specialized bioinformatic tools. They then validated some of the circular reads by reverse transcriptase polymerase chain reaction. Next, they enriched the circRNA content of their samples by selectively digesting linear RNA with ribonuclease R, to which circRNAs are resistant and that has been used to define them.

Using this approach, the authors observed marked circRNA enrichment in platelets (and erythrocytes) relative to nucleated cells, mainly as a result of differential decay of linear RNAs. Conversely, circRNA enrichment in platelets vs cultured megakaryocytes, in the absence of de novo gene transcription, is consistent with circRNAs being produced in platelets rather than being inherited from their precursor cells. Two circRNA junctions (E4-E2 and E5-E2; E, exon) of one gene, *MAN1A2*, are even more abundant in platelets (and erythrocytes) than the linear mRNA. Based on their findings, the authors proposed that mRNA translation in platelets occurs against the backdrop of a rapidly degrading transcriptome, which would explain the relatively weak association between the human platelet transcriptome and proteome.[3]

At the interface of the platelet transcriptome and proteome is an unusually abundant and diverse array of microRNAs (miRNAs),[4] which are key regulators of gene expression. Considering that (1) platelet mRNAs harbor 3′ untranslated regions (3′UTRs) twice as long as those of nucleated cells (1047 vs 492 nucleotide),[5] (2) 3′UTRs are known to contain microRNA (miRNA) binding sites, and (3) 2 circRNAs from nucleated cells act as miRNA sponges,[6] it would be interesting to determine whether platelet circRNAs are enriched in 3′UTR exons and miRNA binding sites, because this could explain the relative abundance of circRNAs and miRNAs in platelets.

To gain further insights into the biology of circRNAs in platelets, we may want to know if platelets contain RNA binding protein Quaking, an alternative splicing factor regulating the production of more than one-third of abundant circRNAs during human epithelial-mesenchymal transition.[7] The biological relevance of platelet circRNAs, if any, remains unclear: do they play a role inside of platelets, or are they simply byproducts of platelet mRNA degradation? The latter assumption does not necessarily preclude them from mediating biological effects outside of platelets. Indeed, circRNAs may still play a role in other cells, as shown previously for mRNAs and miRNAs,[8] provided that they are expelled within exosomes or larger microparticles, under basal conditions or upon activation, which remains to be seen. Because exon-intron circRNAs regulate transcription in nucleated cells,[9] it is tempting to speculate that circRNAs that accumulate in platelets may end up regulating gene expression and function of other recipient cells of the circulatory system.

The possible clinical implications of platelet circRNAs may soon be studied and revealed, because we will want to know if circRNA abundance and/or sequences vary depending on the race, gender, age, or health status of the subjects. Are circRNAs present and conserved in the platelets of small animal models, such as the mouse? If so, very informative in vivo studies could be performed. The suggestion that platelet lifespan may be determined by translational competence support a possible relationship between circRNA levels and platelet half-life or aging. Could circRNAs be used as biomarkers of platelet-related diseases, or signal shorter platelet lifespan or premature platelet aging? Could these conditions be unveiled simply by measuring the level of specific circRNAs by quantitative PCR?

Produced upon cytoplasmic fragmentation of bone marrow megakaryocytes, circulating platelets contain an impressive array of RNA species (messenger RNA, transfer RNA, microRNA, long noncoding RNA)[10] that now includes circRNAs. These recently described RNA species were identified by Alhasan et al[1] among gigabytes of platelet RNA sequencing data simply by using publicly available bioinformatic tools, as one would experience by looking at the full moon with a telescope instead of binoculars. Has anyone thought that, by analyzing the transcriptome of an anucleate element of the blood, we would go round (and round) in circles?

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

REFERENCES

1. Alhasan AA, Izuogu OG, Al-Balool HH, et al. Circular RNA enrichment in platelets is a signature of transcriptome degradation. *Blood*. 2016;127(9):e1-e11.
2. Denis MM, Tolley ND, Bunting M, et al. Escaping the nuclear confines: signal-dependent pre-mRNA splicing in anucleate platelets. *Cell*. 2005;122(3):379-391.
3. Londin ER, Hatzimichael E, Loher P, et al. The human platelet: strong transcriptome correlations among individuals associate weakly with the platelet proteome. *Biol Direct*. 2014;9:3.
4. Plé H, Landry P, Benham A, Coarfa C, Gunaratne PH, Provost P. The repertoire and features of human platelet microRNAs. *PLoS One*. 2012;7(12):e50746.
5. Dittrich M, Birschmann I, Pfrang J, et al. Analysis of SAGE data in human platelets: features of the transcriptome in an anucleate cell. *Thromb Haemost*. 2006;95(4):643-651.
6. Hansen TB, Jensen TI, Clausen BH, et al. Natural RNA circles function as efficient microRNA sponges. *Nature*. 2013;495(7441):384-388.
7. Conn SJ, Pillman KA, Toubia J, et al. The RNA binding protein quaking regulates formation of circRNAs. *Cell*. 2015;160(6):1125-1134.
8. Laffont B, Corduan A, Plé H, et al. Activated platelets can deliver mRNA regulatory Ago2•microRNA complexes to endothelial cells via microparticles. *Blood*. 2013;122(2):253-261.
9. Li Z, Huang C, Bao C, et al. Exon-intron circular RNAs regulate transcription in the nucleus. *Nat Struct Mol Biol*. 2015;22(3):256-264.
10. Bray PF, McKenzie SE, Edelstein LC, et al. The complex transcriptional landscape of the anucleate human platelet. *BMC Genomics*. 2013;14:1.

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● ● ● THROMBOSIS AND HEMOSTASIS

Comment on O'Regan et al, page 1192

The heads and the tails of malaria and VWF

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In this issue of *Blood*, O'Regan et al have extended our understanding of von Willebrand factor (VWF) in the pathogenesis of malaria.[1] According to the World Health Organization (http://www.who.int/gho/malaria/en/), malaria affects 3.2 billion people in 97 countries with 198 million cases having occurred in 2013,

and of those, 584 000 died. Ninety percent of those deaths in 2013 were children under the age of 5. The most devastating form of the disease is cerebral malaria, which occurs most frequently in young children. Although blood coagulation changes such as disseminated intravascular coagulation have been recognized since the 1970s,[2] recent studies have focused on markers of these hemostatic changes as being most prevalent in cerebral malaria caused by *Plasmodium falciparum*. Cerebral malaria is more lethal in children than adults. Exchange transfusion has been used as an aggressive adjunct therapy for this condition.[3]

Studies of VWF and its propeptide, VWFpp, demonstrated activation of endothelial cells with release of VWF and VWFpp from the Weibel Palade bodies of endothelial cells into plasma with an increase in the VWFpp/VWF:antigen ratio in patients with cerebral malaria.[4] Recently, a donkey antibody has been developed that recognizes the "activated" form of VWF that exists in a conformation that spontaneously binds to platelets.[5] Using this approach, activated VWF has been identified in the plasma of patients with cerebral malaria.[6] Recently, additional hemostatic abnormalities have been identified including a reduction of VWF-cleaving protease, ADAMTS13,[7] in plasma and a loss of endothelial protein C receptor (EPCR) in the cerebral vessels in patients with cerebral malaria.[8] One other historical observation is of interest: severe *P falciparum* cerebral malaria is less common in blood group O individuals,[9] and this finding holds up even with more recent genome-wide association studies. In addition, VWF levels are 25% to 30% higher in non–O blood groups,[10] which might suggest an association for VWF levels in the pathogenesis of cerebral malaria.

O'Regan et al help clarify whether VWF levels are the "effect" or the "cause" of decreased malaria resistance. The authors developed a model of cerebral malaria in the mouse using *Plasmodium berghei* ANKA that recapitulates cerebral malaria in humans. When this infection is carried out on the $VWF^{-/-}$ mouse knockout background, clinical experimental cerebral malaria progression is delayed and the survival of these mice is prolonged. Because platelet counts and parasitemia are identical to controls, this suggests VWF is an active participant in cerebral malaria pathogenesis. This effect might partially explain why malaria resistance could be greater in blood group O individuals who have lower VWF levels. As shown (see figure), endothelial cells are activated and (1) release ultralarge VWF multimers that (2) sequester platelets, (3) recruit malaria-infected RBCs, (4) alter endothelial cell permeability, and (5) progress to microvascular thrombosis.

Although it is likely that other mechanisms interplay with this pathogenic pathway, VWF is not just a simple secondary marker of endothelial cell activation (tails), but a potential regulator (heads) of CNS malaria. There will undoubtedly be more to this story in the future.

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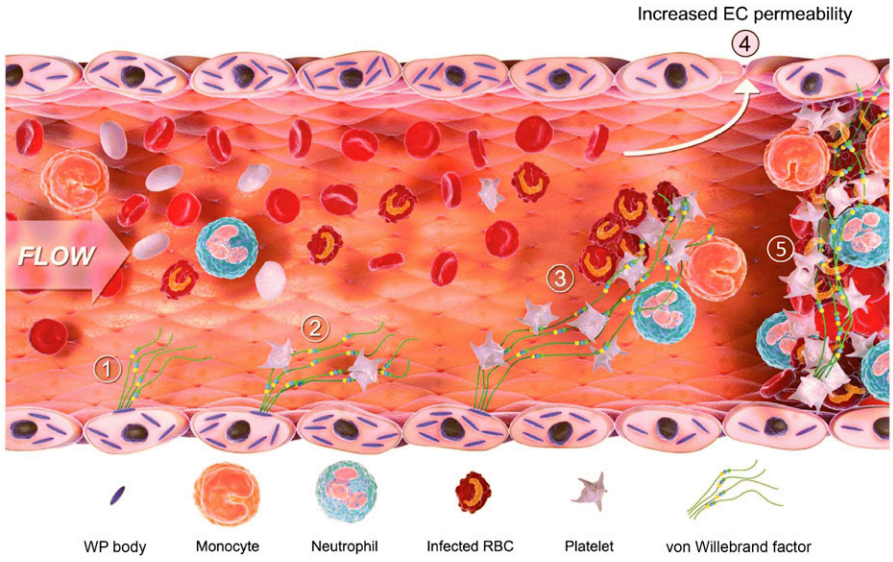

This demonstrates the sequential role of VWF in the process of central nervous system (CNS) malaria. Endothelial cells are activated and release VWF from the Weibel Palade bodies into circulating blood, platelets adhere, parasite-infected red blood cells (RBCs) are recruited, and VWF induces increased endothelial cell permeability and leads to CNS thrombosis. EC, endothelial cell; WP, Weibel Palade. The figure has been adapted from Figure 6 in the article by O'Regan et al that begins on page 1192.

REFERENCES

1. O'Regan N, Gegenbauer K, O'Sullivan JM, et al. A novel role for von Willebrand factor in the pathogenesis of experimental cerebral malaria. *Blood*. 2016;127(9):1192-1201.

2. Moxon CA, Chisala NV, Mzikamanda R, et al. Laboratory evidence of disseminated intravascular coagulation is associated with a fatal outcome in children with cerebral malaria despite an absence of clinically evident thrombosis or bleeding. *J Thromb Haemost*. 2015;13(9):1653-1664.

3. Barman H. Exchange transfusion in complicated pediatric malaria: a critical appraisal. *Indian J Crit Care Med*. 2015;19(4):214-219.

4. Hollestelle MJ, Donkor C, Mantey EA, et al. von Willebrand factor propeptide in malaria: evidence of acute endothelial cell activation. *Br J Haematol*. 2006;133(5):562-569.

5. Hulstein JJ, de Groot PG, Silence K, Veyradier A, Fijnheer R, Lenting PJ. A novel nanobody that detects the gain-of-function phenotype of von Willebrand factor in ADAMTS13 deficiency and von Willebrand disease type 2B. *Blood*. 2005;106(9):3035-3042.

6. de Mast Q, Groot E, Lenting PJ, et al. Thrombocytopenia and release of activated von Willebrand factor during early *Plasmodium falciparum* malaria. *J Infect Dis*. 2007;196(4):622-628.

7. Larkin D, de Laat B, Jenkins PV, et al. Severe *Plasmodium falciparum* malaria is associated with circulating ultra-large von Willebrand multimers and ADAMTS13 inhibition. *PLoS Pathog*. 2009;5(3):e1000349.

8. Aird WC, Mosnier LO, Fairhurst RM. *Plasmodium falciparum* picks (on) EPCR. *Blood*. 2014;123(2):163-167.

9. Pathirana SL, Alles HK, Bandara S, et al. ABO-blood-group types and protection against severe, *Plasmodium falciparum* malaria. *Ann Trop Med Parasitol*. 2005;99(2):119-124.

10. Gill JC, Endres-Brooks J, Bauer PJ, Marks WJ Jr, Montgomery RR. The effect of ABO blood group on the diagnosis of von Willebrand disease. *Blood*. 1987;69(6):1691-1695.

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