

PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA

A pilgrimage of many steps

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Despite its rarity, haemolytic PNH has exercised a fascination for physicians and scientists over the past 125 years or more since the publication in 1882 of Paul Strubing's description of paroxysmal haemoglobinuria, occurring after sleep, in a 29-year-old male. My own interest in PNH was stimulated by having the good fortune to work with two of the more notable clinician scientists on this journey, Professors John Dacie and Lucio Luzzatto. A further stimulus was my opportunity to study aplastic anaemia and its pathogenesis, including the link with PNH (Dacie and Lewis, 1961).

Clinical features

The clinical features of haemolytic PNH are well known. It is an acquired disorder, characterised by intravascular haemolysis, manifested by episodes of haemoglobinuria and with a high incidence of venous thrombosis. The association of increased haemoglobinuria during sleep is not fully explained, but the hypothesis that it was related to increased CO₂ during sleep, associated with a fall in serum pH, led to the development of the acidified serum lysis test, the Ham's test. Typically the episodes of haemoglobinuria wax and wane and may be aggravated by infections, stimulation of the immune system and even by strenuous exercise. Intravascular haemolysis is, however, continuous and, in the absence of frank haemoglobinuria, haemosiderinuria is always present, identified by Perle's stain in epithelial cells shed into urine. Venous thrombosis, often affecting the hepatic or mesenteric veins, is a major cause of morbidity and mortality in PNH in reports from Western countries. Thrombosis seems

to be less of a problem in the East where the development of bone marrow failure appears to be a more common end point. Thrombosis and the relationship to bone marrow failure remain intriguing problems in the pathophysiology of PNH. The natural history of PNH has been studied in two major series. In the follow up of Dacie's patients (Hillman et al 1995) the median actuarial survival was 10 years. Venous thrombosis, particularly of the hepatic vein, was the principal cause of death followed by haemorrhage associated with bone marrow failure. It was noted that the severity of PNH diminished with time in some patients, with ultimate restoration of apparently normal haematopoietic function although small populations of cells sensitive to complement lysis remained in some patients for many years. In a larger study from France (Socié et al 1996) the Kaplan-Meier survival estimate was 65% at 10 years and 48% at 15 years after diagnosis. In this study also thrombosis was the major factor contributing to poor survival followed by the development of pancytopenia, associated with aplastic anaemia, myelodysplastic syndrome or acute leukaemia. Interestingly patients who presented with aplastic anaemia and later developed PNH had a better survival than those in whom bone marrow failure followed the diagnosis of haematopoietic PNH. In both these series supportive care, coupled where necessary with anticoagulation, was the only form of treatment.

Haematology

Classical PNH has the haematological features of acute and chronic intravascular haemolysis. The Ham's test, and subsequent complement

binding sensitivity studies showed that there are at least two populations of red cells in PNH, those susceptible to lysis and those with a more normal pattern. In some cases there may be cells with intermediate sensitivity to lysis. These populations are known as PNH III, I and II respectively. The different populations may now be recognised by flow cytometry which can identify the absence of glycosylphosphatidylinositol (GPI) anchored proteins in PNH III and II populations. The bone marrow in typical haemolytic PNH is often cellular with erythroid hyperplasia but the association with aplastic anaemia means that a third or more of cases of bone marrow is hypocellular. In these cases the presence of reticulocytes and anisocytosis in the peripheral blood raises the suspicion of PNH.

Mosaicism

The mosaicism of the red cell population in PNH is reflected in the haematopoietic stem cells, granulocytes and platelets. Lymphocytes are also affected but the presence long lived normal lymphocytes dilutes the deficient populations. The success of creating lymphocyte cell lines from deficient and normal populations was central to unravelling the pathophysiology of PNH. The susceptibility of PNH cells to complement lysis is caused by deficiency of two main inhibitors of complement activation on the cell surface, namely decay accelerating factor (DAF, CD55) and membrane inhibitor of reactive lysis (MIRL, CD59). The loss of these and other surface protein was explained by the brilliant demonstration by Dr Kinoshita and colleagues in his laboratory that deficiency of the GPI anchor was caused by somatic mutation in the X-linked phosphatidylinositol, complementation group A (PIG-A), gene, essential for the assembly of the GPI anchor (Miyata et al 1993; Takeda et al 1993). The lysis sensitive PNH cells are the result of a mutation in the PIG-A gene, each population being the result of a single mutation. Over 100 different mutations have been described, sometimes several in the same patient. The mechanisms by which the PNH clones are able to expand and produce a stable mosaicism

are not fully understood, but it is thought that the PNH clones escape an immunological inhibition of the non-PNH cells. The PNH stem cells do not have a proliferative growth advantage compared with normal stem cells but do have a growth advantage compared with the apparently unaffected stem cells in the haemolytic PNH marrow. This suggests that PNH cells only expand in the presence of a “damaged” marrow. Credence is given to this view by the finding of small PNH clones from time to time in normal marrow that do not expand. The nature of the immunological attack which the PNH clones evade is not known, though in both PNH and aplastic anaemia there is evidence of expansion of specific cytotoxic T-cell clones (Karadimitris et al 2000; Risitano et al 2004) though these are not directed against GPI anchored proteins.

Pathophysiology and Treatment

Haemolysis in PNH is clearly caused by a failure to inactivate complement on the surface of red cell that are destroyed by the membrane-attack complex (MAC). Whether other manifestations of PNH are caused by complement activation is not certain. It seems likely that the thrombotic tendency is related to complement activation but not proven. The expansion of PNH clones might be related to a differential sensitivity to apoptosis compared with the GPI + stem cell population in the affected marrow (Ismail et al 2003).

Inhibition of complement activation

The haemolysis is result of the effect of the MAC. Eculizumab is a humanised monoclonal antibody which inhibits the C5 convertase enzyme responsible for the formation of the MAC. The antibody greatly reduces haemolysis and the need for transfusion in patients with haemolytic PNH particularly those without significant thrombocytopenia. Since the PNH cells are not lysed the proportion of PNH III cells actually increases, although clinically they are much improved. Where bone marrow failure is more of the part of the clinical syndrome, the effects of the monoclonal antibody are less well marked

(Hillman et al 2004). For these latter patients, immunosuppressive treatment, either with antilymphocyte globulin or cyclosporine may result in some improvement. The new treatment for haemolytic PNH is yet another example of exciting developments that have taken place as the understanding of the disease has advanced step by step.

References

- Dacie JV, Lewis SM. Paroxysmal nocturnal haemoglobinuria: variation in clinical severity and association with bone marrow hypoplasia. *Brit J Haematol* **7**: 442-457, (1961)
- Hillmen P, Hall C, Marsh J et al. Effect of Eculizumab on hemolysis and transfusion requirements in patients with paroxysmal nocturnal hemoglobinuria *New Engl J Med* **350**: 552-559, (2004)
- Hillmen P, Lewis SM, Bessler M, Luzzatto L, Dacie JV. Natural History of Paroxysmal Hemoglobinuria *N Engl J Med* **333**: 1253-1258, (1995)
- Ismail MM, Tooze J, Flynn JA et al. Differential apoptosis and Fas expression on GPI-negative and GPI-positive stem cells: a mechanism for the evolution of paroxysmal nocturnal haemoglobinuria *Brit J Haematol* **123**: 545-551, (2003)
- Karadimitris A, Manavalan JS, Thaler HT et al. Abnormal T-cell repertoire is consistent with immune process underlying the pathogenesis of paroxysmal nocturnal hemoglobinuria. *Blood* **96**: 2613-2620, (2000).
- Miyate T, Takeda J, Iida J et al. The cloning of PIG-A, a component in the early step in GPI-anchor synthesis. *Science* **259**: 1318-1320, (1993)
- Socié G, Mary JY, de Gramont A, Rio B et al. Paroxysmal Nocturnal Haemoglobinuria: long term follow up and prognostic factors *Lancet* **348**: 573-577, (1996)
- Risitano AM, Maciejewski JP, Green S et al. In vivo dominant immune responses in aplastic anaemia patients: molecular tracking of putatively pathogenic T cell clones by TCR β -CDR3 sequencing. *Lancet* in Press (2004)
- Takeda J, Miyate T, Kawagoe K et al. Deficiency of GPI anchor caused by a somatic mutation of the PIG-A gene in paroxysmal nocturnal haemoglobinuria *Cell* **73**: 703-711, (1993)