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The role of the complement system in ocular diseases

Rola układu dopełniacza w chorobach oczu

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Summary: The complement system is a group of proteins involved in the innate immune response. Its activation works as a cascade, in which each component activates the next one, and results in the formation of the membrane attacking complex initiating lytic cell death. Thus, the complement system plays an important role in immune defense against infections.
The complement system is constantly activated in a healthy eye, releasing small amounts of active products. Regulatory proteins of the system maintain the low level of activation, thereby preventing autodestruction of ocular tissues. Recent studies have shown that excessive and uncontrolled activation of the complement system may play a key role as a cause of various widespread ophthalmic diseases, such as inflammatory corneal diseases, glaucoma, uveitis, and age-related macular degeneration.
The article presents a review of current literature on the involvement of the complement system in the pathogenesis of the abovementioned diseases.

Key words: complement system, inflammatory diseases of the cornea, glaucoma, uveitis, age-related macular degeneration.

Streszczenie: Układ dopełniacza jest grupą kilkudziesięciu białek biorących udział w nieswoistej odpowiedzi immunologicznej organizmu. Aktywacja tego układu zachodzi w sposób kaskadowy, czyli każdy kolejny składnik aktywuje następny i prowadzi do powstania kompleksu atakującego błonę i inicjującego śmierć lityczną komórki. W ten sposób układ dopełniacza spełnia ważną funkcję obronną w walce z zakażeniami.
W prawidłowej gałce ocznej jest on stale aktywowany, uwalnia zatem niewielkie ilości aktywnych produktów. Białka regulatorowe tego układu utrzymują tę aktywację na niskim poziomie, zapobiegając tym samym autodestrukcji tkanek oka. Wyniki badań z ostatnich lat pokazały, że nadmierna i niekontrolowana aktywacja tego układu może również odgrywać kluczową rolę wśród przyczyn szeroko rozpowszechnionych chorób oczu, takich jak choroby zapalne rogówki, jaskra, zapalenia błony naczyniowej czy też zwyrodnienie plamki związane z wiekiem.
W niniejszym artykule przedstawiono aktualny przegląd piśmiennictwa dotyczący udziału układu dopełniacza w patogenezie ww. jednostek chorobowych, a także roli, jaką odgrywa w prawidłowej gałce ocznej.

Słowa kluczowe: układ dopełniacza, choroby zapalne rogówki, jaskra, zapalenia błony naczyniowej, zwyrodnienie plamki związane z wiekiem.

Introduction

The complement system is an important component of the innate immune response. It is a collection of proteins and its associated receptors, which play a key role in the defence of the body against microorganisms and the control of the inflammatory process (1). The activation of this system takes place in a cascade reaction, where each further component activates the next one. The three pathways involved in the activation of the complement system (i.e. the classical, lectin and alternative pathways) all lead to the formation of a number of anaphylatoxins and eventually membrane attack complexes (MAC), which can damage cells thus causing their death (1) (Fig. 1). The activation of the classical complement pathway is triggered by specific antibody binding to antigens, which is the key factor in virus inactivation and elimination of the infected cells, and which begins with the formation of antigen-antibody complex present in the serum of the C1q molecule. The Lectin pathway activation takes place via a mannose-binding lectin (MBL) molecule, which binds to the oligosaccharides on pathogen surface (1). The alternative pathway, which is activated spontaneously as a result of the direct microbial exposure of the C3 component molecules, is a lot faster. It is the key element of the antibacterial and antifungal activity (1).

The low specificity of the complement system leads to a range of side effects such as damage to own tissues and cells. Complement regulatory (CREG) proteins are responsible for limiting such damage by inhibiting complement system cascade at different stages, which enables the body to effectively fight an infection without damaging its own tissues (1). CREG proteins are divided into two classes – the soluble, present in tissue fluids, and those expressed on the surface of host cells. The first category includes complement factor H (CFH), Factor H-like pro-

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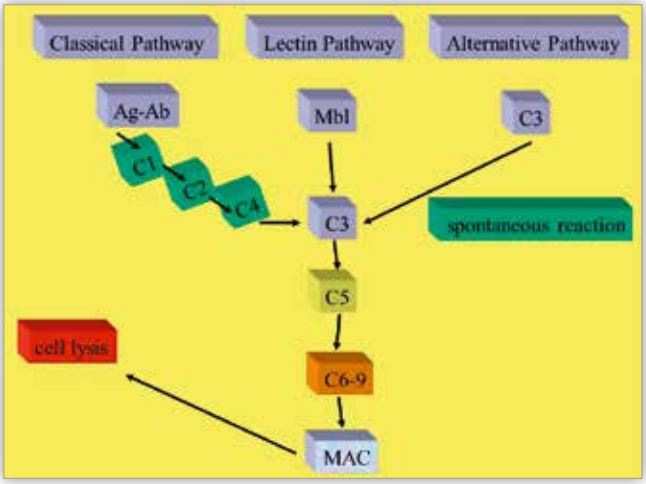

Fig. 1. Schematic diagram of the complement cascade. Explanation of abbreviations: Ag-Ab (antigen-antibody complex), MBL (mannose binding lectin), MAC (membrane attacking complex).

Ryc. 1. Schemat aktywacji układu dopełniacza. Wyjaśnienie skrótów: Ag-Ab (kompleks antygen-przeciwciało), MBL (lektyna wiążąca mannozę), MAC (kompleks atakujący błonę).

tein-1 (FHL-1) and C4 binding protein (C4BP), whereas the second category includes decay-accelerating factor – CD55 (DAF) and membrane cofactor protein – CD46 (MCP). which all are membrane factors, as well as homologous restriction factor – CD59 (HRF 20), complement receptor 1 (CR1), CD35, and complement receptor 2 (CR2). The disturbances in the concentration or membranous expression of regulatory proteins leads to a number of pathologies, which affect the eye, as well. A significant part of this publication, which refers to the activation process and complement system involvement in various human diseases, was obtained from the Department of Transplantology and the Central Tissue Bank of the Medical University of Warsaw (2–5).

This article provides an overview of the current literature on the role of the complement system in a healthy eye and in eye diseases, with particular emphasis on: keratitis, uveitis, glaucoma and Age-related Macular Degeneration (AMD).

Complement system – its role in a healthy eye

Just like the tissues of the central nervous system, ocular tissue is considered an immunologically privileged site, due to the lack of lymphatic system, corneal avascularity, the presence of the blood-retina barrier and specific mechanisms inhibiting the complement system (6). In a healthy eye, the complement system is constantly maintained at a low activation level, as it acts as the first line of defense against pathogens, additionally being a key mediator of the immune response (7). Numerous studies have confirmed the presence of classical and alternative pathway complement fragments (the activation effect) and of regulatory proteins in tears, aqueous humor, vitreous body and eye tissues (7, 8). Along with their direct antimicrobial effect, active fragments of the complement system formed as a result of its constant activation in the eye, inhibit excessive T-cell response and protect ocular tissues against autodestruction (9). The complement system present in tears is an important element of antimicrobial protection, as it prevents bacterial growth on the tear film (10).

The complement system and inflammatory diseases of the cornea (keratitis)

The cornea is the part of the eye directly contacting the outside environment, thus constantly exposed to dangerous microorganisms as well as biological and chemicals substances. The body is equipped with a number of mechanisms to protect the cornea against microbial growth, such as the loss of transparency and the complement system. It has been proven that fragments of the classical and alternative activation pathways (including C3, C3 degradation products and MAC) are constantly present in normal cornea at low concentrations (7, 11). A massive activation of the complement system does, however, occur during an infection and as a part of an immune response (8). Studies have shown that the complement system plays a key protective role against the infection of the cornea by *Pseudomonas aeruginosa* and also against corneal ulcers caused by the Gram-negative bacteria (12).

On the other hand, excessive activation of the complement system could lead to tissue damage and, as a consequence, the loss of its transparency. Hence, the role of the discussed regulatory proteins is of great importance. It has been shown that membrane proteins, such as MCP, DAF, CD59 and Crry, are frequently found in the corneal epithelium of the limbus and its central portion (8). During the continued exposure of the cornea to external factors including pathogens, a high expression of regulatory proteins in the epithelium prevents damage to own tissues (7, 8, 13). Some bacterial enzymes, such as phospholipase, can eliminate membrane proteins from the corneal surface, which can in turn lead to its damage, through excessive and uncontrolled activation of the complement cascade (13).

The complement system and uveitis

Uveitis is defined as an inflammation of the uvea. Inflammation of the retina and its vessels also belongs to this group of diseases, due to the integration of that tissue or layer with the choroid. The most common type is anterior uveitis, which is usually of an idiopathic nature. It is estimated that each year, approximately 17% of patients with an active disease process will progress into either transient or permanent loss of vision (14).

For many years, T-cells were considered a key mediator of uveitis. Recent studies have, however, indicated an important role of complement activation. Animal studies have shown a significant increase of iC3b (a marker, the presence of which indicates the activation of the complement system) in the eye during the severe symptomatic phase of the disease (15). On the other hand, complement cascade inhibition in rats reduced disease symptoms and decreased inflammation. The same was also evident in laboratory *in vitro* studies, where decreased expression of inflammatory cytokines was demonstrated (16). Furthermore, polymorphisms in complement genes increases the risk of uveitis (17).

The membrane proteins also play an important role in this group of diseases. The expression of Crry and CD59 proteins is upregulated in response to active inflammation, which in turn reduces inflammatory response and protects ocular tissue against damage (Fig. 2). It was also observed that inhibiting membrane proteins or reducing their surface expression causes a rapid initial onset, a more severe course and finally a delayed resolution of uveitis (18).

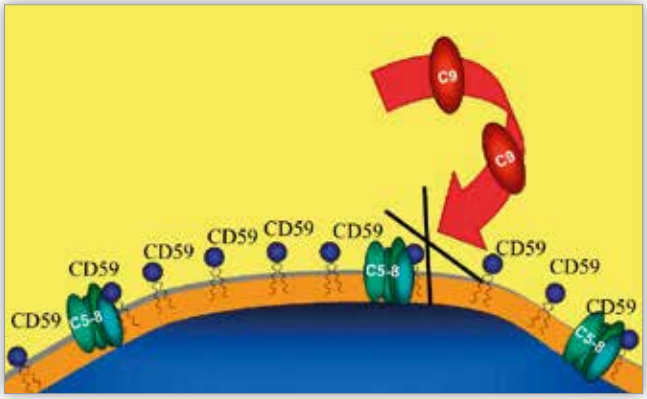

Fig. 2. Inhibition of the complement cascade system by increased expression of a regulatory protein CD59 on the cell surface.
Ryc. 2. Hamowanie kaskady układu dopełniacza przez wzrost ekspresji białka regulatorowego CD59 na powierzchni komórki.

The complement system also seems to play a major role in posterior uveitis. The study in mice demonstrated that the animals devoid of the C3 protein presented with a less severe course of the disease and less often suffered from uveitis (19). In the same experiment, it was confirmed that the Crry protein reduced the incidence and severity of the disease (19). In another study, C5 fission contributed to the total expression of experimental autoimmune uveoretinitis, and the selective C5 blockage suppressed the disease (20).

The complement system and glaucoma

Glaucoma is a group of diseases characterised by the progressive and irreversible damage to the optic nerve, caused by degeneration of retinal ganglion cells and their axons. The end-stage of the disease leads to blindness (21). Although the exact cause of the disease is not known, clinical and experimental studies in recent years have shown an important role of immune processes in glaucomatous optic neuropathy, demonstrating that uncontrolled complement activation, overactive T-cells as well as the presence of autoantibodies can promote damage to ganglion cells and their axons in glaucoma (22). Histology studies of human eye tissues and *in vivo* animal research confirmed the presence of C1q and C3 fragments as well as MAC in the retina of glaucomatous eyes (23). Furthermore, mice devoid of C1q fragment showed no loss of optic nerve ganglion cells, which further supports the crucial role of the C1q fragment in the etiopathogenesis of the disease (23). Also, C1 inhibition was sufficient to maintain dendritic and synaptic architecture (24). The C5 complement fragment essential for the formation of the complex initiating lytic cell death appears to be an important element (25). Although its actual mechanism of action in glaucoma is not entirely clear, significant MAC deposits have been found in retinal ganglion cells of eyes with glaucoma, which confirms possible significant impact of C5 on the development of the disease (25). The activation of the classical complement pathway in mice corroborated its harmful effect on the optic nerve (25).

The role of the complement system in the pathogenesis of glaucomatous optic neuropathy is complex. The effects of its activation has been shown to depend to a large extent on disease severity and on the cells, which the active fragments act on. In early stages of glaucoma, the complement system can play a positive role in protecting the optic nerve by inhibiting ganglion cell death. It also helps remove apoptotic cells, thus reducing inflammation (26).

The complement system and Age-related Macular Degeneration

Age-related Macular Degeneration (AMD) is a disease involving a loss of central vision as a result of damage to the macula, which is responsible for fine vision. There is a dry (atrophic) and wet (neovascular/exudative) form of the disease. The etiology of the disease is multifactorial. Along with age and environmental factors, the involvement of genetic, immune and inflammatory risk factors has been suggested (27).

Current research shows that excessive complement activation via its alternative route, which occurs in patients with AMD, plays a crucial role in the development of the disease (28, 29). The presence of the single nucleotide polymorphism of the gene encoding for the complement factor H (Y402H) has been demonstrated to be an important element in the pathogenesis of AMD (30). CFH prevents uncontrolled activation of the complement system by inactivating C3 convertase of the alternative pathway, thus inhibiting MAC formation and inflammation (Fig. 3). Conversion of tyrosine to histidine at locus 402 of the CHF encoding gene is associated with excessive and uncontrolled complement activation, the onset of inflammation and further increase of AMD risk (31).

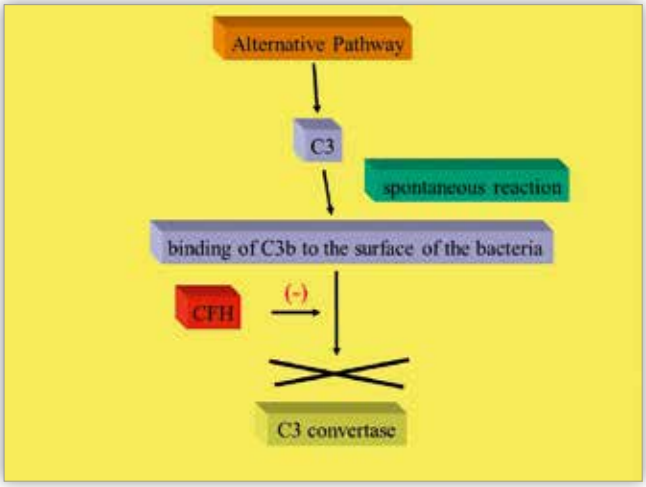

Fig. 3. Inactivation of the alternative pathway C3 convertase by factor H (CFH).
Ryc. 3. Inaktywacja konwertazy C3 drogi alternatywnej przez czynnik H (CFH).

Later studies have identified other variants of genes encoding for the complement pathway, including complement factor I (CFI) and complement factor B (CFB) and complement components 2, 3 and 9 (C2, C3, C9) (31, 32).

One of the early signs of dry AMD is the presence of drusen, yellowish deposits located between Bruch's membrane and retinal pigment epithelium (RPE), the composition of which can be studied using immunohistochemical methods. Drusen consist of retinal pigment epithelium residues and a number of immune cells and molecules, such dendritic cell fibres, major histocompatibility complex (MHC) class II molecules and com-

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plement cascade components: activators (C3a, C5a), inhibitors (CFH) and MAC (33). The composition of drusen tends to suggest a significant role of inflammation in the pathogenesis of this disease. Unfortunately, at present no cure is available for dry AMD. However, there are some anti-complement drugs in clinical trials (34).

The exudative form of AMD leads to choroidal neovascularization (CNV) within choroidal capillaries, the detachment of the serous or fibrovascular pigment epithelium and the final stage involving disciform scar formation. Newly formed abnormal vessels are a source of exudate and hemorrhage, which impair the photoreceptor cell layer. Neovascularisation is stimulated by vascular growth factors, with Vascular Endothelial Growth Factor (VEGF) being the key regulator. Murine model of laser-induced CNV demonstrated the key significance of alternative complement pathway activation in this process. It has also been proven that the activation of the complement cascade is due to increased activity of the complement factor B and a reduction in the expression of CD59 and factor H (35). A recently published study confirmed the excessive activation of complement system in patients with exudative AMD (36). The study, however, failed to determine a possible cause of this excessive and uncontrolled activation. Eventually, experimental research has demonstrated that inhibition of complement system can suppress laser-induced CNV (37, 38).

Summary

The complement system plays an important role in the etiopathogenesis of eye diseases. Its excessive activation, along with regulatory protein deficiencies or abnormalities can contribute to the development of corneal inflammatory diseases, uveitis, glaucoma and AMD, which has been confirmed by numerous studies discussed in the current paper.

Despite plethora of research data available, the exact mechanisms leading to the uncontrolled complement activation still remain unknown. Their understanding will enable effective prevention and treatment of eye diseases.

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