A New Hint to Clonal Dominance in PNH

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Hematol Oncol Stem Cell Ther 2012; 5(3): 162-164
DOI: 10.5144/1658-3876.2012.162

Paroxysmal nocturnal hemoglobinuria (PNH) arises in a hematopoietic stem cell due to an acquired somatic mutation in the phosphatidylinositol glycan class A (PIGA) gene that resides in the short arm of the X chromosome. Affected progeny cells are incapable of biosynthesis of the glycosylphosphatidylinositol (GPI)-anchor and lack from their surface all GPI-anchored proteins (GPI-AP). However, expansion of a PNH clone is not the simple result of the mutational event, as PIGA-mutant cells exist in small numbers in normal individuals. In PNH, clonal expansion of GPI-AP-deficient cells leads to a syndrome characterized by hemolytic anemia, marrow failure, and venous thrombosis. Intravascular hemolysis results from failure of complement inactivation by CD59 (MIRL) and CD55 (DAF), missing from the surface of GPI-AP-deficient erythrocytes. In vivo expansion of dominant T-cell clones can reflect an antigen-driven immune response. Clinical overlap with immune-mediated aplastic anemia (AA), association with particular HLA-DR1501 and abnormalities of the immune system such as oligoclonality of the TCR repertoire support the hypothesis of an autoimmune pathophysiology of PNH. Recently, increased susceptibility to apoptosis of normal GPI+ cells has been shown to be responsible for preferential growth of GPI- cells in PNH patients. Apoptosis of targets may be a consequence of T-cell-mediated cytotoxicity.

In this issue of Hematology/Oncology and Stem Cell Therapy, Kunyaboon et al report results of a study designed to explore the roles of differential apoptosis and CD8+ lymphocytes in the selection of PNH clones in patients with PNH to demystify the mechanisms of PNH clonal dominance. They have elegantly demonstrated that increased apoptosis of GPI+ blood cells is probably an important factor in selection and expansion of PNH clones. In addition, they concluded that mononuclear cells and possibly CD8+ lymphocytes may play a role in this phenomenon. Using an annexin-V based apoptosis assay, they demonstrated that in PNH, CD55+ granulocytes exhibited significantly more apoptosis than their CD59- counterparts in liquid growth culture system at various time points \(P=.037\). The presence of mononuclear cells in the culture system caused more difference in apoptosis and correlated with the size of PNH clone. As intuitively expected, their results (as depicted in Figure 4 of the article) showed that the addition of autologous CD8+ lymphocytes inhibited CFU-GM and BFU-E colony formation significantly more in PNH patients compared to normal controls \(P=.037\).

The strong association of PNH with aplastic anemia, in which immune (T cell)-mediated marrow destruction has been inferred as the underlying pathophysiology, suggests an immune mechanism of PNH clonal selection. Until recently there had been no in vitro experiment which directly demonstrated both the cellular and molecular mechanism of immune escape of the PNH clone. Two recent studies suggested that differences in apoptosis of GPI+ and GPI- cells may be responsible for the clonal expansion of the GPI-negative (PNH) clone. The currently reported study findings of Kunyaboon et al, however, do not exclude the role of natural killer (NK) cells in immune selection of PNH blood cells as suggested by Hanaoka et al since CD8 may also be expressed on NK cells. As noted in patients with the closely linked disorder of aplastic anemia, who sustain immune-mediated marrow injury putatively induced by cytotoxic cells, GPI-deficient blood cells often expand, hence suggesting that the injury allows PNH clones to expand selectively. Potential candidates for the GPI-linked membrane proteins, which activate NK cells include stress-inducible ULBP and CD48. Hanaoka et al reported earlier that leukemic K562 cells preferentially survived NK cell-mediated cytotoxicity in vitro when they acquired PIGA mutations. Hanaoka et al reported later that the survival is ascribable to the deficiency of stress-inducible GPI-linked membrane proteins ULBP1 and ULBP2, which activate NK and T cells. The ULBPs were detected on GPI-expressing, but not on GPI-deficient K562 cells. NK cells spared

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ULBP-deficient cells in vitro. The ULBPs were identified only on GPI-expressing blood cells of a proportion of patients with PNH, but none of healthy individuals. Granulocytes of the patients partly underwent killing by autologous cytotoxic cells, implying ULBP-associated blood cell injury. In this setting, the lack of ULBPs may allow immunoselection of PNH clones. Nevertheless, another study has shown that NK cells may be less efficient in targeting GPI-deficient cells.

Another factor implicated in clonal expansion of hematopoiesis in PNH is the high mobility group A2 (HMGA2) gene that encodes the HMGA2 chromatin remodeling protein, which orchestrates the assembly of protein complexes to regulate gene expression. HMGA2 is expressed predominantly during embryonic development with low or undetectable expression in normal, differentiated tissues. In humans, HMGA2 is located at chromosome 12q13 and chromosomal rearrangements causing a truncation in the 3'UTR of the HMGA2 gene has been reported in two PNH patients.

Overall, the findings of inhibition of progenitor cell growth by CTLs and increased apoptosis of CD59+ granulocytes convince us that the role of CTLs in preferential growth of GPI hematopoietic stem cells through cytotoxicity-induced apoptosis. Elevated Fas receptor expression in GPI+ stem cells, found by some investigators, may render more CTL-induced apoptosis of these cells and enhance more growth advantage of GPI- stem cells leading to PNH clonal dominance. The mechanism and phases of PNH clonal dominance are illustrated in Figure 1.

In summary, Kunyaboon et al have shown that preferentially more apoptosis of the GPI+ blood cells than their GPI-counterparts is responsible for the selection of PNH clones with subsequent clonal expansion in

Figure 1A. Early phase of PNH-PIGA mutation leading to loss of GPI-anchored proteins, including CD55 (DAF), CD59 (MIRL) that leads to complement-mediated hemolysis and deficiency of other GPI-anchored proteins like stress-inducible ULBP (not shown in this figure) that may help in escaping elimination by NK cells and CD8+ T-cells.

Figure 1B. Immune mechanisms of PNH clonal selection. Implicated role of CD8+ T-cells in preferential killing of GPI AP+ CFU-GM and GPI AP+ BFU-E is depicted. Also shown are possible role of NK cells that may spare PIGA mutated GPI AP- CFU-GM and PIGA mutated GPI AP- BFU-E as they lack GPI-anchored stress-inducible ULBP. Truncated HMGA2 gene has also been described effecting hematopoiesis in a few PNH patients.

Figure 1C. Clonal dominance of PIGA mutated GPI-AP deficient CFU-GM and BFU-E leading to a later phase of full blown disease manifestations of PNH or cytopenia as seen in aplastic anemia or myelodysplastic syndrome (Drawings in Figure 1A-C by AR Zaidi).
PNH. Mononuclear cells, especially the auto reactive CD8+ lymphocytes may play a role in this phenomenon by selective suppression of non-PNH hematopoiesis, through cytotoxicity-induced apoptosis. These findings will help us in better understanding of the mechanisms of clonal dominance in PNH.

REFERENCES