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Review

Complement deficiency and disease: An update

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Abstract

Complement deficiencies are probably vastly under-diagnosed within clinical medicine. Judging from a Swedish study of C2 deficiency, a deficiency with an estimated prevalence of about 1/20,000 in Western countries, less than 10% of the deficiencies of the classical and alternative pathways and the late complement components are identified in Sweden. C1 inhibitor deficiency and deficiencies of MBL and MASP-2 were not included in the assessment. The introduction of new screening methods should facilitate detection of complement deficiencies in clinical practice. In our study of C2 deficiency ($n = 40$), 57% of the patients had a history of invasive infection with encapsulated bacteria, mainly *Streptococcus pneumoniae*. This emphasizes the importance of the classical and/or the lectin pathway in defence against severe infection. Rheumatological disease, mainly systemic lupus erythematosus was present in 43% of the patients. In addition, a significant association was found between C2 deficiency and atherosclerosis. Complement-dependent disease mechanisms are discussed together with the potential importance of non-complement genes for disease expression in complement deficiencies. Analysis of larger patient groups is required in order to establish guidelines for investigation and treatment of patients with complement deficiency.

Keywords: Immunodeficiency; Complement; Systemic lupus erythematosus; Septicemia; Atherosclerosis

1. Introduction

Awareness of complement deficiency states as a cause of disease is not strongly established within health care. Hereditary angioedema, systemic lupus erythematosus (SLE) and recurrent meningococcal disease are probably the most common clinical settings in which complement deficiency tends to be suspected, even if this is not always the case. One implication of this is the vast majority of complement-deficient patients that remains to be identified. Most of these patients would clearly benefit from receiving a correct diagnosis, even if specific treatment regimes related to complement deficiencies are limited. With regard to other immunodeficiencies, the availability of immunoglobulin treatment is a major reason why immunoglobulin deficiencies are more systematically sought for in patients with susceptibility to infection. It is noteworthy in this context that the prevalence of common variable immunodeficiency in Western countries (Hermaszewski and Webster, 1993) is similar to the estimated prevalence of about 1/20,000 for C2 deficiency (Pickering et al., 2000).

Observations in complement-deficient patients are an important source of information about immune defence (Figueroa and Densen, 1991; Pickering et al., 2000) and have strongly contributed to development of new concepts concerning pathogenetic mechanisms in SLE and other disease conditions (Figueroa and Densen, 1991; Jönsson et al., 2005; Ochs et al., 1993; Pickering et al., 2000; Rugonfalvi-Kiss et al., 2002). Experiments with complement-deficient and genetically engineered animals have been used in order to address several specific issues (Brown et al., 1983, 2002; Buono et al., 2002; Carroll, 2004; Mitchell et al., 2002; Pickering et al., 2000). On the other hand, the impact of complement function on human disease must be tried and verified by clinical studies, even if difficulties to obtain conclusive results are taken into account. An advance during recent years is that comparatively large groups of complement-deficient

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patients are becoming available providing a basis for more versatile analysis. Among research areas that have brought increased attention to consequences of complement deficiency, lectin pathway deficiencies and complement control protein mutations associated with atypical hemolytic uremic syndrome should be mentioned. These conditions are specifically dealt with elsewhere in this issue (see Thiel et al., 2005; Zipfel et al., 2005).

Hemolytic assays for detection of complement deficiencies have been available for many years, but their use has mostly been restricted to specialized laboratories. As a result of a European collaboration project, a new enzyme-linked immunoassay (ELISA) procedure has now been developed for detection of complement deficiencies including assessment of lectin pathway function (Seelen et al., 2005). A more wide-spread availability of complement screening assays will hopefully facilitate future clinical studies of complement deficiency and provide a basis for more profound knowledge and improved procedures for investigation and treatment of the patients.

The present overview is focused on clinical questions, and discusses mechanisms that may be involved, particularly with regard to infectious diseases.

2. Prevalence of complement deficiencies

Estimates of the prevalence of inherited complement deficiencies are difficult to make due to the fact that most deficiencies have a heterogeneous genetic background and that prevalence varies in different populations (Figueroa and Densen, 1991; Pickering et al., 2000; Sjöholm, 2002; Würzner, 2003). In general, only complete, subtotal or functional complement deficiencies on a homozygous or X-linked basis are taken into account. The significance of some subtotal deficiencies is unclear (Würzner, 2003).

C2 deficiency is unusual in that the deficiency is nearly always caused by a 28 bp deletion in the C2 gene of the *HLA-B*18,S042,DRB1*15* MHC haplotype (Johnson et al., 1992; Yu, 1998). This means that a likely prevalence of homozygous C2 deficiency can be estimated by determining the prevalence of heterozygous carriers in the general population. As summarized by Pickering et al. (2000), results of several studies indicate a C2 deficiency prevalence of about 5 per 100,000 persons in Western countries. We recently described a cohort comprising the 40 C2 deficiency patients, who were identified in Sweden between 1977 and 2002 (Jönsson et al., 2005). Sweden has about 9 million inhabitants. The estimated prevalence of C2 deficiency would yield a total of 450 C2-deficient persons in Sweden, which in turn suggests that less than 10% were identified. The identification of C2 deficiency was based on screening with hemolytic gels, a procedure likely to detect all known deficiencies of the classical and the alternative activation pathways and the late complement components (Sjöholm et al., 2001). Table 1 summarizes the various complement deficiencies identified in Sweden by this means, a total number of 78. With this background and with reservation for some uncertainty in the estimation, the data would suggest at least 800 cases of inherited complement deficiency. As the majority of patients with hereditary angioedema are likely to be investigated and diagnosed without screening, C1 inhibitor deficiency is an exception in the context. The number of hereditary angioedema patients in Sweden is not known, but may be assumed to be close to 200. This would suggest about 1000 cases of complement deficiency in Sweden without inclusion of lectin pathway deficiencies.

Table 1
Inherited complement deficiencies in Sweden identified 1977–2002 by basic screening procedures (C3, C4, hemolytic gels) and by other means

Deficiency	No. of patients	Estimated no. of Swedish cases
C1q	2	
C4	1	
C2	40	>450
C3	3	
C6	3	
C7	12	
C8	2	
C9	1	
Properdin	9	
Factor I	3	
Factor H	1	
Total	78	>800
Deficiencies identified by other means		
MBL	>50	1.3×10^6
MASP-2	1	1350
C1 inhibitor	>100	180

All but three of the 78 patients were diagnosed at the Department of Clinical Immunology, Lund University Hospital. The C7-deficient patients include cases of subtotal deficiency of unknown significance. The Swedish population is about 9 million.

Homozygous MASP-2 deficiency was fairly recently identified in a Danish patient (Stengaard-Pedersen et al., 2003). Initial analysis suggested an allele frequency for the mutation of 5.5% indicating that the deficiency might be very common. Analysis of Swedish blood donors gave an allele frequency of 1.3% (Carlsson et al., 2005), which would correspond to an MASP-2 deficiency prevalence of about 15 per 100,000 persons. In the same study, the prevalence of genetically defined MBL deficiency among the healthy blood donors was found to be 14%.

3. Autoimmune disease and atherosclerosis

The association between SLE or SLE-like disease and inherited C2 deficiency was recognized early in the 1970s as an unexplained and partly paradoxical observation (Agnello et al., 1972; Day et al., 1973). According to current data, the majority of patients with deficiencies of C1 or C4 develop SLE; for C2 deficiency this association is much less pronounced (Pickering et al., 2000). The reason why impaired classical pathway function predisposes to development of SLE has been subject to extensive investigation. The major

theories involve impaired immune complex handling, inefficient clearance of apoptotic cells, and loss of complement-dependent B-cell tolerance (Carroll, 2004; Pickering et al., 2000).

In a recent study of 40 patients with C2 deficiency (Jönsson et al., 2005), invasive infection was found to be the predominant clinical manifestation (57%) followed by SLE (25%) and other rheumatic diseases (18%). There was no history of major infections in 25% of the patients. In addition, a statistically significant increase of cardiovascular disease associated with atherosclerosis was present in the cohort. Interestingly, atherosclerosis appeared to be more related to C2 deficiency than to the presence of SLE in the patients. Other studies have shown that MBL deficiency is associated with cardiovascular disease (Ohlenschlaeger et al., 2004; Rugonfalvi-Kiss et al., 2002). Furthermore, C3 deficiency in genetically engineered mice promotes atherosclerosis (Buono et al., 2002). This implies that a dysfunctional lectin pathway might promote development of atherosclerosis.

Our findings together with those of Lipsker et al. (2000) indicate that the SLE associated with C2 deficiency resembles genuine SLE in many regards. Atypical clinical features, mainly characterized by skin manifestations, were emphasized in early studies of C2 deficiency (Agnello et al., 1972; Day et al., 1973). A bias towards identification of C2 deficiency associated with mild SLE or undifferentiated connective tissue disease might have been present. The reason is not known why antinuclear antibody titers are low or undetectable in most SLE patients with C2 deficiency (Jönsson et al., 2005; Pickering et al., 2000). We found no antibodies to dsDNA in our C2-deficient patients, and antibodies to dsDNA may be unusual in classical pathway deficiencies in spite of severe disease (Pickering et al., 2000; Yang et al., 2004). By contrast, the C2-deficient patients often showed autoantibodies to the collagenous fragment of C1q (to be published), an autoantibody specificity that is usually associated with the presence of anti-dsDNA in SLE (Sjöholm et al., 1997). C2 deficiency might promote formation of anti-C1q autoantibodies providing a partly new aspect on the possible origin of these antibodies.

4. Invasive infections

Most inherited complement deficiencies are associated with susceptibility to invasive bacterial infections (Figueroa and Densen, 1991). The reason why susceptibility appears to be restricted to a limited spectrum of bacteria, mainly encapsulated bacteria, is not entirely clear. *Streptococcus pyogenes* and several other microorganisms (Lindahl et al., 2000, 2005; Würzner, 1999) have elaborated specific mechanisms to circumvent complement-dependent defence, which might partly explain why complement deficiency lacks importance as a susceptibility factor for these pathogens.

Late complement component deficiencies (LCCD) are typically associated with recurrent invasive infections caused by *Neisseria* (*N.*) *meningitidis* and *N. gonorrhoeae* indicating that the serum bactericidal function of C5b-9_n is important in defence against neisserial infections (Figueroa and Densen, 1991). For reasons unknown, the meningococcal serogroups W-135 and Y are particularly common in LCCD, as well as in properdin deficiency. In the absence of serum bactericidal defense, opsonic functions involving immunoglobulins and C3 gain increased importance in LCCD. This is reflected by the finding of opsonophagocytic responses to vaccination with meningococcal capsular polysaccharides (Fijen et al., 2000; Platonov et al., 2003a), and the influence of Fcγ receptor polymorphism (van Sorge et al., 2003) on susceptibility to meningococcal disease in LCCD (Fijen et al., 2000; Platonov et al., 1998). *Haemophilus (H.) influenzae* type b (Hib) is susceptible to the serum bactericidal effect of C5b-9_n (Selander et al., 2000), but is a rare cause of invasive infection in LCCD (Figueroa and Densen, 1991; Pallares et al., 1996). Most likely, opsonophagocytosis is more important than C5b-9_n in defense against Hib.

Factor D deficiency, which is very rare, and the X-linked properdin deficiencies result in selective impairment of alternative pathway function (Sjöholm, 2002). *N. meningitidis* is the by far most common pathogen encountered in properdin deficiency. In some families with properdin deficiency, fulminant infections have been documented in several patients. It is questionable if susceptibility to other bacteria is increased, which is surprising, since it is thought that the principal role of properdin is to promote activation of C3, which is involved in defence against several pathogens (Figueroa and Densen, 1991). Some data indicate that the alternative pathway is strongly involved in anticapsular antibody-dependent immunity to *N. meningitidis* (Selander et al., 2000; Sjöholm, 2002), which might partly explain the association between properdin deficiency and meningococcal disease. On the other hand, anticapsular antibodies formed in response to vaccination can also activate the classical pathway and support killing of *N. meningitidis* in properdin deficiency (Sjöholm, 2002). Vaccination with tetravalent meningococcal vaccine is particularly important in properdin-deficient persons, who have not had meningococcal disease, and who are likely to be found through family investigation following identification of patients with X-linked properdin deficiency. Properdin-deficient survivors of meningococcal disease should probably be vaccinated, even if repeated meningococcal infections are rare in properdin deficiency, as well as in complement-sufficient patients.

Susceptibility to bacterial infection is also important in deficiencies of the classical pathway (Fasano et al., 1990; Figueroa and Densen, 1991), but this has gained less attention than the association between these deficiencies and autoimmune disease. In our recent study of a C2-deficient cohort ($n = 40$), invasive infection (meningitis, septicemia) was found in 57% of the patients, and was the predominant clinical manifestation (Jönsson et al., 2005). In accord with previous studies (Fasano et al., 1990; Figueroa and Densen,

1991) *S. pneumoniae* was the most common pathogen. Other encapsulated bacteria including *N. meningitidis*, Hib and *S. agalactiae* were also found. In several patients, invasive infections occurred during childhood and did not recur during adulthood. However, invasive infections were also seen in adults. The presence of pyelonephritis in four of our patients is noteworthy.

5. Innate and acquired immunity to encapsulated bacteria

Children with C2 deficiency may experience invasive infections that cease during adolescence suggesting establishment of acquired immunity (Fasano et al., 1990; Figueroa and Densen, 1991; Jönsson et al., 2005). This course of events is primarily consistent with an important role of complement in innate immunity mediated through the classical pathway or the lectin pathway, which is become visible in C2 deficiency. Experiments in genetically engineered mice suggest that innate immunity to *S. pneumoniae* involves natural antibody and a functional classical pathway of complement (Brown et al., 2002). The occurrence of severe pneumococcal disease in MASP-2 deficiency indicates that the lectin pathway could partly account for innate immunity to *S. pneumoniae* (Stengaard-Pedersen et al., 2003). MBL deficiency has also been associated with pneumococcal disease (Roy et al., 2002). Earlier animal studies have emphasized a role of the alternative pathway (Brown et al., 1983). Innate immunity to *S. pneumoniae* also involves Toll-like receptor signaling (Currie et al., 2004; Picard et al., 2003). A role of the classical pathway has been suggested in innate immunity to *S. agalactiae* (Wessels et al., 1995). Recent findings have shown that L-ficolin/MASP complexes contribute to opsonophagocytosis of *S. agalactiae* (Aoyagi et al., 2005). For reasons unknown, the median age for meningococcal disease in LCCD and properdin deficiencies occurs late during adolescence (Figueroa and Densen, 1991).

Splenic marginal zone B cells are a likely source of natural antibodies and can respond rapidly to thymus-independent antigens (Zandvoort and Timens, 2002) such as capsular polysaccharides (Rijkers et al., 1993) and phosphorylcholin (Harnett and Harnett, 1999). Anticapsular antibodies are considered to be of particular importance (Bruyn et al., 1992; Rijkers et al., 1993). In humans, a subset of circulating CD27-positive memory B cells develops early in life and shares properties with splenic marginal zone B cells (Weller et al., 2004). Antibodies to other pneumococcal antigens are likely to contribute to defence (Bruyn et al., 1992; Hollingshead and Briles, 2001; Janulczyk et al., 2000).

6. Antibody responses to vaccination

Persons with deficiencies of the classical pathway or C3 show reduced antibody responses to thymus-dependent antigens and impaired IgM/IgG switching (Ochs et al., 1993). The effect is due to the C3-derived fragment C3d, a specific CR2 ligand that was shown to have a strong dose-dependent adjuvant effect, when injected into mice together with the antigen as a recombinant fusion protein (Dempsey et al., 1996). Responses to thymus-independent antigens such as polysaccharides in classical pathway and C3 deficiencies have been less closely investigated. However, an adjuvant effect of C3d coupled to pneumococcal capsular polysaccharide has been reported (Test et al., 2001) indicating a role of C3 activation.

Patients with LCCD mount normal antibody responses to vaccination with tetravalent meningococcal vaccine with evidence for protection against infection (Drogari-Apiranthitou et al., 2000; Platonov et al., 2003b; Sjöholm et al., 2001). Several studies also indicate that antibody responses to tetravalent meningococcal vaccine in properdin deficiency are not impaired (Sjöholm et al., 2001). Inconclusive results were obtained in two patients with C3 deficiency (Drogari-Apiranthitou et al., 2000).

In preliminary studies, we immunized a few C2-deficient adults with meningococcal, pneumococcal and Hib vaccines. Tetravalent meningococcal vaccine (Menomune®) and a conjugate Hib vaccine (Act-HIB®) were given on day 0, and polyvalent pneumococcal vaccine (Pneumovax®) after 6 weeks. Antibodies of the IgG isotype were measured by ELISA in samples before vaccination and 6 weeks after vaccination. The ELISA procedures were calibrated using international reference sera. As compared with controls, the C2-deficient vaccinated persons showed somewhat low antibody responses to *S. pneumoniae* serotype 7F and to Hib. However, C2 deficiency per se did not appear to result in substantially decreased antibody responses to polysaccharides.

7. Considerations concerning antibody-dependent defence against encapsulated bacteria

Some protective effects of specific antibodies might be mediated through complement-independent mechanisms. However, acquired immunity as studied by clearance of antibody-coated pneumococci from the blood-stream of normal and C4-deficient guinea-pigs was strictly complement-dependent and required an intact classical pathway (Brown et al., 1983). IgG antibodies slightly enhanced clearance in the C4-deficient animals indicating that the alternative pathway did not support significant antibody-mediated immunity. This experimental model predicts that vaccination of patients with classical pathway deficiencies should be expected to be of limited value. On the other hand, some data suggest that acquired immunity might be operative in these conditions, which provides a basis for new studies of immune mechanisms.

In C2 deficiency, anticapsular IgM and IgG antibodies might trigger immune adherence (Nelson, 1953) of *S. pneumoniae* to erythrocyte CR1 by recruitment of C4 (Krych-

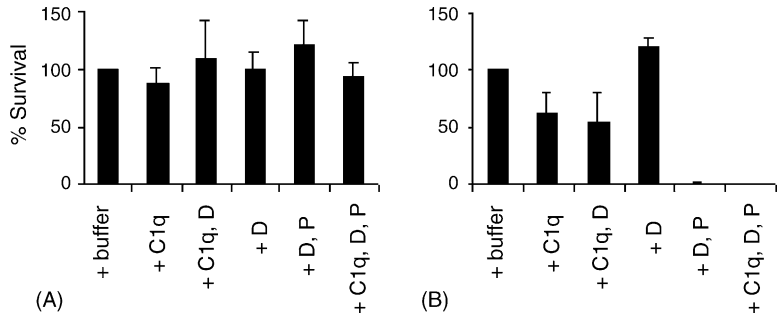

Fig. 1. Anticapsular antibody-dependent killing of *Neisseria meningitidis* serogroup W-135 in serum is mainly mediated through the alternative pathway. Healthy controls ($n = 9$) were vaccinated with tetravalent meningococcal vaccine. Prevaccination and postvaccination (4–6 weeks) pools were made of the sera. The two pools were depleted of C1q, factor D and properdin and were used in bactericidal experiments with 25% serum and 5×10^6 to 5×10^7 log phase bacteria per milliliter of the diluted serum. Incubations were carried out for 30 min at 37 °C. The sera were reconstituted with purified complement proteins at physiological concentrations. Surviving bacteria were assessed by viable counts, and results were given as percentages. The data represent the mean ± SEM from three experiments. (A) Findings in prevaccination serum, and (B) findings in postvaccination serum.

Goldberg and Atkinson, 2001). IgA and IgG_2 antibodies have been shown to activate the alternative pathway (Valim and Lachmann, 1991), and alternative pathway-mediated serum bactericidal responses against *N. meningitidis* and *H. influenzae* type b have been documented in C2 deficiency following immunization with capsular polysaccharide vaccines (Selander et al., 2000). Janoff et al. (1999) have emphasized the potential role of alternative pathway activation by anticapsular IgA antibodies in defence against *S. pneumoniae*. In addition, immune reactions in C2 deficiency could be mediated through the C1q-dependent C2 bypass mechanism (Knutzen Steuer et al., 1989), even if no evidence of this was obtained by bactericidal assay (Selander et al., 2000). It is well established that antibodies to sialic acid residues of the capsular polysaccharide support alternative pathway-mediated opsonophagocytosis of *S. agalactiae* (Wessels et al., 1995). In addition, polymeric IgA has been shown to activate the lectin pathway through MBL (Roos et al., 2001). The importance of this mechanism in defence against encapsulated bacteria remains to be examined.

An experiment was performed in which sera from nine healthy adults were pooled before and after vaccination with tetravalent meningococcal vaccine. The prevaccination and postvaccination pools were each depleted of C1q, factor D and properdin (Sjöholm et al., 1991). Bactericidal assays with *N. meningitidis* serogroup W-135 were performed after addition of purified complement proteins to the complement-depleted sera (Fig. 1). The prevaccination pool did not support bacterial killing after reconstitution of classical and alternative pathway functions. With the postvaccination pool, some bactericidal activity was generated by addition of C1q and C1q and factor D. However, the predominant bactericidal activity was clearly alternative pathway-dependent.

Removal of the IgG from the complement-depleted postvaccination pool by affinity chromatography on protein G Sepharose (Amersham-Pharmacia Biotech, Uppsala, Sweden) resulted in loss of the bactericidal activity generated by reconstitution of complement activity. IgG was eluted from the protein G Sepharose. The concentration of antibodies to

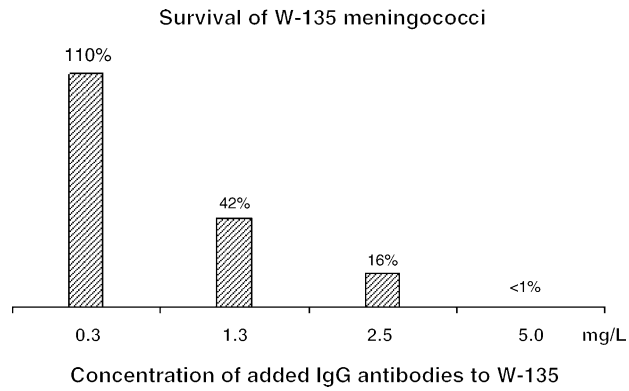

Fig. 2. Anticapsular antibodies that recruit the alternative pathway in killing of *Neisseria meningitidis* serogroup W-135 reside in the IgG fraction. The postvaccination serum pool that was depleted of C1q, factor D and properdin (see legend of Fig. 1) was further depleted of IgG by affinity chromatography on protein G Sepharose. Bactericidal experiments (see legend of Fig. 1) were carried out with reconstitution of the alternative pathway with purified factor D and properdin and with addition of eluted IgG containing anti-W-135 antibodies at specified concentrations.

W-135 polysaccharide was determined in the eluate. Addition of the IgG to the depleted serum in a bactericidal assay with reconstitution of the alternative pathway gave evidence of antibody dose-dependent killing of *N. meningitidis* serogroup W-135 (Fig. 2).

8. Non-complement genes and disease expression

In the C1q-deficient mouse model, the genetic background was found to be a strong determinant of SLE development (Mitchell et al., 2002). The general question may be raised as to what extent non-complement genes modify disease expression and account for phenotypic heterogeneity of the complement deficiencies. C2 deficiency is associated with susceptibility to severe infection, autoimmune disease and atherosclerosis. In addition, many C2-deficient persons remain healthy. An interesting point is that immune func-

tions determined by the major histocompatibility complex may be expected to be much more uniform in C2 deficiency (Jönsson et al., 2005; Pickering et al., 2000; Yu, 1998) than in the other complement deficiencies (Pickering et al., 2000; Sjöholm, 2002; Würzner, 2003).

Deficiencies of the classical pathway and C3 appear to delay maturation of IgG production as indicated by the presence of low concentrations of the IgG_2 and IgG_4 subclasses in the patients (Bird and Lachmann, 1988). Antibodies to polysaccharides mostly belong to the IgG_2 subclass, and in accord with this an increased incidence of infections with encapsulated bacteria has been reported in IgG_2 deficiency (Rijkers et al., 1993). However, investigation of C2-deficient patients revealed no correlation between IgG subclass concentrations and susceptibility to infection (Alper et al., 2003). Current studies indicate that Gm allotypes of the IgG subclasses strongly influence the susceptibility to infection in C2 deficiency (to be published). This implies that infections in C2 deficiency can be overcome through immunoglobulin-dependent mechanisms. Gm allotypes have previously been found to influence susceptibility to meningococcal disease in a family with properdin deficiency (Späth et al., 1999). There is also some evidence for a protective role of certain Gm allotypes (Ambrosino et al., 1985; de Vries et al., 1979). Another interesting example of independent genes that may influence the outcome of complement deficiencies is the polymorphism of the opsonic Fc receptors Fcγ IIa and IIIb (van Sorge et al., 2003). These polymorphisms partly determine the susceptibility to meningococcal disease in LCCD, but do not appear to be of importance in properdin deficiency (Fijen et al., 2000). Essentially no information is available concerning the significance of the lectin pathway (Turner and Hamvas, 2000), when other functional units of the complement system are impaired. MBL has been reported to be an opsonin that does not necessarily require recruitment of other complement proteins (Kuhlman et al., 1989).

9. Conclusions and perspectives

Complement deficiencies are experiments of nature and provide several challenges to our understanding with regard to clinical as well as to immunological issues. Investigation of larger groups of patients will allow more definite conclusions to be made concerning the role of complement in disease and will facilitate analysis of the interplay between different components of the immune system. Guidelines for investigation and treatment of complement-deficient patients should eventually be established. Complement deficiency is mostly overlooked in clinical practice, and efforts should be made to make simple screening methods available and appropriately used. With regard to infections, young patients (<20 years) with invasive infections caused by encapsulated bacteria should be screened for complement deficiency. Complement analysis has a moderately well established diagnostic role within the framework of some rheumatological and nephrological diseases. The potential association between lectin pathway function and cardiovascular disease is interesting, and it could be that complement analysis will be shown to be helpful in some investigations within this field.

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