The Immune System and AMD

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Abstract Age-related macular degeneration is a complex, multifactorial disease that has yet to be completely understood. Significant efforts in the basic and clinical sciences have unveiled numerous areas that appear to be critical in the pathogenesis of this disease. The alternative complement pathway, immune cell activation, and autoimmunity are all emerging as important themes in the suspected immunological origins of this disease. Advancement toward a complete understanding of these processes is important in the development of new techniques for disease monitoring and treatment.

Keywords AMD · Complement pathways · Anti-retinal autoantibodies · Toll-like receptors · Chemokine receptors · Immunology

Introduction

Age-related macular degeneration (AMD) is currently the leading cause of non-reversible central vision loss in the USA. It is a multifactorial disease involving deposition of sub-RPE protein deposits known as drusen and a breakdown of the RPE and Bruch's membrane, which is rarely present in patients under 50 years of age. Ninety percent of patients have "dry" non-neovascular disease, whereas 10 % have "wet" disease characterized by neovascular choroidal membranes and subsequent leakage of fluid into the sub-RPE and subretinal spaces. While the specifics surrounding AMD have yet to be fully elucidated, the body

of research surrounding its biochemistry and pathogenesis has been rapidly expanding with investigations into its genetic, immunological, and inflammatory origins. This section will provide a concise review on the immunological phenomena that have been observed in AMD, including dysregulation of complement cascades, immune cell activation, and anti-retinal antibodies.

Complement Activation

There is a growing body of research implicating a dysregulation of the complement pathway as a contributing factor to the pathogenesis of AMD. The complement system is a major component of the innate (non-specific rapid response) immune system responsible for providing a baseline defense against invading pathogens. It is comprised of an immunochemical cascade that is responsible for immune cell activation, cell lysis, and clearance of pathologic and immune complexes. The complement system accomplishes these goals via three separate pathways: classical, alternative, and lectin. The three pathways all involve the cleavage of complement component 3 (C3) via the enzyme C3 convertase into the subcomponents C3a and C3b. C3b is then able to bind directly to sites on pathogen membranes, enabling further complement activation [1]. The classical pathway is triggered by interaction with antigen-antibody complexes and has not been found to be a significant contributing factor in AMD [2, 3]. Conversely, the alternative and lectin pathways are activated independently of antibody-mediated interaction and have been demonstrated to be major processes in the etiology of AMD [4]. Receptors for C3a and another complement product, C5a, are present within the nerve fiber layer and inner plexiform layer of the retina [5]. The presence of

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Fig. 1 AMD pathogenesis has been linked to dysregulated complement activation, professional inflammatory cell recruitment, most notably macrophages, and possible infectious etiologies with activation of TLRs and the inflammasome leading to cell death. RPE cells stimulated with active complement factors produce increased VEGF-A, which can lead to direct promotion of CNV and recruitment of

macrophages. Similarly, inflamed RPE cells also produce CCL2, which is a chemotactic agent for CCR2 expressing inflammatory macrophages. These molecular and cellular pathways are under intense investigation for possible new therapeutics to target this disease process to prevent and reverse the blinding effects of vascular in-growth and cell death

these receptors suggests that unimpeded activation of the complement system could have direct cytotoxic effects on the retina. This issue was a concern in early clinical trials, in addition to the importance of the complement system in pathogen detection, and a concern for intraocular infections, but no issues were reported with nerve fiber layer or inner-retinal damage with pharmacological complement inhibition. Molecular analyses of macular drusen associated with AMD have confirmed the presence of complement factors, amyloid-B, and apolipoprotein E, which have also been demonstrated in affected tissues from other neurodegenerative disorders [6–8]. Furthermore, animal models have now demonstrated increased complement deposition in sites of choroidal neovascularization (CNV) using a targeted bioimaging approach with anti-C3 monoclonal antibodies, opening the possibility of visualizing and inhibiting complement simultaneously in the human eye [9].

In 2005, a single nucleotide polymorphism (SNP) was discovered in the gene encoding complement factor H (CFH), a complement control protein expressed in the RPE and choroid that significantly elevated the risk of developing AMD. Multiple studies found that the presence of the SNP Y402H alone was enough to significantly elevate risk [10–13]. Mechanistically, this mutation results in a decreased ability of CFH to inhibit C3b, resulting in an uninhibited alternative complement pathway [14]. To date, the CFH data have been repeated in numerous cohorts, confirming its potential as a target for pharmacological blockade in the treatment of AMD. Additional work has identified other SNP associations with confirmatory data for complement factor 2 (C2), C3 (C3), B (CFB), and I (CFI) [15, 16, 17••]. A recent study has identified

complement factor D (CFD) SNPs in patients with AMD, but this finding was nearly confined to females and could not be validated in additional cohorts [18, 19]. However, there are preclinical data to suggest that neutralization of CFD may be protective of photoreceptor cell death [20], providing the rationale for advanced clinical studies of an anti-CFD antibody (FCFD4514S/Lampalizumab, Genentech). Intravitreal administration of lampalizumab was shown to be safe [21•], and in the ongoing Phase II study, MAHALO, the 18-month results demonstrated that progression of geographic atrophy (GA) of the RPE in advanced dry AMD is slowed by 20 % with monthly lampalizumab (10 mg) and by over 40 % in patients with an associated SNP in *Cfi* [22]. Along with the identification of the CFI biomarker for GA progression, these data offer new hope for the treatment of advanced dry AMD and resultant atrophy of the RPE (Fig. 1).

Infectious Associations

Aside from complement, intriguing hypotheses have been proposed regarding pathogen-associated and adaptive antibody-mediated immune activation as a potential driving factor behind AMD onset and progression. One such hypothesis promotes an infectious etiology of AMD. Although no individual pathogen has been explicitly implicated, some laboratories have established associations with cytomegalovirus and *Chlamydia pneumonia* [23–25]. These microbes appear to activate the host immune system via interaction with Toll-like receptors (TLRs). TLRs are a family of membrane-spanning pattern-recognition receptors that are able to bind highly conserved molecules on the

surface of invading pathogens and activate inflammatory cytokine elaboration, thereby activating the host immune system, and are known to be expressed on RPE cells [26]. One particular protein known to be expressed on RPE cells, TLR3 [27], which binds to double-stranded RNA (dsRNA), results in RPE toxicity when activated in animal models and in human tissue culture [28]. Many of the genetic association data regarding TLRs and AMD have been controversial. For example, a link between AMD and two other TLR polymorphisms was described: the TLR3 SNP, L412F, located at 4q35, and the TLR7 SNP, Q11L, located at Xp22 [29]. However, these data were rendered statistically insignificant after correcting for multiple comparisons. Additional studies of the hypofunctional L412F SNP reported a protective effect for advanced dry AMD compared to normal controls [30, 31], but this result could not be corroborated in independent cohorts [32•, 33]. A hypomorphic TLR4 SNP (D299G) has been found to confer increased AMD risk in some populations [34], but not in others [35, 36, 37•]. In spite of inconsistent studies, TLRs may have a partial impact on AMD development, and their involvement bolsters the advancing theory regarding an infectious origin in AMD pathogenesis. Certainly, in animal models, TLR activation appears to induce robust effects in the retina and RPE, with wide-ranging consequences from focal RPE degeneration (TLR3) to frank retinitis (TLR9) [38].

After the discovery of the association of TLR3 with GA, multiple research groups embarked on efforts to identify the effects of dsRNA on the RPE. Molecular investigations revealed that intravitreal delivery of dsRNA induces RPE cell death via TLR3 and a downstream transcription factor, IRF3 [39], as well as through activation of RIP3 kinase pathways resulting in necrosis [40]. Immunolocalization studies in tissue sections of human eyes with GA revealed increased dsRNA levels in the RPE [41••]. This RNA was sequenced and identified as Alu RNA, an archaic non-coding repetitive mobile genetic element with ~1 million copies scattered throughout the human genome. A deficiency in the endogenous dsRNA processing enzyme called DICER1 was found to be the culprit for this rise in Alu RNA. In vivo and in vitro studies confirmed that Alu RNA is cytotoxic to the RPE via induction of the inflammasome through MyD88 and IL18 signaling, leading to caspase activation and eventual apoptosis [42•]. These mediators of Alu RNA retinotoxicity and the inflammasome are being evaluated for therapeutic approaches in the pharmacological rescue of advanced dry AMD.

Although most recent AMD research has focused largely on innate immunity, an adaptive immune response may also be contributory. Histological examination of AMD-affected eyes demonstrated higher levels of anti-retinal autoantibodies [43], which are believed to propagate

AMD progression by way of direct RPE and photoreceptor damage. Anti-astrocyte autoantibodies have also been observed in AMD patients, which possibly interfere with maintenance of the blood-retinal barrier [44]. Additionally, levels of these antibodies parallel anti-VEGF treatment response and may serve as a potentially underutilized biomarker for disease therapy [45]. There are further data to suggest the role of auto-immunity in the development of dry AMD. A protein adduct called carboxyethylpyrrole (CEP) is generated from oxidized lipid in the retina, and with the subsequent formation of anti-CEP antibodies, autoimmune-mediated degeneration of the retina may occur [46•, 47]. This molecular evidence provides another mechanism by which advanced dry AMD may ensue and offers an additional explanation for the presence of anti-retinal antibodies in affected patients.

Immune Cell Recruitment and Activation

Discussions regarding the immuno-pathologic influence in AMD necessitate examining the role of immune cell activation in the affected ocular tissues. Extensive research has been conducted to identify the role of individual immune cells and their respective contributions to the disease process. In mouse models, laser-induced CNVs have revealed that macrophages are particularly important in promoting neovascularization [48, 49]. Furthermore, histological examination of subretinal AMD lesions in human eyes has demonstrated these areas to have increased populations of macrophages among other immune cells [50]. Supporting the importance of macrophages in the proliferation of CNV, blockade of VEGF receptors results in markedly decreased monocyte infiltration into the site of laser-induced CNV [51]. Although some involvement from non-macrophage immune cells may play a role in the neovascular process, these cells (CD4+ and CD8+ lymphocytes, natural killer cells, and neutrophils) do not appear to be foundational in the formation of CNV [52].

Despite the establishment that macrophages are heavily influential in the development of neovascular lesions, several studies have indicated that they are able to impart a contradictory, anti-angiogenic effect as well [53, 54•, 55]. Mouse models have demonstrated this with laser-injury aged mice exhibited a more proinflammatory macrophage phenotype [56]. The phenotypic variation between protective and proinflammatory monocyte-derived cells is not completely understood, but is thought to be triggered by a milieu of environmental and genetic factors, some of which are influenced by age. In a recent study, bone marrow transplantation from old donor mice into young recipient mice transferred susceptibility to laser-induced CNV. Conversely, older mice who received bone marrow from

younger mouse donors showed a dampened response to laser-induced CNV, indicating that age-related changes involving progenitor cells in the bone marrow could be a source of AMD susceptibility [57•]. New data have emerged on the molecular mechanisms of this critical aging effect on macrophage function. Differential macrophage function in normal development and in specific disease models including laser-induced CNV has revealed that neovascular response is regulated by a delicate switch between anti-angiogenic macrophages (M1) and pro-angiogenic macrophages (M2). Specific triggers that drive macrophage polarization to pro-angiogenic subtypes may offer new pharmacological approaches to this disease. Significant attention has been given to the abundance of lipids and associated proteins in drusen from eyes with AMD. Major components of these deposits include esterified cholesterol, phosphatidyl choline, and apolipoprotein B [58]. A subsequent study on aging macrophages in the peripheral blood and eye revealed downregulation of cholesterol trafficking machinery specifically with the transporter ABCA1, which has been linked to AMD in genome-wide association studies [59, 60]. Macrophages with decreased ABCA1 expression are overloaded with free cholesterol, which drives the phenotypic switch to the pro-angiogenic M2 subtype. This molecular mechanism of impaired cholesterol clearance and induction of proinflammatory macrophage differentiation provides a new pathway that links abnormal lipid accumulation in drusen and CNV formation.

In other efforts to identify critical factors that regulate inflammatory macrophage switching, investigators utilized transgenic mice to isolate monocyte cell trafficking mechanisms. A mouse model of AMD was initially described in 2003 and elevated the importance of the hypothesis that macrophage function is impaired in AMD and results in drusen formation. Transgenic mouse models either lacking the gene for chemokine ligand 2 (CCL2) (also known as monocyte chemoattractant protein-1/MCP-1) or chemokine receptor 2 (CCR2) were found to develop drusen-like deposits resembling those found in patients with AMD [53]. The CX3C chemokine receptor 1 (CX3CR1/C-X3-C motif/fractalkine receptor) is also expressed on the cell surface of specific resident macrophage subsets. In support of the previous macrophage-deficient mouse models of AMD, mice strains deficient in CX3CR1 or in combination with genetic ablation of CCR2 or CCL2 develop drusen and phenotypic derangements consistent with AMD [61, 62]. A subsequent study identified a CX3CR1 SNP (T280 M) that is associated with decreased expression of CX3CR1 and increased risk of advanced AMD [63]. These factors influence whether or not macrophage progenitors establish themselves as resident macrophages or transient macrophages directed

toward sites of inflammation. Macrophages expressing CX3CR1 alone are thought to function as resident phagocytic cells, whereas those expressing both CX3CR1 and CCR2 demonstrate proinflammatory features and shorter half-lives [64].

Further studies on mouse models of AMD with altered macrophage chemotaxis have generated considerable controversy about the origin of the subretinal deposits. Fundus imaging and immunofluorescence studies demonstrated lipofuscin and pigment bloated macrophages co-localizing with drusen-like lesions [65]. Several groups have proposed that the associated retinal degeneration in this model may be due to aging alone, and there are now reports that aged C57BL/6N mice develop drusen formation and retinal degeneration because of an rd8 mutation [66]. This issue of inherent retinal degeneration in commercial mouse strains requires serious consideration. Yet, the previously mentioned mouse strains reside on a C57BL/6J and not C57BL/6N background and have not been shown to harbor the rd8 mutation. This collection of findings suggests that a delicate balance between resident scavenging and proinflammatory macrophages is needed to maintain a healthy chorioretinal environment and that therapies directed toward the management of these chemokine receptor imbalances could possibly contribute to delayed disease onset or even disease process reversal.

Conclusions

Although significant scientific progress has been made, the exact mechanisms underlying the immune system's role in AMD pathogenesis remain unclear. A multitude of discoveries have aided our fundamental understanding of the roles of the complement system, immune cell trafficking and inflammation, as well as possible infectious or autoimmune phenomena in this disease. Taken in sum, the data establish AMD as a highly complex, multifactorial disease or perhaps even a spectrum of diseases that ultimately lead to RPE degeneration or choroidal neovascularization. Individual patients likely fall on a gradient of susceptibility to disease development, influenced by a conglomeration of environmental, genetic, and age related risk factors. Working toward an advanced understanding of the biologic systems underlying AMD continues to be an area of enormous scientific interest with the potential to lead to groundbreaking discoveries in both the current management and future treatment of this prevalent vision-threatening disease.

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