

Autoantibodies in haemolytic uraemic syndrome (HUS)

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Summary

Haemolytic uraemic syndrome (HUS) is a severe disease with renal failure, microangiopathic anemia and thrombocytopenia. Several mechanisms leading to HUS have been identified, like infections with enterohaemorrhagic *Escherichia coli*, as well as genetic mutations of complement genes, which result in defective complement control on the surface of host cells. The complement system forms the first defense line of innate immunity and mediates the attack against foreign microorganisms. Defective regulation of this cascade results in attack of self cells and in autoimmune disease. Apparently, the alternative pathway convertase C3bBb is central for the pathophysiology of HUS as

gene mutations of the components (C3 and Factor B) or of regulators (Factor H, Factor I and MCP/CD46) are observed in the genetic form of HUS. Recently, a novel mechanism leading to atypical HUS (aHUS) was identified, in form of autoantibodies that bind the complement inhibitor Factor H. Here we summarize the current concept of HUS and focus in particular on the novel subgroup of aHUS patients with IgG autoantibodies to Factor H which develop on the genetic background of CFHR1/CFHR3 deficiency, and which define a new subform termed DEAP-HUS (deficient for CFHR proteins and Factor H autoantibody positive).

Keywords

Autoimmune diseases, immunity, thrombocytopenia, thrombosis, thrombotic thrombocytopenic purpura (TTP / HUS)

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Haemolytic uraemic syndrome

Haemolytic uraemic syndrome (HUS), is a severe and rare kidney disease, which was first described in 1955 by Gasser et al. (1). HUS is characterized by acute renal failure, microangiopathic anaemia and thrombocytopenia (2, 3). Different types of HUS are defined as (i) a diarrhea-associated form (D⁺HUS; also referred to as typical HUS), and (ii) a non-diarrhea associated form (D⁻HUS, also termed atypical HUS, aHUS). DEAP HUS (deficient for CFHR proteins and Factor H autoantibody positive) has recently been defined as a new form of aHUS, that affects young individuals. At present it is unclear whether these autoantibodies also develop in patients with the typical form of the disease.

Diarrhea-positive HUS is most frequent in young patients. This form accounts for more than 70–90% of cases and is mostly caused by infection with Shiga toxin-producing *Escherichia coli* (STEC) (such as serotype O157:H7). A small subset of typical HUS cases were also reported to be associated with infection by *S. pneumoniae*. Usually patients with the typical D⁺HUS form (or STxHUS) recover, and renal function returns to normal.

Diarrhea-negative HUS is observed in about 10–15% of patients and is frequently caused by genetic mutations of comple-

ment genes. These patients have a poor long-term prognosis and disease recurrence is common. This form is often associated with complement deregulation caused by mutations of complement components and regulators. This diarrhea-negative HUS can be further induced by infections or by the use of certain drugs. This form of aHUS can be recurrent and patients can develop endstage renal disease.

The third form DEAP HUS was recently reported in young patients and has a frequency of 11% of HUS cases. The juvenile patients usually show no signs of EHEC infections and are positive for Factor H autoantibodies. In addition the vast majority of patients have a chromosomal deletion of a 84 kb genomic fragment on chromosome 1q32, which results in the deletion of the *CFHR1* and *CFHR3* genes. Apparently the group of DEAP HUS patients responds well to therapy in form of plasma exchange and immunosuppressive treatment (4–6).

In aHUS, endothelial cell damage and platelet dysfunction represent primary events that lead to microvascular lesions particularly in the kidney, endothelial swelling with retraction of the cells and exposure of the basal lamina or formation of microthrombi that occlude arterioles and capillaries in the kidney (7, 8). The link of HUS as a genetic disease started with the identification of mutations in

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the Factor H gene (9–15), which codes for a central complement inhibitor. Later on, mutations in additional complement genes were identified in HUS patients, including Factor I (16, 17), MCP/CD46 (18, 19), C3 (20) and Factor B (21), as well as a chromosomal deletion of the CFHR1/CFHR3 genes (22). These genes encode proteins which either represent the components of the C3 convertase C3bBb or regulators which control the activity of this enzyme (23). At present the function of the CFHR1 and CFHR3 proteins, which are structurally and immunologically related to Factor H, is not known.

Frequency of Factor H mutations vary depending on the cohort analyzed between 15–30%, MCP mutations between 10–13%, and Factor I mutations are reported in approximately 5% of the aHUS cases. In addition, the presence of autoantibodies in combination with CFHR1/CFHR3 deficiency are observed in approximately 10% of aHUS patients. So far C3b and Factor B mutations are reported for single cases and families, but the frequencies have not been analyzed in larger cohorts. Thus, the current investigations explain about 45–60% of the cases, indicating that additional disease causing mechanisms do exist. Apparently, the type of the affected gene is relevant for the disease outcome. MCP- but not Factor H and Factor I mutations have a good prognosis (12). Similarly, outcome of kidney transplantation for patients with MCP mutations is rather good, but for patients with Factor H and Factor I mutations is poor. These diverse scenarios demonstrate that HUS is a multigenetic disease, and incomplete penetrance suggests the existence of additional predisposing factors. Interestingly most of the identified genes represent components of the alternative pathway complement convertase C3bBb or regulators that control the activity and stability of this important complement enzyme. Therefore an exact understanding of the activation steps and control mechanisms of the complement cascade, in particular how formation, activity and stability of the C3bBb convertase is regulated is central for an understanding of the pathophysiology of HUS.

The alternative complement pathway: A role in HUS

The alternative complement pathway represents an immediately acting defense mechanism of innate immunity that is aimed to recognize and combat invading microbes and modified self cells. This central part of complement cascade normally differentiates between host cell surfaces and microbial surfaces, between self and non-self; thus allowing activation of the cascade on foreign particles and leaving self structures and tissues intact. Complement activation on the surface of microbes results in (i) opsonisation, i.e. C3 deposition, which facilitates uptake of a marked particles and phagocytosis by professional phagocytic cells, (ii) the release of the anaphylatoxins C3a and C5a, which initiate inflammatory reactions, and (iii) the trigger of the terminal complement pathway, which generates the membrane attack complex, forms pores in a cell surface to induce lysis of a pathogen (24, 25). The activated complement system also coordinates acquired immune reactions (26) and is essential for the removal of immun-complexes in the body.

The alternative complement pathway is continuously activated at a low rate, by the spontaneous hydrolysis of the compo-

nent C3 in human plasma. This hydrolyzed C3(H₂O) binds Factor B. Upon cleavage by Factor D the fluid phase convertase C3(H₂O)Bb is formed which cleaves more C3 molecules to C3a and C3b and initiates the amplification pathway by generating additional C3bBb convertases. Any newly generated C3b molecule can covalently bind to macromolecules, cell surfaces and immune complexes in its direct vicinity. Upon binding to foreign surfaces newly generated C3b triggers further amplification and initiates effector functions resulting in the opsonization of the surface with additional C3b components and leading to phagocytosis by host immune effector cells. Binding of an additional C3b molecule to the C3bBb convertase generates the C5 convertase C3bBbC3b, which cleaves C5. C5 cleavage generates the anaphylatoxin C5a as well as C5b, that initiates the terminal complement pathway. Unrestricted formation of the membrane attack complex (MAC) allows formation of pores which cause cell lysis (24, 25).

A newly generated C3b, which is formed during the initial phase of the alternative pathway activation, binds indiscriminately to any surface in its direct vicinity. These initial steps do not differentiate between microbe and host cell, i.e. between foreign and self. Ideally complement activation proceeds unrestricted on foreign surfaces and induces damage and elimination. However, on the surface of self cells complement activation is controlled and restricted by regulators which block the initial steps, the amplification reactions and the terminal steps of the cascade. These regulators either display cofactor activity which favor inactivation of C3b by the serine protease Factor I, facilitate dissociation of a preformed C3 convertase complex, or inhibit formation of the terminal complement pathway and MAC formation on host surfaces. Soluble regulators, such as Factor H and FHL-1, act in the fluid phase and do also attach to cell- and tissue surfaces. The membrane anchored proteins CR1, MCP, DAF and CD59 act on the cell surface. Several of these regulators have overlapping and redundant activities. The relevance of a strong concerted complement regulation for tissue integrity and homeostasis is revealed by the fact that already a heterozygous deficiency of one single regulator may result in cell damage and disease. However, the incomplete penetrance of gene mutations in HUS show that compensation does exist and that the pathophysiology of this disease is multifactorial.

Factor H autoantibodies in HUS

Autoantibodies to complement Factor H were reported in HUS patients and are associated with the disease. However the mechanisms how autoantibodies contribute to endothelial cell damage and to pathophysiology of HUS is an issue of current investigation.

Autoantibodies to Factor H in HUS patients were first reported in 2005 for three children of the French HUS cohort (27). The autoantibody-positive plasma inhibited binding of Factor H to the C3bBb convertase, but did not influence the complement regulatory functions of Factor H in fluid phase which are mediated by the N-terminal SCRs 1–4 of Factor H.

Factor H autoantibodies were further reported in five additional, juvenile HUS patients, and their binding epitopes were localized to the C-terminal cell surface attachment region of Factor H, to SCRs 19–20. These autoantibodies do not inhibit the complement regulatory activity of Factor H in fluid phase but block

cell binding of Factor H. Purified IgG autoantibodies of the patients reduced C3b binding of Factor H, blocked binding of Factor H to cell surfaces and inhibited haemolytic activity (28).

The frequency of Factor H autoantibodies was analyzed in the German HUS cohort of 147 aHUS patients by an ELISA approach. Sixteen patients (i.e. 11%) were positive for Factor H autoantibodies. In addition, Factor H autoantibodies were completely absent in a control group of 100 age-matched healthy individuals.

All autoantibodies identified so far bind to overlapping or to the same C-terminal surface binding region of Factor H, which also represents a hot spot for HUS-associated mutations. This indicates that autoantibodies are linked to the pathophysiology of HUS, suggesting a similar mechanism of autoantibodies and of C-terminal mutations in Factor H. Consequently, it was hypothesized that these autoantibodies inhibit surface attachment and the complement regulatory functions of Factor H on cellular surfaces. Thus, the Factor H autoantibodies described in the French and German cohorts show similar characteristics.

Factor H autoantibodies and CFHR1/CFHR3 deficiency: Genetic association

Protein analyses of the Factor H autoantibody-positive plasma samples demonstrated the complete absence of CFHR1 and CFHR3. Further detailed analyses showed for 14 patients the complete absence of both plasma proteins and for two patients rather low, barely detectable protein levels. Genetic analyses showed for the 14 deficient patients the complete absence of CFHR1 and CFHR3 due to a homozygous deletion of a large 84 kb genomic fragment which includes the *CFHR1* and *CFHR3* genes. Apparently, these patients have the same or very similar chromosomal breakpoints. Deletion of this genomic fragment

appears due to non allelic homologues recombination of two long interspersed repeat elements, located within the Factor H gene cluster on human chromosome 1q32 (22). Family studies revealed homozygous CFHR1/CFHR3 deficiency for all analyzed autoantibody positive patients. In three families all healthy members (n= 11), which showed heterozygous or homozygous CFHR1/CFHR3 deficiency lacked Factor H autoantibodies (29). The strong correlation between the chromosomal deletion and the presence of Factor H autoantibodies indicates that the absence of CFHR1/CFHR3 proteins in plasma represents a predisposing factor for the development of Factor H autoantibodies and for HUS (29). The identification of the exact mechanisms how CFHR1/CFHR3 deficiency results in autoantibody formation is a challenge for future work.

Factor H autoantibodies provide a new link between the two related disorders HUS and thrombotic thrombocytopenic purpura (TTP) with lead to thrombotic microangiopathies. TTP similar to HUS is caused by genetic deficiency and autoantibodies contribute to the diseases (30). The most relevant gene is the van Willebrand cleaving protease ADAMTS13 and autoantibodies bind to a specific region in the ADAMTS13 protein (31).

Role of the C-terminus of Factor H in hereditary HUS

So far all analyzed Factor H autoantibodies of HUS patients bind to the C-terminal recognition region, most likely to SCR 20 of Factor H (28, 29). This C-terminal epitope overlaps or is even identical to the C-terminal surface attachment region which forms a hot spot for HUS associated mutations in the Factor H gene (SCRs 18–20) (14) (Fig. 1).

The N-terminal domains of Factor H (i.e. SCRs 1–4) mediate complement regulatory activity, the C-terminus of the molecule

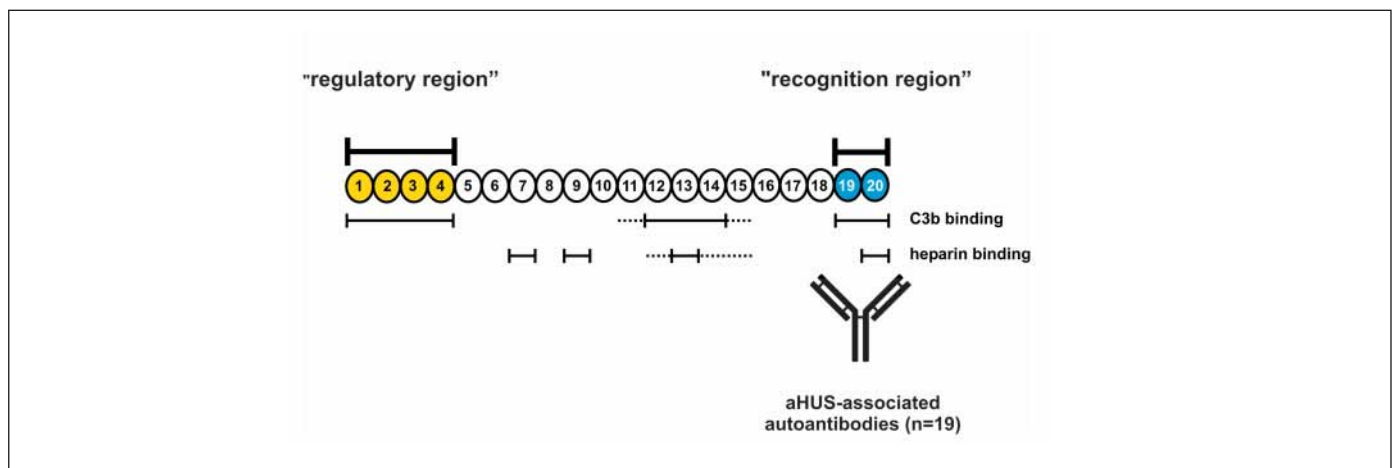


Figure 1: Domain structure of factor H. Factor H is composed of 20 individually folding domains, termed short consensus repeats (SCR) or complement control protein modules. Two major functional regions of Factor H are located at opposite ends of the protein. The N-terminus, i.e. SCRs 1–4 mediates complement regulatory activity (regulatory region, shown in yellow), by (i) acting as a cofactor for C3 degradation for the serine protease Factor I (cofactor activity), and (ii) accelerating the dissociation of the C3bBb complex (decay accelerating activity). The C-terminal SCRs 19–20 display surface-binding activity (shown in blue) as in the native Factor H protein this region makes the first contact to cell surfaces. The Factor H protein has three binding sites for

C3b, which interact with different isoforms of C3b and which are located within SCRs 1–4, SCRs 12–14 and SCRs 19–20. Similarly Factor H has three heparin binding regions, which are localized to SCR 7, to SCR 13 and SCRs 19–20. When binding sites are not exactly defined the dashed lines indicate involvement of other domains. Binding sites of the autoantibodies associated with HUS are localized to SCRs 19–20, the C-terminal surface, C3b and heparin binding region. IgG autoantibodies interfere with surface binding and regulatory functions at the cell surface. HUS-associated autoantibodies have been identified in 16 patients with CFHR1/CFHR3 chromosomal deficiency.

is essential for binding to self cells. The binding via the Factor H C-terminus is mediated by interaction with cell surface polyanionic moieties, such as sialic acids, and also by cell surface deposited C3b (33–40) (Fig. 1).

The C-terminus of Factor H, a surface-binding region

About 75% of the mutations associated with HUS are positioned in the C-terminus of the protein and the vast majority appears in a heterozygous set up. Thus, HUS patients have one defective and one intact allele and consequently the plasma concentration and activity of Factor H is reduced to 50%. The identified gene mutations appear in three major groups: (i) premature stop codons may result in a block of protein secretion and in an intracellular accumulation of the protein, (ii) mutations of structural or architecturally relevant amino acid affect the folding of the individual domains and can interfere with protein secretion and/or ligand binding, (iii) mutations of surface exposed residues generally result in secreted proteins and in normal Factor H plasma levels. However, mutations affect residues positioned within or forming direct contact sites and consequently result in defective ligand binding and functional defects.

Several mutant Factor H proteins were functionally characterized. Biochemical studies of mutant Factor H proteins either purified to homogeneity from patients plasma (37) or expressed recombinantly (34) revealed reduced binding to C3b/C3d, to heparin and most important reduced surface binding to human endothelial cells (32–34). Thus, under conditions of enhanced complement activation, a lower local concentration of Factor H at the cell surface may result in enhanced complement activation leading to endothelial cell damage. This concept is confirmed by haemolytic assays with sera of HUS patients, which showed enhanced erythrocyte damage (28, 33). Structural analyses of the C-terminal SCRs 19–20 of Factor H by homology modelling (34), NMR (35) and by X-ray crystallography (36) confirmed an important role of the aHUS-associated Factor H mutations for the structure and ligand interactions.

Thus, both C-terminally binding autoantibodies as well as HUS associated mutations have very similar or the same effects,

as they inhibit surface binding of Factor H. Consequently autoantibodies interfere with the complement protective role of Factor H at the surface of host cells and tissue (Fig. 2).

Sheep erythrocytes, which represent non activator surfaces are normally protected from lysis by human plasma. However plasma of HUS patients causes haemolysis of sheep erythrocytes. Thus demonstrating that defective complement control and defective Factor H surface activity contributes to this effect (28). Similarly a truncated Factor H protein, which lacked most of SCR 20 and which was purified from plasma of a patient with a E1172stop mutation, displayed reduced complement regulatory activity on the surface of HUVEC cells (37). In addition, a recombinant SCRs 19–20 fragment of Factor H, which competed with Factor H at the surface of sheep erythrocytes further confirms the central role of the C-terminal domains of Factor H for surface attachment and for C3b binding (38).

Role of Factor H C-terminally binding monoclonal antibodies

C-terminally binding mAbs represent a valuable tool to mimic Factor H autoantibodies and these antibodies can be used for functional studies. Factor H-specific mAbs with binding sites in the C-terminal SCRs 19–20 of Factor H (namely mAbs C02, C14, C18 and MH10) showed an inhibitory effect on Factor H binding to C3b, heparin and endothelial cells. These antibodies also reduce Factor H cofactor activity when zymosan was used as a model surface (39). The C-terminally binding mAbs do not influence Factor H cofactor activity in fluid phase, but cause impaired C3b inactivation and enhanced complement activation on the surfaces of endothelial cells. Thus the IgG autoantibodies lead to increased susceptibility of the cells to complement-mediated damage and lysis. Both the autoantibodies and domain mapped monoclonal antibodies bind to overlapping or even the same epitopes of Factor H. This is shown by the fact, that the mAbs inhibit binding of Factor H autoantibodies to SCRs 19–20 and to full-length Factor H (28, 29). In addition Factor H autoantibodies have the same functional effects like the mAbs, they reduce Factor H binding to C3b and enhance lysis of sheep red blood cells (29).

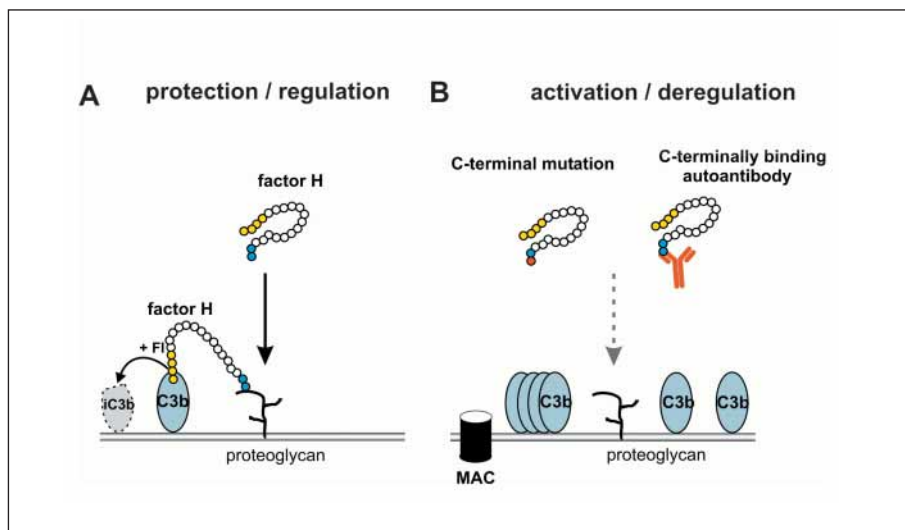


Figure 2: Disease model for Factor H associated HUS. A) Normal scenario: Factor H binds to glycosaminoglycans or deposited C3b on cells (via the C-terminus) and exerts complement regulatory activity (via the N-terminus) on the cell surface. B) Disease scenario: Mutations in the C-terminal cell surface and ligand-binding region or binding of autoantibodies to the same region cause reduced surface attachment of Factor H, e.g. to endothelial cells. Under conditions of complement stress, reduced binding and inappropriate complement inhibition on the surface results in enhanced complement activation, deposition of C3b and generation of terminal components (MAC) leading to cell damage.

Also autoantibodies derived from HUS patients do not influence fluid phase cofactor activity of Factor H (27–28) (Fig. 2).

Animal model: Transgenic Factor HΔSCR16–20 mouse model

The role of the C-terminus of Factor H for HUS pathology was further analyzed in a mouse model. Transgenic mice were generated on a Factor H^{-/-} knock-out background, that express a truncated Factor H protein, which lacks the five C-terminal domains (Factor HΔSCR16–20). These animals develop HUS spontaneously (40). Apparently, the mutant Factor H protein does control complement activation in plasma of the animals in fluid phase, as indicated by almost normal C3 levels. However, complement activation is not properly controlled at the surface of cells and tissues (40). These effects are in contrast to Factor H^{-/-} animals which develop MPGN II. Plasma of these animals shows a lack of complement control due to the absent Factor H inhibitor in fluid phase, resulting in complement consumption and thus leading to inappropriate action on the surface of host tissues, particularly of the glomerular basement membrane. Both the HUS- and the MPGN II animal models demonstrate a crucial role of Factor H in pathophysiology of the two related diseases, show that Factor H-mediated complement regulation is essential for haemostasis, and reveal that defective complement control at the level of C3 results in pathophysiology of both diseases.

Clinical consequences: Diagnosis and therapy of DEAP HUS patients

Clinically, all 24 reported Factor H autoantibody-positive patients presented specific features of aHUS (27–29). Patients with Factor H autoantibody-associated HUS (DEAP-HUS) presented the relapsing form of the disease. However, one major characteristic of this form is the young age (3–17 years) at disease onset, a time when the development of Factor H autoantibodies could represent an essential factor for etiology. Prospective studies, including a systematic screening for Factor H autoantibodies, particularly in juvenile HUS patients, could answer this issue. This screening could directly be performed during the acute phase of the disease.

Diagnosis of Factor H autoantibodies is made by the identification of autoantibodies in the patient's plasma by ELISA. Microtiter plates are coated with purified human Factor H, and diluted plasma samples of the patients are added. Following incubation and washing, the antigen-IgG complexes are measured with a labeled antibody against human IgG. Titers are shown as arbitrary units per ml (AU/ml) and positive samples are compared to a positive reference plasma. This protocol provides a quantitative reference useful to determine the biological follow up.

In case of presence of Factor H autoantibody, a follow up on Factor H autoantibody titers was performed and allowed to monitor the successful outcome of the patient's renal transplantation over a period of 24 months (41). Thus, anti-Factor H IgG titers represent a useful marker to monitor disease progression or disease evolution, as well as treatment efficacy during the acute phase of the disease or after renal transplantation. This test should be combined with further analysis of the complement

status comprising C3, Factor B, Factor H, CFHR1 and CFHR3 plasma levels and also by Factor H functional analysis.

Low C3 and Factor B antigenic levels indicate systemic activation of the alternative complement pathway, as observed in two of three reported French patients (27). A low Factor H antigenic level may be observed in patients with anti-Factor H antibodies and is relevant for the interpretation of the Factor H functional test, as Factor H function is always decreased in plasma derived from autoantibody-positive patients. As previously indicated, different assays exist for functional analysis of Factor H, and most of them are based on haemolytic tests with sheep erythrocytes (31) or measure decay activity of the alternative pathway C3 convertase (27).

Considering the high association of homozygous CFHR1/CFHR3 deletion with the presence of Factor H autoantibodies, plasma screening for the two proteins would be a further valuable diagnostic parameter. The absence of the two proteins may be directly assayed by Western blot analysis, using polyclonal anti-Factor H antibodies reacting with CFHR1 and/or CFHR3, or by ELISA as soon as specific antibodies for each protein are available (22). Diagnosis of the chromosomal deletion can be performed using MLPA (Multiplex Ligation Dependent Probe Amplification) as described (22).

The diagnosis of Factor H autoantibodies associated DEAP HUS is important because it allows to initiate specific treatments based on fresh frozen plasma (FFP) infusion, plasma exchange therapies and/or immunosuppressive therapies. Frequency of plasma exchanges can be adapted to the biological results and can be directly monitored by determination of the autoantibody titer. Different immunotherapies may be proposed comprising steroids, azathioprine or even anti-CD20 (Rituximab[®]) such as in the case of TTP secondary to anti-ADAMST-13 antibodies (42). All these therapies were used in the successful management of the post-HUS renal transplantation in one child exhibiting Factor H autoantibodies (41). Each treatment has now to be prospectively evaluated such as in any autoimmune disease, in order to propose specific therapeutic guidelines.

Future goals

The identification of Factor H autoantibodies with the background of CFHR protein deficiency as a disease-causing condition provided further evidences for the strong requirement of proper complement regulation on self tissues. Thus, additional disease-predisposing factors that lead to reduced complement control will likely be identified in the future. These factors could represent other genetic mutations or the presence of additional autoantibodies for other proteins. The characterization of these autoantibodies and the understanding of their exact functions represents a major challenge to understand the complex disease HUS and thrombotic microangiopathies.

Conclusion

Autoantibodies to the complement inhibitor Factor H represent a novel and important mechanism for the development of HUS. Factor H autoantibodies so far represent an acquired consequence to the absence of CFHR1 and CFHR3 in plasma due to a deletion of a chromosomal DNA fragment. Therefore, the

identification of the two CFHR proteins in plasma of aHUS patients forms one direct diagnostic approach for the identification of patients with this specific form of DEAP HUS; or to identify patients who are at risk for this type of autoimmune disease. At present, it is unclear how Factor H autoantibodies develop, how long Factor H autoantibodies are expressed before disease onset, and how long Factor H autoantibodies persist. Also the autoanti-

body titers vary between patients, and have been followed for one patient during the course of the disease (6, 27). Thus, autoantibody levels may predict specific clinical manifestations, disease severity and progression. In addition, determination of autoantibody titers is relatively easy and allows to monitor the response to therapeutic approaches.

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