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Commentary

Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired disorder characterized by hemolysis, thrombosis, and bone marrow failure caused by defective expression of glycosylphosphatidylinositol-anchored (GPI-anchored) complement inhibitors. Most commonly, PNH is caused by loss of function of *PIGA*, which is required for GPI biosynthesis. In this issue of the *JCI*, Höchsmann et al. report on 4 PNH patients who also had marked autoinflammatory manifestations, including aseptic meningitis. All 4 patients had a germline mutation of the related gene *PIGT* and a somatically acquired myeloid common deleted region (CDR) on chromosome 20q that deleted the second *PIGT* allele. The biochemistry and clinical manifestations indicate that these patients have subtle but important differences from those with PNH resulting from *PIGA* mutations, suggesting PIGT-PNH may be a distinct clinical entity.

Paroxysmal nocturnal hemoglobinuria without GPI-anchor deficiency

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Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired disorder characterized by hemolysis, thrombosis, and bone marrow failure caused by defective expression of glycosylphosphatidylinositol-anchored (GPI-anchored) complement inhibitors. Most commonly, PNH is caused by loss of function of *PIGA*, which is required for GPI biosynthesis. In this issue of the *JCI*, Höchsmann et al. report on 4 PNH patients who also had marked autoinflammatory manifestations, including aseptic meningitis. All 4 patients had a germline mutation of the related gene *PIGT* and a somatically acquired myeloid common deleted region (CDR) on chromosome 20q that deleted the second *PIGT* allele. The biochemistry and clinical manifestations indicate that these patients have subtle but important differences from those with PNH resulting from *PIGA* mutations, suggesting PIGT-PNH may be a distinct clinical entity.

Molecular mechanisms of paroxysmal nocturnal hemoglobinuria

Paroxysmal nocturnal hemoglobinuria (PNH) is a complement-mediated hemolytic anemia caused by expansion of a hematopoietic stem cell harboring a somatic *PIGA* mutation (1). The gene product of *PIGA* is required for the biosynthesis of glycosylphosphatidylinositol (GPI) anchors; thus, *PIGA* mutations lead to a deficiency of GPI-anchored proteins, such as decay-accelerating factor (CD55) and CD59, both complement inhibitors. PNH patients manifest with hemolytic anemia, a propensity for thrombosis, and often evolve from acquired, but not inherited, forms of aplastic anemia (2). The disease can also occur without preceding aplastic anemia.

PIGA is an X-linked gene that is required for the first step in the biosynthesis of GPI (3). GPI anchors attach dozens of proteins to hematopoietic cells, including the complement regulatory proteins CD55 and CD59. The absence of CD55 and CD59 on blood cells leads to complement-mediated hemolysis and a propensity for thrombosis. Complement inhibitors that target C5 rapidly ameliorate these clinical manifestations (4–6). Before 2007, the median survival for PNH patients was roughly 15 years, with thrombosis being the leading cause of death (7, 8). In 2007, eculizumab, a monoclonal antibody that blocks the terminal complement at C5, changed the natural history of PNH by decreasing hemolysis, decreasing or eliminating the need for red cell transfusions, improving quality of life, and mitigating the risk of thrombosis (9).

Interestingly, *PIGA* mutations are not directly responsible for the clonal expansion required to cause PNH (10, 11). The absence of GPI-anchored proteins on PNH stem cells endows them with a conditional survival advantage in the setting of autoimmunity compared with *PIGA* wild-type stem cells. This explains why PNH so often evolves from acquired aplastic anemia (a T cell–mediated autoimmune disease) but not inherited forms of aplastic anemia such as dykeratosis congenital, Fanconi anemia, or Shwachman-Diamond syndrome. Thus, immune escape, alone or in combination with additional somatic mutations, is responsible for the clonal expansion of PNH blood cells. In some cases, mutations associated with clonal hematopoiesis of indeterminate potential (CHIP), such as *JAK2V617F* and *CALR*, have also been found in PNH patients, which may explain how some patients without a history of acquired aplastic anemia develop PNH (12–14). In this issue, Höchsmann et al. present an elegant study investigating the pathogenic mechanism of an extremely rare subtype of paroxysmal hemoglobinuria, PNH, which is caused by mutations affecting *PIGT* rather than *PIGA* (15).

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Distinct genetics cause differing syndromes

GPI anchor biosynthesis takes place in the endoplasmic reticulum and involves more than 24 genes and at least 10 steps. In the 1980s, investigators anticipated finding multiple gene defects in GPI anchor biosynthesis responsible for PNH. However, among the more than 2 dozen genes involved in GPI biosynthesis, only *PIGA* is X-linked; the rest are autosomal. Therefore, while a single inactivating somatic mutation in *PIGA* is sufficient to abolish GPI biosynthesis, for mutations in the autosomal GPI biosynthesis genes to cause disease, two inactivating mutations would have to occur in the same cell (Figure 1). The probability of two hits on different alleles in the same cell is extremely low, explaining why virtually all PNH cases are associated with *PIGA* mutations.

The authors previously reported PNH patients whose GPI anchor protein deficiency was caused by germline and somatic mutations in the *PIGT* gene localized on chromosome 20q (16). Here, Höchsmann et al. describe an additional two patients with germline and somatic mutations affecting *PIGT* (15). The additional two cases allowed them to recognize clinical manifestations that were distinct from

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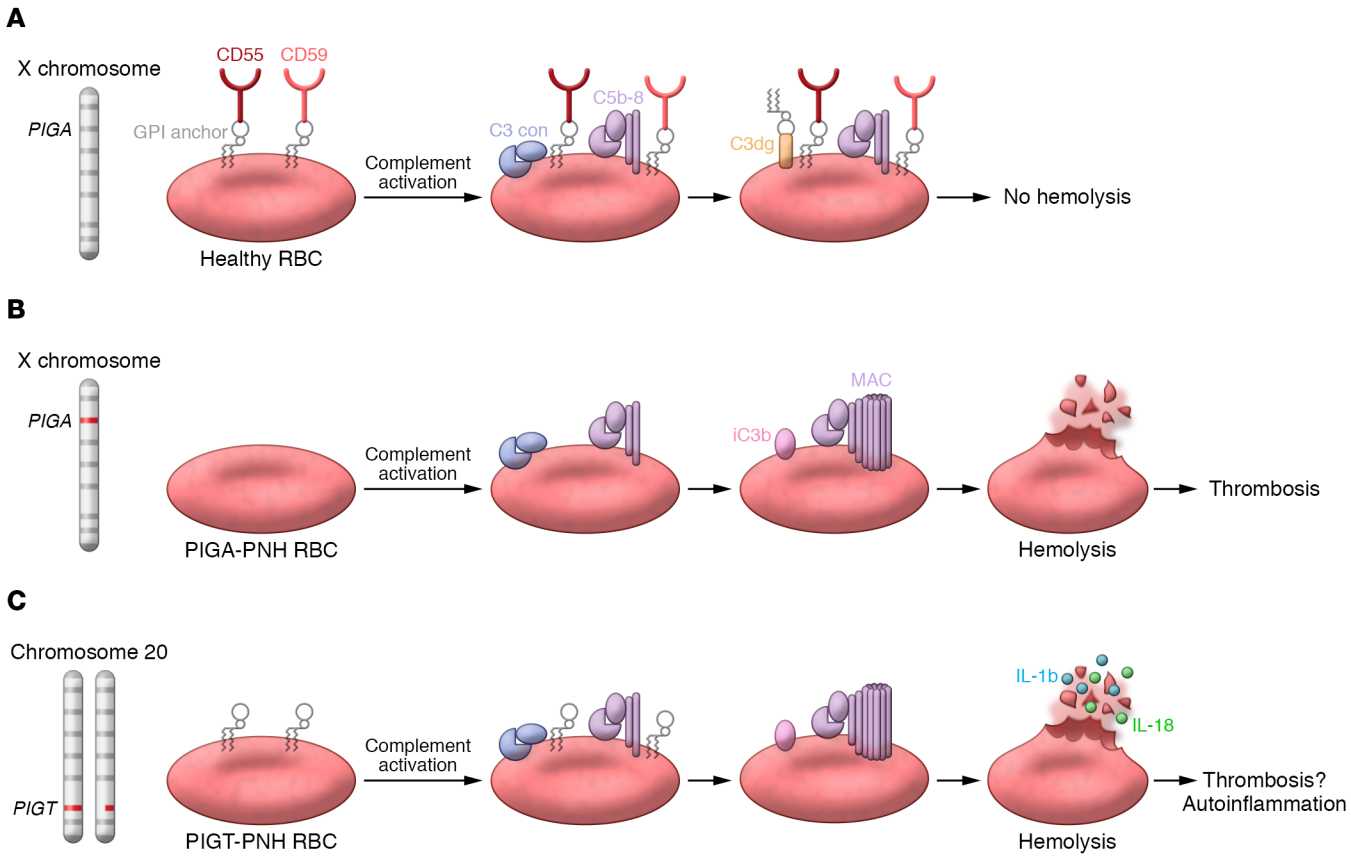

Figure 1. Molecular and cellular differences between PIGA- and PIGT-PNH. (**A**) In healthy subjects, GPI-anchored protein biosynthesis proceeds unperturbed in the endoplasmic reticulum. The full-length GPI anchor with attached protein (e.g., CD55 and CD59) resides in the membrane rafts of blood cells; thus red cells are protected from complement-mediated hemolysis. (**B**) In PIGA-PNH, a somatic mutation in *PIGA* (required for the initial step in GPI-anchored biosynthesis) leads to failure to generate the GPI anchor in hematopoietic cells. After expansion of the PNH clone (often through immunologic escape) the PNH red cells are susceptible to complement-mediated hemolysis due to an absence of the GPI-anchored CD55 and CD59 from the cell surface. (**C**) In PIGT-PNH, the GPI anchor is made in the endoplasmic reticulum (ER), but since PIGT is responsible for transpeptidation of proteins (e.g., CD55 and CD59) to the fully formed GPI molecule, red cells from these patients are susceptible to complement-mediated hemolysis similar to PIGA-PNH. Since *PIGT* is autosomal, two hits to different alleles are required to produce this phenotype. The PIGT-PNH patients have one allele with a germline mutation and one allele with deletion of 20q, which contains *PIGT*. Since 20q also contains putative tumor suppressor genes *L3MBTL1* and *SGK2,* Höchsmann et al. hypothesize that the mechanism of clonal dominance of PIGT-PNH is different from that of PIGA-PNH. Another key difference between these patients is the presence of autoinflammatory symptoms that appear to be a consequence of having free GPI in the plasma membrane.

PNH caused by *PIGA* mutations, and stimulated them to investigate the mechanism for these autoinflammatory symptoms.

All four patients described in the current manuscript had a germline mutation in the *PIGT* gene on one allele and a somatic myeloid common deleted region (CDR) on chromosome 20q of the other allele. The deleted region on 20q is associated with clonal disorders, such as myelodysplastic and myeloproliferative disease (17), and encompasses the *PIGT* locus. This suggests that the mechanism of clonal expansion for PIGT-PNH is different than most PIGA-PNH, as it may not be related to immune escape. Importantly, the authors describe subtle but important clinical and biochemical differences between PIGT-PNH and PIGA-PNH. Patients with PIGT-PNH have marked inflammatory manifestations, including aseptic meningitis, recurrent urticaria, and arthralgia that predated the development of PNH by many years.

The authors further show that the autoinflammatory symptoms were a consequence of the *PIGT* mutation. PIGA is required for the first step in the synthesis of GPI anchors, so *PIGA*-null cells are missing both the anchor and the protein on the cell surface. In contrast, *PIGT* encodes the last protein in the biosynthetic pathway and is required for transpeptidation of proteins to the fully formed GPI molecule (18), so *PIGT*-null cells have free GPI on the cell surface but no attached protein (15) (Figure 1). This was demonstrated by flow cytometric staining using T5 mAb (recognizes free GPI, but not protein-bound GPI), fluorescence-labeled nonlytic aerolysin (FLAER) (recognizes protein-bound GPI), and antibodies to individual GPI-anchored proteins (i.e., CD59) (19). Blood cells from PIGT-PNH patients stained positive for anti-T5 but negative for FLAER and anti-CD59; blood cells from PIGA-PNH patients were negative for all three (anti-T5, FLAER, and anti-CD59), and normal human blood cells stained positive for all three (15).

Free GPI stimulates the inflammasome

The authors next probed the role of the inflammasome and complement-mediated autoinflammation by measuring var-

ious cytokines in patient samples before and after eculizumab treatment. They found that a number of cytokines, including the inflammasome products IL-18 and IL-1β, were increased in PIGT-PNH patients. Using *PIGT* and *PIGA* knockout cell lines, they confirmed that the free GPI on *PIGT*-null cells stimulates the inflammasome. They also found that complement activation was enhanced (compared with PIGA-PNH), leading to heightened sensitivity to damage from the membrane attack complex. Inhibiting C5 with eculizumab ameliorated the complement-mediated damage/hemolysis in vitro and in vivo, improved the autoinflammatory symptoms in patients, and reduced levels of autoinflammatory cytokines.

Clinical implications

PNH is a rare clonal hemolytic anemia with an incidence of 1–2 per million annually in the United States, and is virtually always associated with mutations in the X-linked *PIGA* gene. Thrombosis was the leading cause of death for PNH patients until terminal complement inhibition therapy extended life expectancy and reduced thrombosis risk such that it approaches that of age-matched controls. The meticulous studies presented by Höchsmann et al. describe a novel variant of PNH in which cells have complete loss of *PIGT* due to the combination of a germline mutation in one allele and a somatic chromosome 20q deletion on the other allele. The results have implication for clinicians and researchers. For clinicians, the *PIGT* subtype represents fewer than 1% of PNH patients. In patients experiencing hemolysis, the standard PNH flow cytometry will readily detect the GPI-anchored protein deficiency and the treatment, eculizumab or ravulizumab, will be the same; thus, sequencing is not necessary for clinical care under these circumstances. However, it is important to consider GPI-anchored protein deficiency due to *PIGT* mutations for patients with recurrent autoinflammatory symptoms such as aseptic meningitis even when PNH symptoms are absent, because terminal complement inhibition may be effective for such cases. The authors also show that free GPI enhances complement activation and that the inflammasome response is enhanced by this complement activation. However, the mechanism of inflammasome enhancement and complement activation attributed to free GPI remains unclear, and more research to support these claims and to uncover the mechanism is needed. Nevertheless, the thorough set of experiments by Höchsmann et al. has defined the cellular, molecular, and genetic basis of a fascinating variant of PNH. In addition, this work may give important insight into other inflammasome-associated disorders.

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