Paroxysmal nocturnal hemoglobinuria (PNH) was one of the earliest of the defined hematological diseases to be recognized and, after simple diagnostic tests became available, its protean manifestations became known. Intravascular hemolysis with hemoglobinuria and hemosiderinuria, venous thromboses, particularly of abdominal vessels, and diminished hematopoiesis of variable degree, and possibly increased incidence of infections are all recognized as manifestations of the disease (1).

A major step forward was made in defining and understanding the disorder when it was discovered that the blood cells of patients with PNH lacked all proteins normally tethered to the membrane by the glycosylphosphatidylinositol anchor because of a somatic mutation in a gene responsible for an intermediate needed for the biosynthesis of the anchor (2). This gene was called the *PIGA* gene and its product, although not yet characterized, appears to be a glycosyltransferase necessary in an early step in the biosynthesis of the GPI anchor. As a result of this defect, about 20 proteins are known to be missing from the surface of these cells.

Ideally, all the symptoms of PNH should derive from these biochemical abnormalities, and many do. The hemolysis and the initiation of thrombosis appear to be due to the unregulated activation of complement on the cell surface because of the lack of two proteins that normally control this activity: decay accelerating factor (DAF, CD55) and CD59 (membrane inhibitor of reactive lysis, protectin, etc.). Fibrinolysis, once thrombosis has occurred, may be diminished because of the lack of the urokinase receptor (urokinase–plasminogen activator receptor, uPAR). Infections may be augmented by the lack of the FcγIII receptor on neutrophils and possibly because of the numerous defects that occur in lymphocytes (1). But the explanation for the altered hematopoiesis may be more complex.

Several studies have shown that PNH is a clonal disease and that the abnormal cells with a given defect in the *PIGA* gene arise from a single mutated hematopoietic stem cell (3). The progeny from this cell may come to dominate hematopoiesis; in over 55% of patients, > 80% of the granulocytes (the cell line most reflective of the stem cell population) are of the abnormal phenotype (1). How does this come about? Do the abnormal cells have an intrinsic proliferative advantage? If not, is there some other event that must occur for them to be able to take over the marrow?

To date, no experiments have indicated that the defective cells have an intrinsic proliferative advantage over the normal clone lacking the GPI defect. PNH hematopoietic precursors fail to proliferate as well as normal precursors in in vitro culture conditions in a number of experiments (4). When chimeric animals are made using techniques for inactivating the *pig-a* gene, the proportion of abnormal cells at birth is small (5). Rosti et al. demonstrate in this issue of the *Journal* that the proportion is much larger in earlier fetal life but diminishes with time, suggesting that the defective cells are hematopoietically defective as well (6). Their data, as well as those of Dunn et al. (7), suggest that the number and viability of hematopoietic embryoid bodies is less from the defective cells than from normal cells treated in the same way. This suggests that the defective cells do not have a proliferative advantage in the normal marrow environment.

In PNH, the marrow environment may not be normal. In the first place, at least some of the accessory cells (monocytes, lymphocytes, perhaps even stromal cells) are derived from the abnormal clone and thus may improperly perform their functions in the regulation of hematopoiesis. This defective regulation might lead to a proliferative advantage for the GPI-deficient precursors, although how this might come about is not immediately obvious.

The marrow environment in PNH may be rendered abnormal by an aplastogenic influence such as that causing idiopathic aplastic anemia. Considerable evidence has accumulated suggesting that this influence in aplastic anemia is actually autoimmune in origin (8). The association of PNH and aplastic anemia is well-known; up to 35% of patients recovering from aplastic anemia are found to have the cells characteristic of PNH (1). More important is the fact that the bone marrow in PNH behaves like that of aplastic anemia in in vitro culture, even when the marrow is not hypocellular on microscopic examination. Even the GPI^+ precursors grow poorly in precursor assays (7).

This has led to the suggestion that the development of PNH is a two step process: (*a*) a somatic mutation occurs in a single hematopoietic stem cell but, because of the proliferative disadvantage such cells have in the normal marrow environment, the population remains small—so small as to elude detection in the environment and in the marrow of normal individuals; and (*b*) an aplastogenic influence alters the marrow environment, suppressing GPI^+ cells to a greater degree than GPI^- cells. In this environment, the GPI^- cells have a proliferative advantage and are able to populate the marrow. One might imagine that this favoring of the GPI^- cells might be due to the absence of GPI-linked receptors for inhibitory cytokines (such as TGF-β, of which one receptor is GPI-linked) or ligands (such as LFA-3, a ligand for CD2 on cytotoxic T cells).

According to this paradigm, a very severe aplastogenic influence would result in aplastic anemia and the cells characteristic of PNH would appear as it was ameliorated. On the other hand, complete regression of the aplastogenic influence would again establish a marrow environment in which the GPI^- cells would be at a disadvantage and would disappear. This occurs in many patients who have the disease for a long period of time and who have maintained a GPI^+ population that can be rejuvenated.

Much of this is speculation and, like all speculation, it must be substantiated by facts. The demonstration that the defect in GPI-anchor biosynthesis in PNH is not enough by itself to account for all the manifestations of the disorder frees us to think

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and investigate with imagination; it advances the paradigm another step.

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