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Review

Paroxysmal nocturnal hemoglobinuria and other complement-mediated hematological disorders

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ABSTRACT

The recent availability of eculizumab as the first complement inhibitor renewed the interest for complement-mediated damage in several human diseases. Paroxysmal nocturnal hemoglobinuria (PNH) may be considered the paradigm a disease caused by complement dysregulation specifically on erythrocytes; in fact, PNH is a clonal, non-malignant, hematological disorder characterized by the expansion of hematopoietic stem cells and progeny mature blood cells which are deficient in some surface proteins, including the two complement regulators CD55 and CD59. As a result, PNH erythrocytes are incapable to modulate on their surface physiologic complement activation, which eventually enables the terminal lytic complement leading to complement-mediated intravascular anemia – the typical clinical hallmark of PNH. In the last decade the anti-C5 monoclonal antibody has been proven effective for the treatment of PNH, resulting in a sustained control of complement-mediated intravascular hemolysis, with a remarkable clinical benefit. Since then, different diseases with a proved or suspected complement-mediated pathophysiology have been considered as candidate for a clinical complement inhibition. At the same time, the growing information on biological changes during eculizumab treatment in PNH have improved our understanding of different steps of the complement system in human diseases, as well as their modulation by current anti-complement treatment. As a result, investigators are currently working on novel strategy of complement inhibition, looking at the second generation of anti-complement agents which hopefully will be able to modulate distinct steps of the complement cascade. Here we review PNH as a disease model, focusing on the observation that led to the development of novel complement modulators; the discussion will be extended to other hemolytic disorders potentially candidate for clinical complement inhibition.

Introduction

The complement system is a key component of innate immunity, which has evolved to recognize both exogenous pathogenic microorganisms as well as injured self tissues, and to amplify adaptive immunity. The complement system mainly works in the fluid phase through a number of serum proteins, which may activate along three distinct functional pathways – classical, alternative or lectin –, all finally merging into a common final effector mechanism, the cytolytic membrane attack complex (MAC). Notwithstanding fine mechanisms have evolved to modulate the complement system (including the membrane-bound proteins complement receptor 1 [CR1], membrane cofactor protein [MCP], CD55 and CD59, as well as fluid-phase components such as complement factor I [FI] and factor H [FH]), it is now understood that the complement cascade undergo disease-specific derangements accounting for specific pathological outcomes (Müller-Eberhard 1988; Holers 2008). Different hematological disorders are the most obvious examples of complement-mediated disease, such as distinct hemolytic conditions; they include paroxysmal nocturnal hemoglobinuria (PNH), cold agglutinin disease (CAD) and hemolytic-uremic syndrome (HUS). Indeed, therapeutic complement inhibition has been successfully developed in PNH, with terrific results; more recently, novel data support the concept that complement inhibition may be beneficial in CAD and HUS. Here we briefly review the complement biology underlying hemolysis in PNH – as the paradigm of complement-mediated hemolysis-, as well as the clinical results with the anti-C5 complement inhibitor eculizumab. We also provide some information on the more recent indications to complement inhibition in other hemolytic disorders, as well as the status of art of the pre-clinical development of novel strategies of complement inhibition.

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Complement dysregulation in PNH

PNH is a rare and puzzling hematological disorder characterized by the clinical triad of bone marrow failure, severe thrombophilia and complement-mediated intravascular hemolysis; historically, this latter sign (more specifically the hemoglobinuria consequent to the chronic intravascular hemolysis) has been the most typical for patients and investigators, and accounts for the picturesque name of the disease. PNH is due to the expansion of hematopoietic stem cells (and progeny mature blood cells) which carry the bizarre phenotype of the lack of several proteins from the blood cell surface (Kunstling and Rosse 1969; Nicholson-Weller et al. 1983; Selvaraj et al. 1988). This is due to a mutation in the X-linked *phosphatidylinositol glycan class A* (*PIG-A*) gene (Takeda et al. 1993; Miyata et al. 1993), which is necessary for the biosynthesis of the glycosyl phosphatidyl-inositol (GPI)-anchor (Mahoney et al. 1992; Takahashi et al. 1993); indeed, all the proteins missing from the PNH cell surface are GPI-anchored (Medof et al. 1987). Among these, PNH erythrocytes lack from their surface the two complement regulators CD55 (also known as Decay Accelerating Factor, DAF; Nicholson-Weller et al. 1982; Nicholson-Weller et al.) and CD59 (or Membrane Inhibitor of Reactive Lysis, MIRL; Holguin et al. 1989a, b). CD55, also known as Decay Accelerating Factor (DAF), is a 70-kd protein which inhibits the formation and the stability of the C3 convertase (both C3bBb and C4b2a) (Nicholson-Weller 1992). Historically, CD55 was the first complement regulator reported to be absent on PNH erythrocytes (Pangburn et al. 1983a, b; Nicholson-Weller et al. 1983) possibly accounting for the increased susceptibility of PNH erythrocytes to complement mediated lysis. However, further studies suggested that factors other than CD55 should also be involved, possibly acting downstream on the complement cascade (Medof et al. 1987; Shin et al. 1986). Subsequently, CD59 (also known as Membrane Inhibitor of Reactive Lysis, MIRL) was identified as an additional complement inhibitor which was found deficient on PNH cells (Holguin et al. 1989a). CD59 interferes with the terminal effector complement, blocking the incorporation of C9 onto the C5b-C8 complex, thus preventing MAC formation (Meri et al. 1990). The hierarchical contribution of CD55 and CD59 to hemolysis suggests that CD59 is the key molecule which, if absent, leads to lysis (Wilcox et al. 1991). This is also supported by the observation that subject with isolated deficiency of CD55 (the so-called Inab phenotype) usually do not show any sign of hemolysis (possibly due to redundant regulatory mechanisms, including CD59 itself) (Holguin et al. 1992; Merry et al. 1989), whereas anecdotic cases of inherited CD59 deficiency harbor a clinical phenotype undistinguishable from PNH (Yamashina et al. 1990; Motoyama et al. 1992). The *in vitro* susceptibility of PNH erythrocytes has been initially described by Dr. Ham (who showed that erythrocytes from PNH patients lyse in autologous serum upon complement activation by acidification, the so-called acidified serum assay, also known as the Ham test; Ham and Dingle 1939), and subsequently characterized more in detail by Dr. Rosse and Dr. Dacie, who demonstrated that distinct phenotype of PNH erythrocytes exist, according to their specific sensitivity to complement-mediated lysis *in vitro* (Rosse and Dacie 1966; Rosse 1971). In fact, PNH patients may harbor erythrocytes with a dramatic hypersensitivity to complement-mediated lysis (15–25 times the normal one), or just a moderate hypersensitivity (3–5 times normal). These phenotypes are referred as PNH type III and type II, respectively (Rosse and Dacie 1966; Rosse 1971), and they correspond respectively to a complete (type III) or partial (type II) deficiency of GPI-APs, as documented by flow cytometry. While the *in vitro* susceptibility of PNH erythrocytes has been extensively elucidated, the actual mechanisms leading to complement activation *in vivo* and subsequent hemolysis have not been definitely demonstrated. However, it is conceivable that chronic hemolysis of PNH is due to a continuous steady-state complement activation coming from the low-grade spontaneous C3 *tick over*, with subsequent continuous activation of the complement alternative pathway (CAP) on PNH erythrocyte surface (Pangburn et al. 1981; Pangburn and Müller-Eberhard 1983). Infections or inflammatory status usually result in hemolytic crises (the so-called paroxysms), eventually as a result of massive complement activation. At the moment, it is not clear which pathway accounts for complement activation in each of these specific conditions, even if it is conceivable that all the three pathways may co-operate, possibly with some hierarchical dominance of the CAP, which is specifically uncontrolled due to CD55 deficiency, and may amplify any initial complement activation. As stated above, hemolytic anemia is just one of the three main clinical manifestations of PNH, together with thrombophilia and bone marrow failure (Risitano 2012); as a consequence, a role for the complement system has been hypothesized in these conditions. While the data supporting a complement-mediated pathophysiology of marrow failure are quite weak, it is conceivable that the lack of complement regulators on PNH platelets (GPI-anchored proteins are absent on all PNH mature blood cells) may results in uncontrolled platelet activation with subsequent platelet aggregation and thrombosis. However, additional mechanisms have also been postulated, and the occurrence of thrombosis in PNH likely results from several concomitant pathogenic mechanisms (Risitano 2012; Risitano et al. 2012) (Fig. 1).

Anti-complement treatment in PNH

Given its well-proven complement-mediated pathophysiology, PNH was thought a perfect disorder for investigating candidate complement inhibitors; indeed, after an initial translation plan in autoimmune diseases, the first complement inhibitor eculizumab (Soliris®, Alexion Pharmaceuticals) has been extensively tested in PNH patients, leading to a dramatic change in current treatment of this disease. Eculizumab (h5G1.1-mAb), is a humanized monoclonal antibody (mAb) (Rother et al. 2007) derived from the murine anti-human C5 mAb which bind to the complement component 5 (C5) and inhibits its further cleavage into C5a and C5b, thus disabling the progression to the terminal effector complement MAC (Matis and Rollins 1995). The prediction for PNH was that eculizumab, by preventing MAC assembly, could compensate for the absence of CD59 on PNH erythrocytes, preventing their intravascular lysis upon complement activation. One phase II pilot study (Hillmen et al. 2004) as well as in two multi-national phase III clinical studies (TRIUMPH [Hillmen et al. 2006] and SHEPHERD [Brodsky et al. 2008]) established safety and efficacy of eculizumab; these data were subsequently confirmed in a common open-label extension study (Hillmen et al. 2007). Eculizumab was administered intravenously dosed at 600 mg weekly for four weeks (loading phase), followed one week later by 900 mg fortnightly (maintenance phase); all patients were vaccinated against *Neisseria Meningiditis* at least two weeks before starting the treatment (because of a possible increased frequency and severity of infections by capsulated bacteria). The initial pilot study provided the proof-of-principle of effective blockade of intravascular hemolysis, as shown in eleven PNH patients with a heavy transfusion requirement (Hillmen et al. 2004). The subsequent study was a double-blind, placebo-controlled, multinational randomized trial which enrolled 86 transfusion-dependent PNH patients (Hillmen et al. 2006). Treatment with eculizumab resulted in a dramatic reduction of intravascular hemolysis, as measured by LDH, leading to hemoglobin stabilization and transfusion independence in about half of the patients. Control of intravascular hemolysis was found in all patients, and even cases not achieving transfusion independence showed a reduction of their transfusional needs.