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UPDATE ON THE ROLE OF THE COMPLEMENT SYSTEM IN THE PATHOGENESIS OF THROMBOTIC MICROANGIOPATHIES

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Abstract

In *thrombotic microangiopathies* (TMA) pathological changes of the small vessels are present, which lead to ischaemia of the affected tissues, low platelet-count and intravascular haemolytic anaemia with fragmentocytes. Two main clinical syndromes belong to the group of TMAs: the *haemolytic uraemic syndrome* (HUS) with kidney failure, mainly affecting children, and the thrombotic thrombocytopenic purpura (TTP), starting primarily in adulthood. HUS can be clinically classified into two forms: typical and atypical HUS, the latter being caused by defective regulation of the complement system. However, acccording to recent studies, complement activation is also present in other TMAs. Complement activation products (C3a, C5a, MAC) are able to activate endothelial cells, which results in loss of their antiinflammatory and antithrombotic potential. Activation of the complement system can also lead to direct activation of platelets and granulocytes. The consequent endothelial damage and thrombosis forms the pathological basis of the TMAs. Exploring the exact pathogenetic role of the complement system in these diseases makes the development of new therapeutic methods possible.

Key words: TMA, aHUS, D+ HUS, TTP, complement system, endothelial cell activation.

The thrombotic microangiopathies

Thrombotic microangiopathy (TMA) is a descriptive name for the pathological changes of small vessels (arterioles and capillaries) with swelling of endothelial cells, their detachment from the basal membrane and thickening of the subendothelial layer, leading to thrombosis of the affected vessels [1].

The clinical features of TMAs consist of the following:

- ischaemic injury caused by the thrombotic occlusion of small vessels,
- low platelet count due to platelet consumption,
- haemolytic anaemia caused by the mechanical injury of red blood cells passing through the narrowed small vessels [1].

The group of TMAs consists of two main clinical syndromes: the *haemolytic uraemic syndrome* (HUS) and the *thrombotic thombocytopenic purpura* (TTP).

In HUS, the pathological changes affect mostly the kidney, the glomerular thrombosis often leading to cortical necrosis of the kidney [2]. Thus, besides the thrombocytopenia and microangiopathic haemolytic anaemia, kidney failure is characteristic of HUS. The typical form of the disease, associated with infection with shiga-like toxin producing microbes, affects mainly children [3, 4].

In the case of TTP, lesions in multiple organs are charateristic [2], thrombocytopenia and microangiopathic haemolytic anaemia are often accompanied by gastrointestinal and/or

neurological symptoms, but severe kidney failure seldom occurs. In contrast to typical HUS, the disease manifests mainly in young adulthood, predominantly in females [1, 3, 4].

The growing knowledge concerning the molecular patomechanism of these diseases has allowed a new, more detailed classification of TMAs (Table 1).

Table 1

Classification of TMAs

Advanced etiology	**Unknown etiology, TMA secondary to underlying disease**
• **TTP** as a result of ADAMTS13 deficiency (on account of inhibitor or ADAMTS13-mutation) • **Atypical HUS** as a result of impaired complement regulation • **D+ HUS** as a result of infection caused by verocytotoxin- or shiga-toxin-producing bacteria • HUS as a result of neuraminidase-producing *Streptococcus pneumoniae* or Influenza A infection • HUS on the grounds of defective cobalamine metabolism • Quinine induced HUS	• Malignancy-associated HUS/TTP • Transplantation-associated HUS/TTP • HUS/TTP caused by drugs • TTP/HUS associated with autoimmune diseases (SLE, APLS) • Pregnancy-associated TTP/HUS • HUS/TTP associated with DIC, sepsis • HIV-associated HUS/TTP • Other forms

(Based on*: Besbas, N., Karpman, D., Landau, D. et al.:* A classification of haemolytic uremic syndrome and thrombotic thrombocytopenic purpura and related disorders. Kidney Int., 2006, *70*, 423–431.)

In this article, we are going to discuss the most important forms of TMAs with advanced etiology (D+ HUS, aHUS, TTP).

D+ (diarrhoea positive) *HUS* is associated with microorganisms producing verocytotoxin 2 (shiga-like toxin, Stx) or shiga-toxin. In Europe, the most common cause of the disease is infection with Stx-producing enterohaemorrhagic *E. coli* (EHEC) of the O157:H7 serotype, although the outbreak in 2011, Germany, in which many adults were affected, was caused by the strain O104:H4. The D+ HUS is referred clinically as typical. The disease usually manifests after (bloody) diarrhoea and affects mostly children above the age of 2 years. Its prognosis is relatively good with supportive therapy, relapse is extremely rare [3, 4].

In contrast to the typical, D+ HUS, the *atypical HUS* (aHUS) evolves without a preceding diarrhoea episode, or other atypical factors are present, such as age less than 6 months, slow, insidious onset, asynchronous onset in family members, suspicion of a previous episode (unexplained anaemia or kidney failure in the anamnesis). Atypical HUS is caused by defective regulation of the complement activation, which is most frequently caused by mutations of genes encoding complement proteins or regulators. In a small number of cases, anti-factor H autoantibodies are the main causes of defective complement regulation. The first line therapy of atypical HUS is plasmapheresis with FFP substitution; however, a complement C5 inhibiting monoclonal antibody based drug, eculizumab (Alexion), was registered recently for this disease. Relapses are frequent, therefore the prognosis is poor, the disease often leads to end-stage kidney failure or even death [3, 4].

In the majority of cases clinically classified as *TTP*, the activity of the enzyme ADAMTS13 is severely decreased or absent. This enzyme processes the ULvWF (unusually large von Willebrand factor) macromolecules, which can bind platelets with high affinity. In the less frequent congenital form of the disease the ADAMTS13 gene is defective (mainly in the form of compound heterozygous mutations), resulting in abnormal structure or impaired secretion of the enzyme. In the acquired form accountable for most TTP cases, the deficient enzyme activity is due to inhibitory autoantibodies against ADAMTS13. The acquired form develops mainly in early adulthood, its prevalence is higher in women. The congenital form begins mostly in newborns, but can manifest at any age, and, interestingly, pregnancy is an important precipitating factor for disease develop-

ment in affected women. The first line therapy for TTP is plasmapheresis with FFP substitution. Steroid or other immunosuppressant therapy (i.e. cyclophosphamide or rituximab, as B-cell inhibitory therapies) can be used in addition to reduce the amount of inhibitors. Relapses in congenital TTP can be prevented by FFP infusions every 2 to 3 weeks [1, 3, 4].

The complement system plays an important role in the pathomechanism of atypical HUS; moreover, recent studies have revealed its importance in almost every form of TMAs. Therefore in the next part we summarize its structure and function, and then we review the linkage between this enzyme system and the various forms TMAs.

The complement system

The complement system is a system of recognition molecules, zymogen proteases, regulators and receptors, which turn into a cascade reaction after the specific stimuli and exert an important biological function, such as facilitation of fagocytosis, antigen presentation, inflammation and cell lysis (Figure 1). Constituting the humoral branch of the inborn immune response, its primary role is to protect against pathogens and altered self structures (5).

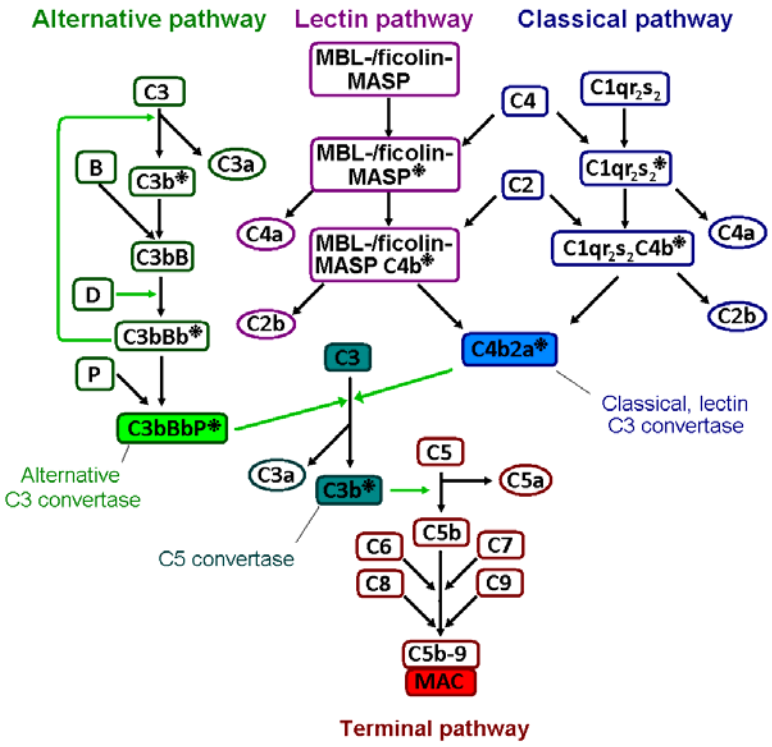

Figure 1 – Schematic presentation of the complement system

The central molecule of the complement system, *C3* is capable of *spontaneous activation*, forming activated C3b. C3b binds to extrinsic surfaces and becomes able to bind complement factor B. The latter molecule is then cleaved by factor D, resulting in the formation of the C3bBb enzyme complex, which is stabilized by a protein called properdin. This complex, known as the alternative C3 convertase, is able to cleave and activate further C3 molecules. The *alternative pathway* is in this manner not only able to start the complement activation but serves also as a positive feedback loop, amplifying the process [5].

Besides spontaneus activation, C3 can be cleaved by other enzyme complexes, too. The *classical pathway* is activated via the binding of the complement factor C1 to antibodies of the IgG or IgM type, apoptotic bodies, CRP molecules, bacteria or viruses. The conseqent conformational changes enable the $C1qr_2s_2$ enzyme complex to cleave C4 and then C2, thus forming the classical C3 convertase, the C4b2a complex [5].

The initial step of MBL-*lectin pathway*s is the binding of the MBL (mannose binding lectin) molecule to foreign carbohydrate structures (mannose, fucose, N-acetylglucosamin). Ficolins are recently discovered carbohydrate-detecting molecules also capable of starting the (ficolin)-lectin pathway. MBL or ficolins then activate the MASPs (MBL associated serine proteases 1 and 2), followed by the activation of C4 and C2. Therefore activation of C1 and MBL/Ficolin complexes lead jointly along a final, common pathway to the activation of C3 [5, 6].

Thus, each of the above recognition pathways leads to the activation of the C3 molecule, which, through binding of more C3b, forms the enzyme C5-convertase complex. This step and the following events are referred to as the *terminal complex*. The C5b fragment binds C6, C7 and C8 forming a pore on the marked surface. Binding of C9 molecules makes the pore bigger, leading to osmotic destabilisation and lysis of the affected cell. The C5b-9 complex is therefore called the membrane attack complex (MAC) [5].

The C3b and C4b fragments bound to the foreign surfaces facilitate the *antigen-presentation* and phagocytosis of the marked structures (*opsonisation*) [5].

The soluble complement products, C3a, C4a and C5a provoke inflammation by increasing the permeability of blood-vessels, recruiting inflammatory cells and activating them. Inflammation provides a milieu ideal for the eradication of pathogens, but can also lead to tissue injury [5].

As excessive complement activation can damage the structures of the organism and can lead to the consumption of the complement system, it is important to keep the process under control. Because of the self-enhancing nature of the reaction this is only possibe with tight regulation [5].

The C1 esterase inhibitor arrests the classical and lectin pathways by causing the dissociation and inhibition of the activated C1 and MBL-MASP (and supposedly the ficolin associated MASPs, too) complexes. Factor I is able to inactivate C3b and C4b in the presence of its cofactors. In the regulation of the alternative pathway factor H plays a role as a cofactor, while the analogous molecules of the classical and lectin pathways is C4b-binding protein. Besides the above soluble cofactors, membrane-bound cofactors also exist, these are the membrane cofactor protein (MCP) and the complement receptor 1 (CR1). The decay-accelerating factor (DAF) is also a membrane-bound complement regulator that facilitates the disintegration of the C3 convertase enzyme complexes [5]. Specific regulators of the terminal pathway are vitronectin and CD59.

How may complement activation provoke the development of TMA in general?

The key step in the development of TMAs is damage to the endothelial layer in small vessels and the subsequent loss of the antithrombotic properties of the endothelium. Activation of the complement system can provoke endothelial damage and prothrombotic changes in multiple ways.

The soluble complement activation products, the C3a and C5a are able to *directly* activate endothelial cells via their cell-surface receptors [5]. Sublytic MAC deposition can also evoke endothelial activation [7]. As a result of the activation, the amount of heparan sulphate molecules (responsible mainly for the antithrombotic character) decreases on the cell surface, the number of adhesion molecules responsible for the binding of leukocytes and platelets (P-selectin, ULvWF) increases in turn [7, 8]. The activated endothelial cells may also express tissue factor (TF) [7], which, besides the reduction in the amount of thrombomodulin [8], leads to loss of anticoagulant properties of the endothelium.

Complement activation may lead to endothelial damage in an *indirect* way as well, as it activates leukocytes, and increases the emission of free radicals. The complement-mediated over-expression of P-selectin makes the endothelial cells more vulnerable to the harm caused by the leukocytes [9]. Moreover, the activated endothelial cells contribute to the activation of leukocytes by secreting leukotrienes and cytokines [7].

Besides the prothrombotic changes to the endothelium, complement activation can also lead to thrombosis by *activating platelets*. C3a

and C5a can activate platelets via anaphylatoxin receptors C3aR and C5aR, sublytic MAC deposition is able to do so by destabilising the membrane potential [5, 10]. Microparticles and platelet-leukocyte complexes formed of activated platelets bear complement factors (C3, MAC) [11], and provide an ideal surface for complement activation, thus further enhancing the thrombotic processes detailed above.

The role of the complement system in the pathomechanism of different TMAs

Atypical HUS

In the development of aHUS the impaired regulation of the alternative pathway plays the primary role. The spontaneous activation of C3 amplified by the self-activating loop of the unregulated alternative pathway results in excessive activation and consumption of the complement system [3], which leads to manifestation of TMA through the mechanisms described above. The aHUS can therefore be regarded as the model-disease of complement over-activation on cell surfaces.

The number of identified mutations exceeds 120 in total, these are identifiable in approximately 50–60% of aHUS cases [12]. The most common among them, with even the worst prognosis in certain cases, are the alterations to the factor H gene, usually affecting the protein regions responsible for binding to the cell membrane or to the C3 molecule [4]. The second most frequent variants are the mutations of the MCP. These have a relatively good prognosis, only seldom leading to end-stage kidney failure, and, if so, the risk of relapse after the transplantation is the lowest among mutations leading to aHUS. This is not suprising however, taking into account that in the transplanted kidney the MCP molecules of the donor are expressed [4]. Factor I mutations usually affect the catalytic regions of the enzyme [12]. Less frequently, gain-of-function mutations of alternative pathway complement activators (C3, factor B) can be identified in the background of the disease [3]. In a further part of the cases, the factor H deficiency is caused by inhibiting autoantibodies, which, like the mutations, perturb the binding regions of the molecule [12].

The penetrance of mutations is generally low, around 50%, indicating that further triggering factors are needed to provoke the disease [3]. TMA occurs often following infectious diseases or pregnancy. These states can cause complement activation, and, if it reaches a critical level, the impaired regulation becomes unable to control the reaction, leading to over-activation of the complement system resulting in aHUS [12].

D+ HUS

Activation of the complement system is also detectable in D+ HUS [8].

During the EHEC infection causing D+ HUS, the pathogen itself does not reach the circulation, but the Stx produced by it does and is able to activate complement. Furthermore, the Stx enters the cells following the binding to the glycolipid called globotriosylceramide (Gb_3). This glycolipid is expressed especially by glomerular endothelium, which helps to explain the involvement of the kidneys in D+ HUS [13]. A sublethal amount of Stx induces typical changes of the endothelial cells. The expression of some adhesion molecules, like P-selectin, increases. P-selectin supports complement activation, as it is able to bind C3 to the cell surface [8].

C3a and C5a produced during complement activation in response to Stx also lead to inflammation and prothrombotic changes of the endothelium via previously delineated mechanisms [8].

The role of the complement system in the pathogesesis of the disease is supported by *in vivo* studies as well. In a murine model of D+ HUS, severe kidney involvement was absent in factor B deficient mice, and administration of C3a receptor antagonists limited glomerular thrombosis [8].

TTP

According to the new classification of TMAs based on molecular etiology, TMA forms caused by deficiency of the enzyme ADAMTS13 belong to this group [3, 4]. The function of ADAMTS13 is the cleavage of the ULvWF. ULvWF is a macromolecule secreted by endothelial cells and platelets upon certain stimuli. As this molecule is highly adhesive to GPIb receptors of platelets, its permanent presence on the endothelial surface as a conseqence of defective cleavage leads to platelet adhesion and aggregation [14].

Severe deficiency of the ADAMTS13 enzyme, however, does not always cause TTP, as is confirmed by the case of the many TTP patients in remission despite of extremely low or deficient enzyme activity. Impaired or deficient ADAMTS13 activity is a predisposing factor, but further triggering events are needed for the evolvement of the disease. TTP develops freqently following conditions with complement activation and inflammation, which suggests the causal role of the complement system in the pathogenesis of the disease [15].

In a study investigating complement activation in TTP, levels of certain complement activation products such as C3a and sMAC (soluble MAC) were found to be elevated during the acute phase of the disease [15]. In another research, C3b and MAC deposition was observed on human microvascular endothelial cells following treatment with serum of acute TTP patients [9].

In the latter experiment it was also shown that the serum taken in the acute phase of the disease was capable of activating leukocytes and diminishing the antithrombotic and antiinflammatory properties of endothelial cells. All of these effects could be prevented by inactivating the complement system, which confirms its role in the process [9].

P-selectin expression was also increased on the surface of endothelial cells in response to acute TTP serum administration. [9] This molecule is able to enhance the complement activation by binding C3 [8]. As P-selectin and ULvWF are stored together in the Weibel-Palade bodies of endothelial cells, an increase of P-selectin expression means that the ULvWF secretion is also increased [16]. The primary role of ULvWF in the prothrombotic changes of endothelial cells was experimentally proven [9].

If an increase in ULvWF secretion caused by complement activation exceeds the cleavage rate by the deficient ADAMTS13 enzyme, the adhesive macromolecules accumulate, which results in platelet-aggregation. The activated platelets and platelet aggregates provide an ideal surface for further complement-activation, enhancing the process [11]. The forming of platelet thrombi throughout the vasculature leads to the evolvement of TTP.

New perspectives in TMA therapy

In the light of the above facts, the complement system takes part in the development of the characteristic pathological changes in TMAs via its ability to cause endothelial injury and platelet activation. Therefore the modulation or inhibition of the complement system seems to be a promising possibility in the treatment of TMAs.

Eculizumab is a monoclonal antibody, which binds to C5 promoting its cleavage, and thus blocking the terminal pathway and forming of C5a. It was shown to be effective in the treatment of atypical HUS, even in cases resistant to plasmapheresis. Therefore, this drug now belongs to the registered therapies of aHUS in the US and EU, although it is not widely used because of its high price and limited availability [17]. Although eculizumab is not registered for the treatment of other TMAs, it was still proven to be useful in some cases of D+ HUS and TTP resistant to routine therapy [18, 19]. We must add, though, that the results acquired during the German EHEC outbreak in 2011 indicate only restricted effectiveness of eculizumab in the treatment of D+ HUS patients (as compared to standard, guideline-based therapy) [12].

Abbreviations

ADAMTS13	von Willebrand cleaving protesase (a disintegrin-like and metalloprotease with thrombospondin type 1 repeats 13)
aHUS	atypical haemolytic uraemic syndrome
APLS	antiphospholipid syndrome
C3aR	C3a receptor
CR1	Complement receptor 1
D+ HUS	diarrhoea positive haemolytic uraemic syndrome
DAF	decay accelerating factor
DIC	disseminated intravascular coagulation

EHEC	enterohaemorrhagic Escherichia coli
FFP	fresh frozen plasma
Gb3	globotriosylceramide
GPIb	glycoprotein Ib
HIV	human immundeficiency virus
HUS	haemolytic uraemic syndrome
MAC	membrane attack complex
MASP	MBL-associated serin-protease
MBL	mannose binding lectin
MCP	membrane cofactor protein
P-HUS	Pneumococcus associated haemolytic uraemic syndrome
SLE	systemic lupus erithematodes
sMAC	soluble membrane attack complex
Stx	shiga-like toxin, verocytotoxin
TF	tissue factor
TMA	thrombotic microangiopathy
TTP	thrombotic thrombocitopenic purpura
ULvWF	unusually large von Willebrand factor

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Р е з и м е

НОВИ СОЗНАНИЈА ЗА УЛОГАТА НА КОМПЛЕМЕНТНИОТ СИСТЕМ ВО ПАТОГЕНЕЗАТА НА ТРОМБОТИЧНА МИКРОАНГИОПАТИЈА

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Кај тромботична микроангиопатија (ТМА) патолошките промени во малите садови се присутни и тие водат до исхемија на афицираните ткаења, тромбопенија и интраваскуларна хемолитична анемија со фрагментоцитоза. Два главни клинички синдрома припаѓаат на групата на ТМА: хемолитично уремичен синдром (ХУС) со бубрежна слабост којшто, главно, ги афицира децата и тромботична тромбоцитопенична пурпура (ТТП), која се манифестира кај возрасните. ХУС може клинички да се класифицира во 2 форми: типичен и атипичен ХУС со тоа што последниот е предизвикан поради дефект во регулација на комплементарниот систем. Меѓутоа, според наодите на неодамнешните студии, активација на комплементот, исто така, е присутна и кај други ТМА. Активационите продукти на комплементот (C3a, C5a, MAC) се способни да ги активираат ендотелните клетки, што резултира во губење на нивниот антиинфламаторен и антитромботичен потенцијал. Активација на комплементниот систем исто може да води до директна активација на тромбоцитите и гранулоцитите. Последователното ендотелно оштетување и тромбозата ја сочинуваат патолошката основа на ТМА. Испитувањето на егзактната патогенетска ролја на комплементниот систем кај овие заболувања овозможува развој на нови терапевтски методи.

Клучни зборови: ТМА, aHUS, D+ HUS, ТТР, комплементарен сиистем, ендотелна клеточна активација.