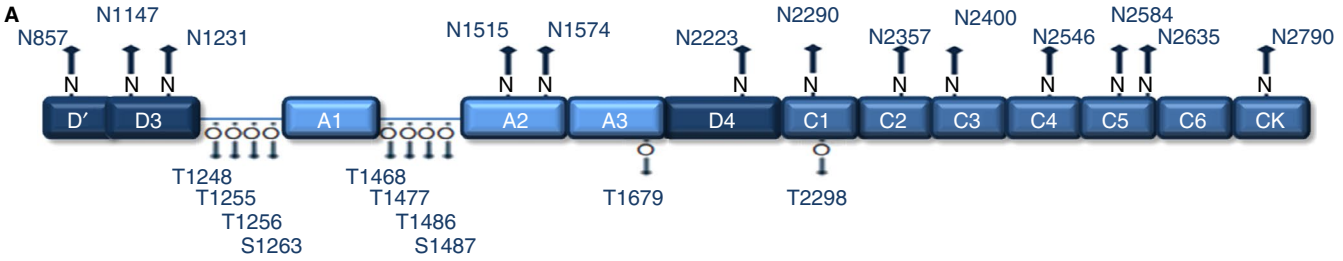
2 | VWF BIOSYNTHESIS AND POST-TRANSLATIONAL MODIFICATION

Under normal physiologic conditions, in vivo biosynthesis of VWF is restricted to vascular endothelial cells (ECs) and megakaryocytes only.[7] VWF synthesized within ECs can be secreted into the plasma or stored within intracellular Weibel-Palade (WP) bodies.[1] Subsequently, acute EC activation by different secretagogues (including thrombin, fibrin, and histamine) can trigger secretion of this stored VWF into the plasma. In contrast, VWF synthesized within megakaryocytes is stored within α-granules.[8] Consequently, under steady-state conditions, plasma VWF is predominantly derived from ECs. Within both EC and megakaryocytes, VWF is initially synthesized as a 2813 amino acid monomer that consists of a series of homologous repeating domains (in the order D′-D3-A1-A2-A3-D4-C1-C2-C3-C4-C5-C6-CK).[1] This initial VWF monomer is subjected to extensive post-translational modification, which includes multimerization and significant glycosylation.[6,9] As a result, VWF circulates in normal plasma as a series of heterogeneous oligomers, with the largest multimers having molecular weights in excess of 20 000 kDa.

3 | SIALYLATION OF HUMAN PLASMA-DERIVED VWF

Initial analysis of the VWF amino acid monomer sequence identified 13 potential N-glycosylation consensus sequons (N857, N1147, N1231, N1515, N1574, N2223, N2290, N2357, N2400, N2546, N2585, N2635, and N2790) (Figure 1A).[10] Recent mass spectrometry (MS) studies on human plasma-derived VWF have confirmed occupancy of all of these sites except N1147.[11] Importantly, these studies also highlighted that the glycans expressed at individual positions across the VWF monomer differed significantly. For example, smaller N-glycan structures were observed at sequons N857 and N1147, whereas larger and more complicated sugar structures were present at N1515 and N1574.[11] Overall, complex-type carbohydrates predominated, such that less than 1% of the chains were high-mannose in type. The most common structures observed were monosialylated and disialylated biantennary chains. Recent studies have demonstrated that approximately 80% of the total sialic acid on VWF is expressed on its N-linked glycans, and that the majority of this N-linked capping sialic acid is present in α2-6 linkage (Figure 1B).[11,12] Finally, terminal ABO(H) blood group determinants have also been identified on approximately 15% of the N-linked carbohydrates of human plasma-derived VWF (Figure 1B).[11] These ABO(H) structures were not localized to specific N-glycan sites on plasma-derived VWF, but, rather, were disseminated across all of the occupied sequons. Importantly, Canis et al observed evidence of concentrated ABO(H) expression at some specific N-glycan sites (e.g., N1515 and N1574),[11] and also that ABH determinants were present only on non-sialylated antennae.

Besides its extensive N-glycosylation, each VWF monomer also contains 10 O-glycosylation sites (Figure 1A). Unlike the N-glycans, which are distributed across multiple different domains of VWF, eight of the 10 O-linked glycans (T1248, T1255, T1256, S1263, T1468, T1477, S1486, and T1487) are clustered in two groups at either side of the A1 domain.[13] The other O-glycan chains are located within the A3 and C1 domains (T1679 and T2298, respectively). In contrast to the complexity of its N-glycans, the O-glycans of plasma-derived VWF predominantly exist as short mucin-type carbohydrates (Figure 1C).[13] Nevertheless, recent MS analyses have again significantly improved our understanding of the structures of these O-glycan structures.[14,15] The predominant structure seen was a disialylated core 1 structure, also referred to as the Thomas Friedenreich (T) antigen, and accounted for approximately 70% of the total O-glycan population (Figure 1C). Altogether, O-linked sialic acid expression has been estimated to account for less than 20% of the total sialylation on human plasma-derived VWF, and may be present in either α2-3 or α2-6 linkage.[11,14,15] It is of note


A
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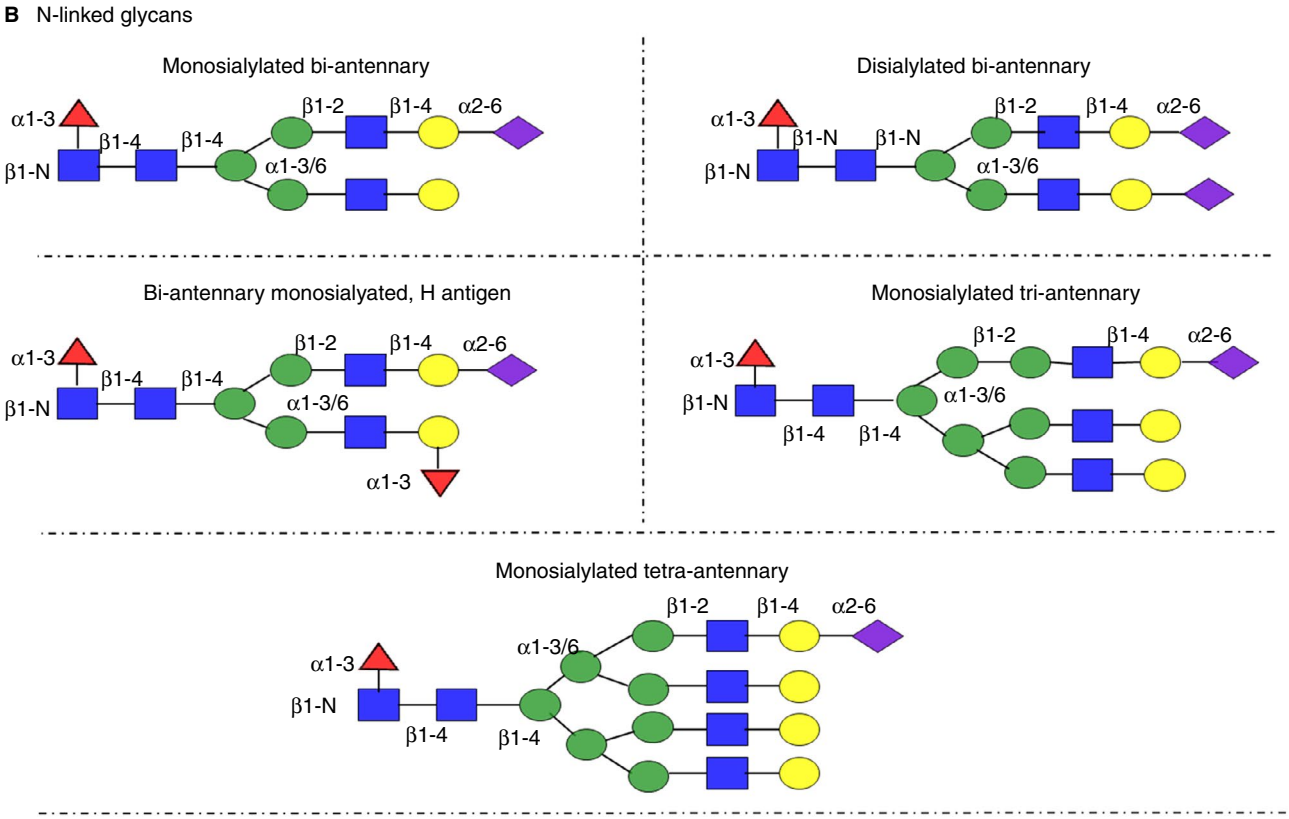

B N-linked glycans
Monosialylated bi-antennary
Disialylated bi-antennary
Bi-antennary monosialyated, H antigen
Monosialylated tri-antennary
Monosialylated tetra-antennary
α1-3
β1-4
β1-N
β1-2
α2-6
α1-3/6

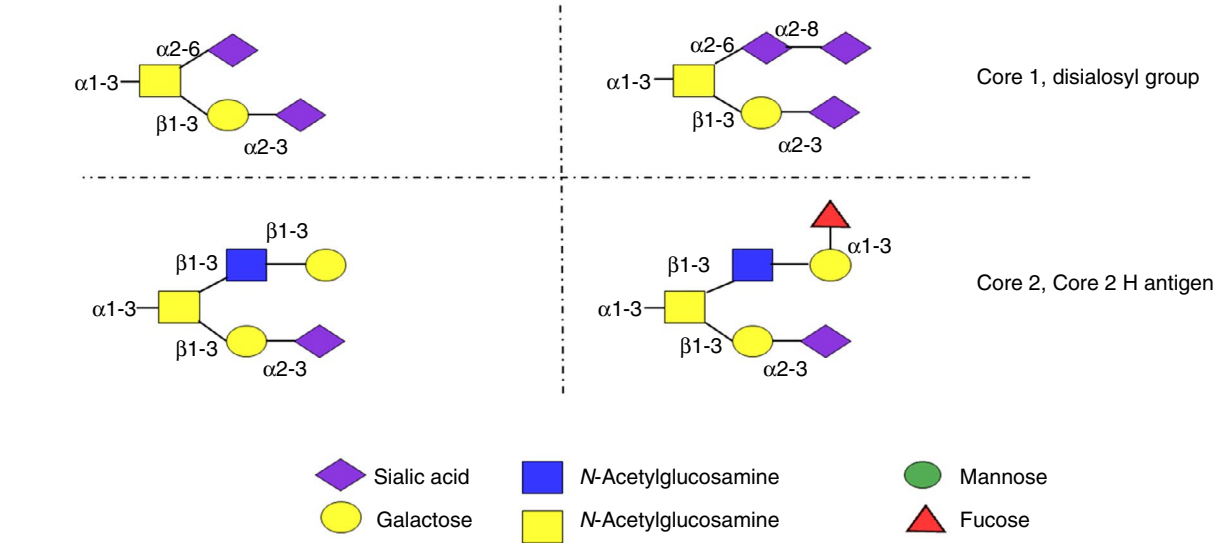

C O-linked glycans
α2-6
α1-3
β1-3
α2-3
α2-8
Core 1, disialosyl group
Core 2, Core 2 H antigen
Sialic acid
N-Acetylglucosamine
Mannose
Galactose
N-Acetylglucosamine
Fucose

FIGURE 1 (A) The von Willebrand factor (VWF) amino acid sequence includes 13 potential N-glycosylation consensus sequons that span the entirety of the VWF domains. Mass spectrometry has revealed that all of these glycosites are occupied, with the exception of N1147 and N2635, which show only partial occupancy. VWF also contains 10 O-linked glycosylation sites, eight of which flank the A1 domain in two clusters. (B) The commonest N-linked glycan structures are illustrated. Monosialylated bi-antennary complex structures account for approximately 80% of VWF N-linked glycans, with tri-antennary and tetra-antennary sugars also being present in the minority, and high-mannose structures representing less than 1% of VWF N-linked glycan structures. The N-linked glycans are highly sialylated, with 50% of the overall complex antennae being capped with α2-6–linked sialic acid. (C) In contrast, VWF O-linked glycans are of the short mucin type, with the disialylated core 1 T-antigen structure accounting for 70% of the total VWF O-linked glycans. Core 2 structures are also present. Both VWF N-glycans and O-glycans express ABO blood group determinants (13% and 1%, respectively)

that disialosyl groups, which are uncommon among human glycans, were identified on core 1 glycan structures (Figure 1C).[14,15] Finally, terminal ABO(H) determinants were also identified on O-linked glycans, but were restricted to approximately 1% of core 2 O-glycan structures.

4 | SIALYLATION AND VWF AGING IN PLASMA

Emerging evidence suggests that, like that of other plasma glycoproteins, VWF glycosylation varies with protein aging. Importantly, Yang

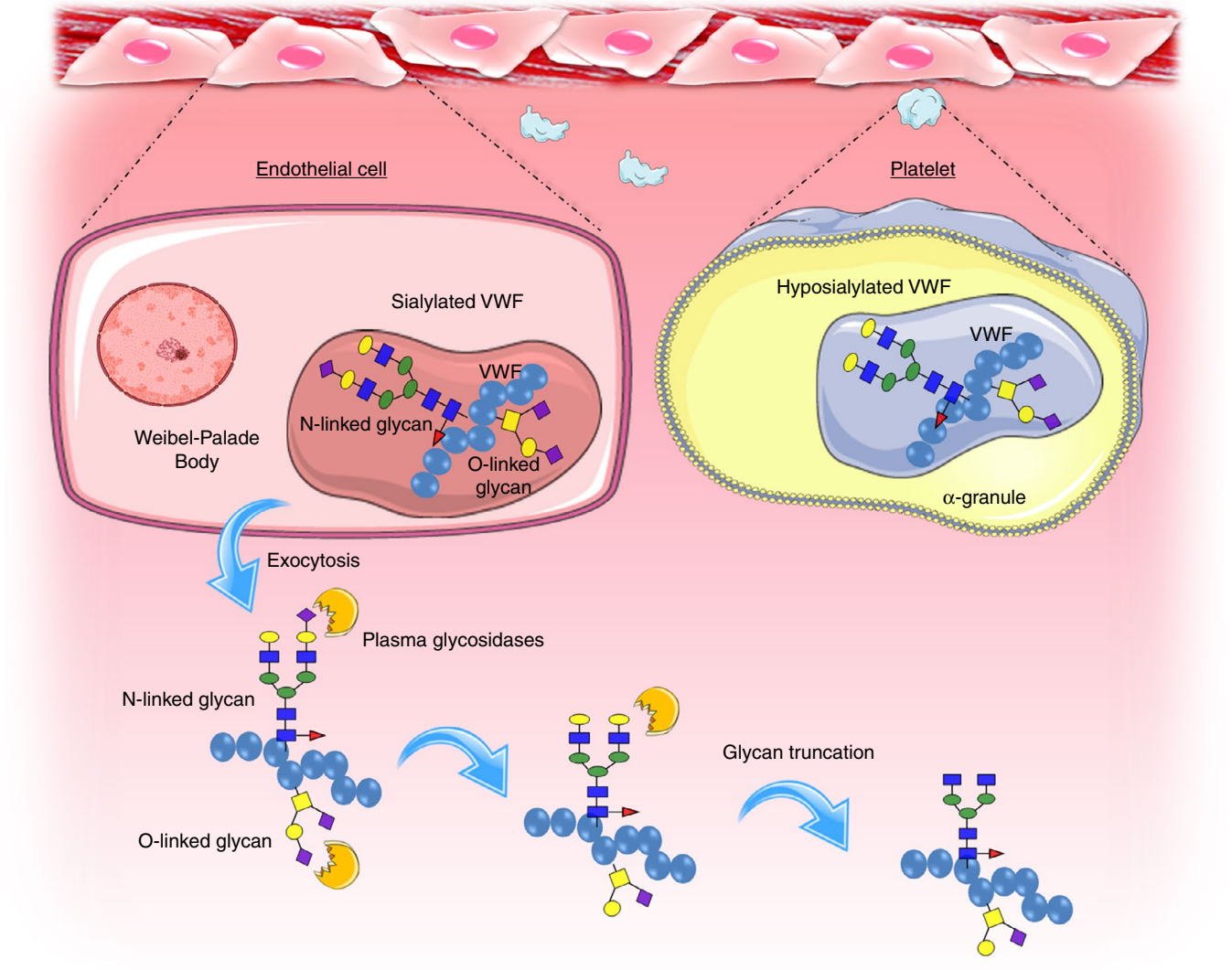


FIGURE 2 The sialylation status of von Willebrand factor (VWF) is heterogeneous and dynamic. VWF of endothelial cell (EC) origin is fully sialylated, whereas platelet-derived VWF has been shown to have significantly reduced VWF N-linked sialylation, probably because of differential sialyltransferase activity in ECs and megakaryocytes. Once secreted, VWF is exposed to circulating plasma glycosidases, which have been reported to perform stepwise removal or trimming of sialic acid/galactose from various plasma glycoproteins

et al recently showed that aging of secreted plasma glycoproteins is associated not only with a loss of sialic acid, but also with a progressive stepwise loss of terminal sugar residues from N-glycan chains.[16] Loss of terminal α2-6–linked sialic acid (catalyzed by plasma neuraminidases) constitutes the first step in this process. Subsequently, through the actions of other plasma glycosidase enzymes, further progressive N-glycan trimming ensues (Figure 2).[16] In keeping with these data, lectin studies performed on plasma samples collected before and after DDAVP administration have demonstrated significant differences in VWF glycan expression. For example, Aguila et al showed that binding of *Sambucus nigra* agglutinin (SNA), a plant lectin that specifically binds α2-6–linked sialic acid, to VWF secreted following DDAVP administration was significantly elevated as compared with binding to circulating steady-state plasma VWF, suggesting that VWF high molecular weight multimers (HMWMs) stored within WP bodies are more highly sialylated than VWF in the circulation.[17] In addition, decreased peanut agglutinin (PNA), a plant lectin with affinity for the short mucin T antigen structure, binding and increased blood group antigen expression on VWF have also been described in post-DDAVP samples.[18,19] Thus, although the majority of the N-linked and O-linked glycans of VWF initially secreted from ECs are capped with terminal sialic residues, the level of plasma VWF sialylation is likely to progressively diminish with glycoprotein aging in the plasma (Figure 2).

5 | SIALYLATION OF HUMAN PLATELET VWF

Platelet α-granules contain an estimated 10%-20% of the total VWF present in platelet-rich plasma.[20,21] This pool of platelet-derived VWF is discrete from plasma-derived VWF, there being no interchange between the two compartments. Previous studies have demonstrated that sialic acid expression on platelet-derived VWF is markedly reduced as compared with that on plasma-derived VWF (Figure 2).[22,23] Using HPLC analysis, McGrath et al further showed that this reduced sialic acid expression was mainly attributable to a specific decrease (>50%) in N-linked sialylation on platelet-derived VWF.[24] In contrast, O-linked sialylation on platelet-derived VWF and plasma-derived VWF were similar. Again in contrast to plasma-derived VWF, studies have also consistently shown that A and B blood group determinants are not present on platelet-derived VWF.[8,24] These glycosylation differences presumably reflect differences in the post-translational modification of platelet-derived VWF (synthesized within megakaryocytes) and that of plasma-derived VWF (synthesized within ECs).[8]

6 | VWF SIALYLATION INFLUENCES FUNCTIONAL ACTIVITY

Previous studies conducted under both static and shear-based conditions have demonstrated that VWF glycan structures significantly influence functional properties.[25–27] A specific role for sialylation in regulating aspects of VWF activity has also been defined. Federici et al showed that neuraminidase treatment of VWF removed more than 95% of total sialic acid from VWF, neuraminidase-treated VWF (Neu-VWF).[25] In the presence of protease inhibitors, desialylation had no direct effect on the multimer pattern.[28] However, Neu-VWF was shown to induce spontaneous platelet aggregation in platelet-rich plasma, and also to be more effective in modulating platelet adhesion to a collagen surface under shear.[25] Desialylation of VWF is associated with exposure of penultimate galactose (Gal) residues. Importantly, removal of these Gal moieties significantly attenuated the increased adhesion and aggregation properties of Neu-VWF.[25]

As previously discussed, N-linked sialylation on platelet-derived VWF is markedly reduced as compared with that on plasma-derived VWF. Given this reduction in sialic acid expression, it is interesting that previous studies have reported functional differences between platelet-derived VWF and plasma-derived VWF. [8,29] For example, although platelet-derived VWF is enriched in HMWMs, it binds glycoprotein (GP) 1bα with significantly reduced affinity as compared with plasma-derived VWF.[22] In contrast, platelet-derived VWF shows significantly enhanced binding to both GPIIb/IIIa and heparin.[22] Further studies will be necessary to elucidate the molecular mechanisms underlying these differences in specific activity, and, in particular, to determine the contribution of differences in VWF glycosylation.

7 | VWF SIALYLATION REGULATES SUSCEPTIBILITY TO PROTEOLYSIS

VWF multimer distribution is a key determinant of its functional activity.[1,3] HMWMs bind collagen and GPIbα with increased affinity as compared with low molecular weight multimers, and are thus more effective in facilitating platelet plug formation. In normal plasma, VWF multimer distribution is regulated by ADAMTS-13, which cleaves VWF at a specific Tyr1605-Met1606 bond within the A2 domain.[30,31] Recent studies have suggested that other proteases, including plasmin, may also play roles in regulating VWF multimer distribution under specific circumstances.[32,33] The clinical importance of regulating VWF multimer distribution is illustrated by the fact that ADAMTS-13 deficiency in patients with thrombotic thrombocytopenic purpura (TTP) results in accumulation of ultralarge VWF (UL-VWF) multimers and life-threatening thrombotic microvascular occlusion.[31] Conversely, loss of HMWMs because of enhanced proteolysis in patients with type 2A VWD is associated with significant bleeding.[34] It is well recognized that terminal sialic acid expression on glycoproteins plays a key role in protecting against proteolytic destruction. In keeping with this concept, previous studies demonstrated that VWF desialylation resulted in significantly enhanced proteolysis by a number of proteases, including cathepsin B, trypsin, and chymotrypsin.[12,28] Paradoxically, however, more recent studies have shown that removal of sialic acid from VWF causes it to be significantly more resistant to cleavage by ADAMTS-13.[12] In particular,

enzymatic desialylation of plasma-derived VWF with α2-3,6,8,9 neuraminidase markedly impaired ADAMTS-13-mediated VWF proteolysis in a dose-dependent manner. Interestingly, treatment with α2-3 neuraminidase to specifically digest α2-3–linked sialic acid from the O-glycans of plasma-derived VWF had no significant effect on ADAMTS-13 proteolysis.[12] Together, these data suggest that α2-6–linked sialylation on plasma-derived VWF may play a critical role in enhancing the susceptibility of VWF to proteolysis by ADAMTS-13. Although the molecular mechanism underpinning this sialylation effect remains unclear, site-directed mutagenesis studies have suggested an important role for the N-linked glycans at Asn1574 in the VWF A2 domain in regulating ADAMTS-13 proteolysis.[35]

As previously discussed, levels of N-linked sialylation are significantly reduced on platelet-derived VWF as compared with plasma-derived VWF.[8] Interestingly, as a result of this quantitative change in terminal α2-6–linked sialic acid expression, platelet-derived VWF shows specific resistance to ADAMTS-13-mediated proteolysis as compared with plasma-derived VWF.[24] Hence, not only are high local concentrations of platelet-derived VWF released at sites of vascular injury, but, because it has undergone different post-translational modification within megakaryocytes, this released platelet-derived VWF exists as a discrete natural glycoform that is at least partially resistant to ADAMTS-13 proteolysis.

Recent studies have suggested that plasmin-induced cleavage of VWF may also be of both physiologic and potential pharmacologic significance.[32,36] For example, significant activation of plasminogen to plasmin has been reported in patients during acute TTP.[32] Furthermore, a role for plasmin in preventing the accumulation of pathologic UL-VWF multimers in the absence of ADAMTS-13 regulation has been proposed.[36] Interestingly, VWF glycans also play a role in regulating susceptibility to plasmin-mediated proteolysis.[28,33] However, in contrast to ADAMTS-13 proteolysis, ABO blood group does not influence VWF cleavage by plasmin,[33] and Berkowitz et al previously showed that desialylation of VWF is associated with enhanced plasmin-mediated proteolysis.[28]

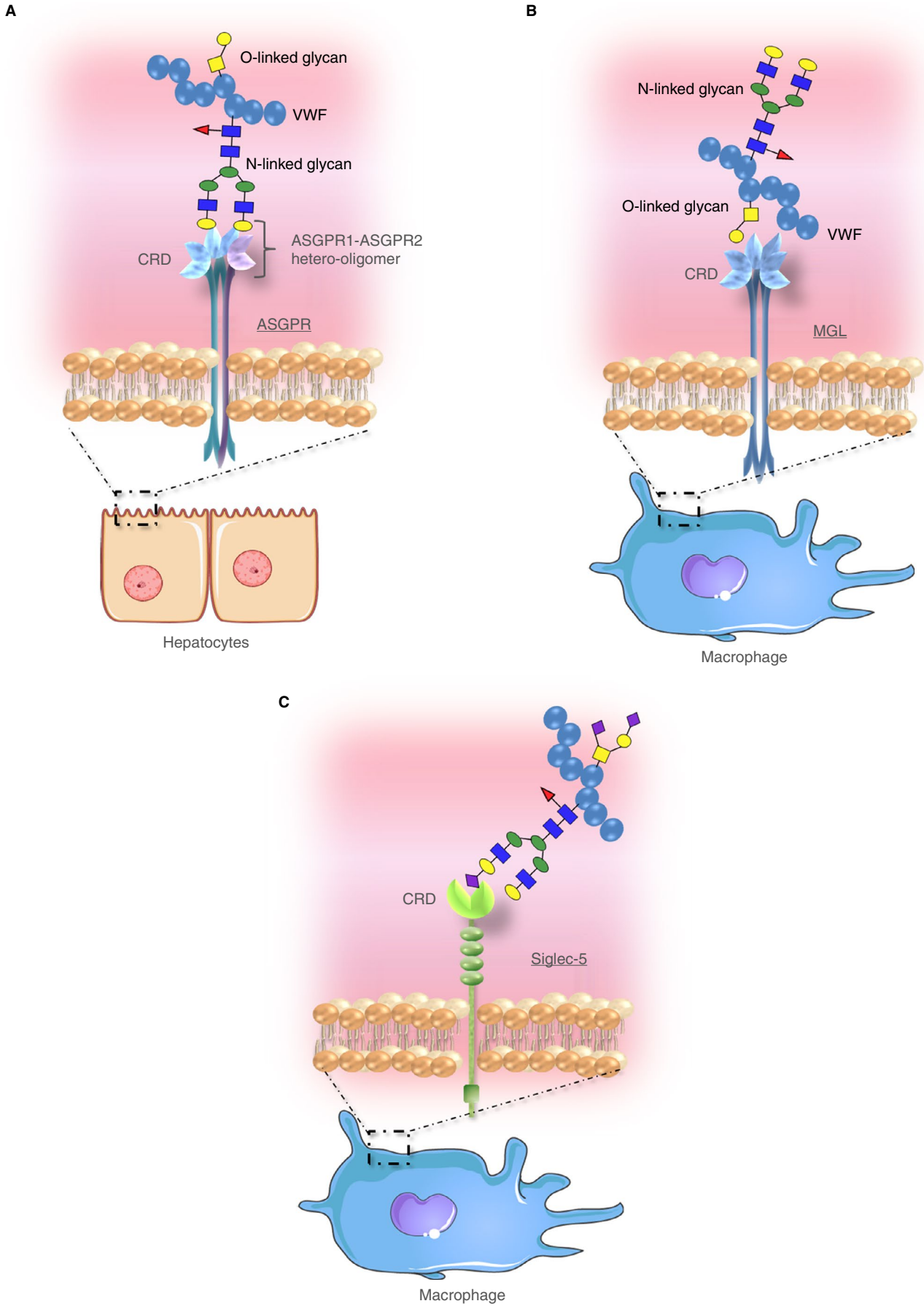
8 | VWF SIALYLATION DETERMINES CLEARANCE THROUGH MULTIPLE PATHWAYS

In addition to influencing the susceptibility of VWF to proteolysis, sialic acid expression has been shown to play a critical role in modulating VWF clearance.[37] Sodetz et al first demonstrated that enzymatic removal of terminal sialic acid residues was associated with a significant reduction in VWF half-life in rabbits.[38] This observation was subsequently confirmed in a number of other independent studies in other animals.[39–41] Furthermore, genetic inactivation of the sialyltransferase ST3Gal-IV in a transgenic mouse model was shown to result in enhanced VWF clearance and a significant reduction in plasma VWF levels.[42] In contrast to the specific effect of α2-6–linked sialylation in enhancing VWF proteolysis by ADAMTS-13, current evidence suggests that both α2-6–linked and α2-3–linked linked sialylation play roles in protecting VWF against clearance.[37,38,40,43,44] Similarly to their specific role in modulating susceptibility to ADAMTS-13 proteolysis, the N-linked glycans at positions 1515 and 1574 within the A2 domain also play a key role in protecting VWF against in vivo clearance.[45] The major effect of α2-3–linked sialylation in regulating clearance is interesting,[43] as this sialic acid accounts for less than 20% of the total sialylation on human plasma-derived VWF, and is expressed predominantly on O-glycans.[14]

A number of lectin receptors have been implicated in the enhanced clearance of hyposialylated VWF (Figure 3).[37] The first receptor proposed to be important in this context was the Ashwell-Morrell receptor or asialoglycoprotein receptor (ASGPR).[44] ASGPR is a C-type lectin receptor expressed predominantly on hepatocytes, and consists of two transmembrane protein subunits (Asgpr-1 and Asgpr-2).[46,47] The ASGPR carbohydrate recognition domain preferentially binds to GPs expressing β-D-galactose (β-Gal) or *N*-acetyl-D-galactosamine (GalNAc) residues.[47] These residues are typically present on VWF as subterminal moieties on glycan chains capped by sialic acid residues.[11,14] Consequently, loss of terminal sialylation results in enhanced exposure of these β-Gal and GalNAc residues, which can then trigger ASGPR-mediated clearance (Figure 3A). In keeping with the hypothesis that ASGPR plays a role in VWF clearance, Grewal et al reported significantly elevated plasma VWF levels in *Asgr-1* knockout mice, and further demonstrated that VWF clearance was reduced in these mice.[44] More recent studies have implicated a role for the macrophage-galactose–type lectin (MGL) in also modulating the enhanced clearance of hyposialylated VWF (Figure 3B).[43] MGL is another C-type lectin receptor that binds to β-Gal and GalNAc residues exposed following desialylation.[48,49] Interestingly, given that loss of 2-3–linked sialylation may be of particular importance in triggering enhanced VWF clearance, emerging data suggest that MGL may have specific affinity for the O-glycan of plasma-derived VWF.[44] This observation is in keeping with previous studies showing that MGL preferentially binds to the T antigen structure.[18] Finally, specific members of the sialic acid–binding immunoglobulin-like lectin (Siglec) family have also been shown to bind to human VWF (Figure 3C). In particular, Pegon et al observed that Siglec-5 bound to human plasma-derived VWF in a dose-dependent manner, and could regulate its endocytosis into early endosomes, suggesting that this receptor may also contribute to VWF clearance.[50] Importantly, in contrast to ASGPR and MGL, which both bind with increased affinity to hyposialylated VWF, Siglec-5 binding to VWF is sialic acid–dependent.[37]

9 | VWF SIALYLATION IN HEALTHY NORMAL INDIVIDUALS

In light of the important effects of sialic acid expression in regulating VWF biology, it is interesting that variation in VWF sialylation has been reported between normal individuals. In a study of 68 healthy blood donors, Aguila et al observed significant interindividual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O-linked glycan
VWF
N-linked glycan
CRD
ASGPR1-ASGPR2
hetero-oligomer
ASGPR
Hepatocytes
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O-linked glycan
VWF
CRD
MGL
Macrophage
C
CRD
Siglec-5
Macrophage

variation in VWF binding for all three lectins that recognize terminal sialic acid residues, namely SNA, *Maackia amurensis* lectin II (MAL-II) and wheat germ agglutinin (WGA).[17] In keeping with these findings, exposure of subterminal β-Gal (*Ricinus communis* agglutinin I [RCA-I] and *Erythrina cristagalli* lectin [ECA] binding) also varied significantly between different normal individuals.[17] Collectively, these data suggest that, even among normal individuals, there may be marked interindividual heterogeneity in quantitative N-linked and O-linked sialic acid expression on plasma-derived VWF. Further studies will be required to determine the biological mechanisms underpinning this interindividual heterogeneity in VWF sialylation, and to define whether the changes in sialic acid expression may contribute to quantitative and qualitative variations in plasma VWF levels in the normal population.[51]

10 | ABNORMAL VWF SIALYLATION IN PATIENTS WITH VWD

Perhaps unsurprisingly, given the key roles for sialylation in regulating VWF biology, several groups have investigated VWF glycosylation in patients with VWD. Two independent studies reported significantly increased binding of the lectin RCA-I to plasma-derived VWF (suggestive of increased Gal or GalNAc exposure) in patients with VWD as compared with controls.[42,52] Importantly, these studies both included patients with different types of VWD, although the total numbers enrolled were limited (n = 19 and n = 26, respectively). Furthermore, significantly enhanced binding of the lectin PNA (suggesting increased O-linked T antigen expression) has also been observed in patients with type 1 VWD as compared with healthy controls.[18] Collectively, these data raise the intriguing question of whether alteration in VWF sialylation could be important in the pathogenesis of VWD, particularly in patients with low VWF levels in whom the disease is not linked to the *VWF* gene locus (Figure 4A).[53]

To further address this question, Aguila et al developed a novel panel of lectin assays to assess VWF sialylation in 110 patients with low VWF levels (plasma VWF levels in the range of 30-50 IU/dL) as compared with ABO-matched healthy controls.[17] For each patient and control, plasma-derived VWF sialylation was assessed with the lectins SNA, MAL-II, and WGA. Significant interindividual variation in VWF sialylation was observed among the cohort of patients with low VWF levels. Importantly, however, binding SNA to VWF was significantly reduced in the cohort with low VWF levels as compared with controls, suggesting a specific reduction in terminal α2-6–linked sialic acid expression in these patients.[17] In keeping with this reduction in terminal α2-6–linked sialylation, a significant increase in RCA-I binding (consistent with increased Gal exposure) was also seen in the cohort with low VWF levels as compared with healthy controls. Interestingly, the highest levels of RCA-I binding were seen in patients with low VWF levels who did not have any *VWF* mutations to explain their reduced plasma VWF levels. Finally, an inverse correlation was observed between enhanced RCA-I binding and estimated VWF half-life in patients with low VWF levels. Together, these findings support the idea that loss of terminal sialylation may contribute to the underlying pathophysiology in at least a subgroup of patients with low VWF levels by promoting enhanced clearance.[17]

11 | ALTERED VWF SIALYLATION IN MISCELLANEOUS PHYSIOLOGIC AND PATHOLOGIC CONDITIONS

In addition to the evidence that VWF sialylation may be abnormal in some patients with VWD, several studies have suggested that a number of other conditions may also be associated with alterations in sialic acid expression on plasma-derived VWF (Figure 4). Several pathogens, including *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Pseudomonas aeruginosa*, have been shown to express neuraminidase enzymes that can target host sialoglycoproteins.[54,55] Furthermore, platelet desialylation by bacterial NanA sialidase has been implicated in causing enhanced platelet clearance and thrombocytopenia in patients with *S. pneumonia* infection.[44] Desialylation of VWF resulting in enhanced clearance and reduced plasma VWF levels has also been observed in mice infected with *S. pneumonia* (Figure 4B).[44]

Several studies have shown that plasma VWF levels are markedly elevated in patients with liver cirrhosis.[18,56] Furthermore, an inverse correlation between PNA binding to VWF and plasma VWF levels has also been described in patients with cirrhosis (Figure 4C).[18] In addition, a correlation between the severity of cirrhosis and relative PNA binding was also observed.[18] As PNA is known to preferentially bind to the desialylated O-linked T antigen carbohydrate structures on VWF, these data suggest that abnormal VWF glycosylation, and in particular reduced presence of the sialylated T antigen, may be common in cirrhotic patients. Reduced VWF sialylation has been reported in patients with pulmonary hypertension.[57] In a study of 16 patients with moderate to severe pulmonary hypertension, Lopes et al observed reduced binding of WGA to patient VWF as

FIGURE 3 A number of lectin receptors have been proposed to play roles in mediating von Willebrand factor (VWF) clearance in vivo. Glycoprotein binding to these receptors is mediated by carbohydrate recognition domains (CRDs) that specifically interact with terminal glycan structures expressed on circulating plasma glycoproteins. (A) Asialoglycoprotein receptor (ASGPR) is expressed on hepatocytes as a heterotrimer composed of the major (ASGPR-1) and minor (ASGPR-2) subunits, with the CRD having specificity for glycoproteins terminating in galactose (Gal) and *N*-acetyl-D-galactosamine (GalNAc) moieties following N-linked sialic acid removal. (B) Macrophage-galactose–type lectin (MGL) is expressed as a homotrimer on macrophages and dendritic cells. The MGL CRD is also specific for exposed Gal/GalNAc terminal moieties; however, MGL binds preferentially to desialylated O-linked glycan structures, with particular affinity for the T antigen. (C) Sialic acid–binding immunoglobulin-like lectin-5 (Siglec-5), which is also expressed on macrophages, preferentially binds sialylated glycan structures

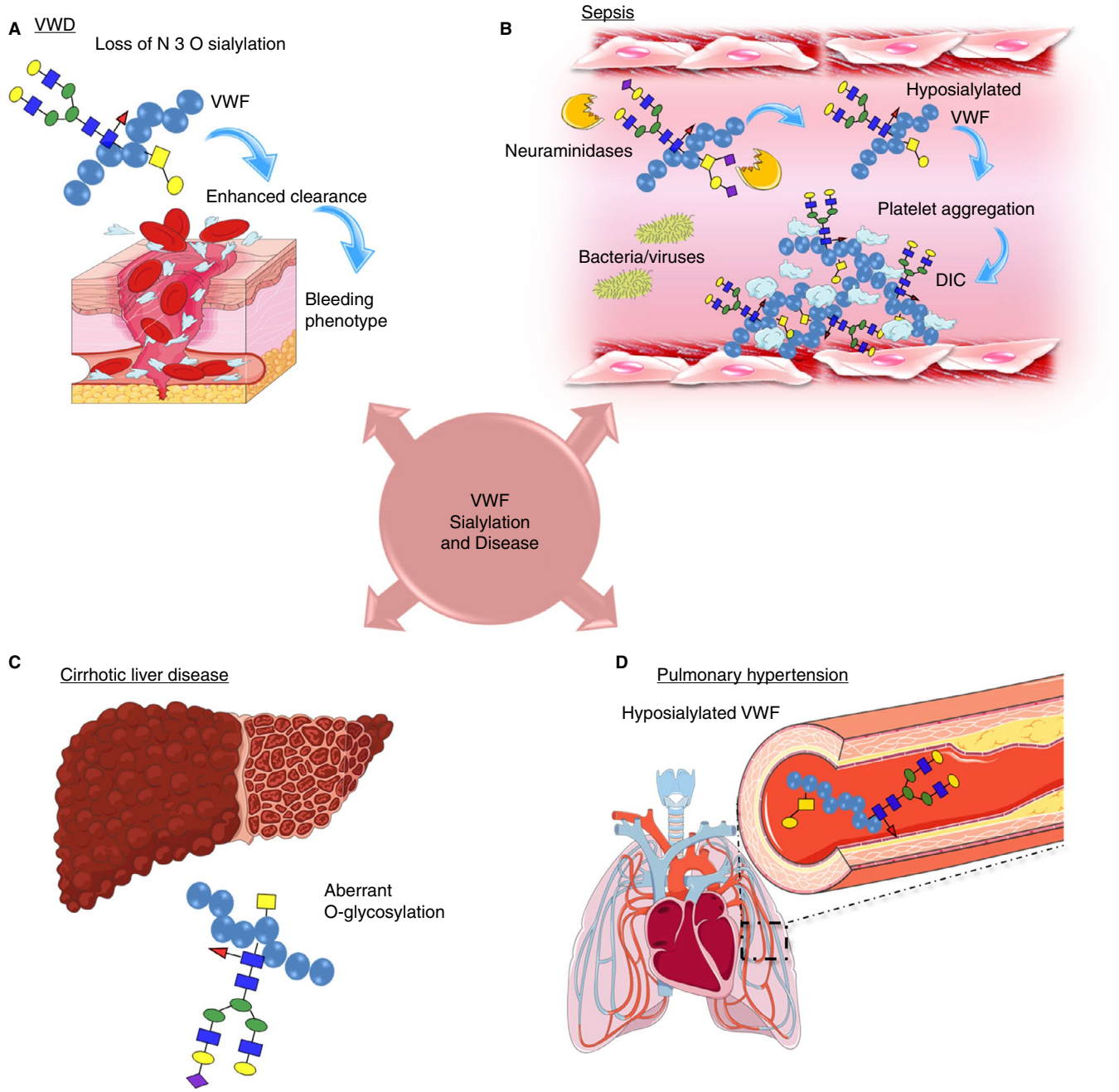

FIGURE 4 (A) A number of studies have demonstrated that subgroups of patients with von Willebrand disease (VWD) show reduced terminal sialic acid expression and subsequent increased galactose exposure on von Willebrand factor (VWF) glycan structures. Galactose exposure is correlated with an increase in VWF clearance in vivo and decreased VWF antigen (VWF:Ag) levels, contributing to the pathophysiology of VWD. (B) Viral and bacterial neuraminidases can mediate the desialylation of circulating VWF and platelets during sepsis. Desialylated VWF has been shown to trigger platelet aggregation and thus contribute to the coagulopathy of sepsis. (C) VWF:Ag levels are markedly elevated in patients with cirrhotic liver disease. Peanut agglutinin binding has been shown to inversely correlate with VWF:Ag in these patients, suggesting reduced presence of the T antigen on VWF disseminated intravascular coagulation (DIC). (D) Hyposialylated plasma VWF and loss of HMW multimers have been reported in patients with pulmonary hypertension

compared with VWF from healthy controls.[57] Subsequent studies demonstrated that this decrease in WGA binding was attributable to a significant reduction in VWF sialic acid expression. Overall, the authors estimated that VWF sialylation in patients with pulmonary hypertension was reduced by 20%-25% (Figure 4D).[57] However, in some of the patients studied, VWF sialic acid expression was actually reduced by up to 70%. These findings are of clinical relevance, as pulmonary hypertension has also been associated with a significant reduction in HMWM VWF. It seems likely that other diseases (e.g., cancer) may also be associated with quantitative and/or qualitative alterations in VWF sialylation. Further studies will be required to define the nature of these glycan changes and determine

how they may impact upon: (*a*) VWF structure and function; and (*b*) disease pathogenesis.

In addition to these pathologic conditions, current evidence suggests that VWF glycosylation and sialylation may also undergo physiologic variations, for example, during pregnancy or with increasing age. It is well established that plasma VWF levels increase progressively during the course of normal pregnancy. For example, Druary-Smith et al recently showed that mean plasma VWF antigen levels were 120.3 IU/dL in the first trimester, 139.9 IU/dL in the second trimester, and 191.3 IU/dL in the third trimester.[58] This increase in levels appears to relate in part to a reduction in VWF clearance. Alterations in plasma VWF multimer distribution (including a reduction in HMWMs and an altered triplet pattern) have also been associated with pregnancy.[58] Although the biology underpinning these changes in VWF clearance and multimer distribution remains unclear, changes shown in isoelectric focusing studies raise the possibility that VWF glycan expression may also differ during pregnancy.[58]

12 | VWF SIALYLATION AND THERAPEUTIC DEVELOPMENT

Recent studies have investigated the glycosylation and sialylation of VWF in a number of different therapeutic concentrates.[59] As all of these concentrates were plasma-derived, it is perhaps unsurprising that Riddell et al observed no significant differences in SNA (which prefers α2-6–linked over α2-3–linked sialic acid) or RCA-1 (terminal Gal residues) binding between 10 commercially available VWF-containing concentrates. In addition to the plasma-derived VWF concentrates available for clinical use, a first recombinant VWF (rVWF) concentrate has recently been licensed (VONVENDI; Shire).[60] This rVWF is expressed in Chinese hamster ovary cells, and consequently shows differences from plasma-derived VWF in glycosylation and sialylation. In particular, ABO(H) blood group antigens are not expressed on the rVWF product, and sialylation levels are increased.[61] Given these differences in glycosylation, it is interesting that data from the phase 1 and phase 3 studies suggest that the half-life of infused rVWF (19.6 hours) is considerably longer than that of plasma-derived VWF (range of 12.8-15.8 hours).[62,63] Moreover, the data further suggest that rVWF may be more effective in stabilizing endogenous FVIII than plasma-derived VWF. Additional studies will be required to confirm these initial observations and to define the biological mechanisms underlying these findings.

13 | CONCLUSIONS

In conclusion, despite the fact that sialic acid is only present as a single terminal capping residue on the end of the complex branching glycans of VWF, it nevertheless plays significant roles in regulating VWF structure and function. Further studies will be required to define the molecular mechanisms through which N-linked and/or O-linked sialylation impacts on the multiple biological activities of multimeric VWF. Nonetheless, the elucidation of these mechanisms may offer new insights into how VWF contributes to the pathogenesis of a range of different pathologies. In addition, these studies may provide exciting opportunities to develop novel recombinant VWF therapeutics with targeted glyco-engineering. In this context, it is interesting that initial studies suggest that polysialylation of VWF may provide a strategy through which to develop a long-acting recombinant VWF product.

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CONFLICT OF INTERESTS

J. S. O'Donnell has served on speaker's bureau for Baxter, Bayer, Novo Nordisk, Boehringer Ingelheim, Leo Pharma, and Octapharma. He has also served on the advisory boards of Baxter, Bayer, Octapharma CSL Behring, Daiichi Sankyo, Boehringer Ingelheim, Shire, and Pfizer. J. S. O'Donnell has also received research grant funding awards from Baxter, Bayer, Pfizer, Shire, and Novo Nordisk. The other authors state that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

All authors drafted the first version of different sections of the manuscript, and all critically reviewed the final manuscript.

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