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Thrombosis in Paroxysmal Nocturnal Hemoglobinuria – insights into the role of complement in thrombosis

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Paroxysmal Nocturnal Hemoglobinuria (PNH) is a rare clonal disorder of the bone marrow, which is the result of an acquired mutation in the PIG-A gene leading to decreased production of the glycosylphosphotiydalinositol (GPI) anchors [1]. As a consequence, membrane proteins, such as the membrane inhibitors of reactive lysis (MIRL) designated as CD59, CD 55, are missing or decreased [2]. Classical PNH is characterized by chronic red blood cell hemolysis punctuated with episodes of acute exacerbations due to complement hypersensitivity. However, because this is a stem cell disorder, white blood cells and platelets are also affected.

PNH is associated with a markedly increased risk of venous thrombosis which can occur in usual (DVT/PE) as well as unusual sites such as hepatic veins (Budd-Chiari syndrome), cavernous sinus, and mesenteric veins [3,4]. While arterial events occur in about 15% of patients, venous thrombosis predominates and thrombosis is the most common cause of death in PNH. The etiology of the hypercoaguability is multifactorial. Although often attributed to intravascular hemolysis, thrombosis may occur in patients with minimal or no evidence of hemolysis. The risk of thrombosis increases 4 fold for every 10% increase in the size of the WBC clone (granulocyte/monocyte) [5]. In response to complement injury, granulocytes and monocytes participate in the hemostatic cascade through several mechanisms. Activation of granulocytes leads to release of inflammatory molecules damaging endothelium. Complement damaged leukocytes adhere to endothelial cells and may induce tissue factor expression and release of inflammatory cytokines. Red cell vesicles scavenged by monocytes can up regulate tissue factor expression.

CD 59 deficient platelets also increase the risk of thrombosis. The platelet clone size parallels that of the WBC clone. In contrast to the RBC, platelets bundle the C9 molecules, forming thrombogenic microvesicles [6]. Platelet microvesicles expressing P selectin can induce additional tissue factor expression by the monocyte/macrophage. Although thrombocytopenia has always been attributable to marrow failure recent evidence suggests that in some patients, the thrombocytopenia may be due to thrombin mediated platelet aggregation further activating coagulation [7].

The role of the endothelial cell is unclear. Endothelial cell vesiculation has been reported [8] and endothelial cell activation with increased expression of von Willebrand factor has been noted in PNH [9]. The role of bone marrow derived circulating endothelial precursors in PNH is actively being investigated.

Eculizumab is a monoclonal antibody to C5 of the complement cascade, which has recently received FDA approval for the treatment of PNH. As a consequence of C5 inhibition the membrane attack complex, C9, cannot be formed. In addition, the production of the complement protein C5a, is blocked. Clinically, this results in a significant reduction in hemolysis, transfusion requirements and fatigue in patients with PNH [10,11]. In addition, recent data demonstrates a 92% reduction in thrombotic events with the use of eculizumab [12]. Previously published data demonstrated high levels of TFMP in patients with PNH, which decreased after bone marrow transplantation. We hypothesized that the reduction in the rate of thromboembolic events seen with eculizumab is due to a decrease in leucocyte complement injury with a reduction in leucocyte-derived tissue factor microparticles, a decrease in systemic thrombin generation, and a reduction in inflammatory cytokines. Markers of hemostatic activation, D-Dimers, TAT were elevated in all but 2 of the 11 patients prior to beginning eculizumab. There was a highly significant decrease in markers of hemostatic activation (TAT, D-dimers) as well as inflammation (IL-6) during the 4 weeks of the inductions and remained low over the 90 days of the study [13]. The reduction of D-Dimer and Fragment 1.2 was also confirmed by Helle, et.al looking at changes at 5 weeks and 11 weeks of treatment [9]. In addition we observed

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a strong correlation between changes in D-Dimers, TAT and IL-6, but found no correlation with the LDH, a surrogate for hemolysis, suggesting a prothrombotic mechanism independent of hemolysis. In addition, platelet counts improved in 57% of patients and were inversely correlated with reduction of D-Dimers, TAT and IL6 but not correlated with the reduction in LDH [7]. Antigenic TFMP also showed a statistically significant decrease over the 4 weeks of the induction and remain lower over the 90 days of the study. However, these did not correlate with other markers of thrombin generation such as D-Dimers and TAT but did show correlation with functional TFMP activity.

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