Molecular Immunology xxx (2009) xxx–xxx

Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

Review

Complement in human diseases: Lessons from complement deficiencies

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ARTICLE INFO

Article history:
Received 17 April 2009
Accepted 28 April 2009
Available online xxx

Keywords:
Complement
Immunodeficiency
Bacterial infections
Immune-complex diseases
Diagnosis
Registry

ABSTRACT

Complement deficient cases reported in the second half of the last century have been of great help in defining the role of complement in host defence. Surveys of the deficient individuals have been instrumental in the recognition of the clinical consequences of the deficiencies. This review focuses on the analysis of the diseases associated with the deficiencies of the various components and regulators of the complement system and their therapeutic implications. The diagnostic approach leading to the identification of the deficiency is discussed here as a multistep process that starts with the screening assays and proceeds in specialized laboratories with the characterization of the defect at the molecular level. The organization of a registry of complement deficiencies is presented as a means to collect the cases identified in and outside Europe with the aim to promote joint projects on treatment and prevention of diseases associated with defective complement function.

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1. Introduction

The research activity of several groups spanning over 100 years has led to the recognition of complement (C) as a multi-functional complex system comprising more than 30 proteins. These include constitutive components involved in the activation process and regulators, which control this process at various steps of the activation sequence. The persistence of this system during evolution and its progressive development to reach the complexity observed in primates and humans is justified by the important role played by C in host defence against infectious agents, in the removal of apoptotic cells and immune complexes, and in the modulation of the adaptive immune system.

In vitro experiments provided the initial information on C function and clarified the contribution of the early components to recognize the targets and that of the late components to act as effectors both as split products or as multi-molecular complexes. However, the *in vitro* data do not entirely reflect the highly complex and dynamic situations that are encountered *in vivo*. This issue has been addressed examining animals with spontaneous deficiencies of both early and late C components, and more recently animals, mainly mice, that have been depleted of most C components and regulators by gene deletion.

The discovery of individuals with C deficiencies has greatly contributed to understand the importance of the C system in host defence. The identification of an increasing number of individuals with selective deficiencies of C components and regulators has led to the recognition that bacterial infections and autoimmune diseases are the clinical conditions most frequently associated with C defects. Other diseases observed in C deficient patients include atypical haemolytic uraemic syndrome, membranoproliferative glomerulonephritis, paroxysmal nocturnal haemoglobinuria and the relatively more frequent hereditary angioedema. With the availability of screening assays that are much easier to perform than the standard haemolytic tests, more and more individuals with C deficiency are expected to be found and new ways should be devised to make them available to the scientific community.

The aim of this review is to provide an update on the inherited C deficiencies with particular reference to their clinical associations, the diagnostic approach used for their recognition and the tools currently available to prevent and treat the associated diseases. In addition, the organization of a registry of C deficiencies will be presented as a useful way to collect information on a large number of individual with these defects and to favour close collaborations and scientific interactions among groups interested in the study of these deficiencies.

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0161-5890/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.molimm.2009.04.029

2. Deficiencies of the classical pathway

It is well documented that homozygous hereditary deficiency of each of the early proteins of the classical pathway of C activation is very strongly associated with the development of a lupus-like disease whose predominant features are rash and glomerulonephritis with low incidence of anti-DNA antibodies (Pickering et al., 2000). Indeed such deficiencies remain the strongest disease susceptibility genes for the development of systemic lupus erythematosus (SLE) in humans. The association shows a hierarchy of prevalence and disease severity according to the position of the protein in the activation pathway. The most prevalent and severe disease is associated with deficiency of the proteins of the C1 complex and with total C4 deficiency. More than 75% of all individuals with deficiency of one of these proteins have SLE, which is commonly severe. By contrast, C2 deficiency is associated with a much lower prevalence of disease, estimated at approximately 10% (Pickering et al., 2000). Interestingly, C3 deficiency, discussed in more detail below, is only very rarely associated with the development of SLE, and one could speculate that this weaker association is because deficiency of C3 also results in reduced generation of effector products of the C system including anaphylatoxins (e.g. C3a and C5a) and the membrane attack complex (MAC). The cases of SLE associated with inherited C deficiency are extremely rare and only account for a tiny minority of the population of patients with SLE. However, they provide an important clue to the aetiology of the disease. They illustrate that there is an important activity of the early classical pathway of C that protects against the development of SLE. Data obtained from animal models have shown that C deficiency may lead to abnormal *in vivo* processing of dying cells (Taylor et al., 2000) that, in the context of an inflammatory response, could initiate and drive an autoimmune response leading to the development of SLE. Despite growing evidence in favour of a clearance defect as the core mechanism in the pathogenesis of SLE associated with C deficiency, it is still unclear precisely how this defect results in an autoimmune response and which further steps are required to lead to the development of disease. An alternative hypothesis linking C deficiency with SLE is the suggestion that C may be involved in the recognition of self by B cells and thereby defects in C might result in failure of B cell negative selection, allowing autoreactive B cells to survive and propagate when they would normally undergo apoptosis or anergy (Carroll, 2004). However tantalising these two hypotheses may be, one should not forget that one of the essential roles of C is to maintain immune complexes in solution. In the absence of C, the clearance of immune complexes is abnormal, a phenomenon that has been demonstrated in many studies of patients with SLE and C deficiency (Davies et al., 1992). In the absence of efficient C fixation, immune complexes may escape efficient clearance, deposit in tissues and cause tissue injury via ligation of Fc receptors on neutrophils and other cell types.

Given that deficiency of classical pathway C proteins is a potent cause for the development of SLE, one obvious therapeutic approach, when standard regimes have failed to control the disease, is the replacement of the missing protein. However, there are potential drawbacks to this approach. The first is that purified or engineered C proteins are not easily available for treatment purposes. Therefore, whole plasma preparations have to be used, which entail all of the complications of plasma treatment, including hypersensitivity reactions and the potential for transmitted viral infections. The second pitfall is that, following exposure to a protein that is genetically deficient and therefore "foreign", antibodies may develop preventing treatment by replenishment. There are several anecdotal reports of the treatment of C deficient patients with plasma, with benefit being reported in two patients with C2 deficiency (Hudson-Peacock et al., 1997; Steinsson et al., 1989). There was no significant clinical response in a patient with C1q deficiency who had received briefly fresh frozen plasma, but antibodies developed to C1q (Bowness et al., 1994). However, in another C1q-deficient patient long-term clinical remission with no complications has been achieved with fresh frozen plasma administrated every 4 weeks (Pickering, unpublished data). As the bone marrow and not the liver is the major source of C1q (Petry et al., 2001), this raises the possibility that C1q replenishment in patients with C1q deficiency and severe SLE might be achieved by bone marrow transplantation. To our knowledge this has not been attempted in humans as yet. In addition to the SLE-specific therapies, it is also extremely important to keep in mind that patients with hereditary C deficiencies are at particular risk of developing serious infections with encapsulated organisms such as *Streptococcus pneumoniae* and *Neisseria meningitidis* and thus should receive prophylactic penicillin therapy and be considered for both pneumococcal and meningococcal vaccination.

3. Deficiency of the lectin pathway

The frequency of MBL deficiency in the general Caucasian population has been estimated between 5% and 10% (Turner, 1991) and may, therefore, be considered the most common congenital immunodeficiency. Interestingly, even after almost two decades of research, there is no real consensus on the definition of MBL deficiency with respect to the cut-off plasma level. Due to a high rate of haplotype variation between different ethnic groups and within these groups, MBL concentrations vary considerably. Since most individuals with a MBL deficiency genotype are healthy, additional immunologic dysfunctions appear to be required for clinical manifestation.

There is, however, an increasing number of clinical studies indicating that deficiency of the lectin pathway, which most often results from low concentrations of MBL, and only sporadically of MASP-2 (a congenital ficolin deficiency has not been described), has been associated with an increased risk, severity, and frequency of infections but also autoimmune disorders (Neth et al., 2001; Sorensen et al., 2005). In particular, three single nucleotide polymorphisms (SNPs) in codons 52, 54 and 57 (D, B and C variants, respectively) of exon-1 lead to reduced functional plasma MBL concentrations which usually rank 1000-fold (about 5 ng/ml to 5 μg/ml) in the normal population. Comparing those studies, disease association is most prevalent if plasma levels are below 100 ng/ml (potentially already below 500 ng/ml).

MBL concentration positively correlates with gestational and postnatal age (Lau et al., 1995). Here, recurrent infections at low MBL levels reflect the importance of the lectin pathway before acquisition of a mature immunologic repertoire. This is especially evident in neonates who are prone to develop partly life-threatening infections as reflected by a high prevalence of MBL deficiency in premature neonates.

MBL deficiency also poses a problem for other immunocompromised individuals, such as cancer patients undergoing chemotherapy (Peterslund et al., 2001; Vekemans et al., 2007). In these patients, there was a strong correlation between low MBL levels and the occurrence of clinically significant infections. Due to increased bacterial colonization of the lung, cystic fibrosis patients with MBL insufficiency have a significant shorter life expectancy than MBL sufficient individuals.

MBL therapy is an area of current research. The usefulness of replacement MBL therapy in an attempt to prevent or ameliorate infections has shown promising results in enhancing recovery from bacterial disease (Garred, 2008; Summerfield, 2003). In a recent phase I study administration of recombinant human MBL restored the ability to activate the MBL pathway of the C system without non-specific activation of the C cascade and no signs of immunogenicity were detected (Petersen et al., 2006). Future studies of MBL

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therapy should provide more definitive answers to the effectiveness of short-term and long-term therapy.

4. Deficiencies of the alternative pathway

The alternative pathway (AP) has long been viewed as an amplification loop of C activation leading to the assembly of the C3 convertase C3bBb stabilized by properdin on the cell surface. More recently, properdin has been found to bind directly to various targets and to trigger the AP of C activation (Hourcade, 2006) confirming previous observation made more than 50 years ago by Pillemer et al. (1954).

Generation of mice with selective deficiencies of components of the AP by gene deletion has been of great help to elucidate the contribution of the AP to various models of human diseases. Thus, MLR/lpr mice deficient in factor B (fB) developed reduced proteinuria and renal alterations and had increased survival as compared to C sufficient MLR/lpr mice. Renal disease was also significantly reduced in factor D deficient MLR/lpr mice, although the life span of these mice did not markedly improve (Elliott et al., 2004; Watanabe et al., 2000).

Similar observations were made in a model of arthritis induced by passive transfer of anti-type II collagen antibodies in fB deficient mice that exhibited a milder clinical disease as compared to wild-type mice, while the disease activity was not different between C4 deficient and control mice (Banda et al., 2006).

Our knowledge on the role of AP in host defence has received great impetus from the identification of individuals with deficiencies of AP components, which include fB, factor D (fD), properdin and C3. The discussion will be restricted here to the deficiencies of fB, fD and properdin because the clinical consequences of C3 deficiency will be considered elsewhere in this review. Despite the great deal of information obtained from fB knockout mice on the importance of fB as a critical component of the AP in the protection from bacterial infection and in the contribution to the onset and development of autoimmune diseases, it is rather surprising that similar information is not available in man. There is only one report of a putative fB deficiency in a patient with a history of meningococcemia and presence of a dysfunctional protein in his serum resulting in undetectable AP activity (Densen et al., 1996).

Three families with fD deficiency have been reported in the literature (Biesma et al., 2001; Hiemstra et al., 1989; Sprong et al., 2006). All the deficient individuals suffered from *Neisserial meningitidis* complicated by septic shock in one of them (Sprong et al., 2006) and presented as recurrent Neisserial infections in the case reported by Hiemstra et al. (1989). The increased susceptibility to septic shock observed in one of these patients is consistent with the *in vitro* observation that an intact AP is required for maximal C activation by meningococci (Sprong et al., 2003). Association of fD deficiency with autoimmune diseases has never been reported in contrast with the observation that fD knock-out mice develop mesangial immune-complex glomerulonephritis (Abrera-Abeleda et al., 2007).

The deficiency of properdin is the most common genetic defect of the components of the AP inherited in an X-linked manner and manifests with complete absence of the molecule (type I), partial deficiency (type II) and normal level of a dysfunctional properdin (type III) (Truedsson et al., 1997). With the exception of one single patient who presented with chronic discoid lupus erythematosus, all the other properdin-deficient individuals now identified in a substantial number are susceptible to meningococcal disease (MD) again emphasizing the important role of the AP to control the growth of meningococci. The disease is frequently complicated by sepsis, occurs preferentially at teen, and is often caused by uncommon serogroups, but recurrent infections are rare (Fijen et al., 1999). Vaccination with the tetravalent vaccine is recommended to prevent Neisserial infections in individuals with no history of MD who are at risk of infection (Fijen et al., 1998). The aim is to promote synthesis of anti-capsular antibodies that are capable of activating the classical pathway.

5. C3 deficiency

Homozygous C3 deficiency is a rare autosomal disease strongly associated with recurrent and life-threatening bacterial infections particularly those caused by encapsulated organisms such as *N. meningitidis*, *S. pneumoniae* and *Haemophilus influenzae* (Pickering et al., 2000). Recurrent otitis media, meningitis and pneumonia are particularly characteristic infections. Major infections in patients with C3 deficiency are most prominent in childhood and are less of a clinical problem in adults. This emphasizes the key role of C3 as an opsonin for bacteria in early childhood and reflects the lesser importance of C in host defence to pyogenic bacteria when protective antibodies and anamnestic responses have developed in adulthood. Unlike inherited deficiency of the classical pathway components, the incidence of SLE among the C3-deficient patients reported in the literature is low. Membranoproliferative glomerulonephritis has been described in a few cases and only three patients from two Japanese families were reported to have a lupus-like syndrome without detectable autoantibodies. Detailed immunisation studies in C3-deficient patients are rare and the findings were not always comparable. Overall C3 deficiency in humans appears to be associated with impaired primary and secondary immune responses, abnormal IgG switch and reduced IgG2 and IgG4 production. However, immunisation with high doses of antigens can largely surmount these defects. Recently an elegant study by Ghannam et al. (2008) showed that in addition to a defective B cell memory, human C3 deficiency may be associated with important functional defects of dendritic and regulatory T cells. These observations, though limited to a single patient, highlight the importance of C3 as a key regulator of cell-mediated immunity.

The optimal management of C3-deficient patients, as well as of other C deficient individuals, remains uncertain. The standard therapeutic strategies (prophylactic antibiotics; active immunisation, and C3 substitution therapy) have been adopted with variable results. The majority of the patients receive prophylactic antibiotics and this may help to reduce the incidence of infection. The use of vaccines directed against encapsulated pathogens has been advocated as beneficial and it is usually adopted in these patients. On the other hand the benefit of replacing C3 with fresh frozen plasma remains controversial with anecdotal reports suggesting a possible therapeutic role for replacement of C3 and others showing no effect. In this context it is worth keeping in mind that one potential drawback of C3 replenishing therapy is the possibility of exacerbating an existing immune-complex disease by reconstituting C activity with C3 as reported in C3-deficient dogs (Cork et al., 1991). However, if the renal disease in C3-deficient patients is caused by the impaired disposal of immune complexes, it would sound logical to replace the missing component, even if this may be associated with a transient disease exacerbation usually controllable with other medications.

6. Deficiencies of the terminal complement components

Deficiency of a single terminal C component is usually compatible with life, and the majority of homozygous carriers remain even undetected until they are old and medical care is intensified (Ross and Densen, 1984; Tedesco et al., 1993; Wurzner et al., 1992). This is interesting as there is no efficient bypass at this stage of the C cascade responsible for multiple functions executed by the terminal C complex (TCC). Nevertheless, the terminal pathway is apparently an Achilles heel with respect to immune defence against

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Gram-negative cocci, as terminal C deficient subjects are highly susceptible to MD, indicating that its cytolytic property is of particular importance in host defense against *Neisseria* (Ross and Densen, 1984; Tedesco et al., 1993; Wurzner et al., 1992). The affected subjects are typically adolescents or young adults who suffer from recurrent infections often caused by rare serogroups, though not always (Ross and Densen, 1984; Tedesco et al., 1993; Wurzner et al., 1992). Neutrophils can only provide insufficient protection, although they can kill meningococci when incubated in terminal C deficient serum. This effect increases after vaccination (Platonov et al., 2003) so that this may represent an alternative treatment option in addition to, or instead of, vigilance and antibiotic prophylaxis. Unlike properdin-deficient patients, those lacking the late components may benefit from vaccination even with a past history of MD as suggested by the observation by Fijen et al. (1998) that the immunized patients with tetravalent vaccine were found to be disease free for over 3 years. Unfortunately, protection against *N. meningitidis* of serogroup B was not available until the discovery that a factor H (fH) binding protein GNA1870 expressed on the surface of these bacteria may represent a good vaccine candidate (Pizza et al., 2000) now in phase III clinical trials.

Due to the clear association of autoimmune disease, such as SLE, and deficiency of classical pathway components (see above), a large number of patients has been assayed for C deficiency and only a few cases with terminal C deficiencies and SLE or SLE-like disease have been found (Abel and Agnello, 2004). Given that roughly 1 out of 10,000 individuals is deficient in one of these components, although there are huge geographical and ethnical differences (Wurzner et al., 1992) (e.g. C9D in Japan: 1 in 1,000 subjects (Khajoee et al., 2003)), it is now well accepted that there is no association between autoimmune disease and terminal C deficiency (Wurzner et al., 1992).

The recognition of combined deficiencies of terminal component (C5, C7 or C8β) and early components (C1q or C4B) offers a unique opportunity to assess their relative contribution to the disease susceptibility in these patients (Chapel et al., 1987; Gianella-Borradori et al., 1990; Segurado et al., 1992; Wuillemin et al., 1991). With only one exception (Segurado et al., 1992), MD remains the hallmark of the terminal component deficiencies even in the presence of defective early components. The feature of immune-complex disease may be somewhat different in these patients, as is the case of the combined deficiencies of C1q and C8β observed in a patient with a history of recurrent MD and SLE-like disease, which surprisingly was less severe and developed at an age (49 years) considerably later than that reported among individuals lacking C1q alone (median onset 6 years) (Pickering et al., 2008). It could therefore be hypothesised that the inability to develop MAC-mediated tissue injury, due to genetic deficiency of C8β, ameliorated the extent and degree of organ damage driven by her SLE-like illness.

Deficiencies of C7 and C8 can also be considered as deficiencies of C modulators, as their local absence may have a more profound effect than the absence of the "well known" C-inhibitors (Nemerow et al., 1979; Wurzner, 2000). C7, for example, is primarily synthesised extrahepatically at the site of inflammation by granulocytes (Hogasen et al., 1995) and endothelial cells (Tedesco et al., 1997), thereby modulating lytic or sublytic membrane attack (Hogasen et al., 1995; Langeggen et al., 2000; Wurzner, 2000). Furthermore, the C7 expressed on endothelial cells acts as a trap for the assembling TCC downregulating inflammation (Bossi et al., 2009).

Heterozygosity of terminal C deficiency does not lead to any disease (Wurzner et al., 1992), and deficiencies can be traced within families via informative haplotypes (Wurzner et al., 1998). About three dozen mutations, including deletions, single nucleotide exchanges and intron–extron boundary mutations leading to complete, sometimes compound, terminal C deficiencies have been reported for C5 (Wang et al., 1995), C6 (Dragon-Durey et al., 2003), C7 (Rameix-Welti et al., 2007), C8 alpha-gamma (Kojima et al., 1998), C8 beta (Kaufmann et al., 1993), and C9 (Witzel-Schlomp et al., 1997).

Subtotal deficiencies of C6, C7, C8 and C9 have also been described with approx. 1–5% of the normal concentration, where the affected protein was still able to incorporate into the TCC and was bactericidal and haemolytically active, but in most cases structurally abnormal (smaller size, abnormal folding, detected, e.g., via isoelectric focusing) (Rameix-Welti et al., 2007; Wurzner et al., 1995). Unrestricted C activation, however, can lead to consumption of functionally active C7, C8 or C9, if present in low amounts, by circulating C5b6, so that no protein can be found in circulation. Thus, these genotypically subtotal deficiencies appear phenotypically complete (Wurzner et al., 1996), which is not observed in subtotal C6 deficiencies where the low C6 is not consumed. Plasma transfusion in a subtotal C7 deficient patient, for example, led to a marked clinical improvement and the appearance of his own C7 in the circulation (Wurzner et al., 1996) due to the lack of C5b6 in the non-activated transfused plasma and the sufficient amount of exogenous C7 binding to patient C5b6. This allowed restoration and maintenance of a low but detectable intermittent level of the patient's own de novo synthesised C7 for a few days until his C5b6 level rose again as a result of his chronic infection (Wurzner et al., 1996). Detection of very low levels of C7, C8 or C9 may, of course, be due also to a subtotal deficiency with a dysfunctional protein (Tedesco et al., 1990) not consumed by the ongoing complement activation.

The inability to generate a functionally active MAC may, however, also be of advantage as suggested by the clinical observation that meningococcal disease is generally less severe in patients with deficiencies of the terminal C components (Platonov et al., 2003; Tedesco et al., 1993). Animal experiments even point towards a protection from severe cerebral malaria for C5 deficiency (Patel et al., 2008), although this has not yet been demonstrated in humans. MD may be less severe in terminal C deficient subjects (Ross and Densen, 1984), due to a reduced extent of microbial damage and thus less severe endotoxin shock (Lehner et al., 1992). This has earlier been considered to be possibly of selective advantage, especially in areas where infantile gastro-enteritis is relatively common (Lachmann, 1987), and may account for the high frequency of a nonsense mutation at codon 95 (R95X) in the C9 gene in Japan (Khajoee et al., 2003). A possible advantage is however lost when the deficiency is accompanied by additional immune defects (Debard et al., 2005). The high proportion of deceased siblings with symptoms typical for MD in affected families also argues against an advantage. However, in view of the finding that a C6 level of less than 10% of normal is providing sufficient C activity (Wurzner et al., 1995) and that none of the 10 C6SD subjects suffered from MD, at least the subtotal C6 deficiency may indeed be beneficial by providing enough, but not too much, lytic activity.

7. Complement regulators

The key classical pathway regulators are C1 inhibitor (C1INH, also termed SERPING1) and C4-binding protein (C4 bp). Deficiency of C1INH results in episodic angioedema and this may be inherited (hereditary angioedema, HAE) or acquired. The molecular genetics and therapy of C1INH deficiency have been reviewed recently so will not be discussed in detail here (Cugno et al., 2009). C1INH deficiency results in angioedema not as a consequence of dysregulated classical pathway regulation, but due to abnormal regulation of the contact system. C1INH, in addition to its role as an inhibitor of C1r and C1s of the classical pathway and MASP-1 and MASP-2 of the lectin pathway, is the major inhibitor of factor XIIa and kallikrein of the contact system. The lack of inhibition of these

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enzymes by C1INH results in inappropriate bradykinin generation. This in turn mediates the increased vascular permeability characteristic of angioedema (Cugno et al., 2009). Genetic deficiency of C4bp has only been reported in one individual who developed an illness with similarities to Behçet's syndrome (a syndrome characterized by oral and genital ulceration, vascular thrombosis and ocular inflammation) (Trapp et al., 1987).

Complement fH (CFH) and factor I (CFI) represent the key regulators of the AP. Complete deficiency of either of these regulators results in uncontrolled activation of the AP with consequent depletion of plasma C3. Hence, CFI and CFH deficiency are associated with secondary C3 deficiency (Pickering and Cook, 2008; Vyse et al., 1994). In CFI deficiency circulating C3 is present as C3b since further physiological cleavage of C3b to iC3b is not possible in the absence of CFI. Furthermore, in CFI deficiency secondary reduction in circulating CFH levels occurs which is thought to be a consequence of CFH–C3b interactions (Naked et al., 2000). In contrast CFI levels remain normal in CFH deficiency.

Complete deficiency of CFH was first identified in an individual with atypical haemolytic uraemic syndrome (aHUS) (Thompson and Winterborn, 1981). aHUS comprises renal failure due to glomerular thrombotic microangiopathy, thrombocytopenia and microangiopathic haemolytic anaemia. CFH deficiency has also been associated with a glomerular inflammatory lesion termed membranoproliferative glomerulonephritis or dense deposit disease (DDD) (Pickering and Cook, 2008). The latter association was of particular interest since it was known that acquired AP dysregulation was associated with the development of DDD. This was most commonly due to the presence of an auto-antibody that stabilized the AP C3 convertase termed C3 nephritic factor, but rarer causes that result in abnormal stabilisation of the AP C3 convertase have also been reported (Smith et al., 2007). Complete deficiency of CFI has also been associated with renal disease, albeit less commonly than that seen for CFH deficiency (Amadei et al., 2001; Genel et al., 2005; Sadallah et al., 1999). In these reports the renal lesion appears to be distinct from dense deposit disease and represents immune-complex mediated glomerular inflammation. In both CFI and CFH deficiency the secondary C3 deficiency renders individuals susceptible to infection.

The association between AP dysregulation and increased susceptibility to aHUS is now well established. Thus, loss-of-function mutations affecting CFH, CFI and the membrane-bound regulator CD46 (membrane cofactor protein) have been associated with aHUS in many studies (Kavanagh et al., 2008). Furthermore, gain-of-function mutations in the AP activation proteins, factor B and C3 have also been associated with aHUS (Fremeaux-Bacchi et al., 2008; Goicoechea de Jorge et al., 2007). aHUS can therefore be seen as one of the major human diseases associated with AP dysregulation. Interestingly, mutations in CFH, MCP and CFI have been reported in C3 glomerulonephritis, a renal disease distinct from both DDD and aHUS (Fakhouri et al., 2008; Fang et al., 2008) and in the HELLP syndrome of pregnancy (Fakhouri et al., 2008; Fang et al., 2008) suggesting that AP dysregulation is important in these conditions.

Animal models of CFH and CFI deficiency have increased our understanding of the mechanisms underlying renal disease and AP dysregulation (Pickering and Cook, 2008). aHUS is thought to develop when there is abnormal activation of C along the renal endothelium. The majority of human CFH aHUS-associated mutations specifically impair the ability of CFH to target to sites of C3 activation (surface recognition activity) leaving the ability of the protein to regulate C3 activation in plasma intact. Thus individuals with these mutations typically have normal C3 levels. Consistent with these observations was the finding that mice engineered to express a mutated CFH molecule that lacks surface recognition domains developed spontaneous aHUS yet retained the ability to regulate C3 in plasma (Pickering et al., 2007). In contrast, mice with complete absence of CFH develop a renal lesion similar to DDD in the setting of marked loss of plasma C3 regulation (Pickering et al., 2002). In these animals, similar to human DDD, there is abnormal accumulation of C3 along the basement membrane of the glomerulus. As expected mice with complete CFI deficiency have low C3 levels (with plasma C3 circulating as C3b) and secondary reduction in CFH levels (Rose et al., 2008). These animals do not develop DDD which is consistent with the lack of a reported association between human CFI deficiency and DDD. Two experimental manoeuvres have prevented the development of DDD in the CFH-deficient mouse model: preventing AP activation by introducing factor B deficiency (Pickering et al., 2002) and preventing cleavage of C3b by introducing CFI-deficiency (Rose et al., 2008). The latter was unexpected and demonstrated that the nature of the plasma activation fragment is important in the development of DDD, at least in the mouse. The implication of these animal studies is that DDD therapies should aim to control plasma C3 activation whilst aHUS therapies should target C3 activation along the renal endothelium.

CD59 is the key regulator of the terminal pathway whilst decay accelerating factor (DAF, CD55) is a membrane-bound regulator that dissociates both classical and alternative C3 convertases. Both are anchored to cell membranes by a glycosyl phosphatidylinositol (GPI) molecule. Paroxysmal nocturnal haemoglobinuria (PNH) is a clonal disorder of red cells due to an acquired mutation in the PIGA gene. As a result of defective PIGA function, affected red cells lack all GPI-linked membrane proteins, including CD59 and DAF. The lack of CD59 and DAF renders PNH-red cells susceptible to autologous C-mediated lysis with consequent haemolytic anaemia. An important advance in the management of PNH has been the demonstration that transfusion requirements in PNH patients can be ameliorated by therapy with a monoclonal antibody that inhibits complement C5 activation (Eculizumab) (reviewed in (Parker, 2009). Only a single case of CD59 deficiency has been reported and this individual developed a PNH-like illness (Yamashina et al., 1990). In contrast, deficiency of DAF, denoted by Cromer blood group antigen phenotypes Inab and DR(a-), is not associated with haemolytic anaemia. This suggests that in PNH it is the lack of CD59 and not DAF that renders red cells susceptible to lysis.

Whilst outside the scope of a discussion on C deficiencies it is important to mention the increasing associations between C regulator polymorphisms and human diseases. Human genetic studies have shown strong associations for polymorphic variations in CFH (reviewed in (de Cordoba and de Jorge, 2008)) and age-related macular degeneration, a major cause of acquired blindness. Polymorphic variants in factor B (Gold et al., 2006), C3 (Yates et al., 2007), CFH-related genes (Hughes et al., 2006) and C1INH (Ennis et al., 2008) have also been associated with AMD. Clearly these associations suggest that C dysregulation is important in the pathogenesis of AMD. Consistent with this is the recent demonstration that a polymorphic variant of factor B that protects against AMD is associated with reduced C3 convertase formation (Montes et al., 2009). Hence, it is likely that mutations or polymorphisms affecting C regulators will be detected in a range of human pathologies characterized wholly or in part by C-mediated tissue injury.

8. Diagnosis of complement deficiencies

The development of new diagnostic tools (Table 1) over the last years has significantly contributed to our current knowledge on the molecular origin of C deficiency states (Mollnes et al., 2007; Mollnes and Kirschfink, 2006).

As in all immunodeficiency cases, the diagnostic approach should be organized in different levels:

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Table 1
Main complement assays.

Functional assays	Total complement activity (screening for complement deficiency): (a) CH50 and APH50 haemolytic assays for CP and AP activity (b) Enzyme immunoassays (EIA) for specific evaluation of CP, LP and AP (WIELISA) activity using C5b-9 as readout Functional activity of single components: (a) Haemolytic assays for single components (e.g. C3) using corresponding deficient sera as test system (b) EIA for MBL/MASP functional activity using deposition of C4 as readout (c) C1 inhibitor assay (chromogenic substrate or EIA) for diagnosis of hereditary angioedema type 2
Protein quantification	Measuring the concentration of single components by immunoprecipitation (RID, nephelometry), EIA or WB, e.g.: (a) C3 and C4 to detect "hypocomplementemia" (b) Follow-up of a low activity detected in total complement activity screening (any component) (c) Properdin, MBL for recurrent neisserial infections (d) C1 inhibitor for diagnosis of HAE type I and acquired angioedema
Activation products	EIAs preferentially based on antibodies to neoepitopes expressed selectively on the activation products: (a) Split products from components after proteolytic cleavage (e.g. C3a, C4a, C5a) (b) Complexes between the activated component and its inhibitor (e.g. C1rs-C1 inhibitor) (c) Macromolecular complexes (e.g. the AP convertase C3bBbP and the terminal SC5b-9 complex)
Auto-antibody analysis	EIA (anti-C1q – SLE; anti-C1Inh – angioedema; anti-fH – atypical haemolytic-uraemic syndrome (aHUS) or functional assay (C3 nephritis factor – MPGN)
Surface proteins	Flowcytometric quantification of CD55/DAF and CD59 for diagnosis of paroxysmal nocturnal haemoglobinuria
Genetic mapping	Detection of disease associated genetic variants; e.g. factor H, MCP, factor I, factor B, C3 in aHUS or MPGN (only factor H), including family analysis

Table 2
Complement deficiency states and complement analysis.

Disease condition	Recommended analysis
Recurrent bacterial infections	CH50, APH50, WELISA, C3, C3a/C3d[a] C5–C9 (especially in *Neisserial* infections) Properdin (especially in *Neisserial* infections) MBL (especially in *Neisserial* infections)
Systemic lupus erythematosus	CH50 C4 (C4A/B) C3a/C3d[a] Anti-C1q autoantibodies
Angioedema	C1 inhibitor antigenic and functional assay C4 C1q Auto-anti-C1 Inh (acquired autoimmune form)
Atypical haemolytic uraemic syndrome	CH50, APH50 C3 C3a/C3d[a] Factor H Factor I Factor B Anti-factor H antibodies
Membranoproliferative Glomerulonephritis	CH50, APH50 C3 C3a/C3d[a] C3 nephritic factor Factor H Factor I Anti-factor H antibodies
Paroxysmal nocturnal haemoglobinuria	CD55, CD59 (FACSCAN) Acid lysis test

[a] Useful to exclude deficiency due to excessive complement consumption.

(I) Global assays identifying lack of pathway function.

This analysis is performed either by haemolytic assays or by a recently developed ELISA with IgM, mannan and lipopolysaccharide coated to the well allowing the simultaneous analysis of all three pathways (Seelen et al., 2005).

This first-step evaluation should be complemented by assays for at least one C activation product (C3a, C3d, SC5b–9) to distinguish primary from secondary deficiencies. Today neoepitope based ELISAs have replaced the older generation of tests which were hampered by pre-assay precipitation or fractionation steps. Furthermore, neoepitope-specific antibodies are particularly suitable to detect in situ C activation by immunohistochemistry since they discriminate between activation products and passively trapped native components.

The initial screening often points to the deficiency of candidate C protein, which should then be verified by quantifying the individual components.

(II) Proteinchemical, cytofluorometric and functional assays for individual components and regulators, including autoantibodies (e.g. anti-C1q, anti-fH, C3Nef, anti-C1INH)Individual C components, irrespective of their functional activity, can be measured by radial immunodiffusion (RID), nephelometry or by ELISA.

(III) Molecular analysis to identify genetic alterations (mutation, polymorphism). Certain clinical symptoms are suggestive of deficiencies of specific C components or regulators and may be used for the selection of the assays to perform (Table 2).

Recurrent bacterial infections is one of the clinical conditions for which it is important to screen for C deficiencies by haemolytic titration of the classical (CH50) and alternative pathway (APH50) or by the recently developed ELISA-based assays for the three C pathways. Patients with late component deficiencies, which are often associated with recurrent Neisserial infections, are readily identified by assays of total complement activity and further identified by selective C5–C9 determination. Patients with meningococcal disease should also be analyzed for the level and functional activity of properdin. As properdin defect is rarely detected by conventional haemolytic assay (APH50), the ELISA-based total C activity test shows a low AP activity in sera with the deficiency of this protein. If a low value of the lectin pathway is detected, the immunochemical level of MBL should also be evaluated.

Autoimmune diseases, and in particular SLE, represent another indication for C analysis given the high frequency of deficiencies of the early C components associated with these diseases.

It should be pointed out, however, that low total C activity and low C4 in active SLE, particularly with renal involvement, are more often due to increased *in vivo* activation, which can be verified by the detection of C activation products. In patients with severe clinical outcome, such as lupus nephritis, auto-antibodies to C1q are often found and may be of prognostic value.

Membranoproliferative glomerulonephritis (*MPGN*), *haemolytic uraemic syndrome* (*HUS*), *and age-related macular degeneration* (*AMD*) are associated with AP dysregulation and patients with

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Table 3
Registry of complement deficiencies[a].

1/5 Laboratory information						
Your lab code	Head of the group	Affiliation	Address	Tel.	Fax	E-mail
2/5 – Patient						
Name (1st letter)	Surname (1st letter)		Your internal patient code			Publication
Ethnic origin	Gender		Year of birth	Age first infection		State of birth
3/5 – Characterization of complement deficiency						
Pathways involved						
1st complement deficiency		Protein	DNA			
2nd complement deficiency		Protein	DNA			
3rd complement deficiency		Protein	DNA			
4/5 – Associated diseases						
1st infection	Agent					
2nd infection	Agent					
3rd infection	Agent					
Other disease						
Other disease						
Other disease						
5/5 – Family study						
Family member	Complement deficiency		Homozygous or heterozygous			

[a] Final details will be subject to ethical approval, patient consent and data confidentiality laws.

these diseases should be tested for C activity and C activation products.

In the case of MPGN type 2, low C3 values and consequently low activity in all pathways results from a continuous C3 activation either triggered by the presence of a C3-convertase stabilizing auto-antibody termed nephritic factor, or by factor H deficiency. These two factors should be analyzed when a patient presents with a very low C3 value.

C analysis in atypic HUS should focus on search for mutations of factor H > MCP > factor I mutation, occasionally also of factor B and C3 (gain of function) resulting in dysregulation of the AP (URL: http://www.fh-hus.org/). Molecular analysis is required as those mutations are only occasionally associated with low/absent regulator expression, but may result in lack of fH binding to tissues. Cell binding assays may be helpful to identify mutations in SCRs expressing the respective binding site. Analysis should also include fH auto-antibody concentration by ELISA often associated with a deficiency of fH related protein-3.

Patients with AMD should be tested for C activation products and for mutations of fH and other C regulators.

Hereditary angioedema associated with deficiency of C-inhibitor is diagnosed by measuring the level of C1 inhibitor and C4. It is important to include both antigenic and functional assays for C1INH, since 15% of the patients have type 2 HAE with normal or even increased antigen concentration of C1 inhibitor. C4 is usually low in both cases even between the attacks and serves as a valuable marker to evaluate in addition to the analysis of C1 inhibitor and in presence of normal level of C3. To identify the acquired form of HAE characterized by normal or even elevated concentration of C1INH, analysis of auto-antibodies to C1INH (ELISA) is mandatory. Besides reduced C1INH and C4 levels, these patients show low concentration of C1q, which in contrast is normal in patients with hereditary HAE. Identification of this form of acquired angioedema is important as its acute treatment requires higher doses of C1INH.

Paroxysmal nocturnal haemoglobinuria (*PNH*) is dependent on a somatic mutation in the gene coding for the phosphatidylinositol (PI) anchor (PIG-A), which results in decreased expression of membrane proteins linked to this structure including decay accelerating factor (DAF, CD55) and CD59. The absence or the reduced expression of DAF and/or CD59 render the red cells susceptible to C mediated lysis, which is the hallmark of this condition. Diagnosis was traditionally made by Ham's test/acid lysis test, but now is specifically assessed by flow cytometric analysis of the respective cell surface proteins.

9. Registry of inherited complement deficiencies

The first case of C deficiency published by Silverstein in 1960 in an healthy individual lacking C2 raised the question whether the C system was really important in host defence (Silverstein, 1960). The issue of the critical role played by C was clarified in the following 20–25 years by several reports that appeared in the literature starting from the paper by Donaldson and Evans (Donaldson and Evans, 1963) who reported the association of HAE oedema with C1INH. It became soon apparent that a functionally defective C system may have serious clinical consequences depending on the components and the regulators involved. Thus, repeated infections were observed in patients with primary deficiency of C3 or secondary to the defect of factor I (Alper and Rosen, 1972; Alper et al., 1972) emphasizing the importance of C as a defence barrier against bacterial infections. The paper published by Hauptmann in the early 1970 (Hauptmann et al., 1974) disclosed the association of lupus erythematosus with C4 deficiency, which was later extended to the deficiencies of the other early components (see above).

With the increasing number of C deficient individuals identified and reported in several publications the associations with selective clinical conditions were recognized. This was the case of Neisserial infections found by Petersen and colleagues to occur particularly in individuals lacking one of the late components (Petersen et al., 1979). A major advance in our knowledge in this field was made by Densen and associates (Figueroa and Densen, 1991; Ross and Densen, 1984) with their survey of all published cases of C deficiencies up to 1990, which provided an overall picture of the diseases associated with the deficiencies of the C components and regulators in a large number of

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patients. Unfortunately, the flow of information was substantially reduced in the following years and was limited to occasional reports and to surveys restricted to deficiencies of some C components (Jonsson et al., 2005; Pickering et al., 2000)

A renewed interest in C deficiencies was stimulated in the late 90s and at the beginning of this century by two European Concerted Actions within the Biomed 2 and PF5 program dealing with the role of C in infectious and chronic diseases. The participants from various European Countries agreed that the C deficient patients recognized in different C laboratories were a precious resource to exploit for future studies. The idea was supported by the European Complement Network and prompted the organization of a registry containing both published and unpublished cases of C deficiencies.

The aim of the registry is to provide information on the number and types of C deficiencies involving both C components and regulators, the prevalence in different populations and ethnic groups, the distribution of the defect in family members, and the association with diseases.

Researchers and clinicians will have an open access to these data and a good opportunity to cooperate in joint projects involving a reasonably large group of patients. The access to the registry to include new cases will be restricted to the representative of the C laboratories listed in the webpage of ECN and will also be extended to members of the International Complement Society.

Table 3 schematically presents the structural organization of the registry and the data that are strictly required and others that are optional. The first section provides information on the head and address of the laboratory where the diagnosis of C deficiency was made followed by a second section reporting personal particulars of the index case. The type of deficiency and the associated disease will be listed in the next two sections and the results of the family study will be finally reported in the last section. The program of the registry has been built in such a way to provide various information including the prevalence of C deficiency and their association with diseases in different populations and geographic areas.

Acknowledgments

This work was supported by grants from the Wellcome Trust (grant number 071467) to MB, Italian Ministero dell'Istruzione, Universita'e Ricerca (PRIN 2007) to FT, and EU NoE grant EuroPathoGenomics (LSHB-CT-2005-512061) to RW. MCP is a Wellcome Trust Senior Fellow in Clinical Science (WT082291MA).

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Please cite this article in press as: Botto, M., et al., Complement in human diseases: Lessons from complement deficiencies. Mol. Immunol. (2009), doi:10.1016/j.molimm.2009.04.029

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Please cite this article in press as: Botto, M., et al., Complement in human diseases: Lessons from complement deficiencies. Mol. Immunol. (2009), doi:10.1016/j.molimm.2009.04.029

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