

THE COMPLEMENT SYSTEM AND GLOMERULAR KIDNEY DISEASES: ETIOPATHOGENESIS, DIAGNOSTICS AND TREATMENT

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SISTEM KOMPLEMENTA I GLOMERULSKE BOLESTI BUBREGA: ETIOPATOGENEZA, DIJAGNOSTIKA I LEČENJE

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ABSTRACT

The human immune system consists of the innate and the acquired immunity. The complement system is part of the innate immune response and it can be activated through one of the three pathways: the classical pathway, the alternative pathway and the lectin pathway. Complement activation forms the C3-convertase complex. The C3-convertase proteolytically cleaves the C3 component into C3a and C3b fragments. The C3b fragment binds to the C3-convertase to form the C5-convertase. The C5-convertase is an enzyme complex that cleaves the C5 component into C5a and C5b fragments. The C5b component binds to C6, C7, C8, C9 thus forming the terminal complex of the complement activation – the membrane attack complex (MAC) which is responsible for effector functions of the complement system. The serum complement is a mediator of C3 glomerulopathy (C3G), a group of diseases that includes membranoproliferative glomerulonephritis type II (MPGN II) or dense deposit disease (DDD) and C3 glomerulonephritis (C3GN). These diseases are caused by a dysregulation of the alternative pathway of the complement activation system. The C3 nephritic factor (C3NeF) is an autoantibody to the alternative pathway C3-convertase, which can cause dysregulation of the pathway. Newly acquired knowledge has enabled therapeutic applications of a specific antibody to the C5 complement component (eculizumab). Eculizumab is a recombinant monoclonal antibody that exhibits high affinity for the complement component C5, with the aim to inhibit its cleavage and activation of the terminal complement pathway.

Key words: complement system proteins; glomerulonephritis; antibodies, monoclonal, humanized.

INTRODUCTION

The human immune system consists of the innate and the acquired immunity. The complement system represents a part of the innate immune response (1). It consists of 30-40 proteins, some of which are soluble, circulating proteins in blood plasma or other body fluids, and some are integral or membrane-bound proteins (2, 3,

SAŽETAK

Imunski sistem čoveka sastoji se od urođenog i stečenog imunskog odgovora. Sistem komplementa predstavlja deo urođenog imunskog odgovora i može se aktivirati na jedan od tri načina: klasičnim, alternativnim i lektinskim putem. Aktivacijom komplementa nastaje kompleks C3-konvertaza. C3-konvertaza proteolitičkim putem cepa C3 komponentu na fragmente C3a i C3b. Spajanjem fragmenta C3b sa C3-konvertazom nastaje C5-konvertaza. C5-konvertaza je enzimski kompleks koji cepa C5 komponentu na C5a i C5b fragmente. Komponenta C5b se vezuje sa komponentama komplementa C6, C7, C8, C9 i tako nastaje završni kompleks aktivacije komplementa – MAC (Membrane Attack Complex) koji je odgovoran za efektorske funkcije sistema komplementa. Serumski komplement je medijator C3 glomerulopatija (C3G), grupe bolesti koja obuhvata membranoproliferativni glomerulonefritis tip II (MPGN tip II) ili bolest gustih depozita (DDD) i C3 glomerulonefritis (C3GN). Obe bolesti nastaju zbog poremećaja regulacije alternativnog puta aktivacije sistema komplementa. C3-nefritički faktor (C3-NeF) predstavlja autoantitelo na C3-konvertazu alternativnog puta i može biti uzrok poremećaja njegove regulacije. Nova saznanja omogućila su terapijsku primenu specifičnog antitela na C5 komponentu komplementa (eculizumab). Eculizumab je rekombinantno monoklonsko antitelo sa visokim afinitetom za C5 komponentu komplementa, čiji je cilj da spreči cepanje komponente C5 i aktivaciju završnog puta sistema komplementa.

Ključne reči: sistem proteina komplementa; glomerulonefritis; antitela, monoklonska, humanizovana.

4). The complement system is a proinflammatory system of the proteolytic cascade in which enzymatic cleavage of one component activates the next one. This way, the whole process is significantly amplified (2). Deposition of complement fragments at sites of tissue damage leads to the process of opsonization, as well as liberation of peptides whose role is to stimulate the inflammatory response and cell lysis (1, 2, 5). This way, the complement

system plays an important physiological role in the innate immunity response and inflammation with the aim to eliminate cellular debris, microbial pathogens and apoptotic host cells (3).

The complement system activation mechanisms

The three pathways can activate the complement system: the classical pathway, the alternative pathway and the lectin pathway. These pathways are activated by different mechanisms (1, 2, 6). Complement components C1q, C1r, C1s, C4 and C2 form the classical pathway (7). Antibodies of immunoglobulin classes G and M (*IgG* or *IgM*) are traditionally known as activators of the classical pathway (1). Activation of the classical complement pathway (CP) starts when the component C1q binds to the Fc-fragment of the antibody (in an immune complex) at the site of damage (2, 9). After binding to specific sites on the activator, the complement component C1q induces activation of the C1r and C1s subcomponents (1). The activated C1r serine-protease proteolytically cleaves C4 and C2 into fragments C4a, C4b, C2a and C2b (2, 7, 8). The fragments C4b and C2b bind to form the complex C4b2b which is a classical pathway C3-convertase, an enzyme that cleaves the C3 component into C3a and C3b by proteolytic activity. The fragment C3b binds to C4b2b (classical pathway C3-convertase) to form the complex C4b2b3b which is a classical pathway C5-convertase, an enzyme that proteolytically cleaves C5 into the fragments C5a and C5b (2, 8). The components C5b, C6, C7, C8, and C9 bind to form the terminal complex of the complement activation - MAC (*Membrane Attack Complex*) (8). Lupus nephritis is the best studied human model of a chronic renal disease in which immune complexes activate the classical complement pathway (CP) (10). In lupus nephritis patents, it is diagnostically important to determine serum levels of C3 and C4, immune complex (IK), as well as antinuclear antibody titre (ANA), anti-dsDNA-antibodies (whose target antigen is double stranded DNA) and anti-Sm-antibodies (antibodies against the Smith antigen) (10, 11, 12, 13). Antibodies binding to the complement component C1q can also be detected in lupus nephritis patents (13).

The circulating molecules, mannose-binding lectin - MBL (a protein similar to C1q) and ficolins 1, 2 and 3 bind to the terminal mannose or N-acetylglucosamine on the bacterial surface and thus initiate the lectin or mannose-binding pathway (7). These molecules have their target structures which they identify and to which they bind. After this binding, two enzymes that are attached to the mannose-binding lectin (MBL) are activated. These enzymes are known as MBL-associated serine-proteases, MASP-1 and MASP-2 (8, 9). The serine-protease MASP-

2 cleaves the components C4 and C2. This further leads to formation of C3-convertase and C5-convertase complexes. Serine-protease MASP-1 can directly cleave the component C3 (1, 8). Immunoglobulin A nephropathy (*IgAN*) or Berger disease is a relatively common form of chronic glomerulonephritis. The main characteristics of this disease include deposition of immune complexes containing immunoglobulin class A (*IgA*) in the kidney mesangium, inflammation, mesangial proliferation and interstitial damage. In approximately 33% of the affected people, the disease gradually progresses to sclerosis and to the end-stage-renal-failure (ESRF). In vitro studies have shown that polymeric serum IgA can bind to mannose-binding lectin (MBL) present in serum, as well as that binding of the serum complex MBL-MASP-2 to immunoglobulin class A (IgA) in the kidney mesangium can activate the component C4, which may present the pathogenic basis for IgA nephropathy (*IgAN*) (14).

The alternative pathway (AP) is constitutively active and its increased activity is the result of the loss of regulation. In contrast, the classical and lectin complement pathways (CP and LP) are not constitutively active and they need a trigger to stimulate their activity (6). The phylogenetically oldest (but last discovered and described), the alternative complement pathway (AP) is composed of three components: fragment C3b and factors B and D (4, 7). The term 'tickover' refers to a physiologically low level of the alternative pathway (AP) activity. This constitutively low activity is controlled by hydrolysis of the internal thioester bond of the central protein complement C3. In this case, the process of the internal thioester bond hydrolysis takes place spontaneously (16). The hydrolysis of the internal thioester bond of the protein C3 leads to a conformational change in the molecule thus yielding the molecule C3(H₂O), which is similar to the complement fragment C3b (4, 17). The serine-protease factor D proteolytically cleaves the factor B into the fragments Ba and Bb (8). The fragment C3b binds to the fragment Bb to form the complex C3bBb which is the C3-convertase of the alternative complement pathway (AP), and which is stabilized by binding to the plasma protein properdin (5, 9, 18, 17). The C3-convertase of the alternative pathway (AP) leads to cleavage of other C3 molecules and the increase in the number of C3b fragments, thus amplifying the production of the complex C3bBb by a process referred to as the C3b amplification loop of the alternative complement pathway (AP) (17, 18). C3b molecules bind to C3bBb, which is an alternative pathway C3 convertase, to generate the complex C3bBb3b, which is a C5-convertase of the alternative pathway (AP). This enzyme complex proteolytically cleaves C5 into the fragments C5a and C5b (3).

Terminal pathway of the complement system activation

The component C5b binds to components C6, C7, C8, C9 thus forming the terminal complex of the complement activation – the membrane attack complex (MAC) which is responsible for effector functions of the complement system (7, 20). Conformational changes in C5b lead to exposure of the binding site for the complement component C6. The ability of the C5b fragment (within the C5-convertase) to bind C6 at the site of damage is short-term and decays rapidly. If these two components bind, the newly formed complex C5b-6 becomes stable. Binding of the complex C5b6 to the component C7 causes disclosure of the membrane binding site and a consequential incorporation of the newly formed complex into the cell membrane. However, if the concentration of component C7 at the site of activation is limited and the binding does not occur, the complex C5b-6 stays soluble (unbound). If this soluble C5b6 complex subsequently binds to the component C7 circulating in plasma and fluid liquids, the so-called fluid-phase C5b-7 is formed.

The time that the fluid phase C5b-7 has to bind to the membrane is limited. The fluid phase C5b-7 has the same affinity as the membrane-bound C5b-7 complex that can bind to the C8 component. The nascent C5b-7 complex binds to the C8 β chain via the component C5b. The newly formed complex C5b-8 binds to the first molecule of C9 via the C8 α chain. Due to major structural changes, binding of the first C9 molecule enables binding of further C9 molecules. This way the C9 cylinder is formed that can be inserted into the target cell membrane. The C9 component is polymerized at the site of insertion. This polymerization process causes a distortion in continuity of the phospholipid bilayer of the cell membrane resulting in 'leaky patches' or hydrophilic channels ('pores') through the membrane. Formation of these structures further leads to processes of disruption, lysis and necrosis of the cell (20). The activation of the complement system can be proven based on the increased levels of the MAC in plasma (normal level is below 0.30 mg/dl) (27).

Regulation of the complement activation system

The complement system activation can have bad effects on the organism cells. Therefore the activation of the complement system is strictly controlled (2). The classical pathway (CP) is activated by binding of antibodies to the C1q component and by activation of C1r and C1s subcomponents (7). Protein C1-inhibitor (C1-INH), a member of a diverse serine-protease inhibitors family, inhibits this reaction by binding to activated enzymes C1r and C1s and inactivating them (7, 8, 19).

Activation of the alternative complement pathway (AP) is regulated by two plasma proteins, the complement

factor H (CFH) and the complement factor I (CFI) (17). The complement factor H (CFH) is a 150-kDa glycoprotein whose serum concentration ranges from 110 to 615 micrograms/ml (5, 17). The complement factor H (CFH) accelerates the decay of the complex C3bBb (the alternative pathway C3-convertase). It is also a cofactor to the complement factor I (CFI) in the proteolytic conversion of C3b to iC3b which is inactive, thus inhibiting the binding of the factor B and the further process (3, 17, 18). The complement factor I (CFI) is serine-protease whose molecular weight is 88-kDa. Serum concentration of this enzyme ranges approximately from 39 to 100 micrograms/ml (17). The complement factor I (CFI) cleaves the fragment C3b (in presence of the cofactor) into fragments iC3b and C3dg (3, 7, 17). Unlike C3b, the fragments iC3b and C3dg are inactive and cannot participate in the C3b-amplification loop (3). Vitronectin or S-protein and clusterin or SP-40,40 are plasma glycoproteins that bind to the metastable site inside the complex C5b-7. This way further incorporation of the C5b-7 complex into the cell membrane is made impossible (2, 7, 20). The molecule CD59 binds to the C9 component binding site on the alpha-chain of the C8 component. Formation of the terminal complex, insertion and polymerization of the component C9 and cell lysis are thus made impossible (7, 20).

Complement and renal damage

The complement system is a mediator of multiple forms of renal diseases which can all be classified into two groups (22). Increased activation of the classical complement pathway (CP) is the pathogenic basis for the first group of diseases which includes lupus nephritis, membranous glomerulonephritis and rapidly progressive glomerulonephritis (6, 19, 23-25). A dysregulation of the alternative complement pathway (AP) presents the pathological basis for the second group of diseases that include C3 glomerulopathy (C3G) – membranoproliferative glomerulonephritis type 2 or dense deposits disease (DDD) and C3 glomerulonephritis (C3GN), and atypical hemolytic uremic syndrome (aHUS) (3, 6, 16, 24-26)

C3 glomerulopathy (C3G)

Membranoproliferative glomerulonephritis (MPGN) is traditionally classified as primary or idiopathic and secondary. The primary or idiopathic type includes membranoproliferative glomerulonephritis type I and type III (MPGN I and III). The secondary membranoproliferative glomerulonephritis is usually developed after a viral infection (hepatitis B and C) (27). Membranoproliferative glomerulonephritis type I and type III (MPGN I and III) are diseases whose pathogenesis

involve immune complexes, in contrast to membranoproliferative glomerulonephritis type II (MPGN II) (5, 18).

Membranoproliferative glomerulonephritis (MPGN) is traditionally classified according to histological and ultrastructural findings on electronic microscopy (EM) (6, 16, 28). The typical findings include an increased number of cells in the kidney mesangium, changes in capillary walls like proliferation and remodeling, presence of immune deposits, as well as doubling of the glomerular basement membrane (GBM) (16, 21, 28). Membranoproliferative glomerulonephritis (MPGN) was previously classified on the basis of the electronic microscopy findings. According to this classification scheme, the first type of the disease or membranoproliferative glomerulonephritis type I (MPGN I) is characterized by the presence of subendothelial and mesangial deposits. The second type of the disease, membranoproliferative glomerulonephritis type II (MPGN II) or dense deposit disease DDD is characterized by intramembranous and mesangial deposits with high electron density. Finally, there are two subtypes of membranoproliferative glomerulonephritis type III (MPGN III). In Burkholder subtype, deposits can be subendothelial and subepithelial, while in Strife and Anders subtype, deposits are subendothelial, subepithelial and intramembranous with lamina densa damage (6, 21, 28).

The use of immunofluorescence microscopy (IF) has brought about major advances in our understanding of the pathogenic basis for membranoproliferative glomerulonephritis (MPGN) and the subsequent reclassification of the disease (16, 28). The old classification has been replaced with a new classification based on the presence of the complement component C3 and/or immunoglobulin (Ig) deposits, assessed by immunofluorescence microscopy (IF) (21). The deposits in membranoproliferative glomerulonephritis type I (MPGN I) contain immunoglobulin IgG and/or IgM as well as C1 and/or C3 components. The deposits in patients with membranoproliferative glomerulonephritis type II or dense deposit disease (MPGN II/DDD) contain only the C3 component. The membranoproliferative glomerulonephritis type III (MPGN III) (Burkholder subtype) usually has deposits of complement components and immunoglobulins (Igs), while Strife and Anders subtype contains either the C3 component alone or the C3 component and the immunoglobulins (Ig) together (16, 28).

According to the findings obtained using immunofluorescence microscopy (IF), Sethi and colleagues proposed a new classification of membranoproliferative glomerulonephritis (MPGN). The new classification includes two main groups. The first group includes diseases with deposits of the C3 component and immunoglobulins (Igs) (Ig-mediated glomerulonephritis).

The second group includes diseases with presence of the C3 component only, which suggests the hyperactivity of the alternative complement pathway (AP) (16).

At about the same time, Pickering and colleagues proposed the term C3 glomerulopathy (C3G) for all types of glomerulonephritis characterized by accumulation of the C3 component, with no deposits of immunoglobulins or classical pathway components (16, 21, 29). The term C3 glomerulopathy (C3G) describes a new group of diseases and includes membranoproliferative glomerulonephritis type II or dense deposit disease (MPGN II/DDD) and C3 glomerulonephritis (C3GN) (16, 21). Both diseases can involve histological changes like mild mesangial proliferation or endocapillary proliferative or extracapillary proliferative forms of glomerulonephritis (GN) (29). According to a new understanding, both of these diseases are the consequence of dysregulation of the alternative pathway (AP) (16, 21). Inspected by electronic microscopy (EM), dense deposit disease DDD shows characteristic highly electron-dense deposits localized within the glomerular basement membranes (GBM), mesangial matrix, Bowman's capsule and tubular basement membrane, that form sausage-shaped or ring forms. Assessed by the same technique, C3 glomerulonephritis (C3GN) shows moderately electron-dense deposits in mesangial and/or subendothelial, intramembranous, and subepithelial locations (21, 27, 29). Both diseases are diagnosed by renal biopsy (27).

Functional and genetic studies have shown that causes of human C3 glomerulopathy (C3G) include mutations in genes for complement factors H (CFH) and I (CFI), as well as the presence of antibodies that lead to disorders in the alternative pathway (AP) (21, 29). Defects in activation or regulation of the C3-convertase enzyme of the alternative complement pathway (AP) lead to shift from low-grade activity (tickover) to hyperactivity of this pathway; and this hyperactivity is considered the pathophysiological basis for C3 glomerulopathy (C3G) (16, 19).

The C3 nephritic factor (C3NeF) is an autoantibody of immunoglobulin class G (IgG) that binds to neoepitopes on the C3-convertase of the alternative complement pathway (AP), but not to individual enzyme components (3, 19). The C3 nephritic factor (C3Nef) stabilizes both the fluid phase and the membrane-bound C3-convertase. The reason for generation of C3 autoantibodies is unknown. There may be a genetic background for generation of this autoantibody, but no studies have addressed this matter so far (30). The C3 nephritic factor binds to the C3-convertase (C3bBb) of the alternative complement path (AP), and makes this enzyme complex resistant to inactivation by the complement factor H (CFH) (5, 19, 30). The C3 nephritic factor (C3NeF) and the complement factor H (CFH) control C3 convertase (C3bBb) with

opposite effects (18). The complement factor H (CFH) and the fragment Bb (factor B decay product) compete for the binding site on the fragment C3b, and if the factor H (CFH) binds to C3b, the complex C3bH is formed (3, 8). The fragment C3b is further cleaved by the complement I (CFI) into small breakdown products, like a fragment iC3b (5, 8). The fragment iC3b can bind to the regulatory molecule CR1 (5). In contrast, the autoantibody C3 nephritic factor (C3NeF) binds to the complex C3bBb, and prolongs the half life of this enzyme approximately 10 times (up to 60 minutes) (16, 18). The presence of the C3 nephritic factor (C3NeF), the absence or defective function of the factor H (CFH) and/or inhibition of the complement factor H (CFH) function by anti-factor-H-autoantibody result in the loss of control of the alternative pathway C3 convertase and unrestricted activation (18).

The C3 nephritic factor (C3NeF) is present in the serum of about 80% of patients with dense deposit disease DDD, and in about 40–50% of patients with C3 glomerulonephritis (C3G), and it can also be detected in the serum of patients with membranoproliferative glomerulonephritis type I and type III (MPGN I and III), as well as in the serum of healthy individuals, which indicates that it is not specific only for C3 glomerulopathies (C3G) (16, 18, 30). An autoantibody that can bind to the native complement factor B (CFB) and thus stabilize the C3 convertase has been recently found in patients with dense deposit disease DDD (3).

Clinical features and prognosis

Clinically, C3 glomerulopathies (C3G) present with proteinuria as urinary protein excretion greater than 150 mg/1.73m²/24h, hematuria and some degree of renal failure (31, 32). The annual incidence is 1 to 2 per million (32). Both sexes are equally affected (5, 32). The disease is usually diagnosed around the age of 21 (32). Dense deposit disease DDD can be diagnosed even earlier (usually around the age of 14) (5, 35). However, the onset of the disease has been recorded even in patients older than 60 (one-fifth of the patients) (3, 5, 32). Patients with dense deposit disease DDD can even have an acquired partial lipodystrophy (loss of subcutaneous fat from the face and the upper body), and ocular disorders (3, 32). Early identification of patients at risk of developing acute renal dysfunction and the timely treatment strategy can slow down the progression of the disease and decrease the mortality rate (33).

Despite treatment, the disease progresses to the end-stage-renal-failure (ESRF) in 30-50% of patients after 10 years from diagnosis. Prognosis is worse for patients with dense deposit disease DDD (36-50%) compared to patients with C3 glomerulonephritis (C3GN) (25%) (3, 16, 32). Therefore, dense deposit disease DDD is more

aggressive than C3 glomerulonephritis (C3GN) (16, 28). One possible explanation is the much higher rate of C3 nephritic factor (C3Nef) positivity in patients with dense deposit disease DDD than in patients with C3 glomerulonephritis (C3GN) (28). In the final stage of both diseases, renal transplantation is the treatment method of choice although the rate of disease recurrence and the rate of allograft failure (50-75%) are very high (3, 16, 32).

Atypical hemolytic uremic syndrome (aHUS)

Hemolytic uremic syndrome (HUS) represents a clinical syndrome comprising of microangiopathic hemolytic anemia, thrombocytopenia and acute renal insufficiency due to endothelial lesion and microvascular thrombosis. In a majority of patients with hemolytic uremic syndrome (HUS), thrombotic microangiopathy is diagnosed a week after a bacterial infection such as gastroenteritis (often hemorrhagic). Gastroenteritis is usually caused by verocytotoxin-producing bacteria, like *E. coli* or *Shigella dysenteriae*. In approximately 10% of patients, hemolytic uremic syndrome (HUS) occurs without any apparent bacterial infection and it is known as atypical hemolytic uremic syndrome (aHUS). aHUS is a disease with high morbidity and mortality rate, which usually occurs as the familial or sporadic atypical form. During the acute stage, the mortality rate is 25 %, while in 50% of the cases, the disease progresses to the end-stage (19,34). Genetic disorders associated with atypical hemolytic uremic syndrome (aHUS) are predisposing rather than directly causal (19). Specific mutations have been found in 50% of patients with atypical hemolytic uremic syndrome (aHUS) (19, 34). In over 60% of these patients, genetic studies have revealed the presence of mutations in genes encoding complement factors H, I, B (CFH, CFI, CFB) and the complement component C3 (19, 34, 35). Genetic mutations for the complement factor H (CFH) are observed in 25-30% of patients, autoantibodies to the complement factor H are found in 6-10% of patients, while mutations in the gene for the complement factor I (CFI) are found in 5-10% of patients (19). Autoantibodies to complement regulatory proteins are also found (34).

Some scientists believe that atypical hemolytic uremic syndrome (aHUS) is part of a spectrum of diseases comprising the entity of C3 glomerulopathy (C3G), since there are several diseases with the same dysfunctions in proteins regulating the activities of the complement system (gene mutations, presence of autoantibodies) (6). Despite all the similarities, atypical hemolytic uremic syndrome (aHUS) should not be considered a C3 glomerulopathy (C3G) due to different mechanism of damage. In atypical hemolytic uremic syndrome (aHUS), thrombotic microangiopathy is the major mechanism that leads to kidney glomerular damage (endothelial cell

damage, formation of blood clots in the glomerular capillaries). Deposition of the C3 component and formation of deposits in the kidney glomerular basement membrane (GBM) is the main mechanism of damage in C3 glomerulopathies (C3Gs). (6, 16).

Treatment

In treatment of C3 glomerulopathy (C3G), it is important to control blood pressure using ACE inhibitors or AR blockers (although these medications may affect renal function), and antiproteinuric diet (3, 30, 36). In patients in whom C3 glomerulopathy (C3G) is due to defective function of the complement factor H (CFH), repletion of the factor H (CFH) by plasma infusion (the recombinant factor H is still not used) can be beneficial (16, 18). In patients with dense deposit disease DDD with moderate complement factor H (CFH) deficiency, treatment with plasma in the volume of 10-20 ml/kg/TM in 14-day intervals (half-life of the H factor is about 6 days) can have favorable effects (18). In the case of dense deposit disease (DDD) caused by the C3 nephritic factor (C3NeF) plasmapheresis is used for treatment. This treatment can considerably delay the disease progression towards the end stage (5, 18, 30).

New knowledge has enabled application of specific anti-complement therapy (15, 30). Eculizumab is the first anti-complement therapy, for therapy of paroxysmal nocturnal hemoglobinuria (PNH) and atypical hemolytic uremic syndrome (aHUS) (6, 18). Eculizumab or the anti-C5-antibody, is a monoclonal antibody obtained by recombinant technology, with high affinity for C5, whose therapeutic goal is to prevent cleavage of the C5

component, and in the further course, formation of the lytic MAC complex (6, 27, 29, 35). In order to obtain the drug, the second and third constant regions of the immunoglobulin subclass G4 (IgG4) heavy chains (domains CH2 and CH3) are first fused to the hinge region and the CH1 domain of the immunoglobulin subclass G2 (IgG2). Then they are paired with the kappa (κ) light immunoglobulin chain. The variable regions both of the light and the heavy chains of immunoglobulin are murine-derived sequences. The most important property of these sequences is that they possess high affinity for the C5 component. Then these sequences bound to the human germline framework regions. This way, a hybrid immunoglobulin molecule (Ig), which has high affinity for the complement component C5, is obtained using recombinant technology. This hybrid molecule does not have the ability to activate the complement system or to bind to antibody Fc-receptors (29). The CH1 domain and the hinge region of the immunoglobulin subclass G2 (IgG2) were chosen because they are not able to bind to antibody Fc-receptors and thus initiate the complement activation, while the CH2 and CH3 domains of the immunoglobulin IgG4 (IgG4) were chosen because they cannot activate the complement (6, 29). The drug is administered parenterally and intravenously, and it has a half-life of about 11+3 days. The conventional sustaining dose is once a day, every other week. In order for the drug to achieve the complete blockage of the complement, it is necessary to reach the drug serum concentration of 35 micrograms/ml (6). A single chain version of eculizumab of 25kD (scFv) lacking the whole constant region is also applied (15).

Table 1. Investigation of the alternative pathway of the complement system

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| Investigation of the alternative pathway cascade of the complement system |
| 1. Measurement of the complement serum proteins |
| • C3 complement component |
| • complement factor H (CFH) |
| • complement factor I (CFI) |
| • complement factor B (CFB) |
| 2. Test(s) for C3 nephritic factor (C3Nef) |
| 3. Test(s) for auto-antibodies: anti-CFH antibodies, anti-CFB antibodies |
| T 4. Test(s) for expression of MCP MCP/CD46 on mononuclear peripheral blood cells |
| Screening for gene mutations of regulatory proteins and C3 convertase components encoding genes |
| • complement factor H (CFH) |
| • complement factor I (CFI) |
| • complement factor B (CFB) |
| • membrane cofactor protein (MCP/CD46) |
| • proteins related to the complement factor H (CFHR1, 2, 3, 4, 5) |
| • component C3 |

Modified according to the reference (3)

The treatment method of choice for atypical hemolytic uremic syndrome (aHUS) is administration of plasma, either intravenously or by plasmapheresis protocol. Plasma infusion restores deficient proteins or improves impaired function of the existing ones, while plasmapheresis removes autoantibodies that inhibit complement regulatory proteins. However, long-term treatment with this form of therapy can be difficult, while in some patients there is no adequate therapeutic response to this treatment (34). Eculizumab has also been tested for treatment of patients with this disease (6, 34). During the first four weeks of the treatment, microangiopathic hemolytic anemia and thrombocytopenia persisted, while renal function deteriorated until there were symptoms for initiation of hemodialysis. However, further treatment with eculizumab led to significant improvement in parameters relevant for hemolytic anemia, platelet count and renal function (ten weeks after its initiation, hemodialysis was discontinued). In studies, after five months of treatment, the patients' serum creatinine levels were significantly decreased, and the sustaining dose was administered every other week (34).

In pathophysiological sense, there is a difference between atypical hemolytic uremic syndrome (aHUS) and C3 glomerulopathies (C3Gs) in the level of dysregulation. In aHUS, endothelial damage is the result of dysregulation of the alternative pathway (AP) in the solid phase, while in C3G, dysregulation occurs in the fluid phase. Dysregulation of the alternative complement pathway (AP) at the solid phase level makes atypical hemolytic uremic syndrome (aHUS) a more homogenous disease compared to C3 glomerulopathies (C3Gs). That is why the response to the anti-C5-antibody and the blockage of the C5 component by the drug are better in patients with aHUS. Variety of disorders in regulation of the alternative pathway (AP) fluid phase in C3 glomerulopathies (C3G) reflects in the fact that dysregulation of the C3-convertase is predominant in some cases, while in others the alternative pathway C5-convertase was dysregulated. A positive response to eculizumab can be expected only in the latter case (6).

Conclusion

The study of deposits by immunofluorescence microscopy (IF) has resulted in a new classification of membranoproliferative glomerulonephritis. This new classification scheme is based on the presence of the complement component C3 and/or immunoglobulins in the deposits. The entity of C3 glomerulopathy (C3G) involves membranoproliferative glomerulonephritis type II or dense deposit disease (MPGN II/DDD) and C3 glomerulonephritis (C3GN). Understanding the role that the complement alternative pathway (AP) and its dysregulation have in the pathogenesis of these diseases

has enabled the use of a new type of specific anti-complement therapy. Eculizumab is a recombinant monoclonal antibody to the complement component C5. The aim of eculizumab is to prevent cleavage of the complement component C5, and in further course, formation of the MAC complex and subsequent lysis of target cells. The present studies on use of eculizumab are giving positive, encouraging results, which raises hope for further treatment of these patients.

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