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# A Perspective of AMD Through the Eyes of Immunology

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Despite strong genetic associations, compelling human histological data and numerous hypotheses generated with supportive animal data, the mechanisms of inflammation or inflammatory control of cell health during progression of age-related macular degeneration arguably remain elusive. This perspective delivers a view that maintaining tissue health requires active immune cellular and tissue pathways, but when responses are perturbed or exaggerated, chronic inflammation is destructive. There are potential pathways and processes to enable understanding and determine how potential causative factors including altered cellular metabolism, senescence, oxidative stress disrupt tissue homeostasis are engaged. Establishing differences in the immune phenotype between normal aging and AMD, and how the inter-relatedness of these triggers contribute to pathobiology is integral for future therapeutic success.

Keywords: inflammation, dry AMD, immunotherapy, microglia

The relentless and increasing burden of AMD is confounded, in common with other chronic, insidious neuro-inflammatory disorders by many factors. Genetic–environmental interactions, the aging cell, tissue, and the immune system are implicated, but causality has yet to be established.[1,2] Unquestionably, the anti-VEGF renaissance has revolutionized treatments for neovascular AMD and prevents (at least in the short term) acute visual loss for millions of individuals.[3] However, we are still treating late in the disease course; therefore, unsurprisingly, atrophy ensues over time. Our focus needs to address and develop new approaches that halt the progressive loss of RPE and photoreceptors that characterize macular degeneration and understanding immune responses in AMD is a tractable target for such therapy.

Although clinical and genetic data support the association of chronic low-grade inflammation in the pathogenesis of AMD, the underlying mechanisms responsible are not fully appreciated, and we still have no current inroads to therapeutically redress the ocular inflammatory environment and prevent progression. Genetic studies strongly imply the central role of immune responses and particularly innate immunity in pathogenesis and disease severity, not least the compelling association with complement regulation.[4] Chronic accumulation of immune cells (infiltrating monocytes and tissue resident microglia) at either the subretinal or choroid RPE interface are associated with early, intermediate, and advanced forms of AMD and likely contribute to disease progression.[5–10] Similarly, increased numbers of activated choroidal macrophages and mast cells (MCs) are observed around atrophic lesions of human donor eyes, implicating complement-mediated MC degranulation.[11,12] Notwithstanding the idiosyncrasies of animal models not truly reflecting the human state, functional studies do indicate that the recruitment and accumulation of activated immune cells are critical for both neovascular and degenerative responses that characterize AMD.[5,13–17] Non-resolving, persistent low-grade inflammation is a major drive of many chronic, age-related diseases, including metabolic disorders, neurodegenerative diseases, and cancer. What unifies these conditions? The persistence of myeloid-derived cells that may amplify inflammation and tissue damage.[18]

Complicating our understanding is the lack of a clear sequence for the events and triggers driving initiation and progression of AMD from the very early stage. Drusen contain immunologically active deposits such as lipids, complement, and other immune activating components. However, is a druse the potential trigger of the initial inflammatory responses or do drusen simply accentuate inflammation? Additional inflammatory triggers include oxidative stress and secondary mediators (e.g., reactive oxidative species [ROS], cytokines, complement effectors, and C-reactive protein [CRP]), which will also dysregulate active pathways controlling immune homeostasis. Constitutive retinal immune regulation serves to preserve tissue and cellular function and is delivered via canonical hemopoietic (or yolk sac) derived immune cells (microglia), as well as nontraditional immune cells (Müller Glia, endothelium, RPE). Despite constant lifetime stress, the retina in many individuals continues to perform adequately late in life, which suggests to some degree that either inflammatory responses are essential and beneficial or intrinsic regulatory mechanisms are not perturbed. Thus, an alternative hypothesis may consider that heightened parainflammatory responses (both cellular and physiologic) required for sterile inflammation are also likely to act as metabolic sensors to regulate and maintain retinal health and homeostasis. Finally, an untapped area of research questioning is understanding how the retina/choroid responds to systemic challenges and determining the roles infection (or noninfective systemic immune activation) and immuno-senescence play; both can alter immune-surveillance or directly stimulate immune-competent cells in the retina to provoke or progress degenerative disorders.[19,20]

## The Status Quo

Maintaining tissue health requires active protective inflammatory responses. However, when responses are heightened, or dysregulated, chronic inflammation is destructive.[21] Inflammation is an adaptive response to tissue stress, regulated at the level of the tissue, but also by local (tissue-resident nonimmune and immune cells) and systemic immune pathways. When tissues are exposed to noxious stresses (environmental factors), cell-autonomous responses (e.g., inflammasome activation or autophagy) are initiated to repair the damage and promote survival to restore homeostasis. The tissue response to stress also elicits inflammatory signals and changes in the tissue microenvironment, which, monitored by the local immune network (tissue resident macrophages and the complement system), trigger transient inflammatory responses. Termed, para-inflammation, these responses are considered an important immunologic mechanism that serves to maintain tissue homeostasis and monitor for tissue malfunction.[22] Varying magnitudes of tissue stress or malfunction are exhibited as a spectrum and accordingly parainflammatory responses are matched: they range from low level or basal housekeeping functions through to unchecked proinflammatory responses. Failure to restore the optimal homeostatic threshold results in excessive parainflammatory responses that manifest into chronic immune-mediated degenerative conditions.[23,24]

In the retina, immune activation and initial recruitment of monocyte-derived macrophages (in addition to microglia) are required to help process photoreceptor and RPE byproducts, thus controlling overt inflammation. Infiltrated monocytes and macrophages respond to the triggers and cues that the environment exposes them to. Hence, the current and useful paradigm of monocytes[25] and macrophage subsets[26] is worthy but does not fully assist our understanding of cell functionality or purpose, nor the kinetics or dynamic nature of cell influx, residency, or efflux within the tissue. The continued understanding through animal models is therefore useful to at least glean knowledge of fate, function, and turnover[27–29] (discussed further in *The "Sick" Activated Microglia* section). For example, oxidized lipoproteins and free radicals are major causes of tissue stress, serving as local triggers for parainflammation and exhibited by the activation and increased motility of resident microglia.

The sick activated microglia is a notion documented in dementia,[30] and potentially contributes to breakdown of the blood retinal barrier and promotion of inflammation. One example of para-inflammation in the eye is complement activation and accumulation of both microglia and infiltrated macrophages (mononuclear phagocytes) to the subretinal space, mast cell activation, and morphologic changes to the melanocytes in the choroid, as well as fibrosis. To prevent rapid progression and curtail inflammation, there are several levels of immune checkpoints, including innate immune regulators. As stated earlier, if drusen drive immune responses (as they contain immune activating components), they will target the coaligned RPE, microglia, and macrophages, driving consequent inflammatory cytokine and angiogenic mediators.[31–34] To date, the most tractable therapy given the genetic variant association is against complement.[35]

## Innate Immunity, Complement, and Gene Association

The most widely studied genetic risk factor for AMD is complement factor H (CFH).[36–39] CFH regulates complement activation in plasma, host cells, and tissue, in particular at sites of tissue inflammation supporting myeloid cell adhesion and migration facilitate phagocytosis of microbes and cellular debris, following injury, or during degeneration.[40] The genetic variant substitutes histidine for tyrosine (Y402H) in the cellular binding domain of CFH. The CFH(H402) variant does not regulate the alternative pathway (AP) of complement activation as efficiently and is associated with early and advanced AMD, suggesting CFH(H402) may drive disease onset.[4] How does this relate to dysregulating immune homeostasis?

Recent data offer new insight into the role of CFH in AMD that is as surprising as it is compelling, revealing a noncanonical role of CFH in AMD distinct from the complement pathway.[41] *Cx3cr1*-deficient animals and *TRE2* mice (a humanized transgenic mouse expressing the AMD-risk APOE2 isoform) model the age-dependent subretinal accumulation of both monocytes and resident microglia and the associated photoreceptor degeneration observed in human AMD.[5,16,42] Contrary to the expectation that perturbed complement regulation would augment disease, CFH genetic deficiency in these models instead inhibits the progressive cellular accumulation, supporting the similar age- and CFH-dependent increase reported in the choroid of CFH heterozygous vs homozygous mice.[43] Through iterative experiments, this elegant study demonstrates that CFH binding to CD11b perturbs the mechanism of CD47-mediated elimination of mononuclear phagocytes (MPs) required to maintain homeostasis in the subretinal space. Normally, during resolution of inflammation, MPs are eliminated through CD11b–CD47 interaction and signaling with its cognate ligand thrombospondin-1 (TSP-1), which is secreted by RPE cells. This finding may also explain previous reports describing increased and prolonged subretinal inflammation observed in TSP-1–deficient mice.[44–46] The clinical relevance of this mechanism was highlighted where the risk variant of AMD CFH(Y402H) binds more efficiently than the nonrisk isoform to CD47-CD11b, thereby inhibiting the immunosuppressive effects of TSP-1 (i.e., elimination of MPs from the subretinal space). These findings raise important questions and new avenues of investigation, as well as insights into disease etiology. Are functional polymorphisms related to CD11b or CD47 conferring similar risks for AMD? Does the association of the high-risk variant with the CD11b–CD47 complex share similarities in its complement-inhibiting activity? The work highlights that development of therapeutic agents directed toward cellular clearance strategies may be of benefit in preventing progression.

CRP is the prototypical acute-phase reactant and an active regulator of the innate immune system. It is a serum biomarker for chronic inflammatory conditions, heart disease, and, more recently and arguably, AMD.[47] CRP has been identified in drusen[48] and the choroid. Among the multiple functions ascribed to CRP are activation of the classical complement pathway and inactivation of the AP.[49] However, CRP exists in at least two conformational and functionally distinct forms: the native pentameric CRP (pCRP) and a modified/monomeric CRP (mCRP).[50,51] The mCRP isoform induces proinflammatory responses in endothelial and innate immune cells,[52,53] is localized within retinal tissue, and exhibits binding affinity for CFH.[54] In relation to AMD CFH isoforms, binding affinity to mCRP of the non–risk-associated variant CFHY402 is stronger than the H402 variant,[55] and individuals who are homozygous for the latter show 2.5-fold higher CRP levels in the RPE–choroid layer compared with those homozygous for the nonrisk variant.[56]

Mechanistic insights into how CRP contributes to the development of AMD demonstrate that mCRP upregulates IL-8 and CCL2 levels in RPE,[57] and CFH binding to mCRP suppresses this proinflammatory activity. CFH from patients carrying the "risk" CFHY402H polymorphism displays im-

paired binding to mCRP and is unable to restrain these proinflammatory effects of CRP. Understanding the structural basis of the CFH–mCRP complex will give further insight into resolution of inflammation and novel approaches to augment these effects in patients. An additional hypothesis for our consideration is that mCRP plays a role in the inflammatory response and tissue damage at the level of RPE. Data showing impairment of RPE functionality (increased paracellular permeability through the disruption of tight junction proteins) suggest that mCRP could induce RPE barrier breakdown and promote chronic inflammation.[58]

Other genetic variants associated with AMD include CFI, CFB, and C3.[59–61] The complement hypothesis for the pathoetiology of AMD describes the notion that certain individuals carry a "complement hyperinflammatory phenotype," resulting in uncontrolled AP activation in response to cellular damage and debris in the retina.[62,63] The polymorphism rs10490924 identified in the *ARMS2* gene loci is highly associated with AMD and induces mRNA instability.[64] A recent study illuminates a role of ARMS2 as a complement activator, binding directly to apoptotic and necrotic cells via properdin complex and augmenting C3b surface opsonization for phagocytosis. Notably, ARMS2 protein appears absent in both blood monocytes and inducible pluripotent stem cells (iPS)–microglia, derived from patients homozygous for the ARMS2 AMD risk variant. One conclusion drawn is that ARMS2 is likely involved in complement-mediated clearance of cellular debris, and ARMS2 protein deficiency may therefore drive drusen formation.[65]

The complement cascade terminates in the cell surface assembly of the membrane attack complex (MAC), promoting inflammation by causing aberrant signal transduction and cell lysis. Age-related accumulation of MAC is observed in the choroid from healthy individuals, with levels significantly elevated in individuals with the high-risk CFH(H402) variant.[66,67] Thus, alterations in complement regulatory mechanisms leading to increased MAC may accelerate choroidal atrophy in early stages of disease, representing a potential trigger for subsequent inflammatory responses and AMD progression.[68,69] As the critical interface between the choroid and outer retina, the basal aspect of RPE is also a recognized site for age-related increases in MAC deposition, with higher levels reported in donor tissues from patients with risk-associated AMD genotypes.[66,67,70] In vitro, MAC assembly on the surface of RPE elevates levels of IL-6, IL-8, and MCP-1 creating a proinflammatory environment.[71] To maintain cellular integrity, intrinsic mechanisms enable the RPE to regulate formation and elimination of MAC through recycling of CD59 (MAC inhibitor), lysosome exocytosis, and endocytic pathways for MAC clearance.[72,73] Disruption of these critical homeostatic mechanisms compromises the protective responses, leading to mitochondrial damage and oxidative stress following sublytic MAC attack. Despite direct evidence this occurs in AMD, it seems plausible that oxidative stress may establish a self-propagating cycle (mitochondrial damage, inflammation, and dysfunction in the RPE). Thus, efforts to identify focused strategies to boost these innate protective mechanisms may help preserve RPE health and function.[73]

The emerging relationship of complement and its contribution to cellular metabolism machinery has generated a conceptual framework referred to as the Complosome. Complement activation regulates key metabolic pathways and thus can impact fundamental cellular processes, such as survival, proliferation, and autophagy. Newly identified functions of intracellular complement include a key role in shaping metabolic reprogramming, which underlies T-cell effector differentiation and a role as a nexus for interactions with other effector systems, including the inflammasome and the Notch transcription-factor network.[74] Therefore, if such mechanisms translate to RPE or macrophages/microglia, this emphasizes the essential role of complement activation to maintain tissue health.

## Loss of Regulatory Control: Sick Cells and Altered Immune Responses

### The "Sick" Activated Microglia

Yolk sac–derived microglia regulate tissue immune responses and are central players in many neuro-inflammatory disorders of the brain including multiple sclerosis and Alzheimer's disease (AD).[75,76] Recognized as a long-lived and self-renewing population, tissue resident microglia are distinct both in terms of origin and phenotype to that of infiltrating monocyte-derived macrophages that are recruited to the inflamed CNS from the circulation.[77–80] In the healthy CNS, microglial immune activity is restrained by dedicated immune inhibitory pathways that suppress undesirable inflammatory responses and tissue destruction that are often associated with immune activation.[81] These homeostatic checkpoint mechanisms include direct interaction of microglia with neurons through receptor–ligand pairs (CD200-CD200R and CX3CL1-CX3CR1), soluble mediators such as TGF-β, or intracellular regulators.[82] Similarly, the coordinated and divergent regulation of triggering receptor expressed on myeloid cells (TREM) signaling pathways (TREM1 versus TREM2) influence microglial inflammatory and homeostatic activity.[83] The complex functional roles of microglia are both beneficial and detrimental to disease pathogenesis, including phagocytosing/degrading toxic proteins (i.e., amyloid plaques in the brain) and promoting neurotoxicity through excessive inflammatory cytokine release. Alterations in the normal homeostatic functions such as surveillance, synaptic pruning, and plasticity may also contribute to excessive synapse loss and cognitive dysfunction in AD and other neurodegenerative diseases.[30]

The contribution of systemic immunity, recruitment of monocytes, and tissue-resident microglia to AD onset and disease progression remains controversial. To expand on this further, an understanding of fate and function gives inroads to dissecting mechanisms. In addition to classification of monocyte and macrophage subsets, fate-mapping techniques now allow us to reliably determine function and distinguish in animal models between resident microglia versus infiltrating cells (whether newly recruited from bone marrow or replacement from choroidal populations).[16,28] One concept suggests that chronic proinflammatory responses of microglia are associated with disease escalation.[84,85] Microglial priming (responding to environmental or systemic cues) and the subsequent exaggerated response of these cells to a secondary systemic trigger highlight treatment approaches for neurodegenerative diseases that target systemic disease or block signaling pathways that mediate the CNS response to systemic inflammation.[20,86] The crucial role of the host microbiome in shaping and controlling the innate immune function of microglia must also be acknowledged. Altering the composition or eradication of gut microbes results in global defects in maturation, differentiation, and functional responses of microglia, again reminding us of the important inter-relationship between the periphery and CNS in both health and disease.[87,88] This may also apply to AMD, supported by emerging data indicating that the modified intestinal microbiome composition of patients may be associated with development of neovascular disease.[89] Collectively, these concepts could equally apply to retinal microglia and explain an early event and trigger toward AMD.

However, there remains contention as to whether microglial function in neurodegenerative diseases is beneficial but insufficient or whether these cells are effective at early stages of disease but lose their efficacy? One aspect of potentially translating this to the retina and AMD is to consider current understanding in dementia. Extrapolating from other diseases reminds us that we must acknowledge the structural differences between brain and ocular tissue and that AMD occurs at the interface between a highly vascularized connective tissue, a layer of neuroepithelium, and a multilayered neural tissue. Nevertheless, increasing recognition of photoreceptor damage and reduced function is noted in AMD with loss of dark adaption.[90] Harnessing multi-omic technology and engaging in single cell RNA-seq analysis have identified a subpopulation of disease-associated microglia (DAM), which are found in the brains of AD (in both human and mouse), amyotrophic lateral sclerosis (ALS), and aged mice.[91] These cells are spatially localized in proximity to amyloid plaques and exhibit markers and activated pathways (previously associated with AD risk factors) but were not specifically attributed to microglia. To become DAM, microglia undergo an initial TREM2-independent step of activation, followed by a second step that is TREM2 dependent. Consequently, microglia lose their homeostatic gene signature and end up by upregulating genes involved in lipid metabolism and phagocytosis, eliciting a protective phenotype. In another study, the TREM2-mediated APOE signaling pathway mediates a switch from homeostatic to DAM phenotype, and targeting this pathway restored the resting signature of microglia in ALS and AD mouse models.[92] There is a tradeoff in homeostasis between the number of DAM cells with phagocytic activity and checkpoint mechanisms that keep them under tight control (e.g., CX3CR1 inhibitory signaling). Thus, checkpoint mechanisms, essential on one hand for function of microglia to ensure risk-free immune activation, may become negative factors when the threshold is altered, and a strong phagocytic activity is needed in aging or under detrimental genetic backgrounds. In this scenario, DAMs are not considered the primary disease effectors, but mediate the removal and clearance of the misfolded and aggregated proteins (drusen in AMD) that accumulate in neurodegenerative diseases and general aging induced damage.[93] Perhaps and counterintuitively, this may suggest that blocking microglia-specific checkpoints may provide a therapeutic approach to trigger the ability of resident microglia to combat neuroinflammation.

The dual functional roles of immune surveillance and tissue maintenance provided by microglia are equally important to the ocular environment. They are critical as regulators of immune health within the retina, acting as phagocytic sentinels to detect initial danger signals (DAMPs), and then rapidly activate to process both pathogens and dying cells. Equally, microglia secrete neurotrophic factors, which impact the physiology and survival of photoreceptors, contributing to the maintenance and integrity of the neuronal network and tissue function.[94] There are checkpoint regulators through ligand–receptor interactions that mediate tonic inhibitory signals maintaining the resting phenotype of resident microglia and regulate myeloid cell inflammatory and angiogenic responses. These include CD200 (expressed on neurons) and myeloid-expressing inhibitory CD200R[95] to maintain normal homeostatic control and limit immune-mediated damage and can be exploited therapeutically.[96] For example, RPE destruction in the model of laser-induced choroidal neovascular membranes (CNV) polarizes infiltrating myeloid cells toward a proangiogenic phenotype. Augmentation of the inhibitory CD200R signaling pathway or administration of Th2 cytokines could suppress macrophage activation or drive antiangiogenic function respectively.[32,97] Similarly, the CX3CR1 signaling pathway modulates cell activation and migration. With age, deficiency in the CX3CR1 receptor was considered linked to increased microglial activation, subretinal migration, and retinal degeneration,[5] supporting GWAS data that *Cx3cr1* polymorphisms are a potential risk factor for AMD. However, conflicting reports raised questions as to whether the accumulation of dysfunctional subretinal macrophages was directly attributable to retinal degeneration.[98–101] Determining genomic background differences between C57BL/6 substrains in these studies revealed the *Crb1* mutation (*rd8*), now firmly recognized to impact and enhance degenerative phenotypes in ocular inflammatory models.[101,102] Thus, subsequent studies using rd8-ve transgenic strains demonstrate that deficiency of CX3CR1 (but also CCL2-CCR2) signaling pathways differentially affects the trafficking of microglia and macrophages with age in the retina but do not cause retinal degeneration per se.[103] However, Cx3CR1 signaling does play an important role in controlling retinal inflammation, as *Cx3cr1*$^{-/-}$mice (rd8-) are susceptible to oxidative stress-induced retinal inflammation and photoreceptor loss.[104]

## An Imbalance of Physiologic Regulation

### Inflammasome Activation

The NLRP3 inflammasome complex is recognized as a sensor that monitors cellular stress through pattern recognition receptors (e.g., Toll-like receptors [TLRs]), activating inflammatory caspases.[105] Activation of NLRP3 (through either foreign or endogenous danger signals) drives caspase-1–mediated liberation of two proinflammatory cytokines, IL-1β and IL-18, and ultimately pyroptosis or apoptosis.[106] The NLRP3 response is almost certainly considered a protective response initially, providing a rapid response to danger to preserve tissue function and integrity. However, the corollary is that sustained inflammasome activation will also damage tissues, as seen in the pathogenesis of autoinflammatory disorders and implicated in AD, cancer, diabetes, and AMD.[33,107,108]

Understanding the regulatory mechanisms controlling inflammasome activation, and specifically how signals from multiple stimuli (cellular damage and stress) are integrated and processed, is essential to progress. To date, increasing evidence has highlighted how different triggers implicit for NLRP3 activation generate effector molecules that are implicated in AMD pathogenesis. Whole drusen extracts, as well as the complement component C1q, isolated from donor AMD eyes activates NLRP3 lead to the secretion of IL-1β and IL-18.[33] Moreover, carboxyethylpyrrole (CEP) proteins that accumulate in the retina in age and serve as a biomarker of AMD can also prime the macrophage inflammasome.[109] In other important studies, repetitive element-derived *Alu* RNA transcripts are recognized as an endogenous activator of the inflammasome, present in the RPE of patients with geographic atrophy (GA).[108] As noncanonical targets of DICER1-mediated enzymatic degradation, accumulation of these transcripts is associated with the loss of DICER1 expression (which might result from oxidative stress in the RPE) and functions as both priming and activating signals to stimulate NLRP3 signaling pathways.[110] Human drusen extracts induce NLRP3-dependent IL-1β secretion from lipopolysaccharide-primed peripheral blood mononuclear cells. Inflammasome-mediated IL-1β release from RPE is supported by evidence of increased *IL-1β* mRNA in RPE donor eyes with GA,[108] release following lysosomal destabilization,[111] and activation of RPE with A2E (lipofuscin) components.[112] It has also been shown that the proinflammatory cytokine IL-17A can induce IL-1β from RPE via NLRP3 activation.[113]

Although blockade of the NLRP3 inflammasome appears an attractive concept and considered the next-generation therapeutic target for dry AMD,[114] we must also consider that effectors perceived as deleterious may also have the capacity to exert protective effects within a tissue. The complexity of the underlying immunobiology should also remind us of the compelling but contrary evidence that altered immune responses (which increase with age) can also provide a capacity to maintain and preserve tissue function.[23] Evidence of NLRP3 activation and IL-18 upregulation in the RPE of human atrophic AMD donor eyes[108,111] supports a putative role for NLRP3 inflammasome in the development of atrophic disease. However, and despite controversy, it is conceivable that inflammasome activation may also exert protective effects through production of IL-18 to protect RPE and attenuate pathologic neovascularization.[115–117] The therapeutic adjuvant effects suggest IL-18 has capacity to modulate multiple pathways triggered from inherent inflammasome activation in AMD, and its expression is a mechanism to augment aspects of inflammatory responses in a bid to protect the tissue.

A recent publication, both pertinent and timely to this perspective, now requires us to re-evaluate our interpretation of the role that NLRP3 plays as the driving force behind RPE dysfunction in nonexudative AMD.[118] A robust and iterative study, it demonstrates NLRP3 is not expressed by human primary or human established RPE cell lines subjected to multiple inflammasome priming conditions or ex vivo macular RPE from AMD patients. Furthermore, it highlights the importance of validating and authenticating the specificity of commercial NLRP3 reagents. Thus, although these observations challenge current dogma, they do not fully negate the contribution that NLRP3 brings to AMD but instead suggest its influence more likely relates to the resident microglia and infiltrating immune cells.[33] Thus, further evidence is still required to characterize the presence, origin, and activation leading to secretion of the IL-1β and IL-18 effectors in AMD. Cognizant that NLRP3 is just one member of a wider NLR inflammasome family, including NLRP1, NLRC4, and AIM2 (all implicated in pathogenesis of ocular diseases), determining whether these complexes are also expressed by RPE and therefore capable of contributing to AMD pathogenesis will be important.[119] Accepting that alternative pathways may in fact drive these effectors is further emphasized in a recent study showing the link between how NLRP1 functions as an innate immune sensor in the context of metabolic stress to produce IL-18, preventing obesity and metabolic syndrome.[120]

Recent investigations of IL-33 in relation to neovascular ocular disease extends current understanding into the functional diversity of members of the IL-1 cytokine family. IL-33 is unique as it is active without caspase-1 cleavage and does not require inflammasome activation for secretion and bioactivity.[121] Monocyte recruitment, contributing to photoreceptor loss in a mouse model of retinal degeneration,[122] infers a pathogenic role of endogenous IL-33 and an a priori for neutralizing IL-33 to reduce myeloid cell accumulation as a possible intervention. However, in consideration of the emerging role of IL-33 in inflammatory disorders[123] and in the absence of progressive cell death, IL-33 also regulates tissue responses. Administration of recombinant IL-33 protects against fibrosis and CNV development.[124] IL-33 is released from activated Müller glia (without cell death), implicating inflammasome-mediated release and inactivation of IL-33.[125] As TLR-dependent upregulation of IL-33 by RPE does not influence cell viability, it is likely that this is an adaptive response to maintain homeostasis. Colocalization of IL-33 within both membrane-bound cytoplasmic vesicles and nuclear euchromatin suggests an interorganelle dynamic of IL-33 trafficking and release in the absence of cellular necrosis.[126]

### Autophagy

Despite the knowledge of risk immune response–related genotypes, these have not led to an understanding of how and when immune regulation is impaired. Whereas oxidative stress serves as a primary environmental factor that induces altered mitochondrial activity, impaired intracellular RPE processing pathways (autophagy, phagolysosome, and protein trafficking) and induction of RPE senescence[35,104,109,127] are tractable pathways that can modulate and determine immune responses. Furthermore, oxidative stress elicits a proinflammatory response in RPE cells with increased IL-1β, IL-1R1, IL-18, and IRAK-1 gene expression. Increased levels of inflammatory cytokines are observed systemically and in the eyes of patients with AMD, and when normal RPE homeostasis is perturbed (e.g., when autophagy is impaired), the regulatory intracellular IL-1 receptor–associated kinase-M (IRAK-M) is inhibited, increasing cell susceptibility to IL-1β–mediated cytotoxicity.[31]

Impaired autophagy associated with age-related degenerative disorders is highlighted by studies in which pharmacologic or genetic manipulation of autophagy pathways can induce cellular and tissue degeneration in vitro and in vivo.[128] In RPE, this leads to RPE transcytosis and exocytosis and early signs of RPE degeneration.[129] Furthermore, human RPE cultured from AMD donors is functionally impaired, demonstrating increased susceptibility to oxidative stress, increased ROS production, and reduced mitochondrial activity.[130] Therefore, regulation of homeostatic mechanisms, whether at the level of the tissue (autophagy or inflammasome activation) or the local immune network (para-inflammation), involves precise and complex signaling cascades and negative feedback mechanisms,[131] but may offer pathways for redressing ongoing degeneration. For example, the negative regulator, IRAK-M, expressed by immune and epithelial cells, serves as a key inhibitor for MyD88/NFκB-mediated inflammatory pathways, and low IRAK-M expression is associated with chronic inflammation, obesity, and metabolic syndrome or when autophagy is inhibited in RPE cells.[31,132–134]

## Future Perspectives

By its nature, a perspective cannot address all aspects of inflammation that may equally underpin AMD development and progression. Unquestionably, the primary focus for AMD research has been innate immunity as the central driver for disease, but compelling evidence also implicates adaptive immune responses. Understanding whether adaptive pathways elicit proinflammatory or regulatory functions or represent bystander effects remains limited, in part due to the lack of functional intraocular lymphatics and tangible evidence of direct B- or T-cell involvement at sites of neovascular or geographic lesions. Notwithstanding, reports confirm the presence of T-cell subsets in human choroid,[135,136] as well as the potential for interplay between innate and adaptive immunity, including increased levels of C5a[137] and elevated levels of IL-22 and IL-17.[138] Furthermore, autoantibodies (AAbs) targeting proteins involved in autophagy, immunomodulation, and protection from oxidative stress and apoptosis are detected in AMD patient sera, including during early stage disease.[139] Tantalizingly, this offers potential biomarkers for diagnosis and prognosis of AMD, but we still do not appreciate or understand whether AAbs are causative or pathogenic or simply represent secondary products generated during AMD progression. One potential hypothesis is that AAbs present early in subsets of AMD patients are the pathogenic drivers of disease. An emerging concept is that autoantibodies may create further complement-mediated damage or activate innate cells

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to switch from protective parainflammatory to pathogenic responses, but this still warrants further investigation.[139–142]

Also requiring our consideration are the factors of aging, senescence, and bioenergetics, equally important and often nascent in our exploration of the disease process. The role of the Warburg effect in the pathogenesis of metabolic disorders, such as diabetes and atherosclerosis, and its contribution to inflammatory processes relevant for disease is recognized.[143,144] The Warburg effect rapidly provides ATP and enhances metabolic pathways to support the need for increased biosynthetic demands and rapid energy production. Extrapolating from observations that tumor cells undergo a bioenergetic switch (permissive for survival and proliferation) to aerobic glycolysis, we now appreciate that such a bioenergetic switch occurs in the aging and early AMD RPE. With age, there is increasing strain on mitochondrial function, autophagy, and mitophagy to maintain cellular and tissue health. A response for the good is to divert energy sources, the Warburg effect, to maintain function against the stress. Cell senescence may also drive immune-mediated degenerative disorders such as AMD. Mitochondrial dysfunction is seen in aging cells and senescent cells, and increasing evidence highlights that a decline in mitochondrial health plays a prominent role in the pathogenesis of AMD.[145] Cybrid models indicate that mitochondrial variants mediate not only energy production but also determine the cell's ability to switch energy source, in turn impacting signaling pathways and phenotype of immune activation.[146] Furthermore, decreased mitochondrial and glycolytic function in AMD donor RPE suggests a bioenergetic crisis contributes to AMD pathology.[147]

In combination with big data and genetic determination of possible causality, unraveling the biological and molecular complexity underlying immune dysregulation also requires an understanding of the potential drivers of immune activation and at each inflection time point of disease progression. However, this is further compounded by how immune responses change with age. To this end, understanding the behavior of resident and infiltrating immune cells within tissues themselves is of growing importance. Appreciating that, although other immune-mediated ocular inflammatory animal models are not fully comparable (because the inciting disease mechanisms differ), we may still contrast the underlying cellular mechanisms and tissues responses. In the context of uveitis, the experimental autoimmune uveoretinitis (EAU) model[148] demonstrates how chronic remodeling of the tissue is associated with the persistence of inflammatory immune infiltrate.[149–153] Thus, two aspects of note that may display convergence of mechanisms to AMD: first, there is remodeling and vascular changes associated with microglial activation and macrophage infiltrate,[44,154] and second, there remains a persistent T-cell response, although assumed to be highly regulated in the chronic phases and be involved in both antibody and complement engagement.[155,156]

We now recognize that immune responses within tissues are localized and heterogenous, where only small regional subsets of immune cells adopt either inflammatory or protective phenotypes. Awareness of functional diversity (either beneficial and detrimental) exerted by microglia in tissue health and disease challenges dogma and means neurodegenerative diseases are considered the consequence of aberrations in the physiologic and homeostatic responses of these cells. Thus, targeting divergent microglial functions, thereby redressing homeostasis, will provide novel paradigms for therapies and biomarkers. Success will require us to rethink and develop new models of disease, including human iPS cells derived microglia and organoids to fully understand which findings may translate to provide microglia-targeted approaches. New molecular multi-omics techniques will enable us to further explore the concept that tissues (RPE, Müller cells, and microglia) are sick, meaning normal function and regulation are either perturbed or exaggerated. Thus, targeted therapeutic approaches through modulation of cellular bioenergetics, inflammasome activation, or autophagy pathways may serve to protect normal immunity and tissue health. Establishing what the differences in immune phenotype between normal aging and AMD are, and how the inter-relatedness of senescence and inflammation contributes to pathobiology, is integral to this approach. Altered epigenetic mechanisms and post-transcriptional control of microRNAs regulating gene expression are also known to impact the immune and tissue homeostasis in the retina.[17]

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## References

1. Miller JW, Bagheri S, Vavvas DG. Advances in age-related macular degeneration understanding and therapy. *US Ophthalmic Rev*. 2017;10:119–130.
2. Wong WL, Su X, Li X, et al. Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. *Lancet Glob Health*. 2014;2:e106–e116.
3. Miller JW. Beyond VEGF: The Weisenfeld lecture. *Invest Ophthalmol Vis Sci*. 2016;57:6911–6918.
4. Fritsche LG, Fariss RN, Stambolian D, et al. Age-related macular degeneration: genetics and biology coming together. *Annu Rev Genomics Hum Genet*. 2014;15:151–171.
5. Combadiere C, Feumi C, Raoul W, et al. CX3CR1-dependent subretinal microglia cell accumulation is associated with cardinal features of age-related macular degeneration. *J Clin Invest*. 2007;117:2920–2928.
6. Levy O, Calippe B, Lavalette S, et al. Apolipoprotein E promotes subretinal mononuclear phagocyte survival and chronic inflammation in age-related macular degeneration. *EMBO Mol Med*. 2015;7:211–226.
7. Gupta N, Brown KE, Milam AH. Activated microglia in human retinitis pigmentosa, late-onset retinal degeneration, and age-related macular degeneration. *Exp Eye Res*. 2003;76:463–471.
8. Lad EM, Cousins SW, Van Arnam JS, et al. Abundance of infiltrating CD163+ cells in the retina of postmortem eyes with dry and neovascular age-related macular degeneration. *Graefes Arch Clin Exp Ophthalmol*. 2015;253:1941–1945.
9. Cherepanoff S, McMenamin P, Gillies MC, et al. Bruch's membrane and choroidal macrophages in early and advanced age-related macular degeneration. *Br J Ophthalmol*. 2010;94:918–925.
10. McLeod DS, Bhutto I, Edwards MM, et al. Distribution and quantification of choroidal macrophages in human eyes with age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2016;57:5843–5855.
11. McLeod DS, Bhutto I, Edwards MM, et al. Mast cell-derived tryptase in geographic atrophy. *Invest Ophthalmol Vis Sci*. 2017;58:5887–5896.
12. Bhutto IA, McLeod DS, Jing T, et al. Increased choroidal mast cells and their degranulation in age-related macular degeneration. *Br J Ophthalmol*. 2016;100:720–726.
13. Tsutsumi C, Sonoda KH, Egashira K, et al. The critical role of ocular-infiltrating macrophages in the development of choroidal neovascularization. *J Leukoc Biol*. 2003;74:25–32.

14. Liu J, Copland DA, Horie S, et al. Myeloid cells expressing VEGF and arginase-1 following uptake of damaged retinal pigment epithelium suggests potential mechanism that drives the onset of choroidal angiogenesis in mice. *PLoS One*. 2013;8:e72935.
15. Cruz-Guilloty F, Saeed AM, Echegaray JJ, et al. Infiltration of proinflammatory m1 macrophages into the outer retina precedes damage in a mouse model of age-related macular degeneration. *Int J Inflammat*. 2013;2013:503725.
16. Sennlaub F, Auvynet C, Calippe B, et al. CCR2(+) monocytes infiltrate atrophic lesions in age-related macular disease and mediate photoreceptor degeneration in experimental subretinal inflammation in Cx3cr1 deficient mice. *EMBO Mol Med*. 2013;5:1775–1793.
17. Sene A, Khan AA, Cox D, et al. Impaired cholesterol efflux in senescent macrophages promotes age-related macular degeneration. *Cell Metab*. 2013;17:549–561.
18. Nathan C, Ding A. Nonresolving inflammation. *Cell*. 2010; 140:871–882.
19. Akbar AN. The convergence of senescence and nutrient sensing during lymphocyte ageing. *Clin Exp Immunol*. 2017;187:4–5.
20. Perry VH, Holmes C. Microglial priming in neurodegenerative disease. *Nat Rev Neurol*. 2014;10:217–224.
21. Franceschi C, Campisi J. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. *J Gerontol A Biol Sci Med Sci*. 2014;69(suppl 1):S4–S9.
22. Medzhitov R. Origin and physiological roles of inflammation. *Nature*. 2008;454:428–435.
23. Xu H, Chen M, Forrester JV. Para-inflammation in the aging retina. *Prog Retin Eye Res*. 2009;28:348–368.
24. Okin D, Medzhitov R. Evolution of inflammatory diseases. *Curr Biol*. 2012;22:R733–R740.
25. Saban DR. New concepts in macrophage ontogeny in the adult neural retina [published online ahead of print April 22, 2018]. *Cell Immunol*. https://doi.org/10.1016/j.cellimm.2018.04.008.
26. Geissmann F, Gordon S, Hume DA, et al. Unravelling mononuclear phagocyte heterogeneity. *Nat Rev Immunol*. 2010;10:453–460.
27. Xu H, Chen M, Mayer EJ, et al. Turnover of resident retinal microglia in the normal adult mouse. *Glia*. 2007;55:1189–1198.
28. O'Koren EG, Mathew R, Saban DR. Fate mapping reveals that microglia and recruited monocyte-derived macrophages are definitively distinguishable by phenotype in the retina. *Sci Rep*. 2016;6:20636.
29. Reyes NJ, O'Koren EG, Saban DR. New insights into mononuclear phagocyte biology from the visual system. *Nat Rev Immunol*. 2017;17:322–332.
30. Salter MW, Stevens B. Microglia emerge as central players in brain disease. *Nat Med*. 2017;23:1018–1027.
31. Liu J, Copland DA, Theodoropoulou S, et al. Impairing autophagy in retinal pigment epithelium leads to inflammasome activation and enhanced macrophage-mediated angiogenesis. *Sci Rep*. 2016;6:20639.
32. Wu WK, Georgiadis A, Copland DA, et al. IL-4 regulates specific Arg-1(+) macrophage sFlt-1-mediated inhibition of angiogenesis. *Am J Pathol*. 2015;185:2324–2335.
33. Doyle SL, Campbell M, Ozaki E, et al. NLRP3 has a protective role in age-related macular degeneration through the induction of IL-18 by drusen components. *Nat Med*. 2012; 18:791–798.
34. Ambati J, Atkinson JP, Gelfand BD. Immunology of age-related macular degeneration. *Nat Rev Immunol*. 2013;13: 438–451.
35. Fritsche LG, Igl W, Bailey JN, et al. A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants. *Nat Genet*. 2016;48:134–143.
36. Klein RJ, Zeiss C, Chew EY, et al. Complement factor H polymorphism in age-related macular degeneration. *Science*. 2005;308:385–389.
37. Edwards AO, Ritter R III, Abel KJ, et al. Complement factor H polymorphism and age-related macular degeneration. *Science*. 2005;308:421–424.
38. Haines JL, Hauser MA, Schmidt S, et al. Complement factor H variant increases the risk of age-related macular degeneration. *Science*. 2005;308:419–421.
39. Hageman GS, Anderson DH, Johnson LV, et al. 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*. 2005;102:7227–7232.
40. Langford-Smith A, Keenan TD, Clark SJ, et al. The role of complement in age-related macular degeneration: heparan sulphate, a ZIP code for complement factor H? *J Innate Immun*. 2014;6:407–416.
41. Calippe B, Augustin S, Beguier F, et al. Complement factor H inhibits CD47-mediated resolution of inflammation. *Immunity*. 2017;46:261–272.
42. Levy O, Lavalette S, Hu SJ, et al. APOE isoforms control pathogenic subretinal inflammation in age-related macular degeneration. *J Neurosci*. 2015;35:13568–13576.
43. Toomey CB, Kelly U, Saban DR, et al. Regulation of age-related macular degeneration-like pathology by complement factor H. *Proc Natl Acad Sci U S A*. 2015;112:E3040–E3049.
44. Chen M, Copland DA, Zhao J, et al. Persistent inflammation subverts thrombospondin-1-induced regulation of retinal angiogenesis and is driven by CCR2 ligation. *Am J Pathol*. 2012;180:235–245.
45. Wang S, Sorenson CM, Sheibani N. Lack of thrombospondin 1 and exacerbation of choroidal neovascularization. *Arch Ophthalmol*. 2012;130:615–620.
46. Ng TF, Turpie B, Masli S. Thrombospondin-1-mediated regulation of microglia activation after retinal injury. *Invest Ophthalmol Vis Sci*. 2009;50:5472–5478.
47. Seddon JM, George S, Rosner B, et al. Progression of age-related macular degeneration: prospective assessment of C-reactive protein, interleukin 6, and other cardiovascular biomarkers. *Arch Ophthalmol*. 2005;123:774–782.
48. Anderson DH, Mullins RF, Hageman GS, et al. A role for local inflammation in the formation of drusen in the aging eye. *Am J Ophthalmol*. 2002;134:411–431.
49. Black S, Kushner I, Samols D. C-reactive protein. *J Biol Chem*. 2004;279:48487–48490.
50. Wu Y, Potempa LA, El Kebir D, et al. C-reactive protein and inflammation: conformational changes affect function. *Biol Chem*. 2015;396:1181–1197.
51. Ji SR, Wu Y, Zhu L, et al. Cell membranes and liposomes dissociate C-reactive protein (CRP) to form a new, biologically active structural intermediate: mCRP(m). *FASEB J*. 2007;21:284–294.
52. Vilahur G, Hernandez-Vera R, Molins B, et al. Short-term myocardial ischemia induces cardiac modified C-reactive protein expression and proinflammatory gene (cyclo-oxygenase-2, monocyte chemoattractant protein-1, and tissue factor) upregulation in peripheral blood mononuclear cells. *J Thromb Haemost*. 2009;7:485–493.
53. Khreiss T, Jozsef L, Potempa LA, et al. Conformational rearrangement in C-reactive protein is required for proinflammatory actions on human endothelial cells. *Circulation*. 2004;109:2016–2022.

54. Hakobyan S, Harris CL, van den Berg CW, et al. Complement factor H binds to denatured rather than to native pentameric C-reactive protein. *J Biol Chem*. 2008;283:30451–30460.
55. Laine M, Jarva H, Seitsonen S, et al. Y402H polymorphism of complement factor H affects binding affinity to C-reactive protein. *J Immunol*. 2007;178:3831–3836.
56. Johnson PT, Betts KE, Radeke MJ, et al. Individuals homozygous for the age-related macular degeneration risk-conferring variant of complement factor H have elevated levels of CRP in the choroid. *Proc Natl Acad Sci U S A*. 2006; 103:17456–17461.
57. Molins B, Fuentes-Prior P, Adan A, et al. Complement factor H binding of monomeric C-reactive protein downregulates proinflammatory activity and is impaired with at risk polymorphic CFH variants. *Sci Rep*. 2016;6:622889.
58. Molins B, Pascual A, Mendez, et al. C-reactive protein isoforms differentially affect outer blood-retinal barrier integrity and function. *Am J Physiol Cell Physiol*. 2017; 312:C244–C253.
59. van de Ven JP, Nilsson SC, Tan PL, et al. A functional variant in the CFI gene confers a high risk of age-related macular degeneration. *Nat Genet*. 2013;45:813–817.
60. Montes T, Tortajada A, Morgan BP, et al. Functional basis of protection against age-related macular degeneration conferred by a common polymorphism in complement factor B. *Proc Natl Acad Sci U S A*. 2009;106:4366–4371.
61. Zhan X, Larson DE, Wang C, et al. Identification of a rare coding variant in complement 3 associated with age-related macular degeneration. *Nat Genet*. 2013;45:1375–1379.
62. Tuo J, Grob S, Zhang K, et al. Genetics of immunological and inflammatory components in age-related macular degeneration. *Ocul Immunol Inflamm*. 2012;20:27–36.
63. Lachmann PJ. The amplification loop of the complement pathways. *Adv Immunol*. 2009;104:115–149.
64. Fritsche LG, Loenhardt T, Janssen A, et al. Age-related macular degeneration is associated with an unstable ARMS2 (LOC387715) mRNA. *Nat Genet*. 2008;40:892–896.
65. Micklisch S, Lin Y, Jacob S, et al. Age-related macular degeneration associated polymorphism rs10490924 in ARMS2 results in deficiency of a complement activator. *J Neuroinflammation*. 2017;14:4.
66. Mullins RF, Schoo DP, Sohn EH, et al. The membrane attack complex in aging human choriocapillaris: relationship to macular degeneration and choroidal thinning. *Am J Pathol*. 2014;184:3142–3153.
67. Mullins RF, Dewald AD, Streb LM, et al. Elevated membrane attack complex in human choroid with high risk complement factor H genotypes. *Exp Eye Res*. 2011;93:565–567.
68. Chirco KR, Sohn EH, Stone EM, et al. Structural and molecular changes in the aging choroid: implications for age-related macular degeneration. *Eye (Lond)*. 2017;31:10–25.
69. Whitmore SS, Sohn EH, Chirco KR, et al. Complement activation and choriocapillaris loss in early AMD: implications for pathophysiology and therapy. *Prog Retin Eye Res*. 2015;45:1–29.
70. Seth A, Cui J, To E, et al. Complement-associated deposits in the human retina. *Invest Ophthalmol Vis Sci*. 2008;49:743–750.
71. Lueck K, Wasmuth S, Williams J, et al. Sub-lytic C5b-9 induces functional changes in retinal pigment epithelial cells consistent with age-related macular degeneration. *Eye (Lond)*. 2011;25:1074–1082.
72. Georgiannakis A, Burgoyne T, Lueck K, et al. Retinal pigment epithelial cells mitigate the effects of complement attack by endocytosis of C5b-9. *J Immunol*. 2015;195:3382–3389.
73. Tan LX, Toops KA, Lakkaraju A. Protective responses to sublytic complement in the retinal pigment epithelium. *Proc Natl Acad Sci U S A*. 2016;113:8789–8794.
74. Hess C, Kemper C. Complement-mediated regulation of metabolism and basic cellular processes. *Immunity*. 2016; 45:240–254.
75. Matcovitch-Natan O, Winter DR, Giladi A, et al. Microglia development follows a stepwise program to regulate brain homeostasis [published online ahead of print August 19, 2016]. *Science*. https://doi.org/10.1126/science.aad8670.
76. Ginhoux F, Greter M, Leboeuf M, et al. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science*. 2010;330:841–845.
77. Shemer A, Jung S. Differential roles of resident microglia and infiltrating monocytes in murine CNS autoimmunity. *Semin Immunopathol*. 2015;37:613–623.
78. Ginhoux F, Prinz M. Origin of microglia: current concepts and past controversies. *Cold Spring Harb Perspect Biol*. 2015;7:a020537.
79. Ajami B, Bennett JL, Krieger C, et al. Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. *Nat Neurosci*. 2007;10:1538–1543.
80. Prinz M, Priller J, Sisodia SS, et al. Heterogeneity of CNS myeloid cells and their roles in neurodegeneration. *Nat Neurosci*. 2011;14:1227–1235.
81. Hanisch UK, Kettenmann H. Microglia: active sensor and versatile effector cells in the normal and pathologic brain. *Nat Neurosci*. 2007;10:1387–1394.
82. Ransohoff RM, Cardona AE. The myeloid cells of the central nervous system parenchyma. *Nature*. 2010;468:253–262.
83. Owens R, Grabert K, Davies CL, et al. Divergent neuroinflammatory regulation of microglial TREM expression and involvement of NF-kappaB. *Front Cell Neurosci*. 2017;11:56.
84. Heppner FL, Ransohoff RM, Becher B. Immune attack: the role of inflammation in Alzheimer disease. *Nat Rev Neurosci*. 2015;16:358–372.
85. Yamasaki R, Lu H, Butovsky O, et al. Differential roles of microglia and monocytes in the inflamed central nervous system. *J Exp Med*. 2014;211:1533–1549.
86. Perry VH, Teeling J. Microglia and macrophages of the central nervous system: the contribution of microglia priming and systemic inflammation to chronic neurodegeneration. *Semin Immunopathol*. 2013;35:601–612.
87. Erny D, Hrabe de Angelis AL, Jaitin D, et al. Host microbiota constantly control maturation and function of microglia in the CNS. *Nat Neurosci*. 2015;18:965–977.
88. Erny D, Hrabe de Angelis AL, Prinz M. Communicating systems in the body: how microbiota and microglia cooperate. *Immunology*. 2017;150:7–15.
89. Zinkernagel MS, Zysset-Burri DC, Keller I, et al. Association of the intestinal microbiome with the development of neovascular age-related macular degeneration. *Sci Rep*. 2017;7:40826.
90. Owsley C, McGwin G Jr, Clark ME, et al. Delayed rod-mediated dark adaptation is a functional biomarker for incident early age-related macular degeneration. *Ophthalmology*. 2016;123:344–351.
91. Keren-Shaul H, Spinrad A, Weiner A, et al. A unique microglia type associated with restricting development of Alzheimer's disease. *Cell*. 2017;169:1276–1290.
92. Krasemann S, Madore C, Cialic R, et al. The TREM2-APOE pathway drives the transcriptional phenotype of dysfunctional microglia in neurodegenerative diseases. *Immunity*. 2017;47:566–581.
93. Yerbury JJ, Ooi L, Dillin A, et al. Walking the tightrope: proteostasis and neurodegenerative disease. *J Neurochem*. 2016;137:489–505.


Investigative Ophthalmology & Visual Science

94. Wang X, Zhao L, Zhang J, et al. Requirement for microglia for the maintenance of synaptic function and integrity in the mature retina. *J Neurosci*. 2016;36:2827–2842.
95. Broderick C, Hoek RM, Forrester JV, et al. Constitutive retinal CD200 expression regulates resident microglia and activation state of inflammatory cells during experimental autoimmune uveoretinitis. *Am J Pathol*. 2002;161:1669–1677.
96. Copland DA, Calder CJ, Raveney BJ, et al. Monoclonal antibody-mediated CD200 receptor signaling suppresses macrophage activation and tissue damage in experimental autoimmune uveoretinitis. *Am J Pathol*. 2007;171:580–588.
97. Horie S, Robbie SJ, Liu J, et al. CD200R signaling inhibits pro-angiogenic gene expression by macrophages and suppresses choroidal neovascularization [published online ahead of print October 30, 2013]. *Sci Rep*. https://doi.org/10.1038/srep03072.98.
98. Ambati J, Anand A, Fernandez S, et al. An animal model of age-related macular degeneration in senescent Ccl-2- or Ccr-2-deficient mice. *Nat Med*. 2003;9:1390–1397.
99. Chen M, Forrester JV, Xu H. Dysregulation in retinal para-inflammation and age-related retinal degeneration in CCL2 or CCR2 deficient mice. *PLoS One*. 2011;6:e22818.
100. Chinnery HR, McLenachan S, Humphries T, et al. Accumulation of murine subretinal macrophages: effects of age, pigmentation and CX3CR1. *Neurobiol Aging*. 2012;33:1769–1776.
101. Luhmann UF, Lange CA, Robbie S, et al. Differential modulation of retinal degeneration by Ccl2 and Cx3cr1 chemokine signalling. *PLoS One*. 2012;7:e35551.
102. Mattapallil MJ, Wawrousek EF, Chan CC, et al. The Rd8 mutation of the Crb1 gene is present in vendor lines of C57BL/6N mice and embryonic stem cells, and confounds ocular induced mutant phenotypes. *Invest Ophthalmol Vis Sci*. 2012;53:2921–2927.
103. Luhmann UF, Carvalho LS, Robbie SJ, et al. Ccl2, Cx3cr1 and Ccl2/Cx3cr1 chemokine deficiencies are not sufficient to cause age-related retinal degeneration. *Exp Eye Res*. 2013;107:80–87.
104. Chen M, Luo C, Penalva R, et al. Paraquat-induced retinal degeneration is exaggerated in CX3CR1-deficient mice and is associated with increased retinal inflammation. *Invest Ophthalmol Vis Sci*. 2013;54:682–690.
105. Lamkanfi M. Emerging inflammasome effector mechanisms. *Nat Rev Immunol*. 2011;11:213–220.
106. Gross O, Thomas CJ, Guarda G, et al. The inflammasome: an integrated view. *Immunol Rev*. 2011;243:136–151.
107. Mankan AK, Kubarenko A, Hornung V. Immunology in clinic review series: focus on autoinflammatory diseases: inflammasomes: mechanisms of activation. *Clin Exp Immunol*. 2012;167:369–381.
108. Tarallo V, Hirano Y, Gelfand BD, et al. DICER1 loss and Alu RNA induce age-related macular degeneration via the NLRP3 inflammasome and MyD88. *Cell*. 2012;149:847–859.
109. Hollyfield JG, Bonilha VL, Rayborn ME, et al. Oxidative damage-induced inflammation initiates age-related macular degeneration. *Nat Med*. 2008;14:194–198.
110. Kaneko H, Dridi S, Tarallo V, et al. DICER1 deficit induces Alu RNA toxicity in age-related macular degeneration. *Nature*. 2011;471:325–330.
111. Tseng WA, Thein T, Kinnunen K, et al. NLRP3 inflammasome activation in retinal pigment epithelial cells by lysosomal destabilization: implications for age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2013;54:110–120.
112. Anderson OA, Finkelstein A, Shima DT. A2E induces IL-1ss production in retinal pigment epithelial cells via the NLRP3 inflammasome. *PLoS One*. 2013;8:e67263.
113. Zhang S, Yu N, Zhang R, et al. Interleukin-17A induces IL-1beta secretion from RPE cells via the NLRP3 inflammasome. *Invest Ophthalmol Vis Sci*. 2016;57:312–319.
114. Marneros AG. NLRP3 inflammasome blockade inhibits VEGF-A-induced age-related macular degeneration. *Cell Rep*. 2013;4:945–958.
115. Doyle SL, Ozaki E, Brennan K, et al. IL-18 attenuates experimental choroidal neovascularization as a potential therapy for wet age-related macular degeneration. *Sci Transl Med*. 2014;6: 230ra44.
116. Doyle SL, Lopez FJ, Celkova L, et al. IL-18 Immunotherapy for neovascular AMD: tolerability and efficacy in nonhuman primates. *Invest Ophthalmol Vis Sci*. 2015;56:5424–5430.
117. Campbell M, Doyle S, Humphries P. IL-18: a new player in immunotherapy for age-related macular degeneration? *Exp Rev Clin Immunol*. 2014;10:1273–1275.
118. Kosmidou C, Efstathiou NE, Hoang MV, et al. Issues with the specificity of immunological reagents for NLRP3: implications for age-related macular degeneration. *Sci Rep*. 2018;8:461.
119. Yerramothu P, Vijay AK, Willcox MDP. Inflammasomes, the eye and anti-inflammasome therapy. *Eye (Lond)*. 2018;32:491–505.
120. Murphy AJ, Kraakman MJ, Kammoun HL, et al. IL-18 Production from the NLRP1 inflammasome prevents obesity and metabolic syndrome. *Cell Metab*. 2016;23:155–164.
121. Cayrol C, Girard JP. The IL-1-like cytokine IL-33 is inactivated after maturation by caspase-1. *Proc Natl Acad Sci U S A*. 2009;106:9021–9026.
122. Xi H, Katschke KJ Jr, Li Y, et al. IL-33 amplifies an innate immune response in the degenerating retina. *J Exp Med*. 2016;213:189–207.
123. Liew FY, Pitman NI, McInnes IB. Disease-associated functions of IL-33: the new kid in the IL-1 family. *Nat Rev Immunol*. 2010;10:103–110.
124. Theodoropoulou S, Copland DA, Liu J, et al. Interleukin-33 regulates tissue remodelling and inhibits angiogenesis in the eye. *J Pathol*. 2017;241:45–56.
125. Cayrol C, Girard JP. The IL-1-like cytokine IL-33 is inactivated after maturation by caspase-1. *Proc Natl Acad Sci U S A*. 2009;106:9021–9026.
126. Kakkar R, Hei H, Dobner S, et al. Interleukin 33 as a mechanically responsive cytokine secreted by living cells. *J Biol Chem*. 2012;287:6941–6948.
127. Marazita MC, Dugour A, Marquioni-Ramella MD, et al. Oxidative stress-induced premature senescence dysregulates VEGF and CFH expression in retinal pigment epithelial cells: implications for age-related macular degeneration. *Redox Biol*. 2016;7:78–87.
128. Mizushima N, Yoshimori T, Levine B. Methods in mammalian autophagy research. *Cell*. 2010;140:313–326.
129. Kaarniranta K, Sinha D, Blasiak J, et al. Autophagy and heterophagy dysregulation leads to retinal pigment epithelium dysfunction and development of age-related macular degeneration. *Autophagy*. 2013;9:973–984.
130. Golestaneh N, Chu Y, Xiao YY, et al. Dysfunctional autophagy in RPE, a contributing factor in age-related macular degeneration. *Cell Death Dis*. 2017;8:e2537.
131. Medzhitov R, Horng T. Transcriptional control of the inflammatory response. *Nat Rev Immunol*. 2009;9:692–703.
132. Jain A, Kaczanowska S, Davila E. IL-1 receptor-associated kinase signaling and its role in inflammation, cancer progression, and therapy resistance. *Front Immunol*. 2014;5:553.
133. Miyata M, Lee JY, Susuki-Miyata S, et al. Glucocorticoids suppress inflammation via the upregulation of negative regulator IRAK-M. *Nat Commun*. 2015;6:6062.

134. Geng S, Chen K, Yuan R, et al. The persistence of low-grade inflammatory monocytes contributes to aggravated atherosclerosis. *Nat Commun*. 2016;7:13436.
135. Zhao Z, Liang Y, Liu Y, et al. Choroidal gammadelta T cells in protection against retinal pigment epithelium and retinal injury. *FASEB J*. 2017;31:4903–4916.
136. Ezzat MK, Hann CR, Vuk-Pavlovic S, et al. Immune cells in the human choroid. *Br J Ophthalmol*. 2008;92:976–980.
137. Scholl HP, Charbel Issa P, Walier M, et al. Systemic complement activation in age-related macular degeneration. *PLoS One*. 2008;3:e2593.
138. Liu B, Wei L, Meyerle C, et al. Complement component C5a promotes expression of IL-22 and IL-17 from human T cells and its implication in age-related macular degeneration. *J Transl Med*. 2011;9:1–12.
139. Iannaccone A, Giorgianni F, New DD, et al. Circulating autoantibodies in age-related macular degeneration recognize human macular tissue antigens implicated in autophagy, immunomodulation, and protection from oxidative stress and apoptosis. *PLoS One*. 2015;10:e0145323.
140. Crabb JW, Miyagi M, Gu X, et al. Drusen proteome analysis: an approach to the etiology of age-related macular degeneration. *Proc Natl Acad Sci U S A*. 2002;99:14682–14687.
141. Gu X, Meer SG, Miyagi M, et al. Carboxyethylpyrrole protein adducts and autoantibodies, biomarkers for age-related macular degeneration. *J Biol Chem*. 2003;278:42027–42035.
142. Gu J, Pauer GJ, Yue X, et al. Assessing susceptibility to age-related macular degeneration with proteomic and genomic biomarkers. *Mol Cell Proteomics*. 2009;8:1338–1349.
143. Palsson-McDermott EM, O'Neill LA. The Warburg effect then and now: from cancer to inflammatory diseases. *Bioessays*. 2013;35:965–973.
144. Wen H, Ting JP, O'Neill LA. A role for the NLRP3 inflammasome in metabolic diseases: did Warburg miss inflammation? *Nat Immunol*. 2012;13:352–357.
145. Udar N, Atilano SR, Memarzadeh M, et al. Mitochondrial DNA haplogroups associated with age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2009;50:2966–2974.
146. Kenney MC, Chwa M, Atilano SR, et al. Mitochondrial DNA variants mediate energy production and expression levels for CFH, C3 and EFEMP1 genes: implications for age-related macular degeneration. *PLoS One*. 2013;8:e54339.
147. Ferrington DA, Ebeling MC, Kapphahn RJ, et al. Altered bioenergetics and enhanced resistance to oxidative stress in human retinal pigment epithelial cells from donors with age-related macular degeneration. *Redox Biol*. 2017;13:255–265.
148. Prendergast RA, Iliff CE, Coskuncan NM, et al. T cell traffic and the inflammatory response in experimental autoimmune uveoretinitis. *Invest Ophthalmol Vis Sci*. 1998;39:754–762.
149. Kerr EC, Raveney BJ, Copland DA, et al. Analysis of retinal cellular infiltrate in experimental autoimmune uveoretinitis reveals multiple regulatory cell populations. *J Autoimmun*. 2008;31:354–361.
150. Copland DA, Wertheim MS, Armitage WJ, et al. The clinical time-course of experimental autoimmune uveoretinitis using topical endoscopic fundal imaging with histologic and cellular infiltrate correlation. *Invest Ophthalmol Vis Sci*. 2008;49:5458–5465.
151. Chu CJ, Herrmann P, Carvalho LS, et al. Assessment and in vivo scoring of murine experimental autoimmune uveoretinitis using optical coherence tomography. *PLoS One*. 2013;8:e63002.
152. Chen X, Kezic JM, Forrester JV, et al. In vivo multi-modal imaging of experimental autoimmune uveoretinitis in transgenic reporter mice reveals the dynamic nature of inflammatory changes during disease progression. *J Neuroinflamm*. 2015;12:17.
153. Kielczewski JL, Horai R, Jittayasothorn Y, et al. Tertiary lymphoid tissue forms in retinas of mice with spontaneous autoimmune uveitis and has consequences on visual function. *J Immunol*. 2016;196:1013–1025.
154. London A, Benhar I, Mattapallil MJ, et al. Functional macrophage heterogeneity in a mouse model of autoimmune central nervous system pathology. *J Immunol*. 2013;190:3570–3578.
155. Boldison J, Chu CJ, Copland DA, et al. Tissue-resident exhausted effector memory CD8+ T cells accumulate in the retina during chronic experimental autoimmune uveoretinitis. *J Immunol*. 2014;192:4541–4550.
156. Zhou R, Horai R, Silver PB, et al. The living eye "disarms" uncommitted autoreactive T cells by converting them to Foxp3(+) regulatory cells following local antigen recognition. *J Immunol*. 2012;188:1742–1750.

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