Where next with atypical hemolytic uremic syndrome?

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On behalf of The European Working Party on the Genetics of HUS

Abstract

Hemolytic uremic syndrome (HUS) is a systemic disease characterized by damage to endothelial cells, erythrocytes and kidney glomeruli. A “typical” form of HUS follows gastrointestinal infection with enterohemorrhagic E. coli (e.g. O157:H7). Atypical HUS (aHUS) is not associated with gastrointestinal infections but is sporadic or familial in nature. Approximately 50% of aHUS cases are associated with a mutation in one or more genes coding for proteins involved in regulation or activation of the alternative pathway of complement. The link between the disease and the mutations shows the important balance of the alternative pathway between activation and regulation on host cell surfaces. It also demonstrates the power of this pathway in destroying cellular targets in general. In this review we discuss the current knowledge on pathogenesis, classification, diagnostics and management of this disease. We indicate a comprehensive diagnostic approach for aHUS based on the latest knowledge on complement dysregulation to gain both immediate and future patient benefit by assisting in choosing more appropriate therapy for each patient. We also indicate directions in which therapy of aHUS might improve and indicate the need to re-think the terminology and categorization of the HUS-like diseases so that any advantage in the understanding of complement regulatory problems can be applied to patients accurately.

Keywords: Complement; Hemolytic uremic syndrome; Innate immunity; Human disease; Thrombotic microangiopathy; TMA

1. Introduction

Hemolytic uremic syndrome (HUS) is a systemic disease characterized by damage to endothelial cells and erythrocytes, thrombocytopenia, microthrombosis and kidney failure. In many cases the alternative pathway of complement is activated (Ruggenenti et al., 2001). The majority of human HUS cases are associated with a gastrointestinal infection with enterohemorrhagic E. coli such as the serotype O157:H7 and sometimes these cases are seen as epidemics (so called “typical” HUS). In approximately 10% of the HUS cases there is no evidence of an E. coli infection. These cases are found sporadically or in a familial pattern, follow a more aggressive course than the typical HUS cases, and respond poorly to medical treatment. Based on these differences this group has been named atypical HUS (aHUS, OMIM 235400) or diarrhoea-negative HUS (D-HUS). HUS is part of the disease cluster called thrombotic microangiopathies (TMA) and classification of these diseases is discussed later in this review.

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During the last few years it has become evident that aHUS is strongly associated with mutations in proteins needed either for activation or regulation of the alternative pathway of complement. The complement system is part of innate immunity that consists of a set of plasma and membrane-bound proteins that protect the body against invading organisms. In addition, the complement system is involved in removal of debris from plasma and tissues, and enhancement of cell-mediated immune responses. Complement is activated through three cascade-like activation pathways. Activation of the classical and lectin pathways requires binding of antibody or a pattern recognition molecule to the target. By contrast, the alternative pathway (AP) is initiated spontaneously in plasma at a low level and if left uncontrolled leads to attack against all the particles, membranes, and cells which are plasma-exposed but not specifically protected from it (Fearon and Austen, 1977). The published aHUS associated mutations of complement genes represent proteins involved in activation or regulation of the alternative pathway, namely complement factor H (CFH), factor I (CFI), membrane cofactor protein (MCP, CD46), C3 and factor B (CFB). Therefore, we first introduce the alternative pathway activation and regulation followed by a description of the mutations so far described in these molecules.

The key molecule in AP activation is C3. It is spontaneously hydrolyzed in plasma at a low rate leading to covalent deposition of a low amount of C3b molecules onto practically all surfaces in contact with plasma (Pangburn et al., 1981). CFB binds to C3b and this complex acts as a substrate for a protease factor D (CFD) that releases Ba fragments and generates an active C3-convertase of AP, C3bBb. This complex has a relatively short half-life but is stabilized by plasma protein properdin (CFP). In the presence or absence of CFP, the C3bBb complex cleaves more C3 to C3b and this leads to the so-called amplification cascade and efficient deposition of C3b onto the target surface (Fig. 1).

The aim of AP activation is to label and attack harmful invaders or debris. That is why, in the absence of regulators, activation proceeds via an amplification cascade and leads to effective opsonization of the foreign structures with C3b together with simultaneous formation of complement membrane attack complexes leading to cell lysis. It is essential that activity is down-regulated on self cells and other tissue surfaces while efficient activation is permitted on foreign targets. Activation also needs to be controlled in the fluid-phase of plasma since activation on any surface also leads to generation of fluid-phase C3b (Fig. 1).

Both plasma proteins and membrane-bound proteins are involved in regulation of the alternative pathway. CFH and CFI are the key plasma regulators of the alternative pathway of complement. If one of them is missing, or totally dysfunctional, AP activation in plasma is vigorous and leads to secondary deficiency of active complement via over-consumption of C3 and some other complement components. CFI is able to permanently inactivate C3b by proteolysis but needs a cofactor. CFH is such a cofactor and is composed of 20 short consensus repeat domains (SCRs or complement control protein units, CCPs) each consisting of approximately 60 amino acids. CFH regulates AP activation by competing with CFB in binding to C3b, by acting as a cofactor for CFI leading to proteolytic inactivation of C3b, and by enhancing dissociation of the C3bBb complex (Farries et al., 1990; Weiler et al., 1976; Whaley and Ruddy, 1976). In addition to its regulatory activity in plasma CFH is practically the only regulator that is involved in down-regulating AP activation on host structures that lack other membrane-bound regulators such as basement membranes in kidney glomeruli (Meri et al., 1992; Zipfel et al., 2006); CFH also contributes to protection of cellular surfaces as discussed later.

The membrane-bound regulators of the alternative pathway include MCP, decay-accelerating factor (DAF, CD55), and complement receptor 1 (CR1, CD35). In the same way as CFH, MCP acts as a cofactor for CFI, but it only protects those cells on which it is expressed. DAF enhances dissociation of the C3bBb complex similarly to CFH but again, only on expressing cells. CR1 has both cofactor and decay-accelerating activities. Functional sites of all of these molecules are composed of SCR domains.

The first link between complement and aHUS came from identification of mutations in the CFH encoding gene CFH. The mutations cause either truncation of CFH or amino acid substitu-
tions mainly within domains 19–20 of CFH (Caprioli et al., 2001; Dragon-Durey et al., 2004; Manuelian et al., 2003; Neumann et al., 2003; Perez-Caballero et al., 2001; Sanchez-Corral et al., 2002; Warwicker et al., 1998). Since aHUS is characterized by endothelial cell damage, microthrombosis, kidney failure, and alternative pathway activation it appeared that protection of endothelial cells and/or kidney glomeruli from complement attack was impaired by malfunction of the carboxy-terminus of CFH. The next complement gene in which mutations were detected in aHUS patients was the MCP gene (Fremeaux-Bacchi et al., 2006, 2007b; Noris et al., 2003; Richards et al., 2003). Lately mutations in genes coding for CFI (Fremeaux-Bacchi et al., 2004; Kavanagh et al., 2005), CFB (Goicoechea de Jorge et al., 2007), and C3 (Fremeaux-Bacchi et al., 2007a) have also been reported and recently deficiency of CFH-related molecules 1 and 3 (CFHR1 and CFHR3) has been found in aHUS (Zipfel et al., 2007). A comprehensive and continuously updated list of published (and unpublished) mutations of CFH, CFI and MCP is found in the aHUS mutation database (www.fh-hus.org) (Saunders et al., 2007).

In this review we concentrate on the current knowledge of complement-associated aHUS, compare this disease with the other types of aHUS on classification level and highlight some new aspects and areas of research into the role of complement in pathogenesis, diagnostics and management of aHUS.

2. Molecular pathogenesis of complement-related aHUS

The functional consequences of genetic abnormality in complement genes have been studied for a number of mutations by in vitro mutagenesis. The defects can be classified into two types. In Type I, the mutations lead to quantitative deficiency indicating that the mutant protein is either absent from the plasma or is present in lower amounts than normal. In Type II, the mutations lead to functional changes. The mutant protein is present in normal amounts in plasma but one or more of its functions are affected (Saunders et al., 2007). Type II mutations in the negative regulators of the alternative pathway activation (CFH, CFI, MCP) are lack-of-function mutations while mutations in the activation components (C3, CFB) are gain-of-function mutations.

Inheritance of aHUS (especially those involving Type II mutations) follows usually a dominant pattern with variable penetrance. Therefore it is possible that in addition to the first identified mutation additional mutations or polymorphisms and/or an environmental factor are necessary for clinical disease to develop. This so-called multiple hit theory is supported by aHUS cases where several mutations or polymorphisms have been found (Esparza-Gordillo et al., 2005, 2006; Fremeaux-Bacchi et al., 2005).

2.1. Lesson from CFH mutations in aHUS

At present, over 80 distinct aHUS cases with CFH mutations have been reported (Zipfel, 2006). Nearly all of these mutations are heterozygous and so far only a few homozygous mutations or cases of total CFH deficiency have been reported in aHUS (Cho et al., 2007; Landau et al., 2001; Ohali et al., 1998; Rougier et al., 1998; Thompson and Winterborn, 1981). As with aHUS mutations in general, CFH mutations are also divided into two groups. The first type of mutation affects the CFH plasma level and the second type of mutation impairs function of the protein without any effect on CFH plasma level. In the first type, protein secretion is blocked and the product of the mutant allele is retained in the cytoplasm. This has been demonstrated particularly for mutations of the framework cysteine residues but also for other type of mutations (Ault et al., 1997; Hegasy et al., 2002; Vaziri-Sani et al., 2006). In aHUS several mutations have been reported which introduce a premature stop codon. So far truncated forms of CFH with a premature stop in the middle region (i.e. SCRs 6–17) have not been detected in plasma of patients. Thus these truncated proteins are either retained in the cytoplasm or, if expressed, are highly unstable. Due to the heterozygous situation, one allele is affected. The normal allele is expressed and its product is present in plasma. Consequently in these cases plasma levels of CFH are reduced, theoretically by 50%.

In contrast to the more common heterozygous CFH mutations homozygous CFH mutations can cause aHUS but are observed predominantly in membranoproliferative glomerulonephritis type II (MPGN II; also termed mesangiocapillary glomerulonephritis or intramembranous dense deposit disease). These mutations cause either a block in protein secretion resulting in the complete absence of CFH in plasma, or result in a functionally defective protein (Appel et al., 2005; Licht et al., 2005). In addition, one case has been described where autoantibodies block the regulatory function of CFH (Jokiranta et al., 1999; Meri et al., 1992). As a consequence of these scenarios, uncontrolled C3 activation in plasma results in severely reduced C3 levels which is unusual in aHUS. In total CFH deficiency it is believed that the alternative pathway activation is so vigorous that insufficient alternative pathway activity or active C3 remains to trigger aHUS with generalized microvascular damage on top of the local kidney damage typical for MPGN II.

The second type of CFH mutation represents single amino acid exchanges, and most of these (~75%) are clustered in the carboxyl-terminal region of CFH (domains 19–20). This results in a mutant CFH protein that is expressed and detected in plasma. Based on the location of the mutation, such carboxyl-terminal CFH mutants retain regulatory activity and have full function in inactivation of C3b and C3bBb in the fluid phase but lack proper activity on cell-bound C3b or C3bBb (Heinen et al., 2007; Pangburn, 2002). Most of these point mutations have been reported to impair both the carboxyl-terminal functions of CFH, i.e. binding to both heparin or cell surface glycosaminoglycans and to the C3d part of C3b (Herbert et al., 2006; Jokiranta et al., 2006; Jozsi et al., 2006; Manuelian et al., 2003; Sanchez-Corral et al., 2002). All of these mutations seem to impair regulatory activity of CFH on cell surfaces, especially endothelial cell surfaces (Ferreira et al., 2006; Heinen et al., 2007; Jokiranta et al., 2005; Manuelian et al., 2003; Sanchez-Corral et al., 2004). Therefore it is clear that CFH has a physiological action both in the fluid phase and at the cell surface, particularly at the surface of endothelial cells.
2.2. *Incomplete penetrance of aHUS*

There are two unresolved questions in the pathogenesis of aHUS associated with the lack-of-function mutations of CFH, CFI and MCP: (1) why is penetrance low, and (2) what trigger is needed for disease expression? Incomplete penetrance is demonstrated by the fact that not every individual with the mutation develops the disease. Within family members carrying the same mutation, the disease may occur in infancy or not until adulthood. Therefore, it seems that under certain conditions patients with pathological mutations remain unaffected. An explanation for this situation is that under favourable conditions the reduced activity of CFH, CFI or MCP is still sufficient to control complement activation in plasma and on the cell surface. As other membrane-bound complement regulators are expressed at the cell surface there is redundant capacity in the control of the alternative pathway and control is maintained even when activity of one regulator is reduced. This could explain the incomplete penetrance of the disease and the reasons why aHUS caused by mutations in complement regulators occurs sometimes late in life.

2.3. A trigger for aHUS?

Since aHUS can occur in adulthood although the genetic predisposing factors are present from infancy, it would be logical to suppose that there is one or more trigger for aHUS. So far no general triggering event has been identified, although variable shigatoxin or verocytotoxin independent infections have been reported from some aHUS patients prior to the onset of the disease (Berner et al., 2002; Cho et al., 2007; Olie et al., 2005). Pregnancy or drugs have also been suggested to trigger the clinical disease in the presence of mutations in complement proteins. A general concept is that under conditions of excessive complement activation in plasma, endogenous cells require more protection than under normal conditions. In such situations, the concerted action of all the regulators, plasma regulators attracted to the cell surface together with resident membrane-bound regulators, is required for efficient protection. Therefore, aHUS could follow increased complement activation if any of the key regulators of the alternative pathway, CFH, CFI or MCP, has subnormal activity. Such a scenario is in agreement with the reported aHUS cases with gain-of-function mutations in genes coding for either of the components of the alternative pathway convertase C3bBb, i.e. C3 and CFB (Fremeaux-Bacchi et al., 2007a; Goicoechea de Jorge et al., 2007).

If AP C3-convertase activity is enhanced by the mutations of C3 or CFB, either by stabilization of the enzyme or restricted control by the regulators, the effect is similar to the situation when one or more regulators are defective. Therefore the presence of a loss-of-function mutation in one of the regulators (CFH, CFI, MCP) or gain-of-function mutation activation components (C3, CFB) of the alternative pathway can make the homeostatic balance more labile. This could lead to the pathological aHUS process following any event that leads to enhanced alternative pathway activation. Therefore it is possible that no single trigger for aHUS will be found but that any stimulus that initiates local activation of the complement cascade, for example a systemic infection, can be considered a potential trigger for HUS. The lesson learned from this complex scenario is that under condition of enhanced complement challenge complement activation at the cell surface requires tight control (Fig. 2).

3. Should aHUS be divided into distinct subtypes?

The pathological finding of thrombotic microangiopathy (TMA) appears to be common not only to complement associated aHUS and the typical HUS associated with enterohemorrhagic *E. coli* but to several other clinical disorders. As our understanding of the molecular mechanisms involved in the pathogenesis of HUS increases should we be modifying the diagnostic classification for this disease? Currently hemolytic uremic syndrome is included as a single entity in the World Health Organization International Classification of Diseases (ICD-10 (http://www.who.int/classifications/apps/icd/icd10online/). The code for HUS (D59.3) comes under the sub-heading of acquired hemolytic anemia (D59) within the section on hemolytic anemias in chapter 3 (“Diseases of the blood and blood-forming organs and certain disorders involving the immune mechanism”). It is cross-referenced within chapter 14 (“Diseases of the genitourinary system”) in the section on glomerular disease (N00-N08) under the sub-heading of “Glomerular disorders in diseases classified elsewhere” (N08) within N08.2 (“Glomerular disorders in blood diseases and disorders involving the immune mechanism”).

3.1. History of TMA classification

The term hemolytic uremic syndrome was first used by Conrad von Gasser in 1955 in an article titled “Hamolytisch-uramische Syndrome: Bilateral Nierindennekrosen bei akuten erworbenen hamolytischen anamien” (Gasser et al., 1955). Gasser was a hematologist with a particular interest in hemolytic anemia. He reported five children who in addition to hemolysis presented with acute renal failure and thrombocytopenia (Gasser et al., 1955). One of these had a dysentery-like illness and two had pneumonia prior to HUS. Following this it was recognised that for a small number of patients the disease was familial (Kaplan et al., 1975) or recurrent (Kaplan, 1977). Later it was shown that in children, HUS that was associated with a prodrome of diarrhoea had a different clinical course from those without diarrhoea (Dolislager and Tune, 1978). The demonstration of a role for verotoxin (shiga-like toxin)-producing *E. coli* (Karmali et al., 1983) confirmed the differentiation between diarrhoea-associated (D+) and non-diarrhoal (D−) forms (Barratt et al., 1987). The D−form subsequently also became known as atypical HUS (aHUS).

3.2. Recently introduced TMA classification

A recent publication from the European Paediatric Research Group for HUS has suggested that a new classification based on etiology be adopted (Besbas et al., 2006). Using this clas-
sification it may be more appropriate to consider disease phenotypes such as HUS and thrombotic thrombocytopenic purpura (TTP) under the broad heading of TMA. Circumstances where the etiology is well understood include induction by an infection, disorders of complement regulation, ADAMTS13 abnormalities, defective cobalamine metabolism and quinine induced TMA. The infection-induced form includes disease associated with shigatoxin or verocytotoxin producing bacteria (classically associated with D+ HUS) and Streptococcus pneumoniae. Neuraminidase produced by S. pneumoniae cleaves sialic acid residues from cell surface glycoproteins exposing the Thomsen-Freidenreich antigen (T-antigen). Binding of naturally occurring IgM antibodies to the exposed T-antigen on platelets and endothelial cells has been proposed to induce thrombotic microangiopathy (Klein et al., 1977).

TMA-disorders associated with complement regulation include mutations and copy number variation in the proteins CFH, MCP, CFI, C3 and CFB (and putative complement regulators CFHR1 and CFHR3) as described above. Similarly HUS cases with anti-CFH autoantibodies belong to this category. ADAMTS13 abnormalities (associated with a TTP phenotype) include autoantibodies against ADAMTS13 and mutations leading to deficiency. Disorders of defective cobalamine metabolism include Cobalamin C disease (cblC) (Geraghty et al., 1992) and the gene responsible for cblC (MMACHC) has recently been identified (Lerner-Ellis et al., 2006). This is characterized by methylmalonic aciduria and homocystinuria, and is the most common inborn error of cobalamin metabolism. It has also been associated with a CFH mutation (Guigonis et al., 2005). Quinine induced TMA is associated with antibodies against glycoproteins on platelets. Diseases associated with TMA where the etiology is not so well defined include HIV, malignancy, drugs (including cytotoxics, immunosuppressants, oral contraceptives and antiplatelet agents) (Dlott et al., 2004), pregnancy, systemic lupus erythematosus and antiphospholipid antibody syndrome. Table 1 shows this new classification and those diseases which might currently be considered under the heading of aHUS are highlighted.

3.3. Future of aHUS classification

Although the TMA-disorders associated with genetic defect in complement regulation are considered as a single entry (1.ii.a) in Table 1, it is worth noting that both the therapeutic approach and prognosis of MCP-mutation associated disease is different from the disease where a complement plasma protein such as
CFH and CFI is defective. Therefore in future it will probably be considered to divide the genetic complement defects into two categories: defects in membrane-bound complement regulators and defects in complement plasma proteins. Before this kind of division will be made it is necessary to perform further studies on the clinical course of patients with the recently found CFB and C3 mutations.

It is possible that in future some of the separate entities shown in Table 1 will merge into each other. For example the pregnancy- or malignancy-associated aHUS might actually be connected to complement dysfunction, although for the time being this is speculative.

4. Diagnostic measures for aHUS

4.1. Current diagnostic approaches in aHUS

In the past 10 years we have learned that the introduction of molecular tests into clinical practice provides a tool for diagnosis of susceptibility factors in aHUS. In practice in 2007 we propose a comprehensive screening test based on protein expression levels (either serum levels or surface expression), CFH autoantibody detection and genetic testing of at least three of the known susceptibility genes (CFH, CFI and MCP) as shown in Table 2.

Patients suspected of having aHUS should be initially screened by measuring complement C3, C4, CFB, CFH, CFHR1, and CFI antigenic levels. Serum C3 and C4 levels will generally form part of a basic complement screen. For the assessment of complement components CFH, CFI, CFB and MCP a variety of different complement assays are being undertaken but they remain within the specialized diagnostic laboratories. CFH, CFI and CFB levels are measured by immunochemical methods (radial immunodiffusion, ELISA or nephelometry) or by functional analysis. In the absence of international standards, the normal ranges and units vary from laboratory to laboratory. Anti-CFH autoantibody screening is currently performed by ELISA assays. From the reports available to date, plasma measurement of CFH or CFI will detect less than 50% of patients with mutations in CFH or CFI, respectively. Severe complement consumption through the alternative pathway, as indicated by very low plasma levels of C3 and CFB or isolated low C3 level, is frequently seen in patients with mutations in CFH or CFI, particularly if the mutations are homozygous and cause total or subtotal deficiency of the protein. The C3 level may be also within the normal range and this is more often seen with heterozygous mutations.

Membrane expression of MCP is usually analyzed using granulocytes or peripheral blood mononuclear cells (PBMC) in a flow cytometry analysis (FACS). We recommend the mea-

<table>
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<th>Table 1</th>
<th>Classification of thrombotic microangiopathies adapted from (Besbas et al., 2006) with permission</th>
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<tr>
<td>Advanced understanding of etiology</td>
<td>Infection induced</td>
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<tr>
<td>1.i</td>
<td>(a) Shiga and verocytotoxin (shiga-like toxin)-producing bacteria</td>
</tr>
<tr>
<td>1.i</td>
<td>(b) Streptococcus pneumoniae</td>
</tr>
<tr>
<td>1.ii</td>
<td>Disorders of complement regulation,</td>
</tr>
<tr>
<td>1.ii</td>
<td>(a) Genetic disorders of complement regulation</td>
</tr>
<tr>
<td>1.ii</td>
<td>(b) Acquired disorders of complement regulation, for example anti-FH antibody</td>
</tr>
<tr>
<td>1.iii</td>
<td>ADAMTS13 abnormalities</td>
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<tr>
<td>1.iii</td>
<td>(a) ADAMTS13 deficiency secondary to mutations</td>
</tr>
<tr>
<td>1.iii</td>
<td>(b) Autoantibodies against ADAMTS13</td>
</tr>
<tr>
<td>1.iv</td>
<td>Defective cobalamine metabolism</td>
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<tr>
<td>1.v</td>
<td>Quinine induced</td>
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| Etiology not fully understood | HIV |
| 2.i | Malignancy |
| 2.ii | Drugs |
| 2.iii | Pregnancy |
| 2.iv | Systemic lupus erythematosus and antiphospholipid antibody syndrome |

Those conditions which might be included under the heading of aHUS are underlined.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Suggestion for a comprehensive diagnostic approach to analyse involvement of complement in aHUS cases</th>
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<tr>
<td>Analyses of protein expression levels from plasma</td>
<td>CFH</td>
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<tr>
<td>Analyses of protein expression level from PBMC</td>
<td>MCP (CD46)</td>
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<td>Analysis of autoantibodies</td>
<td>Anti-CFH</td>
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<tr>
<td>Genetic analyses of susceptibility genes</td>
<td>CFH</td>
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<td>Analyses of protein expression levels from plasma</td>
<td>CFI</td>
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<td>Analyses of protein expression level from PBMC</td>
<td>MCP (CBF)</td>
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<td>Analysis of autoantibodies</td>
<td>(C3)</td>
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<td>Genetic analyses of susceptibility genes</td>
<td>(CFHR1)</td>
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<td>Analyses of protein expression levels from plasma</td>
<td>(CFHR3)</td>
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measurement of MCP expression with anti-MCP phycoerythrin (PE)-conjugated antibodies because it has been our experience that these are more likely to discriminate normal from abnormal expression of MCP compared to anti-MCP fluorescein isothiocyanate (FITC)-conjugated antibodies (Fremeaux-Bacchi et al., 2006). From currently available data, screening in this manner will detect around 80% of MCP deficient patients. No MCP expression is detected by FACS in patients with homozygous MCP deficiency (approximately 20% of cases). The mean fluorescence intensity in patients with heterozygous deficiency should be around 50% of the normal median. In interpretation of the flow cytometry results it needs to be considered that MCP expression can decrease transiently in the absence of any mutations. This effect on PBMCs has been detected after a typical HUS episode with normalization within a few days after the onset (Fremeaux-Bacchi et al., unpublished observations).

Functional CFH complement regulatory defects can be detected in the patients’ sera with a simple hemolytic assay (Sanchez-Corral et al., 2004). Functional assays of specific AP proteins from patient plasma are generally not very sensitive and may not provide accurate information. Therefore the assessment of complement protein level or function in plasma is insufficient and genetic analyses are necessary. As various mutations and small deletions represent more than 95% of genetic abnormalities, direct sequencing analysis is now becoming the method of choice for mutation screening. Primers for mutation screening for CFH, CFI and MCP have already been reported by several groups experienced in this field (Caprioli et al., 2006; Dragon-Durey et al., 2004; Fremeaux-Bacchi et al., 2004, 2006).

Based on the identification of mutations in several parts of the genes it may be prudent to screen all exons of the genes including the CFH gene. As recently proposed, multiplex ligation dependent probe amplification (MLPA) can detect large genetic rearrangement (Venables et al., 2006). Since 10% of patients have mutations in two complement regulators, screening should be made to interrogate all the known aHUS predisposing genes. The functional consequences of each genetic abnormality could be determined in vitro by mutagenesis but effects of most of the mutations can be assumed based on the experimentally verified structures of the most frequently mutated domains of CFH (Herbert et al., 2006; Jokiranta et al., 2006) or MCP (Casasnovas et al., 1999), or the full C3b (Janssen et al., 2005) or CFB (Milder et al., 2007) structures.

Currently, genetic testing is indicated for all subjects waiting for renal replacement due to aHUS. In addition we suggest that all aHUS patients should be tested for involvement of the complement system in their disease, preferably with a comprehensive analysis as shown in Table 2. Involvement of complement should be studied even if another risk factor has been found, as compound risk factors are reported.

Despite an improvement in prognosis following the introduction of plasma therapy, the outcome following diagnosis of aHUS remains poor, with 25% mortality in the acute phase, and an evolution to end stage renal disease (ESRD) in an additional 50% of patients. Early diagnosis and treatment are crucial in the management of the first episode of aHUS in order to modify the outcome. For clinicians, the challenge is now to identify the susceptibility factor in order to propose specific and aggressive therapy.

4.2. aHUS diagnostics in the future

Based on the complexity of single genes and various multiple genetic traits associated with aHUS, there is a need to establish rapid and inexpensive methods of mutation screening and genotyping. A microarray-based resequencing technology may become a highly competitive tool, which would open the prospect of evaluating all known susceptibility genes on patient’s DNA or RNA as a routine clinical diagnostic procedure. Children with a parent who has aHUS have an unknown risk of inheriting the disease since penetrance is incomplete and several genetic factors may be involved (according to the multiple hit theory). The same uncertainty for the exact risk of developing clinical aHUS exists in the case of the other relatives. Thus the systematic screening for the mutations in healthy relatives is debatable. According to the overlay of clinical presentation of the sub-groups of patients with TMA as described above, it is most important to establish the pathogenic mechanism that leads to TMA in the patients and to eliminate other forms of TMA, such as ADAMTS-13 deficiency.

4.3. Further approaches for the patients who do not have any known mutations

Despite the recent advances in the comprehension of the etiology of aHUS, the underlying genetic or functional alterations remain unknown in approximately half of the patients. In the group of “unexplained HUS”, recovery of renal function is uncommon and many patients require long-term renal replacement therapy and recurrence of the disease in renal allografts is seen in approximately 50% of patients. Recently, genetic variants of CFB and C3 have been identified as aHUS risk factors but the frequency is currently unknown. Testing for the predisposing mutations in these genes will be necessary in the near future. Also deficiency of CFHR1 and CFHR3 is associated with aHUS and detecting plasma level of CFHR1 and possibly CFHR3 seems to be indicated from aHUS patients in future (Zipfel et al., 2007). Based on the current understanding of the pathophysiology of aHUS, several candidate genes can be defined based on their role as regulators of the complement amplification convertases. These candidate genes include properdin, which stabilizes the amplification convertase, and CR1, which has overlapping functions with the already identified regulators CFH and MCP.

Microvascular endothelial cell activation is an important feature in aHUS. Genetic analyses on aHUS have focused on complement genes and we could not exclude the identification of other groups of proteins of the endothelial cells which could be implicated in the microcirculatory diseases. For example, polyanionic molecules such as heparin are involved in acquisition of CFH from plasma onto the endothelial cells and therefore it is possible that in aHUS some defects will be found in synthesis or expression of these molecules on cell surfaces.
5. Therapeutic approaches

5.1. Plasma treatment

Some, although not all, patients with aHUS respond to plasma treatment (Caprioli et al., 2006; Lara et al., 1999; Licht et al., 2005). It has been proposed that plasma exchange might be relatively more effective than plasma infusion since it might remove potentially toxic substances from the patient’s circulation and exchange was found to have superior efficacy to plasma infusion in one study (Ruggenenti et al., 2001). Moreover there are risks attached to infusion of plasma in patients who are already hypertensive and whose vascular volume is already expanded because of renal impairment. In patients with acquired CFH deficiency, plasma exchange may offer the advantage of rapidly removing the anti-CFH antibodies. In one report, response to treatment of monozygotic twins suggested that long-term plasma exchange may have benefits over plasma infusion alone (Davin et al., 2006).

Usually between 40 and 100 mL/kg is exchanged per session. Treatment can be intensified by increasing the volume of plasma replaced. If plasma exchange is not available plasma infusion, 30–40 mL/kg on day 1 followed by 10–20 mL/kg per day can be tried. Platelet count and serum lactate dehydrogenase (LDH) are the most sensitive markers for monitoring the response to plasma therapy and treatment should be continued until they are persistently normalized. However, no clinical parameter predicts the needed duration for plasma therapy. Prompt exacerbation of disease activity, principally manifested by a falling platelet count and requiring the resumption of daily plasma therapy, is common after treatment discontinuation with reported frequencies of 29–82%.

Plasma infusion or exchange has been used in patients with aHUS and CFH mutations, with the rationale to provide the patients with normal CFH to correct the genetic deficiency. In a large series (Caprioli et al., 2006), around 50% of patients with CFH mutations treated with plasma underwent either complete or partial remission (hematological normalization with renal sequelae). Half of patients, however, did not respond at all to plasma treatment and 20% died during the acute episode. The rationale for using plasma in patients with MCP mutations is not so clear, since MCP is a transmembrane protein and theoretically plasma infusion or exchange would not correct the MCP defect. Published data (Caprioli et al., 2006; Richards et al., 2003) indicate that the majority (70–80%) of patients with MCP mutations undergo remission following plasma infusion or exchange, however complete recovery from the acute episode was also observed in 70–80% of patients not treated with plasma.

5.2. Transplantation

Fifty percent (in sporadic forms) to 60% (in familial forms) of patients with aHUS (Caprioli et al., 2003, 2006; Ruggenenti et al., 2001) progress to end-stage renal disease (ESRD). Renal transplantation is not necessarily an option for aHUS, at variance with typical HUS. Around 50% of transplanted aHUS patients had a recurrence of the disease in the grafted organ (Artz et al., 2003). Live-related renal transplant should also be avoided since it carries an additional risk to precipitate the disease onset in the healthy donor relative, as recently reported in two families (Donne et al., 2002). New knowledge from genetic studies predicts more accurately the risk of recurrence. In patients with CFH mutations the graft outcome is poor, the recurrence rate ranges from 30 to 100% in different surveys, and is significantly higher than in patients without CFH mutations. Similarly, most patients with mutations in other plasma proteins CFI and CFB have poor kidney transplant outcome similar to those with a CFH mutation. On the other hand, kidney graft outcome is favourable in patients with MCP mutations as found in four patients who have been successfully transplanted with no disease recurrence.

Two CFH deficient patients were treated by combined liver and kidney transplantation in order to substitute defective CFH. However, these patients developed either severe responses (Remuzzi et al., 2002) or died within a short period following treatment (Remuzzi et al., 2005). Also an auxiliary partial orthotopic liver transplantation with poor outcome has been reported in aHUS with a CFH mutation (Cheong et al., 2004). Thereafter a report has been published from a successfully treated patient who received extensive plasma therapy before combined kidney and liver transplantation in order to substitute the defected or absent plasma CFH (Saland et al., 2006). In addition, a report from additional two successful cases is under preparation (Jalanko et al., manuscript in preparation). So far all the three patients who received this plasma treatment prior to the combined transplant responded well and survived with well functioning grafts.

5.3. Other therapeutic approaches

Splenectomy has been found to induce remission in some plasma-resistant cases, but was ineffective and actually increased morbidity and mortality in others. Currently the subset of patients who would benefit from splenectomy is not clear and therefore a general recommendation about splenectomy cannot be given.

In a few patients with extensive microvascular thrombosis at renal biopsy accompanied by refractory hypertension and signs of hypertensive encephalopathy, bilateral nephrectomy has been performed when conventional therapies were not enough to control the disease. This has led to remission in some patients with excellent follow-up (Remuzzi et al., 1996). Other treatments, including anti-platelet agents, prostacyclin, heparin or fibrinolytic agents, steroids and intravenous immunoglobulins have been attempted, with no consistent benefit (Ruggenenti et al., 2001).

Those patients who develop HUS upon challenge with cyclosporin or tacrolimus have to stop the medication. Sirolimus has been used as an alternative in occasional patients (Franco et al., 2003).

5.4. Therapeutic approaches in future?

Research efforts are aimed at identifying more specific approaches that may interfere with the primary cause of microan-
giopathy in the different forms of aHUS. There are two main targets in these efforts, either to replace the missing or mutated protein or to inhibit alternative pathway activation with a non-physiological molecule.

Replacement of the missing or mutated plasma component can be achieved either by liver transplantation or infusion of recombinant or plasma purified protein. For example, for aHUS associated with CFH mutations, specific replacement therapies with recombinant CFH or plasma fractions enriched in CFH could provide the patient with enough active molecules while minimizing the risk of allergy and fluid overload. The problem with CFH replacement therapy is that the concentration of CFH in plasma is high and the half-life of the protein is relatively short (approximately 8 days) (Licht et al., 2005). A second approach for replacement therapy is utilization of gene therapy. Advances in vector safety and transfection efficiency will hopefully soon render gene therapy a realistic option for these patients. In the case of CFH mutations the problem again is the high plasma concentration needed.

Inhibition of the alternative pathway with nonphysiological therapeutics is an interesting future option. The discovery of mutations in three different complement regulatory genes provides enough evidence to undertake clinical trials using complement inhibitors that block the activation of C3 (Kirschfink, 2001).

6. New scenarios and conclusions

Mutations in most, but not all, of the alternative complement pathway proteins have so far been searched for in aHUS cases. One protein that has not been reported to be mutated in aHUS but, at least theoretically, could cause similar effects to the reported CFH, CFI, C3, or CFB proteins, is properdin. In searching for new aHUS associated mutations – as well as in interpretation of the previously published mutations – it must be considered that screening of mutations or polymorphisms in several proteins can lead to finding of mutations that are not functionally relevant to the disease pathogenesis but are accidentally found in one or a few aHUS patients. A few such cases have been thoroughly investigated and published from CD55 (DAF) and CFI (Kavanagh et al., 2007; Nilsson et al., 2007).

Very recently deficiency of CFH-related proteins 1 (CFHR1) and 3 has been associated with aHUS (Zipfel et al., 2007). In addition, at least some correlation between a CFHR5 polymorphism and aHUS has been found (Monteferrante et al., 2007). Association of mutations, polymorphisms, or deficiencies of the CFH-related proteins 1–5 with aHUS is a very interesting result based on the previously published mutations – it must be considered that screening of mutations or polymorphisms in several proteins can lead to finding of mutations that are not functionally relevant to the disease pathogenesis but are accidentally found in one or a few aHUS patients. A few such cases have been thoroughly investigated and published from CD55 (DAF) and CFI (Kavanagh et al., 2007; Nilsson et al., 2007).

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One more poorly explored topic is the possible role of autoantibodies in pathogenesis of those aHUS cases where mutations cannot be found. Anti-FH antibodies have already been reported in aHUS (Dragon-Durey et al., 2005) but no search for other anti-complement antibodies from aHUS patients has yet been published.

In conclusion, aHUS associated with complement defects has turned out to be caused by heterogenous loss-of-function or gain-of-function mutations or polymorphisms in complement regulators and components. Identification of the mutation in each patient has practical consequences, at least in considering suitability to renal transplantation, but might also help in rational problem-solving regarding other management problems. Further studies on pathogenesis of complement-associated aHUS in general are important for identifying novel therapeutic approaches in this disease.

Acknowledgements

Work of TSJ has been financially supported by the Academy of Finland (projects #201506 and #202529), the Helsinki University Central Hospital Funds, The Finnish Cultural foundation and the Sigrid Jusélius Foundation. Work of VF-B is supported by the Délégation Régionale à la Recherche Clinique, Assistance Publique—Hôpitaux de Paris (PHRC AOM 05130). THG is supported by the Foundation for children with atypical HUS. Work form PFZ is funded by the Deutsche Forschungsgemeinschaft and Kidneeds, Iowa City, USA.

References


