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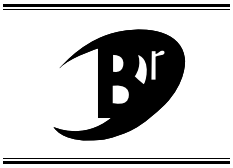

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REVIEW

Advances in the diagnosis and therapy of paroxysmal nocturnal hemoglobinuria

Robert A. Brodsky *

The Division of Hematology, Johns Hopkins, University School of Medicine, 720 Rutland Avenue, Ross Research Building, Room 1025, Baltimore, MD 21205, United States

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Summary PNH is an uncommon acquired hemolytic anemia that often manifests with hemoglobinuria, abdominal pain, smooth muscle dystonias, fatigue, and thrombosis. The disease results from the expansion of hematopoietic stem cells harboring a mutation in a gene, *PIG-A*, that is required for the biosynthesis of a lipid moiety, glycosylphosphatidylinositol (GPI), that attaches dozens of different proteins to the cell surface. Thus, PNH cells are deficient in cell surface GPI anchored proteins; this deficiency on erythrocytes leads to intravascular hemolysis since certain GPI anchored proteins normally function as complement regulators. Free hemoglobin released from intravascular hemolysis leads to circulating nitric oxide depletion and is responsible for many of the clinical manifestations of PNH, including fatigue, erectile dysfunction, esophageal spasm, and thrombosis. Interestingly, rare *PIG-A* mutations can be found in virtually all healthy control subjects leading to speculation that *PIG-A* mutations in hematopoietic stem cells are common benign events. However, recent data reveals that most of these mutations in healthy controls are not derived from stem cells. The recently FDA approved complement inhibitor eculizumab has been shown to decrease hemolysis, decrease erythrocyte transfusion requirements, decrease the risk for thrombosis and improve quality of life for PNH patients.

Introduction

Paroxysmal nocturnal hemoglobinuria (PNH) is a clonal hematopoietic stem cell disorder that has fascinated hematologists for more than a century due to its protean clinical manifestations and fascinating pathophysiology.[1] One of the earliest descriptions of PNH was by Dr. Paul Strübing, who in 1882 described a 29-year-old cartwright who presented with fatigue, abdominal pain, and severe nocturnal paroxysms of hemoglobinuria that were exacerbated by excess alcohol, physical exertion, and iron salts.[2] Later reports by Marchiafava and Micheli led to the eponym, Marchiafava-Micheli syndrome, but it was Enneking, in 1925,

* Tel.: +1 410 502 2546; fax: +1 410 955 0185.
E-mail address: brodsro@jhmi.edu

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who introduced the term ''paroxysmal nocturnal hemoglobinuria''.[3]

In 1937, Thomas Ham found that PNH erythrocytes were hemolyzed when incubated with normal, acidified serum.[4] This seminal discovery resulted in the first diagnostic test for PNH, the acidified serum or Ham test. Red cell lysis following acidified serum appeared to be complement dependent since heat inactivation abrogated the reaction; however, it wasn't until 1954, with the discovery of the alternative pathway of complement activation, that increased complement sensitivity was formally proven to cause the hemolysis of PNH red cells.[5] Following the emergence of specific diagnostic tests, additional disease manifestations such as venous thrombosis, smooth muscle dystonias and other clonal stem cell disorders were found to be associated with PNH. These non-erythroid manifestations of the disease foreshadowed the discovery that PNH results from the clonal expansion of a mutated hematopoietic stem cell.

In the 1980s, it was discovered that PNH cells display a global deficiency in a group of proteins attached to the cell surface by a GPI moiety. Interestingly, two of these missing proteins (e.g., CD55 and CD59) are important complement regulatory proteins. A few years later, the genetic mutation (*PIG-A*) responsible for the GPI-anchor protein deficiency was discovered[6] and most recently, a humanized monoclonal antibody that inhibits terminal complement activation has been shown to ameliorate hemolysis and disease symptoms in PNH patients.[7,8] Although the pathophysiology of many manifestations of PNH are now understood, the mechanism of clonal dominance, and the close association of PNH and acquired aplastic anemia continue to be areas of intense investigation.

GPI-anchor biosynthesis

Covalent linkage to GPI is an important means of anchoring dozens of cell surface glycoproteins to the cell membrane. GPI is synthesized in the endoplasmic reticulum and transferred *en bloc* to the carboxyl terminus of a protein that has a GPI-attachment signal peptide. Biosynthesis of GPI anchors involves at least 10 reactions and more than 20 different genes (Fig. 1 and Table 1).[9] The common core structure of GPI consists of a molecule of

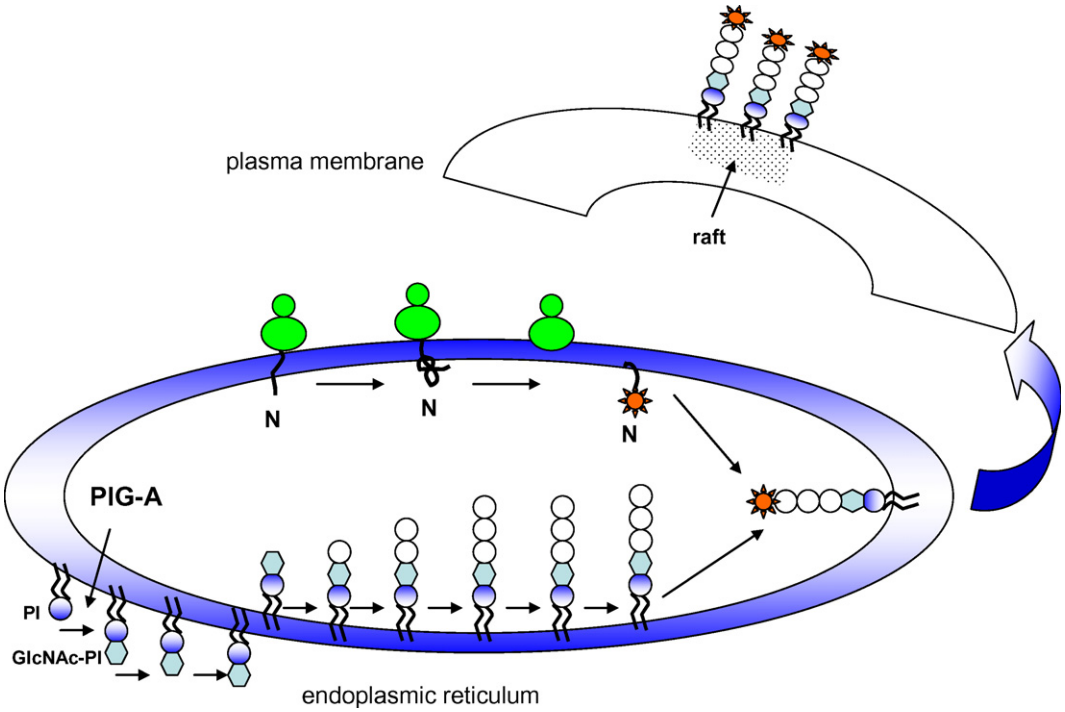

Figure 1 Biosynthesis of GPI-anchored proteins. GPI-anchor biosynthesis takes place in the endoplasmic reticulum. *PIG-A* is one of 7 genes required for the first step (see Table 1), the transfer of N-acetylglucosamine (GlcNAc) from uridine 5′-diphospho-N-acetylglucosamide (UDP-GlcNAc:PI) to phosphatidylinositol (PI) to yield GlcNAc-PI. After synthesis of the mature GPI precursor, the cognate protein is attached and then transported to the plasma membrane where the GPI-anchored protein resides in membrane rafts. *PIG-A* mutations lead to a defect in the first step in GPI anchor biosynthesis resulting in intracellular degradation of the cognate protein and a lack of cell surface GPI anchored proteins.