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Review

Paroxysmal nocturnal hemoglobinuria (PNH) and primary p.Cys89Tyr mutation in CD59: Differences and similarities

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ABSTRACT

CD59 encodes a 77 amino acid glycosylphosphatidylinositol (GPI)-anchored cell surface glycoprotein that inhibits the final step of membrane attack complex (MAC) formation. CD59 deficiency is a common finding in adult patients with paroxysmal nocturnal hemoglobinuria (PNH). In this condition, there is a clonal expansion of hematopoietic stem cells that have acquired a mutation in the PIGA gene (phosphatidylinositol glycan anchor biosynthesis, class A). PIGA encodes a GPI biosynthesis protein, phosphatidylinositol N-acetylglucosaminyltransferase subunit A, and erythrocytes deficient in GPI-anchored membrane proteins, including CD59, undergo complement-mediated hemolysis. We have recently described a primary homozygous Cys89Tyr CD59 deficiency in humans that resulted in the amino acid substitution p.Cys89Tyr with resulting failure of proper localization of the CD59 protein to the cell surface. The Cys89Tyr mutation in CD59 was clinically manifested in infancy, and associated with chronic hemolysis and relapsing peripheral demyelinating disease resembling recurrent Guillain–Barré syndrome (GBS) or chronic inflammatory demyelinating polyneuropathy (CIDP). In this review we describe differences and similarities in the pathogenesis and clinical manifestations of PNH and primary CD59 Cys89Tyr mutation with the aim of tracking the contribution of CD59 deficiency to the pathophysiology and perhaps deepening our understanding of both diseases.

1. Introduction

CD59 encodes a 77-amino acid glycosylphosphatidylinositol (GPI)-anchored cell surface glycoprotein, synthesized as 128 amino acids protein including a signal sequence and the sequence for GPI-anchor replacement. The CD59 protein inhibits the final step of membrane attack complex (MAC) formation. MAC is a C5b-9 complex that is generated after activation of the complement system. It is formed by the sequential binding of C6, C7, C8, and multiple C9 molecules to C5b. The insertion of C8 and C9 into lipid bilayers causes cell lysis. Host cells are protected from MAC-mediated injury by CD59 (protectin), which prevents pore formation (Hadders et al., 2007).

CD59 deficiency is a common finding in adult patients with paroxysmal nocturnal hemoglobinuria (PNH). In this condition, there is clonal expansion of hematopoietic stem cells that have acquired a mutation in the PIGA gene (phosphatidylinositol glycan anchor biosynthesis, class A). PIGA encodes a GPI biosynthesis protein, phosphatidylinositol N-acetylglucosaminyltransferase subunit A (Miyata et al., 1993; Takeda et al., 1993), and erythrocytes deficient in GPI-anchored membrane proteins, including CD59, undergo complement-mediated hemolysis. CD59, formerly known as membrane inhibitor of reactive lysis (MIRL) was identified by several groups (Davies et al., 1989; Holguin et al., 1989a; Okada et al., 1989; Sugita et al., 1988) and its relationship to the erythrocyte phenotypes of PNH was soon established (Holguin et al., 1989b). Later, in a murine model, Holt, Boto, and Morgan created targeted deletion of the CD59 gene that caused spontaneous intravascular hemolysis and hemoglobinuria (Holt et al., 2001).

We have recently described a primary homozygous Cys89Tyr CD59 deficiency in humans that resulted in the amino acid substitution p.Cys89Tyr and thus a failure of the proper localization of CD59 protein to the cell surface (Nevo et al., 2013). Cys89Tyr mutation in CD59 is a founder mutation in Jewish subjects of North African ancestry. Sequence determination of CD59 exon 3 in 197 anonymous Jewish subjects of North African origin identified three carriers, indicating a carrier rate of 1:66 in this community. The mutation was not found in 5379 exomes from healthy subjects available through the Exome Variant Server of the National Heart, Lung, and Blood Institute Exome Sequencing Project. The Cys89Tyr mutation in CD59 was clinically manifested in infancy by chronic hemolysis and relapsing peripheral demyelinating disease resembling recurrent Guillain–Barré syndrome (GBS) or chronic inflammatory demyelinating polyneuropathy (CIDP).

PNH and primary Cys89Tyr mutation in CD59 have differences and similarities in their clinical presentation (summarized

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Table 1
Clinical and laboratory manifestations in PNH and primary Cys89Tyr CD59 deficiency.

	CD59 expression	CD55 expression	Intravascular hemolysis	Thrombophilia	Bone marrow dysfunction	Recurrent Guillain–Barré-like disease
PNH	Variable	Variable	+	+	+	–
Primary Cys89Tyr CD59 deficiency	100% missing	100% present	+	+	–	+

in Table 1) and underlying molecular biology that can deepen our understanding of both diseases.

1.1. Intravascular hemolysis

PNH, a disease of hematopoietic stem cells, arises as a consequence of a clonal expansion of one or more hematopoietic stem cells that have acquired a somatic mutation of the X chromosome gene PIGA. The protein encoded by PIGA is essential for synthesis of the GPI moiety that serves as the membrane anchor for a functionally diverse group of cellular proteins. As a consequence of mutant PIGA, all GPI-anchored proteins (several dozen) are deficient on progeny of the affected stem cells (Hillmen et al., 1995). The complement regulatory proteins CD55, decay-accelerating factor (DAF), which inhibits complement activation, and CD59, which inhibits MAC formation, are among the proteins that are not expressed on the membrane due to this mutation.

The clinical hallmarks of PNH, intravascular hemolysis and the resulting hemoglobinuria, were thought to be a direct consequence of deficiencies in CD55 and CD59, since peripheral blood erythrocytes derived from the mutant clone lack the capacity to restrict cell surface activation of the alternative complement pathway due to CD55 deficiency, and to block formation of the cytolytic MAC due to CD59 deficiency. Under conditions of acidification, activation of the alternative complement pathway does occur (Hansch et al., 1983) but under these conditions, the amount of CD55 and CD59 on normal erythrocytes is sufficient to inhibit activity of the alternative complement pathway. Indeed, studies have shown that susceptibility of PNH erythrocytes to acidified serum lysis is due to CD55 (DAF) and CD59 (MIRL) deficiency (Wilcox et al., 1991). Accordingly, following incubation in acidified serum, normal erythrocytes bear only small amounts of C3 activation products and hemolysis is not observed, but when CD55 (DAF) and CD59 (formerly known as MIRL) are deficient, the capacity of PNH erythrocytes to inhibit activity of the alternative complement pathway is limited, alternative pathway complement activity proceeds on the cell surface, and PNH erythrocytes are hemolyzed.

Two questions are posed by these relationships: What is the relative contribution of each of the regulatory complement proteins CD55 and CD59 to hemolysis, and are additional GPI proteins involved in PNH manifestations?

It has been predicted based on laboratory investigations (Holguin et al., 1989a, 1989b) that, although CD55 and CD59 act in concert to control susceptibility to acidified serum lysis, CD59 is the main complement regulatory protein responsible for intravascular hemolysis. However, mice with targeted deletion of the CD59 gene that had spontaneous intravascular hemolysis and hemoglobinuria in these studies were not anemic (Holt et al., 2001), and in order to support the critical role of CD59, a "natural" human knockout was lacking. This was provided with the recognition of primary homozygous Cys89Tyr CD59 deficiency, where all patients exhibit intravascular hemolysis manifested by Coombs-negative hemolytic anemia with reticulocytosis, low haptoglobin, and high LDH and MAC deposition on red blood cell (RBC) membranes. The marked intravascular hemolysis observed in Cys89Tyr CD59 deficiency suggests that the previous observations were indeed correct and CD59 is the main complement-regulating GPI membrane protein responsible for intravascular hemolysis.

CD55-deficient erythrocytes were needed to finally verify this premise. Indeed, it has been shown that antigens of the Cromer blood group complex are located on CD55, and rare cases of a null phenotype called Inab have been reported (Telen et al., 1988). Apparently Inab erythrocytes are completely deficient in CD55, but, unlike PNH erythrocytes, other GPI-linked proteins that have been studied are expressed normally on Inab. Interestingly, Inab cells are resistant to reactive lysis, suggesting that CD59 or additional unknown GPI-linked proteins are needed for lysis in PNH. Inab cell susceptibility to acidified serum lysis has been examined by two groups. Telen and Green (Telen and Green, 1989) reported that Inab cells were resistant to acidified serum lysis, whereas Merry et al. (1989) reported that approximately 5% of Inab cells were hemolyzed in acidified serum. Although it has been reported that CD55 renders RBCs more susceptible to acidified serum lysis (Medof et al., 1985; Wilcox et al., 1991), we could conclude that the contribution of CD55 to hemolysis in the null CD55 phenotype Inab is very low and perhaps negligible.

What about other GPI-linked proteins? Additional GPI-linked proteins may participate in susceptibility to hemolysis; however, no other specific protein has been seriously suggested.

In conclusion, CD59 is the membrane complement regulatory protein that is the main and perhaps the only protein responsible for the massive intravascular hemolysis observed in PNH and primary Cys89Tyr CD59 deficiency.

1.2. Thrombophilia

Thrombosis is the prognostic factor with the greatest effect on survival in PNH patients (de Latour et al., 2008; Poulou et al., 2007). Data from several retrospective studies in the pre-eculizumab era showed that the cause of death was related to thrombosis in 22.2–37.2% of PNH patients. The extremely high incidence of thrombosis in PNH and its major effects on morbidity and mortality underline its clinical importance. The cumulative 10-year incidence of thrombosis in a retrospective study of 460 PNH patients with larger clones was 31–39% (de Latour et al., 2008; Hillmen et al., 1995; Van Bijnen et al., 2012). The risk of venous thrombosis correlates with PNH granulocyte clone size and was 44% in patients with a PNH granulocyte clone of >50% (Hall et al., 2003; Moyo et al., 2004).

In the PNH population with a classic presentation, such as the patients who participated in the various eculizumab trials, 15% of pretreatment thrombotic events were arterial, located in either the cerebral vasculature (13.6%) or coronary arteries (1.4%) (Hillmen et al., 2007); thus, risk of arterial thrombosis is probably also increased in this group in comparison with age-matched healthy controls. Ziakas et al. (2007) described 38 reports of arterial thrombosis, mainly in the central nervous system or coronary arteries. Thrombosis occurred in relatively young patients, with a median age of 35 years (range 22–47) for acute coronary syndromes, and 39 years (range 11–76) for stroke. In primary Cys89Tyr CD59 deficiency, thrombophilia was only the second most important factor in the pre-eculizumab era.

Hemostasis is achieved via a balance of pro- and antithrombotic forces, maintained by coagulation and fibrinolysis, and is influenced by blood physiology, serum factors as well as factors derived from vessel walls and blood cells. In PNH, prothrombotic