Parainflammation, chronic inflammation, and age-related macular degeneration

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ABSTRACT

Inflammation is an adaptive response of the immune system to noxious insults to maintain homeostasis and restore functionality. The retina is considered an immune-privileged tissue as a result of its unique anatomic and physiologic properties. During aging, the retina suffers from a low-grade chronic oxidative insult, which sustains for decades and increases in level with advancing age. As a result, the retinal innate-immune system, particularly microglia and the complement system, undergoes low levels of activation (parainflammation). In many cases, this parainflammatory response can maintain homeostasis in the healthy aging eye. However, in patients with age-related macular degeneration, this parainflammatory response becomes dysregulated and contributes to macular damage. Factors contributing to the dysregulation of age-related retinal parainflammation include genetic predisposition, environmental risk factors, and old age.

Dysregulated parainflammation (chronic inflammation) in age-related macular degeneration damages the blood retina barrier, resulting in the breach of retinal-immune privilege, leading to the development of retinal lesions. This review discusses the basic principles of retinal innate-immune responses to endogenous chronic insults in normal aging and in age-related macular degeneration and explores the difference between beneficial parainflammation and the detrimental chronic inflammation in the context of age-related macular degeneration.


Introduction

The central role of the immune system is to protect the host from exogenous and endogenous insults and to maintain tissue homeostasis. Dysfunction or dysregulation of the immune system may lead to various immune-related diseases, such as infection and autoimmune disorders. In addition to the classic inflammatory diseases, compelling evidence suggests that a low-grade chronic inflammation contributes critically to many human diseases that were previously not considered as inflammatory disorders, including obesity [1, 2], atherosclerosis [2–4], and various neurodegenerative disorders [3, 5–8]. It is now clear that chronic inflammation is involved in almost all age-related degenerative diseases, including those that occur in “immune-privileged” tissues, such as the brain (e.g., Alzheimer disease and Parkinson disease) [3, 5, 8] and the retina (e.g., AMD) [9, 10]. Aging involves the accumulation of oxidative insults, and the initial triggers for age-related degenerative diseases are believed to be the oxidative damage. Inflammation is secondary to the tissue damage and is thought to be part of a protective response of the immune system. Why this protective response becomes detrimental has been a puzzle for many years.

In 2008, Medzhitov [11] discussed the origin of inflammation in his essay published in Nature, where he further extended the concept of the “danger theory” of inflammation. He suggests that between basal homeostatic conditions and true inflammation, a “parainflammation” state exists [11]. Parainflammation is an adaptive response of the immune system to low levels of tissue stress (i.e., a low-degree of “danger” stimuli), such as in aging, whereby oxidative stress accumulates bit by bit for many decades. The physiologic role of parainflammation is to maintain homeostasis (or reset the homeostatic threshold of the tissue) and restore tissue functionality [11]. This parainflammation theory helps to explain many phenomena observed in various chronic disease conditions, an example of which is “inflamming” [12, 13]. This concept centers on a well-controlled parainflammation, which is beneficial and dysregulated parainflammation that is detrimental. A lot of studies since have focused on how the parainflammatory response becomes dysregulated in disease conditions. In this review, we will discuss parainflammation in the aging eye and will present our understanding, based on published data from us and others, on how parainflammation is dysregulated in AMD, a sight-threatening disease that affects >170 million people worldwide [14].

Abbreviations: AGE = advanced glycation end-product, AMD = age-related macular degeneration, AP = alternative pathway (of the complement system), BM = Bruch’s membrane, BRB = blood retina barrier, CFB/H/I = complement factor B/H/I, Crb1 = Crumbs homolog 1, CRP = C-reactive protein, DAF = decay-acceleration factor, DAMP = danger-associated molecular pattern, DTR = diphtheria toxin receptor, FasL = Fas ligand, GA = geographic atrophy, (continued on next page)

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AMD
AMD is a disease involving progressive degeneration of the macula, the central part of the neuroretina, in the elderly (Fig. 1A). In Western nations, ~22% of people >70 yr and 34%, >80 yr may suffer from AMD in at least one eye [14]. With demographic shifts and trends toward increasing longevity in the developing world, the number of people suffering from AMD is projected to reach 190 million in 2020 and 288 million in 2040 [14].

The pathologies of AMD are restricted to the retina-choroid interface of the macula [15, 16] (Fig. 1). The macula, in particular, the fovea, has a unique structure, whereby cone cells constitute the majority of photoreceptors, and no blood vessels are present (Fig. 1B). Nutrients and oxygen are supplied to the macula by choroidal circulation through BM and a monolayer of RPE cells, which form the oBRB (Fig. 1B). The metabolic waste materials of the retina are disposed through the RPE/BM to the choroid and then removed by choroidal macrophages or the choroidal circulation (Fig. 1B). During aging, 2 processes contribute to macular damage: 1) the thickness of the BM increases, and permeability decreases [17], and 2) the RPE function declines [18, 19], and the density of chorocapillaris is reduced [17]. During the early stages of AMD, there is an accumulation of extracellular deposits, called “drusen,” between the RPE and BM, consisting of various lipid-, carbohydrate-, and protein-rich debris (Fig. 1C) [20]. AMD can progress into 2 late sight-threatening stages: GA (or dry AMD; Fig. 1D) and nAMD (or wet AMD; Fig. 1E). GA is characterized by the death of RPE and photoreceptors, whereas nAMD is typified by the growth of abnormal blood vessels into the sub-RPE or subretinal space (Fig. 1D and E). GA and nAMD are not mutually exclusive, ~12% of AMD patients may develop both GA and nAMD [21, 22], and GA often develops in nAMD eyes following anti-VEGF therapy [23–25].

AMD is a multifactorial disease, and old age and environmental and genetic risk factors all contribute to disease pathogenesis [15, 26]. Exactly how these multiple factors cause macular damage is poorly understood. Whereas we appreciate that multiple pathways may contribute to macular damage, it is now clear that inflammation plays a major role in AMD pathogenesis [10, 26, 27]. Evidence supporting the role of inflammation in AMD includes the following: 1) inflammatory molecules, including vitronectin, amyloid A/P, Factor X, prothrombin, and in some instances, Ig, HLA-DR, and complement proteins (C3, C5, C5b-9, CFH, and CRP) have been detected in drusen, the hallmark of early AMD [9]; 2) immune cells, including macrophages, lymphocytes, and mast cells, have been detected in AMD lesions or the choroid adjacent to macular lesions [28, 29]; 3) polymorphisms of various immune-related genes, such as CFH, C2/CFB, C5, CXcr1, and TLR3/4, are associated with AMD risk (reviewed by Tuo et al. [30]); and 4) AMD-like lesions can be modeled in experimental animals by manipulating immune-related genes [31–35]. The question is why this “protective” response becomes detrimental in AMD. To address this question, it is essential to review the basic principle of the immune response to age-related chronic insults and how the immune system uses the principle to protect the eye, particularly under aging conditions.

INFLAMMATION—AN ADAPTIVE RESPONSE TO TISSUE STRESS

Inflammation is an adaptive response to tissue stress [11, 36]. The response can occur at 3 levels: tissue cells, the immune system of local tissue, and the systemic immune system. At the tissue level, when cells suffer from chronic noxious insults (e.g., a change of microenvironmental parameters, such as temperature, nutrients, oxygen, and growth factors), and the insults are not strong enough to cause cell death, a cell-autonomous response, including the up-regulation of heat shock proteins [37–39] and the activation of the autophagy pathways [40–43], may ensue. The purpose of the cell-autonomous response is to repair the damage so that the cells can return to basal homeostasis. The cell-autonomous response can also result in the production of inflammatory cytokines and chemokines; therefore, this represents a tissue cell-autonomous inflammatory response. The subtle change in microenvironment related to the cell-autonomous response is monitored by the immune system of the tissue, such as resident macrophages and the complement system, which in turn, may release cytokines and growth factors to promote further the repair/recuperation of stressed cells. The difference between the cell-autonomous response and the response of local immune cells is that the former promotes the survival of self, whereas the latter assists in the survival of other cells and ensures the integrity of tissue structure and functionalities. If the damage is restricted to a limited number of cells, and the insult is transient, tissue repair can be achieved with minimal disturbance of the local or the systemic immune system (“minimal inflammation”).

When more cells or the whole tissue are stressed, and/or the insult persists for a sustained period of time, such as in aging, the autonomous response may not be able to return stressed cells to healthy, where they may undergo senescence or even death. Senescent cells can secrete a number of proinflammatory cytokines and chemokines, a phenomenon known as SASP [44]. Examples of SASP-associated factors include cytokine, such as IL-6, IL-8, TNF-α, and IL-1α/β; chemokine MCP-1/2 and CX3CL1 [45]; IGF/IGFR [46]; and CSFs (G-CSF and GM-CSF) [47, 48]. These proinflammatory mediators further stimulate resident macrophages and the tissue complement system (local tissue inflammation), promoting tissue repair and remodeling to maintain homeostasis or reset the threshold of homeostasis and restore functionality. If the level of tissue stress exceeds the reparatory capacity of resident macrophages, then they may release additional cytokines and chemokines to recruit circulating monocytes [36]. When tissue factors are released into the circulation, they may activate the systemic immune system (systemic inflammation). The stress may also initiate other
innate-immune pathways, such as the complement pathway to promote tissue repair/remodeling. This adaptive response of the innate-immune system to tissue malfunction was called parainflammation by Medzhitov [11]. The physiologic purpose of the parainflammatory response is to help the tissue to adapt to the stressful conditions and to restore functionality [11].

When tissues suffer from acute insults that cause substantial necrotic cell death, overt inflammation may ensue. Dead cells may release large amounts of inflammatory stimuli, such as uric acid [49], high-mobility group box 1 protein [50–52], and S100B [53], resulting in the aggressive activation of resident immune macrophages, as well as recruitment of circulating leukocytes, typically neutrophils and macrophages.

An inflammatory response requires 4 elements: the inducers, sensors, mediators, and effectors [36]. According to the “danger model” of inflammation [54], the inducers are the danger molecules (exogenous and endogenous DAMPs), whereas the sensors are the PRRs, expressed by tissue or immune cells. Upon engaging with DAMP ligands, PRR-expressing cells secrete cytokines and chemokines (mediators) that further recruit and activate immune cells (effectors) to sites of inflammation [36].

**RETINA—AN IMMUNE-PRIVILEGED TISSUE**

**Immune privilege property of the retina**

The retina has a highly complex, sophisticated structure, and even a minor perturbation may cause devastating visual impairment. However, the eye has developed a special mechanism to protect the retina from exogenous and endogenous insults. This protective mechanism not only reduces the risk of pathogenic insult but also prevents circumvent, inappropriate immune reactions to insults, thereby reducing the risk of inflammation-mediated retinal damage. Therefore, the retina represents an immune-privileged tissue for several reasons [55–57]. Firstly, the retina is protected by physical barriers. The BRB that is formed by tight junctions between vascular endothelial cells (inner BRB) and RPE cells (outer BRB) ensures that circulating cells and molecules do not freely pass into the retinal parenchyma. The BRB also sequesters retinal antigens within the intraocular compartment, avoiding T cell activation, a phenomenon called immunologic ignorance [56, 58–60]. In addition, the retina has no lymphatic system. Therefore, when the retina suffers from any sort of insult, the endogenous alarmins are unlikely to be detected by circulating or choroidal/extraocular APCs if the BRB is intact. The second mechanism of retinal-immune privilege involves a sophisticated immune-regulatory system, orchestrated by retinal cells, including various neurons and RPE cells [55, 61, 62]. These retinal cells express various immune modulators that can suppress myeloid cell (microglia/macrophage) activation via CD200-CD200R [63] or CX3CL1-CX3CR1 [32], reduce T cell activation, induce T regulatory cell formation (through thrombospondin-1, TGF-β, CTLA4, or CTLA2 [64–69]), even induce the death of infiltrating immune cells through FasL and TRAIL [70–72], or suppress complement activation via CD55, CD46, and the DAF [73, 74]. In addition, ocular fluids contain a number of immunoinhibitory molecules, such as α-melanocyte-stimulating hormone; and vasoactive intestinal peptide [75, 76].
Importantly, despite being an immune-privileged tissue, when the retina suffers from noxious insults, an immune response can still be mounted by a local defense system, involving retinal innate-immune cells and the complement system.

**Retinal immune system**

Microglial cells form an important part of the immune defense of the retina. These cells are located in the inner layers of the retina and are distributed into 3 layers: the GL, IPL, and OPL (Fig. 2A). Our data have shown that in the mouse eye, the density of microglial cells in the IPL is higher than that in the OPL (~260 cells/mm² vs. ~98 cells/mm²; Fig. 2B and C). The pathophysiologic role of microglia in retinal health and disease has been reviewed extensively elsewhere [77–80]. Microglia express various TLRs [81, 82] that allow them to monitor the surrounding microenvironment. Upon engaging with danger signals, the microglia may convert from a resting surveillance state to an active form, specialized to operate within the diseased environment. Microglial activation is classically characterized by 2 major changes. First, the cell shape transforms from a highly branched (Fig. 2D) and ramified morphology to an ameboid form (Fig. 2F) [80]. Secondly, these ameboid cells become active phagocytes (Fig. 2F) [80]. Microglia may also undergo a low level of intermediate activation, characterized by shorter dendrites and larger somas compared with resting cells (Fig. 2E).

Perivascular macrophages are another important subset of retinal resident-immune cells that have a distinct morphology and phenotype. Although both perivascular macrophages and microglia express CD11b and F4/80, the former express high levels of CD14 (LPSR) and CD45, whereas microglial cells are negative for CD14 and express low levels of CD45 [83–85]. In addition, bone marrow chimeric studies have shown that brain perivascular macrophages are regularly replaced by circulating monocytes [86], suggesting that they may originate from bone marrow hematopoietic stem cells.

Whether the retina contains professional APCs (dendritic cells) has been a subject of debate for many years. Early work by Zhang and colleagues [87] reported a small population of MHC-II⁺ cells in rat retina. With the use of flow cytometry analysis, Gregerson and Yang [88] detected a small population of CD11c⁺ DEC205⁺ dendritic cells in normal mouse retina. This group further confirmed the existence of retinal CD11c cells by use of CD11c-DTR transgenic mice [89]. However, another study by Chen et al. [90] suggested that the rd8 mutation in the Crb1 gene may contribute to the abnormal number of CD11c⁺ cells in the retina in CD11c-enhanced yellow fluorescent protein transgenic mice. These CD11c⁺ cells had the characteristics of activated microglia but not dendritic cells, and they were virtually absent in the CD11c-DTR/GFP mice that did not have a Crb1 mutation [90]. Previously, our group identified a small population of MHC-II⁺33D1⁺ dendritic cells in mouse retina, which are located strategically around the optic disc and peripheral retinal margin area [91]. The function of these cells is unclear, but their location suggests that they may be “gatekeepers” of the retina.

When activated T cells were injected intravenously to mice with early uveitis, an inflammatory condition of the retina and choroid, early infiltration of T cells, was observed around the
optic disc and retinal periphery [91], allowing possible initial contacts with dendritic cells. It is possible that in the normal physiologic state, these retinal dendritic cells promote tolerance ("privilege") rather than immunity. Other immune cells, such as T/B cells, NK cells, and Mast cells have not been detected in the normal, healthy retina.

In addition to these immune cells, a complement regulatory system exists in the retina. The complement system is an important part of the innate-immune system, consisting of >30 small proteins and protein fragments. Complement proteins are normally synthesized by hepatocytes in the liver and released into the circulation in a latent form. Upon stimulation, complement proteins are cleaved by appropriate proteases, resulting in amplifying cascades involving further cleavage and ultimately, the formation of the MAC, a potent molecule that can kill cells [92]. The complement system can be activated through the classical pathway (mediated by an antibody-antigen complex), the AP (spontaneous tick-over), and the lectin pathway (mediated by mannose-binding lectin or ficolin binding to certain sugars) [92]. In addition to MAC, complement activation generates various complement fragments, including C3a, C5a, and C4a, which are actively involved in various immune responses [92].

Complement activation is involved in various retinal diseases, including uveoretinitis [93–95], diabetic retinopathy [96], and AMD [27], suggesting that the complement system is also an important part of retina innate-immune defense. Retinal cells can produce various complement proteins and regulators. For example, the mRNAs of C1qa/b, C1s, Crr1, C2, C4, C6b, C6d, C5, and C7, as well as complement regulatory genes, including Serping-1, MCP (CD46), DAF (CD55), CFH, CFI, and CD59, were detected in neuroretina of human [27] and mouse [97–99]. Furthermore, in vitro studies have shown that microglia and RPE cells are the major cellular sources of complement in the retina [97]. These results confirm that a local complement regulatory system exists in the retina and plays a role in retinal health and disease.

**PARAINFLAMMATION IN THE AGING RETINA**

Aging involves the accumulation of oxidative stress, and significant expression of oxidized lipids/proteins can be detected in the aging retina [100]. Other altered metabolic products, such as AGEs [101–103], β-amyloid [104, 105], pyridinium bisretinoid (A2E) [106], and hyaluronan fragments [107], may also accumulate in the aging retina. Oxidative or metabolic stress can damage retinal cells, including various neurons and RPE cells. As a result, a parainflammatory response may be initiated to repair damage and maintain homeostasis. Increased inflammatory gene and protein expression has been observed in various models of retinal aging [108, 109], and both the retinal cell-autonomous response and activation of the retinal immune system may contribute to age-related retinal parainflammation.

**Parainflamatory autonomous responses by RPE cells**

Over the years, many studies have investigated the age-related inflammatory response of RPE cells, but little has been published on the response of retinal neurons. Therefore, we will focus on the autonomous response of RPE cells under aging conditions. The RPE cells express various PRRs, including TLRs and nucleotide-binding oligomerization domain-like receptors, which can detect various stresses intracellularly or at the cell surface. Gene array technology has helped to define the overall gene-expression profile of RPE cells during aging, and inflammation is one of the major functional pathways that has been identified in these studies [109–111]. Many of the age-induced immune gene-expression changes identified in the array studies have also been observed in vitro. Treatment of RPE cells with the age-related DAMPs, such as AGEs [103], amyloid-β [112–114], or oxidized photoreceptor outer segments [98, 99], induces the up-regulation of proinflammatory genes, such as CCL2, IL-6, TNF-α, and CFB but also reduces immune-regulatory genes (such as CFH [98]). The SASP is a well-known phenomenon in all senescent cells [48]. The age-related autonomous response of RPE cells may be another example of SASP.

**Parainflammatory response by retinal immune system**

Microglia, perivascular macrophages, and a small number of dendritic cells constitute the cellular component of the retinal immune system. Like any other innate-immune cells, these cells express various PRRs that can detect various DAMPs in the aging retina. A number of studies have shown that retinal innate-immune cells, in particular, microglia, undergo low levels of activation during aging. Chan-Ling et al. [115] have detected ED2+ MHC-II+ cells in the normal aging rat retina. With the use of flow cytometry analysis, our group has found that expression of TLR3/4, CD11c, 33D1, and MHC-II in retinal CD11b+ CD45low cells (resident retinal myeloid-lineage cells) was increased significantly in the aging mouse retina [100]. Furthermore, an age-dependent increment in the number of microglial cells was present in the mouse retina [35], as well as subretinal migration and accumulation [116]. Evidence suggests that microglia in the aging retina appear to be activated at low levels. Initially, they do not acquire an ameboid shape, characteristic of fully activated microglia, suggestive of an mild activation state (Fig. 2F). Instead, the dendrites become shorter and less symmetric (Fig. 2E) compared with resting microglial cells (Fig. 2D). Secondly, unlike microglia from the young retina that are often confined within the IPL or OPL, microglial cells in the aging retina can migrate to the subretinal space [100, 116] (Fig. 3A and B). The role of microglia in the aging neuroretina has been reviewed extensively previously [79, 100, 117]. The subretinal space is devoid of any immune cells under normal, healthy conditions. The presence of microglia suggests tissue insult/damage at the retina-choroidal interface. What role do the subretinal microglia play in the aging eye? Although the morphology of subretinal microglia varies markedly, even in the same eye, these cells generally have larger cell bodies and shorter dendrites (Fig. 3C) compared with those in the inner retina (Fig. 2). Previously, we have shown that they express Iba-1, P2Y12, arginase-1, as well as low levels of MHC-II [34], suggesting a tissue repair/remodeling function. In support of this concept, melanin-loaded subretinal microglia were frequently observed in the normal, aging eye (Fig. 3D). Exocytosis of melanin granules is
Figure 3. Retinal microglia in the aging eye. (A and B) Reconstructed z-stack confocal images from a 16 (A)- and 27 (B)-mo-old mouse retina stained for Iba-1 (green for microglia) and lectin B4 (red for blood vessels). (A) At 16 mo, few Iba-1+ microglial cells were detected at the subretinal space (thick arrows), and some were still connected to cells in the OPL layer (thin arrows). (B) At 27 mo, many more Iba-1+ cells were detected at the subretinal space (thick arrows), and no Iba-1+ cells were detected between the OPL and subretinal space. (C) Heterogeneous morphology of Iba-1+ cells at the subretinal space in an 18-mo-old mouse. Most of the cells have larger cell bodies and shorter dendrites, and a few cells display a relatively small cell body and long dendrites (arrow). (D) Subretinal Iba-1+ cells from a 27-mo-old mouse showing a pigmented cell body (arrowheads).

A characteristic feature of stressed RPE cells and indeed, of melanin-containing cells generally [118].

In addition to a scavenger role, subretinal microglia may interact with RPE cells, facilitating cell–cell regulation. We have shown recently that the expression of complement components by RPE cells is regulated by activated macrophages [119]. The classically activated M1 macrophages up-regulate CFB/C3 expression by RPE cells, whereas the alternative M2 macrophages up-regulate the expression of complement inhibitors by RPE cells [119]. Whether subretinal macrophages can also modulate other RPE functions, such as phagocytosis, the expression of tight junctions and ion/water channels remains to be defined.

The complement system is an important part of the innate-immune system, and retinal cells, in particular, RPE cells, express various complement proteins. During aging, the expression of complement components, such as CFB and C3d, is increased in the retina-chordal interface [99], whereas the expression of complement regulators, such as CFH, is decreased [98]. In addition, an age-dependent expression of MAC in RPE/choroid has been observed in both human [120] and rat eyes [121]. A low level of complement activation may participate in retinal homeostasis in a number ways. The complement fragments C3a and C5a are known anaphylatoxins and may promote inflammation through the receptors C3aR and C5aR on immune cells, whereas C3b/C3c may opsonize dead cells/debris and promote phagocytosis. Although C5b-9 (MAC) can promote cell lysis, sublytic assembly of MAC induces cell-cycle activation and survival [122] and may be neuroprotective [123].

**DYSREGULATED PARAINFLAMMATION AND AMD**

Why does the protective retinal parainflammatory response become detrimental in AMD? Perhaps there is a balance between the level of age-mediated retinal stress and the capacity of the parainflammatory response to repair the damage. On the one hand, if the level of retinal stress exceeds the repair capacity of the immune system, then tissue damage is unavoidable. On the other hand, if the parainflammatory response becomes dysregulated, then it may transform into chronic inflammation and contribute to tissue damage. Parainflammatory response in the aging retina includes the cell-autonomous response, the response by the retinal innate-immune system (i.e., microglia/macrophage and the complement system), and the response of the systemic immune system. Theoretically, AMD may occur when any or all of these inflammatory responses become dysregulated.

**Dysregulated cell-autonomous response in AMD**

The autonomous response in the context of AMD predominantly concerns RPE cells. A typical example of an age-related RPE inflammatory response is the activation of NLRP3 inflammasome. NLRP3, PYCARD, and caspase-1 have been detected in RPE cells at lesion sites in both GA (dry AMD) and nAMD (wet AMD) [124, 125]. In vitro studies have shown that the NLRP3 inflammasome in RPE cells can be activated by various intracellular/extracellular stimuli that may exist in the aging eye, such as AluRNA [124], amyloid-β [126], A2E [127], lipofuscin [128], or oxidized lipoproteins [129]. Depending on the stimuli, activation of the NLRP3 inflammasome in RPE cells may result in IL-1β production [124] or IL-1β production [127] or both [126, 128]. Inflammasome activation, in particular, AluRNA-induced NLRP3 inflammasome activation, often leads to RPE cell death and the development of GA-like lesions [124, 130]. Despite the presence of TLRs on RPE cells, AluRNA-induced NLRP3 inflammasome activation does not involve P2Y7 and MyD88 [124, 130].

What causes the uncontrolled inflammasome activation in AMD? One obvious cause is that the levels of macular stress in AMD patients differ from that experienced by healthy aged...
people. For example, AluRNA was detected in AMD eyes but not in healthy controls [124], and retinal A2E levels were higher in AMD compared with controls [131]. Dysfunction of the autophagy pathway may also be involved in RPE inflammasome activation in AMD. Autophagy is the self-clearance machinery of a cell [43] and is important for cells to dispose of damaged organelles or waste molecules [43]. An imbalance in the autophagy system may result in the intracellular accumulation of toxic molecules and the generation of ROS [42], which may lead to progressive inflammasome activation [42]. Increased autophagosome numbers and expression of autophagy proteins have been observed in RPE cells of the normal, aging eye [132, 133]. Whereas in AMD eyes, autophagy proteins, autophagosomes, and autophagy flux were reduced [133, 134]. With the onset of AMD, the excessive accumulation of lipofuscin in RPE cells may impair lysosomal enzyme activity, resulting in autophagy dysfunction [133, 135]. Accumulation of ROS and lipofuscin in RPE cells may lead to inflammasome activation [41].

**Dysregulated retinal innate-immune activation in AMD**

Activation of retinal microglia and the complement system features the parainflammatory response of the retinal innate immune system in the aging eye [100]. Malfunction in the immune regulatory system or the innate-immune component of the retina may lead to the dysregulation of the parainflammatory response. RPE cells produce various immune-suppressive factors (both membrane and soluble forms) to maintain the immune-privileged state of the retina. The production and/or function of these regulators may be altered in AMD. For example, FasL, expressed by RPE cells, is important in maintaining retinal immune privilege by inducing the death of infiltrating immune cells [70, 72]. During aging, the matrix metalloprotease activity is increased, resulting in the cleavage of FasL and the loss of immune-regulatory function in RPE cells [136]. It would be interesting to know if this age-related cleavage of FasL is accelerated in AMD. RPE cells also express/produce various complement regulators, such as CFH, CD46, and CD59 [27, 97]. Local production of these regulators may protect retinal cells, including RPE and photoreceptors, from complement attack. Immunohistochemistry studies have detected MAC in drusen and macular lesions in AMD [9, 27], suggesting that RPE cell death in AMD may be related to uncontrolled complement activation. The expression of CFH [137], CD46, and CD59 [138] in RPE cells was reduced in AMD. In addition, the functional change of innate-immune cells (as a result of genetic or epigenetic regulation) may confer a detrimental effect on aging insults. Studies in the function of CFH protein have shown that the variant CFH 402His has reduced binding affinity to CRP [139], BM, heparin [139, 140], and oxidized phospholipids [141]. These functional alterations may result in a reduced ability of CFH to protect the retina, in particular, RPE cells, and ultimately contribute to AMD development.

In addition to the retinal immune-regulatory system, malfunction of microglia and macrophages in the retina and choroid may lead to dysregulated parainflammation in AMD. The CCL2/CCR2 and CX3CL1/CX3CR1 pathways are 2 major chemokine axes involved in monocyte/macrophage migration. CCL2 critically controls the trafficking of CX3CR1-expressing monocytes to sites of inflammation [142], whereas CX3CL1 regulates the trafficking of CX3CR1-expressing resident monocytes under homeostatic conditions [142]. A study by Ambati et al. [31] have shown that mice deficient in CCL2 or CCR2 age dependently develop retinal pathologies akin to human AMD, although it is unclear whether the retinal phenotype was affected by the Crb1 rd8 mutation [143]. The result suggests that CCL2/CCR2 pathway-mediated subretinal inflammation may have a protective role in retinal aging. Interestingly, the CX3CL1/CX3CR1 pathway-mediated subretinal inflammation also appears to be beneficial. Mice deficient in CX3CR1 developed retinal degeneration during aging [32]. More recent studies have shown that CCR2+ mononuclear phagocytes from CX3CR1-deficient mice can induce neuronal apoptosis through IL-1β secretion [144], and the production of IL-1β in CX3CR1-deficient phagocytes is mediated, at least in part, by the up-regulation of P2X purinoreceptor 7 [145]. It appears that CX3CR1+ monocytes and CCR2+ monocytes are necessary for retinal homeostasis during aging, and disruption in either pathway may result in age-dependent retinal degeneration.

How can these data be interpreted? The CCL2/CCR2 pathway and the CX3CL1/CX3CR1 pathway may be involved in different stages of subretinal inflammation during aging. Microglial cells are CCR2/CX3CR1+ [146], although circulating monocytes or choroidal macrophages may express both CCR2 and CX3CR1 [142]. At the early stages of aging, when RPE and photoreceptors are mildly stressed from age-related oxidative insults, and the BRB is intact, the CX3CR1+ microglial cells may migrate from the inner retina to the subretinal space to remove debris and maintain homeostasis. As the aging progresses, and age-related macular stress accumulates, microglial cells may not be able to maintain macular homeostasis. Activated subretinal microglial cells and stressed RPE cell may release chemokines, such as CCL2, to recruit CCR2+ macrophages from the choroidal tissue to maintain homeostasis. BRB breakdown has been observed in the normal rat-aging eye [115]. In mouse eyes, migrating microglial cells were observed frequently at the photoreceptor layer (OPL) at the early stages (12–18 mo) but not late stages (24–29 mo) of aging, despite the fact that more subretinal macrophages exist at the late stages (Fig. 3). The increased number of subretinal macrophages during the late stages of aging may be related to the recruitment of CCR2+ macrophages from the choroid or circulation. In addition, the lack of function of the CCR2+ or CX3CR1+ monocyte (as a result of genetic or epigenetic modification) may lead to malfunction of the other subset, resulting in a dysregulated parainflammatory response. We have shown recently that macrophages from CCL2−/− mice or CCR2−/− mice produce excessive amounts of inflammatory cytokines TNF-α and IL-1β when stimulated with LPS compared with cells from wild-type mice [34]. Interestingly, macrophages from the CCL2/CX3CR1 double-knockout mice had a more aggressive response to LPS or IL-1 stimulation under hypoxic conditions but reduced phagocytosis [33]. These mice develop localized retinal atrophies in an age- and light-dependent manner [33]. Our results may suggest that mononuclear phagocytes from CCL2−/− or CX3CR1−/− mice or CCL2−/−CX3CR1−/− dual-knockout mice are genetically predisposed to a proinflammatory phenotype. When these macrophages are
recruited to the subretinal space in the aging eye, they may do more harm than good. This may be particularly relevant to AMD patients with CX3CR1 polymorphisms [147]. The results from animal models highlight the importance of the CCL2/CCR2 and CX3CL1/CX3CR1 pathways in the development of age-related retinal degeneration. Further studies are necessary to investigate these pathways in human AMD.

**Dysregulated systemic inflammation and AMD**

Aging is associated with a low-grade activation of the systemic immune system, and the term inflamming is frequently used to describe this phenomenon [12, 13]. Previous studies have shown that plasma levels of complement fragments C3a, Bb, C4a, and C5a were increased in AMD patients [148, 149]. Increased serum levels of CRPs [150–152] and pentraxin 3 [152] have been reported in AMD. In addition, retinal autoantibodies [153, 154] and higher levels of circulating WBCs were reported in AMD patients [155–157]. More recently, it has been shown that AMD patients have higher levels of blood neutrophils, with an increased neutrophil/lymphocyte ratio [158, 159]. In addition, increased serum levels of inflammatory cytokines, such as IL-1β, TNF-α, and IL-17, have been detected in AMD patients [160], with IL-17 production potentially related to higher C5a levels in these patients [161, 162]. These data suggest that the level of systemic, low-grade immune activation (inflamming) is more severe in AMD patients compared with age-matched controls.

Interestingly, apart from retinal autoantibodies, most of the inflammatory mediators are generic markers of systemic immune activation. Is this nonspecific systemic chronic inflammation biologically linked to AMD pathology, which affects only a small area (~5.5 mm) of the neuroretina? It is unlikely that chronic damage in this tiny tissue would affect the levels of cytokines/growth factors in the whole circulation (~5 liters of blood [163]). The increased systemic immune activation in AMD patients may reflect an intrinsic over-reactivity of the immune system to age-related insults, i.e., dysregulated age-related systemic parainflammation. When immune cells are recruited to the macula in the aging eye, they may contribute to macular damage by producing various proinflammatory cytokines and chemokines. If this is the case, the use of nonsteroidal anti-inflammatory drugs or other anti-inflammatory drugs would benefit AMD patients. Indeed, epidemiologic evidence demonstrates that the use of anti-inflammatory medication reduces the risk of AMD [164]. Rheumatoid arthritis patients with regular immunosuppressive medications also had a lower prevalence of AMD [165]. Furthermore, systemic immunosuppression (e.g., dactlizumab or rapamycin) [166] or topical use of bromfenac can significantly reduce the number of intravitreal anti-VEGF injection in nAMD patients [167, 168]. However, a number of meta-analysis of various clinical trials suggest that the use of aspirin may increase the incidence of nAMD [169, 170]. However, most patients with AMD take only low-dose aspirin (75–100 mg/day) to reduce the risk of cardiovascular disease, and such low doses are likely to have minimal effects on the immune system.

What causes the dysregulation of systemic parainflammation (inflamming) in AMD? So far, the evidence indicates that the activation of the systemic immune system in AMD is unlikely to be related to autoimmunity. The nature of the systemic immune activation should be considered as an adaptive response to age-related insults, with increased magnitude in AMD patients. The amplified response in AMD patients may be related to the nature/amount of insults accumulated during aging as a result of genetic predisposition or epigenetic/environmental influence. For example, if the antioxidant system does not function as well as it should, as a result of genetic predisposition, or the patient has an unhealthy lifestyle, age-related oxidative insults may accumulate more rapidly and to a greater extent. Smoking and high-fat diet are known environmental risk factors of AMD, and both can directly impact on the immune system [171–173]. Aging can also affect the immune system [174, 175], and it is possible that immune senescence is accelerated in AMD.

Another possible cause of dysregulated systemic parainflammation is that the immune-regulatory system may not function properly in AMD patients as a result of genetic predisposition or epigenetic modifications. The polymorphisms in the Cfh gene (encoding the CFH protein that negatively regulates AP complement activation) are typical examples of dysfunction of immune regulators in AMD. In addition, insufficient expression of other complement regulatory proteins, such as CD46 and CD59, has been observed in AMD patients [176]. Complement activation not only results in the cell-killing MAC, but it also generates various complement fragments, such as C3a, C3b/c, C4a, and C5a, which can participate in various other immune responses [92].

Malfunction of the effector arm of the immune response, e.g., monocytes/macrophages and T cells, may also lead to dysregulated systemic parainflammation. Increased expression of CCR2 in monocytes [177] and lower levels of CX3CR1 expression in CD8 T cells [178] have both been observed in AMD patients. In addition, increased CD200 expression in CD11b+ monocytes has been observed in AMD [179]. Although the functional significance of these changes remains to be elucidated, the observations suggest that malfunction in monocytes or T cells may lead to increased systemic inflammation in AMD patients.

**CONCLUSIONS AND FUTURE DIRECTIONS**

Studies in the past decade have revealed some causal links between low-grade chronic inflammation and AMD. As we age, oxidative insults accumulate, and such insults persist and increase in magnitude with age. The systemic parainflammation is the adaptive response of the immune system to age-related insults to the whole body, whereas local parainflammation is the response of the eye to macular insults. A healthy immune system should initiate an effective parainflammatory response and keep it under control, as part of a healthy aging eye (Fig. 4). The parainflammatory response may become dysregulated as a result of genetic predisposition, epigenetic modification, or environmental intervention, and dysregulated parainflammation (chronic inflammation) is detrimental and contributes to macular pathology (i.e., AMD; Fig. 4).

Further studies are necessary to understand how and why the parainflammatory response becomes dysregulated in AMD. More knowledge on how the systemic and local retinal immune
responses are connected in AMD will help to understand whether AMD is a disease with systemic immune dysregulation. In addition, inducers of subretinal para-inflammation in the aging eye and in AMD remain to be fully characterized. The immune response in the choroid in AMD is understudied. A para-inflammation response also presents in the aging choroid [100], and the degeneration of choroidal vasculature has been proposed as an early event in both GA and nAMD [180]. It is possible that choroidal neovascularization in nAMD might be initiated by dysregulated choroidal para-inflammation. More knowledge about the inflammatory mediators released by RPE cells, photoreceptors, and subretinal/choroidal macrophages in the aging eye and in AMD may help to identify novel targets for anti-inflammatory therapy. The communication among subretinal macrophages, RPE cells, photoreceptors, and choroidal macrophages in the aging eye and in AMD will be a challenging but important topic of future research and may uncover new insights into AMD pathogenesis.

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AUTHORSHIP
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Inflammation in age-related macular degeneration


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