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The Immune System and AMD

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Abstract

Age related macular degeneration (AMD) is a complex, multifactorial disease that has yet to be completely understood. Significant efforts in the basic and clinical sciences have unveiled numerous areas which 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 to the suspected immunologic origins of this disease. Advancement toward a complete understanding of these processes is important in development of new techniques for disease monitoring and treatment.

Keywords

AMD; complement pathways; anti-retinal autoantibodies; toll-like receptors; chemokine receptors; immunology; drusen; macrophage; choroidal neovascularization

Introduction

Age-related macular degeneration (AMD) is currently the leading cause of non-reversible central vision loss in the United States. 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 that 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, immunologic and inflammatory origins. This section will provide a concise review on the immunologic phenomena that have been observed in AMD, including dysregulation of complement cascades, immune cell activation, and anti-retinal antibodies.

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Conflicts of Interest

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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 as 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 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 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 pharmacologic 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] [7] [8]. Furthermore, animal models have now demonstrated increased complement deposition in sites of choroidal neovascularization 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 responsible for 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 presence of the SNP Y402H alone was enough to significantly elevate risk [10] [11] [12] [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 has been repeated in numerous cohorts confirming its potential as a target for pharmacologic blockade in the treatment of AMD. Additional work has identified other SNP associations with associations confirmed for Complement Factor 2 (C2), C3 (C3), B (CFB), and I (CFI) [15–17]. However, there still has been no successful translation of complement inhibition approaches in the treatment of AMD.

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 labs have established associations with cytomegalovirus and *Chlamydia pneumonia*[18] [19] [20]. 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 [21]. One particular protein known to be expressed on RPE cells, TLR3 [22], results in RPE toxicity when activated in animal models [23]. Much of the genetic association data regarding TLRs and AMD has been controversial. For example, an 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 [24]. However, this data was 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 [25, 26], but this result could not be corroborated in independent cohorts [27, 28]. A hypomorphic TLR4 SNP (D299G) has been found to confer increased AMD risk in some populations [29], but not in others [30] [31] [32]. 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 (TLR) [33].

Although research has focused largely on innate immunity, an adaptive immune response may also be contributory. Histologic examination of AMD affected eyes demonstrates that affected eyes contain higher levels of anti-retinal autoantibodies [34], which are believed to propagate AMD progression by way of RPE and photoreceptor damage. Anti-astrocyte autoantibodies have also been observed in AMD patients, which possibly interfere with astrocyte mediated maintenance of the blood-retinal barrier [35]. Additionally, levels of these antibodies parallel anti-VEGF treatment response, and may serve as a potentially underutilized biomarker for disease therapy [36]. There has further data to suggest the role of auto-immunity in the development of dry AMD. A protein adduct known as 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 [37, 38]. This molecular evidence provides another mechanism by which advanced dry AMD may ensue and offers an additional explanation of 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 choroidal neovascular lesions have revealed that macrophages are particularly important in promoting neovascularization [39] [40]. Furthermore, histologic examination of subretinal AMD lesions in human eyes has demonstrated these areas to have increased populations of macrophages, among other

immune cells [41]. Supporting the importance of macrophages in the proliferation of CNV, blockage of VEGF receptors results in markedly decreased monocyte infiltration into the site of laser induced CNV lesions [42]. 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 lesions [43].

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 [44] [45] [46]. Mouse models have demonstrated that, in response to laser-injury, aged mice exhibit a more pro-inflammatory macrophage phenotype [47]. The phenotypic variation between protective and pro-inflammatory 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 CNV, indicating that age related changes involving progenitor cells in the bone marrow could be a source of AMD susceptibility [48].

In other efforts to identify critical factors that regulate inflammatory macrophage switching, investigators utilized transgenic mice to isolate monocyte cell trafficking mechanisms. CCR2 (chemokine receptor 2/C-C motif) and CX3C chemokine receptor 1 (CX3CR1/C-X3-C motif/fractalkine receptor) are both cytokine receptor proteins expressed on the cell surface of many leukocytes. These two proteins influence whether or not macrophage progenitors establish themselves as resident macrophages or transient macrophages directed toward sites of inflammation. Macrophages expressing CX3CR1 alone as are resident whereas those expressing both CX3CR1 and CCR2 demonstrate pro-inflammatory features and shorter half-lives [49].

There have been multiple studies involving alterations of these two chemokine receptors and their resulting effects on macrophages. The discovery of a CX3CR1 SNP (T280M) has been associated with decreased expression of CX3CR1 and a higher risk of AMD [50]. In laboratory models, mice deficient in CCR2, as well as CX3CR1 and CCR2/CX3CR1 knockout mice exhibit ocular phenotypic and fundoscopic appearances similar to that of human AMD [44] [51] [52]. Microscopically, CCL2/CX3CR1 knockout mice exhibit early photoreceptor degeneration, as well as aberrant growth of rod bipolar and horizontal cells [53]. This collection of findings suggests that a delicate balance between resident scavenging and pro-inflammatory macrophages is needed to maintain a health 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.

Conclusion

In short, the exact mechanisms underlying the immune system's role in AMD pathogenesis remain unclear. Great strides have been made in characterizing the roles of the complement system, immune cell trafficking and inflammation, as well as possible infectious or autoimmune phenomena underlying 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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