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REVIEW

The complement system and age-related macular degeneration

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Abstract

Purpose **Age-related macular degeneration (AMD) is the leading cause of blindness in the developed world. There are increasing evidences to suggest the complement system may play a significant role on the pathogenesis of AMD. In this review, we summarise the current research in this area.**
Methods **Review of literature.**
Results **The complement system is a complex system with several activation pathways. Complement factor H (CFH) polymorphisms has been associated with increase risk of AMD. CFH is an inhibitor protein; the polymorphisms might cause uncontrolled activation by initiation events.**
Conclusion **Further studies on the molecular basis of the complement-mediated pathogenesis of AMD may offer novel therapy to AMD**
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Keywords: complement; complement factor H; CFH; age-related macular deneration; angiogenesis; oxidation stress

Introduction

The complement system and age-related macular degeneration (AMD)

AMD is the leading cause of blindness in the elderly.[1–3] For decades, the accumulation of drusen in the Bruch's membrane was considered the most important finding for the pathogenesis of the disease. Data accumulated in the last decade suggest that AMD may involve chronic inflammatory processes.[4–6] Immune mechanism and cellular interactions in AMD are similar to those seen in other diseases characterised by the accumulation of extracellular deposits such as atherosclerosis and Alzheimer's disease.[7] Evidence is growing that the complement system may play a significant role in the pathogenesis of AMD. Inflammatory and immune-mediated events involving complement proteins have been implicated in the biogenesis of drusen.[6] Recent studies have also identified multiple genetic variants of Complement factor H (CFH), a regulator protein that can confer elevated risk of AMD.[8–11]

The complement system

The complement system comprises of a complex of at least 30 enzymes and regulators and provides an innate immune defence mechanism. The liver is the main source of complement synthesis and the complement molecules constitute approximately 5% of the total serum proteins. Many extrahepatic cells such as monocytes, endothelial cells, epithelial cells, glial cells, and neurons also produce complements[12] presumably as part of a defence mechanism, though they can be counterproductive leading to local tissue damage. The multiple biological activities of this cascade include control of inflammatory reactions and chemotaxis, clearance of immune complexes, cellular activation and antimicrobial defence.

Three principle pathways are involved in complement activation, all of which converge on the activation of the third component C3 (Figure 1). These are the classical pathway which is activated by antibody bound to antigen, the lectin pathway activated by carbohydrates and oxidative stress and the alternate pathway triggered by the presence of microbial pathogens, cellular debris or multimolecular aggregates. The convergence of these pathways to C3 initiates the final common pathway that results in the formation of terminal complexes such as C5b-9 that promotes

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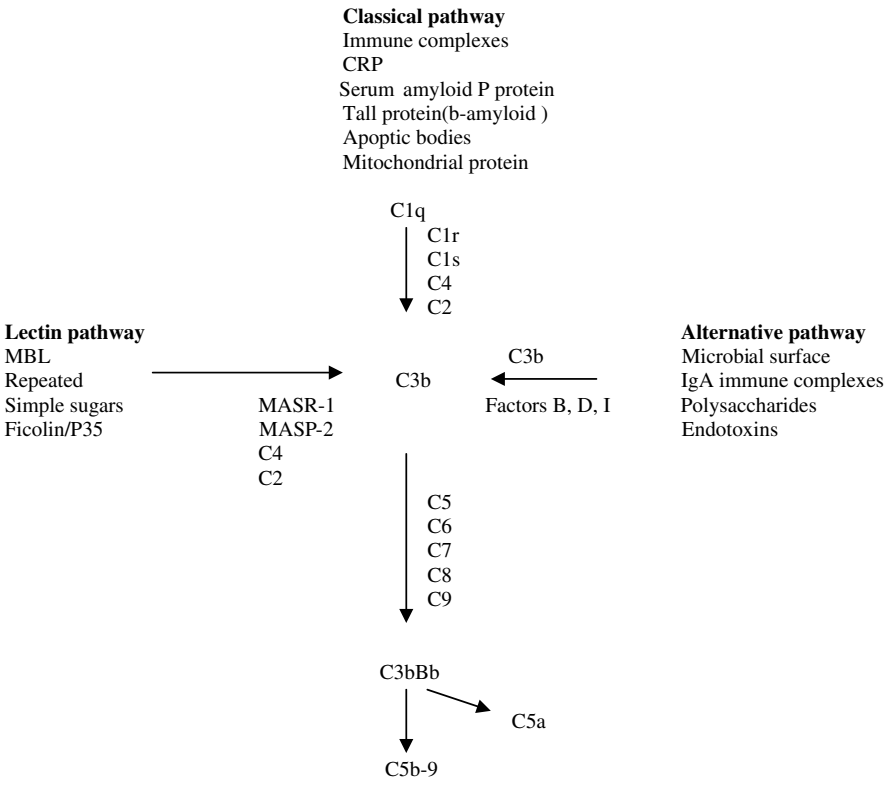

Figure 1 The complement system.

cell lysis of the target cell whether it is an invader or bystander cell exposed to the complement activation. Other by-products of the complement cascade such as C3b and anaphylatoxins (C3a, C4a, and C5a may also serve as proinflammatory polypeptides further amplifying the injury or inflammation.

The mRNAs of a few components of the complement system such as C3 and terminal complement component, C5 have been identified in the retina and retinal pigment epithelial (RPE)–choroid complex of adult human donor eyes and cultured human RPE cells.[6,13–15] Although they may serve as a protective function in innate immunity, it also suggests the presence of chronic low-grade local complement activation that is controlled by complement regulatory proteins.

Complement regulatory proteins

The complement system is regulated by both fluid phase and membrane-bound regulatory proteins to prevent inadvertent damage as a consequence of complement activation. Fluid phase proteins, vitronectin and clusterin (apolipoprotein J) bind C5b67 complement complexes thereby preventing cell lysis. Membrane-bound cofactor proteins (MCP, CD46), decay acceleration factor (DAF, CD55), membrane inhibitor of reactive lysis (MIRL, CD59), and complement receptors (CR1, CD35) help in clearance of soluble immune complexes.[16]

Both vitronectin and clusterin have been identified in healthy donor RPE–choroid complex[13,14] indicating their role in containing proinflammatory local stimuli. The co-precipitation of CD46 with β1 integrin in the basolateral membrane of healthy RPE cells suggest that this complement regulatory protein may be useful both in adhesion of RPE cells and in integrin-signalling pathways.[17]

Complement factor H

Another important regulatory protein is the CFH. Recent evidence suggests that CFH may play a significant role in the pathogenesis of AMD.[8–11] CFH is a single polypeptide chain with a molecular weight of 155 kD that is present in the plasma at a concentration of 110–615 μg/ml. It is constitutively produced by the liver. Extrahepatic synthesis of CFH occurs in a wide variety of cells such as lymphocytes, glomerular mesangial cells, neurons and glial cells. In the context of AMD, CFH is synthesized in the RPE cells and accumulates in drusen. The CFH controls the complement system in the fluid phase and on cellular surfaces. It inhibits the activation of C3 to C3a and C3b and also directly binds to C3b

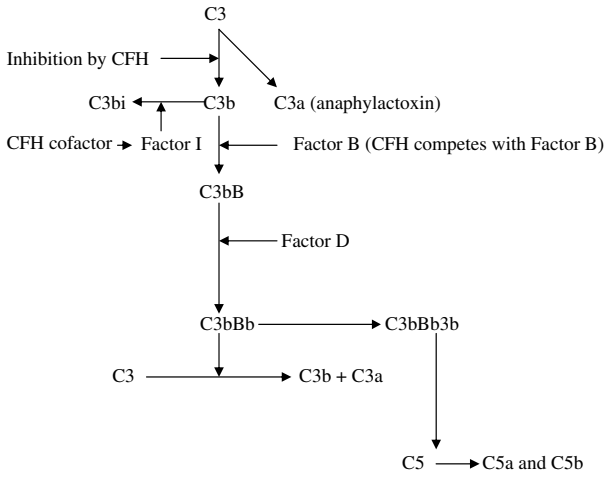

Figure 2 The role of CFH in the alternate pathway.

(Figure 2). It is a major inhibitor of the alternate complement pathway. CFH can also interact with polyanionic molecules sialic acid and heparin on cell surfaces conferring them resistance to damage induced by complement activation. It also binds to C-reactive protein (CRP) which may help to arrest CRP-dependent complement activation induced by damaged tissues.[18] The protein is composed of 20 short consensus repeats (SCR) directed towards different fragments of C3. The SCR7 and SCR 19-20 are the most likely sites involved in the interaction with C3b, heparin and CRP.[19] This complement regulatory protein also displays functions outside the complement system such as binding to cellular integrin receptor and interaction with cell surface glycosaminoglycans.[16]

CFH polymorphism

Familial aggregation, segregation analysis and twin studies have suggested that there is a significant genetic contribution to the pathogenesis of AMD and up to six regions of our genome have been identified as potentially harbouring AMD genes. Linkage studies and candidate gene screening suggest that one such locus associated with AMD may exist at 1q25–32.[20–22] A recent breakthrough in this research is the identification of the gene within the interval of chromosome 1 that is involved in AMD. This gene encodes CFH.[8–11]

The mutational screening of CFH gene in independent series of patients with AMD resulted in the identification of a polymorphism in the CFH gene. The four studies used distinct but complementary methods to screen the genomes from nonoverlapping groups of AMD patients and all the studies identified the risk allele as a tyrosine-histidine substitution at amino acid 402 of the CFH gene.[8–10] While studies in additional ethnic groups will be required, as well as prospective studies on disease risk associated with this variant, current data suggest that a single histidine allele (heterozygous) confers a two- to four-fold increased risk of developing AMD, while two histidine alleles (homozygous) confer a five- to seven-fold increased risk.

Although the genetic studies have clearly demonstrated the involvement of CFH in AMD, the precise role the CFH plays in this heterogeneous condition is unknown. Polymorphism confers disease susceptibility only and can be present in the normal population while a mutation almost always causes the disease.

The cause of AMD is a probably still multifactorial. Inherited complement defects may represent a predisposing condition (permissive gene) that increases the risk of the condition in combination with other intercurrent environmental or acquired factors. Established risk factors for AMD such as smoking has been shown to decrease the plasma CFH levels.[23,24] In addition, smoke-modified C3 has diminished binding to CFH.[25]

The exposure of the RPE–choroid complex to these risk factors may initiate local tissue response that activates complement. In normal conditions, CFH may effectively limit the complement activation and extension of tissue damage. When the bioavailability or activity of CFH is congenitally defective, it may lead to uncontrolled complement activation and further tissue damage. The implication of CFH also renders other complement components candidates as risk factors for AMD.

CFH deficiency can also cause type II membranoproliferative glomerulonephritis (MPGN II), a rare renal disease with coexistent drusen that share similar molecular composition to those in AMD.[26] A high proportion of these patients have been shown to harbour the AMD at-risk haplotype of CFH suggesting that both diseases may be the result of uncontrolled trigger of the alternate complement pathway.[11] However, CFH mutations have also been identified in haemolytic uremic syndrome (HUS), a syndrome that has never been linked to AMD to date. The CFH mutations in HUS do not result in decreased plasma CFH levels and is thought to be due to the inefficient protection of host cells to complement activation while those with MPGN II results in hypocomplementemia. Therefore, the two renal conditions associated with CFH mutations differ in the mode of complement activation. Further studies are required to understand how AMD is linked with MPGN II and not HUS.

Immunolocalisation of complement components in drusen

Drusen is the hallmark for AMD. Several components of the complement cascade including C3 complement

fragments, C5 and the membrane attack complex C5b-9 have been identified in drusen, sub-RPE space and within the capillary pillars of the choroid.[13–15] A recent study demonstrated that the deposition of C5b-9 in drusen is associated with degeneration of the macular choriocapillaris.[27] In addition, fluid phase regulatory proteins such as vitronectin and clusterin have been localised in drusen. Vitronectin has been isolated from all drusen phenotypes while clusterin is readily identified in large drusen. Vitronectin binds to C5b-9 complexes at the site of their generation.[13]

Membrane-bound regulatory proteins such as MCP and CR1 have also been identified in drusen. MCP tends to colocalize with C3 fragments in drusen. The recent evidence that transcripts for CFH and FHL1 (truncated isoform gene) in RPE–choroid complex approaches levels observed in liver indicates that drusen may be the result of a local imbalance between the activators and regulators of the complement system.[11]

Activators of the complement system

The complement system seems to play a crucial but nonspecific role in the amplification of chronic inflammation in diseases associated with accumulation of extracellular deposits such as AMD, atherosclerosis, and Alzheimer's disease.[28,29] Several components of the chronically sequestrated debris in AMD may be potential activators of the proteolytic cascade including apoptotic cells, nuclear fragments, and membrane-bound vesicles. Metabolic end products such as lipofuscin, phospholipids, advanced glycation end products, cholesterol, and microfibrillar proteins may also serve as powerful chemotactic stimuli for leucocytes via the complement cascade.[15]

Other than the abnormal tissue deposits, other acute phase proteins such as CRP and serum amyloid-P can also augment the inflammatory cascade. CRP activates the classic pathway but inhibits binding of C5b-9 through the direct binding of CFH. *Chlamydia pneumoniae* which is also an activator of complement system was recently implicated in the pathogenesis of AMD.[30] All these data are suggestive of the existence of multiple activators of complement in the RPE–choroid complex which mediate complement activation in AMD.

Complement activation and oxidative stress

Oxidative stress is thought to play an important role in the pathogenesis of AMD.[31] Oxidative stress results in the activation and deposition of complement on the vascular endothelium. It is still unclear which specific complement pathways are involved in the primary mechanism of initial complement activation in oxidative stress. Most studies are focused in oxidative stress in ischaemia–reperfusion injury of the myocardium. Collective data suggest that the lectin pathway plays an important role in the myocardium and the gastrointestinal system. However, it is unsure at this stage whether the contribution of complement pathways may be organ dependent. Further studies using specific inhibitors of the classical, alternative or lectin pathway, in addition to using genetically modified mice will be needed to better comprehend the role of complements in oxidative stress.[32] The role of these pathways in AMD remains to be investigated.

The only prospective, controlled, clinical trial providing proven benefit of antioxidant supplementation for AMD is the Age-Related Eye Disease Study (AREDS).[33] Patients at high risk for AMD as defined by the AREDS classification were shown to have a significant benefit with regard to disease progression by supplementing with high-dose antioxidants and zinc. Zinc affects the complement system at multiple sites and has both enhancing and inhibitory effects on the complement proteins. It strongly inhibits activity of C3-convertases of both the classical and alternative pathways. It has additive effect to CFH and inhibits the cofactor activities of CFH thereby inhibiting the degradation of C3b. On the other hand, it also increases activation of the alternative pathway on nonactivator surfaces.[34] The exact mechanism by which zinc and the complement system interacts in AMD is unclear.

Complement activation and angiogenesis

Immunostaining of laser-induced choroidal neovascularisation (CNV) in primates have demonstrated the presence of C3 and C5b-9 in the neovascular complex.[35] The CNV was inhibited by complement depletion using cobra venom factor and did not develop in C3(−/−) mice. It was also noted that elevated levels of angiogenic factors in mice with laser-induced CNV were marked reduced after complement depletion. In addition, other complement proteins, C3a and C5a also promote CNV.[36]

Conclusion

A growing body of clinical data strongly suggest that genetic variants of CFH is associated with increased risk of AMD complimenting previous reports of proinflammatory pathways being involved in the pathogenesis of AMD. AMD is a downstream consequence of complement-mediated deposition of extracellular debris akin to atherosclerosis and Alzheimer's disease. Further studies on the molecular basis of complement-mediated pathogenesis of AMD

may offer some promise towards the search for preventive and therapeutic options for this condition.

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