New insight into the role of the complement in the most common types of retinopathy-current literature review

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Abstract

Pathological neovascularisation, which is a critical component of diseases such as age-related macular degeneration (AMD), diabetic retinopathy (DR) and retinopathy of prematurity (ROP), is a frequent cause of compromised vision or blindness. Researchers continuously investigate the role of the complement system in the pathogenesis of retinopathy. Studies have confirmed the role of factors H and I in the development of AMD, and factors H and B in the development of DR. Other components, such as C2, C3, and C5, have also been considered. However, findings on the involvement of the complement system in the pathogenesis of ROP are still inconclusive. This paper presents a review of the current literature data, pointing to the novel results and achievements from research into the role of complement components in the development of retinopathy. There is still a need to continue research in new directions, and to gather more detailed information about this problem which will be useful in the treatment of these diseases.

Key words: age-related macular degeneration; diabetic retinopathy; factors of the complement system; retinopathy of prematurity; single nucleotide polymorphism

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INTRODUCTION

Pathological neovascularisation is a critical component of retinopathic diseases, including age-related macular degeneration (AMD), retinopathy of prematurity (ROP) and diabetic retinopathy (DR)\(^1\)\(^-\)\(^3\). These conditions are primarily caused by immune system disorders, particularly those affecting anti-inflammatory mechanisms and innate immunity. The main role of the immune system is to protect the host against exogenous and endogenous factors, and to maintain homeostasis. Abnormal function or dysregulation of the immune system may lead to various immune diseases such as infections and autoimmune disorders\(^4\). The complement system is an important element of innate immunity. The role of the complement system in the development of immune response is well known. However, the latest scientific discoveries also emphasize its involvement in the processes of tissue remodelling. Nevertheless, there is little evidence of the regulatory effect of complement components on neovascularisation. Studies suggest that the components of the complement system may stimulate or inhibit pathological angiogenesis\(^5\).

The complement system is an integral part of the nonspecific, innate immune response. It consists of more than 30 plasma proteins, membrane-bound receptors, and regulatory proteins\(^6\), whose main role is to defend the host against infections and modulate antigen-specific immune and inflammatory response\(^7\). The complement system can be activated in three pathways: classical, alternative and lectin-dependent. Cascade activation of components of individual pathways leads to the synthesis of the membrane attacking complex (MAC), which is able to build into the cell membrane and form a channel therein, resulting in cell lysis (death).

Under physiological conditions the alternative pathway is activated continuously by low-grade hydrolysis of C3, while undesired effects are controlled by various endogenous soluble and membrane-bound inhibitory molecules. The key steps in the full activation of the complement system is the cleavage of its C3 and C5 components. The three pathways of complement system activation rely on different mechanisms in which C3 and C5 convertases are generated. The classical pathway can be triggered by immune complexes or by substances such as C-reactive protein, and complement components including C1, C2, C4 and C3\(^7\). The alternative pathway is rapidly activated and does not rely on antibodies and complement amplification. This pathway is activated directly by C3 interacting with certain activating surfaces (for example,
zymosan, lipopolysaccharides) and involves components such as C3b, factor B, factor D and properdin[8]. The lectin pathway does not rely on antibodies and the formation of immune complexes; it is activated by collectins present in plasma, for example, by the binding of mannose-binding lectin (MBL) and pulmonary surfactant proteins A and D to mannose and N-acetylgalcosamine residues present in bacterial cell walls[9]. C3 and C5 convertases in the classical and lectin pathways consist of components C4bC2a and C4bC2aC3b, respectively, while in the alternative pathway C3 convertase consists of C3bBb, and C5 convertase of C3bBbC3b[10].

Under normal conditions, activation of the complement system is strictly controlled by plasma and membrane-bound regulatory proteins. Membrane-bound proteins include the decay-accelerating factor, DAF (CD55), the complement cofactor protein, MCP (CD46), the complement receptor, CR1 (CD35) and the membrane inhibitor of reactive lysis, MIRL (CD59). Plasma factors include C1-inhibitor (C1INH), C4-binding protein (C4bp), factor H (CFH), factor I (CFI) and protein S[11]. Research on the human retina and retinal pigment epithelium (RPE)/choroid identified the presence of mRNA for complement system components such as C1q, C1r, C2, C3, C4, factor B and factor H[12]. Cells of retinal microglia and the RPE seem to be the major source of the expression of complement system components in the retina[13]. The retina/RPE/choroid complex also expresses regulatory proteins: the presence of MCP has been confirmed in the basal part of RPE, CR1 in ganglion cells and photoreceptors, MIRL in retinal nerve fibres, and factor H in pigment epithelial cells and choriocapillaries[14-16].

The retina is an anatomical structure that plays an important role in the mechanism of vision, and has immune privilege. There are special mechanisms in the eye to protect the retina from exogenous and endogenous hazards, decreasing the risk of infection but also preventing abnormal immune response, thus reducing the risk of damage to the retina. These include the blood-retina barrier (BRB) formed by the close connection between the endothelial cells and RPE[17], as well as an advanced mechanism regulating the immune system, which involves retinal cells, including various neurons and RPE cells[18-19], able to express immunomodulators inhibiting the activation of immune cells. An additional factor is the absence of lymph vessels. When the BRB is intact, the circulating complement proteins are unable to penetrate to the retinal neurons. Nevertheless, retinal cells, including neurons, microglia cells and RPE cells, can produce various complement proteins (for example, Clq/a/b, C1s, Cr1, C2, C4, Cfb, Cfd, C5 and C7) and complement regulatory molecules [for example, Serping-1, MCP (CD46), DAF (CD55), CFH, CFI and CD59][13,16,20-21].

Retinal neovascularisation, defined as the formation of new retinal blood vessels in an abnormal configuration, occurs in various ischaemic disorders of the retina, such as DR, ROP[22], and AMD. Vascular proliferation is associated with serious complications, often leading to vitreous haemorrhage, retinal detachment or neovascular glaucoma with subsequent loss of vision. The Complement System and Age-related Macular Degeneration AMD is the most common cause of irreversible blindness in developed countries[23]. It is a multifactorial disease, and aging and genetic and environmental factors are involved in its pathogenesis[24]. Despite the fact that macular damage can occur in many pathways, it is known today that inflammation plays a major role in the pathogenesis of AMD[12,25]. There is growing evidence that the complement system, the main humoral component of the innate immune system, plays a crucial role in the pathology of AMD[26]. Significant activation of the complement system via the alternative pathway contributing to the progression of AMD was reported[27]. Other studies confirmed the genetic link between the classical activation pathway and AMD. Different complement components, including C3, C5b-9, CFB and CFH, were found both in drusen and in AMD lesions[28]. Complement regulatory proteins, i.e. CR1, MCP and vitronectin, were detected in drusen[29], and factor H and FHL-1 protein in the macular region in patients with the early stage of AMD[20]. Studies have also revealed increased plasma levels of C3a, C3d, Bb and C5a in patients with AMD[30-31].

Edwards et al.[32] demonstrated a significant association between polymorphism in the gene encoding factor H of the complement system and the development of AMD (SNP rs 1061170; p.402Y>H). The presence of at least one histidine at amino acid position 402 is associated with a 2.7-fold increase in the risk of AMD, and may account for up to 50% of risk of AMD[32]. The Y402H variant is also associated with the risk of AMD and the development of AMD (SNP rs 1061170; p.402Y>H). The presence of at least one histidine at amino acid position 402 is associated with a 2.7-fold increase in the risk of AMD, and may account for up to 50% of risk of AMD[32]. The Y402H mutation may reduce the affinity of CFH to heparin, which suppresses the inhibition of C3b and enhances the alternative pathway of complement activation[31]. The Y402H variant is also associated with reduced neutralization of the pro-inflammatory product of lipid peroxidation, malondialdehyde, leading to the induction of IL-8 and TNF-α[34]. The concentration of CRP, an acute-phase protein and the marker of inflammation, involved in complement-mediated phagocytosis, is elevated in AMD patients, and specifically in CFH homozygotes with the “at-risk” 402HH variant[35-36]. Recent findings indicate that the short consensus repeat region of CFH (SCR7) binds with fibulin-3 (EFEMP-1, 2p16), a protein detected in drusen and an established locus for an inherited type of familial drusen[37]. The alternative splicing to FHL (factor H-like 1), also encoding SCR7, as well as the associated CFHR1 and CFHR3 genes involved in the pathogenesis of AMD, additionally supports the relationship between CFH and the development of AMD[38].
The complement system and retinopathy

Lauer et al \cite{40} demonstrated that the Y402H polymorphism affects CFH surface recruitment by monomeric C-reactive protein (123260) to necrotic RPE cells. Reduced monomeric CRP binding of the CFH H402 variant results in complement activation, generation of anti-inflammatory mediators, onset of inflammation, and pathology \cite{39}.

Raychaudhuri et al \cite{40} showed that a rare variant of factor H, Arg1210Cys, is strongly linked with AMD, regardless of Tyr402His polymorphism. Moreover, carriers of this variant had an earlier onset of AMD. The Arg1210Cys variant is associated with a 47-fold greater risk of developing AMD \cite{41}. Seddon et al \cite{42} investigated the role of rare variants of genes linked with advanced AMD. They sequenced the exons of 681 genes associated with AMD in subjects from the study group and in controls. The study revealed that 7.8% of subjects with AMD and 2.3% of controls were carriers of rare missense CFI variants. The researchers also found a significant association for missense variants of rare alleles in genes other than CFI, in addition to associations between alleles of the C3 and C9 genes. The allele of C3 encoding Gln155 is responsible for resistance to proteolytic inactivation by CFH and CFI. These findings indicate the loss of C3 protein regulation and excessive activation of the alternative complement pathway in the pathogenesis of AMD \cite{43}. The Lys155Gln variant is associated with a 3-fold greater risk of developing AMD \cite{41}. Analysis of C9 gene sequences in a study involving 1676 cases and 745 controls showed that the Pro167Ser variant is associated with increased risk of developing AMD. The involvement of the Pro167Ser variant in AMD was also confirmed in another study \cite{44}, where it was associated with a 1.7-fold greater risk of developing AMD \cite{41}. Carriers of this mutation have a higher serum concentration of factor C9, and it is suggested that this may lead to increased complement activation, which through cell lysis contributes to the degenerative process observed in AMD \cite{41}. The authors of this study also concluded that reduced serum concentrations of complement components are linked with the degradation of C3b in carriers of rare CFI variants (Gly119Arg, Leu131Arg) but not carriers of CFH variants, suggesting the influence of CFH variants on the functional activity of factor H but not its serum concentration. Carriers of CFH (Arg175Gln and Ser193Leu) and CFI (Gly119Arg and Leu131Arg) variants have impaired ability to regulate complement activation, and they may benefit more from a therapy inhibiting the complement system than patients with AMD in general. Other researchers also confirmed the association between polymorphisms of factor C3 (p.102R>G, p.155K>Q, p.314P>L) and the incidence of AMD \cite{45}. A research team led by van de Ven \cite{46} investigated a rare highly penetrant missense mutation in the CFI gene (p.119Gly>Arg) associated with high risk of AMD. The study found that the degradation of the C3b factor in plasma and serum was lower in carriers of this mutation than in controls. The Gly119Arg variant is associated with a 5-fold greater risk of developing AMD \cite{41}. An increased effect of the gene encoding factor H (including the non-coding variant in the CFH gene) on the development of AMD was found by Maller et al \cite{47}. Gold et al \cite{48} demonstrated a relationship between genes encoding factor B and component C2 and the development of AMD. Haplotype analysis identified a statistically significant risk haplotype (H1) and two protective haplotypes. The L9H variant of factor B (rs 4151667 SNP) and the E318D variant of component C2 (rs9332739 SNP) (H7) significantly reduce the risk of AMD. A combined analysis of the factor B and component C2 variants, as well as variants of factor H, indicates that change in two loci can predict the clinical outcome in 74% of subjects affected by the disease and in 56% of controls. Findings from these studies suggest that defective components of the alternative pathway provide protection, while defective regulatory proteins of this pathway, such as factor H (CFH), increase the risk of AMD. This information expands and improves the understanding of the genetic risk of AMD \cite{49}. Butler et al \cite{40} also reported the protective nature of alleles of component C2 (E318D) and factor B (R32Q).

Genetic variants with minor allele frequency (MAF) >5% of the population of genes encoding CFH, C2/CFB, C3 and CFI of the complement system are together responsible for 40%-60% of AMD heritability \cite{50}. Ennis et al \cite{41} indicated a genetic link between the SNP C1-inhibitor (also known as SERPING1) with AMD, suggesting that the classical complement activation pathway at least in part contributes to the progression of AMD. This has been confirmed by experimental animal studies on laser-induced CNV, where the functional alternative pathway is necessary to promote angiogenesis, but is not independently sufficient to induce a phenotype \cite{52-53}.

The formation of proinflammatory by-products of the complement system, in particular C3a and C5a, is responsible for initiating the progression of AMD. The presence of these potent chemotactic agents is specific for drusen found in AMD but not for peripheral drusen in ageing human eyes without AMD \cite{54}. C3a and C5a increase the expression of VEGF-A, a factor stimulating the pathological neovascularisation in the course of AMD.

Experimental studies have demonstrated that the inhibition of complement activation via the systemic or local pathways can suppress laser-induced CNV. The inhibition of C3a, C5a, CFB and MAC, or the administration of complement regulatory molecules CD59 and CFH, can suppress the development of CNV in animal models \cite{54-57}.
The Complement System and Diabetic Retinopathy

Diabetes mellitus (DM) can affect the production of complement system proteins and regulatory proteins. Decreased levels of membrane-bound regulators, including CD55 and CD59, in the retina of diabetic patients were reported. Furthermore, CD59 glycoprotein, which inhibits C9 polymerization, and thus also the formation of MAC, may be inactivated by non-enzymatic glycation. Interestingly, C1q, C4 and MBL were not detected in the eyes of patients with DR, indicating that the complement system may be activated in the alternative pathway. The involvement of the alternative pathway was confirmed in another independent study through the detection of factor B in the vitreous body of patients with proliferative DR. Patients with proliferative diabetic retinopathy (PDR) had elevated levels of factor B, but also other complement proteins in the vitreous humour, such as C3, C4b and C9, compared to non-diabetic patients, and patients with DR had significantly higher levels of C3d and MAC. These findings indicate the importance of the alternative activation pathway at the early stage of DR, whereas the classical pathway may be involved in the later stages of the disease. Other researchers have also reported increased levels of C5a, C3 and CFI in the vitreous body of patients with PDR.

When the BRB is damaged, serum proteins, including complement and immunoglobulins, can be released and accumulate in the retina of diabetic patient and activate the complement. Retinal pericyte-reactive autoantibodies were detected in the plasma of patients with DR, suggesting that the classical complement pathway mediated by antibodies can lead to the death of pericytes and vascular degeneration in DR. Fragments of complement, such as C3a and C5a, can bind to respective receptors on retinal cells, causing inflammation or synthesis of angiogenic growth factors. In Müller cells C5aR is constitutively expressed, which can be upregulated by hyperglycaemia and proinflammatory factors, for example, PGE2. Binding C5aR to C5a in Müller cells leads to the release of IL-6 and VEGF, involved in the pathology of DR. Studies also show that autoantibodies against glycated and glycol-oxidized proteins can activate the classical pathway of the complement system in diabetes. Increased plasma levels of C3, associated with vascular thrombosis, were found in patients with type 1 and type 2 diabetes. Elevated level of soluble MAC in the blood is associated with increased risk of cardiovascular events in patients with type 2 diabetes. Higher plasma levels of MBL, positively correlated with DR, have been reported in patients with types 1 and 2 diabetes. In conclusion, patients with diabetes have increased levels of complement activators (C3, MBL and autoantibodies), and decreased activity of regulators, for example, CD59, which results in uncontrolled complement activation, tissue damage, and the development of diabetes complications.

The role of genetic factors in the development of DR was analysed in a study by Wang et al., who investigated the association between factor H (CFH) and factor B (CFB) gene polymorphisms and DR. The study revealed a significant increase in the frequencies of the A allele and AA genotype for rs1048709 (factor B) in patients with DR compared to controls with diabetes. However, there was a significant decrease in the frequencies of the A allele and AA genotype for rs800292 (factor H) in patients with DR compared to diabetic controls. In addition, the study found that the rs800292/AA genotype was related with delayed progression of DR.

The same researchers also investigated the relationship between complement pathway genes and susceptibility to DR. Complement component C5 and SERPING1 (the human C1-inhibitor gene) were genotyped in 570 patients with type 2 diabetes: 295 patients with DR (138 nonproliferative and 157 proliferative) and 275 control subjects with diabetes. Among the six C5 gene polymorphisms an association was detected between rs17611 and DR. The study revealed a significant reduction in the frequencies of the G allele and GG homozygosity for rs17611 in patients with PDR as compared to diabetic controls, and it was related to disease progression. The authors found no significant association between DR and other tagged SNPs of C5 and SERPING1 genes. Polymorphism rs1410996 CFH may also be associated with both PDR and coronary disease in patients with type 1 diabetes.

Xu et al. investigated the association between C5 gene polymorphisms and PDR in type 2 diabetes in a Chinese Han population. Four C5 gene polymorphisms, including rs2269067, rs7040033, rs1017119 and rs7027797, were genotyped in 400 patients with PDR (study group) and 600 patients with nonproliferative diabetic retinopathy (NPDR) (controls). The study revealed that the C5 rs2269067 GG genotype increased the risk of PDR in patients with type 2 diabetes in the Chinese Han population and was linked with elevated C5 mRNA expression and increased IL-6 synthesis. A chronic mild inflammatory process is a feature of type 2 diabetes. Adipocytes activated in subjects with obesity release adipocytokines that induce pro-inflammatory cytokine secretion, resulting in vascular endothelial dysfunction and organ damage. Complement component C3a is considered a factor inducing tissue inflammation. An example adipocytokine is adipsin (ASP), a serine protease responsible for the activation of the alternative complement pathway.

A research team led by Fujita investigated the relationship between diabetic microangiopathy and the complement system in 32 obese patients with type 2 diabetes and 32 healthy...
also resulted in pathological angiogenesis of retinal vessels. OIR. The receptor deficiencies, mainly for C5a and C3a, by increased neovascularisation in 17-day-old mice with deficiency of the complement component C3 is manifested angiogenesis. This process is mediated by macrophages the complement system does not have a direct effect on significant neovascularisation. The study demonstrated that Deficiency of complement component C5 also caused i.e. components of the classical complement pathways, of OIR. They found that the expression of genes encoding complement pathway in the pathogenesis of a murine model angiogenesis. The role of the complement system in ROP has been primarily investigated in experimental studies in a murine model of oxygen induced retinopathy (OIR). Langer et al[85] demonstrated that deficiency of the complement component C3 is manifested by increased neovascularisation in 17-day-old mice with OIR. The receptor deficiencies, mainly for C5a and C3a, also resulted in pathological angiogenesis of retinal vessels. Deficiency of complement component C5 also caused significant neovascularisation. The study demonstrated that the complement system does not have a direct effect on angiogenesis. This process is mediated by macrophages[83], and cells expressing high levels of IL-6, IL-12, tumour necrosis factor alpha (TNF-α), and the soluble form of vascular endothelial growth factor receptor 1 (sVEGFR1), and low levels of IL-10 elicit an antiangiogenic effect[84]. Tao et al[85] demonstrated the activation of the classical complement pathway in the pathogenesis of a murine model of OIR. They found that the expression of genes encoding components of the classical complement pathways, i.e. C1qb and C4b, in 17-day-old mice was significantly higher in the retina of mice with OIR compared to mice in the control group. The expression of mannose-associated serine protease 1 (MASP1) and mannose-associated serine protease 2 (MASP2), genes encoding the components of the lectin pathway, was low and did not differ significantly from that measured in the control group. Moreover, the expression of CFB in the alternative pathway was significantly lower in the retina of mice with OIR. However, different correlations were found for the expression of the gene encoding CFH. In another study Lau et al[86] analysed the interferon-stimulated in vitro expression of CFH in human RPE cells and observed increased levels of this protein in cultures. Sweigard et al[87] identified an increased number of newly formed vessels in the retina of alternative complement pathway-deficient mice (knock-out FB- mice). They demonstrated that vascularisation is not a result of increased concentration of VEGF, but compromised the ability of removing the formed neovessels. Kim et al[88] analysed components of the alternative complement pathway at phase 1 of proliferative retinopathy in 8-day-old mice and found significantly lower vascular loss in FB- mice with OIR. Luo et al[89] also showed that in inflammatory conditions the alternative pathway of complement activation could be activated in the retinal cells, and is manifested by a significant increase in the concentrations of CFB and C3.

**Complement System and Idiopathic Macular Hole** Idiopathic macular hole (IMH) is a full-thickness hole in the retina that arises due to traction on the vitreoretinal interface. It can cause significant loss of visual acuity because of the mechanical disruption of the anatomical structure of the fovea. The peak incidence of IMH is observed in subjects aged between 60 and 80y, and with three-fold higher frequency in women than in men. There are reports supporting the involvement of the complement system in the pathogenesis of this medical condition. For example, Zhang et al[90] investigated the expression of various proteins in the vitreous body of patients with IMH and control subjects, including complement pathway proteins (CFH, CFB, C3, C4-A). Their study revealed elevated expression of two proteins of the alternative complement pathway, i.e. factor B and H, in the vitreous body in the eyes with IMH compared to the control group. In addition, an increased expression of the C3 component, which plays a key role in the activation of the complement system, was observed in subjects with IMH. The increased expression of C4-A (non-enzymatic component of C3 and C5 convertase) in IMH also suggests the involvement of the classical complement pathway in the pathogenesis of IMH[90].

**CONCLUSIONS** Recent reports still do not provide clear conclusions on the involvement of the complement system in the development of retinopathy. However, the effect of complement components on this process has been most thoroughly investigated in relation to the development of AMD. For example, a relationship between mutations in the genes encoding CFH and CFI and increased risk of developing AMD has been proven. CFH and CFI are plasma factors regulating the complement system, and they elicit an inhibiting effect protecting against excessive pathological activation of the complement system. Researchers have also identified a relationship between alleles of genes encoding C3 (determining resistance to inactivation by CFH and CFI), C9, C2 and CFB.
Features of the complement system linked with DR include polymorphisms of genes encoding CFH, CFB and C5. The relationship between the activation of the alternative complement pathway (C3, C4 and CFB) has also been highlighted. The least evidence for the effect of the complement system on the development of abnormal neovascularisation relates to ROP, and so far studies have been conducted mainly on animal models. Research demonstrated an association of C3 and C5 deficiency with abnormal angiogenesis, as well as the activation of the classical complement pathway (C1 and C4). There is also some evidence for disorders within the alternative pathway (lower expression of the CFB gene, higher expression of the CFH gene).

In this paper we present a review of the current literature data, pointing out the novel results and achievements from research into the role of complement components in the development of retinopathy. There is still a need to continue research in new areas, and to gather more detailed information about this problem which will be useful in the treatment of these diseases.

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