Review

Paroxysmal nocturnal hemoglobinuria and other complement-mediated hematological disorders

Antonio M. Risitano*
Hematology, Department of Biochemistry and Medical Biotechnologies, Federico II University, Naples, Italy

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.

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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.
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 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 (Merry 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 analogous cases of inherited CD55 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 SHEEHYD [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 Meningitidis 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.
The effects of eculizumab on hemolysis were evident since the initial administration, and lasted for the whole study period. In comparison to placebo, eculizumab also resulted in a significant improvement in fatigue and quality of life, as measured by validated questionnaires. The study also showed that eculizumab treatment was extremely safe, with negligible side effects and incidence of adverse events comparable to that of the placebo. These data were confirmed in the open-label phase III study SHEPHERD, which enrolled a broader PNH population including patients with moderate marrow failure and minimal transfusion requirement. Based on the 96 patients enrolled in the study, treatment with eculizumab once again led to a remarkable control of intravascular hemolysis, regardless of the pretreatment transfusion requirement; transfusion independence was achieved in half of the patients, with significant improvement in fatigue and quality of life in all treated patients (Brodsky et al. 2008). These two initial studies were continued in a common open-label extension study, which included a total of 187 patients who have previously completed one of the parent clinical trials (Hillmen et al. 2007). The extension study confirmed the efficacy and the safety of eculizumab with a longer follow up, demonstrating that the effects of eculizumab treatment on intravascular hemolysis were retained over time. The extension study also looked to additional clinically relevant endpoint, such as the incidence of thromboembolic events. By comparing the rate of thrombosis between the pretreatment and treatment periods in the same patients, the extension study showed...
Learning from current anti-complement

Eculizumab represented a unique treatment option for many PNH patients, resulting in a remarkable breakthrough in PNH therapy. On the other hand, eculizumab also gave a considerable contribution to the understanding of PNH pathophysiology: indeed, our recent observations in PNH patients on anti–C5 therapy allowed the dissection of the different phases of complement cascade which remain uncontrolled in PNH. Our main finding was that during eculizumab treatment PNH erythrocytes survive to complement cleavage even in presence of the terminal complement complex (C5b-9), which may eventually work as opsonins with subsequent MAC assembly-; however, they may progressively bind on their surface C3 fragment C3b, which binds predominately to glycoporphin A and activates (now in a membrane-bound phase) the CAP amplification loop (Parker et al. 1982; Pangburn et al. 1983c; Müller-Eberhard 1988). As stated above, this process is finely tuned by CD55, which is absent on PNH erythrocytes; as a result this process, which is self-limiting on normal cells, leads to progressive CAP-mediated amplification, regardless of the presence of eculizumab (which acts downstream C3). The reasons why only a fraction of PNH erythrocytes has membrane-bound C3, and why the proportion varies among patients, are not fully understood, even if in vitro data support the concept that PNH erythrocytes are all susceptible to C3 deposition once exposed to conditions causing complement activation (Sica et al. 2010). Our current understanding is that each individual erythrocyte has to reach a specific threshold needed to start CAP activation, and that this may occur stochastically, or as a result of to specific microenvironmental conditions generating in particular vascular districts (this may open a possible similarity with the site-specific risk of thrombotic events).

We have also postulated that inter-individual genetic differences in other physiological inhibitors (such as CR1, complement FH and complement FI) may lead to a patient-specific tuning of the complement activation, possibly accounting for distinct patterns of C3 deposition. This may also work downstream, in driving the subsequent fate of C3-bound PNH erythrocytes; in fact, it is not clear why some patients do not show clinically relevant extravascular hemolysis regardless of a large proportion of C3-bound PNH erythrocytes (Risitano et al. 2010). In addition, we cannot exclude that surface C3 binding may hamper deformability and other physical properties of erythrocytes, possibly leading to enhanced clearance independently by any specific complement-receptor, similar to that observed for senescent erythrocytes (Karnchanaphanurach et al. 2009). In conclusion, we have identified C3-mediated extravascular hemolysis as a novel mechanism of disease accounting for suboptimal response in many PNH patients on eculizumab. At the moment the clinical impact of this finding is limited, because we are not able either to predict in advance the patients who will develop this condition, or to provide a possible solution once C3-mediated extravascular hemolysis has developed. In fact, steroids are not useful and should be avoided (Risitano et al. 2010), and splenectomy may represent an option (Risitano et al. 2008), but carries considerable medical risks, such as intra- or peri-operative thrombotic complications and life-long infectious events (Brodsky 2009).

Developing future anti-complement strategies in PNH

The observation that early phases of the complement cascade, and in particular C3 activation, represent a common biological observation as well as a possible clinically meaningful event led
to the hypothesis that alternative strategies of complement inhibition may be useful in PNH. Indeed, it has to be remarked once again that all these observations have been described during terminal complement inhibition by the anti-C5 eculizumab, making the point that eculizumab may be ineffective in preventing all the biological events leading to decreased erythrocyte survival in PNH. On the other hand, C3 opsonization and possible extravascular hemolysis cannot be considered a side effect of anti-C5, but rather a disease-related event which is simply unmasked by eculizumab given its downstream inhibition of the complement cascade. Thus, investigators have started to think about alternative strategies to target early events of the complement cascade, possibly directly at the initial C3 activation. Ideally, one would like delivering a complete inhibition of the complement activation occurring on erythrocyte surface due to the lack of the complement inhibitors CD55 and CD59, possibly retaining at least part of the physiological role of the complement in the clearance of microorganisms and injured tissues. In keeping with the terrific clinical results of the anti-C5, different anti-C3 mAb have been initially considered, aiming to bind native C3 in the fluid phase preventing its activation along all the three pathways; however, this approach was quickly abandoned because of the concerns on possible infectious and autoimmune complications secondary to a complete disabling of the complement cascade. More recently, a different strategy was developed, aiming to target activated C3 (C3b/iC3b) rather than the native circulating C3. Indeed, the anti-C3b/iC3b murine mAb 3E7 and its chimeric-deimmunized derivative H17 were shown to selectively inhibit the activity of C3 and C5 convertases of the CAP only, providing the opportunity for a selective inhibition of different complement pathway (Lindorfer et al. 2010). These antibodies were tested in vitro on PNH erythrocytes, and were shown effective in preventing complement-mediated hemolysis of CD55/CD59 deficient erythrocytes (Lindorfer et al. 2010). This finding was elegant and conceptually innovative, but likely still far from a clinical translation, because given that in PNH C3 and C5 convertases localized on erythrocyte surface, these antibodies would finally work as additional opsonins which should even increase PNH erythrocyte clearance by the reticuloendothelial cells, through both the C3- and Fc-specific macrophage receptors. Indeed, anti-C3 mAbs may have further clinical development only provided that their Fc is removed by further molecular engineering, likely preventing any Fc-mediated clearance. More recently, we and others have developed a novel strategy of complement inhibition, which aims to deliver a selective (for the CAP) and targeted (on PNH erythrocytes) inhibition of early phases (C3 activation) of the complement cascade, while retaining intact the functioning of the other two complement pathways. This innovative agent has been designed by creating a recombinant fusion protein between two endogenous complement-related proteins complement FH and complement receptor 2 (CR2). FH is a physiological complement inhibitor that modulates the initial CAP activation in the fluid phase by preventing C3 convertase activity and by promoting C3b inactivation into iC3b (Whaley and Ruddy 1976). Indeed, FH defuses the CAP amplification loop (which starts regardless the initial pathway of complement activation), and it has been demonstrated protective from lysis for PNH erythrocytes in vitro (Ferreira et al. 2007). In the aim to deliver FH activity locally at the site of complement activation, FH was fused with the iC3b/C3d-binding domain of CR2 (Fridkis-Hareli et al. 2011). Using C3 fragments as target seem quite appropriate given our observation that complement activation in PNH starts with surface C3-deposition, eventually leading to intravascular hemolysis or, in presence of eculizumab, to possible extravascular hemolysis. This novel compound named TT30 was recently investigated by our group in an in vitro model based on exposure of PNH erythrocytes to CAP-activated serum. We have demonstrated that TT30 completely inhibits complement-mediated hemolysis of PNH erythrocytes; in addition, it effectively prevents initial C3 activation and further C3 deposition on PNH erythrocytes (Risitano et al. 2009b; Risitano et al. 2012). We were able to demonstrate the presence of TT30 on PNH erythrocyte surface, as well as the reversion of the protective effect if anti-CR2 antibodies are added, consistent with the initial assumption that TT30 mainly work at the surface level, and cell membrane targeting is required for full inhibitory effect. Mechanistically, as a FH derivative, TT30 is able to act as a cofactor for FI to convert nascent C3b into iC3b, thus generating more of its own target and disabling the CAP amplification loop. At the same time, TT30 serves a CD55 surrogate on PNH erythrocytes, preventing the formation and promoting the decay of the CAP C3 convertase (and possibly C5 convertase) by displacing factor B and factor Bb, respectively, from the C3b-bound state. These in vitro data support the concept that TT30 in vivo could be useful to prevent both intravascular and extravascular hemolysis of PNH erythrocytes. Based on this robust background, a phase I clinical trial (NCT01335165) has just started to enroll PNH patients in a first-in-human study (ClinicalTrials.gov 2011). This initial trial will also be useful to rule our possible concerns about this more intensive strategy of complement inhibition, as well as to provide initial pharmacokinetic data in humans: the hope is that physiological immune-complex clearance and microorganism elimination may be preserved because of the CAP-selective and membrane-preferential action of TT30. If this anticipation will be confirmed, one may hypothesize that TT30 will be extensively investigated in PNH, given that based on the in vitro data it is very likely that the anti-hemolytic action on PNH erythrocytes should be extremely effective. Thus, TT30 is at the moment the strategy of C3 inhibition closest to the clinical translation; however, several additional complement inhibitors or modulators are currently under pre-clinical development. They include agents which target the C3 convertase directly through the C3 (such as the small molecule compstatin and its derivatives; Ricklin et al. 2008), or via other physiological proteins involved in the complement cascade (such as anti-FB antibodies or properdin inhibitors). Given that most of them act at the level of C3, they are all potentially effective for treating PNH, representing the next generation agents which will likely improve the clinical results achieved with C5-blockade by eculizumab. Future clinical investigations will also make clear whether these upstream complement inhibitors will make redundant the downstream blockade by eculizumab, or whether these strategies will have to be employed concomitantly for the best clinical efficacy. And of course they will also assess whether these strategies targeting early phase of the complement cascade may carry any increased risk of infectious or autoimmune complications, which on the other hand were quite infrequent in patients receiving long-term inhibition by eculizumab.

**Other complement-mediated hemolytic disorders: a room for therapeutic complement inhibition?**

The complement system has get involved in many human diseases (Holers 2008), for which current treatments are limited or ineffective. Thus, since the success of complement inhibition in PNH, there was a growing interest to investigate complement modulators in diseases other than PNH, including autoimmune (arthritis, vasculitis, systemic lupus erythematosus, psoriasis, dermatomyositis, Crohn’s disease), neurological (myasthenia gravis, multifocal motor neuropathy), renal (C3 nephropathy, membranoproliferative glomerulonephritis, dense deposit disease) and ocular diseases (age-related macular degeneration). For sake of consistency, here we will limit our discussion to a few examples of hematological disorders where the complement has been...
postulated or demonstrated as a mechanism of disease, all sharing hemolysis as a common feature. The first example is HUS, a condition characterized by thrombotic microangiopathy (TMA) which leads to mechanic intravascular hemolytic anemia, platelet consumption and end-stage organ damage, specifically targeting the kidneys (Noris and Remuzzi 2005; Lоорat and Frémeaux-Bacchi 2011). Sporadic (typical) HUS is an acquired disease usually associated with infectious events, typically by Shiga-toxin producing Escherichia coli (STEC). Atypical HUS (aHUS) is instead an inherited condition, where the clinical syndrome is associated with inherited mutations of complement genes, including FH, FI, FB, MCP, thrombomodulin, C3 convertase and C3 (Kavanagh et al. 2008; Noris and Remuzzi 2009; Kavanagh and Goodship 2010). Thus, the pivotal role of complement activation in aHUS has been largely elucidated (Loorat and Frémeaux-Bacchi 2011), and the anti-C5 eculizumab has been investigated in distinct prospective trials, enrolling aHUS patients resistant to plasma exchange (PE) and/or plasma infusion (PI), or requiring PE/PI. The first trial enrolled 17 aHUS patients resistant to PE/PI, and showed a significant increase in platelet counts, as a surrogate measure for TMA. The initial 26-week analysis showed TMA-free status in 15/17 patients (15/15 of those remaining on treatment), with platelet normalization in 13/15 patients; 4/5 patients on dialysis also achieved discontinuation of dialysis. In the long-term follow up of the extension of this study (Greenbaum et al. 2011) these data were confirmed with sustained response (15/17 TMA-free, 13/15 with platelet normalization, 4/5 free of dialysis) after median treatment duration longer than 1 year. A second similar study included 20 aHUS patients on chronic PE/PI; again after the initial 26 weeks of treatment eculizumab led to a progressive improvement of renal function, as well as a TMA-free status. These data were confirmed with a longer follow up in the subsequent extension study (Licht et al. 2011); furthermore, in both the extension study a dramatic improvement in quality of life was demonstrated (Muus et al. 2011). The terrific data of these 2 studies (which enrolled patients with a mid-term life-expectancy lower than 50%) led to the approval of eculizumab as second line treatment for aHUS (even in absence of a definitive proof of the causative mutation). In addition, based on biological data suggesting that even in typical HUS the causative Shiga-toxin may work through complement activation (Orth et al. 2009), it has been postulated that eculizumab may be beneficial even in STEC-HUS (Orth-Höller et al. 2011). Indeed, some anecdotal reports have suggested some efficacy (Lapeyraque et al. 2011); and based on these data, eculizumab was extensively used in patients with severe HUS during the recent outbreak of enterohemorrhagic E. coli in Europe (Orth-Höller et al. 2011; Laursen 2011), with results still pending. The second hemolytic disorder which may benefit from complement inhibition is CAD, which is characterized by the production of IgM autoantibodies specific for some erythrocyte antigens (usually I, more rarely i) (Neff 2003). The feature of these IgM auto-Abs is that they typically bind to erythrocytes at a low temperature, usually in the cooler peripheral circulation, and then they keep bound at a higher temperature, as in the warmer central circulation. Here they may fix complement, mainly through the classical pathway, eventually leading to MAC assembly and subsequent hemolysis. CAD may exist either as a chronic disorder, characterized by mild to moderate hemolysis (both intravascular and extravascular), or as an acute disease, usually associated to specific infections (e.g., Mycoplasma Pneumoniae, mononucleosis) and with a self-limiting course. As proof of principle, eculizumab has been proved effective in blocking intravascular hemolysis in a few cases of CAD (Roth et al. 2009; Roth and Duhrsen 2010; Notaro and Luzzatto, personal communication); however, results were less homogeneous in comparison to PNH, and more reliable data from an ongoing clinical trial (NCT01303952; ClinicalTrials.gov 2011) are awaited for. This may be due to the fact that MAC-mediated intravascular hemolysis may be just one of the pathogenic events leading to anemia, given that some extravascular hemolysis may develop due to the entrapment in the reticuloendothelial cells via the C3 receptors (because of sublytic complement activation and C3 surface deposition) (Jaffe et al. 1976). The contribution of these different mechanisms of disease may vary according to the specific auto-Ab, its specificity and its thermal range, making any single patient quite unique in terms of clinical response to eculizumab in vivo and in vitro. In addition, it has to be remarked that, as for PNH, the terminal complement blockade by eculizumab may unmask extravascular hemolysis even in CAD, even if at a lesser extent, given the proper functioning of CD55 on erythrocytes of CAD patients. Finally, the complement system could be involved in other auto-Ab mediated hemolytic anemias (including that by isoagglutinins or paroxysmal cold hemoglobinuria), through mechanisms similar to those described for CAD (Parker 2003). In fact, even if most warm (and mixed warm and cold) auto-Ab lead to anemia given the recognition of their Fc by tissue macrophages, in some cases they may also fix and activate the complement, leading to both MAC-mediated hemolysis and C3-mediated extravascular hemolysis (Neff 2003). Indeed, eculizumab has been considered in some refractory, life-threatening, severe hemolytic anemia as off-label, compassionate use (Bommer et al. 2011); however, at the moment there are no clinical data supporting this use.

Conclusion

Complement inhibition has started its clinical phase in the last decade with the availability of the anti-C5 eculizumab, initially for PNH and more recently for HUS and CAD, with the main limitation of its considerable cost. Even if a number of diseases are known to harbor a complement-mediated pathophysiology, these hemolytic conditions still represent the best paradigms for understanding complement-mediated mechanisms of disease, as well as dissecting the specific effect of current and novel complement inhibitors. The lesson from PNH is very useful: eculizumab led to a dramatic change in the clinical management of the disease, but additional mechanisms of complement-mediated damages were identified. Regardless the clinical impact in individual patients of these observations, they have led to the design of novel strategies of complement inhibition, which likely will be very useful in the future for the treatment not only of PNH (where after all the current anti-C5 inhibition remains extremely effective) but also of many other complement-mediated disorders. This is not an endless trip, but we are in the very beginning of a new revolution in conceiving biological treatments, which hopefully will lead in a few years in a substantial improvement of our medical practice. The challenge has just started, and the efforts that investigators and possibly companies will put in the development of these novel compounds will determine the length of our trip.

References


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