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Immunology of age-related macular degeneration

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Abstract

Age-related macular degeneration (AMD) is a leading cause of blindness in aged individuals. Recent advances have highlighted the essential role of immune processes in the development, progression and treatment of AMD. In this Review we discuss recent discoveries related to the immunological aspects of AMD pathogenesis. We outline the diverse immune cell types, inflammatory activators and pathways that are involved. Finally, we discuss the future of inflammation-directed therapeutics to treat AMD in the growing aged population.

Optimal vision requires a high-functioning central `retina`, in particular the photoreceptor-dense `macula` (FIG. 1), which is responsible for the fine visual acuity that is required for tasks such as reading, facial recognition and driving. Age-related macular degeneration (AMD) refers to the progressive degeneration of the macula that commonly occurs in people over 60 years of age. More than 30 million individuals worldwide suffer from visual impairment as a result of AMD, which is estimated to account for more than US$300 billion in annual economic costs[1].

The study of AMD has, in many respects, been at the forefront of research into complex diseases. The discovery of the Tyr402His polymorphism of `complement factor H` (CFH) that confers increased statistical risk of developing AMD was the first of its kind for a complex disease[2–5]. Furthermore, multiple biological therapies that target vascular endothelial growth factor A (VEGFA) in `neovascular AMD` (also known as exudative or 'wet' AMD) have recently revolutionized the clinical management of this disease. However, despite advances in the understanding, diagnosis and treatment of AMD in the past decade, disease prevalence increases with the ageing human population. The next decade will see a steady increase in the incidence and economic cost of AMD[1].

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Competing interests statement

The authors declare competing financial interests. See Web version for details.

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Despite the fact that AMD is presumed to have a multifaceted aetiology, immune dysfunction is a recurring theme in its pathogenesis. In this Review, we discuss the fundamental concepts and current ideas of AMD pathogenesis, with a particular focus on several recent advances in the immune aspects of the disease. We begin by describing the normal structure of the retina and the characteristic features of AMD. Next, we focus on the major immunological processes that have important roles in AMD development and we discuss the inflammatory component of neovascular AMD. We then present an integrated model of the immune modulation of AMD pathogenesis. Finally, we summarize the possibility of using immune-based therapeutics to prevent and treat AMD development. The aim of this Review is to present an up-to-date discussion of recent immunological findings in AMD. Consequently, we have condensed the discussion of the complement pathway, which is the most thoroughly studied immune pathway in AMD; for a more comprehensive summary of complement biology in AMD, the reader is referred to other reviews[6–9].

Structure of the retina

The sensory retina is organized into alternating layers of neuronal extensions and cell nuclei. The conversion of light into chemical signals occurs in the outer segments of the cone and rod `photoreceptors` in the posterior of the neural retina (FIG. 1). Encoded visual information is integrated through retinal networks and ultimately travels to the optic nerve. Blood is supplied to the neural retina by retinal blood vessels that originate from the central retinal artery. Transport across retinal blood vessels is controlled by endothelial tight junctions, which constitute the inner `blood–retinal barrier`.

Next to the photoreceptor layer is a highly organized monolayer of `retinal pigmented epithelium` (RPE), which has several roles in supporting the metabolically active photoreceptor layer. These include recycling biochemical by-products of photoreception through the phagocytosis of photoreceptor outer segments, supplying trophic factors such as VEGFA[10] and maintaining the integrity of the outer blood–retinal barrier through tight junctions. Thus, the integrity of the RPE is essential for homeostasis in the retina. Posterior to the RPE is the Bruch membrane — a thick, elasto-collagenous extracellular matrix. Together, the RPE and the Bruch membrane form the outer blood–retinal barrier, which prevents the entrance of macromolecules and immune cells from the underlying choroid into the photoreceptor layer. Located behind the Bruch membrane, the choroid contains a dense network of blood vessels (choriocapillaris), which supplies oxygen and nutrients to the RPE, outer retina and optic nerve. The choroid endothelium is fenestrated, which enables the transport of molecules to the metabolically demanding RPE[11]. The choroid also contains tissue-resident melanocytes, fibroblasts, macrophages, mast cells and dendritic cells.

A brief description of AMD

The first clinical feature of early ‘dry’ AMD is the presence of `drusen` (BOX 1), which are extracellular deposits below the RPE that comprise lipid- and protein-rich debris[12]. Small, isolated drusen do not affect visual function but their expansion and coalescence are hallmarks of AMD progression. The two main advanced forms of AMD are: neovascular AMD, which is characterized by the invasion of abnormal choroidal (or occasionally retinal) blood vessels and fluid leakage into the retina; and `geographic atrophy` (also known as end-stage dry AMD or atrophic AMD).

Neovascular AMD is the leading cause of blindness among the elderly in industrialized nations, affecting more than 1 million individuals over the age of 40 in the United States alone[13]. Although the fundamental aetiology of neovascular AMD remains unclear, therapies that target VEGFA, which is a potent stimulator of angiogenesis and

vasopermeability, have been successful in substantially improving central vision in approximately 30% of patients and in arresting vision loss in 94% of patients (compared with 62% of sham-treated patients)[14,15].

Geographic atrophy is characterized by large, confluent regions of atrophied RPE in the macular area. There are no approved therapies for geographic atrophy, which is mainly due to the lack of suitable molecular targets. Unlike VEGFA in neovascular AMD, a single crucial factor has not been identified as promoting RPE degeneration in geographic atrophy. As geographic atrophy is considered to be the default end point of dry AMD[16], further investigation into the initiating factors that promote the early development of this disease should be useful in the search for new therapies.

Importantly, these advanced stages of AMD are not mutually exclusive — neovascular lesions are often present in the periphery of eyes with geographic atrophy[17]. In addition, long-term treatment of neovascular AMD with VEGFA-targeted therapy is associated with the development of geographic atrophy[18], possibly as a result of atrophy of the choriocapillaris[19] and loss of the neurotrophic activity of VEGFA in the retina[10]. Genetic polymorphisms that are associated with AMD — including those in the genes encoding CFH[20–23] and high-temperature requirement A serine peptidase 1 (HTRA1)[24,25] — confer similar statistical risk of developing both forms of AMD, which indicates that there are many shared underlying pathological mechanisms. The effect of polymorphisms in the *HTRA1* or age-related maculopathy susceptibility 2 (*ARMS2*) gene locus on gene activity or expression, as well as the functional consequences for AMD, are unclear.

AMD is associated with marked changes in normal retinal anatomy. Early AMD is associated with thickening of the Bruch membrane, deposition of drusen, RPE hypertrophy and pigment extrusion[26]. Advanced AMD is associated with photoreceptor degeneration following either invasion of choroidal blood vessels through the Bruch membrane into the retina in neovascular AMD or degeneration of the choroidal vasculature and RPE cells in geographic atrophy[27].

Immune surveillance in maintaining retinal health

Maintenance of the blood–retinal barrier confers a degree of `immune privilege` to the mammalian eye. This immune privilege is characterized by tolerance of foreign antigens owing to the expression of endogenous immunosuppressive factors and the absence of functional intraocular lymphatics[28–30]. However, the introduction of specific foreign or endogenous inflammatory signals in the retina evokes innate immune responses.

The resident inflammatory cells of the retina are the microglia, which are similar to tissue macrophages and central nervous system microglia. The retinal microglia normally reside in proximity to retinal blood vessels in the inner layers of the neural retina. The number of resident retinal microglial cells increases with age in mice[31]. Aged microglia have a branched morphology, which is indicative of a ‘resting’ phenotype, and they show decreased responsiveness to tissue injury[31].

Evidence indicates that the subretinal migration of microglia is necessary to eliminate visual by-products and to maintain vision[31,32]. The impairment of microglial migration into or out of the subretinal space promotes the death of photoreceptor cells[33,34]. In AMD, microglia accumulate in the subretinal space[35]; this is probably both a symptom of inflammatory damage and a beneficial response to injury, and the impairment of this accumulation in the subretinal space exacerbates retinal degeneration. However, infiltration of microglia and macrophages to sites of retinal injury can also promote the growth of neovascular lesions[32,36,37]. Macrophages, together with RPE cells, are a major source of pro-angiogenic

factors such as VEGFA[38]. Macrophages and dendritic cells are not normally present in the retina but reside in the underlying choroid. In cases of breakdown of the blood–retinal barrier, these cells are recruited from the underlying choroid or from the systemic circulation into the retina where they modulate disease.

The use of aged animals in models of impaired immune cell trafficking, such as those with CC-chemokine ligand 2 (CCL2), CC-chemokine receptor 2 (CCR2) and/or CX_3C-chemokine receptor 1 (CX_3CR1) deficiency, results in animals that have AMD-like features, including changes to the Bruch membrane, RPE swelling and retinal thinning[39–41]. Aged mice that are deficient in CCL2 or its receptor CCR2 develop spontaneous hypopigmented subretinal lesions that are visible using `fundus photography`[33,39,42], as well as developing photoreceptor and RPE damage[33,39]; they also infrequently develop spontaneous choroidal neovascular lesions that are similar to those in neovascular AMD. Subretinal lesions of CCL2- or CCR2-deficient mice are comprised of bloated outer segment-laden macrophages[42] (which are similar to foam cells in atherosclerosis), and CCL2- or CCR2-deficient macrophages in the periphery show impaired chemotaxis and phagocytosis[33]. Importantly, CCL2–CCR2-dependent immune cell function prevents photoreceptor apoptosis following amyloid-β treatment[43], which adds further support to the idea that immune cell trafficking to sites of local tissue damage is an essential component of retinal health. Similarly, aged mice that are deficient in CX_3CR1 show subretinal microglial accumulation and retinal degeneration, which have both been attributed to impaired trafficking of immune cells in the retina[40]. As in the CCR2- and CCL2-deficient mouse models that accumulate subretinal macrophages, CX_3CR1-deficient mice acquire large bloated subretinal microglia[44]. Genetic studies also support a role for impaired repair of tissue damage in AMD, as polymorphisms in *CX3CR1* that cause macrophage migratory defects[38,43] are associated with AMD[45].

Whether increased or impaired CCL2–CCR2 signalling is a cause or a symptom of AMD pathogenesis remains to be shown. CCL2 levels are increased in the RPE and the choroid of aged mice[46], as well as in the serum of elderly individuals[47]; it is possible that this is related to the increased burden of tissue damage in the aged retina. The presence of increased systemic chemokine levels in patients with AMD indicates that systemic inflammation manifests itself locally in the retina. In addition, CCL2 expression is upregulated early in laser-induced choroidal neovascularization[48], and inhibition of CCR2 decreases neovascular growth[49]. At first glance, these observations of increased CCL2–CCR2 signalling in AMD are not consistent with the idea that deficiencies in CCL2–CCR2-mediated immune cell trafficking contribute to AMD. However, the distinction between local and systemic chemokine signalling is important; local signalling probably has a protective role in the immune-mediated repair of damaged retinal tissue in early AMD, whereas systemic signalling contributes to the recruitment of pro-angiogenic immune cells to sites of neovascularization in late-stage AMD. It is also important to consider the distinction between immune responses to acute events and those to long-term chronic `para-inflammation` or to persistent low-level inflammation (for example, involving complement activation and cytokine expression) owing to sustained tissue stress in the ageing retina[35]. This distinction probably accounts for some of the discrepancies that have been observed between age-related animal models of retinal degeneration, which rarely involve choroidal neovascularization, and acute injury models (such as light-induced retinal damage), in which retinal degeneration occurs within days. CCL2–CCR2 signalling might modulate AMD development in different directions depending on the disease context; this would be in line with current models of immune cell involvement in AMD development that suggest that immune cells have dual, opposing roles in preventing and promoting disease.

Dysregulated immune activation in AMD

Although a functioning retinal immune system is crucial for visual homeostasis, a substantial amount of evidence also indicates that the overactivation of specific immune processes is important in AMD pathogenesis. Over a lifetime, the retina and RPE are in contact with a large number of innate immune activators that could potentially contribute to vision loss in AMD. Data from a decade of research strongly indicate that improper immune activation is involved in inducing and advancing AMD pathology. Among these immune activation pathways, the complement pathway is the most well-established and widely accepted as contributing to AMD. Genetic evidence from `genome-wide association studies` (GWASs) and rare variant analyses[50] strongly indicates that the `alternative pathway of complement activation` is overactive in AMD (BOX 2). This evidence has been discussed in several excellent reviews[6–9] and, therefore, these data are only summarized in this Review in relation to the general idea that excessive inflammation contributes to AMD pathogenesis.

Complement

In 2005, GWASs showed that approximately 50% of the heritability of AMD could be accounted for by a single nucleotide polymorphism (SNP) in an exon encoding CFH[2–5]. However, it should be noted that the complement system had been implicated in AMD pathogenesis before this discovery[12,51,52]. The risk variant of CFH (402His) does not regulate the alternative pathway of complement activation as efficiently as the main allele (Tyr402) does. The 402His variant binds with lower affinity to numerous constituents of the damaged retina[6,53–56]; therefore, the inhibitory effect of CFH on the complement pathway is decreased, which results in a greater degree of complement activation following retinal injury.

Thus, one hypothesis for the aetiopathogenesis of AMD is that in individuals carrying a ‘complement hyperinflammatory phenotype’ the complement pathway overreacts to cellular damage and debris in the retina[6–9, 160]. In addition to common alleles of CFH that confer greater risk of, or protection from, AMD as well as rare variants of CFH with high penetrance[50], similar findings have been shown for other members of the alternative complement pathway, including activating components C3 and factor B (gain-of-function mutations associated with AMD) and the regulator factor I (loss-of-function mutations associated with AMD). Carrying the 402His CFH variant or these other risk factors enables the alternative pathway to generate undesirable quantities of complement components C3b and C3a, as well as of the downstream effectors C5a and C5b-C9.

Inflammasome activation

The inflammasome is a protein complex that is activated by foreign or endogenous danger signals, which culminate in caspase 1-mediated maturation of the cytokines interleukin-1β (IL-1β) and IL-18 and, ultimately, in pyroptosis or apoptosis. The inflammasome complex is comprised of three protein constituents: a NOD-like receptor family member (the best studied of which is NLRP3 (NOD-, LRR- and pyrin domain-containing 3)), the adaptor protein ASC and the protease caspase 1.

Inflammasome activation has recently been implicated in AMD pathogenesis. One group found that `carboxyethylpyrrole-adducted proteins`, which accumulate in the retina with age[57], prime the NLRP3 inflammasome[58]. They also found that drusen extracts that had been isolated from AMD donor tissue, as well as the complement component C1q (a component of human drusen), activate the NLRP3 inflammasome in macrophages.

In a separate study, we identified another endogenous activator of the inflammasome that is present in the RPE of patients with geographic atrophy[59]. Repetitive element-derived *Alu* RNA transcripts, which are non-canonical targets of DICER1-mediated enzymatic degradation, accumulate in human geographic atrophy following the loss of DICER1 expression (which might result from oxidative stress in the RPE)[60]. These transcripts function as both priming and activating signals to stimulate NLRP3 inflammasome signalling. Importantly, we found evidence of inflammasome activation in the eyes of patients with AMD; this included increased levels of NLRP3, IL-18 and activated caspase 1 in the RPE. Two recent studies have confirmed evidence of inflammasome activation in the eyes of patients with AMD[61] (C. Chan, personal communication).

An important inflammasome effector is IL-1β. Human drusen extracts induce NLRP3-dependent IL-1β secretion from lipopolysaccharide-primed peripheral blood mononuclear cells[58]. In our study we did not detect significant release of mature IL-1β from RPE cells in response to *Alu* RNA[59], although inflammasome-mediated IL-1β release from RPE cells has been more recently found in response to 4-hydroxynonenal[62], amyloid-β[63], A2E (*N*-retinyl-*N*-retinylidene ethanolamine) (D. Shima, personal communication) and lysosomal destabilization[61], which indicates that RPE cells are capable of inflammasome-mediated IL-1β release. In our study we also found increased levels of *IL1B* mRNA in the RPE of donor eyes from individuals with geographic atrophy[59], which indicates that IL-1β might be involved in the development of AMD.

Taken together, these studies show that the retina can respond to diverse ‘danger signals’ that are abundant in AMD through the activation of the NLRP3 inflammasome. However, the consequences of inflammasome activation in AMD pathogenesis are poorly understood. Both IL-1β and IL-18 signal through the adaptor protein myeloid differentiation primary-response protein 88 (MYD88) to induce inflammatory and apoptotic effects. However, although we have found potent cytotoxic effects of IL-18 and IL-1β on the RPE[59], in another study, beneficial effects of inflammasome-mediated IL-18 release were reported to occur through the inhibition of neovascularization in an acute laser-induced injury model of neovascular AMD[58]. These contrasting findings might imply that a single factor (IL-18) or pathway (NLRP3 inflammasome activation) can be simultaneously anti-angiogenic and destructive to the RPE. This model mirrors our findings that Toll-like receptor 3 (TLR3) activation is beneficial in terms of decreasing choroidal neovascularization[64], but that it also promotes RPE degeneration[65] (see below). In contrast to the reported anti-angiogenic effects of IL-18 on laser-induced choroidal neovascularization[58], IL-1β promotes neovascularization[66].

In summary, the extent, the underlying mechanisms and the cumulative effect of inflammasome activation and the secretion of IL-1β and IL-18 in human AMD remain uncertain. Therefore, we caution against pursuing IL-1β- or IL-18-based therapeutic strategies for neovascular AMD.

TLR signalling

Activation of pathogen- or danger-associated molecular pattern-mediated signalling in the retina often results in rapid and vigorous inflammatory responses. Retinal cells express multiple TLR family members, and the RPE in particular expresses most TLRs[67]. The contribution of TLR signalling to AMD is still under intense investigation. Initial reports of the association of polymorphisms in TLR3 (REF. 68) and TLR4 (REF. 69) with AMD susceptibility were controversial[70]. TLR activation in the retina by synthetic or endogenous ligands can have positive or negative effects in AMD pathogenesis and this probably depends on context- and ligand-specific factors. Activation of TLR3 signalling by administration of double-stranded RNAs (dsRNAs) of 21 nucleotides or longer inhibits the

development of choroidal neovascularization after acute laser-induced injury[64]. However, the benefits of TLR3 signalling in terms of preventing neovascular lesions are counteracted by RPE cytotoxicity[65]. Genetic ablation of TLR3 prevents disease development in a mouse model of retinal injury that is induced by impairment of all-*trans*-retinal clearance[71], which indicates that endogenous TLR3 agonists promote retinal degeneration. Our investigations identified strong immunoreactivity with a dsRNA-specific antibody in human drusen, which supports the idea that the TLR3 signalling pathway is potentially involved in the progression of geographic atrophy[60]. The findings of TLR3-induced RPE degeneration probably limit the use of nonspecific TLR3 agonist-based therapies for neovascular AMD.

Unlike experimental TLR3 activation that suppresses the development of choroidal neovascularization, TLR2 ligands (carboxyethylpyrrole-adducted proteins and *Chlamydia pneumoniae* antigen) promote experimental choroidal neovascularization[72,73]. We speculate that TLR activation broadly contributes to AMD pathologies; however, the full range of agonists and outcomes is unknown.

Adaptive immunity

Although AMD research has mostly focused on innate immune processes, a growing body of evidence supports the idea that there is some contribution of adaptive immunity to AMD. The mammalian eye lacks functional intraocular lymphatic vessels, and B cells or T cells have not been widely reported to be present in proximity to either neovascular or geographic atrophy lesions. As B cells and T cells do not have a directly defined role in mediating angiogenesis or tissue damage in human AMD, any contribution of adaptive immunity to AMD development probably involves retinal antigen presentation and indirect autoantibody-mediated retinal degeneration.

Increased titres of autoantibodies recognizing components that are enriched in the retina have been measured in the serum of patients with AMD[74–76] and have led to the hypothesis that AMD might be an autoimmune disorder. Indeed, the *Ccl2*$^{-/-}$*Cx3cr1*$^{-/-}$ mouse model of retinal degeneration involves retina-specific autoantibodies[41], which indicates that there might be at least a circumstantial relationship between AMD and adaptive immune processes.

Immunization of mice with carboxyethylpyrrole-modified albumin induces retinal degeneration that has features similar to those of AMD, including drusen-like deposits, sub-RPE complement deposition and photoreceptor dysfunction[57], and that requires B cells and T cells. Importantly, the authors of this study observed macrophage infiltration into retinal lesions, which indicates that antibody-mediated complement activation can induce a ‘primary’ retinal injury to which macrophages respond and potentially further modulate the disease. The mechanisms involved in antigen presentation and antibody-mediated RPE degeneration (presumably by activation of the classical complement cascade) have not been resolved; they may provide important and interesting insights into AMD pathogenesis. The lack of functional lymphatic vessels and immune cells in the normal mammalian retina indicates that antigen presentation involves either a direct breakdown of the blood–retinal barrier and could be mediated by local dendritic cells, or the leakage of retinal antigens into the circulation. Importantly, some retinal antigens such as docosahexaenoic acid-derived carboxyethylpyrrole protein modifications are not strictly unique to the retina. Thus, the antigen need not be derived from the retina for a damaging antibody-mediated immune response to be mounted against the retina. However, whether lymphocytes have a causal role in AMD pathogenesis is uncertain.

Other immune stimulators

In addition to the pathways highlighted above, several other factors that are linked to immune activation in AMD merit discussion. One of the most important of these factors is A2E, which is a principal component of retinal lipofuscin. Accumulation of A2E owing to mutations in the retinal-specific ATP-binding cassette A4 transporter (ABCA4) causes a form of Stargardt's disease, which is an early-onset, inherited disease that results in retinal degeneration and that has many aetiological similarities to AMD. A2E has multiple damaging effects on RPE cells, including complement activation[77], inflammasome activation (D. Shima, personal communication; J.A. and N. Kerur unpublished observations), cytokine expression[78,79] and RPE degeneration[80,81]. ABCA4-deficient mice also have increased complement activation[82], and subretinal injection of A2E exacerbates experimental neovascularization[83].

Other important immune activators in the retina that might have a role in AMD are lipid oxidation byproducts, which are formed from the oxidation of polyunsaturated fatty acids. The retina is prone to lipid peroxidation because it has an abundance of lipid material and a high metabolic demand[84]. The major by-products of these reactions are malondialdehyde (MDA), carboxyethylpyrrole and 4-hydroxynonenal (4-HNE) protein modifications. Lipid oxidation products have potent degenerative and inflammatory effects: both MDA and 4-HNE induce lysosomal dysregulation[85] and lipofuscin generation[86], which prevent the photoreceptor maintenance function of the RPE; MDA-modified proteins induce VEGFA expression in RPE cells and pro-inflammatory gene expression in macrophages[87], which is prevented by the binding of CFH to MDA; 4-HNE induces inflammasome activation[62] and RPE apoptosis[88]; and carboxyethylpyrrole (as discussed above) directly activates TLR2 signalling[72] and might function as an antigen for retina-specific autoantibody production[57].

Amyloid-β is also abundant in drusen[89]; it promotes RPE and photoreceptor damage[90] as well as retinal inflammation[63,70,91]. Other environmental factors, such as excessive light exposure, hypercholesterolaemia and smoking, damage the RPE and the retina by inducing pro-inflammatory effects that are associated with AMD (reviewed in REF. 92).

In summary, clear evidence supports a role for a range of pro-inflammatory factors that are abundant in the ageing retina as driving forces in AMD pathogenesis. We discuss below the special case of neovascular AMD, in which the breakdown of the blood–retinal barrier allows systemic immune cells unusual access to the retina. In this way, immune regulation of neovascular AMD represents an 'outside-in' phenomenon, in contrast to early dry AMD and geographic atrophy, in which immune activation is generally self-contained and self-inflicted.

Immune cell recruitment in neovascular AMD

Clear evidence from animal models supports dual roles for resident and invading macrophages/microglia in preventing and promoting neovascular AMD. These paradoxical effects have been attributed to two different functions of innate immune cells. First, as discussed above, the surveillance and phagocytosis of damaged cellular components is a necessary function of resident microglia for the maintenance of retinal health. Second, invading macrophages provide potent pro-angiogenic signals that exacerbate choroidal vessel invasion and pathogenesis. Importantly, whereas ample evidence supports the involvement of immune cells in both human neovascular AMD[93–96] and experimental choroidal neovascularization[36,37,97], the evidence for immune cell involvement in early and intermediate dry AMD and geographic atrophy is less impressive. Histological evidence showing relatively few immune cells in the atrophic area of geographic atrophy[98] indicates that local immune cells may not have a substantial role in this form of AMD. However, this

does not rule out immune-mediated tissue damage being involved in geographic atrophy, as emerging evidence highlights RPE-specific inflammasome-mediated degeneration as a driving force for RPE atrophy[59,61,62] (D. Shima and C. Chen, personal communication). In this respect, the role of innate immunity in neovascular AMD compared with geographic atrophy pathogenesis probably diverges at the local tissue level, and might represent both a consequence and a cause of the ultimate pathogenic outcome.

The development of neovascular AMD is accompanied by the recruitment of inflammatory cells to the site of vascularization, the consequence of which is the expression of numerous cytokines and chemokines that induce angiogenesis. Human choroidal neovascular lesions contain inflammatory cell infiltrates[93–96,99], and experimental depletion of macrophages decreases choroidal neovascularization in laser-injured mice[36,37]. Subsequent analyses have also shown that macrophages can have antiangiogenic properties in the context of experimental choroidal neovascularization; indeed, intraocular injection of macrophages before injury decreases laser-induced choroidal neovascularization[100]. Furthermore, mice that are deficient in the anti-inflammatory cytokine IL-10 have both an increased accumulation of macrophages in neovascular lesions and decreased choroidal neovascularization[100]. Indeed, our initial finding of spontaneous choroidal neovascularization in *Ccl2*- or *Ccr2*-deficient mice[39] can be generally interpreted as a systemic impairment of macrophage chemotaxis enhancing the development of choroidal neovascularization.

The contradictory contributions of macrophages that have been observed in choroidal neovascularization might be explained by differential macrophage polarization with respect to `M1 macrophages` (pro-inflammatory) and `M2 macrophages` (pro-angiogenic). So far, the role of macrophage polarization in AMD development has not been well characterized. One report identified an increased expression of the M1 marker CCL22 compared with an M2 marker, CXC-chemokine ligand 11 (CXCL11), in macrophages from individuals with AMD compared with those from the eyes of unaffected individuals[101], which indicates that inflammatory macrophage polarization might contribute to or be symptomatic of AMD development. Furthermore, choroidal M1 (inducible nitric oxide synthase $(iNOS)^{+}$) macrophages have been measured in proximity to subclinical neovascular AMD lesions[98]. Whether M1 macrophages contribute to pathological angiogenesis and whether increased numbers of M2 polarized macrophages are also present in choroidal or retinal neovascular membranes are not yet known but further studies will probably provide insights into the role of macrophage polarization in neovascular AMD. In mice, IL-10 was found to be a crucial factor driving M2 polarization, and inhibition of IL-10 decreased angiogenesis[100]. Although the role of IL-10 remains unclear (two studies show that IL-10 suppresses choroidal angiogenesis in other inflammatory contexts[102,103]), a follow-up study found that both IL-10 levels and M2 polarization are increased in the retinal macrophage population of aged mice[104]. Macrophage polarization as a directing factor in AMD is an intriguing possibility that merits further study.

In addition to macrophages, other analyses have found invading neutrophils to make variable contributions to promoting choroidal neovascularization in animal models. With respect to the number of invading cells, neutrophils constitute a large fraction of the inflammatory cell infiltrate in experimental neovascular lesions of the retina, although their contribution to laser-induced choroidal neovascularization is minimal when macrophage infiltration is impaired[97]. Other studies have found that antibody-mediated depletion of neutrophils prevents laser-induced choroidal neovascularization[97,105], and that they produce VEGF[105] as well as matrix metalloproteinase 9 (MMP9), which promote the loss of RPE barrier integrity[106]. However, neutrophils are not a major constituent of human AMD lesions[107]. The role of neutrophils in animal models of AMD probably represents an acute

injury response and may not reflect the mechanisms that occur in human AMD, which is a progressive disease that typically spontaneously develops over decades.

In addition to the roles of CCL2, CCR2 and CX_3CR1 in immune cell migration and retinal neovascularization, several other cytokines and chemokines have potentially important roles in the development of neovascular AMD (BOX 3).

An integrated model of immunity in AMD

The metabolic and immunological demands of the macular retina are unlike any other tissue in the body. Recent findings have brought diverse immunological aspects of AMD pathogenesis into sharper focus. We propose below a generalized model of AMD progression on the basis of these observations (FIG. 2).

We propose that the fundamental abnormality in AMD is a dysfunction of RPE cells; this can result from multiple types of ‘insult’ but the final common pathway is damage to an important metabolically active cell type that maintains homeostasis in the neuroretina. The importance of individual insults, especially in individual patients, in mediating RPE injury is unclear. Many of these insults, which can be both local and systemic, arise as a result of inappropriate immune activation, such as dysregulated complement activation, production of inflammasome activators and autoantibodies that are specific for retinal antigens.

Under normal conditions, the RPE layer continuously maintains immune suppression through both tight junction-mediated barrier integrity and anti-inflammatory cytokine production. Retinal microglia provide additional immune surveillance by clearing cell debris. Many different hazards can create initial foci of tissue damage and, importantly, also render the retina unable to removing dying, dead or stressed retinal cells. This concept is similar to that of other progressive age-related diseases (BOX 4). The inability to sufficiently cope with increased tissue damage leads to drusen accumulation, and the drusen function as concentrated immunostimulatory punctae. As drusen accumulate, the development of AMD depends on the type of immune response. In the neovascular form of AMD, breakdown of the blood–retinal barrier provides circulating immune cells with unusual access to a highly immunogenic environment, which results in macrophage recruitment and activation. When these macrophages encounter an avascular, metabolically demanding tissue, they initiate a VEGFA-dependent neovascular response. The complex mixture of pro-angiogenic cytokines that they produce results in a leaky, abnormal vascular network that is associated with fluid leakage and, if left untreated, in the eventual development of fibrosis[108]. In the case of geographic atrophy, circulating immune cells are denied access to the degenerating retina. Rather than a proliferative vascular response, the inability to repair the increasing tissue damage leaves large, confluent areas of degenerated RPE and photoreceptors. The degenerating area expands as diseased RPE cells damage neighbouring cells by releasing toxic inflammatory stimulators and cytokines.

In this model, AMD arises from both immune hypoactivity in the case of inadequate tissue repair and immune hyperactivity in the case of complement-mediated tissue damage. One emerging theme from such a model is that pan-immunosuppression could potentially reduce late-stage AMD; however, by preventing endogenous immune-mediated tissue repair processes, new foci of dysfunction and disease might be created in the remaining functional retina. Another prediction from this model is that the most effective immunotherapies must be highly target- and context-specific to inhibit specific pathogenic factors but to only minimally affect tissue repair mechanisms. As the search for new and improved AMD therapeutics continues, we must not turn a blind eye to the Janus-faced nature of ocular immunity.

Immune-based therapy for AMD

Any discussion about the future potential for AMD therapeutics must be placed in the context of the current standard of care. The management of neovascular AMD has undergone a transformation over the past 10 years as a result of the astonishing success of VEGFA-targeted therapies (<10% of patients treated with VEGF-targeted agents exhibit considerable vision loss[14]). The 'off-label' use of bevacizumab (Avastin; Genentech/Roche) — a humanized monoclonal antibody targeting VEGFA — which improves or maintains vision in the majority of patients and is available in doses that cost in the order of tens of dollars[18,109], has somewhat diminished future prospects for the development and commercialization of new drugs to treat neovascular AMD. Nonetheless, in cases in which VEGFA-based treatment is insufficient or ineffective, or in the case of geographic atrophy, combination therapies or immune-based therapies that are directed towards alternative targets are being explored.

As our understanding of the role of immunity in AMD has advanced, we have gained an appreciation of the nuanced and conflicting roles of the immune system as a protector, an instigator and a driver of retinal degeneration. The complex cell type-, pathological context-, temporal- and pathway-specific aspects of immune regulation of retinal health demand targeted medicine. It is perhaps a lack of understanding or appreciation of these facts that has led to the numerous failures of immune-based therapies; for example, steroidal anti-inflammatory drugs have only achieved very modest success as an adjunct to VEGFA-targeted treatment[110,111].

Complement-based diagnostics and therapeutics

Despite the knowledge that complement activation is a genetic risk factor for AMD development, so far the clinical predictive or therapeutic value of these findings has not been realized. Several clinical trials using complement inhibition-based treatment of AMD have produced disappointing primary outcome data that show minimal improvement in visual acuity or reduction in disease progression (TABLE 1). It is also worth noting that, despite millions of dollars having been spent and nearly a decade of intense research, the predictive value of genetic screening for AMD is limited by the simple reality that the relatively inexpensive procedure of taking a patient's history and a routine eye examination provide more relevant information with respect to current and potential future clinical AMD development. Data about whether polymorphisms in complement genes and other AMD risk factor genes can be used to predict the response to VEGFA-targeted therapies are conflicting[112,113]; however, given the lack of alternative treatments that are available, these findings are unlikely to change the management of neovascular AMD. Similarly, genetic models fail to predict progression to geographic atrophy[114,115] but without an approved therapy for geographic atrophy, once again, there is no immediate clinical use for such genetic information.

Despite considerable interest, no complement-based therapeutic has yet progressed to a Phase III clinical trial. The early results from complement-based strategies have dampened enthusiasm for using these as targets and have highlighted our lack of basic understanding about the mechanisms by which complement factors influence AMD development. Indeed, one mechanism that is apparently involved in complement-mediated AMD development is the upregulation of VEGFA[48,116] — this only reminds us that VEGFA-based therapies already do an excellent job in the majority of patients.

Other immune-based clinical trials

So far, immune-based therapies have exclusively evaluated anti-inflammatory agents. The successes of these therapies have been measured in terms of a reduction in the number of VEGFA-targeted injections that are needed for neovascular AMD treatment; it is possible that a positive outcome results from a better response to trauma from repeated intraocular injections rather than prevention of a fundamental disease process. Although reducing the number of injections is an important goal, no trial has reported improvements in visual acuity as a result of anti-inflammatory monotherapy or adjunctive therapy.

Ocular immunity is involved both in maintaining visual homeostasis and in driving AMD pathogenesis, so we support the development of new targeted therapies as the most promising approach to treat this disease; however, this awaits a more thorough investigation of the molecular mediators of AMD. Recent studies have identified new potential targets (such as IL-1β, IL-18 and inflammasome components) that merit further evaluation for potential intervention. The search for new targets will also benefit from a re-evaluation of current animal models of advanced AMD. Laser photocoagulation- induced neovascularization effectively models the VEGFA-dependent angiogenic component but probably does not mimic the inflammatory component of human neovascular AMD and has a timescale that differs by several orders of magnitude. Similarly, no animal model has yet been developed that mimics all of the changes that are associated with advanced geographic atrophy.

We postulate that the future of AMD-based therapies lies in pathomechanism-driven discovery and caution against demanding that putative targets be validated in GWASs. VEGFA is a prime example of a validated target for AMD that arose via mechanistic biology and which would not withstand the scrutiny of statistical genetics. Immunology-based insights are likely to have an important role in the future of clinical AMD management once a more thorough understanding of ‘friend’ and ‘foe’ in the context of AMD pathogenesis is realized.

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Glossary

Retina	A highly organized and specialized neural network where light is converted into electrical impulses. Diseases of the retina such as age-related macular degeneration are leading causes of blindness in the developed world.
Macula	A specialized region of the retina densely populated with cone photoreceptors, which is responsible for fine visual acuity. Degeneration of the macular photoreceptors following either atrophy of the retinal pigmented epithelium (geographic atrophy) or fluid leakage from choroidal neovessels

	(neovascular age-related macular degeneration (AMD)) is the cause of vision loss in AMD.
Complement factor H	(CFH). A negative regulator of alternative complement pathway activation. Single nucleotide polymorphisms in CFH that reduce its inhibitory potential are responsible for a substantial proportion of the genetic risk for the development of age-related macular degeneration.
Neovascular AMD	(also known as exudative or 'wet' age-related macular degeneration). Characterized by degeneration of the macula following fluid leakage from choroidal neovessels that have invaded the retina. The use of vascular endothelial growth factor A-targeted therapies has revolutionized the management of this disease, which accounts for the majority of blindness that results from AMD.
Photoreceptors	Specialized neurons that are responsible for the conversion of light into biochemical signals.
Blood–retinal barrier	A tightly controlled transport barrier that is maintained by tight junctions in the retinal capillary endothelium (comprising the inner blood–retinal barrier), and by the Bruch membrane and the retinal pigmented epithelium monolayer (comprising the outer blood–retinal barrier). Integrity of the blood–retinal barrier is important for control of fluid leakage, solute transport and immune quiescence, all of which support the functional homeostasis of the retina.
Retinal pigmented epithelium	(RPE). A monolayer of epithelial cells that has multiple essential roles in visual function, including recycling components of the visual cycle, secreting trophic factors and maintaining the outer blood–retinal barrier. The RPE is widely considered to be the focal point of age-related macular degeneration pathogenesis, in which breakdown of the RPE leads to secondary photoreceptor degeneration.
Drusen	Discrete extracellular deposits that commonly precede the development of age-related macular degeneration, and that are comprised of numerous cellular and inflammatory factors.
Geographic atrophy	(also known as end-stage 'dry' age-related macular degeneration (AMD) or atrophic AMD). A disease affecting the macula in which the retinal pigmented epithelium can no longer support photoreceptor function owing to spontaneous degeneration of large confluent regions. Although geographic atrophy occurs less frequently than neovascular AMD (approximately 50% as common), there are no currently approved therapies.
Immune privilege	The property of a tissue being tolerant to antigen. Retinal immune privilege is maintained by blood–retinal barrier integrity and the absence of functional lymphatic circulation.
Fundus photography	A common method for visualizing the retinal pigmented epithelium, retina and retinal circulation photographically.

	Funduscopy is used by ophthalmologists to diagnose retinal disorders such as age-related macular degeneration.
Para-inflammation	A state in which tissue homeostasis is maintained by low-grade inflammatory-based clearance of noxious stimuli. In the retina, para-inflammation may persist and ultimately contribute to age-related macular degeneration through increased immune cell infiltration and activation at sites of tissue damage.
Genome-wide association studies	(GWASs). The process by which genetic variations and disease phenotypes are statistically correlated. Linkage studies carried out with markers located across the entire genome that were traditionally carried out with approximately 300 markers of simple sequence-length repeats but that have been more recently carried out with approximately 1–5 million single nucleotide polymorphisms.
Alternative pathway of complement activation	An evolutionarily ancient innate immune process by which microorganisms are destroyed through opsonization and activation of the membrane attack complex. According to the ‘complement hypothesis’, misactivation of, and/or the inability to appropriately inhibit, the alternative pathway results in retinal tissue damage and drives age-related macular degeneration pathology.
Carboxyethylpyrrole-adducted proteins	Proteins that are modified through the oxidation of the fatty acid docosahexaenoic acid. These adducts are abundant in the retina. They can have direct pro-inflammatory effects through pattern recognition, and autoantibodies that recognize them are abundant in the circulation of patients with age-related macular degeneration.
M1 macrophages	A pro-inflammatory or ‘classically activated’ subset of macrophages, which are characterized by phagocytic activity and expression of particular inflammatory cytokines (such as tumour necrosis factor) and inflammatory mediators (such as inducible nitric oxide synthase).
M2 macrophages	A pro-angiogenic or ‘alternatively activated’ subset of macrophages, which are characterized by the expression of particular angiogenic cytokines (such as vascular endothelial growth factor A) and anti-inflammatory mediators (such as arginase).

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Box 1 | The pro-inflammatory components of drusen

Drusen are extracellular deposits that accumulate between the retinal pigmented epithelium (RPE) and the Bruch membrane, or sometimes between the RPE and the photoreceptors[117]. The RPE is a major source of drusen components, and extracellular and serum-derived factors are also highly abundant[12]. RPE monocultures can be made to produce 'drusen-like' deposits and to release factors that are abundant in drusen, such as vitronectin, clusterin, serum amyloid P, complement proteins and apolipoprotein E (APOE)[118]. Local drusen biogenesis probably occurs as a result of a failure of lysosome- and autophagy-mediated digestion of cellular components[119]. The accumulation of waste material is eventually extruded in exosomes and might function as nucleating sites for the deposition of other extracellular factors[51]. Data on the genetic influence of drusen formation have not conclusively determined whether complement dysregulation has a role in this process[22,120,121]. Furthermore, drusen also contain components of dendritic cell processes, which supports a role for local immunity in their biogenesis[122].

Drusen consist of aggregated intracellular, extracellular and secreted proteins, and lipids and cellular components. Proteomic, histological and biochemical analyses show that the major components of human drusen include albumin, APOE, complement factors and related proteins (including complement components C1q, C3, C5 and C5b-9, and vitronectin), immunogloblulins and amyloid-β[123–125]. Interestingly, the protein content of drusen from the eyes of individuals who do not have age-related macular degeneration (AMD) is similar to that from the eyes of individuals with AMD[123]. The proteins in drusen commonly contain carboxyethylpyrrole modifications[123]. Drusen also contain double-stranded RNAs[60].

Drusen extracts can induce inflammasome activation[58], and individual components of drusen can experimentally induce profound cellular effects: for example, C1q induces inflammasome activation[58] and amyloid-β induces retinal degeneration[43]. However, because drusen are solid, insoluble deposits, it is difficult to infer the *in situ* activity of drusen constituents — which may have abnormal conformation and low biological availability — from cell culture treatments of soluble factors.

Thus, the relationship between drusen formation and AMD is entirely correlative — increased numbers and size of drusen correlate with increased risk of developing advanced AMD. Whether drusen are a cause or a symptom of AMD or some combination of the two is unknown. Although AMD almost never occurs in the absence of prior drusen formation, the transition from intermediate AMD to geographic atrophy is associated with drusen regression[126]. Indeed, drusen formation could be an adaptive process to prevent the widespread dispersal of activated complement and other toxic constituents in the retina. One might speculate that the spontaneous release of drusen material might be an immediate cause of RPE loss in geographic atrophy.

Box 2 | Genetics of complement in AMD

The risk of developing age-related macular degeneration (AMD) is influenced not only by the common complement factor H (CFH) polymorphic variant 402His (the minor allele) but also by multiple other variants (common, uncommon and rare)[6–9,50]. The complement factor H-like protein (CFHL1) is generated by alternative splicing of the *CFH* gene[127]. The complement factor H-related proteins 1–5 (CFHR1–5) arose by gene duplication events and are encoded just downstream of *CFH* on the long arm of chromosome 1. Their function is poorly understood. A simple concept is that CFHR1 competes with CFH for binding to substrates and thereby reduces the effectiveness of CFH. This hypothesis is consistent with a deletion of *CFHR1* and *CFHR3* being protective against AMD[128].

These studies of CFH and related proteins, combined with the identification of risk and protective variants in alternative pathway complement activators and other regulators, provide powerful evidence implicating overactivation of the alternative complement pathway in AMD. A finding that is consistent with this idea is that the protective variants result in less alternative pathway activity, whereas risk variants result in more activity[129]. In summary, common and rare variants in multiple members of a pro-inflammatory pathway of innate immunity are associated with the same disease; those that decrease the function of the pathway are protective and those that increase the function create risk.

Box 3 | Cytokine and chemokine signalling in neovascular AMD

In addition to the main molecules and pathways involved in neovascular age-related macular degeneration (AMD), several important secreted factors might also affect disease processes and could be possible therapeutic targets.

Eotaxins

Eotaxins (CC-chemokine ligand 11 (CCL11), CCL24 and CCL26) and their receptor CC-chemokine receptor 3 (CCR3) are highly expressed in neovascular lesions before the retinal invasion by new blood vessels[130]. CCL11 levels are increased in retinal specimens from aged humans and in choroidal endothelial cells from patients with AMD[131], and both CCL11 and CCL24 levels are increased in the circulation of patients with AMD[132,133]. CCR3 antagonism prevents laser-induced choroidal neovascularization, and CCR3 expression may be a useful biomarker of subclinical AMD[130,134]. Studies of eotaxin signalling might provide therapeutic insights that enable neovascular AMD to be diagnosed and treated at early stages.

IL-6

Increased interleukin-6 (IL-6) levels are found in ocular fluids of patients with neovascular AMD and they predict AMD progression[135]. The pro-angiogenic effects of IL-6 are well-described in the context of tumour angiogenesis and involve the upregulation of vascular endothelial growth factor A[136]. Genetic ablation of IL-6 or its receptor decreases laser-induced choroidal neovascularization[137]. IL-6 signalling also promotes degeneration of the retinal pigmented epithelium (RPE) following lipopolysaccharide stimulation[138]. Thus, IL-6 might have dual pathogenic functions by promoting both angiogenesis and RPE degeneration in advanced AMD.

CXCL10

CXC-chemokine ligand 10 (CXCL10), which is a potent anti-angiogenic chemokine, is highly expressed in the retina of patients with AMD[132] and laser-injured mice[139]. Choroidal endothelial cells express CXC-chemokine receptor 3 (CXCR3) — the CXCL10 receptor — and genetic ablation of CXCR3 exacerbates laser-induced choroidal neovascularization in mice[139]. Thus, CXCL10 might be an important endogenous negative regulator of neovascular AMD.

TNF

Tumour necrosis factor (TNF) is abundant in human choroidal neovascular membranes[95], and genome-wide association studies have identified two polymorphisms in the TNF receptor *TNFRSF10* (which encodes the protein TAILR1) that are associated with neovascular AMD[140,141]. Inhibition of TNF by antibodies[142,143] or recombinant receptor fragments[144] decreases experimental choroidal neovascularization in laser-injured rodents. However, TNF-targeted therapy was not well tolerated in human trials of neovascular AMD[145].

CXCL12

CXCL12 is a potent chemoattractant. It drives the recruitment of endothelial progenitor cells and haematopoietic stem cells to regions of neovascularization[146]; these cells constitute as much as 50% of the neovascular lesions in laser injury models. In laser-induced choroidal neovascularization, CXCL12 expression is increased, and inhibition of its receptor CXCR4 decreases angiogenesis[146,147]. Conversely, expression of CXCL12 is variable in neovascular human AMD (two of ten[148] and zero of four[149] neovascular

lesions stained positive for CXCL12 expression). Thus, the contribution of CXCL12 signalling in human AMD has not been firmly established.

Box 4 | Age-dependent degenerative diseases

Most tissues require methods to clear dead cells and intracellular and extracellular waste. Necrosis and apoptosis are two examples of these methods, which can be triggered by 'wear and tear' (so-called normal ageing phenomena), ischaemia–reperfusion injury, trauma, foreign agents (microorganisms) and toxins. The complement system is one of the first (if not the first) responder to such events and is activated by necrosis and apoptosis[150]. It is involved in degenerative arthritis[151] and atherosclerosis[152,153], including complement activation by lipid particles, and there is also a role for the complement pathway in the development of abdominal aortic aneurysm. In this regard, stressed and damaged retinal pigmented epithelium (RPE) could be seen as analogous to cartilage loss predisposing an individual to osteoarthritis[151]. Relative to atherosclerosis, drusen accumulation could be viewed as comparable to extracellular lipid deposits[154]. Indeed, a recent collaborative genome-wide association study identified age-related macular degeneration (AMD) risk variants in loci that are also involved in atherosclerosis and lipid metabolism[155]. Although the nature of the debris, the generation of the inflammatory response and the clearance mechanisms are likely to have unique features in specialized tissues, the innate immune response and the degradative and clearance processes will have overlapping features[92]. Thus, the four great diseases of ageing — Alzheimer's disease, atherosclerosis, AMD and degenerative arthritis — can all be viewed in this perspective: they feature a generally unknown age-dependent degenerative and destructive process, as well as an accumulation of self debris: amyloid (in Alzheimer's disease), lipids (in atherosclerosis), drusen (in AMD) or glycosaminoglycans (in degenerative arthritis). The debris is not adequately eliminated and the dying cells (neurons, RPE cells or chondrocytes) are not easily replaceable. The innate immune response to this damage is insufficient. Initially, the innate immune response is presumably beneficial and promotes longevity, but at later stages in the disease process it is probably detrimental.

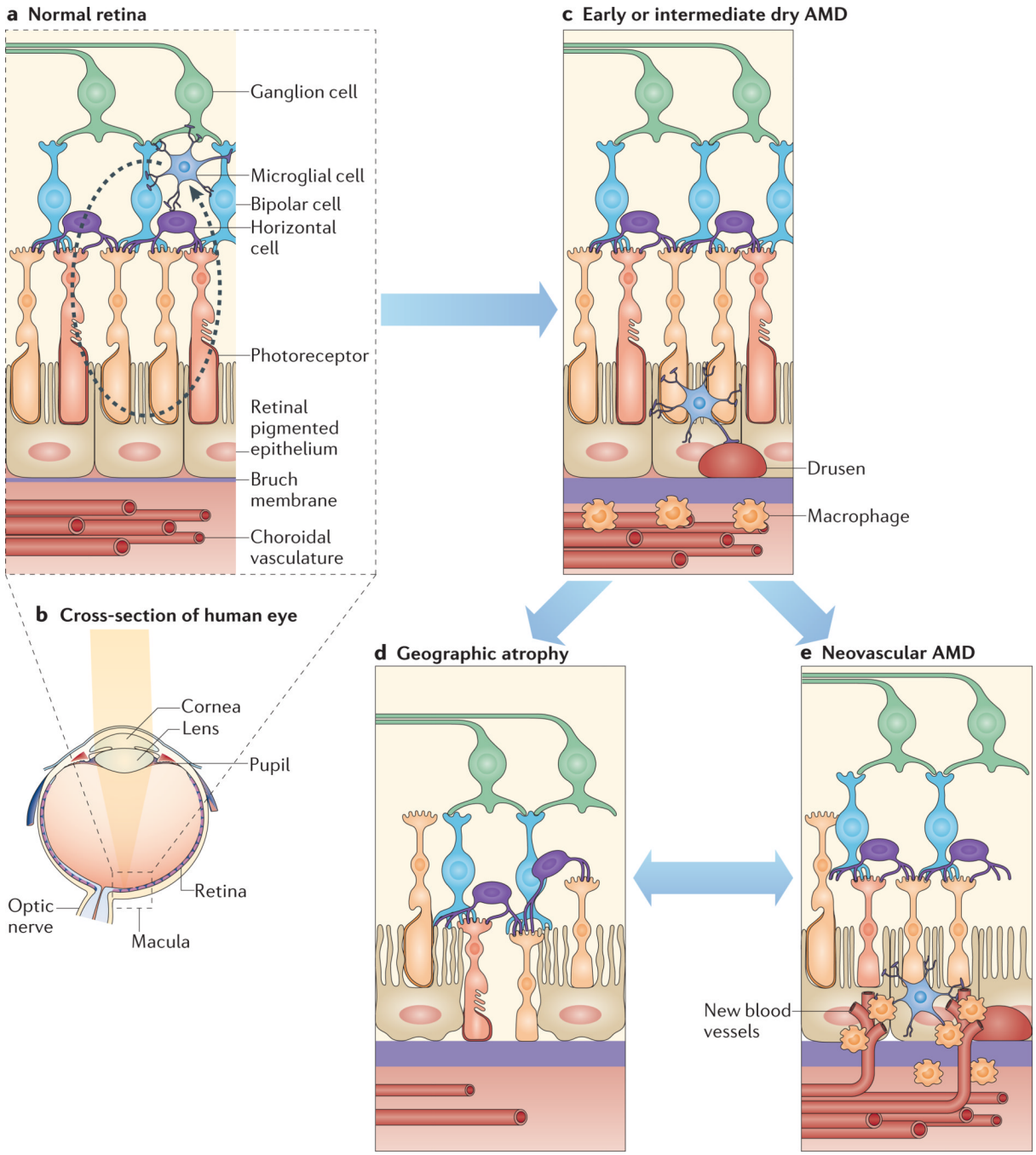

Figure 1. Retinal anatomy in health and disease

The retinal anatomy is composed of several layers (part **a**); and a cross-section of the human eye (part **b**) shows focusing of light into the macular area, which is a dense collection of retinal photoreceptors. Normal retinal architecture (part **a**) is comprised of (from anterior to posterior) a ganglion cell layer, resident retinal microglia, bipolar cells, horizontal cells, the photoreceptor layer, the retinal pigmented epithelium (RPE), the Bruch membrane and a choroidal vascular network. Normal microglia migrate into and out of the subretinal space (as shown by the dashed arrow). Early or intermediate dry age-related macular degeneration (AMD) (part **c**) is associated with the accumulation of subretinal drusen and microglia and

of choroidal macrophages and a thickened Bruch membrane. Geographic atrophy (part **d**) is the advanced form of dry AMD, which is characterized by confluent regions of RPE and photoreceptor degeneration as well as constriction of choroidal blood vessels. Neovascular AMD (part **e**) is characterized by the invasion of abnormal, leaky choroidal blood vessels and accompanying macrophages into the retina through breaks in the Bruch membrane, which leads to photoreceptor degeneration.

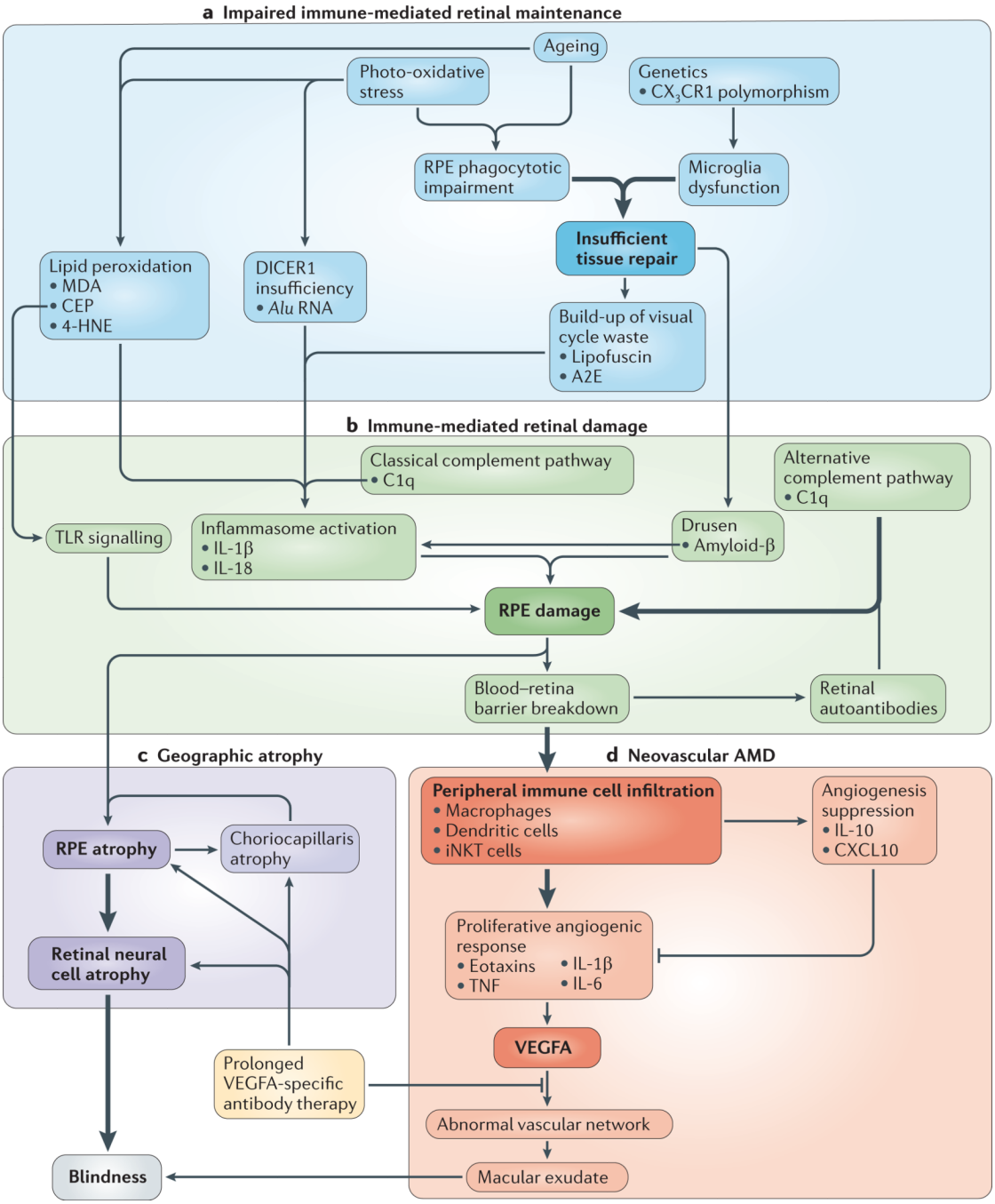

Figure 2. An integrated model of AMD pathogenesis

The cell types, environmental factors and immune pathways that contribute to age-related macular degeneration (AMD) are categorized as either impaired immune-mediated retinal maintenance (part **a**) or immune-mediated retinal damage (part **b**). The combination of these factors leads to either geographic atrophy (part **c**) or neovascular AMD (part **d**). The most important pathways are highlighted in bold. The earliest known steps in AMD pathogenesis (part **a**) reflect a reduced capacity to manage the metabolic demands of the retina. The convergence of genetic, environmental and metabolic factors leads to a state in which the accumulation of toxic elements (such as lipid peroxidation by-products, lipofuscin and *Alu*

RNAs) ultimately tips the balance towards immune activation. Once it is replete with unwanted waste material (part **b**), the retina is the target of inappropriate immune activation. The toxic contents of the retina induce inappropriate activation of diverse immune pathways, including classical and alternative complement pathways, the inflammasome and Toll-like receptor (TLR) signalling. Ultimately, the sustained activation of these pro-inflammatory and damaging pathways leads to advanced AMD. In the case of geographic atrophy (part **c**), sustained damage to the retinal pigmented epithelium (RPE) leads to the development of degeneration of the RPE, the choriocapillaris and, finally, neural retinal cells. In neovascular AMD (part **d**), breakdown of the blood–retinal barrier results in immune cell trafficking into the retina, which drives vascular endothelial growth factor A (VEGFA)-dependent neovascularization, causing blindness. 4-HNE, 4-hydroxynonenal; A2E, *N*-retinyl-*N*-retinylidene ethanolamine; C1q, complement component 1q; CEP, carboxyethylpyrrole; CX_3CR1, CX_3C-chemokine receptor 1; CXCL10, CXC-chemokine ligand 10; IL, interleukin; iNKT, invariant natural killer T; MDA, malondialdehyde; TNF, tumour necrosis factor.

Table 1

Current immune-based clinical trials for AMD

Compound	Target	Structure	Indications	Trial status	Trial number
POT-4 (Alcon)	C3	Cyclic peptide inhibitor	Neovascular AMD	Phase I has been completed 93% of patients had no improvement in visual acuity (S. Bakri, personal communication)	NCT00473928
Eculizumab (Alexion)	C5	Monoclonal antibody	Dry AMD (presence of drusen) and geographic atrophy	Phase II is ongoing There was no improvement in mean visual acuity or area of geographic atrophy in the first 6 months of the trial (P. Rosenfeld, personal communication)	NCT00935883
LFG316 (Novartis)	C5	Monoclonal antibody	Geographic atrophy Advanced neovascular AMD	Phase II is ongoing (Phase I data are unpublished)	NCT00709527 NCT00950638
ARC1905 (Ophthotech)	C5	Aptamer	Neovascular AMD Geographic atrophy	Phase I has been completed (Phase I data are unpublished) Phase I is ongoing	NCT00709527 NCT00950638
FCFD4514S (Genentech)	Factor D	Monoclonal antibody (Fab fragment)	Geographic atrophy	Phase II is ongoing (Phase I data are unpublished)	NCT01602120
Sonepcizumab (Lpath)	Sphingosine-1-phosphate	Monoclonal antibody	Neovascular AMD, ‘subresponders’ to VEGFA-targeted therapy	Phase II is ongoing (Phase I data are unpublished)	NCT01414153
Glatiramer acetate (Teva)	Unknown anti-inflammatory target	Small peptide	Early and intermediate dry AMD	Phase II and Phase III are inactive A small cohort (N = 8) showed reduction in drusen size156	NCT00466076
RN6G (Pfizer)	Amyloid-β	Monoclonal antibody	Geographic atrophy	Phase II is ongoing (Phase I data are unpublished)	NCT01577381
Daclizumab (Hoffman-La Roche)	IL-2 receptor subunit-α	Monoclonal antibody	Neovascular AMD, co-administered with VEGFA-targeted therapy	Phase II has been completed It reduced the frequency of VEGFA-specific injections in four patients[157]	NCT00304954
Bromfenac (ISTA)	Cyclooxygenase	NSAID	Neovascular AMD, co-administered with VEGFA-targeted therapy	Phase II has been completed It reduced the number of VEGFA-specific injections in 16 patients but had no benefit for visual acuity[158]	NCT00805233
Infliximab (Janssen)	TNF	Monoclonal antibody	Neovascular AMD, co-administered with VEGFA-targeted therapy	Phase II has been completed It reduced the number of VEGFA-specific injections in three patients[157]	NCT00304954

Compound	Target	Structure	Indications	Trial status	Trial number
Adalimumab (Abbott)	TNF	Monoclonal antibody	Neovascular AMD, co-administered with VEGFA-targeted therapy	Phase II has been completed (data are unpublished)	NCT01136252
Triamcinolone acetonide (Bristol-Myers Squibb)	Broad anti-inflammatory target	Corticosteroid	Neovascular AMD, co-administered with VEGFA-targeted therapy	Phase III has been completed It reduced the number of VEGFA-specific injections but had no benefit for visual acuity[159]	NCT00370370
Fluocinolone acetonide (Alimera Sciences)	Broad anti-inflammatory target	Corticosteroid	Geographic atrophy	Phase II (Phase I data are unpublished)	NCT00695318

AMD, age-related macular degeneration; C, complement component; IL-2, interleukin-2; NSAID, non-steroidal anti-inflammatory drug; TNF, tumour necrosis factor; VEGFA, vascular endothelial growth factor A.