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**VON WILLEBRAND FACTOR: FROM FIGURANT TO MAIN CHARACTER IN THE SCENE OF
INFLAMMATION**

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Short title: VWF and innate immunity

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VWF: a multi-purpose protein

Von Willebrand factor (VWF) is a peculiar protein, not only by being the largest protein that travels through our vessels, or by the way hemodynamic forces regulate its platelet-binding functions but also because of its pleiotropic influence on a diverse spectrum of physiological and pathological processes. In addition to the well-established mechanisms by which VWF supports primary haemostasis and functions as a chaperone-protein for coagulation factor VIII (FVIII), we now begin to understand in more detail how VWF exerts its activities beyond hemostasis. For instance, the various ways by which VWF may modulate angiogenic processes has been elucidated during the last decade.(1, 2, 3) More recently, it has been shown that VWF promotes smooth muscle cell proliferation via direct interactions with the LRP4- $\alpha_v\beta_3$ receptor complex, thereby inducing signaling pathways that include p38MAP kinase activation.(4).

VWF interacts with macrophages for multiple reasons

Drakeford and colleagues recently reported a new breakthrough in our understanding of VWF function beyond haemostasis, *ie* its role in innate immunity.(5) Ample evidence that VWF contributes to inflammatory responses has been reviewed by Kawecki et al..(6) VWF is able to recruit leukocytes to sites of inflammation via direct interactions and via the formation of platelet-strings that enable platelet-leukocyte interactions.(7, 8, 9, 10, 11, 12) However, little was known about the interaction between VWF and another player in the immune system: the macrophage. In the context of VWF biology, macrophages are mainly known as cells that are part of the catabolic pathway that eliminates VWF from the circulation.(13, 14) In addition, VWF located at the surface of liver macrophages (Kupffer cells) plays a role in the surveillance mechanism that is designed to eliminate platelet/bacteria complexes from the circulation.(15) Drakeford and colleagues now show that VWF has the potential to drive macrophages into a pro-inflammatory M1-phenotype.(5)

How VWF turns macrophages into pro-inflammatory assets

In a series of elegant experiments, Drakeford *et al.* demonstrate that VWF binding to macrophages (either THP-1-derived or bloodborne monocyte-derived) induces p38MAP kinase signaling, forcing a change in gene

expression pattern with 1334 genes displaying modified expression (Figure 1). Notably, pro-inflammatory cytokines and chemokines are upregulated and increased production of Tumor Necrosis Factor (TNF), interleukin (IL)-6, IL-1 β , chemokine C-C ligand (CCL)-2, CCL-3 and CCL-4 was detected. Interestingly, the supernatant of VWF-stimulated macrophages appeared more efficient in recruiting naïve monocytes compared to the supernatant of LPS-stimulated macrophages, illustrating the pro-inflammatory potential of the VWF/macrophage axis.

Perhaps unexpected, VWF not only induced changes in gene associated with the inflammatory response, but also genes involved in metabolic pathways (Fig. 1). These alterations provoked a marked increase in glycolytic activity in combination with changes in the morphology of mitochondria. High glycolytic activity is indeed known to modify the structure of mitochondria, and is characteristic of the inflammatory phenotype of macrophages.(16)

An important finding during their studies was that VWF-induced macrophage reprogramming is transient, in contrast to changes induced by LPS, which proved long-lasting (Figure 1). Knowing that VWF is one of the so-called acute-phase proteins, it seems possible that transient increases in VWF levels (for instance in response to endothelial stimulation) allow for a temporary, reversible stimulation of macrophages towards the pro-inflammatory M1-phenotype. This temporary effect may originate from the notion that VWF is efficiently endocytosed and degraded by macrophages.

VWF and LRP1: a flexible interaction

To identify the receptor that mediates the VWF-induced alterations in the gene expression profile and subsequent phenotype of the macrophages, the authors turned to receptors that were previously identified to be involved in the catabolism of VWF. One of these receptors is the scavenger-receptor LDL-receptor related protein-1 (LRP1).(17) By using dedicated inhibitors of LRP1 (*ie.* anti-LRP1 antibodies and the universal LRP-inhibitor Receptor-Associated Protein), the authors were able to partially neutralize the pro-inflammatory effect of VWF. Signaling via the p38MAP kinase pathway was approximately halved and consequently, macrophages were less willing to turn into the M1-phenotype. This strongly suggests that LRP1 is indeed

involved not only in the endocytosis of VWF but also is a key element in VWF-dependent reprogramming of the macrophages. It should be noted that the role of the VWF-LRP1 interaction is perhaps more complex than one would think at first sight. Previous studies have shown that LRP1 can induce both pro- and anti-inflammatory outcomes, as illustrated by the findings that a loss of macrophage LRP1 has a dual and opposite effect on atherosclerotic plaque biogenesis, depending on whether the plaque is growing or shrinking.(18, 19) Is it possible that different LRP1-mediated signals into the macrophage are dependent on the ligand that binds to LRP1? In favor of this would be the finding that factor VIII (also a ligand for LRP1) does not modify the phenotype of macrophages.(20) Another possibility is that the multimeric nature of VWF allows for oligomerization of LRP1 receptors at the cellular surface, a condition that perhaps could force LRP1-mediated signaling into the pro-inflammatory direction. Again another option is that there is another yet unidentified receptor involved. LRP1 (and other members of the LRP-family) are known to form heterologous-receptor complexes at the surface of cells, and it is not uncommon that this is done in a cell-specific manner. Examples hereof are complexes of LRP1 and the NMDA-receptor on neuronal cells, of LRP1 and the receptor tyrosine kinase EphA2 on glioblastoma cells, and of LRP1 with the PDGF-receptor on smooth muscle cells.(21, 22, 23) Of particular interest in this regard is the capacity of LRP1 to form complexes with β_2 -integrins at the surface of leukocytes, knowing that these integrins (notably $\alpha_b\beta_2$) are associated with a pro-inflammatory phenotype of macrophages.(24, 25) Moreover, β_2 -integrins are similar to LRP1 in that they can bind to VWF, suggesting that a tertiary complex can be formed between VWF, LRP1 and β_2 -integrins.(7) It would be of interest to investigate whether the formation of this tertiary complex is responsible for the pro-inflammatory reprogramming of macrophages.

***In vivo* chemotaxis activity of VWF**

The *in vivo* relevance of the VWF-macrophage axis was tested in a mouse model of chemotaxis. Following peritoneal deposition of VWF, the cellular content of the peritoneum was analyzed at 3h and 24h. No differences were observed between vehicle- and VWF-treated mice after 3h. In contrast, after 24h VWF-treated mice were characterized by an increased number of small peritoneal macrophages, a population of

macrophages that is known to expand in response to inflammatory stimuli (Fig. 1). Macrophages isolated from the peritoneum also manifested increased expression of pro-inflammatory cytokines, like TNF, IL-6 and IL-1 β .

Translational consequences

Having identified VWF as a pro-inflammatory agent, the next question is obviously how this would affect us in daily life? As mentioned above, the link between VWF and inflammation has been fairly well-established in different animal models, including stroke and atherosclerosis. This association is perhaps less clear in the human context, but also less easy to investigate, since confounding variables are often present. However, having more insight into the molecular basis by which VWF exerts its pro-inflammatory activity may help us to further define the conditions in which VWF plays a more or less relevant role in the inflammatory response. In particular, the interplay between lung macrophages and VWF in conditions like systemic inflammatory response syndrome (SIRS) and COVID-19 could be of interest. VWF levels are markedly increased in COVID-19, whereas SIRS is characterized by increased levels of active VWF (a platelet-binding conformation, that also promotes binding to LRP1).(17, 26, 27, 28) Moreover, increased levels of VWF in COVID-19 or active VWF in SIRS are associated with exaggerated inflammatory responses and an increased risk of mortality.(28, 29) These findings may provide a rationale for studies aiming to reduce plasma levels of VWF or to interfere with VWF-LRP1 interactions in order to limit the inflammatory response under such conditions.

The other side of the coin would be conditions in which VWF is deficient or dysfunctional, as in von Willebrand disease (VWD). Also here the difficulty is that VWD is a very heterogenous disease, both from a molecular and clinical point of view. It will be complicated to design studies to explore the connection between VWF and innate immunity in such an heterogenous group of patients, especially if these patients receive prophylactic treatment with VWF concentrates. However, the availability of large national healthcare databases may open new avenues to explore this intriguing relationship in more detail.

In conclusion, Drakeford and colleagues have presented an elegant study that further confirms the multi-functional role of VWF beyond hemostasis. Having identified (at least in part) the molecular pathways that

contribute to the pro-inflammatory action of VWF towards macrophages. This knowledge will help us to better understand the complex relationship between haemostasis and innate immunity.

Author contribution

All authors contributed to the writing and editing of the manuscript

Conflict of interest

The authors declare no conflict of interest

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Legend Figure 1: VWF turns macrophages into a proinflammatory M1-phenotype

Binding of VWF to LRP1 (and probably a yet unidentified second receptor) at the surface of macrophages induces signaling, resulting in transient changes gene expression, driving these macrophages into a pro-inflammatory M1-phenotype. Particularly, increased expression and secretion of pro-inflammatory cytokines and chemokines has been observed, promoting chemotaxis in both *in vitro* and *in vivo* models. These findings further establish a role of VWF as a pro-inflammatory agent.(5) Figure has been created using Biorender.

References

1. Starke RD, Ferraro F, Paschalaki KE, Dryden NH, McKinnon TA, Sutton RE, et al. Endothelial von Willebrand factor regulates angiogenesis. *Blood*. 2011;117(3):1071-80.
2. Randi AM, Smith KE, Castaman G. von Willebrand factor regulation of blood vessel formation. *Blood*. 2018;132(2):132-40.
3. de Vries MR, Peters EAB, Quax PHA, Nossent AY. von Willebrand factor deficiency leads to impaired blood flow recovery after ischaemia in mice. *Thromb Haemost*. 2017;117(7):1412-9.

4. Lagrange J, Worou ME, Michel JB, Raoul A, Didelot M, Muczynski V, et al. The VWF/LRP4/alphaVbeta3-axis represents a novel pathway regulating proliferation of human vascular smooth muscle cells. *Cardiovasc Res.* 2022;118(2):622-37.
5. Drakeford C, Aguila S, Roche F, Hokamp K, Fazavana J, Cervantes MP, et al. von Willebrand factor links primary hemostasis to innate immunity. *Nat Commun.* 2022;13(1):6320.
6. Kawecki C, Lenting PJ, Denis CV. von Willebrand factor and inflammation. *J Thromb Haemost.* 2017;15(7):1285-94.
7. Pendu R, Terraube V, Christophe OD, Gahmberg CG, de Groot PG, Lenting PJ, et al. P-selectin glycoprotein ligand 1 and beta 2-integrins cooperate in the adhesion of leukocytes to von Willebrand factor. *Blood.* 2006;108(12):3746-52.
8. Lerolle N, Dunois-Larde C, Badirou I, Motto DG, Hill G, Bruneval P, et al. von Willebrand factor is a major determinant of ADAMTS-13 decrease during mouse sepsis induced by cecum ligation and puncture. *J Thromb Haemost.* 2009;7(5):843-50.
9. Petri B, Broermann A, Li H, Khandoga AG, Zarbock A, Krombach F, et al. von Willebrand factor promotes leukocyte extravasation. *Blood.* 2010;116(22):4712-9.
10. Hillgruber C, Steingraber AK, Poppelmann B, Denis CV, Ware J, Vestweber D, et al. Blocking von Willebrand factor for treatment of cutaneous inflammation. *J Invest Dermatol.* 2014;134(1):77-86.
11. Adam F, Casari C, Prevost N, Kauskot A, Loubiere C, Legendre P, et al. A genetically-engineered von Willebrand disease type 2B mouse model displays defects in hemostasis and inflammation. *Sci Rep.* 2016;6:26306.
12. Ayme G, Adam F, Legendre P, Bazaa A, Proulle V, Denis CV, et al. A Novel Single-Domain Antibody Against von Willebrand Factor A1 Domain Resolves Leukocyte Recruitment and Vascular Leakage During Inflammation-Brief Report. *Arterioscler Thromb Vasc Biol.* 2017;37(9):1736-40.
13. van Schooten CJ, Shahbazi S, Groot E, Oortwijn BD, van den Berg HM, Denis CV, et al. Macrophages contribute to the cellular uptake of von Willebrand factor and factor VIII in vivo. *Blood.* 2008;112(5):1704-12.
14. Chion A, O'Sullivan JM, Drakeford C, Bergsson G, Dalton N, Aguila S, et al. N-linked glycans within the A2 domain of von Willebrand factor modulate macrophage-mediated clearance. *Blood.* 2016;128(15):1959-68.
15. Wong CH, Jenne CN, Petri B, Chrobok NL, Kubes P. Nucleation of platelets with blood-borne pathogens on Kupffer cells precedes other innate immunity and contributes to bacterial clearance. *Nat Immunol.* 2013;14(8):785-92.
16. O'Neill LA, Kishton RJ, Rathmell J. A guide to immunometabolism for immunologists. *Nat Rev Immunol.* 2016;16(9):553-65.

17. Rastegarlarlari G, Pegon JN, Casari C, Odouard S, Navarrete AM, Saint-Lu N, et al. Macrophage LRP1 contributes to the clearance of von Willebrand factor. *Blood*. 2012;119(9):2126-34.
18. Overton CD, Yancey PG, Major AS, Linton MF, Fazio S. Deletion of macrophage LDL receptor-related protein increases atherogenesis in the mouse. *Circ Res*. 2007;100(5):670-7.
19. Mueller PA, Zhu L, Tavori H, Huynh K, Giunzioni I, Stafford JM, et al. Deletion of Macrophage Low-Density Lipoprotein Receptor-Related Protein 1 (LRP1) Accelerates Atherosclerosis Regression and Increases C-C Chemokine Receptor Type 7 (CCR7) Expression in Plaque Macrophages. *Circulation*. 2018;138(17):1850-63.
20. Kis-Toth K, Rajani GM, Simpson A, Henry KL, Dumont J, Peters RT, et al. Recombinant factor VIII Fc fusion protein drives regulatory macrophage polarization. *Blood Adv*. 2018;2(21):2904-16.
21. May P, Rohlmann A, Bock HH, Zurhove K, Marth JD, Schomburg ED, et al. Neuronal LRP1 functionally associates with postsynaptic proteins and is required for normal motor function in mice. *Mol Cell Biol*. 2004;24(20):8872-83.
22. Gopal U, Bohonowych JE, Lema-Tome C, Liu A, Garrett-Mayer E, Wang B, et al. A novel extracellular Hsp90 mediated co-receptor function for LRP1 regulates EphA2 dependent glioblastoma cell invasion. *PLoS One*. 2011;6(3):e17649.
23. Boucher P, Gotthardt M, Li WP, Anderson RG, Herz J. LRP: role in vascular wall integrity and protection from atherosclerosis. *Science*. 2003;300(5617):329-32.
24. Spijkers P, Martins PD, Westein E, Gahmberg CG, Zwaginga JJ, Lenting PJ. LDL-receptor-related protein regulates beta 2-integrin-mediated leukocyte adhesion. *Blood*. 2005;105(1):170-7.
25. Aziz MH, Cui K, Das M, Brown KE, Ardell CL, Febbraio M, et al. The Upregulation of Integrin alpha(D)beta(2) (CD11d/CD18) on Inflammatory Macrophages Promotes Macrophage Retention in Vascular Lesions and Development of Atherosclerosis. *J Immunol*. 2017;198(12):4855-67.
26. Fogarty H, Ward SE, Townsend L, Karampini E, Elliott S, Conlon N, et al. Sustained VWF-ADAMTS-13 axis imbalance and endotheliopathy in long COVID syndrome is related to immune dysfunction. *J Thromb Haemost*. 2022;20(10):2429-38.
27. Rauch A, Labreuche J, Lassalle F, Goutay J, Caplan M, Charbonnier L, et al. Coagulation biomarkers are independent predictors of increased oxygen requirements in COVID-19. *J Thromb Haemost*. 2020;18(11):2942-53.
28. Hyseni A, Kemperman H, de Lange DW, Kesecioglu J, de Groot PG, Roest M. Active von Willebrand factor predicts 28-day mortality in patients with systemic inflammatory response syndrome. *Blood*. 2014;123(14):2153-6.
29. Philippe A, Gendron N, Bory O, Beauvais A, Mirault T, Planquette B, et al. Von Willebrand factor collagen-binding capacity predicts in-hospital mortality in COVID-19 patients: insight from VWF/ADAMTS13 ratio imbalance. *Angiogenesis*. 2021;24(3):407-11.

Figure 1
VWF and innate immunity
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