HHS Public Access

Author manuscript

Prog Retin Eye Res. Author manuscript; available in PMC 2022 July 01.

Published in final edited form as:

Prog Retin Eye Res. 2021 July ; 83: 100936. doi:10.1016/j.preteyeres.2020.100936.

Targeting complement components C3 and C5 for the retina: Key concepts and lingering questions

Benjamin J. Kim[a,*], **Dimitrios C. Mastellos**[b], **Yafeng Li**[a], **Joshua L. Dunaief**[a], **John D. Lambris**[c]

[a]Scheie Eye Institute, Department of Ophthalmology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

[b]National Center for Scientific Research 'Demokritos', Athens, Greece

[c]Department of Laboratory Medicine and Pathology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Abstract

Age-related macular degeneration (AMD) remains a major cause of legal blindness, and treatment for the geographic atrophy form of AMD is a significant unmet need. Dysregulation of the complement cascade is thought to be instrumental for AMD pathophysiology. In particular, C3 and C5 are pivotal components of the complement cascade and have become leading therapeutic targets for AMD. In this article, we discuss C3 and C5 in detail, including their roles in AMD, biochemical and structural aspects, locations of expression, and the functions of C3 and C5 fragments. Further, the article critically reviews developing therapeutics aimed at C3 and C5, underscoring the potential effects of broad inhibition of complement at the level of C3 versus more specific inhibition at C5. The relationships of complement biology to the inflammasome and microglia/macrophage activity are highlighted. Concepts of C3 and C5 biology will be emphasized, while we point out questions that need to be settled and directions for future investigations.

Keywords

Age-related macular degeneration; Geographic atrophy; Complement; C3; C5; Retina

[*]Corresponding author. Scheie Eye Institute of the University of Pennsylvania, Perelman School of Medicine, 51 North 39th Street Philadelphia, PA, 19104, USA. benjamin.kim@pennmedicine.upenn.edu (B.J. Kim).

CRediT authorship contribution statement

Benjamin J. Kim: Conceptualization, Project administration, Writing - original draft, Writing - review & editing. **Dimitrios C. Mastellos:** Conceptualization, Writing - review & editing. **Yafeng Li:** Writing - original draft, Writing - review & editing. **Joshua L. Dunaief:** Conceptualization, Writing - review & editing. **John D. Lambris:** Conceptualization, Supervision, Writing - review & editing.

Financial disclosures

B.J.K. has been a consultant for Synergy Research, Apellis Pharmaceuticals, and Allergan. J.L.D. has been a consultant for Eyevensys, Mperia, and Abfero. J.D.L. is the founder of Amyndas Pharmaceuticals which develops complement inhibitors for therapeutic purposes, and inventor of patents that describe the therapeutic use of complement inhibitors, some of which are developed by Amyndas. J.D.L. is also the inventor of the compstatin technology licensed to Apellis Pharmaceuticals (i.e., 4 (1MeW)7W/POT-4/APL-1 and PEGylated derivatives such as APL-2/pegcetacoplan and APL-9).

1. Introduction

Age-related macular degeneration (AMD) is the leading cause of blindness in the developed world (World Health Organization, 2020). AMD affects patients ≥55 years of age, and there are an estimated 200 million people with AMD in the world; this may increase to 300 million worldwide by 2040 (Mitchell et al., 2018). Significant vision loss is associated with the two advanced stages of AMD, nonexudative (dry) and exudative (wet) AMD. The advanced stage of nonexudative AMD is characterized by the presence of geographic atrophy (GA), which are lesions with deterioration of the photoreceptors, retinal pigment epithelium (RPE), and choriocapillaris. These lesions are typically located within the macula (central retina), cause scotomas within the central visual field, and relentlessly enlarge in size at an average rate of 1.2–2.8 mm^2 per year (Holz et al., 2014). Exudative AMD is characterized by abnormal choroidal neovascularization (CNV) beneath the macula. The CNV causes central vision loss from bleeding, edema, and scarring of retinal tissue. Wet AMD is typically treated with intravitreal injections of medicines (e.g. ranibizumab, aflibercept, bevacizumab) that inhibit the vascular endothelial growth factor (VEGF) pathway. While exudative AMD would still benefit from more therapeutic options, finding a treatment for GA currently is a very high priority as there is no treatment available for GA. The blinding lesions of GA are estimated to cause 20% of the legal blindness in North America (Holz et al., 2014).

Complement biology plays a key role in AMD pathophysiology. The complement system is a highly interactive protein network that becomes activated in a cascade-like fashion, rapidly neutralizing microbial intruders or endogenous stress signals through potent opsonophagocytic and inflammatory mechanisms (Ricklin et al., 2010). By coordinating both innate and adaptive immune signaling, complement contributes to tissue homeostasis and host immunosurveillance (Hajishengallis et al., 2017; Mastellos et al., 2019). As will be discussed in this article, studies of genetic variants first thrusted AMD into the field of complement biology (see Section 3), and it became clear that AMD can be considered an immune-mediated disease. Ensuing investigations have uncovered vital links between complement dysregulation and other pillars of AMD pathophysiology, such as oxidative stress (Datta et al., 2017) and inflammasome activity (Tarallo et al., 2012).

Of the many complement components, C3 and C5 have major positions within the complement cascade and have risen as the leading therapeutic targets in many inflammatory diseases, including AMD (Mastellos et al., 2019). While C5 inhibition effectively targets the terminal complement pathway and blocks C5a release and MAC assembly, C3 inhibition is emerging as a more comprehensive targeting approach, whereby both upstream C3-derived fragments and signaling mediators and downstream C5-derived proinflammatory effectors can be simultaneously inhibited. Eculizumab (Soliris, Alexion) is a monoclonal antibody that binds C5 and blocks its proteolytic activation (Rother et al., 2007; Wang et al., 1995). Eculizumab was approved in 2007 for paroxysmal nocturnal hemoglobinuria, becoming the first complement inhibitor available to patients. More recently, positive phase III data emerged for the first time for a C3 inhibitor, underscoring both its efficacy and safety in the clinical setting; in these trials, pegcetacoplan, a PEGylated version of a second-generation compstatin analog (APL-2, Apellis Pharmaceuticals) showed efficacy as a treatment for

paroxysmal nocturnal hemoglobinuria with signs of broader therapeutic coverage and clinical gain over eculizumab (Apellis Pharmaceuticals, 2020; Castro et al., 2020). Furthermore, while other complement inhibitors have failed in clinical trials for GA, there are now ongoing phase III trials that will evaluate C3 inhibition (pegcetacoplan, Apellis Pharmaceuticals; NCT03525600, NCT03525613) and C5 inhibition (avacincaptad pegol, Iveric Bio; NCT04435366) as treatments for GA.

Other excellent reviews about AMD and GA (Datta et al., 2017) have focused on the complement cascade as a whole or more specifically on the key involvement of complement factor H in AMD pathophysiology (Boyer et al., 2017; Toomey et al., 2018). While the relationships of complement proteins and regulatory proteins are clearly important, this review will concentrate on the intricacies of C3 and C5 biology as they relate to AMD and especially GA. In addition to holding pivotal positions in the complement cascade, C3, C5, and their fragments have multiple, robust functions that could be important in AMD. Clinical and laboratory data have pointed towards these proteins as therapeutic targets, but many lingering questions have arisen as well.

2. Overview of the complement system

While C1 – C9 may be considered the “backbone” components of the complement system, complement actually involves around 50 different proteins that interact with each other in a highly ordered fashion, engaging in intricate crosstalk with both innate and adaptive immune pathways. The complement components are arranged into a cascade that is poised to amplify and react quickly to various microenvironmental insults or cues (Ricklin et al, 2010, 2016a). Generally, the cascade can start from three different pathways: the classical pathway (CP), the alternative pathway (AP), and the lectin pathway (LP) (Fig. 1). The classical pathway begins with the binding of the C1q fragment of complement factor C1 to antigen-antibody complexes. Lectins are carbohydrate-binding proteins, and the lectin pathway starts by mannose-binding lectin binding to polysaccharide or glycoprotein motifs on the cell surface of foreign cells or injured host cells. In contrast, the AP is characterized by a constitutive, low-level activation of C3. This activation, termed ‘C3 tickover’, is mediated by the spontaneous hydrolysis of C3; it leads to the generation of $C3H_20$ and the formation of an active C3 convertase, $C3H_20Bb$ (Fig. 1). The $C3H_20Bb$ convertase can initiate the AP. Of note, $C3H_2O$ is unable to bind to cell surfaces because of its hydrolyzed thioester bond (Pangburn et al., 1981; Winters et al., 2005). However, recent evidence has suggested that $C3H_2O$ may engage in many biological functions, serving also as a key initiator of intracellular complement activation via its entry into various target cells (Elvington et al., 2017). In addition to the three canonical pathways of complement activation (CP, LP, AP), there is also an extrinsic protease-mediated pathway, whereby various proteases of the coagulation/contact/fibrinolysis pathways can directly cleave C3 and C5 independent of the convertases, leading to the release of bioactive C3 and C5 fragments and anaphylatoxins (Amara et al., 2010; Huber-Lang et al., 2006).

Regardless of the triggering route (CP, LP or AP) all complement pathways lead to the formation of C3 convertases, the enzymatic complexes that bind to and cleave C3. The CP and LP both utilize the same C3 convertase C4b2b. $C3H_20Bb$ serves as the initial convertase

of the AP, with C3bBb being the actual AP convertase that fuels AP amplification on target surfaces opsonized by C3b. In fact, the AP not only serves as a stand-alone activation pathway but can also amplify the complement response initiated through the classical and lectin pathways. C3 is thus a point of convergence of all complement activation pathways and holds a pivotal position within the cascade. The cleavage of C3 by convertases releases C3a and C3b. Then, factor B (FB) binds to C3b forming the so-called C3 proconvertase (C3bB). FB is subsequently cleaved by Factor D (FD) to form Bb, which combines with C3b to form the AP C3 convertase C3bBb. The C3bBb convertase can cleave more C3 molecules, amplifying the cascade. In this way, the AP functions as a significant amplification loop for the classical and lectin pathways at the level of C3 (Lachmann, 2009), contributing about 80% of the downstream terminal (lytic) pathway activation (Harboe et al., 2009). This AP amplification loop is a powerful driver of multiple complement functions. The C5 convertase, C3bBbC3b, cleaves C5, releasing C5b and C5a. C5b initiates the formation of the C5b-9 membrane-attack-complex (MAC), and C5a exerts multiple proinflammatory and immunomodulatory functions. The classical and lectin pathways also give rise to a C5 convertase (C4bC2bC3b) that can cleave C5. The MAC (C5b-9 complex) is a key downstream effector of the complement cascade, as MAC has the capacity to cause lysis of targeted cells by forming pores in cell membranes or it can activate intracellular signaling pathways in sublytic doses (see also Section 6.3) (Triantafilou et al., 2013). Being the initial step in the process of MAC assembly, C5 activation contributes to many pathological processes with inflammatory phenotypes. Intensifying the strength of this system beyond MAC, many complement proteins and their fragments have immunomodulatory and effector functions. A prominent example of complement's effector function is opsonization, which is when target cells are tagged for phagocytosis by immune cells bearing complement and Fc receptors. Phagocytes initiate the phagocytic process by recognizing the so-called opsonins, which are C3 and C4-derived fragments deposited on the surface of the target cell as a result of complement activation (i.e., C3b, iC3b, C4b, and C3dg). Further, the cleavage of C3 and C5 releases the anaphylatoxins C3a and C5a which have several inflammatory functions (see Section 6.1), including the ability to chemotactically attract phagocytes to the site of infection. Additional information on the biology of the complement system and how it relates to various diseases can also be found in recent reviews (Mastellos et al., 2019; Ricklin et al., 2018).

Regulatory proteins can dampen or amplify complement activation, and key regulators and proteases will be mentioned briefly here. The serine proteases FB and FD have important roles in the assembly and activity of the C3 convertase of the alternative pathway. As mentioned above, FB binds C3b, interacts with FD, and this gives rise to the C3bBb convertase. In addition to this, FB binds hydrolyzed C3 ($C3H_2O$) following the spontaneous hydrolysis of native C3 (C3 tick-over). Cleavage of the bound FB by FD then leads to the $C3(H_2O)$Bb convertase of the AP. With these roles, FD is considered the rate-limiting enzyme of the complement AP. AP activity is further controlled by key regulatory proteins, including properdin and Factor H (FH). The C3bBb convertase activity is stabilized by properdin; C3bBb has a half-life of only 90 seconds but is stabilized 5 to 10-fold by properdin (Kouser et al., 2013). Properdin was previously considered to have pattern-recognition ability, enabling it to interact with cell surfaces independent of C3b, but this has

been called into question (Harboe et al., 2017). In contrast to properdin's stabilization of C3bBb, FH is involved in C3b degradation and is one of several endogenous regulatory proteins expressed by host cells to modulate complement activation both in the circulation and on autologous surfaces. Beyond FH, these other regulators include Complement Receptor 1 (CR1), decay accelerating factor (DAF/CD55), and membrane cofactor protein (MCP/CD46), each of which can accelerate the dissociation of C3bBb (Hourcade et al., 2002). It is important to note that, in addition to its role as a regulator of membrane-bound C3b, FH acts as the main fluid phase inhibitor of AP activation. Further, FH has single nucleotide polymorphisms (SNPs) that were the first complement SNPs found to be associated with AMD (see Section 3).

The complement cascade was originally considered to be a pathogen-killing protein system within the blood that "complements" antibodies, but is now known to also have broader functions in immune surveillance, development, and intracellular activities (Freeley et al., 2016; Merle et al., 2015; Ricklin et al., 2010, 2016a). Recognizing the multi-faceted roles of complement is a fundamental concept to consider for therapeutic development.

At its best, the complement cascade is able to be potent and refined, adjusting to each microenvironment. However, it is clear that dysregulation of the complement system leads to excessive inflammation and disease. Dysfunction of the complement system is implicated in the pathophysiology of a variety of diseases including periodontitis, paroxysmal nocturnal hemoglobinuria, sepsis, transplant rejection, C3 glomerulopathy, cancer, and AMD (Mastellos et al., 2019). Mounting clinical evidence has also indicated that complement dysregulation contributes to COVID-19 pathogenesis, driving key thromboinflammatory pathways that culminate in disseminated microvascular injury, endothelial dysfunction, and immunothrombosis (Risitano et al., 2020; Skendros et al., 2020). Of note, a comparative study of the C3-targeted drug candidate AMY-101 (Amyndas Pharmaceuticals) and the anti-C5 monoclonal antibody eculizumab in severe COVID-19 patients revealed divergent laboratory profiles and clinical traits that point to a broader impact of C3 inhibition on NET (neutrophil extracellular traps)-driven thromboinflammation (Mastellos et al., 2020). All of these clinical developments have brought complement therapeutics to the forefront of biopharmaceutical research, expanding the spectrum of indications that may benefit from acute or chronic complement intervention.

3. Evidence for the pathogenic role of C3 and C5 in AMD

Landmark genetic studies in 2005 first established that complement proteins, specifically factor H polymorphisms, are associated with AMD (Edwards et al., 2005; Hageman et al., 2005; Haines et al., 2005; Klein et al., 2005). Genome-wide association studies have since revealed that genetic variations in multiple components of the complement cascade are associated with increased risk for AMD (e.g. C3, FB, Factor I, C9), pointing predominantly to a key pathogenic role of AP dysregulation leading to increased C3 turnover in this disease (Park et al., 2019). In this section, we will focus on evidence for C3 and C5's role in AMD.

Drusen are the hallmark retina finding of the early and intermediate stages of AMD, and it is clear that C3, C5, and other complement proteins are present within drusen deposits

(Mullins et al., 2000; Nozaki et al., 2006). Furthermore, the anaphylatoxins C3a and C5a, which are inflammatory mediators (see also Section 6.1), have been shown to be present in human drusen and Bruch's membrane. In AMD patients, higher plasma levels of bioactive fragments, such as C3a, C3d, Bb, and C5a, have also been reported, suggesting that complement activation plays a role in AMD pathogenesis (Lechner et al., 2016; Reynolds et al., 2009; Scholl et al., 2008). In the laser-induced mouse model of choroidal neovascularization, immunolabeling has shown C3a and C5a in and around RPE cells within hours after laser injury. Interestingly, Nozaki et al. found that C3a and C5a induced vascular endothelial growth factor (VEGF) in retinal pigment epithelium (RPE) cells (Nozaki et al., 2006). When the receptors for C3a (C3aR) or C5a (C5aR1) are knocked out, there is a reduction in VEGF expression, leukocyte recruitment, and CNV formation in the laser-induced model. Bora et al. found C3 and the membrane attack complex (MAC) in CNV from the laser-induced model, and MAC inhibition with anti-C6 antibodies significantly reduced CNV (Bora et al., 2005). This animal model data was produced about 15 years ago and strongly suggested that complement inhibition, especially at the level of C3 or MAC, could be helpful for neovascular AMD (nvAMD). However, clinical trials have yet to show a benefit of complement inhibition for nvAMD for unclear reasons. While the laser-induced CNV model is an established method of studying CNV development and has been valuable for VEGF therapeutic development, this model does not fully represent the complex relationship of complement and nvAMD development in humans.

Genetic association studies focusing on C3 and C5 deserve discussion. There are generally two predominant alleles of the C3 gene. These are known as C3S (slow) and C3F (fast) because of their different mobility on agarose electrophoresis (Delanghe et al., 2014). The frequency of the C3F allele is about 20% in the Caucasian population. Genetic studies by different groups revealed that only the rs2230199 single nucleotide polymorphism (SNP), which leads to C3F, is associated with AMD (Maller et al., 2007; Yates et al., 2007). The consequence of this SNP is the substitution of an uncharged glycine residue for the positively charged arginine at position 80, potentially affecting activity of the C3 thioester-containing domain (Yates et al., 2007). C3F also has been suggested to be associated with other diseases including IgA nephropathy, systemic vasculitis, partial lipodystrophy, and membranoproliferative glomerulonephritis type II (MPGNII, also known as dense deposit disease) (Delanghe et al., 2014). Additionally, a rare variant of C3 (p.Lys155Gln; rs147859257) has been found to be associated with AMD, and this has been confirmed in several populations (Helgason et al., 2013; Seddon et al., 2013; Zhan et al., 2013). This mutation is thought to code for a C3 protein variant that has reduced ability to bind FH, thereby attenuating the C3b breakdown cycle and increasing AP activity. Among a subset of patients with either GA or exudative AMD, Helgason et al. found that the p.Lys155Gln variant had significantly higher frequency among the GA cases (Helgason et al., 2013). In contrast to C3, there presently are no known associations of AMD with C5 polymorphisms. Baas and colleagues did not identify any significant correlation between C5 SNPs and propensity to develop AMD in their study of 2599 AMD cases and 3458 controls (Baas et al., 2010). In this study, there were 8 C5 SNPs associated with AMD for the Amsterdam/Rotterdam-Netherlands study population, but none of these associations were replicated when the analysis was extended to three other study populations. Nevertheless, as noted

here, there is still significant evidence suggesting that C5 has an important role in AMD pathophysiology despite the lack of an AMD associated C5 SNP.

Several laboratory studies have heightened the interest in C3 and C5 as pathogenic drivers and targets for therapeutic intervention in AMD. Oxidative stress related, in part, to bisretinoids and iron overload is considered a pathogenic mechanism of AMD that leads to complement activation (Dunaief, 2006; Zhou et al., 2006). *In vitro* studies suggest that photooxidation products of the bisretinoid A2E activate the complement cascade. A2E is a photoreactive byproduct of the visual cycle that can generate reactive oxygen species; it is derived from vitamin A aldehyde and is a major component of the lipofuscin within RPE. When complement containing human serum was placed over ARPE-19 cells (a human RPE cell line) that contained photooxidized A2E, there were increased levels of iC3b and C3a, suggesting complement activation from fragments of oxidized A2E that either left the cells or were present at the cell surface (Zhou et al., 2006). It is also possible that A2E causes cellular stress that leads to the development of neoepitopes on RPE that activate the complement cascade (Joseph et al., 2013). Li et al. have also shown that iron itself contributes to C3 production by the RPE, indicating a local source of C3 (Li et al., 2015). Further, collaborative work between the Dunaief and Sparrow labs has shown that iron promotes cell death caused by bisretinoids, showing a link between these pathways (Ueda et al., 2018). Other causes of oxidative stress highly implicated in AMD, such as smoking, also cause the RPE to produce C3 and are associated with AP activation (Kunchithapautham et al., 2014). While there is no single animal model that recapitulates the complex mechanisms of AMD, the light-damage mouse model is useful for understanding the role of light-induced oxidative stress within the retina. Several complement proteins are upregulated from light-damage, including C3, C5, and the anaphylatoxin receptors, C3aR and C5aR1 (Song et al., 2017). All together, these data show that multiple research approaches have implicated C3 and C5 in AMD pathophysiology.

Although the data presented in this section strongly implicates C3 and C5 in AMD pathobiology, the differences between C3 and C5 should not be overlooked as they might point to distinct pathogenic pathways that are differentially modulated by these two key components. C3 activation is a converging step in the cascade that could orchestrate multiple pathologic processes in AMD. In this regard, C3 activation triggers the release of opsonic fragments that drive phagocytic responses, controls the activation of the proinflammatory C3a-C3aR axis, and also drives the generation of downstream effectors of the terminal pathway (C5a, MAC) through formation of C3b-containing C5 convertases (see also Section 6). Further investigations that specifically evaluate these C3 functions and distinguish them from C5 are indicated as we consider C3 and C5 therapeutics.

4. Molecular structure and biological functions of C3 and C5

Because of the central location of C3 and the distal location of C5 within the complement cascade, both C3 and C5 play distinct but organized roles in activation and regulation of complement-mediated inflammation and immunity. Delving into biochemical features, this section focuses on the structure – function relationships of both C3 and C5.

4.1. Molecular size and gene of C3 and C5

The human *C3* gene is on the short (p) arm of chromosome 19 at position 13.3 (chr.19p13.3), localized to base pairs 6,677,704 - 6,720,650 (NCBI Homo sapiens Updated Annotation Release 109.20200815, GRCh38.p13, 2020). The primary amino acid sequence and derived cDNA coding sequence have been elucidated. The encoded precursor molecule contains a peptide of 22 amino acid residues, the β-chain (645 residues), and the α-chain (992 residues). Cloning and sequencing studies have revealed several functionally important sites (e.g. thioester site) in the C3 protein (de Bruijn and Fey, 1985). Phylogenetic analyses showed that human *C3* has 79% identity to mouse *C3* at the nucleotide level and 77% identity at the amino acid level (de Bruijn and Fey, 1985). The molecular weight of the mature human C3 protein is 187 kDa (Sahu and Lambris, 2001).

The location of the human *C5* gene is on the long (q) arm of chromosome 9 at position 33.2 (chr.9q33.2; base pairs 120,952,335–121,074,865 (NCBI Homo sapiens Updated Annotation Release 109.20200815, GRCh38.p13, 2020). The mature C5 molecule is comprised of an α- and a β-chain, which are covalently linked by a disulfide bridge (Hughes, 1994). It is proteolytically processed into the small anaphylatoxin polypeptide C5a and a larger fragment termed C5b (Fredslund et al., 2008). The molecular weight of mature C5 protein is 196 kDa (Jore et al., 2016). Phylogenetic analyses showed that the human *C5* gene is approximately 89% synonymous to mouse *C5* at the nucleotide level (Hughes, 1994).

4.2. C3 and C5 structure in the context of their physiologic function

4.2.1. C3 structure—A discussion of the complement system's evolution is relevant to the structural understanding of C3 and C5. Complement is an ancient defense system that likely emerged at least 600–700 million years ago; several of its core components and activities have been described in invertebrates and almost all animal phyla (Oriol Sunyer et al., 1998; Ricklin et al., 2016b). The complement system evolved with a set of primitive C3, FB and MASP-like (mannose-binding lectin associated serine proteases) molecules likely appearing in early metazoans before the divergence of the deuterostome lineage that led to the emergence of all vertebrate phyla. This 'primordial' complement system initially served as a potent phagocytic machinery that mediated effective tissue immunosurveillance while acting as a first line of defense against pathogens. Through millions of years of evolutionary pressure and intense phylogenesis, the complement system was further diversified through gene duplications and domain rearrangements of the C3, FB, and MASP genes, that gradually led to the formation of discrete pathways in more evolved vertebrates (i.e., mammals). Protein domain shuffling, gene duplication events, and copy number variations propelled the sequential emergence of the AP and the subsequent appearance of the CP and the terminal lytic pathway. It is therefore thought that C5 emerged from a common, ancestral, thioester domain (TED)--containing molecule that initially evolved as C3, likely through gene duplication, and then led to the appearance of C5 through additional domain reshuffling. The emergence of the terminal pathway is tightly associated with the divergence of the jawed fishes (gnathostomes/cyclostomes) from a common ancestor of jawed vertebrates. Of note, the presence of multiple isoforms of C3 and C5 proteins within the same teleost fish has been attributed to copy number variations and gene duplication events rather than alternative splicing of a single precursor gene (Ricklin et al., 2016b).

Additionally, C3, along with C5 are closely related to the α_2-macroglobulin (α2M) family (Duval et al., 2020; Law and Dodds, 1997). Thioester motifs are well conserved, biochemically reactive structures, and the α2M family has a TED (Levashina et al., 2001).

C3 is derived from a single-chain precursor protein (Pro-C3) of approximately 200 kDa (Okura et al., 2016). Pro-C3 is proteolytically processed into two polypeptides, the α (115 kDa) and β (70 kDa) subunits, forming the mature C3 protein (Misumi et al., 1984; Morris et al., 1982). Crystallographic structural determination of C3 protein purified from human plasma revealed 13 protein domains: 8 macroglobulin domains (MG 1–8); anaphylatoxin; linker; CUB (complement C1r/C1s, Uegf, Bmp1); TED; and C345C domains (Fig. 2A) (Janssen et al., 2005). Among these domains, TED has a crucial role in C3 activation and regulation. It harbors a highly reactive thioester group that remains protected within the conformation of native C3 but becomes exposed upon spontaneous hydrolysis or proteolytic cleavage of C3 to enable the rapid covalent attachment of C3 to target surfaces (Chen et al., 2016; Janssen et al., 2005). Upon proteolytic conversion to C3b, C3 undergoes drastic conformational changes that expose surfaces for multi-tiered interactions with other complement components and regulators (Janssen et al., 2006). C3b molecules close to cell surfaces can attach via their activated TED and tag them for opsonization. Since nascently activated C3b can bind to hydroxyl and amine groups, C3b can bind to most biological surfaces (Law and Dodds, 1997). C3b activity may be controlled through a rapid cleavage by Factor I (FI), yielding iC3b. Further cleavage of iC3b by FI, in the presence of co-factors such as FH or CR1, leads to the release of C3c and C3dg (see also Section 6.2.1) (Xue et al., 2017). Although iC3b can no longer participate in C3 or C5 convertase assembly, it serves as a crucial effector molecule for downstream host defense processes as it binds to complement receptors on a variety of immune cells. The function of iC3b in contrast to C3b was further understood after a structural analysis using hydrogen–deuterium exchange mass spectrometry, a technique that characterizes a protein's deuterium uptake at peptide resolution, thereby providing insight into the conformational basis of the protein's activity and selectivity (Papanastasiou et al., 2017). This study elucidated the structural dynamics of C3b and iC3b based on the observation that peptides with distinct deuterium contents were found in C3b and iC3b. For instance, an increase in deuterium uptake was observed for CUB domain peptides in iC3b when compared with C3b, indicating that a more structurally disordered CUB domain emerges in the context of iC3b formation. The disordered CUB in iC3b likely impacts the orientation and accessibility of this opsonin via MG-CUB-TED domain arrangement; this explains the differences between C3b and iC3b with regards to convertase formation and the exposure of various surfaces that allow binding to distinct regulators and receptors (Papanastasiou et al., 2017).

A few other domains deserve a brief highlight. The α-chain starts with the anaphylatoxin domain (residues 650–726). When this domain is cleaved off, the C3a anaphylatoxin, forms (Huber et al., 1980). Finally, residues 1496–1641 form a C-terminal C345C domain. This domain is covalently linked to the MG domains, promoting stabilization of the C3bBb convertase by interaction with properdin (Alcorlo et al., 2013).

4.2.2. Molecular structural basis of alternative pathway (AP) activation and regulation—Although there is evidence showing that the two other complement pathways

contribute to the etiology of AMD (Rohrer et al., 2011), dysregulation of the AP, which predisposes individuals to developing disease, is considered most important (Karki et al., 2019). Thus, the structural basis for AP activation and regulation deserves discussion. Under physiological conditions, the AP vigilantly keeps a low level of constitutive activation by the "tick-over" mechanism, whereby there is spontaneous hydrolysis of a labile thioester bond, forming C3(H_2O) and eventually the initial AP convertase C3(H_2O)Bb (Pangburn et al., 1981). The initial AP convertase cleaves C3 into C3a and C3b, and there is ensuing deposition of C3b onto cell surfaces. As C3b binds covalently to a surface via the activated TED, the half-life of the thioester in C3b is less than 1 second with subsequent poor attachment efficiency to surfaces (Law and Dodds, 1997). This very short half-life is mediated by hydrolysis of the thioester and places a time restriction on C3b, limiting the activity to close proximity of the location of activation. Furthermore, bound C3b molecules can also be rapidly inactivated by complement regulators.

Upon activation of the AP, FD binds to and cleaves C3-bound FB, releasing the N-terminal fragment Ba and resulting in the formation of the AP C3 convertase (C3bBb) (Milder et al., 2007). The half-life of C3bBb is short (~90s) (Pangburn and Müller-Eberhard, 1986). Therefore, a stabilization of C3bBb is required to enable adequate host defense. Properdin, a protein secreted by monocytes/macrophages and T lymphocytes (Schwaeble et al., 1993), stabilizes the AP C3 convertase, as mentioned earlier. Properdin also facilitates the assembly of the AP C3 convertase, as properdin can bind FB, Bb, and C3b (Hourcade, 2006). Electron microscopy studies demonstrated that properdin binding induces a large displacement of the TED and CUB domains of C3b (Alcorlo et al., 2013). These structural changes twist the binding site for factor H (Kajander et al., 2011). It makes the AP C3 convertase more resistant to decay by factor H. Of note, factor H, along with decay-accelerating factor (DAF) and complement receptor 1 (CR1), can bind to C3b and accelerate the decay of the AP C3 convertase; this is accomplished by dissociation of Bb from C3b and proteolytic inactivation of C3b (Alcorlo et al., 2015; Hourcade et al., 2002).

4.2.3. C5 structure—In the terminal pathway distal to C3, C5 is the critical component. C5 has some structural similarities to C3 as noted in Section 4.2.1: C5 contains macroglobulin domains (MG1–MG8), the CUB domain, the C5d domain (structurally homologous to the TED of C3), the C5a domain (also called anaphylatoxin) and an extended linker region located between MG1–MG2 and MG4–MG6 (Fig. 2B). The C5d domain is structurally homologous to the TED in C3, but it does not contain a thioester bond. The linker and C5a domains insert into the MG6 domain, whereas C5d is inserted into the CUB domain (Fredslund et al., 2008).

Structural understanding of C5 and its derivatives has propelled development of therapeutics targeting discrete components of the terminal complement pathway. The inhibitory mechanisms may include blocking of key proteins in the effector cascade (e.g., C5), neutralization of complement-derived anaphylatoxins (e.g., C5a), and blockade of complement anaphylatoxin receptors (e.g., C5aR1/CD88) (Emlen et al., 2010). In a crystallographic study of the C5-eculizumab complex, the C5 residues recognized by the antibody are all part of the antiparallel four-stranded β-sheet in the MG7 domain (Schatz-Jakobsen et al., 2016). Eculizumab and other pharmaceutical candidates were designed

based on the understanding of the C5 molecule's MG7 structure (Jore et al., 2016). Binding to the MG7 domain of C5, eculizumab prevents cleavage of C5 by the complement C5 convertases and effectively inhibits the effectors of the terminal complement pathway (Jore et al., 2016; Schatz-Jakobsen et al., 2016).

5. C3 and C5 expression and activity: It's all about location

One of the most important topics in need of additional research is the location and timing of C3 and C5 expression in the context of AMD. Complement proteins, including C3 and C5, are typically produced in the liver, then enter the circulation and can deposit within various tissues. Of note, extrahepatic C3 or C5 expression by both myeloid and non-myeloid cell types has also been described in many tissues, disease models and pathophysiological contexts (Reis et al., 2006). Under physiological conditions, the plasma complement components circulate as inactive zymogens, and activation of complement is effectively controlled by soluble inhibitors such as factor H, C4-binding protein (C4bp), and membrane-associated regulatory proteins such as CD46, CD55, and CD59 (Ferreira et al., 2010; Geller and Yan, 2019).

Patients with AMD-associated complement SNPs may have a genetic background that predisposes them to systemic complement overactivity. Scholl et al. suggested that, compared to controls, patients with AMD do have higher levels of plasma markers of complement activation, especially factor Ba and C3d (Scholl et al., 2008). Data supporting systemic complement activation in AMD patients with risk-associated gene polymorphisms has also been reported by other groups (Heesterbeek et al., 2020; Lorés-Motta et al., 2018). Using the C3d/C3 ratio as a measure of systemic C3 activation (see also Section 6.2.1), Heesterbeek et al. recently reported data suggesting that systemic C3 activity is increased in patients with more advanced stages of AMD, particularly in patients with risk-associated polymorphisms (Heesterbeek et al., 2020). In some patients, the deposition of complement into the eye from systemic complement over-activity may play a role in AMD progression. These observations link dysregulated C3 activation with disease severity and suggest that targeting C3 convertase activity and/or AP amplification may provide an effective means of containing the inflammatory sequelae that deteriorate the clinical status of AMD patients.

If plasma-derived complement deposits into ocular tissues, then the choroid has potential as a key site of deposition for the eye. With immunohistochemistry of non-human primate retinas, we have seen C3 present primarily in the choriocapillaris and Bruch's membrane (Hughes et al., 2020). Others have shown evidence of MAC deposition within the photoreceptor outer segments of an eye with geographic atrophy (Fig. 4E of (Mullins et al., 2014). It is not clear what proportion of these critical complement proteins are deposited in these locations from the systemic circulation or after ocular infiltration by peripheral blood mononuclear cells, as opposed to being locally produced. The blood-retina barrier would normally prevent many serum proteins, including complement components, from entering the subretinal space or the neurosensory retina (Clark et al., 2017). However, recent work from the Dunaief lab at UPenn has shown that there is some passage of plasma proteins into these areas in patients with the intermediate stage of AMD, providing evidence for break-down of the blood-retina barrier in AMD patients (Schultz et al., 2019). Using two different

cohorts and controls matched for age and post-mortem interval, Western blots were performed on human post-mortem neurosensory retinas. Significantly more albumin and complement component C9, two proteins that do not typically cross the blood-retina barrier and are not known to be expressed in the neurosensory retina at the age of AMD susceptibility, were found in the intermediate stage AMD eyes compared to controls (Fig. 3). These data provide important evidence that the blood-retina barrier is moderately compromised in nonexudative ("dry") AMD and plasma components can subclinically "leak" across into the neurosensory retina. Thus, complement in the systemic circulation could directly affect the retina in diseased states, and one would expect that C3, being the most abundant protein in plasma, could leak progressively into the retinal tissue to a greater extent compared to other complement proteins.

As AMD develops, the putative local expression of complement components by retinal cells arises as an important question with many implications for the optimal delivery of therapeutics. An intriguing clinical study by Khandhadia et al. evaluated AMD status in 223 patients ≥ age 55 that had a liver transplant for at least 5 years. In these liver transplant patients, they found that AMD was associated with recipient *CFH Y402H* genotype rather than donor *CFH Y402H* genotype (Khandhadia et al., 2013). Additionally, the concentration of plasma complement components was similar for those with and without AMD. The authors of this study argued that local intraocular complement, rather than systemically produced complement, is more important in AMD pathophysiology. Regarding this local expression, the paper by Anderson et al. provides a wealth of data about complement expression in human retinal and choroidal tissue, and some of this data will be highlighted here (Anderson et al., 2010). They found that the choroid is the primary source of local gene expression of AP components and regulators. C3 expression was found primarily in the choroid; C5 expression in the choroid was detected as well, but to a lesser extent. Using immunohistochemistry of human tissue they found C3 predominantly in the choriocapillaris. In eyes with drusen, the locations around drusen had C3 within the cytoplasm of RPE and between the RPE and Bruch's membrane. Adding to this data, Katchke et al. have reported finding C3 in the choriocapillaris as well as some photoreceptor outer segments outside GA lesions of AMD donor eyes (Katschke et al., 2018). Pauly et al. explored this topic further and recently used single-cell RNA sequencing of mouse retinal cells to demonstrate that different retinal cell types have different complement expression profiles (Pauly et al., 2019). This study evaluated cells of the neurosensory retina and RPE, but did not specifically look at choroidal cells. Müller and RPE cells were the predominant sources of complement proteins, and the expression profile changed depending on the age of the mice. In particular, C3 was mostly expressed by Müller cells with lesser amounts of expression by neurons and RPE cells. Expression of C3 and other complement activators increased in the context of an ischemia – re-perfusion injury model of the mouse retina. C5 and other MAC components were mostly expressed by the RPE rather than the neurosensory retinal cells. All together, these data show that complement activation within the retina can be sustained without significant contribution of the plasma-derived complement stores. Thus, there is an ongoing debate about whether pathologic levels of complement proteins come from locally produced complement, the plasma, or both (Schnabolk et al., 2015). Further, the data by Pauly et al. suggest that complement proteins have cell-type specific expression and activities likely

related to homeostasis within the normal retina (Pauly et al., 2019). This raises a note of caution as to how complement therapeutics should be optimally delivered to the diseased retinal tissue (both in terms of delivery route and optimal timing/duration of treatment).

Age is also a factor in complement expression as complement plays a role in homeostasis of the aging retina, including removal of apoptotic cells and selective elimination of undesirable synapses (Mukai et al., 2018; Stevens et al., 2007). It's highly likely that complement expression patterns found in non-diseased eyes is evidence of local complement activity related to retina homeostasis. When comparing younger mice to older mice that are *C3aR*$^{-/-}$ and/or *C5aR*$^{-/-}$, there is progressive retinal degeneration with electroretinogram abnormalities, as well as loss of different retinal cell types over time in *C3aR*$^{-/-}$ and *C3aR*$^{-/-}$*C5aR*$^{-/-}$ but not *C5aR*$^{-/-}$ mice (Yu et al., 2012). Older mice that are *C3*$^{-/-}$ or *C5*$^{-/-}$ also show electroretinogram abnormalities compared to younger mice (Hoh Kam et al., 2013; Mukai et al., 2018; Yu et al., 2012). These data suggest important roles of C3 and C5 as the retina ages, although it should be noted that reduction of electroretinogram amplitudes has also been seen in aged mice deficient in other AP components such as CFH (Coffey et al., 2007). While these studies from knockout mice raise caution regarding C3 or C5 inhibition, one must keep in mind that genetic loss of C3 or C5 throughout life is quite different than conditional pharmacologic inhibition only later in life. Additional work characterizing complement protein locations with immunohistochemistry of normal eyes and AMD eyes of different stages and age from deeply phenotyped humans would shed further light on this important topic.

Yet another consideration is the intracellular vs. extracellular roles of complement. The complement system is traditionally considered to be made up of proteins present within plasma after synthesis in the liver. In this section, we have also discussed the synthesis of complement proteins by retinal cells. In contrast to the typical roles of complement secreted into the plasma, it has been found that intracellular complement proteins can have several, important functions such as an impact on intracellular metabolic pathways. Complement proteins likely emerged around a billion years ago, and Liszewski et al. suggest that C3 actually began as an intracellular protein that protected cells from pathogens entering cells before it evolved into a secreted protein with functions within the plasma (Liszewski et al., 2017). Intracellular C3a has been found in a range of cell types, indicating an important role for C3a in cellular physiology (Liszewski et al., 2013). In fact, cells can contain intracellular pools of C3 so they are primed for a quick complement response. This intracellular C3 can be produced within cells and stored, or it can enter the cell from the plasma in its hydrolyzed form, $C3H_20$ (Elvington et al., 2016). Intracellular C3 activation and C3a generation can regulate T cell activity and significantly affect metabolic pathways by increasing mTOR (mammalian target of rapamycin) activity (Liszewski et al., 2017). Kaur et al. have shown that the RPE can internalize extracellular C3 via endosomes, and then the RPE cells cleave the C3 to generate fragments that possibly include intracellular C3a (Kaur et al., 2018). There is potential for the C3a to then bind to C3a receptors on lysosomes, thereby increasing mTOR activity (Liszewski et al., 2013). Overactivation of the mTOR pathway has also been suggested as a pathologic mechanism affecting the RPE in AMD (Zhao et al., 2011). Nevertheless, a clinical trial evaluating intravitreal sirolimus, an inhibitor of the mTOR pathway, did not suggest efficacy for GA (Gensler et al., 2018). Interestingly, other

complement components such as C5 also have intracellular functions, and intracellular C5 has a role in NLRP3 inflammasome assembly in $CD4^+$ T cells (Arbore et al., 2016). These intracellular pathways add substantial complexity to the complement system; more work is needed to elucidate the roles of intracellular C3 and C5, especially within retinal cell types. When considering complement therapies, consideration of whether a C3 or C5 inhibitor can access and affect these intracellular complement pathways is warranted.

6. Diving deeper into C3 and C5 fragments

The multiple functions of the fragments of C3 and C5 deserve attention as one considers therapeutic inhibition at these locations of the complement cascade. In particular, the numerous fragments of C3 underscore the intricacy and breadth of C3 functions as compared to C5. This section seeks to be comprehensive in covering the fragments and their receptors for the benefit of those less familiar with this topic. Concurrently, it will emphasize those fragments and multi-protein complexes that have relationships to AMD, especially C3a, C5a, and MAC.

6.1. The anaphylatoxins: C3a and C5a

Cleavage of C3 leads to C3a and C3b, while cleavage of C5 leads to C5a and C5b. C3a and C5a have broad and potent immunomodulatory functions and are known as anaphylatoxins because of their role in anaphylactic allergic reactions.

Given the importance of C3a and C5a, the anaphylatoxin receptors and their locations of expression should be discussed. The receptor for C3a is C3aR, and C5a binds to either C5aR1(CD88, also referred to as C5aR) or C5a-like receptor 2 (C5L2/GPR77, also referred to as C5aR2). Both C3aR and C5aR1 are G-protein coupled receptors, while C5aR2 is not G-protein coupled and was originally thought to act as a decoy receptor by sequestering plasma C5a or C5a desArg (Li et al., 2013). Recent studies have shown that C5aR2 can modulate C5a signaling in myeoloid cells by forming an intracellular complex with β-arrestin, thereby negatively modulating the proinflammatory action of C5aR1. Despite progress in our understanding of its biology, the physiological role of C5aR2 remains both enigmatic and controversial. Both C3aR and C5aR1 are present on neutrophils, basophils, eosinophils, mast cells, monocytes/macrophages, and dendritic cells (Klos et al., 2009). Other cells expressing C3aR include astrocytes, endothelial cells, some epithelial cells, and activated T cells. Studies have suggested that C5aR1 is also expressed on some T cells as well as some astrocytes, endothelial cells, and neurons. C5aR2 is expressed in a variety of cell-types including neutrophils and immature dendritic cells, as well as tissues such as the brain, liver, and heart (Klos et al., 2009). Several studies have evaluated the expression of these receptors within the posterior segment of the eye. With immunohistochemistry of normal eyes, Vogt and colleagues found C3aR in the nerve fiber layer in a pattern that co-localized with a Müller cell marker, suggesting that C3aR was present on Müller cells (Vogt et al., 2006). There is varying data on C5aR1 ocular expression, depending on the experimental approach. Vogt found some inner retina staining with C5aR1 around the inner plexiform layer but could not identify the exact cell that was expressing C5aR1 at this location. Toomey et al. found C5aR1 predominantly in the choroid rather than the RPE

(Toomey et al., 2018), and others have reported that C5aR1 is expressed on some epithelial cells, choroidal endothelial cells, and Müller cells (Cheng et al., 2013; Skei et al., 2010). Of note, APRE-19 cells subjected to oxidative stress potentially increase their expression of C5aR1 (Trakkides et al., 2019). Although C3aR and C5aR1 expression was not seen on RPE by immunohistochemistry of human eyes in one study (Vogt et al., 2006), microarray analysis by Rohrer and colleagues from C57BL/6 J mice suggested RPE expression of both C3aR and C5aR1. Further, the presence of these receptors on RPE has also been demonstrated by functional studies relevant to AMD (Rohrer, 2018).

The anaphylatoxin functions are multiple and include contraction of smooth muscle, increasing vascular permeability, release of histamine (hence the name anaphylatoxin), and promoting phagocytosis. Further, C3a and C5a are chemoattractants for macrophages, monocytes, mast cells, and neutrophils. Over the past decade, other anaphylatoxin functions have been uncovered that diversify their significance even more. For example, both C3a and C5a can independently induce embryonic chick retina regeneration (Haynes et al., 2013). In fact, the broader tissue regenerative potential of these anaphylatoxins has been conserved across the evolutionary ladder (Strey et al., 2003). The anaphylatoxins are inactivated in plasma through the action of proteolytic enzymes, called carboxypeptidases N or R. These enzymes efficiently cleave off the C-terminal arginine of the polypeptide (Campbell et al., 2001), yielding C3a-desArg and C5a-desArg. C3a-desArg is incapable of triggering cell activation or downstream proinflammatory signaling via the C3aR, while C5a-desArg retains about 1–10% of its inflammatory activity (Klos et al., 2009; Reis et al., 2012).

With these various roles, the exact function of C3a and C5a will likely depend on the local micro-environment. Several lines of evidence implicate the anaphylatoxins in the pathophysiology of AMD. First, C3a can contribute to the development of sub-RPE basal deposits that form between the RPE and Bruch’s membrane (Fernandez-Godino et al., 2015), and these RPE basal deposits can be prevented with a C3a receptor antagonist (Fernandez-Godino and Pierce, 2018a). Second, the inflammasome is thought to have an instrumental role in AMD pathophysiology, and C5a can “prime” RPE cells for inflammasome activation (Brandstetter et al., 2015). After inflammasome activation, there is secretion of the inflammatory cytokines IL-18 and IL-1β which can damage the RPE (Tarallo et al., 2012; Wang et al., 2016). Third, the ability of anaphylatoxins to recruit mononuclear phagocytes, including microglia, has particular relevance to AMD as these cells are highly implicated in AMD pathophysiology (further discussed in Section 9) (Liao et al., 2020; Song et al., 2017). It should be stated, however, that Toomey et al. tested an anti-C5a therapy in a mouse model of early/intermediate dry AMD (Toomey et al., 2015) and found that systemic administration of an anti-C5a antibody did not prevent the development of AMD-like findings while it did reduce the recruitment of mononuclear phagocytes to the choroid-RPE interface (Toomey et al., 2018). Fourth, cigarette smoking is the major environmental risk factor for AMD, and cigarette smoke extract increases production of C3a by the RPE via a pathway modulated by the antioxidant transcription factor Nrf2 (Wang et al., 2014). Data has also shown that cigarette smoke extract increases C3 and C3a production by the RPE through an autocrine feedback loop involving C3aR and subsequent lipid accumulation (Kunchithapautham et al., 2014). While the relative contributions of the anaphylatoxins to nonexudative versus exudative AMD need more

clarification, C3a and C5a can contribute to the development of laser-induced CNV (Nozaki et al., 2006; Toomey et al., 2018). C3a and C5a can increase vascular endothelial growth factor (VEGF) production from RPE cells (Rohrer, 2018). On a separate note, others have investigated the presence of C3a and other complement proteins within the aqueous humor of exudative AMD patients as biomarkers and evidence of complement activation (Schick et al., 2017). Significantly increased levels of C3a within the aqueous humor were found compared to controls. This suggests local complement activity as well as the potential for aqueous humor sampling of patients with ophthalmic diseases.

6.2. C3 fragments and receptors

6.2.1. The opsonins: C3b, iC3b, C3dg/C3d—The cleavage of C3 by convertases leads to C3a and C3b. C3b is a multi-functional protein that forges multiple interactions with diverse ligands. It has the ability to interact with multiple effectors, regulatory proteins, and receptors of the complement system. C3b thereby mediates many functions that amplify and regulate the crosstalk of complement with other innate and adaptive immune signaling pathways. When C3b forms, there is a conformational change whereby the TED domain shifts position to expose an activated thioester. The activated thioester can then covalently bind to hydroxyl or amine groups on antigens or cell surfaces, including the surfaces of microorganisms (Hannan, 2016). Opsonization and enhancement of T cell responses are among the functions of C3b (Erdei et al., 2016; Reis et al., 2019). Within the eye, Fernandez-Godino et al. have suggested that an abnormal extracellular matrix leads to increased C3b deposition by the RPE and subsequent activation of the alternative complement pathway (Fernandez-Godino et al., 2018b). Further, CFH is the major regulator of C3b cleavage in the alternative pathway. Thus, C3b has a critical role in AMD and CFH genetic abnormalities may further exacerbate pathology in AMD by fueling AP dysregulation, C3 convertase overactivity and uncontrolled, persistent C3b deposition in the retinal tissue.

Factor I acts on C3b, producing iC3b (“inactivated” C3b) (Fig. 1, right side). Factor H, membrane cofactor protein, and complement receptor 1 (CR1) are cofactors for factor I during this cleavage of C3b (Alcorlo et al., 2011). This inactivates C3b in the context of the C3 or C5 convertases, and the formation of iC3b is therefore a negative regulatory action on the convertases. However, it is vital to realize that the iC3b fragment is far from being “inactivated”; it actually has potent inflammatory effects, can still perform opsonization, and can promote phagocytic clearance of target cells (Toapanta and Ross, 2006). The iC3b fragment can activate complement receptors 2–4 (CR2, CR3, and CR4) (Alcorlo et al., 2011; Bajic et al., 2013; van Lookeren Campagne et al., 2007). Binding to CR3 on neutrophils or monocyte/macrophages, iC3b can cause cell activation and phagocytosis (Lay et al., 2015). Sequential cleavage of iC3b by factor I then leads to C3c and C3dg, the latter of which can be cleaved to C3d and C3g.

C3dg/C3d is a ligand for CR2 on B cells (Fig. 1, right side). The binding of C3dg/C3d to CR2 contributes to B cell activation and antibody formation, thus linking innate immunity to an adaptive immune response (Lyubchenko et al., 2005; Toapanta and Ross, 2006). C3dg/C3d can act as an adjuvant, significantly increasing the immunogenicity of an antigen

and potentially distinguishing harmful from less harmful antigens (Dempsey et al., 1996). Futher, antigen-bound C3dg/C3d can interact with follicular dendritic cells to maintain memory B cells (Bergmann-Leitner et al., 2006). C3dg/C3d is also involved in opsonization and can bind CR3 in addition to CR2. C3dg can opsonize erythrocytes and also bind to CR3 on monocytes, leading to erythrophagocytosis by monocytes (Lin et al., 2015). C3dg has a longer plasma half-life (4 h) compared to C3a and C3c, and it has been investigated as a potential biomarker for systemic lupus erythematosus (SLE) (Troldborg et al., 2018).

C3d also has a relatively long half-life and has been used as a marker of disease state (Shields et al., 2017). In particular, the C3d/C3 ratio is considered as a more reliable marker of ongoing complement activation as compared to measuring activation fragments alone (Ekdahl et al., 2018). By using a ratio of C3d over total C3 levels, the concentration of plasma C3 is taken into account, allowing comparison of the C3d/C3 ratio among different research subjects. This ratio has been used in several studies evaluating complement activation in AMD (Lorés-Motta et al., 2018; Ristau et al., 2014). With some varying results, these studies have associated systemic complement activity, as reflected by an increased C3d/C3 ratio, with specific AMD-associated SNPs in C3 and FH. While utilizing ratios of activated versus total protein can be helpful, some caution is needed when utilizing systemic complement levels to inform about a chronic disease with a local manifestation. Systemic complement measurements can be variable and there are potential measurement differences between laboratories due to the lack of standardized protocols (Mastellos et al., 2019; Prohászka et al., 2016).

6.2.2. Receptors—There are 4 main complement receptors (CR1 – CR4). CR1 (CD35) is a glycoprotein that binds C3b; with lower affinity, it also binds to iC3b, C4b, C1q, and mannan-binding lectin (Leslie et al., 2003; Liu and Niu, 2009). CR1 is expressed predominantly on B cells and follicular dendritic cells. It is also expressed on erythrocytes, monocytes, neutrophils, and some T cells (Liu and Niu, 2009). Importantly, CR1 has decay accelerating activity for the AP convertase (C3bBb) and acts as a co-factor for the Factor I-mediated cleavage of C3b/C4b. Using immunohistochemistry, CR1 has been found on the apical side of the RPE in both normal and early stage AMD eyes (Fett et al., 2012). CR2 (CD21) is a glycoprotein that binds iC3b, C3dg and C3d. It also binds the Epstein-Barr virus, soluble or membrane-bound CD23 (IgE receptor), and interferon alpha (Hannan, 2016). Similar to CR1, CR2 is expressed predominantly on B cells and follicular dendritic cells. It is also expressed on a subset of T cells, thymocytes, basophils, mast cells, keratinocytes, and other epithelial cells. The C3 fragment-binding domains of CR2 (SCRs 1–4) have been exploited for the design of surface-targeted AP therapeutics. For example, an alternative pathway inhibitor consisting of the inhibitory domain of CFH fused to the portion of CR2 that binds opsonins (Huang et al., 2008) can inhibit CNV development in murine models (Rohrer et al., 2009) and has shown benefits in a dry AMD model involving exposure to cigarette smoke (Woodell et al., 2016). CR3 (CD11b/CD18, MAC-1) and CR4 (CD11c/CD18) are members of the integrin family. These receptors are expressed on macrophages, neutrophils, monocytes, and follicular dendritic cells. A subset of lymphocytes, eosinophils, and B cells also express CR3 and CR4 (Liu and Niu, 2009;

Vorup-Jensen and Jensen, 2018). CR3 primarily binds iC3b and can also bind to C3dg, while CR4 binds predominantly to iC3b.

6.3. C5b and the membrane attack complex

Proteolytic cleavage of C5 leads to the generation of C5a and C5b. C5b associates with C6–C9 to form the multi-protein complex MAC (C5b-9), which can lyse cells by inserting into the plasma membrane and is considered to have a key pathogenic role in AMD. MAC is present on the RPE but even more so on the choriocapillaris of AMD patients (Mullins et al., 2014). Interestingly, MAC is found on the choriocapillaris early in life, but increases in amount with age. This suggests that MAC plays a role in choriocapillaris homeostasis but has potential to reach levels that can cause pathological changes to target cells (Mullins et al., 2014). To prevent injury, cells have mechanisms to inhibit and handle MAC. CD59 is a membrane protein found on nucleated cells that inhibits the full assembly of MAC. Using immunohistochemistry, Ebrahimi and colleagues found reduced CD59 expression on RPE cells at the leading edge of GA and dysmorphic RPE in patients with GA (Ebrahimi et al., 2013). Reduced CD59 expression has also been demonstrated on the RPE in the laser-induced CNV mouse model (Schnabolk et al., 2017). Additionally, while a low level of MAC is continuously deposited on cells because of basal complement activity, other mechanisms of control are the removal of this MAC by release of membrane fragments or internalization (endocytosis) of portions of membranes containing MAC (Kumar-Singh, 2019; Xie et al., 2020; Georgiannakis et al., 2015). As both C3 and C5 inhibition are considered as promising therapeutic approaches in ocular diseases, it should be noted that both targeting strategies can interfere with downstream terminal pathway activation and the formation of MAC.

MAC has significant functions that extend beyond cell lysis. When MAC does not lead to cell lysis because of MAC inhibitors such as CD59 or rapid removal, MAC actually has important roles in intracellular signaling and cell activation. Studies have found that sublytic MAC is involved in apoptosis, release of pro-inflammatory cytokines, increased expression of adhesion molecules, increased cell proliferation, and calcium signaling (Busch et al., 2017; Rus et al., 2001). Sublytic MAC can initiate different intracellular signaling in several ways including the formation of sub-lytic membrane pores acting as channels for small molecules or ions, signaling through cell membranes independent of actual pore formation, and signaling pathways activated by endocytosis of membrane fragments containing MAC (Xie et al., 2020). In human endothelial cells, this latter pathway of endocytosis-mediated signaling leads to non-canonical NF-κB signaling and NLRP3 inflammasome activation (Xie et al., 2019), which is relevant to AMD. As another mechanism of sublytic MAC damage in AMD, the internalization of large amounts of sublytic MAC and subsequent lysosome processing can overload the lysosomes of RPE cells. This stress on the lysosomes may reduce the RPE’s ability to degrade damaging macromolecules such as bisretinoids (Cerniauskas et al., 2020). This concept of sublytic MAC’S role in AMD has gained substantial traction and creates a fresh twist on our understanding of ocular complement pathophysiology.

7. C3 and C5 AMD therapeutics in development

Although some animal models have suggested a potential role of complement inhibition for exudative AMD (Rohrer et al., 2009; Bora et al., 2005; Nozaki et al., 2006), complement therapeutics have not progressed to Phase III trials for exudative AMD. This suggests challenges in translating complement therapeutics into the more complex pathophysiology of human AMD. In addition to noting the complexity of exudative AMD compared to laser-induced CNV, Poor and colleagues have pointed out that subtle differences in methodology can affect the reproducibility of data from the laser-induced CNV mouse model (Poor et al., 2014). These differences can include the nature of the laser application and the genetic background of mice. On the other hand, complement-based therapeutics have developed tremendous momentum for GA (advanced nonexudative AMD) with two drugs in phase III clinical trials and several other drugs in earlier phases of development. While there are varied approaches to potential GA treatments, including cell-replacement strategies, these trials evaluating complement therapeutics have strived to slow down the growth of the GA lesions. However, increasing interest about the prospects of complement therapeutics in the ocular space has to be balanced against prudent clinical design, comprehensive patient stratification into trials, and additional longitudinal monitoring of patient outcomes. Previous large phase III trials with the Factor D inhibitor lampalizumab (Roche) had negative results despite positive phase II data (Holz et al., 2018). On top of this, there is a long history of drug failures for GA (Dolgin, 2017). Prior enthusiasm for lampalizumab was, in part, based on an over-reliance on subgroup analyses from the phase II trial (Sun et al., 2014). Rather than representing an inappropriate emphasis on complement pathophysiology in AMD, lampalizumab's failure may partly signify the elusive pathological contribution of a Factor D bypass mechanism that could override FD blockade (Irmscher et al., 2018); drug bioavailability problems; or the potential permeability of Bruch's membrane to FD from systemic sources (Clark and Bishop, 2018). Yet another possibility is pharmacodynamic breakthrough from just enough residual Factor D activity to still amplify the alternative pathway; it has been shown that only a small amount of FD is necessary for AP activity in humans and mice (Wu et al., 2018). The recent renewed and greater interest in the potential of complement inhibition is fueled by the similarly promising results of a C3-inhibition phase II trial and a C5-inhibition phase IIb trial. Here, we will discuss C3- and C5-based therapeutics (Table 1), focusing primarily on GA with an emphasis on the complexities that make GA drug development challenging.

7.1. C3 and C3 convertase-based therapeutics

Compstatins are a family of cyclic peptides that inhibit human and primate C3 and C3b. Compstatins were discovered using the phage display technique to screen a random peptide library for binding to C3b (Sahu et al., 1996). The functional activity of compstatins is based on a cyclic peptide scaffold of 13–14 amino acids that bind to the C3c portion of C3 in a shallow pocket formed between the MG4 and MG5 domains of the β-chain (Fig. 4) (Janssen et al., 2007). Compstatins act as protein–-protein interaction inhibitors that bind both native C3 and C3b, sterically hindering the binding and cleavage of native C3 by C3 convertases. Thus, compstatins are potent inhibitors of C3 activation acting at the convergence point of all three complement pathways, blocking C3 convertase formation, AP amplification, as well

as the generation of all downstream signaling mediators and terminal pathway effectors, including C5a and MAC (Mastellos et al., 2015). Various modifications of the compstatin peptide have been developed over the years leading to more potent analogs with greater inhibitory potency, higher binding affinity for C3 and more favorable pharmacokinetic properties for both systemic and local administration (see below). Some modifications enable cell penetration and internalization, which may even allow inhibition of intracellular C3 activity (Lambris et al., unpublished observations). By blocking the central step of C3 activation, compstatins afford broader and more comprehensive therapeutic control of the complement cascade, attenuating both upstream inflammatory signaling (C3a-C3aR axis) and downstream effector, immune-signaling and cytolytic functions (C5a/C5aR, sublytic or lytic MAC).

POT-4 (Potentia Pharmaceuticals) is a second-generation compstatin derivative that was initially evaluated for exudative AMD. POT-4 was administered with intravitreal injections. No safety concerns were seen in a phase I study (NCT00473928) but a subsequent phase II study (NCT01157065) did not show benefit, among other reasons, possibly due to insufficient dosing of the drug. Furthermore, there were concerns about vitreous deposits from POT-4 that limited its potential. To increase the solubility of this compstatin, a 40 kDa polyethylene glycol (PEG) attachment was made, and this new pegylated compstatin (pegcetacoplan) was studied for GA.

Currently, pegcetacoplan (APL-2, Apellis Pharmaceuticals) is in phase III clinical trials for GA after producing promising phase II results. The phase II trial for pegcetacoplan involved 246 patients randomized to sham, monthly intravitreal injection treatments, or every other month treatments (Liao et al., 2020). The primary outcome was the change in the rate of geographic atrophy growth at one year. Importantly, there was a dose dependent positive finding: GA growth rates were reduced by 29% ($p = 0.008$) and 20% ($p = 0.067$) compared with sham treatment for the monthly and every other month treatment groups, respectively. Regarding safety, there were 3 cases of endophthalmitis (2 culture-proven, 1 sterile) in eyes treated with pegcetacoplan. No conclusion can be drawn as to whether there is an increased risk of endophthalmitis with these small numbers of endophthalmitis cases, and the phase III data will clarify this topic. Considering that POT-4 was previously investigated for exudative AMD, an unexpected and intriguing finding was that more cases of investigator-determined conversion to exudative AMD were seen in the pegcetacoplan monthly (20.9%) and every other month (8.9%) groups compared to sham treatment (1.2%). These exudative AMD conversions were more common among patients with fellow (non-study) eyes that had exudative AMD. These cases of exudative AMD were generally mild, diagnosed early in the disease course, and promptly treated with anti-VEGF therapy. To investigate the possibility that these exudative conversions were from stimulation of existing, subclinical CNV, a retrospective review of baseline OCT data was performed. This revealed that the “double layer sign” was identified in 73% of eyes that converted to exudative AMD as compared to 32% of treated eyes that did not convert (Puliafito and Wykoff, 2020). The “double layer sign” is a pigment epithelial detachment suggestive of subclinical CNV in nonexudative AMD eyes (de Oliveira Dias et al., 2018), and pegcetacoplan may have activated these subclinical lesions (Puliafito and Wykoff, 2020). Although it rightfully draws some concern, this data further suggests that pegcetacoplan was having a real biologic effect on the eyes.

With their publication of these findings, Liao and colleagues hypothesized that C3 inhibition reduces the macrophage and microglia mediated phagocytosis of photoreceptors, RPE, and capillary endothelial cells by reducing deposition of C3 fragments on the surface of these cells (Liao et al., 2020). It is suggested that C3 inhibition leads to increased viability of endothelial cells and possibly a shift of macrophages and microglia from an inflammatory and phagocytic M1 phenotype found in GA towards an angiogenic and reparative M2 phenotype found in exudative AMD. This shift in macrophage and microglia phenotype is likely caused by decreased C5a production (Langer et al., 2010). In this way, C3 inhibition could switch the outer retina/choriocapillaris microenvironment away from progressive atrophy and more towards a state where the outer retina can be nourished by the choriocapillaris (Liao et al., 2020). Overall, these findings are highly suggestive of pegcetacoplan's beneficial role in GA, but the positive findings will need replication in the ongoing Phase III trials.

More recently, fourth generation compstatins have been developed that show promising properties for sustained therapeutic delivery to the eye. By adding lysine residues to the C terminus of the compstatin structure, the inhibitory potency of the cyclic peptide is retained but there is improved solubility and binding affinity for C3 (Berger et al., 2018). We investigated the intraocular residence of fourth generation compstatins with an attached chain of 2 or 3 lysines (Cp40-KK or Cp40-KKK, Amyndas Pharmaceuticals). After intravitreal injection of 500 μg of either of these lysine-modified compstatins into cynomolgus monkey eyes, Cp40-KK and Cp40-KKK were still present in the vitreous at 90 days; (Fig. 5) (Hughes et al., 2020). Recent studies have shown that saturating levels of Cp40-KKK can be detected in the non-human primate vitreous up to 6 months after a single intravitreal injection (Lambris et al., unpublished observations). Both Cp40-KK and Cp40-KKK were still fully active in binding to C3 and were present at concentrations about 100-fold higher than the C3 vitreous concentration in the eye. While both compstatin analogs have subnanomolar affinity for C3, immunohistochemistry was performed with Cp40-KKK, which has better binding affinity to C3 compared to Cp40-KK (K_D of Cp40-KKK is 0.21 nM vs.0.44 nM for Cp40-KK) (Berger et al., 2018). Of note, Cp40-KKK exhibits a 100-fold higher binding affinity for C3 compared to pegcetacoplan (Lambris et al., manuscript in preparation). Immunohistochemistry revealed that Cp40-KKK has C3 target driven localization and was found primarily at the choriocapillaris and Bruch's membrane (Fig. 6). These data also demonstrate that Cp40-KKK, which has a small size of 2.19 kDa, can penetrate the retina and RPE to access the underlying choriocapillaris. Cp40-KKK is not PEGylated and no toxicity was seen with this dose of Cp40-KKK (unpublished observations). With its prolonged intraocular residence and potent ability to inhibit C3, Cp40-KKK has clinical potential and a phase I trial in AMD patients is being developed. A reduced injection frequency of every 5–6 months, or possibly longer, would be highly beneficial for GA patients.

Advancing the number of drug candidates acting on C3, CB2782-PEG (Catalyst Biosciences/Biogen) is a long-acting C3 protease that degrades C3 into non-functional components (Catalyst Biosciences,). Here again, PEGylation was used to extend the vitreous half-life (Furfine et al., 2019). CB2782-PEG may only require intravitreal injection 3 to 4 times a year, which also would be of great benefit for patients. However, a cautionary

note should be made here such that C3-targeted proteases may likely cleave/degrade C3 in a manner similar to Cobra Venom factor (CVF), thereby generating a high burden of C3-derived fragments that could skew the therapeutic effect towards a more inflammatory phenotype (e.g. due to persistent immune stimulation of target cells) (Krishnan et al., 2009). This drug is undergoing additional preclinical studies; the start date for a phase I trial has not been determined.

C3 convertase inhibitors certainly deserve to be mentioned. While FH targeting approaches came to a standstill due to the failure of the FD-targeting Fab lampalizumab (Genentech, Roche) as mentioned above (Holz et al., 2018), other elegant therapeutic strategies focusing on AP C3 regulators or cofactors of FH are being pursued. For instance, an adeno-associated viral (AAV) vector-based gene therapy (GT005) is clinically developed by Gyroscope Therapeutics (UK) for local Factor I (FI) overexpression in the diseased retina and therapeutic containment of AP dysregulation in GA patients (NCT03846193, NCT04437368). GT005 is administered by subretinal injection, and a phase II program evaluating this therapeutic for GA has started. From a different standpoint, recombinant miniaturized forms of FH (mini-FH) encompassing unique surface targeting properties and exhibiting even greater potency than full-length FH have been developed. These could form a basis for local, FH-based therapeutic modulation of the AP in the diseased retina (Schmidt et al., 2013).

FB is cleaved by FD and thereby is a key component of AP C3 convertase formation. IONIS-FB-L_{rx} (Ionis/Roche) uses ligand-conjugated antisense technology to achieve cell-specific delivery of an antisense oligonucleotide. This therapeutic is designed to decrease FB production and is currently investigated in a phase II trial (NCT03815825). In contrast to other therapeutics discussed here, IONIS-FB-L_{rx} is administered by subcutaneous injection every 4 weeks and targets hepatic expression of FB. Although preclinical data has suggested that this systemic treatment will significantly affect ocular FB levels (Grossman et al., 2017), the possibility of enough FB produced by the RPE to counteract this remains (Schnabolk et al., 2015). Additional safety data from the phase II trial will be critical to evaluate the merits of this unique systemic therapy for GA.

Properdin stabilizes C3 and C5 convertases, and CLG561 (Novartis) is an anti-properdin monoclonal antibody. A trial (NCT02515942) evaluating monthly intravitreal injection of CLG561 as monotherapy or in combination with LFG316 was completed. Despite hopes to see benefits from a combination treatment approach to the complement cascade, this trial of 114 patients also had negative results with no benefit for GA growth.

7.2. C5 therapeutics

Iveric Bio’s avacincaptad pegol, which is a pegylated aptamer, has garnered significant attention as a promising C5 inhibitor for GA. Avacincaptad pegol is administered by monthly intravitreal injections and inhibits the cleavage of C5, preventing formation of C5a and C5b. It therefore offers potential for reducing inflammasome activation from C5a and preventing MAC formation. A phase III trial for this drug has recently started. The result of the phase II/III trial for avacincaptad pegol was especially intriguing because of its similarity to the phase II data for the C3 inhibitor pegcetacoplan. The avacincaptad pegol phase IIb

trial randomized 67 patients to the 2 mg dose, 83 patients to the 4 mg dose, and a total of 110 patients to sham injections. Randomization to the 4 mg dose only occurred in the second stage of this trial, and patients receiving the 4 mg dose were compared to the 84 patients randomized to sham injection in this stage of the trial. After 12 months, the GA growth rate was reduced by 27.4% ($p = 0.0072$) with the 2 mg dose and 27.8% ($p = 0.005$) with the 4 mg dose compared to sham treatments (Jaffe et al., 2020). This study also found higher than expected conversions to exudative AMD with 9.0% in the 2 mg group and 9.6% in the 4 mg group, as compared to 2.7% of sham control eyes. Other than the conversions to exudative AMD, there were no significant safety concerns, including no cases of endophthalmitis. Both pegcetacoplan and avacincaptad pegol reduced GA growth rates by close to 30% compared to sham treatment, and both found a higher than expected conversion to exudative AMD. These very promising findings have increased confidence that these complement inhibitors have significant biologic effects on AMD and lead to anticipation that they will successfully reduce GA growth rates in the ongoing phase III trials. It is possible that the inhibition of MAC, whether by C3 or C5 inhibition, may account for a great part of the therapeutic effect as the GA growth rate reductions were comparable with these two drugs. Nevertheless, the broader complement inhibition of C3 therapeutics deserves emphasis, and there are potentially unrealized benefits of this broader inhibition (e.g. direct or indirect protective effects of C3 inhibition on minimizing retinal cell phagocytosis/damage) that will be seen as more clinical data is obtained. Although the conversions to exudative AMD remain concerning, they can be treated with anti-VEGF therapy. If the conversions to exudative AMD are replicated in the phase III trials, then clinicians will ideally diagnose these conversions early as patients return for complement inhibitor injections. Further, there is a question of whether periodic concurrent anti-VEGF therapy would be useful were these drugs to get approved. The data suggest that CNV is potentially nourishing to the retina; there is likely a delicate balance whereby mild CNV could reduce atrophy development, but overactive CNV is vision-threatening. An alternative possibility is that the PEG attachment for these two therapeutics is contributing to CNV, as PEG is not an inert attachment. Potential accumulation of PEG into tissues during chronic treatments with high PEG doses may cause adverse effects (e.g. toxicity, tissue vacuolation), and others have suggested that it can cause CNV (Jevševar et al., 2010; Lyzogubov et al., 2011; McDonnell et al., 2014). Finally, one must wonder if complement inhibition can reduce GA growth rates by more than 30% or if inhibition of other GA pathogenic mechanisms is needed to achieve higher reductions in growth rates. Here as well, the phase III results will be illuminating.

Eculizumab (Alexion) is the first FDA approved complement inhibitor and has indications ranging from paroxysmal nocturnal hemoglobinuria and atypical hemolytic uremic syndrome, to generalized myasthenia gravis and neuromyelitis optica spectrum disorder (Frampton, 2020). Eculizumab is a monoclonal antibody that binds C5 and inhibits its cleavage into C5b and C5a. The clinical development of eculizumab was spearheaded in the 1980's by Frei and colleagues who discovered BB5.1, a monoclonal antibody that selectively bound mouse C5 and enabled the first proof of concept studies for C5 inhibition in inflammatory disease models (Frei et al., 1987). Of note, eculizumab is also one of the most expensive drugs on the market with a cost of about $500,000 per patient per year (Elgin et al., 2017). The COMPLETE trial evaluated intravenous eculizumab for GA. This

small pilot trial randomized 30 patients and measured the change of GA area at 6 months as the primary outcome (Yehoshua et al., 2014). No beneficial effect on GA growth rate was seen, but it is likely that insufficient amounts of the drug reached the eye beyond the choroid. The small sample size and 6 month primary outcome also hindered the ability to observe a positive effect. It is unknown if intravitreal injection of eculizumab is beneficial for GA patients. Interestingly, companies developing eculizumab biosimilars have emerged (Ricklin et al., 2018).

Tesidolumab (LFG316, Novartis) is an IgG1 antibody that prevents C5 cleavage (Roguska et al., 2014). No significant safety events were seen in a phase I study of intravitreal injection of tesidolumab (NCT01255462). A subsequent phase II trial involved 150 patients randomized 2:1 to 5 mg of monthly tesidolumab vs. sham injection. No benefit for GA or vision was seen for tesidolumab (NCT01527500). Limited information is in the public domain about LFG316, making it difficult to compare to other C5 inhibitors.

CD59 is a membrane GPI (glycosylphosphatidylinositol)-anchored regulatory protein that inhibits formation of MAC by acting on C9. Normally, CD59 prevents unwanted damage to cells from MAC. Hemera Biosciences has designed an adeno-associated virus gene therapy that leads to production of soluble CD59 by retinal cells. Known as HMR59 (or AAVCAGsCD59), this therapy is administered as a single intravitreal injection. Phase 1 data for 17 nonexudative AMD patients showed that HMR59 was well tolerated, although results have not been published. A phase II trial for GA has been planned (NCT04358471). Further, a phase I trial will be evaluating HMR59 for exudative AMD patients.

7.3. Complement inhibition and infection

The risk of infections with complement inhibition is a long-debated topic that has largely been based on assumptions rather than actual clinical evidence from the use of complement inhibitors. Safety issues regarding the use of C3 or C5 therapeutics have been extensively discussed in recent reviews (Mastellos et al., 2017, 2019). C3 deficient patients are at increased risk for pyogenic infections, and C5 deficient patients are particularly susceptible to infections by *Neisseria meningitides* (Ricklin et al., 2018). Meningococcal vaccines have therefore been a part of clinical protocols for C5 therapeutics. However, it should be noted that these infections seen in C3 and C5 deficiencies tend to affect younger age patients, suggesting that the adaptive immune system develops compensatory mechanisms that offset the infection risk (Botto et al., 1992). Furthermore, pharmacologic complement inhibition does not recapitulate the wider consequences of a genetic deficiency that may skew ones' immune response in yet unpredictable ways, considering the multitude of interconnected systems and pathways. Appropriate vaccination is needed if a C3- or C5-based therapy is used systemically, but the risk of infection is not as high as once thought and two complement inhibitors (eculizumab and pegcetacoplan) have shown acceptable safety profiles during prolonged and systemic clinical intervention in patients with paroxysmal nocturnal hemoglobinuria (Castro et al., 2020). Additionally, the systemic risk is not much of a concern for therapeutics delivered directly to the eye, and the phase II AMD trials for pegcetacoplan and avacincaptad pegol showed no clear ocular infection risk. While we await phase III results, the risk of endophthalmitis also may not be as high as previously thought.

Lastly, it is worth noting that the ability to inject a medicine that wears off may have benefits as opposed to a gene therapy that persistently blocks the complement cascade.

8. The complotype and AMD subtypes

When considering the AMD associated C3 rs2230199 SNP as well as other polymorphisms of FH and complement FB, the potential for pharmacogenomics arises. It has yet to be determined if complement subtyping, sometimes referred to as the “complotype” (Harris et al., 2012), could be relevant to AMD. Initial thoughts that lampalizumab was most beneficial for patients with a FI risk allele were not demonstrated in the phase III trials (Holz et al., 2018), and the phase II trial for pegcetacoplan found no greater treatment effect associated with a gene polymorphism (Liao et al., 2020). On the other hand, there is evidence that other complement-mediated diseases display variable drug responses based on complement genetics. For example, paroxysmal nocturnal hemoglobinuria patients with a polymorphism in the CR1 gene are seven times more likely to have a suboptimal response to eculizumab and need blood transfusion (Rondelli et al., 2014). In these patients, the CR1 polymorphism leads to lower expression of CR1, thereby causing less decay of the C3 convertase, increased C3 mediated opsonization of erythrocytes, and subsequent extravascular hemolysis.

In contrast to other complement diseases, why is there such limited evidence for the use of complotyping in GA trials? One explanation is that genetic polymorphisms associated with AMD development may have less of a role in the mechanisms of GA progression (Guymer, 2018). Another consideration is that data have suggested that a single disease-associated polymorphism may have a relatively small effect on complement activity, whereas grouping patients by their overall combination of polymorphisms can reveal greater effects of the “complotype” on complement activity (Heurich et al., 2011). In fact, the complotype is meant to refer to the collection of complement gene variations an individual may have and does not focus on a single polymorphism. Looking at drug effects by grouping patients by individual alleles may not be as effective as grouping patients by their overall complotype.

GA trials have thus far recruited patients with the common endpoint of GA growth rates despite some possible heterogeneity in the underlying mechanisms leading to GA. Is subgrouping by complotype enough or should this be combined with other putative GA subtypes to evaluate drug effects? Morphologic subtypes determined by imaging seems graspable, but there are still uncertainties with regards to subtyping GA patients in this way. Actually, it is still debated if the primary pathophysiology of GA is at the choriocapillaris, RPE, or photoreceptors. The choriocapillaris (Whitmore et al., 2015) and RPE are perhaps more commonly implicated as the primary sites of GA pathophysiology. For example, eyes with early AMD and especially those with GA have choriocapillaris loss compared to normal controls (Sohn et al., 2019; Biesemeier et al., 2014), and there is decreased subfovealor blood flow in more advanced stages of AMD (Grunwald et al., 1998, 2005). However, using a histopathological assessment, Bird et al. have demonstrated GA cases where there is significant photoreceptor loss away from the GA lesion edge (Bird et al., 2014). We must also remember that some surgical cases of macular translocation led to subsequent RPE atrophy at the location where photoreceptor-deficient retina was translocated (Khurana et al., 2005). There may be GA subtypes that are driven first by

photoreceptor defects and other subtypes driven first by RPE or choriocapillaris loss. How do complotypes relate to the primary cell type lost in cases of GA? In these potential subtypes, the extent of complement pathophysiology and its timing as GA develops and grows is unclear. Answering these questions would help determine whether complement based pharmacogenomics has untapped benefits for GA. Beyond pharmacogenomics, the potential for personalized AMD risk models to include the complotype along with other risk factors such as the environment (e.g. smoking) deserves more exploration (Handa et al., 2019).

9. Conclusions and future directions

With over 5 million people worldwide with GA (Wong et al., 2014), AMD holds a pivotal position for the field of complement therapeutics, as current inhibitors are approved for diseases that are much less prevalent. Among the potential points of inhibition in the complement cascade, therapeutics aimed at C3 and C5 have advanced as the leading candidates. The promising results in phase II trials for pegcetacoplan and avacincaptad pegol increase confidence that these therapeutics exert real biological effects on AMD that will be confirmed in the phase III trials. As complement inhibitors for GA develop, they open the door for other ophthalmic opportunities. The utility of complement inhibitors for earlier stages of AMD should also be considered, as preventing rather than slowing GA lesions would have an even greater impact. Furthermore, data is surfacing regarding complement's role in other ocular diseases; these include Stargardt disease as well as highly prevalent conditions such as diabetic retinopathy and glaucoma (Bosco et al., 2018; Clark and Bishop, 2018; Hu et al., 2020; Shahulhameed et al., 2020). More exploration of complement mechanisms in these diseases is warranted.

Despite the current pace of clinical development of C3 and C5 inhibitors for GA, there are several remaining questions about complement pathophysiology in AMD. Further studies are needed to determine if pathologic complement activity in the outer retina and choroid of humans with AMD is primarily driven by local production, systemic production, or both. This should include further investigations of ocular expression of complement proteins and their receptors using normal and diseased human ocular tissue categorized by AMD stage. Another unsolved puzzle is the disparity between animal models for CNV and AMD mechanisms in humans that have culminated in limited clinical trial progress of complement inhibitors for exudative AMD. Instead, complement inhibition has heightened its momentum for the GA of non-exudative AMD, and recent clinical data raise intriguing questions about how C3 and C5 relate to CNV activity. Finally, it is critical to realize that despite the grossly similar clinical results for pegcetacoplan and avacincaptad pegol, C3 and C5 inhibition are quite different from a mechanistic standpoint and may impact in non-overlapping ways the pathophysiology of AMD. While inhibiting cleavage of C3 and C5 will both limit C5a and MAC formation, C3 and C5 inhibition are not the same (Mastellos et al., 2020). With multiple bioactive fragments involved in opsonization and immune activation, C3 therapeutics broadly inhibit multiple complement-mediated pathways. On the other hand, C5 therapeutics specifically target the terminal complement pathway, blocking C5a generation and MAC formation. We have not yet determined how these differences could affect eyes with AMD. For example, some patients with paroxysmal nocturnal hemoglobinuria do not

respond well to the C5 inhibitor eculizumab; one of the proposed mechanisms is extravascular hemolysis of C3b-opsonized erythrocytes (Lin et al., 2015). How important is opsonization for eyes with AMD? Preliminary studies in a cynomolgus monkey model of early-onset macular degeneration characterized by drusen development suggest that intravitreal injection of compstatin may cause drusen regression (Chi et al., 2010), and the AP inhibitor CR2-fH causes regression of deposits at Bruch's membrane in a nonexudative AMD mouse model (Woodell et al., 2016). Although the mechanistic role of drusen in AMD is unclear (Maguire, 2012), could human drusen be affected differently by C3 vs. C5 inhibition? Might C3 inhibition have a role in the treatment of earlier stages of AMD? Are there long term effects that will distinguish C3 from C5 inhibition? More research is needed, and ultimately these questions will be addressed by more clinical data. C3 inhibition is wider-ranging in potential effects, C5 inhibition has a narrower therapeutic scope, and different complement-mediated diseases may have different needs for complement inhibition.

We believe that complement therapeutics should have a beneficial role for AMD. We suggest the following complement-focused model of GA pathophysiology (Fig. 7).

- First, there is some basal level of complement activity within the retina for homeostasis. This includes expression of complement proteins within Muller cells, RPE, photoreceptors, choriocapillaris, and microglia within the retina and choroid.
- Second, there is oxidative stress from multiple factors including light damage, iron overload, mitochondrial dysfunction, and, in some cases, cigarette smoking. Over time, there is buildup of metabolic waste products, including bisretinoids within the RPE. Photooxidation of bisretinoids can contribute to complement activation (Zhou et al., 2009). A stressed microenvironment then leads to increased production and deposition of complement proteins within the RPE and choriocapillaris, including local activation and deposition of MAC. Iron overload increases expression of C3 by the RPE (Li et al., 2015), and also contributes to activation of the NLRP3 inflammasome in the RPE (Gelfand et al., 2015), which recruits complement-producing macrophages to the retina. These macrophages can produce C3 and then deposit C3 fragments on the photoreceptors, further activating the complement cascade and likely amplifying the opsonophagocytic clearance of retinal cells.
- Additionally, some patients have a genetic predisposition to AMD based on complement polymorphisms (Heurich et al., 2011; Paun et al., 2016). While key complement factors have been found on the choriocapillaris, RPE, and photoreceptors, these factors may come from both local production and systemic production. In patients with systemically overactive complement, such as those with pathogenic complement polymorphisms, deposition into the choriocapillaris and deposition into the outer retina via passage through an incompetent blood-retina barrier may further exacerbate local complement activity (Schultz et al., 2019). The choriocapillaris is likely a key site of GA pathophysiology, and human tissue studies found C3, C5, and MAC predominantly in the choroid (see

Sections 5 and 6.3). The potentially important role of the choriocapillaris in GA suggests that the ability of a therapeutic to access the choriocapillaris deserves consideration. We have shown that Cp40-KKK (see Section 7.1) can access the choriocapillaris after intravitreal injection, but evaluating complement inhibition with suprachoroidal injection approaches (Yeh et al., 2020) could be explored. Nevertheless, there may be subtypes of AMD for which the RPE or photoreceptors have an equal or more important role in pathophysiology (Bird et al., 2014).

- Finally, dysregulated complement activity may lead to increased cell death in several ways including MAC'S ability to lyse cells, sublytic MAC mechanisms, and the actions of C3 and C5 fragments (Fig. 8). Uncontrolled lysis of outer retina cells by MAC has been considered a key pathogenic pathway. However, emerging evidence strongly suggests that sub-lytic MAC causes significant pathology as well. Sub-lytic MAC stimulates inflammation with release of cytokines such as MCP-1/CCL2 (monocyte chemoattractant protein/chemokine (C—C motif) ligand 2) by the RPE, which recruits phagocytes (Lueck et al., 2011; Mulfaul et al., 2020). Further, RPE cells can alleviate the plasma membrane damage from MAC by endocytosis of MAC (Georgiannakis et al., 2015). Increased MAC formation can therefore overload the lysosomes of RPE, impairing their ability to process damaging macromolecules and exacerbating these pathways. *In vitro* evidence suggests that C3 inhibition with compstatin Cp40 alleviates the overload of lysosomes in the RPE by lowering C3 turnover and attenuating internalization and deposition of MAC on lysosomal vesicles (Cerniauskas et al., 2020). This study not only supports the clinical potential of C3 inhibition in AMD patients carrying risk-associated FH polymorphisms but also reveals an important link between complement activation in the retinal space and the autophagy-lysosome pathway in the RPE compartment.

  In this article, we have also discussed the potent functions of C3 and C5 fragments, which should not be underestimated. C3a and C5a can also recruit microglia/macrophages to the subretinal space. The accumulation of these subretinal phagocytes may also be related to decreased clearance from a non-canonical FH dependent mechanism whereby FH, and especially the Y402H FH variant, competitively bind to CD47 and CR3, preventing the clearance mediated by thrombospondin-1's interaction with these receptors (Calippe et al., 2017). Uncontrolled opsonization by C3 fragments and subsequent phagocytosis may cause loss of photoreceptors, RPE, and choroidal endothelium. Primary phagocytosis (phagoptosis) is cell death from phagocytosis of cells that are stressed but viable and has been shown in neurons (Brown and Neher, 2014). Primary phagocytosis of photoreceptors by microglia has been shown in the rd10 mouse model of retinitis pigmentosa, and more data evaluating primary phagocytosis in AMD is needed (Zhao et al., 2015). While inflammasome activity can be stimulated by different signals including oxidized lipoproteins (Gnanaguru et al., 2016) and lysosome dysfunction (Tseng et al., 2013), C5a can prime the RPE for inflammasome activity (Brandstetter et al., 2015). Once

activated, the inflammasome also contributes to RPE cell death (Tarallo et al., 2012). In addition to recruiting microglia/macrophages, C5a can shift their phenotype towards proinflammatory and phagocytic activity (Liao et al., 2020) with further damage to photoreceptors and RPE as stated above. Lastly, a reduction in C5a activity may stabilize or stimulate choroidal neovascular tissue that nourishes the outer retina; this possible relationship cannot be excluded and deserves further investigation (Capuano et al., 2017; Wong, 2020).

In summary, in advanced nonexudative AMD, there is a complex microenvironment with several crucial complement-mediated pathways, some of which likely run in parallel to cause outer retina cell damage. C3, C5, and their fragments play critical roles, and clinical data thus far shows a promising future for C3 and C5 based therapies for GA. Ongoing investigations should help discern whether the broader inhibitory profile of C3 therapeutics may translate into greater clinical gains in AMD patients, clinically distinguishing itself from the terminal pathway-specific nature of C5 therapeutics.

Acknowledgements

Funding: This work was supported by the National Institutes of Health [R01EY028916 (J.L.D.)]; funding was also provided in the form of block research grants to the Scheie Eye Institute from Research to Prevent Blindness (New York, NY) and the Paul and Evanina Bell Mackall Foundation (Chicago, IL).

References

Alcorlo M, Martínez-Barricarte R, Fernández FJ, Rodríguez-Gallego C, Round A, Vega MC, Harris CL, Rodríguez De Cordoba S, Llorca O, 2011. Unique structure of iC3b resolved at a resolution of 24 Å by 3D-electron microscopy. Proc. Natl. Acad. Sci. U. S. A 108, 13236–13240. 10.1073/pnas.1106746108. [PubMed: 21788512]

Alcorlo M, Tortajada A, Rodriguez de Cordoba S, Llorca O, 2013. Structural basis for the stabilization of the complement alternative pathway C3 convertase by properdin. Proc. Natl. Acad. Sci. Unit. States Am 110, 13504–13509. 10.1073/pnas.1309618110.

Alcorlo M, López-Perrote A, Delgado S, Yébenes H, Subías M, Rodríguez-Gallego C, Rodríguez de Córdoba S, Llorca O, 2015. Structural insights on complement activation. FEBS J. 282, 3883–3891. 10.1111/febs.13399. [PubMed: 26250513]

Amara U, Flierl MA, Rittirsch D, Klos A, Chen H, Acker B, Brückner UB, Nilsson B, Gebhard F, Lambris JD, Huber-Lang M, 2010. Molecular intercommunication between the complement and coagulation systems. J. Immunol 185 (9), 5628–5636. 10.4049/jimmunol.0903678. [PubMed: 20870944]

Anderson DH, Radeke MJ, Gallo NB, Chapin EA, Johnson PT, Curletti CR, Hancox LS, Hu J, Ebright JN, Malek G, Hauser MA, Bowes Rickman C, Bok D, Hageman GS, Johnson LV, 2010. The pivotal role of the complement system in aging and age-related macular degeneration: hypothesis re-visited. Prog. Retin. Eye Res 29 (2), 95–112. [PubMed: 19961953]

Apellis Pharmaceuticals. Pegcetacoplan News Releases, https://investors.apellis.com/news-releases/news-release-details/apellis-announces-submission-pegcetacoplan-marketing accessed 21 September 2020.

Arbore G, West EE, Spolski R, Robertson AAB, Klos A, Rheinheimer C, Dutow P, Woodruff TM, Yu ZX, O'Neill LA, Coll RC, Sher A, Leonard WJ, Köhl J, Monk P, Cooper MA, Arno M, Afzali B, Lachmann HJ, Cope AP, Mayer-Barber KD, Kemper C, 2016. T helper 1 immunity requires complement-driven NLRP3 inflammasome activity in CD4+ T cells. Science 352 (6292), aad1210. 10.1126/science.aad1210. [PubMed: 27313051]

Baas DC, Ho L, Ennis S, Merriam JE, Tanck MWT, Uitterlinden AG, de Jong PTVM, Cree AJ, Griffiths HL, Rivadeneira F, Hofman A, van Duijn C, Smith RT, Barile GR, Gorgels TGMF,

Vingerling JR, Klaver CCW, Lotery AJ, Allikmets R, Bergen AAB, 2010. The complement component 5 gene and age-related macular degeneration. Ophthalmology 117 (3), 500–511. 10.1016/j.ophtha.2009.08.032. [PubMed: 20022638]

Bajic G, Yatime L, Sim RB, Vorup-Jensen T, Andersen GR, 2013. Structural insight on the recognition of surface-bound opsonins by the integrin i domain of complement receptor 3. Proc. Natl. Acad. Sci. U. S. A 110 (41), 16426–16431. 10.1073/pnas.1311261110. [PubMed: 24065820]

Berger N, Alayi TD, Resuello RRG, Tuplano J.v., Reis ES, Lambris JD, 2018. New analogs of the complement C3 inhibitor compstatin with increased solubility and improved pharmacokinetic profile. J. Med. Chem 61 (14), 6153–6162. 10.1021/acs.jmedchem.8b00560. [PubMed: 29920096]

Bergmann-Leitner ES, Leitner WW, Tsokos GC, 2006. Complement 3d: from molecular adjuvant to target of immune escape mechanisms. Clin. Immunol 121 (2), 177–185. 10.1016/j.clim.2006.07.001. [PubMed: 16914381]

Biesemeier A, Taubitz T, Julien S, Yoeruek E, Schraermeyer U, 2014. Choriocapillaris breakdown precedes retinal degeneration in age-related macular degeneration. Neurobiol. Aging 35 (11), 2562–2573. [PubMed: 24925811]

Bird AC, Phillips RL, Hageman GS, 2014. Geographic atrophy: a histopathological assessment. JAMA Ophthalmology 132 (3), 338–345. 10.1001/jamaophthalmol.2013.5799. [PubMed: 24626824]

Bora PS, Sohn J-H, Cruz JMC, Jha P, Nishihori H, Wang Y, Kaliappan S, Kaplan HJ, Bora NS, 2005. Role of complement and complement membrane attack complex in laser-induced choroidal neovascularization. J. Immunol 174 (1), 491–497. 10.4049/jimmunol.174.1.491. [PubMed: 15611275]

Bosco A, Anderson SR, Breen KT, Romero CO, Steele MR, Chiodo VA, Boye SL, Hauswirth WW, Tomlinson S, Vetter ML, 2018. Complement C3-targeted gene therapy restricts onset and progression of neurodegeneration in chronic mouse glaucoma. Mol. Ther 26 (10), 2379–2396. 10.1016/j.ymthe.2018.08.017. [PubMed: 30217731]

Botto M, Fong KY, So AK, Barlow R, Routier R, Morley BJ, Walport MJ, 1992. Homozygous hereditary C3 deficiency due to a partial gene deletion. Proc. Natl. Acad. Sci. U. S. A 89 (11), 4957–4961. 10.1073/pnas.89.11.4957. [PubMed: 1350678]

Boyer DS, Schmidt-Erfurth U, Van M, Campagne L, Henry EC, Brittain C, 2017. The pathophysiology of geographic atrophy secondary to age-related macular degeneration and the complement pathway as a therapeutic target. Retina 37 (5), 819–835. [PubMed: 27902638]

Brandstetter C, Holz FG, Krohne TU, 2015. Complement component c5a primes retinal pigment epithelial cells for inflammasome activation by lipofuscin-mediated photooxidative damage. J. Biol. Chem 290 (52), 31189–31198. 10.1074/jbc.M115.671180. [PubMed: 26565031]

Brown GC, Neher JJ, 2014. Microglial phagocytosis of live neurons. Nat. Rev. Neurosci 15 (4), 209–216. 10.1038/nrn3710. [PubMed: 24646669]

Busch C, Annamalai B, Abdulsalamova K, Reichhart N, Huber C, Lin Y, Jo EAH, Zipfel PF, Skerka C, Wildner G, Diedrichs-Mohring M, Rohrer B, Strauβ O, 2017. Anaphylatoxins activate Ca 2+, akt/PI3-kinase, and FOXO1/FoxP3 in the retinal pigment epithelium. Front. Immunol 8, 703. [PubMed: 28663750]

Calippe B, Augustin S, Beguier F, Charles-Messance H, Poupel L, Conart JB, Hu SJ, Lavalette S, Fauvet A, Rayes J, Levy O, Raoul W, Fitting C, Denèfle T, Pickering MC, Harris C, Jorieux S, Sullivan PM, Sahel JA, Karoyan P, Sapieha P, Guillonneau X, Gautier EL, Sennlaub F, 2017. Complement factor H inhibits CD47-mediated resolution of inflammation. Immunity 46 (2), 261–272. 10.1016/j.immuni.2017.01.006. [PubMed: 28228282]

Campbell W, Okada N, Okada H, 2001. Carboxypeptidase R is an inactivator of complement-derived inflammatory peptides and an inhibitor of fibrinolysis. Immunol. Rev 180, 162–167. [PubMed: 11414358]

Capuano V, Miere A, Querques L, Sacconi R, Carnevali A, Amoroso F, Bandello F, Souied EH, Querques G, 2017. Treatment-naïve quiescent choroidal neovascularization in geographic atrophy secondary to nonexudative age-related macular degeneration. Am. J. Ophthalmol 182, 45–55. 10.1016/j.ajo.2017.07.009. [PubMed: 28734811]

Castro C, Grossi F, Weitz IC, Maciejewski J, Sharma V, Roman E, Brodsky RA, Tan L, Di Casoli C, El Mehdi D, Deschatelets P, Francois C, 2020. C3 inhibition with pegcetacoplan in subjects with

paroxysmal nocturnal hemoglobinuria treated with eculizumab. Am. J. Hematol. ajh 25960 10.1002/ajh.25960.

Catalyst Biosciences. Pipeline, Complement, https://www.catalystbiosciences.com/pipeline/complement/ accessed 25 September 2020.

Cerniauskas E, Kurzawa-Akanbi M, Xie L, Hallam D, Moya-Molina M, White K, Steel D, Doherty M, Whitfield P, Al-Aama J, Armstrong L, Kavanagh D, Lambris JD, Korolchuk VI, Harris C, Lako M, 2020. Complement modulation reverses pathology in Y402H-retinal pigment epithelium cell model of age-related macular degeneration by restoring lysosomal function. Stem Cells Translational Medicine. Advance online publication. 10.1002/sctm.20-0211.

Chen ZA, Pellarin R, Fischer L, Sali A, Nilges M, Barlow PN, Rappsilber J, 2016. Structure of complement C3(H_2O) revealed by quantitative cross-linking/mass spectrometry and modeling. Mol. Cell. Proteomics 15, 2730–2743. 10.1074/mcp.M115.056473. [PubMed: 27250206]

Cheng L, Bu H, Portillo JAC, Li Y, Subauste CS, Huang SS, Kern TS, Lin F, 2013. Modulation of retinal Müller cells by complement receptor C5aR. Investig. Ophthalmol. Vis. Sci 54 (13), 8191–8198. 10.1167/iovs.13-12428. [PubMed: 24265019]

Chi ZL, Yoshida T, Lambris JD, Iwata T, 2010. Suppression of drusen formation by compstatin, a peptide inhibitor of complement C3 activation, on cynomolgus monkey with early-onset macular degeneration. Adv. Exp. Med. Biol 703, 127–135. 10.1007/978-1-4419-5635-4_9. [PubMed: 20711711]

Clark SJ, Bishop PN, 2018. The eye as a complement dysregulation hotspot. Semin. Immunopathol 40 (1), 65–74. 10.1007/s00281-017-0649-6. [PubMed: 28948331]

Clark SJ, McHarg S, Tilakaratna V, Brace N, Bishop PN, 2017. Bruch's membrane compartmentalizes complement regulation in the eye with implications for therapeutic design in age-related macular degeneration. Front. Immunol 8, 1778. 10.3389/fimmu.2017.01778. [PubMed: 29312308]

Coffey PJ, Gias C, McDermott CJ, Lundh P, Pickering MC, Sethi C, Bird A, Fitzke FW, Maass A, Chen LL, Holder GE, Luthert PJ, Salt TE, Moss SE, Greenwood J, 2007. Complement factor H deficiency in aged mice causes retinal abnormalities and visual dysfunction. Proc. Natl. Acad. Sci. Unit. States Am 104 (42), 16651–16656.

Datta S, Cano M, Ebrahimi K, Wang L, Handa JT, 2017. The impact of oxidative stress and inflammation on RPE degeneration in non-neovascular AMD. Prog. Retin. Eye Res 60, 201–218. 10.1016/j.preteyeres.2017.03.002. [PubMed: 28336424]

de Bruijn MH, Fey GH, 1985. Human complement component C3: cDNA coding sequence and derived primary structure. Proc. Natl. Acad. Sci. Unit. States Am. 82, 708–712. 10.1073/pnas.82.3.708.

de Oliveira Dias JR, Zhang Q, Garcia JMB, Zheng F, Motulsky EH, Roisman L, Miller A, Chen CL, Kubach S, de Sisternes L, Durbin MK, Feuer W, Wang RK, Gregori G, Rosenfeld PJ, 2018. Natural history of subclinical neovascularization in nonexudative age-related macular degeneration using swept-source OCT angiography. Ophthalmology 125 (2), 255–266. 10.1016/j.ophtha.2017.08.030. [PubMed: 28964581]

Decanini A, Nordgaard CL, Feng X, Ferrington DA, Olsen TW, 2007. Changes in select redox proteins of the retinal pigment epithelium in age-related macular degeneration. Am. J. Ophthalmol 143 (4), 607–615. 10.1016/j.ajo.2006.12.006. [PubMed: 17280640]

Delanghe JR, Speeckaert R, Speeckaert MM, 2014. Complement C3 and its polymorphism: biological and clinical consequences. Pathology 46 (1), 1–10. 10.1097/PAT.0000000000000042. [PubMed: 24300728]

Dempsey PW, Allison ME, Akkaraju S, Goodnow CC, Fearon DT, 1996. C3d of complement as a molecular adjuvant: bridging innate and acquired immunity. Science 271 (5247), 348–350. [PubMed: 8553069]

Dolgin E, 2017. Age-related macular degeneration foils drugmakers. Nat. Biotechnol 35, 1000–1001. 10.1038/nbt1117-1000. [PubMed: 29121027]

Dunaief JL, 2006. Iron induced oxidative damage as a potential factor in age-related macular degeneration: the Cogan lecture. Investig. Ophthalmol. Vis. Sci 47 (11), 4660–4664. 10.1167/iovs.06-0568. [PubMed: 17065470]

Duval D, Pichon R, Lassalle D, Laffitte M, Gourbal B, Galinier R, 2020. A new assessment of thioester-containing proteins diversity of the freshwater snail Biomphalaria glabrata. Genes 11, 69. 10.3390/genes11010069.

Ebrahimi KB, Fijalkowski N, Cano M, Handa JT, 2013. Decreased membrane complement regulators in the retinal pigmented epithelium contributes to age-related macular degeneration. J. Pathol 229 (5), 729–742. 10.1002/path.4128. [PubMed: 23097248]

Edwards AO, Ritter R, Abel KJ, Manning A, Panhuysen C, Farrer LA, 2005. Complement factor H polymorphism and age-related macular degeneration. Science 308 (5720), 421–424. 10.1126/science.1110189. [PubMed: 15761121]

Ekdahl KN, Persson B, Mohlin C, Sandholm K, Skattum L, Nilsson B, 2018. Interpretation of serological complement biomarkers in disease. Front. Immunol 9, 2237. 10.3389/fimmu.2018.02237. [PubMed: 30405598]

Elgin B, Bloomfield D, Chen C, 2017. When the Patient Is a Goldmine: the Trouble with Rare-Disease Drugs. Bloomberg Businessweek, https://www.bloomberg.com/news/features/2017-05-24/when-the-patient-is-a-gold-mine-the-trouble-with-rare-disease-drugs. accessed 25 September 2020.

Elvington ML, Liszewski MK, Kulkarni HS, Atkinson JP, 2016. A C3(H2O) recycling and degradation pathway of the intracellular complement system. Immunobiology 221 (10), 1197. 10.1016/j.imbio.2016.06.160.

Elvington M, Liszewski MK, Bertram P, Kulkarni HS, Atkinson JP, 2017. A C3 (H20) recycling pathway is a component of the intracellular complement system. J. Clin. Invest 127 (3), 970–981. 10.1172/JCI89412. [PubMed: 28192370]

Emlen W, Li W, Kirschfink M, 2010. Therapeutic complement inhibition: new developments. Semin. Thromb. Hemost 36, 660–668. 10.1055/s-0030-1262888. [PubMed: 20865643]

Erdei A, Sándor N, Mácsik-Valent B, Lukácsi S, Kremlitzka M, Bajtay Z, 2016. The versatile functions of complement C3-derived ligands. Immunol. Rev 274 (1), 127–140. 10.1111/imr.12498. [PubMed: 27782338]

Fernandez-Godino R, Garland DL, Pierce EA, 2015. A local complement response by RPE causes early-stage macular degeneration. Hum. Mol. Genet 24 (19), 5555–5569. 10.1093/hmg/ddv287. [PubMed: 26199322]

Fernandez-Godino R, Pierce EA, 2018a. C3a triggers formation of sub-retinal pigment epithelium deposits via the ubiquitin proteasome pathway. Sci. Rep 8 (1), 9679. 10.1038/s41598-018-28143-0. [PubMed: 29946065]

Fernandez-Godino R, Bujakowska KM, Pierce EA, 2018b. Changes in extracellular matrix cause RPE cells to make basal deposits and activate the alternative complement pathway. Hum. Mol. Genet 27 (1), 147–159. 10.1093/hmg/ddx392. [PubMed: 29095988]

Ferreira VP, Pangburn MK, Cortés C, 2010. Complement control protein factor H: the good, the bad, and the inadequate. Mol. Immunol 47 (13), 2187–2197. 10.1016/j.molimm.2010.05.007. [PubMed: 20580090]

Fett AL, Hermann MM, Muether PS, Kirchhof B, Fauser S, 2012. Immunohistochemical localization of complement regulatory proteins in the human retina. Histol. Histopathol 27 (3), 357–364. 10.14670/HH-27.357. [PubMed: 22237713]

Frampton JE, 2020. Eculizumab: a review in neuromyelitis optica spectrum disorder. Drugs 80 (7), 719–727. 10.1007/s40265-020-01297-w. [PubMed: 32266705]

Fredslund F, Laursen NS, Roversi P, Jenner L, Oliveira CLP, Pedersen JS, Nunn MA, Lea SM, Discipio R, Sottrup-Jensen L, Andersen GR, 2008. Structure of and influence of a tick complement inhibitor on human complement component 5. Nat. Immunol 9, 753–760. 10.1038/ni.1625. [PubMed: 18536718]

Freeley S, Kemper C, le Friec G, 2016. The “ins and outs” of complement-driven immune responses. Immunol. Rev 274 (1), 16–32. 10.1111/imr.12472. [PubMed: 27782335]

Frei Y, Lambris JD, Stockinger B, 1987. Generation of a monoclonal antibody to mouse C5 application in an ELISA assay for detection of anti-CS antibodies. Mol. Cell. Probes 1 (2), 141–149. 10.1016/0890-8508(87)90022-3. [PubMed: 3453897]

Furfine E, Rao A, Baker S, Connacher M, Kazanstev A, Kumar B, Blouse G, Stanton M, Traylor M, 2019. Pegylated CB2782: a complement factor C3-inactivating protease and potential long-acting treatment for dry AMD. Invest. Ophthalmol. Vis. Sci 60, 374.

Gelfand BD, Wright CB, Kim Y, Yasuma T, Yasuma R, Li S, Fowler BJ, Bastos-Carvalho A, Kerur N, Uittenbogaard A, Han YS, Lou D, Kleinman ME, McDonald WH, Núñez G, Georgel P, Dunaief JL, Ambati J, 2015. Iron toxicity in the retina requires alu RNA and the NLRP3 inflammasome. Cell Rep. 11 (11), 1686–1693. 10.1016/j.celrep.2015.05.023. [PubMed: 26074074]

Geller A, Yan J, 2019. The role of membrane bound complement regulatory proteins in tumor development and cancer immunotherapy. Front. Immunol 10, 1074. 10.3389/fimmu.2019.01074. [PubMed: 31164885]

Gensler G, Clemons TE, Domalpally A, Danis RP, Blodi B, Wells J, Rauser M, Hoskins J, Hubbard GB, Elman MJ, Fish GE, Brucker A, Margherio A, Chew EY, 2018. Treatment of geographic atrophy with intravitreal sirolimus: the age-related eye disease study 2 ancillary study. Ophthalmology Retina 2 (5), 441–450. 10.1016/j.oret.2017.08.015. [PubMed: 29806044]

Georgiannakis A, Burgoyne T, Lueck K, Futter C, Greenwood J, Moss SE, 2015. Retinal pigment epithelial cells mitigate the effects of complement attack by endocytosis of C5b-9. J. Immunol 195 (7), 3382–3389. 10.4049/jimmunol.1500937. [PubMed: 26324770]

Gnanaguru G, Choi AR, Amarnani D, D'Amore PA, 2016. Oxidized lipoprotein uptake through the CD36 receptor activates the NLRP3 inflammasome in human retinal pigment epithelial cells. Investig. Ophthalmol. Vis. Sci 57 (11), 4704–4712. 10.1167/iovs.15-18663. [PubMed: 27607416]

Grossman TR, Carrer M, Shen L, Johnson RB, Hettrick LA, Henry SP, Monia BP, McCaleb ML, 2017. Reduction in ocular complement factor B protein in mice and monkeys by systemic administration of factor B antisense oligonucleotide. Mol. Vis 23, 561–571. [PubMed: 28855795]

Grunwald JE, Hariprasad SM, Dupont JC, Maguire MG, Fine SL, Brucker AJ, Maguire AM, Ho AC, 1998. Foveolar choroidal blood flow in age-related macular degeneration. Investig. Ophthalmol. Vis. Sci 39 (2), 385–390. [PubMed: 9477998]

Grunwald JE, Metelitsina T, Dupont JC, Ying GS, Maguire MG, 2005. Reduced foveolar choroidal blood flow in eyes with increasing AMD severity. Investig. Ophthalmol. Vis. Sci 46 (3), 1033–1038. [PubMed: 15728562]

Guymer RH, 2018. Geographic atrophy trials: turning the ship around may not Be that easy. Ophthalmology Retina 2 (6), 515–517. 10.1016/j.oret.2018.03.004. [PubMed: 31047603]

Hageman GS, Anderson DH, Johnson L.v., Hancox LS, Taiber AJ, Hardisty LI, Hageman JL, Stockman HA, Borchardt JD, Gehrs KM, Smith RJH, Silvestri G, Russell SR, Klaver CCW, Barbazetto I, Chang S, Yannuzzi LA, Barile GR, Merriam JC, Smith RT, Olsh AK, Bergeron J, Zernant J, Merriam JE, Gold B, Dean M, Allikmets R, 2005. A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration. Proc. Natl. Acad. Sci. U. S. A 102 (20), 7227–7232. 10.1073/pnas.0501536102. [PubMed: 15870199]

Haines JL, Hauser MA, Schmidt S, Scott WK, Olson LM, Gallins P, Spencer KL, Shu YK, Noureddine M, Gilbert JR, Schnetz-Boutaud N, Agarwal A, Postel EA, Pericak-Vance MA, 2005. Complement factor H variant increases the risk of age-related macular degeneration. Science 308, 419–421. 10.1126/science.1110359. [PubMed: 15761120]

Hajishengallis G, Reis ES, Mastellos DC, Ricklin D, Lambris JD, 2017. Novel mechanisms and functions of complement. Nat. Immunol 18 (12), 1288–1298. 10.1038/ni.3858. [PubMed: 29144501]

Handa JT, Bowes Rickman C, Dick AD, Gorin MB, Miller JW, Toth CA, Ueffing M, Zarbin M, Farrer LA, 2019. A systems biology approach towards understanding and treating non-neovascular age-related macular degeneration. Nat. Commun 10 (1), 3347. 10.1038/s41467-019-11262-1. [PubMed: 31350409]

Hannan JP, 2016. The structure-function relationships of complement receptor type 2 (CR2; CD21). Curr. Protein Pept. Sci 17 (5), 463–487. [PubMed: 26916158]

Harboe M, Garred P, Karlstrøm E, Lindstad JK, Stahl GL, Mollnes TE, 2009. The down-stream effects of mannan-induced lectin complement pathway activation depend quantitatively on alternative pathway amplification. Mol. Immunol 47, 373–380. 10.1016/j.molimm.2009.09.005. [PubMed: 19800125]

Harboe M, Johnson C, Nymo S, Ekholt K, Schjalm C, Lindstad JK, Pharo A, Hellerud BC, Ekdahl KN, Mollnes TE, Nilsson PH, 2017. Properdin binding to complement activating surfaces depends on initial C3b deposition. Proc. Natl. Acad. Sci. U. S. A 114 (4), E534–E539. 10.1073/pnas.1612385114. [PubMed: 28069958]

Harris CL, Heurich M, Cordoba S.R. de, Morgan BP, 2012. The complotype: dictating risk for inflammation and infection. Trends Immunol. 33 (10), 513–521. 10.1016/j.it.2012.06.001. [PubMed: 22749446]

Haynes T, Luz-Madrigal A, Reis ES, Echeverri Ruiz NP, Grajales-Esquivel E, Tzekou A, Tsonis PA, Lambris JD, del Rio-Tsonis K, 2013. Complement anaphylatoxin C3a is a potent inducer of embryonic chick retina regeneration. Nat. Commun 4, 2312. 10.1038/ncomms3312. [PubMed: 23942241]

Heesterbeek TJ, Lechanteur YTE, Lorés-Motta L, Schick T, Daha MR, Altay L, Liakopoulos S, Smailhodzic D, den Hollander AI, Hoyng CB, de Jong EK, Jeroen Klevering B, 2020. Complement activation levels are related to disease stage in AMD. Investig. Ophthalmol. Vis. Sci 61 (3), 18. 10.1167/iovs.61.3.18.

Helgason H, Sulem P, Duvvari MR, Luo H, Thorleifsson G, Stefansson H, Jonsdottir I, Masson G, Gudbjartsson DF, Walters GB, Magnusson OT, Kong A, Rafnar T, Kiemeney LA, Schoenmaker-Koller FE, Zhao L, Boon CJF, Song Y, Fauser S, Pei M, Ristau T, Patel S, Liakopoulos S, van de Ven JPH, Hoyng CB, Ferreyra H, Duan Y, Bernstein PS, Geirsdottir A, Helgadottir G, Stefansson E, den Hollander AI, Zhang K, Jonasson F, Sigurdsson H, Thorsteinsdottir U, Stefansson K, 2013. A rare nonsynonymous sequence variant in C3 is associated with high risk of age-related macular degeneration. Nat. Genet 45 (11), 1371–1374. 10.1038/ng.2740. [PubMed: 24036950]

Heurich M, Martínez-Barricarte R, Francis NJ, Roberts DL, Rodríguez De Córdoba S, Morgan BP, Harris CL, 2011. Common polymorphisms in C3, factor B, and factor H collaborate to determine systemic complement activity and disease risk. Proc. Natl. Acad. Sci. U. S. A 108 (21), 8761–8766. 10.1073/pnas.1019338108. [PubMed: 21555552]

Hoh Kam J, Lenassi E, Malik TH, Pickering MC, Jeffery G, 2013. Complement component C3 plays a critical role in protecting the aging retina in a murine model of age-related macular degeneration. Am. J. Pathol 183, 480–492. 10.1016/j.ajpath.2013.04.008. [PubMed: 23747511]

Holz FG, Strauss EC, Schmitz-Valckenberg S, van Lookeren Campagne M, 2014. Geographic atrophy: clinical features and potential therapeutic approaches. Ophthalmology 121 (5), 1079–1091. 10.1016/j.ophtha.2013.11.023. [PubMed: 24433969]

Holz FG, Sadda SR, Busbee B, Chew EY, Mitchell P, Tufail A, Brittain C, Ferrara D, Gray S, Honigberg L, Martin J, Tong B, Ehrlich JS, Bressler NM, 2018. Efficacy and safety of lampalizumab for geographic atrophy due to age-related macular degeneration: chroma and spectri phase 3 randomized clinical trials. JAMA Ophthalmology 136 (6), 666–677. 10.1001/jamaophthalmol.2018.1544. [PubMed: 29801123]

Hourcade DE, 2006. The role of properdin in the assembly of the alternative pathway C3 convertases of complement. J. Biol. Chem 281, 2128–2132. 10.1074/jbc.M508928200. [PubMed: 16301317]

Hourcade DE, Mitchell L, Kuttner-Kondo LA, Atkinson JP, Edward Medof M, 2002. Decay-accelerating factor (DAF), complement receptor 1 (CR1), and factor H dissociate the complement AP C3 convertase (C3bBb) via sites on the type A domain of Bb. J. Biol. Chem 277 (2), 1107–1112. 10.1074/jbc.M109322200. [PubMed: 11694537]

Hu J, Pauer GJ, Hagstrom SA, Bok D, DeBenedictis MJ, Bonilha VL, Hollyfield JG, Radu RA, 2020. Redox Biology (Online ahead of print).

Huang Y, Qiao F, Atkinson C, Holers VM, Tomlinson S, 2008. A novel targeted inhibitor of the alternative pathway of complement and its therapeutic application in ischemia/reperfusion injury. J. Immunol 181 (11), 8068–8076. [PubMed: 19017999]

Huber R, Scholze H, Paques EP, Deisenhofer J, 1980. Crystal structure analysis and molecular model of human C3a anaphylatoxin. Hoppe-Seylerś Zeitschrift für physiologische Chemie 361, 1389–1400. 10.1515/bchm2.1980.361.2.1389.

Huber-Lang M, Sarma JV, Zetoune FS, Rittirsch D, Neff TA, McGuire SR, Lambris JD, Warner RL, Flierl MA, Hoesel LM, Gebhard F, Younger JG, Drouin SM, Wetsel RA, Ward PA, 2006. Generation of C5a in the absence of C3: a new complement activation pathway. Nat. Med 12 (6), 682–687. 10.1038/nm1419. [PubMed: 16715088]

Hughes AL, 1994. Phylogeny of the C3/C4/C5 complement-component gene family indicates that C5 diverged first. Mol. Biol. Evol 11 (3), 417–425. 10.1093/oxfordjournals.molbev.a040123. [PubMed: 8015436]

Hughes S, Gumas J, Lee R, Rumano M, Berger N, Gautam AK, Sfyroera G, Chan AL, Gnanaguru G, Connor KM, Kim BJ, Dunaief JL, Ricklin D, Hajishengallis G, Yancopoulou D, Reis ES, Mastellos DC, Lambris JD, 2020. Prolonged intraocular residence and retinal tissue distribution of a fourth-generation compstatin-based C3 inhibitor in non-human primates. Clin. Immunol 214, 108391. 10.1016/j.clim.2020.108391. [PubMed: 32229292]

Irmscher S, Döring N, Halder LD, Jo EAH, Kopka I, Dunker C, Jacobsen ID, Luo S, Slevogt H, Lorkowski S, Beyersdorf N, Zipfel PF, Skerka C, 2018. Kallikrein cleaves C3 and activates complement. Journal of Innate Immunity 10 (2), 94–105. 10.1159/000484257. [PubMed: 29237166]

Jaffe GJ, Westby K, Csaky KG, Monés J, Pearlman JA, Patel SS, Joondeph BC, Randolph J, Masonson H, Rezaei KA, 2020. C5 inhibitor avacincaptad pegol for geographic atrophy due to age-related macular degeneration: a randomized pivotal phase 2/3 trial. Ophthalmology Advance online publication. 10.1016/j.ophtha.2020.08.027.

Janssen BJC, Huizinga EG, Raaijmakers HCA, Roos A, Daha MR, Nilsson-Ekdahl K, Nilsson B, Gros P, 2005. Structures of complement component C3 provide insights into the function and evolution of immunity. Nature 437, 505–511. 10.1038/nature04005. [PubMed: 16177781]

Janssen BJC, Christodoulidou A, McCarthy A, Lambris JD, Gros P, 2006. Structure of C3b reveals conformational changes that underlie complement activity. Nature 444, 213–216. 10.1038/nature05172. [PubMed: 17051160]

Janssen BJC, Halff EF, Lambris JD, Gros P, 2007. Structure of compstatin in complex with complement component C3c reveals a new mechanism of complement inhibition. J. Biol. Chem 282 (40), 29241–29247. 10.1074/jbc.M704587200. [PubMed: 17684013]

Jevševar S, Kunstelj M, Porekar VG, 2010. PEGylation of therapeutic proteins. Biotechnol. J 5 (1), 113–128. 10.1002/biot.200900218. [PubMed: 20069580]

Jore MM, Johnson S, Sheppard D, Barber NM, Li YI, Nunn MA, Elmlund H, Lea SM, 2016. Structural basis for therapeutic inhibition of complement C5. Nat. Struct. Mol. Biol 23, 378–386. 10.1038/nsmb.3196. [PubMed: 27018802]

Joseph K, Kulik L, Coughlin B, Kunchithapautham K, Bandyopadhyay M, Thiel S, Thielens NM, Holers VM, Rohrer B, 2013. Oxidative stress sensitizes retinal pigmented epithelial (RPE) cells to complement-mediated injury in a natural antibody-, lectin pathway-, and phospholipid epitope-dependent manner. J. Biol. Chem 288 (18), 12753–12765. [PubMed: 23493397]

Kajander T, Lehtinen MJ, Hyvarinen S, Bhattacharjee A, Leung E, Isenman DE, Meri S, Goldman A, Jokiranta TS, 2011. Dual interaction of factor H with C3d and glycosaminoglycans in host-nonhost discrimination by complement. Proc. Natl. Acad. Sci. Unit. States Am 108, 2897–2902. 10.1073/pnas.1017087108.

Karki RG, Powers J, Mainolfi N, Anderson K, Belanger DB, Liu D, Ji N, Jendza K, Gelin CF, mac Sweeney A, Solovay C, Delgado O, Crowley M, Liao S-M, Argikar UA, Flohr S, la Bonte LR, Lorthiois EL, Vulpetti A, Brown A, Long D, Prentiss M, Gradoux N, de Erkenez A, Cumin F, Adams C, Jaffee B, Mogi M, 2019. Design, synthesis, and preclinical characterization of selective factor D inhibitors targeting the alternative complement pathway. J. Med. Chem 62, 4656–4668. 10.1021/acs.jmedchem.9b00271. [PubMed: 30995036]

Katschke KJ, Xi H, Cox C, Truong T, Malato Y, Lee WP, McKenzie B, Arceo R, Tao J, Rangell L, Reichelt M, Diehl L, Elstrott J, Weimer RM, van Lookeren Campagne M, 2018. Classical and alternative complement activation on photoreceptor outer segments drives monocyte-dependent retinal atrophy. Sci. Rep 8 (1), 7348. 10.1038/s41598-018-25557-8. [PubMed: 29743491]

Kaur G, Tan LX, Rathnasamy G, Cunza N. la, Germer CJ, Toops KA, Fernandes M, Blenkinsop TA, Lakkaraju A, 2018. Aberrant early endosome biogenesis mediates complement activation in the retinal pigment epithelium in models of macular degeneration. Proc. Natl. Acad. Sci. U. S. A 115 10.1073/pnas.1805039115, 9014–9019. [PubMed: 30126999]

Khandhadia S, Hakobyan S, Heng LZ, Gibson J, Adams DH, Alexander GJ, Gibson JM, Martin KR, Menon G, Nash K, Sivaprasad S, Ennis S, Cree AJ, Morgan BP, Lotery AJ, 2013. Age-related macular degeneration and modification of systemic complement factor H production through

liver transplantation. Ophthalmology 120 (8), 1612–1618. 10.1016/j.ophtha.2013.01.004. [PubMed: 23562165]

Khurana RN, Fujii GY, Walsh AC, Humayun MS, de Juan E, Sadda SR, 2005. Rapid recurrence of geographic atrophy after full macular translocation for nonexudative age-related macular degeneration. Ophthalmology 112, 1586–1591. 10.1016/j.ophtha.2005.04.016. [PubMed: 16051364]

Klein RJ, Zeiss C, Chew EY, Tsai JY, Sackler RS, Haynes C, Henning AK, Sangiovanni JP, Mane SM, Mayne ST, Bracken MB, Ferris FL, Ott J, Barnstable C, Hoh J, 2005. Complement factor H polymorphism in age-related macular degeneration. Science 308 (5720), 385–390. [PubMed: 15761122]

Klos A, Tenner AJ, Johswich KO, Ager RR, Reis ES, Köhl J, 2009. The role of the anaphylatoxins in health and disease. Mol. Immunol 46 (14), 2753–2766. 10.1016/j.molimm.2009.04.027. [PubMed: 19477527]

Kouser L, Abdul-Aziz M, Nayak A, Stover CM, Sim RB, Kishore U, 2013. Properdin and factor H: opposing players on the alternative complement pathway “see-saw. Front. Immunol 4, 93. 10.3389/fimmu.2013.00093. [PubMed: 23630525]

Krishnan V, Ponnuraj K, Xu Y, Macon K, Volanakis JE, Narayana SVL, 2009. The crystal structure of Cobra Venom factor, a cofactor for C3- and C5-convertase CVFBb. Structure 17 (4), 611–619. 10.1016/j.str.2009.01.015. [PubMed: 19368894]

Kumar-Singh R, 2019. The role of complement membrane attack complex in dry and wet AMD - from hypothesis to clinical trials. Exp. Eye Res 184, 266–277. 10.1016/j.exer.2019.05.006. [PubMed: 31082363]

Kunchithapautham K, Atkinson C, Rohrer B, 2014. Smoke exposure causes endoplasmic reticulum stress and lipid accumulation in retinal pigment epithelium through oxidative stress and complement activation. J. Biol. Chem 289 (21), 14534–14546. [PubMed: 24711457]

Lachmann PJ, 2009. The amplification loop of the complement pathways. Adv. Immunol 104, 115–149. 10.1016/S0065-2776(08)04004-2. [PubMed: 20457117]

Langer HF, Chung KJ, Orlova V.v., Choi EY, Kaul S, Kruhlak MJ, Alatsatianos M, DeAngelis RA, Roche PA, Magotti P, Li X, Economopoulou M, Rafail S, Lambris JD, Chavakis T, 2010. Complement-mediated inhibition of neovascularization reveals a point of convergence between innate immunity and angiogenesis. Blood 116 (22), 4395–4403. 10.1182/blood-2010-01-261503. [PubMed: 20625009]

Law SKA, Dodds AW, 1997. The internal thioester and the covalent binding properties of the complement proteins C3 and C4. Protein Sci. 6 (2), 263–274. 10.1002/pro.5560060201, 1997. [PubMed: 9041627]

Lay E, Nutland S, Smith JE, Hiles I, Smith RAG, Seilly DJ, Buchberger A, Schwaeble W, Lachmann PJ, 2015. Complotype affects the extent of down-regulation by Factor I of the C3b feedback cycle in vitro. Clin. Exp. Immunol 181 (2), 314–322. 10.1111/cei.12437. [PubMed: 25124117]

Lechner J, Chen M, Hogg RE, Toth L, Silvestri G, Chakravarthy U, Xu H, 2016. Higher plasma levels of complement C3a, C4a and C5a increase the risk of subretinal fibrosis in neovascular age-related macular degeneration. Immun. Ageing 13, 4. 10.1186/s12979-016-0060-5. [PubMed: 26884800]

Leslie RGQ, Prodinger WM, Nielsen CH, 2003. Complement receptors type 1 (CR1, CD35) and 2 (CR2, CD21) cooperate in the binding of hydrolyzed complement factor 3 (C3i) to human B lymphocytes. Eur. J. Immunol 33 (12), 3311–3321. 10.1002/eji.200324330. [PubMed: 14635039]

Levashina EA, Moita LF, Blandin S, Vriend G, Lagueux M, Kafatos FC, 2001. Conserved role of a complement-like protein in phagocytosis revealed by dsRNA knockout in cultured cells of the mosquito, Anopheles gambiae. Cell 104, 709–718. 10.1016/S0092-8674(01)00267-7. [PubMed: 11257225]

Li R, Coulthard LG, Wu MCL, Taylor SM, Woodruff TM, 2013. C5L2: a controversial receptor of complement anaphylatoxin, C5a. FASEB (Fed. Am. Soc. Exp. Biol.) J 27 (3), 855–864.

Li Y, Song D, Song Y, Zhao L, Wolkow N, Tobias JW, Song W, Dunaief JL, 2015. Iron-induced local complement component 3 (C3) up-regulation via non-canonical transforming growth factor

(TGF)-β signaling in the retinal pigment epithelium. J. Biol. Chem 290, 11918–11934. 10.1074/jbc.M115.645903. [PubMed: 25802332]

Liao DS, Grossi F. v, el Mehdi D, Gerber MR, Brown DM, Heier JS, Wykoff CC, Singerman LJ, Abraham P, Grassmann F, Nuernberg P, Weber BHF, Deschatelets P, Kim RY, Chung CY, Ribeiro RM, Hamdani M, Rosenfeld PJ, Boyer DS, Slakter JS, Francois CG, 2020. Complement C3 inhibitor pegcetacoplan for geographic atrophy secondary to age-related macular degeneration: a randomized phase 2 trial. Ophthalmology 127 (2), 186–195. 10.1016/j.ophtha.2019.07.011. [PubMed: 31474439]

Lin Z, Schmidt CQ, Koutsogiannaki S, Ricci P, Risitano AM, Lambris JD, Ricklin D, 2015. Complement C3dg-mediated erythrophagocytosis: implications for paroxysmal nocturnal hemoglobinuria. Blood 126 (7), 891–894. 10.1182/blood-2015-02-625871. [PubMed: 26082452]

Liszewski MK, Kolev M, le Friec G, Leung M, Bertram PG, Fara AF, Subias M, Pickering MC, Drouet C, Meri S, Arstila TP, Pekkarinen PT, Ma M, Cope A, Reinheckel T, Rodriguez de Cordoba S, Afzali B, Atkinson JP, Kemper C, 2013. Intracellular complement activation sustains T cell homeostasis and mediates effector differentiation. Immunity 39, 1143–1157. 10.1016/j.immuni.2013.10.018. [PubMed: 24315997]

Liszewski MK, Elvington M, Kulkarni HS, Atkinson JP, 2017. Complement's hidden arsenal: new insights and novel functions inside the cell. Mol. Immunol 84, 2–9. 10.1016/j.molimm.2017.01.004. [PubMed: 28196665]

Liu D, Niu Z-X, 2009. The structure, genetic polymorphisms, expression and biological functions of complement receptor type 1 (CR1/CD35). Immunopharmacology and Immunotoxicology 00. 10.1080/08923970902845768.

Lorés-Motta L, Paun CC, Corominas J, Pauper M, Geerlings MJ, Altay L, Schick T, Daha MR, Fauser S, Hoyng CB, den Hollander AI, de Jong EK, 2018. Genome-wide association study reveals variants in CFH and CFHR4 associated with systemic complement activation: implications in age-related macular degeneration. Ophthalmology 125 (7), 1064–1074. 10.1016/j.ophtha.2017.12.023. [PubMed: 29398083]

Lueck K, Wasmuth S, Williams J, Hughes TR, Morgan BP, Lommatzsch A, Greenwood J, Moss SE, Pauleikhoff D, 2011. Sub-lytic C5b-9 induces functional changes in retinal pigment epithelial cells consistent with age-related macular degeneration. Eye 25, 1074–1082. 10.1038/eye.2011.109. [PubMed: 21597483]

Lyubchenko T, Dal Porto J, Cambier JC, Holers VM, 2005. Coligation of the B cell receptor with complement receptor type 2 (CR2/CD21) using its natural ligand C3dg: activation without engagement of an inhibitory signaling pathway. J. Immunol 174 (6), 3264–3272. 10.4049/jimmunol.174.6.3264. [PubMed: 15749857]

Lyzogubov V.v., Tytarenko RG, Liu J, Bora NS, Bora PS, 2011. Polyethylene glycol (PEG)-induced mouse model of choroidal neovascularization. J. Biol. Chem 286 (18), 16229–16237. 10.1074/jbc.M110.204701. [PubMed: 21454496]

Maguire MG, 2012. Drusen volume as a study endpoint. Ophthalmology 119 (7), 1501–1502. 10.1016/j.ophtha.2012.03.012. [PubMed: 22749098]

Maller JB, Fagerness JA, Reynolds RC, Neale BM, Daly MJ, Seddon JM, 2007. Variation in complement factor 3 is associated with risk of age-related macular degeneration. Nat. Genet 39 (10), 1200–1201. 10.1038/ng2131. [PubMed: 17767156]

Mastellos DC, Yancopoulou D, Kokkinos P, Huber-Lang M, Hajishengallis G, Biglarnia AR, Lupu F, Nilsson B, Risitano AM, Ricklin D, Lambris JD, 2015. Compstatin: a C3-targeted complement inhibitor reaching its prime for bedside intervention. Eur. J. Clin. Invest 45 (4), 423–440. 10.1111/eci.12419. [PubMed: 25678219]

Mastellos DC, Reis ES, Ricklin D, Smith RJ, Lambris JD, 2017. Complement C3-targeted therapy: replacing long-held assertions with evidence-based discovery. Trends Immunol. 38 (6), 383–394. 10.1016/j.it.2017.03.003. [PubMed: 28416449]

Mastellos DC, Ricklin D, Lambris JD, 2019. Clinical promise of next-generation complement therapeutics. Nat. Rev. Drug Discov 18 (9), 707–729. 10.1038/S41573-019-0031-6. [PubMed: 31324874]

Mastellos DC, Pires da Silva BGP, Fonseca BAL, Fonseca NP, Auxiliadora-Martins M, Mastaglio S, Ruggeri A, Sironi M, Radermacher P, Chrysanthopoulou A, Skendros P, Ritis K, Manfra I,

Iacobelli S, Huber-Lang M, Nilsson B, Yancopoulou D, Connolly ES, Garlanda C, Ciceri F, Risitano AM, Calado RT, Lambris JD, 2020. Complement C3 vs C5 inhibition in severe COVID-19: early clinical findings reveal differential biological efficacy. Clin. Immunol 220, 108598. 10.1016/j.clim.2020.108598. [PubMed: 32961333]

McDonnell T, Ioannou Y, Rahman A, 2014. PEGylated drugs in rheumatology-why develop them and do they work? Rheumatology (United Kingdom) 53 (3), 391–396. 10.1093/rheumatology/ket278.

Merle NS, Noe R, Halbwachs-Mecarelli L, Fremeaux-Bacchi V, Roumenina LT, 2015. Complement system part II: role in immunity. Front. Immunol 6, 257. 10.3389/fimmu.2015.00257. [PubMed: 26074922]

Milder FJ, Gomes L, Schouten A, Janssen BJC, Huizinga EG, Romijn RA, Hemrika W, Roos A, Daha MR, Gros P, 2007. Factor B structure provides insights into activation of the central protease of the complement system. Nat. Struct. Mol. Biol 14, 224–228. 10.1038/nsmb1210. [PubMed: 17310251]

Misumi Y, Takami N, Ikehara Y, 1984. Biosynthesis and processing of pro-C3, a precursor of the third component of complement in rat hepatocytes: effect of secretion-blocking agents. FEBS Letters 175, 63–67. 10.1016/0014-5793(84)80570-0. [PubMed: 6479339]

Mitchell P, Liew G, Gopinath B, Wong TY, 2018. Age-related macular degeneration. Lancet 392 (10153), 1147–1159. 10.1016/S0140-6736(18)31550-2. [PubMed: 30303083]

Morris K, Goldberger G, Colten H, Aden D, Knowles B, 1982. Biosynthesis and processing of a human precursor complement protein, pro-C3, in a hepatoma-derived cell line. Science 215, 399–400. 10.1126/science.7199205. [PubMed: 7199205]

Mukai R, Okunuki Y, Husain D, Kim CB, Lambris JD, Connor KM, 2018. The complement system is critical in maintaining retinal integrity during aging. Front. Aging Neurosci 10, 15. 10.3389/fnagi.2018.00015. [PubMed: 29497373]

Mulfaul K, Ozaki E, Fernando N, Brennan K, Chirco KR, Connolly E, Greene C, Maminishkis A, Salomon RG, Linetsky M, Natoli R, Mullins RF, Campbell M, Doyle SL, 2020. Toll-like receptor 2 facilitates oxidative damage-induced retinal degeneration. Cell Rep. 30 (7), 2209–2224. 10.1016/j.celrep.2020.01.064. [PubMed: 32075760]

Mullins RF, Russell SR, Anderson DH, Hageman GS, 2000. Drusen associated with aging and age-related macular degeneration contain proteins common to extracellular deposits associated with atherosclerosis, elastosis, amyloidosis, and dense deposit disease. Faseb. J 14 (7), 835–846. 10.1096/fasebj.14.7.835. [PubMed: 10783137]

Mullins RF, Schoo DP, Sohn EH, Flamme-Wiese MJ, Workamelahu G, Johnston RM, Wang K, Tucker BA, Stone EM, 2014. The membrane attack complex in aging human choriocapillaris: relationship to macular degeneration and choroidal thinning. Am. J. Pathol 184, 3142–3153. 10.1016/j.ajpath.2014.07.017. [PubMed: 25204844]

NCBI Homo sapiens Updated Annotation Release 109.20200815, GRCh38.p13. C5 gene. https://www.ncbi.nlm.nih.gov/genome/gdv/browser/genome/?id=GCF_000001405.39 accessed 25 September 2020.

NCBI Homo sapiens Updated Annotation Release 109.20200815, GRCh38.p13. C3 gene. https://www.ncbi.nlm.nih.gov/genome/gdv/browser/gene/?id=718 accessed 25 September 2020.

Nozaki M, Raisler BJ, Sakurai E, Sarma JV, Barnum SR, Lambris JD, Chen Y, Zhang K, Ambati BK, Baffi JZ, Ambati J, 2006. Drusen complement components C3a and C5a promote choroidal neovascularization. Proc. Natl. Acad. Sci. U. S. A 103 (7), 2328–2333. 10.1073/pnas.0408835103. [PubMed: 16452172]

Okura Y, Kobayashi I, Yamada M, Sasaki S, Yamada Y, Kamioka I, Kanai R, Takahashi Y, Ariga T, 2016. Clinical characteristics and genotype-phenotype correlations in C3 deficiency. J. Allergy Clin. Immunol 137, 640–644. 10.1016/j.jaci.2015.08.017e1. [PubMed: 26435005]

Oriol Sunyer J, Zarkadis IK, Lambris JD, 1998. Complement diversity: a mechanism for generating immune diversity? Immunol. Today 19, 519–523. 10.1016/S0167-5699(98)01341-3. [PubMed: 9818547]

Pangburn MK, Müller-Eberhard HJ, 1986. The C3 convertase of the alternative pathway of human complement. Enzymic properties of the bimolecular proteinase. Biochem. J 235, 723–730. 10.1042/bj2350723. [PubMed: 3638964]

Pangburn MK, Schreiber RD, Muller-Eberhard HJ, 1981. Formation of the initial C3 convertase of the alternative complement pathway: acquisition of C3b-like activities by spontaneous hydrolysis of the putative thioester in native C3. J. Exp. Med 154 (3), 856–867. 10.1084/jem.154.3.856. [PubMed: 6912277]

Papanastasiou M, Koutsogiannaki S, Sarigiannis Y, Geisbrecht B.v., Ricklin D, Lambris JD, 2017. Structural implications for the formation and function of the complement effector protein iC3b. J. Immunol 198, 3326–3335. 10.4049/jimmunol.1601864. [PubMed: 28258193]

Park DH, Connor KM, Lambris JD, 2019. The challenges and promise of complement therapeutics for ocular diseases. Front. Immunol 10, 1007. 10.3389/fimmu.2019.01007. [PubMed: 31156618]

Pauly D, Agarwal D, Dana N, Schäfer N, Biber J, Wunderlich KA, Jabri Y, Straub T, Zhang NR, Gautam AK, Weber BHF, Hauck SM, Kim M, Curcio CA, Stambolian D, Li M, Grosche A, 2019. Cell-type-specific complement expression in the healthy and diseased retina. Cell Rep. 29, 2835–2848. 10.1016/j.celrep.2019.10.084e4. [PubMed: 31775049]

Paun CC, Lechanteur YTE, Groenewoud JMM, Altay L, Schick T, Daha MR, Fauser S, Hoyng CB, den Hollander AI, de Jong EK, 2016. A novel complotype combination associates with age-related macular degeneration and high complement activation levels in vivo. Sci. Rep 6, 26568. 10.1038/srep26568. [PubMed: 27241480]

Poor SH, Qiu Y, Fassbender ES, Shen S, Woolfenden A, Delpero A, Kim Y, Buchanan N, Gebuhr TC, Hanks SM, Meredith EL, Jaffee BD, Dryja TP, 2014. Reliability of the mouse model of choroidal neovascularization induced by laser photocoagulation. Investig. Ophthalmol. Vis. Sci 55 (10), 6525–6534. [PubMed: 25205860]

Prohászka Z, Nilsson B, Frazer-Abel A, Kirschfink M, 2016. Complement analysis 2016: clinical indications, laboratory diagnostics and quality control. Immunobiology 221 (11), 1247–1258. 10.1016/j.imbio.2016.06.008. [PubMed: 27475991]

Puliafito CA, Wykoff CC, 2020. New Frontiers in Retina: highlights of the 2020 angiogenesis, exudation and degeneration symposium. International Journal of Retina and Vitreous 6, 18. 10.1186/s40942-020-00221-4. [PubMed: 32477592]

Reis ES, Barbuto JAM, Isaac L, 2006. Human monocyte-derived dendritic cells are a source of several complement proteins. Inflamm. Res 55 (5), 179–184. 10.1007/S00011-006-0068-y. [PubMed: 16830104]

Reis ES, Chen H, Sfyroera G, Monk PN, Kohl J, Ricklin D, Lambris JD, 2012. C5a receptor-dependent cell activation by physiological concentrations of desarginated C5a: insights from a novel label-free cellular assay. J. Immunol 189 (10), 4797–4805. [PubMed: 23041570]

Reis ES, Mastellos DC, Hajishengallis G, Lambris JD, 2019. New insights into the immune functions of complement. Nat. Rev. Immunol 19 (8), 503–516. 10.1038/s41577-019-0168-x. [PubMed: 31048789]

Reynolds R, Hartnett ME, Atkinson JP, Giclas PC, Rosner B, Seddon JM, 2009. Plasma complement components and activation fragments: associations with age-related macular degeneration genotypes and phenotypes. Investig. Ophthalmol. Vis. Sci 50 (12), 5818–5827. 10.1167/iovs.09-3928. [PubMed: 19661236]

Ricklin D, Hajishengallis G, Yang K, Lambris JD, 2010. Complement: a key system for immune surveillance and homeostasis. Nat. Immunol 11 (9), 785–797. 10.1038/ni.1923. [PubMed: 20720586]

Ricklin D, Reis ES, Lambris JD, 2016a. Complement in disease: a defence system turning offensive. Nat. Rev. Nephrol 12 (7), 383–401. 10.1038/nrneph.2016.70. [PubMed: 27211870]

Ricklin D, Reis ES, Mastellos DC, Gros P, Lambris JD, 2016b. Complement component C3 – the “Swiss Army Knife” of innate immunity and host defense. Immunol. Rev 274 (1), 33–58. 10.1111/imr.12500. [PubMed: 27782325]

Ricklin D, Mastellos DC, Reis ES, Lambris JD, 2018. The renaissance of complement therapeutics. Nat. Rev. Nephrol 14 (1), 26–47. 10.1038/nrneph.2017.156. [PubMed: 29199277]

Risitano AM, Mastellos DC, Huber-Lang M, Yancopoulou D, Garlanda C, Ciceri F, Lambris JD, 2020. Complement as a target in COVID-19? Nat. Rev. Immunol 20 (6), 343–344. 10.1038/s41577-020-0320-7. [PubMed: 32327719]

Ristau T, Paun C, Ersoy L, Hahn M, Lechanteur Y, Hoyng C, de Jong EK, Daha MR, Kirchhof B, den Hollander AI, Fauser S, 2014. Impact of the common genetic associations of age-related macular degeneration upon systemic complement component C3d levels. PloS One 9 (3), e93459. 10.1371/journal.pone.0093459. [PubMed: 24675670]

Roguska M, Splawski I, Diefenbach-Streiber B, Dolan E, Etemad-Gilbertson B, Rondeau J-M, Keating M, 2014. Generation and characterization of LFG316, A fully-human anti-C5 antibody for the treatment of age-related macular degeneration. Invest. Ophthalmol. Vis. Sci 55, 3433.

Rohrer B, 2018. Anaphylatoxin signaling in retinal pigment and choroidal endothelial cells: characteristics and relevance to age-related macular degeneration. Adv. Exp. Med. Biol 1074, 45–51. 10.1007/978-3-319-75402-4_6. [PubMed: 29721926]

Rohrer B, Long Q, Coughlin B, Wilson RB, Huang Y, Qiao F, Tang PH, Kunchithapautham K, Gilkeson GS, Tomlinson S, 2009. A targeted inhibitor of the alternative complement pathway reduces angiogenesis in a mouse model of age-related macular degeneration. Investig. Ophthalmol. Vis. Sci 50 (7), 3056–3064. [PubMed: 19264882]

Rohrer B, Coughlin B, Kunchithapautham K, Long Q, Tomlinson S, Takahashi K, Holers VM, 2011. The alternative pathway is required, but not alone sufficient, for retinal pathology in mouse laser-induced choroidal neovascularization. Mol. Immunol 48, e1–e8. 10.1016/j.molimm.2010.12.016. [PubMed: 21257205]

Rondelli T, Risitano AM, de Latour RP, Sica M, Peruzzi B, Ricci P, Barcellini W, Iori AP, Boschetti C, Valle V, Frémeaux-Bacchi V, de Angioletti M, Socie G, Luzzatto L, Notaro R, 2014. Polymorphism of the complement receptor 1 gene correlates with the hematologic response to eculizumab in patients with paroxysmal nocturnal hemoglobinuria. Haematologica 99 (2), 262–266. 10.3324/haematol.2013.090001. [PubMed: 24038027]

Rother RP, Rollins SA, Mojcik CF, Brodsky RA, Bell L, 2007. Discovery and development of the complement inhibitor eculizumab for the treatment of paroxysmal nocturnal hemoglobinuria. Nat. Biotechnol 25, 1256–1264. [PubMed: 17989688]

Rus HG, Niculescu FI, Shin ML, 2001. Role of the C5b-9 complement complex in cell cycle and apoptosis. Immunol. Rev 180, 49–55. 10.1034/j.1600-065X.2001.1800104.x. [PubMed: 11414362]

Sahu A, Lambris JD, 2001. Structure and biology of complement protein C3, a connecting link between innate and acquired immunity. Immunol. Rev 180, 35–48. 10.1034/j.1600-065X.2001.1800103.x. [PubMed: 11414361]

Sahu A, Kay BK, Lambris JD, 1996. Inhibition of human complement by a C3-binding peptide isolated from a phage-displayed random peptide library. J. Immunol 157 (2), 884–891. [PubMed: 8752942]

Schatz-Jakobsen JA, Zhang Y, Johnson K, Neill A, Sheridan D, Andersen GR, 2016. Structural basis for eculizumab-mediated inhibition of the complement terminal pathway. J. Immunol 197, 337–344. 10.4049/jimmunol.1600280. [PubMed: 27194791]

Schick T, Steinhauer M, Aslanidis A, Altay L, Karlstetter M, Langmann T, Kirschfink M, Fauser S, 2017. Local complement activation in aqueous humor in patients with age-related macular degeneration. Eye 31 (5), 810–813. 10.1038/eye.2016.328. [PubMed: 28128795]

Schmidt CQ, Bai H, Lin Z, Risitano AM, Barlow PN, Ricklin D, Lambris JD, 2013. Rational engineering of a minimized immune inhibitor with unique triple-targeting properties. J. Immunol 190 (11), 5712–5721. 10.4049/jimmunol.1203548. [PubMed: 23616575]

Schnabolk G, Coughlin B, Joseph K, Kunchithapautham K, Bandyopadhyay M, O'Quinn EC, Nowling T, Rohrer B, 2015. Local production of the alternative pathway component factor B is sufficient to promote laser-induced choroidal neovascularization. Investig. Ophthalmol. Vis. Sci 56 (3), 1850–1863. 10.1167/iovs.14-15910. [PubMed: 25593023]

Schnabolk G, Beon MK, Tomlinson S, Rohrer B, 2017. New insights on complement inhibitor CD59 in mouse laser-induced choroidal neovascularization: mislocalization after injury and targeted delivery for protein replacement. J. Ocul. Pharmacol. Therapeut 33 (5), 400–411.

Scholl HPN, Issa PC, Walier M, Janzer S, Pollok-Kopp B, Börncke F, Fritsche LG, Chong N. v., Fimmers R, Wienker T, Holz FG, Weber BHF, Oppermann M, 2008. Systemic complement activation in age-related macular degeneration. PloS One 3 (7), e2593. 10.1371/journal.pone.0002593. [PubMed: 18596911]

Schultz H, Song Y, Baumann BH, Kapphahn RJ, Montezuma SR, Ferrington DA, Dunaief JL, 2019. Increased serum proteins in non-exudative AMD retinas. Exp. Eye Res 186, 107686. 10.1016/j.exer.2019.05.026. [PubMed: 31158383]

Schwaeble W, Dippold WG, Schafer MKH, Pohla H, Jonas D, Luttig B, Weihe E, Huemer HP, Dierich MP, Reid KBM, 1993. Properdin, a positive regulator of complement activation, is expressed in human T cell lines and peripheral blood T cells. J. Immunol 151 (5), 2521–2528. [PubMed: 8360474]

Seddon JM, Yu Y, Miller EC, Reynolds R, Tan PL, Gowrisankar S, Goldstein JI, Triebwasser M, Anderson HE, Zerbib J, Kavanagh D, Souied E, Katsanis N, Daly MJ, Atkinson JP, Raychaudhuri S, 2013. Rare variants in CFI, C3 and C9 are associated with high risk of advanced age-related macular degeneration. Nat. Genet 45 (11), 1366–1370. 10.1038/ng.2741. [PubMed: 24036952]

Shahulhameed S, Vishwakarma S, Chhablani J, Tyagi M, Pappuru RR, Jakati S, Chakrabarti S, Kaur I, 2020. A systematic investigation on complement pathway activation in diabetic retinopathy. Front. Immunol 11, 154. 10.3389/fimmu.2020.00154. [PubMed: 32117292]

Shields KJ, Mollnes TE, Eidet JR, Mikkelsen K, Almdahl SM, Bottazzi B, Lyberg T, Manzi S, Ahearn JM, Hollan I, 2017. Plasma complement and vascular complement deposition in patients with coronary artery disease with and without inflammatory rheumatic diseases. PloS One 12 (3), e0174577. 10.1371/journal.pone.0174577. [PubMed: 28362874]

Skei JM, Fingert JH, Russell SR, Stone EM, Mullins RF, 2010. Complement component C5a activates ICAM-1expression on human choroidal endothelial cells. Investig. Ophthalmol. Vis. Sci 51 (10), 5336–5342. 10.1167/iovs.10-5322. [PubMed: 20484595]

Skendros P, Mitsios A, Chrysanthopoulou A, Mastellos DC, Metallidis S, Rafailidis P, Ntinopoulou M, Sertaridou E, Tsironidou V, Tsigalou C, Tektonidou MG, Konstantinidis T, Papagoras C, Mitroulis I, Germanidis G, Lambris JD, Ritis K, 2020. Complement and tissue factor-enriched neutrophil extracellular traps are key drivers in COVID-19 immunothrombosis. Journal of Clinical Investigation. Advance online publication. 10.1172/jci141374.

Sohn EH, Flamme-Wiese MJ, Whitmore SS, Workalemahu G, Marneros AG, Boese EA, Kwon YH, Wang K, Abramoff MD, Tucker BA, Stone EM, Mullins RF, 2019. Choriocapillaris degeneration in geographic atrophy. Am. J. Pathol 189 (7), 1473–1480. [PubMed: 31051169]

Song D, Sulewski ME, Wang C, Song J, Bhuyan R, Sterling J, Clark E, Song WC, Dunaief JL, 2017. Complement C5a receptor knockout has diminished light-induced microglia/macrophage retinal migration. Mol. Vis 23, 210–218. [PubMed: 28442885]

Stevens B, Allen NJ, Vazquez LE, Howell GR, Christopherson KS, Nouri N, Micheva KD, Mehalow AK, Huberman AD, Stafford B, Sher A, Litke AMM, Lambris JD, Smith SJ, John SWM, Barres BA, 2007. The classical complement cascade mediates CNS synapse elimination. Cell 131 (6), 1164–1178. 10.1016/j.cell.2007.10.036. [PubMed: 18083105]

Strey CW, Markiewski M, Mastellos D, Tudoran R, Spruce LA, Greenbaum LE, Lambris JD, 2003. The proinflammatory mediators C3a and C5a are essential for liver regeneration. J. Exp. Med 198 (6), 913–923. 10.1084/jem.20030374. [PubMed: 12975457]

Sun X, Ioannidis JPA, Agoritsas T, Alba AC, Guyatt G, 2014. How to use a subgroup analysis users' guides to the medical literature. JAMA - Journal of the American Medical Association 311 (4), 405–411. 10.1001/jama.2013.285063. [PubMed: 24449319]

Tarallo V, Hirano Y, Gelfand BD, Dridi S, Kerur N, Kim Y, Cho WG, Kaneko H, Fowler BJ, Bogdanovich S, Albuquerque RJC, Hauswirth WW, Chiodo VA, Kugel JF, Goodrich JA, Ponicsan SL, Chaudhuri G, Murphy MP, Dunaief JL, Ambati BK, Ogura Y, Yoo JW, Lee DK, Provost P, Hinton DR, Núñez G, Baffi JZ, Kleinman ME, Ambati J, 2012. DICER1 loss and Alu RNA induce age-related macular degeneration via the NLRP3 inflammasome and MyD88. Cell 149, 847–859. 10.1016/j.cell.2012.03.036. [PubMed: 22541070]

Toapanta FR, Ross TM, 2006. Complement-mediated activation of the adaptive immune responses: role of C3d in linking the innate and adaptive immunity. Immunol. Res 36 (1–3), 197–210. 10.1385/IR:36:1:197. [PubMed: 17337780]

Toomey CB, Kelly U, Saban DR, Bowes Rickman C, 2015. Regulation of age-related macular degeneration-like pathology by complement factor H. Proc. Natl. Acad. Sci. Unit. States Am 112 (23), E3040–E3049.

Toomey CB, Johnson L. v, Bowes Rickman C, 2018a. Complement factor H in AMD: bridging genetic associations and pathobiology. Prog. Retin. Eye Res 62, 38–57. 10.1016/j.preteyeres.2017.09.001. [PubMed: 28928087]

Toomey CB, Landowski M, Klingeborn M, Kelly U, Deans J, Dong H, Harrabi O, Van Blarcom T, Yeung YA, Grishanin R, Lin JC, Saban DR, Bowes Rickman C, 2018b. Effect of anti-C5a therapy in a murine model of early/intermediate dry age-related macular degeneration. Investig. Ophthalmol. Vis. Sci 59 (2), 662–673. [PubMed: 29392311]

Trakkides TO, Schäfer N, Reichenthaler M, Kühn K, Brandwijk RJMGE, Toonen EJM, Urban F, Wegener J, Enzmann V, Pauly D, 2019. Oxidative stress increases endogenous complement-dependent inflammatory and angiogenic responses in retinal pigment epithelial cells independently of exogenous complement sources. Antioxidants 8 (11), 548. 10.3390/antiox8110548.

Triantafilou K, Hughes TR, Triantafilou M, Morgan PP, 2013. The complement membrane attack complex triggers intracellular Ca2+ fluxes leading to NLRP3 inflammasome activation. J. Cell Sci 126 (Pt 13), 2903–2913. 10.1242/jcs.124388. [PubMed: 23613465]

Troldborg A, Jensen L, Deleuran B, Stengaard-Pedersen K, Thiel S, Jensenius JC, 2018. The C3dg fragment of complement is superior to conventional C3 as a diagnostic biomarker in systemic lupus erythematosus. Front. Immunol 9, 581. 10.3389/fimmu.2018.00581. [PubMed: 29632534]

Tseng WA, Thein T, Kinnunen K, Lashkari K, Gregory MS, D'Amore PA, Ksander BR, 2013. NLRP3 inflammasome activation in retinal pigment epithelial cells by lysosomal destabilization: implications for age-related macular degeneration. Investig. Ophthalmol. Vis. Sci 54 (1), 110–120. 10.1167/iovs.12-10655. [PubMed: 23221073]

Ueda K, Kim HJ, Zhao J, Song Y, Dunaief JL, Sparrow JR, 2018. Iron promotes oxidative cell death caused by bisretinoids of retina. Proc. Natl. Acad. Sci. U. S. A 115 (19), 4963–4968. 10.1073/pnas.1722601115. [PubMed: 29686088]

van Lookeren Campagne M, Wiesmann C, Brown EJ, 2007. Macrophage complement receptors and pathogen clearance. Cell Microbiol. 9 (9), 2095–2102. 10.1111/j.1462-5822.2007.00981.x. [PubMed: 17590164]

Vogt SD, Barnum SR, Curcio CA, Read RW, 2006. Distribution of complement anaphylatoxin receptors and membrane-bound regulators in normal human retina. Exp. Eye Res 83 (4), 834–840. 10.1016/j.exer.2006.04.002. [PubMed: 16764856]

Vorup-Jensen T, Jensen RK, 2018. Structural immunology of complement receptors 3 and 4. Front. Immunol 9, 2716. 10.3389/fimmu.2018.02716. [PubMed: 30534123]

Wang Y, Rollins SA, Madri JA, Matis LA, 1995. Anti-C5 monoclonal antibody therapy prevents collagen-induced arthritis and ameliorates established disease. Proc. Natl. Acad. Sci. Unit. States Am 92, 8955–8959.

Wang L, Kondo N, Cano M, Ebrahimi K, Yoshida T, Barnett BP, Biswal S, Handa JT, 2014. Nrf2 signaling modulates cigarette smoke-induced complement activation in retinal pigmented epithelial cells. Free Radic. Biol. Med 70, 155–166. 10.1016/j.feeradbiomed.2014.01.015. [PubMed: 24440594]

Wang L, Cano M, Datta S, Wei H, Ebrahimi KB, Gorashi Y, Garlanda C, Handa JT, 2016. Pentraxin 3 recruits complement factor H to protect against oxidative stress-induced complement and inflammasome overactivation. J. Pathol 240 (4), 495–506. 10.1002/path.4811. [PubMed: 27659908]

Whitmore SS, Sohn EH, Chirco KR, Drack AV, Stone EM, Tucker BA, Mullins RF, 2015. Complement activation and choriocapillaris loss in early AMD: implications for pathophysiology and therapy. Prog. Retin. Eye Res 45, 1–29. [PubMed: 25486088]

Winters MS, Spellman DS, Lambris JD, 2005. Solvent accessibility of native and hydrolyzed human complement protein 3 analyzed by hydrogen/deuterium exchange and mass spectrometry. J. Immunol 174 (6), 3469–3474. 10.4049/jimmunol.174.6.3469. [PubMed: 15749882]

Wong WT, 2020. C3 function and inhibition in geographic atrophy: interesting insights from a phase 2 study. Ophthalmology 127 (2), 196–197. 10.1016/j.ophtha.2019.09.008. [PubMed: 31973832]

Wong WL, Su X, Li X, Cheung CMG, Klein R, Cheng CY, Wong TY, 2014. Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. Lancet Glob. Heal 2 (2), e106–116. 10.1016/S2214-109X(13)70145-1.

Woodell A, Jones BW, Williamson T, Schnabolk G, Tomlinson S, Atkinson C, Rohrer B, 2016. A targeted inhibitor of the alternative complement pathway accelerates recovery from smoke-induced ocular injury. Investig. Ophthalmol. Vis. Sci 57 (4), 1728–1737. [PubMed: 27064393]

World Health Organization, 2020. Priority Eye Diseases. https://www.who.int/blindness/causes/priority/en/index7.html. accessed 2 June 2020.

Wu X, Hutson I, Akk AM, Mascharak S, Pham CTN, Hourcade DE, Brown R, Atkinson JP, Harris CA, 2018. Contribution of adipose-derived factor D/adipsin to complement alternative pathway activation: lessons from lipodystrophy. J. Immunol 200 (8), 2786–2797. [PubMed: 29531168]

Xie CB, Qin L, Li G, Fang C, Kirkiles-Smith NC, Tellides G, Pober JS, Jane-Wit D, 2019. Complement membrane attack complexes assemble NLRP3 inflammasomes triggering IL-1 activation of IFN-γ-Primed human endothelium. Circ. Res 124 (12), 1747–1759. 10.1161/CIRCRESAHA.119.314845. [PubMed: 31170059]

Xie CB, Jane-Wit D, Pober JS, 2020. Complement membrane attack complex: new roles, mechanisms of action, and therapeutic targets. Am. J. Pathol 190 (6), 1138–1150. 10.1016/j.ajpath.2020.02.006. [PubMed: 32194049]

Xue X, Wu J, Ricklin D, Forneris F, Di Crescenzio P, Schmidt CQ, Granneman J, Sharp TH, Lambris JD, Gros P, 2017. Regulator-dependent mechanisms of C3b processing by factor i allow differentiation of immune responses. Nat. Struct. Mol. Biol 24 (8), 643–651. 10.1038/nsmb.3427. [PubMed: 28671664]

Yates JRW, Sepp T, Matharu BK, Khan JC, Thurlby DA, Shahid H, Clayton DG, Hayward C, Morgan J, Wright AF, Armbrecht AM, Dhillon B, Deary IJ, Redmond E, Bird AC, Moore AT, 2007. Complement C3 variant and the risk of age-related macular degeneration. N. Engl. J. Med 357 (6), 553–561. 10.1056/NEJMoa072618. [PubMed: 17634448]

Yeh S, Khurana RN, Shah M, Henry CR, Wang RC, Kissner JM, Ciulla TA, Noronha G, Peachtree Study Investigators, 2020. Efficacy and safety of suprachoroidal CLS-TA for macular edema secondary to noninfectious uveitis: phase 3 randomized trial. Ophthalmology 127 (7), 948–955. [PubMed: 32173113]

Yehoshua Z, Alexandre De Amorim Garcia Filho C, Nunes RP, Gregori G, Penha FM, Moshfeghi AA, Zhang K, Sadda S, Feuer W, Rosenfeld PJ, 2014. Systemic complement inhibition with eculizumab for geographic atrophy in age-related macular degeneration: the COMPLETE study. Ophthalmology 121, 693–701. 10.1016/j.ophtha.2013.09.044. [PubMed: 24289920]

Yu M, Zou W, Peachey NS, McIntyre TM, Liu J, 2012. A novel role of complement in retinal degeneration. Investig. Ophthalmol. Vis. Sci 53, 7684–7692. 10.1167/iovs.12-10069. [PubMed: 23074214]

Zhan X, Larson DE, Wang C, Koboldt DC, Sergeev Y.v., Fulton RS, Fulton LL, Fronick CC, Branham KE, Bragg-Gresham J, Jun G, Hu Y, Kang HM, Liu D, Othman M, Brooks M, Ratnapriya R, Boleda A, Grassmann F, von Strachwitz C, Olson LM, Buitendijk GHS, Hofman A, van Duijn CM, Cipriani V, Moore AT, Shahid H, Jiang Y, Conley YP, Morgan DJ, Kim IK, Johnson MP, Cantsilieris S, Richardson AJ, Guymer RH, Luo H, Ouyang H, Licht C, Pluthero FG, Zhang MM, Zhang K, Baird PN, Blangero J, Klein ML, Farrer LA, DeAngelis MM, Weeks DE, Gorin MB, Yates JRW, Klaver CCW, Pericak-Vance MA, Haines JL, Weber BHF, Wilson RK, Heckenlively JR, Chew EY, Stambolian D, Mardis ER, Swaroop A, Abecasis GR, 2013. Identification of a rare coding variant in complement 3 associated with age-related macular degeneration. Nat. Genet 45 (11), 1375–1379. 10.1038/ng.2758. [PubMed: 24036949]

Zhao C, Yasumura D, Li X, Matthes M, Lloyd M, Nielsen G, Ahern K, Snyder M, Bok D, Dunaief JL, LaVail MM, Vollrath D, 2011. mTOR-mediated dedifferentiation of the retinal pigment epithelium initiates photoreceptor degeneration in mice. J. Clin. Invest 121 (1), 369–383. 10.1172/JCI44303. [PubMed: 21135502]

Zhao L, Zabel MK, Wang X, Ma W, Shah P, Fariss RN, Qian H, Parkhurst CN, Gan WB, Wong WT, 2015. Microglial phagocytosis of living photoreceptors contributes to inherited retinal degeneration. EMBO Mol. Med 7 (9), 1179–1197. [PubMed: 26139610]

Zhou J, Jang YP, Kim SR, Sparrow JR, 2006. Complement activation by photooxidation products of A2E, a lipofuscin constituent of the retinal pigment epithelium. Proc. Natl. Acad. Sci. U. S. A 103 (44), 16182–16187. 10.1073/pnas.0604255103. [PubMed: 17060630]

Zhou J, Kim SR, Westlund BS, Sparrow JR, 2009. Complement activation by bisretinoid constituents of RPE lipofuscin. Investig. Ophthalmol. Vis. Sci 50 (3), 1392–1399. 10.1167/iovs.08-2868. [PubMed: 19029031]

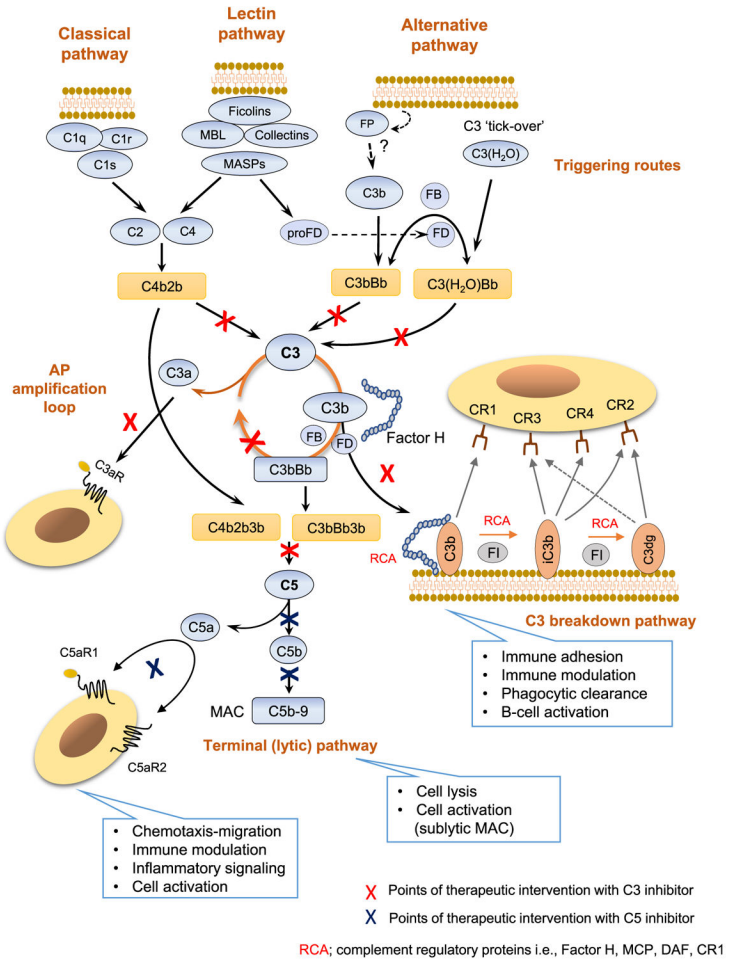

Fig. 1.

Overview of the complement system. The complement cascade involves many protein interactions that occur within the plasma, on cell surfaces, and within cells. It begins with the classical, lectin, and alternative pathways. The three pathways converge on C3, and C3 mediates multiple functions. Cleavage of C5 brings about the terminal lytic pathway with formation of the membrane attack complex. Shown on the right are key components of the C3 breakdown pathway, including C3 fragments, the complement receptors, and associated functions. This portion of the schematic intends to illustrate the multiplicity of interactions between C3 fragments and their receptors on a generic cellular scaffold and does not imply the concomitant presence of all complement receptors on the same cell type. Shown on the left are the anaphylatoxins (C3a and C5a), their receptors, and associated functions. Red X's highlight pathways blocked by C3 inhibition, and black X's highlight pathways blocked by C5 inhibition. Convertases are shown in yellow boxes. Important interactions are discussed in more detail within the text. For simplicity, the schematic does not depict intracellular or extrinsic protease-mediated routes of complement activation, but these important aspects of complement biology are discussed in the text. Abbreviations: MBL, mannose-binding lectin; MASPs, mannose-binding lectin associated serine proteases; FP, factor properdin; FB, factor B; FD, factor D; FI, factor I; CR, complement receptor; C3aR, C3a receptor; CSaR1, C5a

receptor 1; C5aR2, C5a receptor 2; MAC, membrane attack complex; RCA, regulator of complement activation; MCP, membrane cofactor protein; DAF, decay accelerating factor.

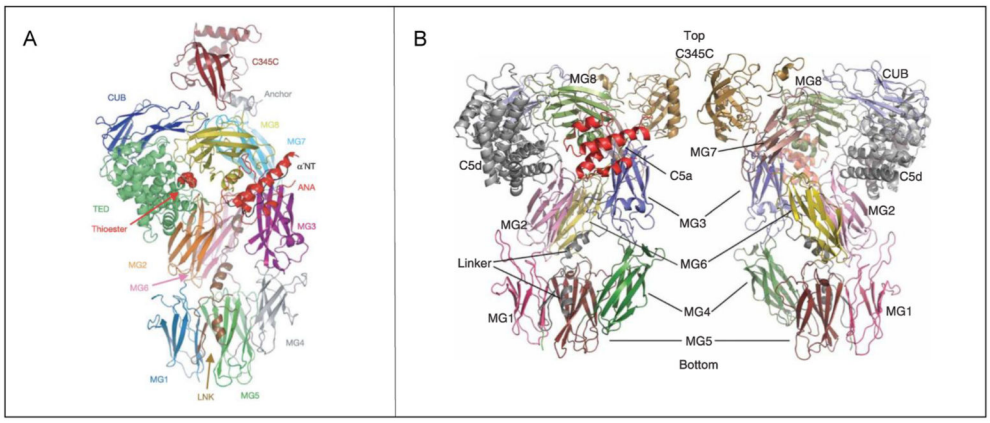

Fig. 2.

Crystal structures of C3 and C5. The domains of C3 (A) and C5 (B) are labeled. Figure and caption adapted from Janssen et al., 2005 (C3) and Fredslund et al., 2008 (C5) with permission of the copyright holder. Abbreviations: MG, macroglobulin; TED, thioester domain; ANA, anaphylatoxin; LNK, linker; CUB (complement C1r/C1s, Uegf, Bmp1).

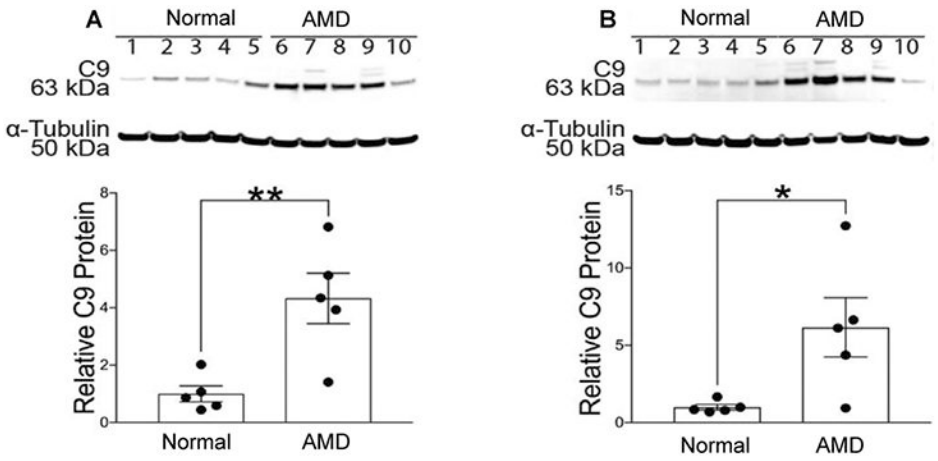

Fig. 3.

Passage of complement component 9 (C9) across the blood retina barrier of nonexudative AMD (age-related macular degeneration) patients. Western analysis of post mortem neurosensory retina protein from macula (A) with corresponding pixel density graphs and from nasal periphery (B) with corresponding pixel density graphs. Tissue was obtained from the Minnesota Lions Eye Bank. Immunoblots were done with anti-C9 (R & D Systems, Minneapolis, MN) and α-tubulin (Sigma-Aldrich, Inc., St. Louis, MO). Loading control α-tubulin (50 kDa) bands are shown below each set of lanes. Graphs show band densitometry normalized to loading control calculated using Image J software. Numbers represent mean values (±SEM). Grading of eyes was done using the Minnesota Grading System (Decanini et al., 2007; Olsen and Feng, 2004). There were 5 normal eyes, and 5 intermediate-stage AMD eyes. Statistical analysis was performed using student's two-tailed unpaired t-test. *$p < 0.05$, **$p < 0.01$. Normal and AMD eyes were matched for age and post-mortem interval. These findings were replicated with a second cohort of eyes from the Alabama Eye Bank. Figure and caption adapted from Schultz et al., 2019 with permission of the copyright holder.

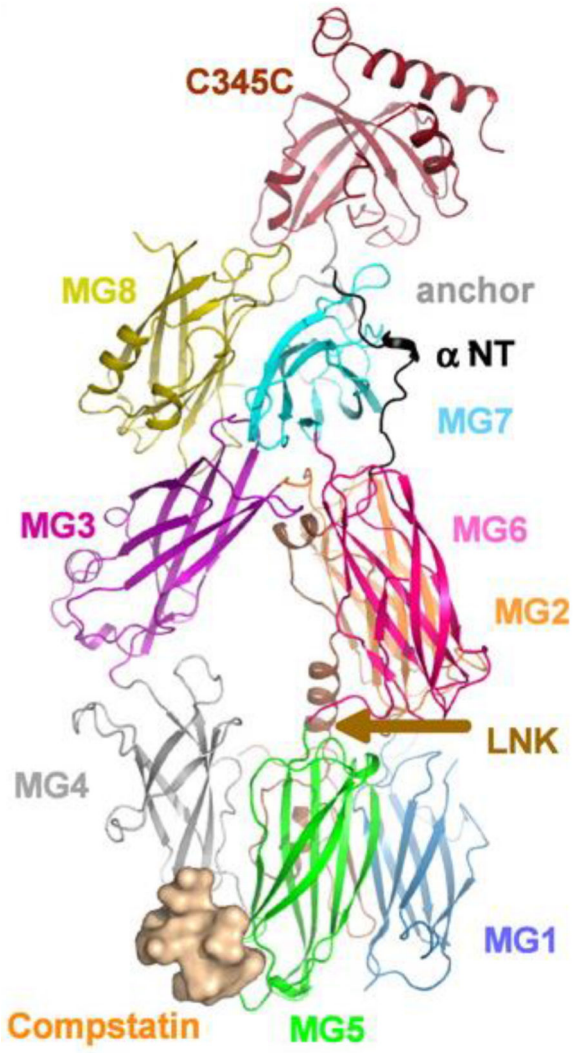

Fig. 4.

Crystal structure of C3c bound to compstatin. Compstatin (analog 4W9A) binds to the C3c portion of C3 between MG4 and MG5. Compstatin sterically prevents C3 from interacting with C3 convertases. Figure and caption adapted from Janssen et al., 2007 with permission of the copyright holder. Abbreviations: MG, macroglobulin; LNK, linker.

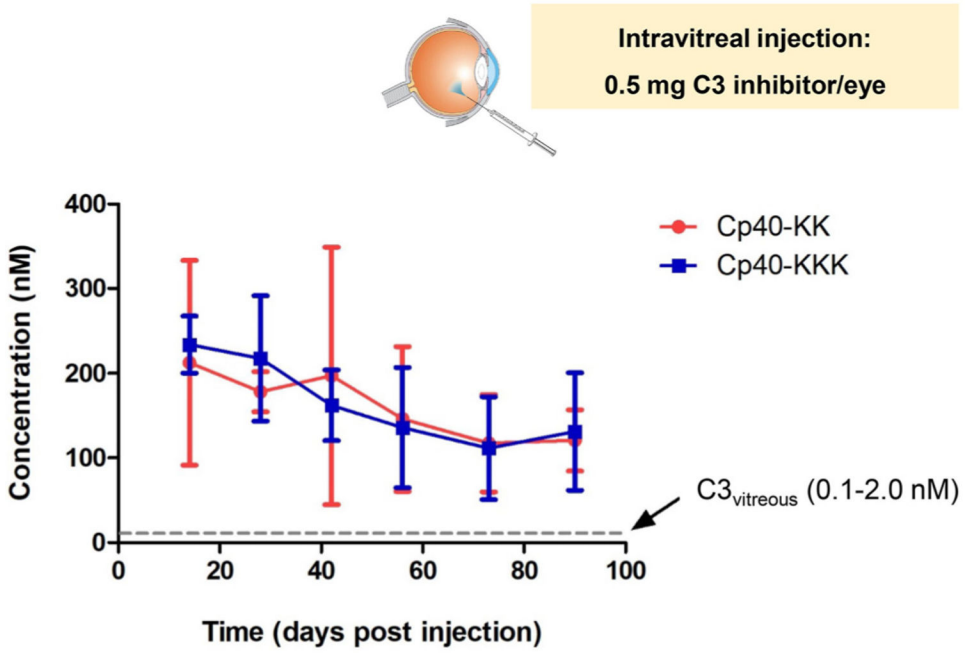

Fig. 5.

Prolonged intraocular residence of Cp40-KKK. Pharmacokinetic profile of compstatin-based analogs Cp40-KK and Cp40-KKK in the vitreous of non-human primates. The intravitreal concentration of the lysine modified Cp40 analogs, Cp40-KK and Cp40-KKK, was determined by surface plasmon resonance-based quantification at various time points (i.e., 14, 28, 42, 56, 73, 90 days) following a single intravitreal injection of 500 μg of inhibitor in cynomolgus monkeys. A total of three eyes were used per treatment and each data point in the curve represents the mean value ± SD from N = 3 animals per group. Each curve represents successive measurements (intraocular inhibitor levels) from the same animal, averaged for a group of three treated eyes per inhibitor. Figure and caption adapted from Hughes et al., 2020 with permission of the copyright holder.

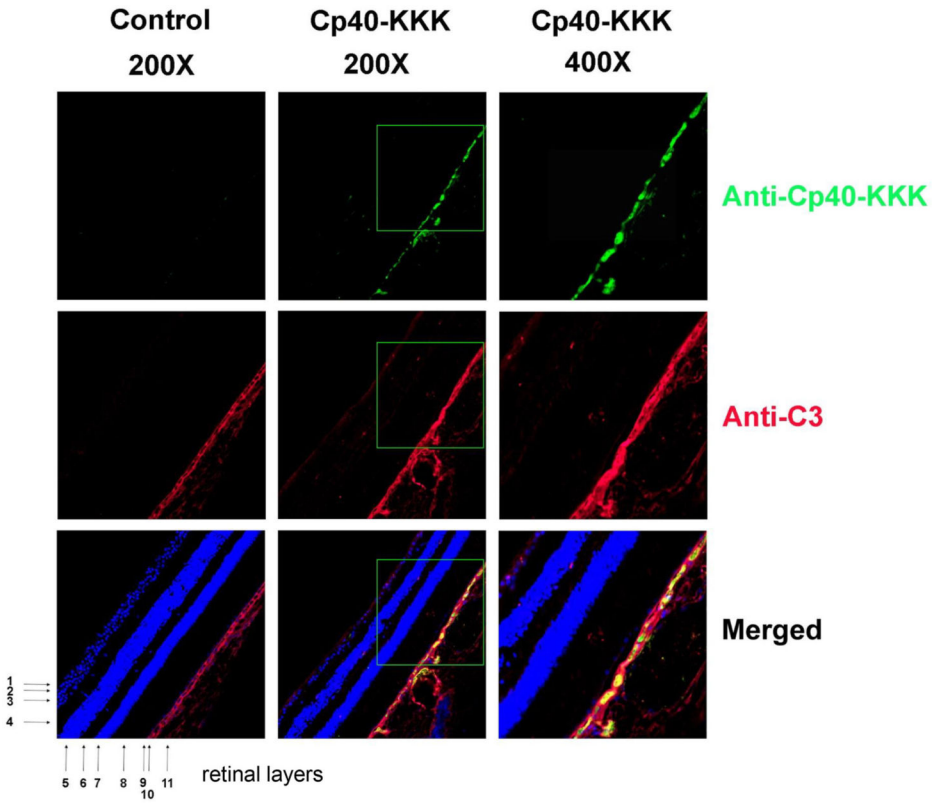

Fig. 6.

Retinal tissue distribution of Cp40-KKK and its co-localization with C3 in non-human primates. Non-human primates (NHPs) (6–7 years old) were treated with one dose of 500 μg of Cp40-KKK peptide via intravitreal Injection. One month later following the injection, the NHPs were euthanized. Both right and left eyes of NHPs were collected, fixed in 4% paraformaldehyde solution in PBS and held at 4 °C until dissection. The eyecup was carefully dissected into two halves; the eyecup half with the optic nerve head was cryopreserved overnight in 30% sucrose (prepared in PBS) at 4 °C. The following day, the sample was embedded in OCT, frozen in isopropanol (precooled in dry ice), and the tissue was sectioned at 12-μm thick sections and stored at −80 °C. The sections were then stained with both goat anti-human complement C3 (1:200 dilution) and anti-Cp40 antibody (final concentration of 2 μg/ml). The Cp40-KKK staining signal was amplified by using the Alexa Fluor™ 555 Tyramide SuperBoost kit (Thermo Fischer, B40923). The images were taken using a Nikon Eclipse Ti2 Confocal Microscope. Note: Control Retina tissue was from untreated NHPs. The middle (Cp40-KKK, 200X) and right panel (Cp40-KKK, 400X) of photomicrographs display retinal tissue sections from NHPs being treated with one dose of 500 μg of Cp40-KKK peptide at x200 and x400 magnification, respectively; the green-colored squares in the middle panel denote the magnified region of the retina. Red color: represents the C3 staining; Green color: represents the Cp40-KKK staining. Blue color: represents DAPI staining of nuclei. Yellow color: represents the co-localization of both C3 and Cp40-KKK. The retinal tissue consists of a total of 11 layers: 1. Internal limiting membrane; 2. Nerve fiber layer; 3. Ganglion cell layer; 4. Inner plexiform layer; 5. Inner nuclear layer; 6. Outer plexiform layer; 7. Outer nuclear layer; 8. Layer of rods and cones; 9.

Retinal pigment epithelium; 10. Bruch’s membrane and choriocapillaris; 11. Choroid. Figure and caption adapted from Hughes et al., 2020 with permission of the copyright holder.

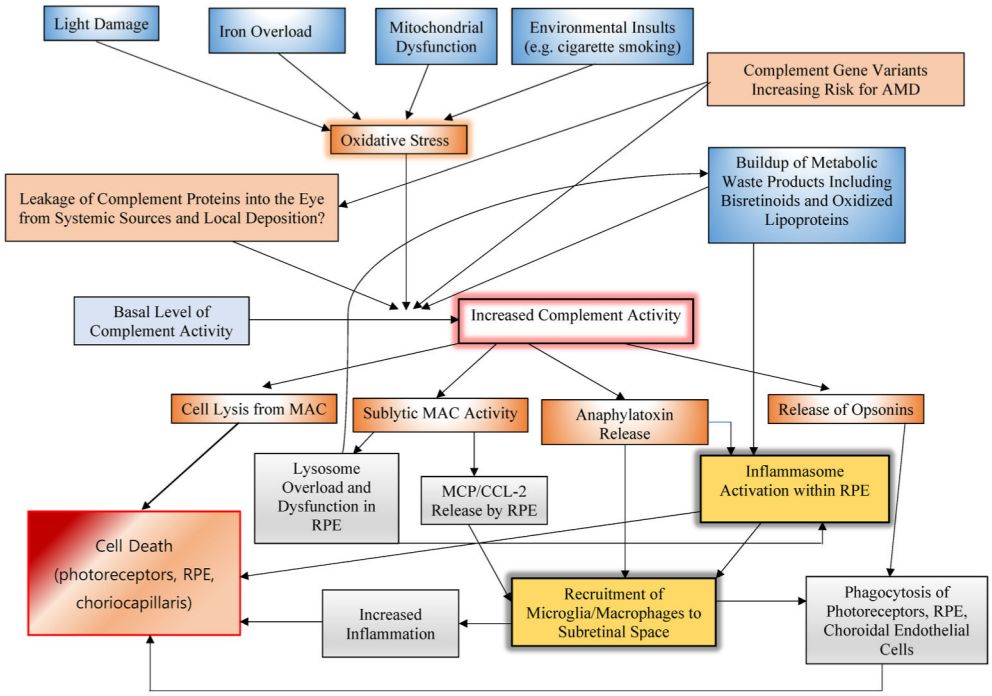

Fig. 7.

Complement focused model of major pathways in geographic atrophy pathophysiology. Several parallel pathways contribute to oxidative stress. Buildup of metabolic byproducts, oxidative stress, the impact of complement gene variants, and perhaps systemic complement deposition within the eye cause abnormally increased complement activity. This in turn contributes to several pathways including membrane attack complex activity, microglia/macrophage infiltration into the subretinal space, increased inflammation, and inflammasome activation. These potential pathways, acting in concert, contribute to cell death of photoreceptors, retinal pigment epithelium, and choriocapillaris. Abbreviations: AMD, age-related macular degeneration; MCP, monocyte chemoattractant protein; CCL-2, chemokine (C—C motif) ligand 2; RPE, retinal pigment epithelium.

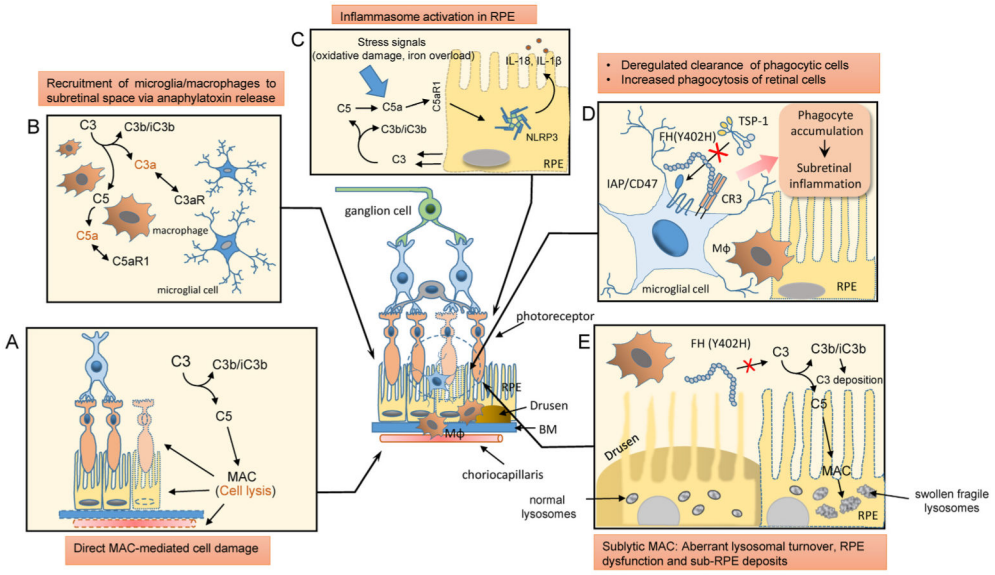

Fig. 8.

Potential pathological consequences of deregulated complement activity in the AMD (age-related macular degeneration) retina. Several complement mediated mechanisms may act in parallel to bring about geographic atrophy. A. Increased membrane attack complex activity may cause cell lysis of retinal pigment epithelium, choriocapillaris, and photoreceptors. B. The anaphylatoxins C3a and C5a cause microglia/macrophages to migrate to the subretinal space. C. Complement dysregulation contributes to NLRP3 inflammasome activity within the RPE with secretion of IL-18 and IL-1β. D. The Y402H FH variant can competitively block TSP-1 mediated phagocyte clearance, leading to phagocyte accumulation, and subsequent inflammation (Calippe et al., 2017). There is also potential for phagocytosis of photoreceptors, RPE, and choroidal endothelium. E. Increased sublytic membrane attack complex activity overloads lysosomes leading to RPE dysfunction and sub-RPE deposits (Cerniauskas et al., 2020). Abbreviations: MAC, membrane attack complex; RPE, retinal pigment epithelium; BM, Bruch's membrane; Mφ, macrophage; c3aR, C3a receptor; C5aR1, C5a receptor 1; NLRP3, NOD-, LRR- and pyrin domain-containing protein 3 inflammasome; FH(Y402H), Factor H Y402H variant; IAP, integrin associated protein; CR3, complement receptor 3; TSP-1, thrombospondin-1.

Table 1

C3 and C5 relevant therapeutics for geographic atrophy.

Scientific Name (Sponsor)	Target	Mechanism of Action	Mode of Administration	Current Phase in Clinical Trial (Clinicaltrials.gov ID)
Pegcetacoplan/APL-2 (Apellis)	C3	Pegylated peptide (second generation compstatin), inhibits cleavage of C3	Intravitreal injection	Phase III (NCT03525600, NCT03525613)
Cp40-KKK/AMY-106 (Amyndas)	C3	4th generation compstatin, inhibits cleavage of C3	Intravitreal injection	Phase I trial in development.
CB2782 – PEG (Catalyst Biosciences/Biogen)	C3	Pegylated C3 protease, degrades C3	Intravitreal injection	Preclinical
Avacincaptad Pegol (IVERIC bio)	C5	Pegylated aptamer, inhibits cleavage of C5	Intravitreal injection	Phase III (NCT04435366)
Eculizumab (Alexion)	C5	Monoclonal antibody, inhibits C5 cleavage	Intravenous (systemic)	Stopped after phase II (NCT00935883)
Tesidolumab/LFG316 (Novartis)	C5	Monoclonal antibody, inhibits C5 cleavage	Intravitreal injection	Stopped after phase II (NCT01527500)
Ionis-FB-L_{RX} (Ionis/Roche)	Factor B	Antisense oligonucleotide, inhibits Factor B, reducing AP C3 convertase formation	Subcutaneous injection (systemic)	Phase II (NCT03815825)
Lampalizumab (Genentech/Roche)	Factor D	Fragment of humanized monoclonal antibody, inhibits Factor D and blocks AP C3 convertase formation	Intravitreal injection	Stopped after phase III (NCT02247531, NCT02247479)
GT005 (Gyroscope Therapeutics)	Factor I	Adeno-associated virus, produces Factor I locally and reduces AP C3 convertase activity	Subretinal injection	Phase II (NCT04437368)
CLG561 (Novartis)	Properdin	Monoclonal antibody, inhibits properdin and reduces AP C3 convertase activity	Intravitreal injection	Stopped after phase II (NCT02515942)

AP = alternative pathway.