Immunological Aspects of Age-Related Macular Degeneration

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Michael J. Allingham, Anna Loksztejn, Scott W. Cousins, and Priyatham S. Mettu

Abstract

Increasing evidence over the past two decades points to a pivotal role for immune mechanisms in age-related macular degeneration (AMD) pathobiology. In this chapter, we will explore immunological aspects of AMD, with a specific focus on how immune mechanisms modulate clinical phenotypes of disease and severity and how components of the immune system may serve as triggers for disease progression in both dry and neovascular AMD. We will briefly review the biology of the immune system, defining the role of immune mechanisms in chronic degenerative disease and differentiating from immune responses to acute injury or infection. We will explore current understanding of the roles of innate immunity (especially macrophages), antigen-specific immunity (T cells, B cells, and autoimmunity), immune amplifications systems, especially complement activity and the NLRP3 inflammasome, in the pathogenesis of both dry and neovascular AMD, reviewing data from pathology, experimental animal models, and clinical studies of AMD patients. We will also assess how interactions between the immune system and infectious pathogens could potentially modulate AMD pathobiology via alterations in in immune effector mechanisms. We will conclude by reviewing the paradigm of "response to injury," which provides a means to integrate various immunologic mechanisms along with nonimmune mechanisms of tissue injury and repair as a model to understand the pathobiology of AMD.

Keywords

Age-related macular degeneration · Drusen · Choroidal neovascularization · Geographic atrophy · Pathobiology · Immunology · Innate immunity · Macrophages · Monocytes · T cells · B cells · Autoimmunity · Complement · NLRP3 inflammasome

6.1 Introduction

Age-related macular degeneration (AMD) is a progressive degenerative disorder, involving the

M. J. Allingham · A. Loksztejn · P. S. Mettu (✉)
Department of Ophthalmology, Duke Center for Macular Diseases, Duke Eye Center, Duke University School of Medicine, Durham, NC, USA
e-mail: prithu.mettu@duke.edu

S. W. Cousins
Department of Ophthalmology, Duke Center for Macular Diseases, Duke Eye Center, Duke University School of Medicine, Durham, NC, USA

Department of Immunology, Duke University School of Medicine, Durham, NC, USA

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retinal pigment epithelium (RPE), neurosensory retina, Bruch's membrane, and choriocapillaris, and represents the leading cause of vision loss among the elderly [1, 2]. AMD pathogenesis is complex and multifactorial and includes aging, genetic, systemic health, and environmental risk factors [3–11]. While the specific mechanisms mediating disease onset and progression remain unknown, increasing evidence over the past two decades points to a pivotal role for immune mechanisms in AMD pathobiology.

This chapter will explore immunological aspects of AMD, with a specific focus on how immune mechanisms modulate disease phenotype and severity and how components of the immune system may serve as triggers for disease progression. We will briefly review the biology of the immune system, defining the role of immune mechanisms in chronic degenerative disease and differentiating from immune responses to acute injury or infection. We will explore current understanding of the roles of innate immunity, adaptive immunity, and immune amplifications systems, especially complement activity and the NLRP3 inflammasome, in pathogenesis of the various stages of AMD. Note that the role of resident retinal microglia is beyond the scope of this chapter and will be discussed in a separate dedicated chapter. Finally, we will conclude by reviewing the paradigm of "response to injury," which provides a means to integrate various immunologic mechanisms along with nonimmune mechanisms of tissue injury and repair as a model to understand the pathogenesis of AMD.

6.2 Biology of Immunology Relevant to AMD

In general, there are two types of immune responses: innate and adaptive immunity [12–14]. Innate immunity includes stereotyped, nonspecific responses to various components, including microbes, foreign substances, products of cellular injury, or other potentially offending stimuli. Adaptive immunity includes antigen-specific responses that are designed to rid the body of very specific foreign (i.e., not "self") components, using targeted and coordinated antibody and cellular responses. While innate and adaptive immune mechanisms are typically studied in the context of acute responses to infection injury, both types of immune responses have potential relevance to chronic degenerative diseases such as AMD. We will review specific biologic aspects of innate and adaptive immunity that are relevant to AMD.

6.2.1 Innate Immunity

Innate immunity includes specific immune cell types, especially macrophages and neutrophils, that respond to various stimuli via a pattern recognition response. Such stimuli include microbe-associated molecules (e.g., pathogen-associated molecular patterns, or PAMPs), toxins, or cellular debris resulting from injury (e.g., damage-associated molecular patterns, or DAMPs) [15–17]. Innate immune cells bind to these stimuli via specific pattern recognition receptors (PRRs), (e.g., families of PRRs such as Toll-like receptors (TLRs)). Activation of PRRs on innate immune cells subsequently triggers a stereotyped, antigen-independent signaling, and cellular response. Innate immune cells then [1] phagocytose such stimuli, sequestering, degrading, and processing stimuli, [2] generate biochemical mediators that affect the surrounding microenvironment and recruit additional inflammatory cells, and [3] serve as antigen-presenting cells (APCs), displaying processed antigen on the cell surface to other immune cells, especially cells of the adaptive immune system [18]. PRRs are also frequently found on the cell surface of many parenchymal (nonimmune) cell types, (e.g., as with TLRs on the surface of RPE cells), so stimuli such as PAMPs and DAMPs can also directly stimulate PRRs on parenchymal cells to initiate a direct cellular response from nonimmune cells [19, 20].

In the innate immune response, the PRR signaling mechanisms in monocytes, macrophages, and neutrophils have been genetically and evolutionarily predetermined to recognize conserved molecular patterns on different triggering stimuli. These molecular patterns frequently include specific amino acid sequences, certain lipoproteins,

certain phospholipids, or molecular components. While there is tremendous heterogeneity for such stimuli, the key feature of innate immune response is that these varied stimuli frequently trigger the same stereotyped cellular response. Thus, PRRs s, and the innate immune response they activate, are generally conserved among individuals within a given species [18].

Classically, the fundamental innate immune response is considered within the context of acute infection. For example, in endophthalmitis, or infection within the eye, microbial-derived molecules (which are frequently toxic to host tissue) or cellular debris arising from damaged host tissue bind PRRs, activating neutrophils and monocytes, which in turn phagocytose stimuli, produce inflammatory cytokines, and stimulate the recruitment of additional immune cells [18]. Microbial-derived stimuli can also activate receptors on retinal neurons, exacerbating cellular injury. The PRR-activated mechanisms and effector responses to bacteria such as *Staphylococcus* are nearly identical to those of other organisms, since they are determined by the recognition of conserved patterns or motifs that may be present across different families of stimuli of microbes or cellular debris in the microenvironment. Of course, the innate immune response in the setting of chronic degenerative diseases such as AMD is substantially different from the response encountered in the setting of acute infection or injury.

6.2.1.1 Monocytes and Macrophages

The monocyte, innate immune cell circulating in the blood and originating from the bone marrow, and the macrophage, phagocytic innate immune cell in the tissue, are vital immune cells [15]. Monocytes are relatively large cells (12–20 μm in suspension, but up to 40 μm in tissues) and traffic through many normal sites. Upon tissue infiltration, they can give rise to blood-derived macrophages. Macrophages include two populations: tissue-resident macrophages and blood-derived macrophages. Tissue-resident macrophages are comprised of either precursor cells that migrated into the tissue during embryonic development (i.e., "yolk-sac" or fetal liver derived) or monocyte-derived cells that migrated into a tissue weeks or months previously; the relative proportion of embryonic precursor-derived vs. monocyte-derived is tissue-dependent and at least partially determined by ease of access of circulating monocytes to the specific tissue compartment [21]. The primary function of tissue-derived macrophages is tissue-resident macrophages typically acquire tissue-specific properties and may be distinguished by specific cellular markers. In many tissues, resident macrophages have been given tissue-specific names (e.g., microglia in the brain and retina) [22–24]. In contrast, blood-derived macrophages represent monocytes that have recently migrated from the blood into a tissue locus, usually within a few days and typically in the setting of inflammation or injury, having transformed into macrophages from the monocyte but still maintaining many properties of the circulating cell [21].

Macrophages serve three primary functions: as scavengers to clear cell debris and pathogens while limiting significant tissue damage, as antigen presenting cells for T lymphocytes, and as inflammatory effector cells. Conceptually, macrophages exist in different levels or stages of metabolic and functional activity, each representing different "programs" of gene activation and mediator synthesis. There are multiple paradigms that have been put forth to understand differential biology of monocyte and macrophages, the majority of which is focused on differences in inflammatory and effector functions. In the *conventional paradigm*, infiltrating "quiescent" M0 monocytes can become differentiated into classically activated ("M1") or alternatively activated ("M2") macrophages within inflamed tissues [25–27]. Typical activational stimuli that promote activation of M1 macrophages include bacterial toxins (such as lipopolysaccharide (LPS)), antibody-coated pathogens, complement-coated debris, or certain cytokines [28–30]. While M1 macrophages are proinflammatory, M2 macrophages mediate tissue repair, angiogenesis, and resolution of inflammation [31–35]. M2 macrophages contribute to physiologic processes

such as fibrosis, wound repair, extracellular matrix turnover, and angiogenesis [36–44]. However, these processes can contribute to chronic injury in the absence of inflammatory cell infiltration or widespread tissue destruction. For example, M2 macrophages play important roles in the pathogenesis of atherosclerosis, glomerulosclerosis, osteoarthritis, keloid formation, pulmonary fibrosis, and other noninflammatory disorders, indicating that the "repair" process is not always beneficial to tissues with complex morphologies with precise structure-function requirements [45–49]. The M1-M2 dichotomy in this conventional paradigm derives primarily from in vitro studies and presupposes that the fate and effector function of recruited monocytes are determined in bulk populations of cells by differentiation factors within an inflamed tissue microenvironment, and that macrophages can be "switched" from M1 to M2, and vice versa, either by manipulating factors in the tissue microenvironment or by altering intracellular signaling or transcriptional activity within macrophages that regulate the cell's M1 or M2 identity.

Investigators from the monocyte ontogeny field have proposed an alternative paradigm: Functionally distinct monocyte subsets can be identified in the blood according to specific cell surface markers [50, 51], which include Ly6C in mice and CD14 and CD16 in humans. In mice, "classical" Ly6C^{hi} monocytes (~80% of normal blood monocytes) have high expression of CCR2, low expression of CX_3CR1, and are inflammatory, expressing high levels of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), nitric oxide synthase 2 (NOS2), and proteases [52]. This subset tends to predominate in the normal physiologic unperturbed "steady state." "Nonclassical" Ly6C^{lo} monocytes (~10%) have low expression of CCR2, high expression of CX_3CR1, and in the setting of infection, injury, or illness, serve a reparative function, expressing profibrogenic factors such as TGF-β, osteopontin, insulin-like growth factor-1 (IGF-1), fibroblast growth factor (FGF), connective tissue growth factor (CTGF), and others [52–54]. This alternative paradigm presupposes that monocytes are pre-programmed to one or the other subset within bone marrow, spleen, or lymphoid tissue. Thus, circulating monocytes in the blood are committed to specific effector functions and are triggered to mediate these functions upon tissue entry. While Ly6C^{hi} monocytes enter tissue early in response to injury or infection, Ly6C^{lo} monocyte tissue entry is delayed (i.e., during a second phase) [52, 55], creating a biphasic response of early inflammatory response followed by later reparative response. In humans, these subsets include classical CD14^{++}CD16^{-}, intermediate CD14^{+} $^{+}$CD16^{+}, and nonclassical CD14^{+}CD16^{+} monocytes [56, 57]. However, considerably less is understood about the biology of these subsets in humans, with investigations suggesting that that nonclassical and intermediate subsets may serve pro-inflammatory roles, and the classical subset serving more reparative roles [56, 57].

To add to the complexity, some studies suggest a third blended paradigm. Dichotomous blood monocyte subsets are present, but tissue Ly6C^{lo} macrophages are presumed to be derived from Ly6C^{hi} infiltrating monocytes, which undergo a poorly understood "phenotype switch" into a Ly6C^{int}-Ly6C^{lo} subset [58, 59].

Importantly, all of these paradigms are not mutually exclusive and may apply variably to different tissues and different disease states, including various stages of AMD disease. For the purposes of simplicity and convenience and to account broadly for these paradigms, this chapter will refer to macrophage subsets as *scavenging, inflammatory, and reparative* macrophage subsets to distinguish macrophages based on effector function and on the potential contributions of each to AMD disease.

6.2.1.2 Dendritic Cells

Dendritic cells (DCs) are antigen-presenting cells (APCs) that serve the primary function of processing antigens and presenting antigen components to T cells DCs are distinct from monocytes and macrophages and comprise approximately 0.1–1% of blood mononuclear cells [60]. Within tissue, DCs grow in size (15–30 μm) that form extensions 23 times the diameter of the cell, similar in appearance to the dendritic structure of neurons (hence the name of

the cell). Nonlymphoid and lymphoid organs recruit DCs by defined migration pathways, and within these sites, DCs function as accessory cells that process and present antigens to T cells. Specifically, DCs serve as APCs for naïve lymphocytes and trigger responses in these cells, and are potent activators of T-cell dependent immune responses. In contrast, to macrophages, the function of DCs is primarily focused on antigen presentation; DCs have limited or no capacity as phagocytic scavenger (repair) cells nor are they major producers of inflammatory mediators. While DCs can be found in the retina and the choroid [61, 62], they are not thought to be significant contributors to AMD pathobiology, as they are not readily detected in histopathology studies of human AMD or in preclinical (e.g., mouse) models of AMD.

6.2.1.3 Basophils and Mast Cells

Basophils are a type of circulating granulocyte that can be recruited from the circulation into the tissue and have been shown to play roles in different types of inflammatory reactions, especially allergic disease. Mast cells are another type of granulocyte that has similar appearance and function; both cells store and release histamine. Once thought to be blood-borne and tissue equivalents, ontogeny studies have demonstrated that basophils and mast cells have distinct hematopoietic origins.

There are two major subtypes of mast cells, connective tissue versus mucosal subtypes. Both subtypes can release preformed granules following activation of receptors on the cell surface, and both subtypes can produce certain mediators de novo [63, 64]. Mast cells of the connective tissue subtype predominantly have granules with histamine and heparin; upon stimulation, they can also produce prostaglandin D2. Mast cells of the mucosal subtype also form granules but need T cell-derived cytokines to enable formation of granules; for this reason, they typically contain lower levels of histamine. Mucosal mast cells are also functionally distinguished in that they synthesize primarily leukotrienes upon cellular stimulation. Specific granule content and effector function can be dependent on tissue location, but the regulatory mechanisms for each are poorly understood. In contrast to other granulocytes such as neutrophils and eosinophils, mast cells have unique granule contents and also have on their cell surface high-affinity Fc receptors for IgE. As such, mast cells are the primary effector cells in in IgE-mediated immune responses, including allergic disease, asthma, atopy, hay fever, and immediate hypersensitivity. However, mast cells can also contribute to other aspects of cell-mediated immunity and wound healing [65, 66], and stimuli beyond IgE, including complement and certain effector cytokines, can mediate mast cell activation and promote cellular degranulation [67]. Mast cells can also secrete other inflammatory cytokines such as TNF-α, thereby mediating cellular injury and acute inflammatory processes. For example, mast cells have been shown to promote neuronal degeneration and death in the setting of thiamine deficiency and certain metabolic diseases [64]. Mast cells have also been detected in atheromatous plaques have been found to be co-localized with angiogenic mediators, such as platelet-derived endothelial growth factor (PDGF) [67–73].

Mast cells (of the connective tissue subtype) have been found in abundance in the choroid [61, 74]. The contributions of mast cells in AMD are not fully understood, though there is some evidence to suggest they may play limited roles. In histopathology studies, degranulated mast cells have been identified near foci of geographic atrophy (GA) [75]. Mast cells have also detected at sites of incipient new vessel formation and have been detected near Bruch's membrane in all stages of AMD [76]. Release of mediators such as heparin, metalloproteinases, and VEGF from mast cells may also activate endothelial cells and promote the early steps of angiogenesis [77–79]. Oral tranilast, a drug that inhibits mast cell degranulation, has been shown to suppress CNV in the rat model of laser-induced CNV [80].

6.2.2 Adaptive Immunity

Adaptive immunity, also referred to "antigen-specific" or "acquired" immunity, is a distinct aspect of the immune response, in which there is a generated response to a specific portion, or

epitope, of an antigen; this is fundamentally distinct from the broad, genetically pre-determined, and stereotyped response of innate immunity [12–14]. In adaptive immunity, there is specific "recognition" by cells of the immune system of a unique antigen as "foreign" to the body and therefore distinct from "self" components. Once recognized and bound, specialized cells of the immune system, called antigen-presenting cells (APCs), display antigenic components on the cell surface and interact with a host of adaptive immune cells. Once the adaptive immune cell (T and B lymphocyte) that recognizes the specific antigenic component is engaged, this subsequently results in the activation of these unique antigen-specific immunologic effector cells (T and B lymphocytes), with production of unique antigen-specific soluble effector molecules (antibodies). The goal of this targeted response a specific antigen is to effect the removal of the specific antigen (and the source from which the antigen derives) from the body in a specific and targeted fashion, other irrelevant antigenic stimuli (including "self" antigens). In the adaptive immune response, these cells must generate, de novo, a specific receptor, which, in turn, must recognize a unique molecular structure in the antigen for which no pre-existing gene was present. As such, the adaptive immune system has evolved a mechanism for the generation of new antigen receptor genes in T and B lymphocytes through recombination, rearrangement, and mutation of the germline genetic structure. As a result, the adaptive immune cells produce a "repertoire" of new antigen receptors, producing a diversity of immune cell recognition capacity within and among individuals.

Classically, the adaptive immune response is vital for response to viral pathogens that can mutate or change over time. The host that is infected by a given virus cannot a priori evolve the requisite receptors to recognize novel mutations in that virus. However, upon infection, novel antigens produced by these mutations in the virus can then stimulate an antigen-specific immune response by the host to the virus, which will recognize the virus producing that antigen, but not other viruses that do not produce this novel antigen.

While adaptive immune mechanisms traditionally have not been thought to contribute to AMD, emerging clinical evidence as well as several preclinical models suggest potential plausible roles for adaptive immunity in various aspects of AMD disease (see following sections below).

Lymphocytes of the adaptive immune system derive from precursors cells within the bone marrow [14, 81, 82]. Full functionality of lymphocytes requires subsequent maturation in peripheral lymphoid organs, where recognition of specific antigens takes place. Lymphocytes are subdivided based upon function and on the presence of specific detectable cell surface proteins (i.e., surface markers). These "markers" are related to functional and molecular activity of individual subsets. Three broad categories of lymphocytes have been determined: B cells, T cells and non-T, non-B lymphocytes.

6.2.2.1 T Lymphocytes

Thymus-derived lymphocytes, which are commonly referred to as T cells, are comprised of several subsets, functioning as effector cells to mediate antigen-specific inflammation and immune responses [83, 84]. Helper T cells (T_h cells), which are recognized as CD4+ T cells, assist in the processing of antigen for antigen-specific immunity within lymph nodes; specifically, CD4+ T cells are vital in assisting B cells to produce antibody in response to specific antigenic components and in sensitizing antigen-specific cytotoxic T cells. Outside the lymph node, CD4+ T cells can also serve as direct effector cells through production and release of certain mediators (e.g., interferon-γ and TNF-β) [85]. In this setting, CD4+ T cells can home into a particular tissue, recognize antigen and APCs, upon recognition fully activate, and then release cytokines and mediators to amplify the immune response. In certain instances, CD4+ T cells can also become activated in an antigen-independent fashion, called *bystander activation* [86–88]. This phenomenon may account for the occasional presence of T lymphocytes detected in CNV specimens from AMD eyes [76, 89].

Cytotoxic T cells (T_c cells), which are recognized as CD8+ T cells, serve as a second subset of T cells, which mediate effector biology to kill virally infected host cells or to recognize and kill tumor cells via release of cytotoxic mediators that are highly injurious or by specialized pore-forming molecules that compromise the structural integrity of target cells. CD8+ T bind MHC class I molecules (which are present on the surface of nearly all nucleated cells) and recognize specific antigenic targets; upon activation, CD8+ T cells produce IL-2 and IFN-γ, cytokines that modulate effector functions of other immune cells (e.g., macrophages, NK cells). It is possible though unlikely that CD8+ T cells contribute to AMD pathobiology.

A third type of T cells, T helper 17 (T_h17) cells, are pro-inflammatory T helper cells that produce IL-17. While T_h17 cells have been shown to play important roles in host defense at mucosal surfaces and in pathogen clearance, they have also been shown to contribute to chronic inflammation and autoimmune disorders, especially in diseases such as rheumatoid arthritis [90]. As will be discussed below, there is some evidence suggesting a contribution of T_h17 cells to AMD.

6.2.2.2 B Lymphocytes and Antibody

B lymphocytes, or B cells, are responsible for the humoral component of the adaptive immune system, producing antibodies that recognize specific antigens. Developmentally, B cells originate from and mature in the bone marrow, though they may complete development to maturity in the spleen. Antibodies, which can include various immunoglobulins isotypes, are soluble antigen-specific effector molecules that mediate the adaptive immune response [14, 81, 82]. B cells express B cell receptors (BCRs) on their cell surface, which enables recognition of specific antigens. Following antigen recognition with the assistance of CD4+ T cells in lymphoid organs (such as lymph nodes), B cells are activated to produce and secrete After appropriate antigenic stimulation with T cell help, B cells secrete antibodies (initially IgM isotype and then later others) at lymphoid organs, which pass into efferent lymph fluid and eventually into the venous circulation. Once in bloodstream or within tissues, antibodies can then mediate a diverse spectrum of immune effector activities by binding to the specific antigen it recognizes and targets.

There are at least four potential mechanisms by which antibodies mediate immune responses within specific tissue microenvironments. Circulating antibodies in the bloodstream (derived from B cells in lymphoid organs) can bind antigen to form circulating immune complexes, which can then deposit into tissues. In a second mechanism, B cells from the circulation can entire specific tissues sites and produce antibodies locally, which then form immune complexes locally following binding to recognized antigens. Third, the Fc portion of a secreted antibody can bind to an innate immune cell, especially macrophage, neutrophil or mast cell (via Fc receptor on cell the surface of that cell), which can result in a combined antibody and cellular effector immune response. None of these three mechanisms are likely to contribute to AMD pathobiology.

It is possible, however, that a fourth antibody-mediated mechanism may contribute to AMD: circulating antibodies (typically the IgG isotype), previously produced and secreted by B cells in lymph nodes and other tissue sites, may passively leak into tissue with fenestrated capillaries, like the choriocapillaris. Antibodies that accumulate within the tissue may then form an immune complex with antigens within the tissue, triggering the onset of a specific effector mechanism [14, 81, 82, 91–94]. We will consider several such effector mechanisms here (Fig. 6.1):

(a) *Immune complexes with extracellular matrix-bound antigens:* Free antibody passively entering the tissue can bind to recognized antigen trapped, or bound, within the extracellular matrix of the tissue, forming antibody-antigen immune complexes. Such complexes can in some instances trigger activation of the complement system, which can produce local cellular injury as well as production of

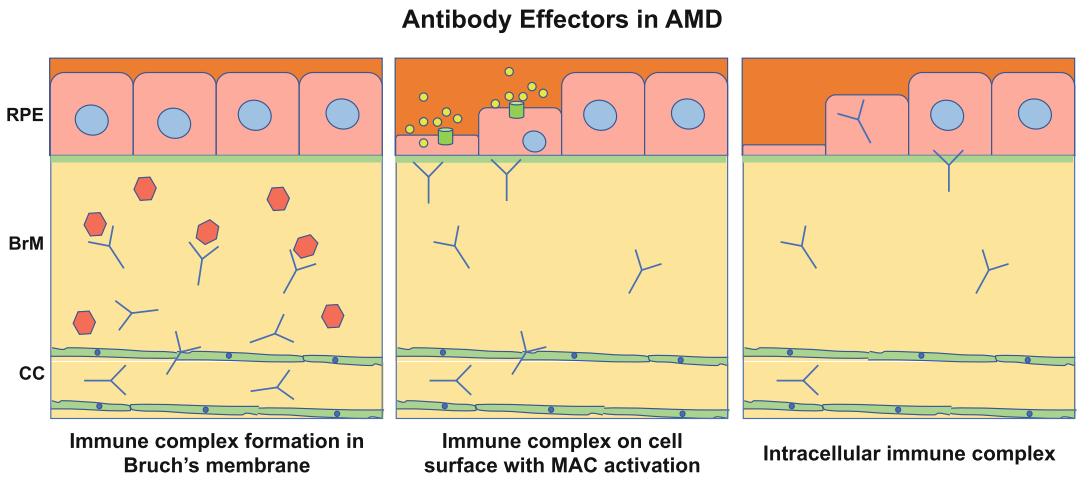

Fig. 6.1 Possible mechanisms for antibody effectors in AMD, including immune complex (IC) formation in Bruch's membrane (BrM), IC formation on retinal pigment epithelial (RPE), or choroidal endothelial, cell surface with membrane activation complex (MAC) activation, or endocytosis of antibody with subsequent intracellular IC formation with antigen within cell. *CC* choriocapillaris

complement components called anaphylatoxins, which serve to recruit additional inflammatory cells. In chronic disease such as AMD, such formation of in situ immune complexes may occur at a low-grade level, which could be consistent with the modest (and variable) immune cellular infiltration observed in AMD.

(b) *Immune complexes with cell-surface antigen:* Free antibody passively entering the tissue might also bind to antigen on the surface of cells within tissue, and this antigen-antibody binding could itself trigger complement activation and specifically formation of the membrane attack complex (MAC), which can then induce cellular injury, a phenomena that has been observed in certain immune-mediated diseases (e.g., hemolytic anemia). Given the genetic associations of AMD with the complement system, it is plausible that this mechanism may contribute to cellular injury (e.g., at RPE cells) in the setting of AMD (Fig. 6.1).

(c) *Immune complex with intracellular antigen:* Another potential means by which free antibody may mediate cellular injury is via immune complex with intracellular antigen [93, 94]. In this setting, an antibody is bound and then internalized into cells independent of antigen biding. The antibody functionally becomes an autoantibody (i.e., antibodies recognizing self-antigens) directed against a specific intracellular antigen, which could be in various organelles, including the ribosome or nucleus (Fig. 6.1). The bound autoantibody-antigen can alter cellular signaling pathways or cellular metabolism, creating cellular injury. Such a mechanism is well described in the setting of systemic lupus erythematosus and has been implicated in cancer-associated retinopathy (CAR), wherein specific autoantibodies directed against intracellular antigens in photoreceptors may promote cellular injury and death in rod and/or cone photoreceptors [95]. This is of direct relevance to AMD since emerging data demonstrates the presence of retinal auto-antibodies in some patients with AMD, suggesting the possibility that this mechanism may also contribute to a subset of AMD disease.

6.2.3 Mechanisms for the Activation of Immune Responses in Degenerative Diseases

6.2.3.1 Activation of Innate Immunity

There are multiple potential mechanisms for activation of innate immunity; we will review several of them here.

(a) *Cellular injury as a trigger of innate immunity:* As noted, the innate immune system is a broad and conserved response to specific stimuli, including injury to parenchymal (nonimmune) cells [14, 81, 82, 96–98]. Broadly, cellular injury can be defined as any exposure or triggering stimulus that perturbs the cell's normal function and elicits a cellular response, evident as activation of signaling pathways, alterations in biochemical mediators, or change in specific cellular functions. Such stimuli may include immune effector mechanisms, cytokines, oxidants, chemical mediators, alterations in ambient pH, hypoxia, increased temperature (heat), light, or mechanical injury [98]. Cellular responses to injury include activation of signaling pathways, morphological changes, cellular migration, proliferation, cytokine production, alteration in gene expression, or alterations in cellular metabolism. These cellular responses to injury can promote the recruitment and activation of immune cells or the activation of immune amplification systems, such as the complement system, via production of inflammatory mediators and/or upregulation of cellular adhesion molecules. A highly relevant cellular response to injury that can promote activation of innate immune mechanisms is extracellular deposit accumulation [98, 99], which can serve as a nidus for macrophage infiltration and phagocytosis, especially in the setting of oxidant injury and modification of deposits by oxidation or other forms of chemical alteration.

(b) *Infection as a trigger of innate immunity:* Microbial invasion is a well-established trigger of innate immunity in active infection, usually by the release of microbial-derived molecules (i.e., endotoxins, exotoxins, cell wall components) that can directly activate receptors expressed on the cell surface of monocytes, macrophages, neutrophils or, in some cases, on parenchymal cells. Active infection is characterized by microbes that invade the tissue microenvironment, replicate, and cause tissue injury via direct effects or indirectly, via activation of the immune response [100].

There has been considerable interest over the past several decades in the concept that certain chronic degenerative diseases may be caused by direct microbial infection at a given locus of disease [97–100]. One such example is peptic ulcer disease, wherein the gram-positive bacterium called *Helicobacter pylori* directly infects the gastric subepithelial mucosa and results in chronic tissue injury [101]. In vascular biology, direct and chronic infection of vascular endothelial cells by bacteria or viruses has been suggested as a potential etiology for certain types of atherosclerotic disease. There is limited data to suggest a similar phenomenon in AMD, including with latent CMV infection and *C. pneumoniae* infection [102–104]. Another possibility is that infection can indirectly promote cellular response to injury. Chronic exposure to microbial components can prime, or partially activate, monocytes in spleen, lymph node, bone marrow, or other sites of exposure or surveillance, altering the expression of certain cytokines and mediators and committing exposed cells to specific effector functions [105–107]. These functions then become fully manifest upon recruitment to the disease locus (i.e., RPE and retina) and transformation into fully activated macrophages. These concepts as they relate to AMD will be discussed in greater detail in Section II, Part D [102–104, 108].

6.2.3.2 Mechanisms for the Activation of Normal and Aberrant Adaptive Immunity

(a) *Activation of adaptive immunity*: In the "immune response arc," interaction between antigen and the adaptive immune system is comprised of three distinct phases: afferent arm (peripheral tissue site of antigen recognition), processing (within the immune system, e.g., lymph node) and effector arm (cellular and humoral response to the antigen at the original site, completing the arc) [14, 81, 82]. The *afferent* arm transmits information on the antigen to the lymph node for processing by two distinct mechanisms. In the first, the APC captures, digests, and presents antigenic fragments, and then carries the fragments to the lymph node, where interaction with T cells occurs [83, 84, 109]. Alternatively, in the second mechanisms, the intact antigen is transmitted directly to the lymph via lymphatic circulation, where it can interact with specific B cells that recognize components of the antigen [14, 81, 82].

Processing of the antigen occurs in the lymph node, where antigenic component, APC, T cell, and B cells interact in a specific fashion to activate the adaptive immune response. The spleen serves as an alternative site of processing for tissues such as the retina and choroid that are not served by draining lymph nodes. Once processing occurs, antigen-specific B cells can be activated to produce antibodies and antigen-specific T cells can be activated to produce cellular mediators. These activated cells and their products are then released into efferent lymphatics and the blood circulation, where they eventually return to the original tissue site and mediated a specific *effector* response (e.g., immune complex formation or delayed hypersensitivity reaction). Compared to most tissue sites, the immune response arc of the retina and choroid is not well characterized is influenced by specific aspects of the retina and choroid, including immune privilege, blood-retinal barrier, unique anatomical features, and these are discussed elsewhere [110, 111].

(b) *Aberrant activation of adaptive immunity:* Aberrant activation of adaptive immunity may play a role in the pathogenesis of chronic degenerative diseases such as AMD. Among potential autoimmune mechanisms, two in particular may have relevance to AMD: molecular mimicry and desequestration. Additionally, potential third and fourth mechanisms include the generation of "neo-antigens" against which immune responses are directed and true foreign antigens that become trapped within normal tissues.

In *molecular mimicry*, certain regions (epitopes) of an unrelated foreign antigen share similar structure to self-antigens [112]. In this setting, there may be the generation of an appropriate afferent, processing, and efferent immune reaction against a true foreign antigen of a microbe, but antibodies directed against the foreign antigen or antigen-specific T cells may inappropriately cross-react with a self-antigen that shares similarity with this foreign antigen, generating an unwanted autoimmune response. This response would include directed tissue injury where this self-antigen is recognized, causing additional lymphocytes responses that may be directed against other self-antigens and recruiting additional effector immune cells. Molecular mimicry has been identified as a potential mechanism for anti-retinal autoimmunity [113].

In most cases, inappropriate activation of adaptive immune system is prevented by a process of active tolerizing to self-antigens (which occurs by several mechanisms). However, in other cases, antigens must be sequestered within specific cellular or tissue compartments away from exposure to components of the adaptive immune system. *Desequestration* occurs when such molecules are released from such

compartments, where they are mistaken by the immune system to be foreign antigens [114–116]. For example, certain nuclear proteins and ribosomal enzymes are sequestered; in the event of cellular injury, such proteins or enzymes can be extruded into extracellular space and upon exposure to an immune cell, may be recognized as an autoantigen [115]. Of relevance to AMD, nonlethal RPE cell injury is associated with active blebbing or extrusion of cytosolic or membrane components the extracellular space. Upon their release into the extracellular environment, previously intracellular peptides can become desequestered, becoming antigens that can auto-activate the adaptive immune system [110, 116].

Formation of "neo-antigens" represents another mechanism for aberrant activation of adaptive immunity. In this mechanism, there is chemical modification of normal self-proteins that are trapped or deposited within tissues [117]. For example, oxidative modification of peptide components of large proteins (e.g., apolipoproteins) trapped within Bruch's membrane can produce neo-antigens, and citrullination of cellular peptides in the setting of cellular injury or exposure to oxidants is a biochemical modification that is increasingly recognized as a cause of neo-antigen formation [118]. In both cases, resultant neo-antigens can trigger specific T cell responses and antibodies that are reactive to the modified protein.

Antigen trapping represents the fourth and final mechanism for aberrant adaptive immunity activation [119]. In this mechanism, true foreign components of a particular size or charge become inappropriately trapped within the substratum of this tissue, leading to inappropriate activation of the adaptive immune system at the site of trapped antigen. In AMD, this may occur when foreign antigen passes through fenestrated capillaries of the choriocapillaris, becoming lodged within the connective tissue of the choroid or within extracellular matrix of Bruch's membrane. While this mechanism has been put forth as a potential trigger of ocular inflammatory disease and certain conditions such as ocular histoplasmosis [120, 121] and this could be a plausible trigger for AMD, there is minimal supportive evidence for this in AMD pathobiology.

6.2.4 Amplification Systems for Immune Responses in Chronic Degenerative Diseases

Although injury or inflammation can be directly induced by either innate or adaptive immunity, in most cases, a process of amplification is required for overt clinical signs of disease to manifest. Several potential amplifications systems contribute, including: [1] plasma-derived enzyme systems, which include complement, kinins, and fibrin; [2] lipid mediators (prostaglandins, leukotrienes, other eicosanoids and platelet activating factors); [3] vasoactive amines (histamine and serotonin); and [4] cellular inflammatory signaling programs that serve to integrate both immune and nonimmune responses, which includes the NLRP3 inflammasome. This chapter will focus on the complement system and the NLRP3 inflammasome, since both have been identified as potential modulators of the immune response in AMD.

6.2.4.1 Complement System

The complement system serves to amplify the effects of both innate and adaptive immune system and mediate various injury responses [122–124]. Components of the complement system, which include over 30 different protein molecules, are synthesized in the liver and circulate in the blood as inactive precursors and become activated at sites of injury, in response to infection or inflammation. However, some specific complement components can also be produced locally within certain tissues; within the eye, this includes the cornea, sclera and retina [123]. For example, certain complement-related proteins, including C3, factors B, H, H-like

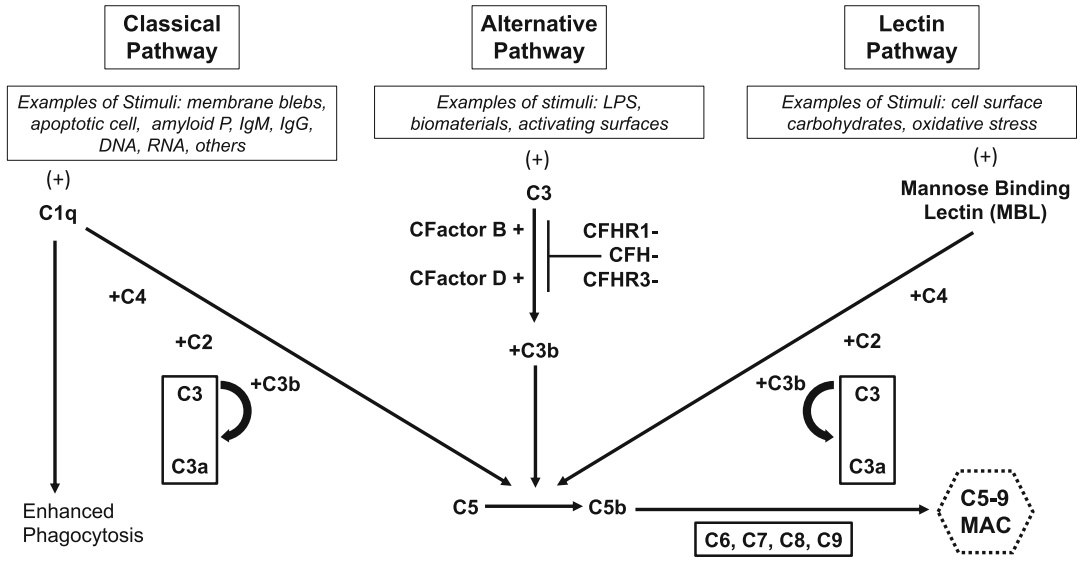

Fig. 6.2 Schematic of the components and fragments of the complement cascade indicating three primary sources of activation via the classical, alternative or lectin pathway

1, CD46, CD55, CD59, and clusterin, can by produced by RPE cells [125].

Components of the complement system serve a collective amplifier function by interacting in a sequential cascade to produce a number of different cellular (and noncellular) phenomena. This may occur by one of three specific pathways that activate the complement system: classical pathway, alternative pathway and the lectin pathway (Fig. 6.2).

The classical pathway can be activated by adaptive immune mechanisms, including by certain antigen/antibody (immune) complexes, referred to as pentraxins, especially those formed by IgM, IgG1 and IgG3 [122–124]. However, activation of the classical pathway, and ultimately C3 and C5 activation, can also be triggered by certain innate immune stimuli, including DNA, RNA, insoluble deposits of abnormal proteins (e.g., amyloid P) or apoptotic cells [126–129].

Activation of the lectin pathway, which includes activation of C2 and C4, occurs via mannose-binding lectin (MBL), or ficolin, that identifies DAMP molecule regions on apoptotic cells or microbial-derived PAMPs, that are typically certain types of sugars [130]. MBL typically does not recognize self-components; however, oxidant modification or glycosylation modification of molecular structures, as can occur in degenerative diseases such as AMD, may alter surface protein expression or the presence of sugar moieties that are not typically present but that are recognized by MBL, leading to MBL deposition and complement activation via C3 [131–134].

Finally, the alternative pathway can be activated by innate immunity, primarily by activating C3. This may occur through innate immune recognition of specific moieties on microbial-associated molecules (e.g., LPS), activated surfaces (e.g., implanted medical devices) [126], or oxidant-modified cellular byproducts (e.g. the photo-oxidative products of A2E, bis-retinoid pigment that may accumulate in the RPE in AMD) [135].

Ultimately, activation of any one of the complement pathways leads to the same common points of activation and generation of the same activated complement byproducts. Activated complement byproducts may amplify injury or inflammation by at least three distinct mechanisms: [1] C3b, which is a specific fragment of C3, can opsonize, or coat, antigenic or pathogenic surfaces, to enhance phagocytosis by

macrophages or neutrophils [2]; the terminal complement components C5–9, called the membrane attack complex (MAC), can be activated to form transmembrane channels that disrupt cell membrane of target cells, leading to cellular injury, loss of cytoplasm (lysis), or death [3]; production of anaphylatoxins (C3a, C4a and C5a), small pro-inflammatory polypeptides, that can induce many inflammatory mediators and recruit inflammatory cells. Complement activation is regulated by specific inhibitors, such as decay accelerating factor (CD55), CD59, CD46, complement factor H (CFH), complement receptor 1 (CR1), and others that serve to block, resist, or modulate the induction of various activation pathways especially in degrading the C5b-9 MAC. As will be discussed below, the role(s) of CFH and potentially other inhibitors (e.g., CR1) may be relevant to pathobiology of AMD [122–124].

As noted, the RPE is capable of producing various complement components locally in situ. Thus, C3 and other complement components can be cleaved to produce activated fragments by various enzyme systems, in the absence of the entire complement cascade, which in turn can activate certain specific cellular functions [136, 137]. Additionally, inhibitors of complement activation, which can also be produced locally by cells such as the RPE, serve to regulate the function of complement, protecting against tissue injury mediated by inappropriate complement activation [138].

6.2.4.2 NLRP3 Inflammasome

The NLRP3 inflammasome is a multimeric complex of cellular cytosolic proteins that assembles in response to specific exogenous and endogenous danger signals, including damaged cell-intrinsic components (e.g., DAMPs such as dsRNA, cytoplasmic DNA), microbial-associated molecules (e.g., PAMPs such as LPS), oxidants, and other moieties, integrating cellular responses to such injury stimuli by specific upregulation of the cellular inflammatory response [139]. The NLRP3 inflammasome has traditionally been described as present in innate immune cells such as macrophages and microglia and may also function in part to integrate aspects of the innate and adaptive immune responses [140]. More recent work has described the role of the NLRP3 inflammasome in nonimmune cells, where its assembly is triggered in response to cellular perturbations such as mitochondrial dysfunction, aberrant ion (e.g., calcium) flux, lysosomal dysfunction, and proteasome insufficiency [141]. Indeed, as will be detailed below, the NLRP3 inflammasome may play a vital role in RPE cellular function and response to injury in the setting of AMD.

6.3 Specific Immune Contributions to AMD Pathobiology

Unlike with acute inflammatory diseases, primary autoimmune diseases, or uveitis, immune mechanisms in the setting of AMD are a secondary response to a primary disease process at the RPE and neurosensory retina, which serves to amplify or alter the disease phenotype. Considerable data from human pathology of AMD and preclinical animal models have clarified specific roles for the immune system in both dry and neovascular AMD, which this section will elaborate in greater detail.

6.3.1 Innate Immunity

6.3.1.1 Macrophages and Dry AMD

Early histopathologic studies using immunofluorescence and electron microscopy noted the presence of macrophages in close proximity to Bruch's membrane, drusen, and the basal aspect of RPE in eyes with dry AMD [142, 143]. However, macrophages were also observed in the choroid of eyes from age-matched controls without AMD, raising questions about whether the presence of these cells was specific to disease or instead representative of age-related alterations in the local immune microenvironment. Sarks and colleagues assessed the presence of macrophages in choroid and outer retina of a broad range of ocular specimens from normal aging, early AMD, NVAMD, and late AMD

with GA and found that thinning of Bruch's membrane, areas of deposition of membranous debris from the RPE, and drusen deposits were all associated with macrophages, which were frequently observed engulfing fragments of outer collagenous zone of Bruch's membrane or membranous debris [142]; they hypothesized that the accumulation of phospholipid membranous debris and focal concentration of lipid in drusen served as attractants to recruit macrophages. Subsequent work found that the presence of extracellular deposits, specifically soft drusen as well as thick continuous basal laminar deposits was associated with increased frequency of macrophages recruited to the inner choroid and Bruch's membrane, and that choroidal macrophages from eyes with the various stages of AMD were characterized by expression of iNOS, while choroidal macrophages from normal eyes did not express iNOS [144]. This observation highlights a potential key difference between choroidal macrophages recruited in the context of dry AMD versus typical resident choroidal macrophages [144]. Interestingly, in this study, macrophages present in Bruch's membrane in AMD eyes did not express iNOS, suggesting either immunomodulatory differences between choroid and Bruch's membrane, or distinct subsets of macrophages present at each site. Subsequent studies have affirmed that early AMD with drusen and basal laminar deposits is associated with a significant increase in IBA1+ macrophages and HLA-DR+ subset of activated macrophage as compared to age-matched control eyes [145]. In addition, AMD eyes with thick basal deposits, CNV, and GA were all associated with a marked increase in the frequency and size of CD163+ cells in the outer retina, subretinal space, and subRPE space in the macula, while such cellular infiltration was not observed in healthy aged controls without AMD [146] (Fig. 6.3). In particular, the findings of the latter study suggest that the frequency of macrophages in AMD may have been underestimated due to use of markers that do not readily detect macrophage cells in tissues, and that macrophages may play a key role in all aspects of AMD disease. Multiple studies have now demonstrated that macrophages, labeled by a variety of markers including RCA-1, CX3CR1, CD18, IBA1, CCR2, CD163, and CD14, are found in association with large drusen, within atrophic areas, or RPE cells adjacent to atrophy [147].

The precise role(s) that macrophages play in the setting of AMD likely depend on the predominant effector function of the infiltrating cells, and whether those cells manifest *scavenging, inflammatory, or reparative* effector biology. Presently, our ability to differentiate specific effector functions is limited because cellular markers do not necessarily distinguish functionally distinct subsets and because presently available techniques to label effector cytokine expression in situ are inconsistent, particularly for postmortem specimens. At the same time, our general lack of understanding of the specific mechanisms that mediate progression to advanced stages of AMD disease, especially GA, limits our ability to interrogate effector biology of macrophages in appropriate context. However, there is considerable inferential evidence from human histopathology and supporting data from preclinical mouse models that suggest potential hypotheses for how macrophages contribute to dry AMD.

(a) *Scavenging and homeostatic function:* As noted above, macrophage phagocytosis of lipid-rich membranous debris along with macrophages associated with accumulated drusen are readily apparent by histopathologic studies of AMD, suggesting that macrophages may serve a housekeeping role in clearing debris from Bruch's membrane and the subRPE space and in dynamic turnover of drusen [142, 143]. Mice with genetic deletion of P2X7 receptor, which has function as scavenger receptor on macrophages, develop progressive thickening of Bruch's membrane, RPE cell loss, retinal functional deficits, and accumulation of inflammatory macrophages in the subretinal space [148]. The development of pathologic features that mimic dry AMD suggest that scavenging function of choroidal macrophages may be essential to provide a homeostatic function. Indeed, patients with

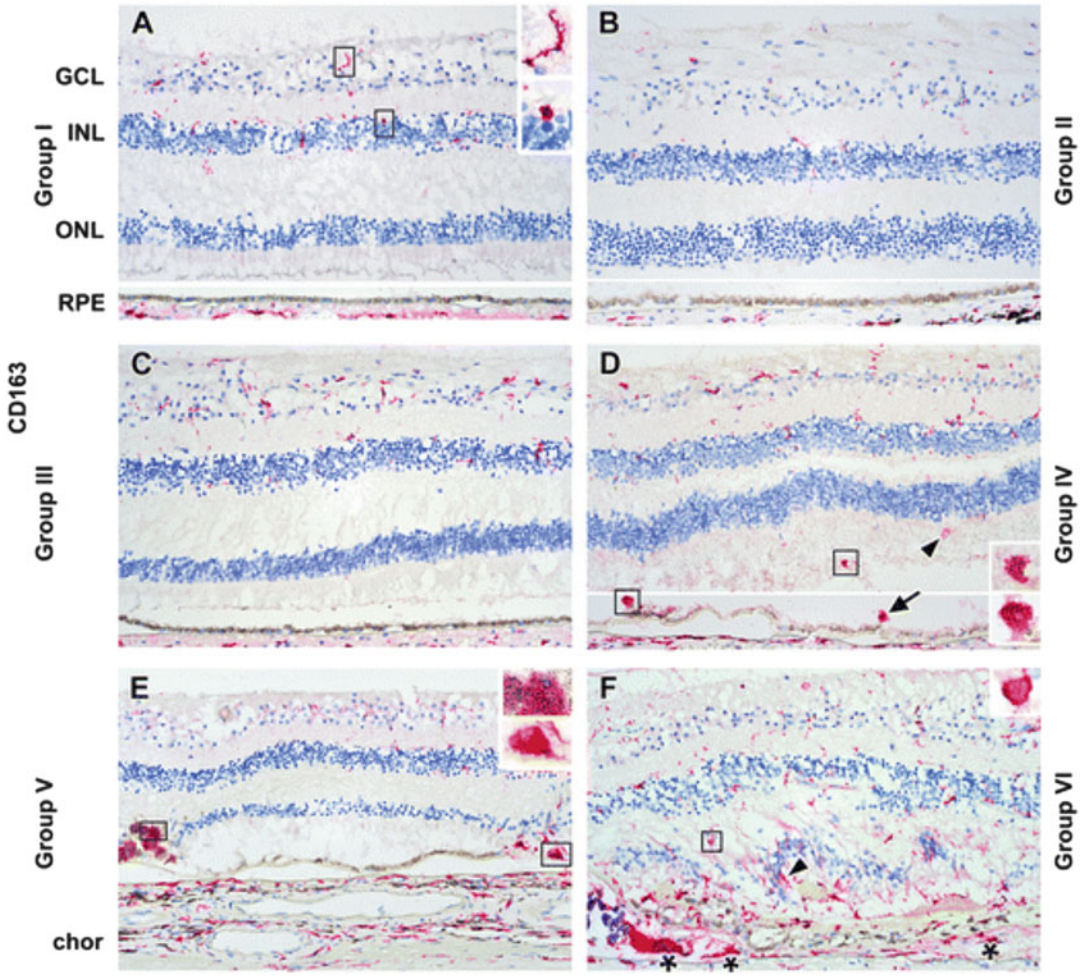

Fig. 6.3 Immunohistochemical localization of CD163+ macrophages in normal, age-matched control eyes and eyes with AMD of various severity grades. CD163+ cells were detected using a red alkaline phosphatase polymer system. The tissue was counterstained with hematoxylin and the nuclei are *blue*. CD163+ cells are present solely in the inner retina in normal, age-matched eyes (**a**) and AMD eyes groups II and III (i.e., early AMD) (**b**, **c**), but were present in the outer retina and subretinal space in eyes with thick subretinal deposits in AMD group IV (**d**), geographic atrophy in group V (**e**), and neovascular AMD with fibrosis in group VI (**f**). *Insets* in **a**: In normal eyes, CD163+ cells in the nerve fiber layer and ganglion cell layer had a dendritic, microglioid phenotype with a small soma and long processes. In the inner plexiform layer and inner nuclear layer, some of the CD163+ cells had a dendritic morphology and others were characterized by a more rounded, epithelioid conformation. *Insets* in **d**, **f**: In eyes from groups IV-VI, CD163+ cells in the outer retina and subretinal space had a rounded morphology with large cell bodies and small processes. *Insets* in **e**: In eyes with geographic atrophy (group V), the CD163+ cells had a larger soma and shorter processes, consistent with an activated macrophage morphology. The edges of geographic atrophy expressed the marker CD163 in cells filled with melanin granules, which most likely represent macrophages that have ingested melanin pigment. Scale bar = 100 μm; *insets* in **a**–**f** are enlarged fourfold. Arrow heads CD163+ macrophages in the outer retina; *arrows*: subretinal CD163+ cells; *: sub-RPE CD163+ cells. The *white line* in panels (**a**, **b**, and **d**) is the break between two separate photomicrographs taken due to postmortem sensory retinal detachment separating photoreceptors from RPE. Reprinted by permission from: Springer Nature, Graefe's Archive for Clinical and Experimental Ophthalmology. Lad EM, et al., Abundance of infiltrating CD163 + cells in the retina of postmortem eyes with dry and neovascular age-related macular degeneration. Graefes Arch Clin Exp Ophthalmol 2015; 253:1941-1945. Copyright Springer Nature 2015

nonfunctional haplotypes of the P2RX7 and P2RX4 genes, which give rise to the P2X7 and P2X4 scavenger receptors, respectively, have circulating monocytes with substantial reduction in phagocytic capacity, and this haplotype was over four times more frequent in AMD patients as compared to controls [149]. Thus, monocytes/macrophages with scavenging function may serve a protective role, and loss of this scavenging function or dysfunction of molecular pathways that mediate scavenging and phagocytosis capabilities of macrophages could thus exacerbate the dry AMD phenotype.

(b) *Inflammatory injury at the RPE and retina:* Other evidence suggests that macrophages exhibiting inflammatory effector function may cause nonlethal and lethal injury to RPE cells and photoreceptors and may directly promote AMD disease progression [150, 151]. As noted above, macrophages having an activated morphology or labeling with markers of activation (e.g., HLA-DR) are found within areas of GA and adjacent to areas of GA [145, 147]. Moreover, the frequency and extent of subretinal macrophage infiltration appears to correlate with stage of disease in dry AMD, with abnormally increased frequency of subretinal macrophages in eyes with large drusen and still greater numbers observed in eyes with GA [146, 147, 152]. Another clinical observation linking macrophages to AMD progression has to do with the subretinal and intraretinal hyperreflective foci which are seen by OCT in AMD patients and which have been associated with progression of GA and photoreceptor loss [153, 154]. RPE which are injured in the context of AMD are known to extrude their melanosomes and histology demonstrates that these melanosomes are frequently phagocytosed by macrophages [146, 155]. Interestingly, in vivo flood-illumination adaptive optics imaging (FIAO) in patients with AMD demonstrate that melanin-containing cells corresponding to subretinal and intraretinal hyperreflective foci on OCT and that these cells demonstrate morphologic features of macrophages [156]. Taken together, these observations suggest an association between disease severity and inflammatory macrophage infiltration in eyes with AMD.

Meanwhile, a growing body of evidence from retina mouse models, including for example, the APOE4 transgenic and the CX3CR1-deficient mouse models of subRPE deposit formation and the photo-oxidative stress model, demonstrate that there is increased and persistent infiltration of macrophages in the subretinal space and that these cells mediate RPE cellular injury and photoreceptor death via direct cellular interaction and paracrine release of inflammatory cytokines [147, 152, 155, 157, 158]. Macrophage infiltration occurs as a result of response to injury and as a result of dysregulated immunosuppressive mechanisms that are meant to limit macrophage access to the subretinal space. Following moderate light challenge in CX3CR1-deficient mice or severe light exposure in the photo-oxidative stress model [159], there is rapid infiltration of blood-derived macrophages into the subretinal space, which account for 50% of the macrophages present in the subretinal space [147, 155, 159]. Inhibition of CCR2 substantially reduced this infiltration and reduced the severity of subsequent photoreceptor degeneration in the CX3CR1-deficient mouse model [155]. Under physiologic conditions, the RPE actively promotes an immunosuppressive environment in the subretinal space to limit injury due to inflammation. This includes the expression of Fas ligand (FasL) by the RPE, which interacts with the Fas receptor on macrophages to induce cell death and eliminate them from the subretinal space [152]. The RPE also produces the complex signaling moiety Thrombospondin-1 (TSP-1), which interacts with CD47 on macrophages to reduce phagocytic capacity of macrophages and

sensitize these cells to FasL-mediated cell death and elimination [160–164]. These systems interact to maintain an immunosuppressive environment and prevent macrophage associated cellular injury at the RPE and the outer retina. Loss of FasL expression or TSP-1 production, or aberrations in their respective signaling mechanisms, allows macrophages to persist in the subretinal space and create a pathologic, persistent inflammatory microenvironment. In an analogous fashion, the CX3CL1 ligand is produced by a variety of neuronal cell types and interacts with CX3CR1 expressed by microglia cells to help maintain retinal homeostasis [152, 155, 158]; perturbations in this system may allow or enable infiltration of the neurosensory retina by blood-derived macrophages.

Infiltrating macrophages likely contribute to AMD pathobiology by secretion of pro-inflammatory effector cytokines, which include IL-1β, TNF-α, and IL-6. IL-1β is initially produced as a proform within macrophages following stimulation of TLRs by PAMPs or DAMPs [165, 166]. Macrophage activation by a second stimulus triggers the assembly of the NLRP3 inflammasome to activate caspase-1 to cleave pro-IL-1β to a mature form that is secreted as an active cytokine [167]. IL-1β is toxic to neuronal cells and its secretion by subretinal macrophages has been shown to induce death of photoreceptors, especially cones [168]. Such a mechanism could contribute to loss of photoreceptors that occurs adjacent to GA in spite intact subjacent RPE. Additionally, IL-1β has been shown to contribute to CNV induction and may serve as a pro-inflammatory mediator that mediates NVAMD onset.

Macrophage-derived TNF-α has been implicated in promoting dysfunction of RPE homeostasis, as it has been shown to repress OTX2, a key transcription factor of the RPE [169]. In the adult RPE, OTX2 regulates RPE expression of a number of genes that are essential for the visual cycle, including transthyretin (TTR), a retinol carrier, and retinol dehydrogenase 5 (RDH5) that re-isomerizes all-trans-retinal into 11-cis-retinal [170]. Thus, reduction in OTX2 within RPE by macrophage TNF-α may impair the visual cycle and cause visual dysfunction by limiting the efficiency of photoreceptor function even at the intermediate stage of AMD, since subretinal macrophage infiltration is readily observed in patients with large drusen [146, 152, 155, 171, 172]. Over time, chronic OTX2 repression may induce or contribute to photoreceptor loss; indeed, ablation of OTX2 in adult mice leads to progressive photoreceptor degeneration, suggesting that macrophage TNF-α could promote dry AMD disease progression in this fashion [170]. TNF-α producing macrophages may also mediate visual dysfunction by infiltration into the neurosensory retina where TNF-α may disrupt and injure photoreceptor bipolar synapses and promote Muller cell activation leading to disruption of Muller cell synaptic support and the cone-Muller cell accessory visual cycle [146, 173, 174]. Additionally, patients with the highest levels of TNF-α expression in circulating monocytes have a greater risk of developing NVAMD [175]. TNF-α may directly contribute to new vessel formation or it may also indirectly contribute to CNV induction by activating RPE cells to upregulate production of VEGF, which consequently drives angiogenesis [176, 177] (see Macrophages in NVAMD section).

IL-6 is another cytokine produced by macrophages that may contribute to AMD, as it antagonizes TGF-β and therefore may disrupt the immunosuppressive microenvironment of the retina and subretinal space [178]. IL-6 has been shown to reduce RPE FasL expression, which interferes with the ability of the RPE to eliminate macrophages from the subretinal space [152], and elevated expression of IL-6 in retina models

correlates with persistent of subretinal macrophage infiltration [152]. Moreover, systemic levels of IL-6 correlate with the rate of progression of GA in patients with AMD [179]. Importantly, IL-6 has also been shown to correlate with the presence of macular edema in the setting of NVAMD [180]. Extrapolating to dry AMD biology, since macular edema may reflect more generally Muller cell injury, it is plausible that IL-6 produced by infiltrating macrophages could mediate Muller cell injury in dry AMD [146].

These cytokines are but a subset of macrophage-derived factors that could affect visual function and disease progression in dry AMD; clearly, other cytokines will contribute, and macrophages may also mediate effects through coordinated biology with other immune and nonimmune mechanisms. For instance, subretinal macrophage-produced C1q may mediate photoreceptor degeneration via NLRP3 inflammasome activation and IL-1β production [181].

(c) *Reparative function:* Considerably less evidence is available to understand how macrophages may manifest reparative functions in the setting of dry AMD. The macrophage marker CD163 is frequently considered to be a marker of reparative macrophages since it serves as a scavenger receptor for hemoglobin-haptoglobin complex, it is upregulated by IL-10 [182], and because CD163+ macrophages are frequently found in association with fibrosis and neovascularization (see NVAMD section—neovascular remodeling). As noted above, CD163+ macrophages are prevalent in in postmortem eyes with dry AMD, found in the subretinal space, within areas of GA, at the margins of GA, and within the retina [146] (Fig. 6.3). It is thus possible that reparative macrophages contribute to AMD disease. TGF-β produced by reparative macrophages may serve an immunoregulatory function, downregulating inflammatory activity, limiting tissue injury and cellular loss, and promoting clearance of cellular debris and limited tissue repair. On the other hand, CD163 may not be specific for reparative function and CD163+ macrophages may more broadly label multiple subgroups of macrophages with different effector function. Indeed, some subgroups of CD163+ macrophages have been found to have inflammatory effector function [183], suggesting that the CD163+ macrophages observed in dry AMD may have heterogeneous functions. Additional investigation is needed to identify specific markers of reparative function and understand how perturbations of this macrophage subset might influence dry AMD disease phenotype.

6.3.1.2 Macrophages and NVAMD

Neovascular AMD (NVAMD) is characterized by the onset, formation, and growth of aberrant choroidal neovascularization (CNV) subjacent to the retina. In spite of the efficacy of intravitreal anti-VEGF medications, it still represents the leading cause of vision loss in the elderly [1]. Loss of vision occurs as a result of plasma exudation, hemorrhage, fibrosis, atrophy of overlying RPE, injury and atrophy of overlying photoreceptors, and synaptic dysfunction in the overlying neurosensory retina. There is now considerable evidence from analyses of NVAMD histopathology, preclinical animal models of CNV, and studies of patients with NVAMD that macrophages contribute to various aspects of NVAMD pathobiology, including the onset of NVAMD disease (i.e. triggering onset and development of CNV), CNV formation and growth, extent of disease activity, and synaptic dysfunction within the overlying neurosensory retina. We will explore potential contributions to each aspect of NVAMD disease, with a specific emphasis on understanding the relative contributions of inflammatory (as defined by high expression of inflammatory mediators) and reparative (as defined by high expression of pro-fibrogenic cytokines) macrophage subsets to various stages of disease.

(a) *Onset of NVAMD:* The specific mechanisms that trigger onset of NVAMD, the initial development of incipient CNV, remain unknown. As such, there are no consensus strategies to identify patients who are highest risk for NVAMD conversion, and there are no effective therapies to prevent progression to NVAMD. Numerous histopathologic studies, including Sarks, et al., have demonstrated the presence of macrophages in association with the leading edge of CNV vascular structures, adjacent to or within a thinned and irregular Bruch's membrane, suggesting that macrophages might promote the initial development of neovessels via release of angiogenic factors and/or inflammatory cytokines that mediate initial response to locus of injured or diseased tissue [184] (Fig. 6.4). Inflammatory macrophages have also been observed in close association with endothelial progenitor cells (EPCs) in surgically excised CNV specimens [185], suggesting that macrophages may directly mediate initial steps of neovessel assembly via recruitment and activation of these EPCs at the inner choroid and Bruch's membrane. Using the experimental model of murine laser-induced CNV, several investigators have demonstrated a key role for CCR2+ inflammatory monocytes (which express TNF-α, IL-1β, NOS2, VEGF, and proteases such as MMP-9) in the early development of CNV, as genetic deletion of CCR2 and reduction of this inflammatory monocyte subset substantially reduces CNV induction [186–189]. Intravitreal corticosteroid treatments inhibit incipient CNV development with decreased inflammatory monocyte/macrophage infiltration in the laser induction model [190–192]. In patients, pro-inflammatory monocyte activation state, as reflected by increased expression of TNF-α in peripheral monocytes, is associated with increased risk of NVAMD as compared to patients with dry AMD [175], and other studies have shown that monocytes from NVAMD patients have altered transcriptomes as compared to controls [193]. In addition, inflammatory cytokine receptors CCR1 and CCR2 are co-upregulated on intermediate CD16+ monocytes from NVAMD patients [194] and CD200 upregulated on circulating CD11b + monocytes in NVAMD patients as compared to controls [195]. To further add to this perspective, the PRO-CON clinical trial found that intravitreal anti-VEGF treatment with aflibercept was not efficacious as prophylactic treatment against progression to NVAMD, as compared to sham injection, in high-risk fellow eyes with dry AMD [196]. Taken together, these data collectively support the concept and hypothesis that the specific biologic triggers of NVAMD progression and incipient CNV development extend beyond VEGF, and that pro-inflammatory macrophages may directly trigger NVAMD onset, promoting incipient CNV induction and formation via release of inflammatory meditator such as TNF-α, IL-1β, and MMP-9. These data also highlight the potential of targeted anti-inflammatory drugs as potential therapies to prevent progression to NVAMD.

(b) *CNV Formation and Growth— Angiogenesis:* Following initial onset of disease, the formation and growth of CNV occurs via coordinated assembly of various cell types to form new vessel structures. The prevailing paradigm for new vessel growth, both broadly within vascular biology and specifically for CNV, is *angiogenesis,* wherein endothelial cells resident in the choroid arise from existing choroidal vasculature and proliferate, invade Bruch's membrane, and assemble into a nascent network of capillary tubes, a process that is regulated primarily by VEGF as well as other angiogenic factors [197, 198] (Fig. 6.5) [199]. *Maturation* occurs when the new capillary CNV acquires a pericyte sheath, a process that is mediated largely by PDGF, stabilizing the CNV and allowing it

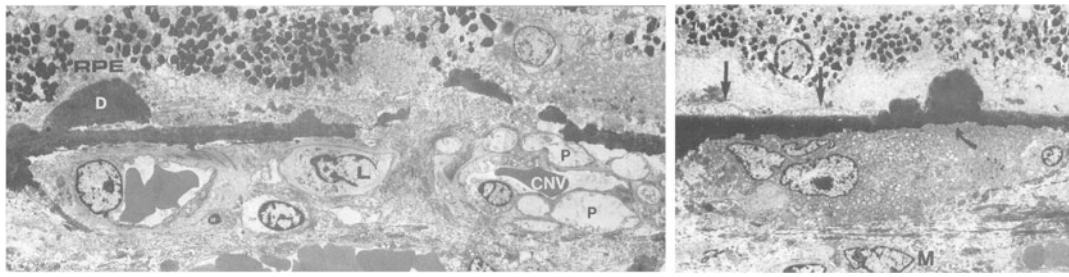

Fig. 6.4 (left) Electron micrograph (×2210) showing activated choroidal neovascularization (CNV) extending into a disrupted Bruch's membrane. Small amorphous hard drusen (D) lie on Bruch's membrane but there is minimal diffuse debris under the retinal pigment epithelium (RPE). (right) Electron micrograph section (×1410) close to CNV, just beyond the vessel leading edge, showing macrophage closely in contact with the outer surface of Bruch's membrane, beneath two hard drusen. The outer collagenous zone appears thinned here (curved arrow). A thin layer of membranous debris lies on the internal surface of Bruch's membrane, external to the basement membrane of the RPE (straight arrows). Adapted with permission from Sarks JP, Sarks SH, and Killingsworth MC. Eye 1997; 11:515-522. Copyright Nature Research 1997

to persist even in the absence of angiogenic factors. The biology of angiogenesis and maturation is fundamental to NVAMD, as the anatomical subtype of capillary CNV comprises approximately 20–25% of all NVAMD cases [200–202]. Moreover, angiogenesis and maturation represent the prevailing biology in the vast majority of studies of experimental laser-induced CNV, since these studies are focusing on the formation and growth of capillary CNV in young (i.e., 2–4 month old) mice. Numerous studies have found that following initial CNV induction, macrophages are recruited to the site of incipient neovessel formation and contribute to capillary angiogenesis and maturation via release of effector growth factors [186, 187]. Inflammatory macrophages are recruited to the incipient CNV beginning at day 2 following induction can promote angiogenesis either directly via release of VEGF or indirectly via release of TNF-α and IL-1β, which stimulate the RPE to produce VEGF and macrophage recruitment factors such as MCP-1 and IL-8 [203]. In young mice, reparative macrophages (frequently identified by expression of cell surface molecules CD163 and/or CD206) begin to infiltrate the lesion at day 4 and express high levels of factors such as VEGF, PDGF, FGF-1, Ang-1, Ang-2, and IL-10, as histopathology with immunostaining of CNV in the laser model demonstrate localization of angiogenic factors with reparative macrophages [203, 204]. Furthermore, multiple studies have demonstrated that depletion of circulating monocytes by systemic clodronate administration or by systemic monocyte or local macrophage depletion using CD11b + -DTR system limits capillary angiogenesis, with reduction in capillary CNV lesion size [205, 206]. Correlative human studies have found that circulating monocytes of NVAMD patients express high levels of VEGF relative to controls [207, 208], suggesting that blood-derived macrophages may directly contribute to angiogenesis in NVAMD, while other data suggest that monocytes from NVAMD patients produce higher levels of MCP-1 and IL-8, both macrophage chemotactic factors, indicating that recruited macrophages may also indirectly amplify this disease process via ongoing recruitment of monocytes to the site of CNV formation [208]. Thus, infiltrating macrophages, predominantly of the reparative subset, may contribute to angiogenesis and maturation of capillary CNV lesions in some patients

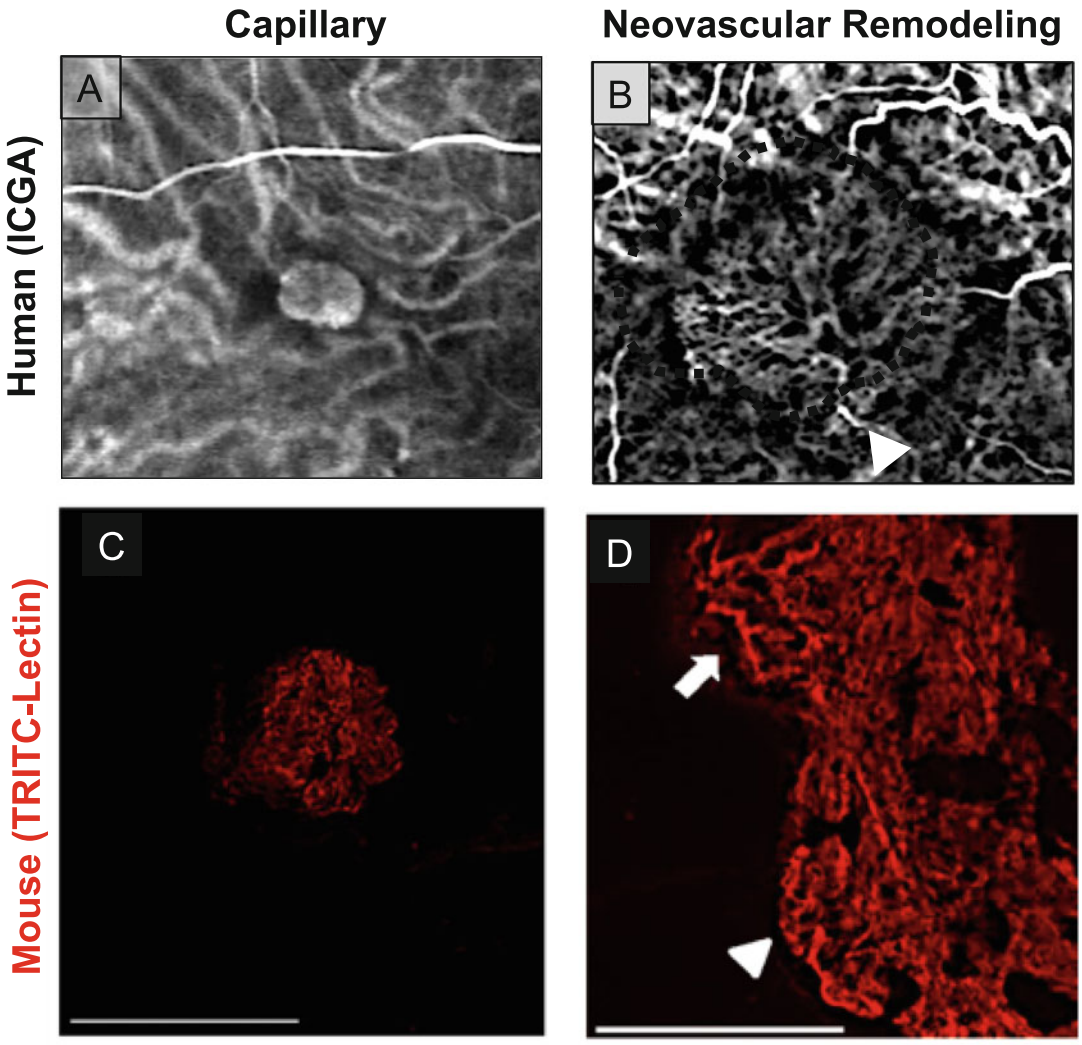

Fig. 6.5 Morphology of choroidal neovascularization (CNV), in human neovascular age-related macular degeneration (AMD) (top, **a**, **b**), and in mouse laser-induced CNV (bottom, **c**, **d**). Representative clinical images of indocyanine green angiography (ICGA) in neovascular AMD demonstrate (**a**) capillary CNV, evident as a small microvascular network; and (**b**) neovascular remodeling (NVR), evident as large caliber feeding artery (arrowhead), many branching arterioles, and minimal capillaries. Representative TRITC-lectin flatmounts of CNV demonstrate analogous morphology features including (**c**) small capillary microvascular network in young (2 month old) mice; and (**d**) branching arterioles (arrow), terminal vascular anastomotic loops (arrowhead) and minimal capillary component (left), all characteristic of NVR. Scale bars = 100 μm. Adapted with permission from Espinosa-Heidmann DG, et al., Invest Ophthalmol Vis Sci. 2013; 54:7439-7449. Copyright Association for Vision and Research in Ophthalmology 2013

via production and secretion of relevant growth factors and cytokines that promote and amplify angiogenesis.

(c) *CNV Formation and Growth—Neovascular Remodeling:* It is increasingly recognized from clinical phenotyping, especially characterization by indocyanine green angiography, that there is a tremendous heterogeneity of CNV morphology among NVAMD patients [200–202, 209, 210]. This

heterogeneity in morphology and anatomical subtypes suggests differential mechanisms of neovascular growth. As noted above, capillary CNV comprise 25% of NVAMD patients. In contrast, patients with arteriolar CNV, characterized by large-caliber feeding artery, many branching arterioles, terminal vascular anastomoses, and minimal capillary component, account for over 50% of NVAMD patients [200–202] (Fig. 6.5) [199]. The arteriolar CNV pattern reflects neovascular remodeling (NVR): the transformation of nascent neovessels into branching arterioles with perivascular fibrosis. As compared to capillary angiogenesis, the pathobiology of NVR is not well understood. Histopathology studies, both from surgically excised CNV and postmortem studies, suggested an increased frequency of macrophages in lesions with arterioles and perivascular fibrosis [145, 211, 212] (Fig. 6.6). Furthermore, CNV with arteriolar morphology are specifically associated with increased infiltration of the CD163+ macrophages [146], which include reparative macrophages, and profibrotic factors produced by macrophages such as TGF-β, FGF, IGF-1, osteopontin, and SPARC localize to macrophages in fibrotic CNV in postmortem studies [203, 213]. Macrophage biology in the setting NVR may share some pathologic overlap with reparative macrophage contributions to atherosclerosis [214] and glomerulosclerosis, where macrophages mediate fibrosis and vascularization of the atheroma [215–218] or mesangial cell proliferation [219–221], respectively. Clinical and pathologic studies demonstrate that macrophages and increased intraocular levels of macrophage-derived cytokines are associated with more active (i.e., exudative or leaking) CNV less frequent in eyes with inactive CNV [145, 180, 222]. Lastly, there is emerging evidence linking biology of reparative macrophages to more severe forms of disease in NVAMD. Patients with NVAMD have increased expression of the transcriptional activator STAT3 in intermediate monocytes, which may contribute to increased arteriolarization and fibrosis in CNV [207]. Additional investigations have observed that the proportion of circulating CD11b+ monocytes directly correlate with the frequency of anti-VEGF injections necessary for disease control [223]. These data collectively suggested that blood-derived macrophages infiltrating the site of CNV may promote NVR (arteriolarization and fibrosis) as well as increased disease activity or exudation, which lead to anti-VEGF resistance in NVAMD.

NVR biology can be modeled in the murine laser-induced CNV model in several different conditions, including mice aged to 16 months of age, mice exposed to cigarette smoke, mice with latent chronic cytomegalovirus (CMV) infection, and mice exposed to low-grade microbial toxins (i.e., PAMPs) [105, 224–226]. In each of these models, CNV lesions exhibit increased size with high-flow, large-caliber arterioles and perivascular fibrosis, along with increased leakage (exudation), as compared to capillary CNV. In the aging model of NVR, depletion of circulating monocytes with clodronate liposomes abrogates the arteriolar phenotype, resulting instead in the formation of capillary CNV, indicating that macrophages drive the development of NVR [227]. Other studies have also demonstrated that increased profibrotic effector function of reparative macrophages in aged mice, and the recruitment and activation of the reparative macrophage subset may be in part mediated by IL-10 signaling, as genetic deletion of IL-10 limits NVR biology, with smaller lesions in association with increased inflammatory macrophage frequency and decreased reparative macrophage infiltration [228, 229]. Furthermore, the NVR-mediating effector function in reparative macrophages may occur via increased STAT3 signaling as well as by

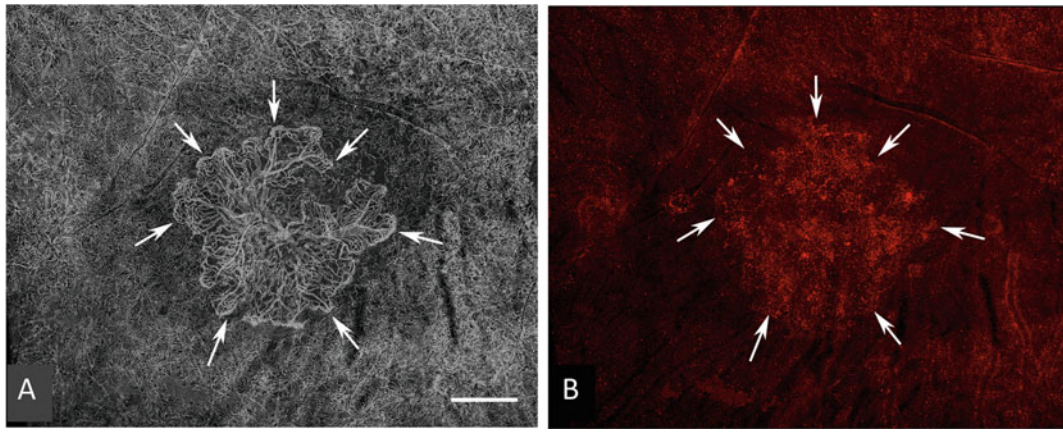

Fig. 6.6 Choroidal flatmount micrograph of a patient with neovascular AMD showing a CNV in the submacular region. (**a**) desaturated Ulex europaeus agglutinin (UEA) lectin stain demonstrates neovascular remodeling (NVR) with central feeder artery, multiple branching arterioles, and terminal vascular loops (arrows). (**b**) Labeling for HLA-DR+ macrophages demonstrates extensive HLA-DR+ macrophage infiltration in association with arteriolarized vessels in NVR CNV lesion. Scale bar = 1 mm. Adapted with permission from McLeod DS, et al., Invest Ophthalmol Vis Sci. 2016; 57:5843-5855. This work is licensed under a Creative Commons Attribution 4.0 International License. Copyright The Authors 2016

alterations in LXR nuclear receptor activity in this macrophage subset, suggesting that these pathways could be targeted to modulate NVR biology [230, 231]. Additionally, CD11b+ reparative macrophages at the site of incipient CNV formation were positive for a variety of factors that mediate fibrovascular growth, including PDGF-β, FGF-1, FGF-2, and TGF-β [204]. Interestingly, local ocular corticosteroids do not prevent perivascular fibrosis and CNV-associated scar formation [232], which is likely due to the fact that corticosteroids have limited effects on the effector function of CD163+ reparative macrophages [233]. The pathobiology of NVR extends beyond macrophages, however, as bone marrow transplantation from aged to young mice transfers age-related susceptibility to NVR independent of macrophage function, as a result of increased frequency of circulating mesenchymal progenitor cells (MPCs) in the bone marrow of aged mice [199]. Collectively, these data support the concept that NVR biology occurs as a result of cross-talk between reparative macrophages and MPCs, wherein macrophages recruit MPCs from the circulation and activate them to become vascular smooth muscle cells (VSMC) and myofibroblasts, establishing the template for arteriolar CNV, early in the process of formation and development [199]. While there is much to be explored and understood about the regulatory mechanisms for NVR, modulation of the cross-talk between reparative macrophages and mesenchymal precursors may represent a novel therapeutic strategy to target NVR and extend benefits of disease control and improved visual function for the large segment of patients with NVAMD and arteriolar CNV.

(d) *Regulation of Macrophage Biology in CNV Formation and Growth:* The specific mechanisms that regulate macrophage biology in the setting of CNV formation and growth are not well understood. In terms of macrophage recruitment and infiltration, as noted above, both MCP-1 and IL-8 [203], as well as IL-6 may mediate the recruitment of monocytes from the circulation to the incipient CNV [234]. There is also evidence to

support a role for the leukotriene B_4 (LTB4)-leukotriene B_4 receptor 1 (BLT1) signaling axis, as blockade of LTB4 reduces macrophage infiltration [235]. Evidence from the laser CNV model indicates that soluble Fas ligand (sFasL) released from injured RPE is a key mediator of monocyte recruitment and infiltration in aged mice [236]. Inhibition of complement factor C3 reduces CNV macrophage infiltration, suggesting that complement components may serve as stimuli of inflammatory or reparative macrophage infiltration and activation [237]. Collectively, these data suggest that there may be multiple pathways, rather than a single master factor, regulating macrophage infiltration to the site of CNV formation. Understanding the regulation of macrophage effector function remains an emerging area of investigation, but several studies have highlighted potential mechanisms. Modulation of macrophage STAT3 signaling [234], inhibition of macrophage Rho-associated kinase (ROCK2) [238], and inhibition of RIP1 kinase [239] may downregulate reparative macrophage effector biology in the setting of CNV formation and growth [228, 229, 234]. Additional investigation into mechanisms of macrophage biology will be essential to develop therapeutic strategies directed against macrophages in NVAMD.

(e) *Macrophage Infiltration in Neurosensory Retina:* While the vast majority of clinical and preclinical studies have centered on the contributions of blood-derived macrophages to CNV induction, formation, and growth, considerably fewer studies have explored the roles and effects of infiltrating blood-derived macrophages to pathology and dysfunction in the overlying neurosensory retina. Histopathology of NVAMD demonstrates significant infiltration of CD163+ macrophages in the neurosensory retina overlying CNV, especially in CNV with arteriolarization and fibrosis [146]. In the murine laser-induced CNV model, using the 810 nm laser that limits injury to the photoreceptors and the overlying retina (as compared to the 532 nm laser), there is substantial infiltration of blood-derived macrophages into the retina overlying CNV, which increases along with the lateral spread of the CNV over time [173] (Fig. 6.7). Retinal macrophage infiltration in this setting is associated with diminished ERG B-wave amplitudes and disruption of photoreceptor-bipolar synapses in the outer plexiform layer and Muller cell injury and activation [174]. Effects on synapses could be mediated by macrophage-derived and secreted TNF-α or IL-6, which may specifically promote Muller injury and activation. Importantly, prevention of retinal macrophage infiltration abrogates the synaptic and visual dysfunction [173, 174], suggesting that specific therapies targeted against retina-infiltrating macrophages in NVAMD could improve visual function independent of leakage control.

(f) *Integrated Hypothesis for Macrophage Biology in NVAMD:* Clearly, there is considerable evidence that macrophages contribute to NVAMD, but much remains to be determined about the specific mechanisms by which macrophages mediate effects on CNV pathobiology. Based on the available clinical, histopathologic, and preclinical evidence to date, we embrace the hypothesis that blood monocytes give rise to distinct macrophage subsets, inflammatory and reparative, and monocytes that give rise to each are preprogrammed for distinct effector biology. We propose that monocytes that are primed to express high levels of TNF-α, IL-1β, NOS2, VEGF, and proteases such as MMP-9 [52], arrive at the locus of disease at the RPE, Bruch's membrane, and choriocapillaris as part of an initial wave of recruitment, where they are transformed into fully activated inflammatory macrophages in situ, secreting effector molecules that contribute to or perhaps directly trigger the induction and initial development of the CNV. Following this initial phase of recruitment, a second

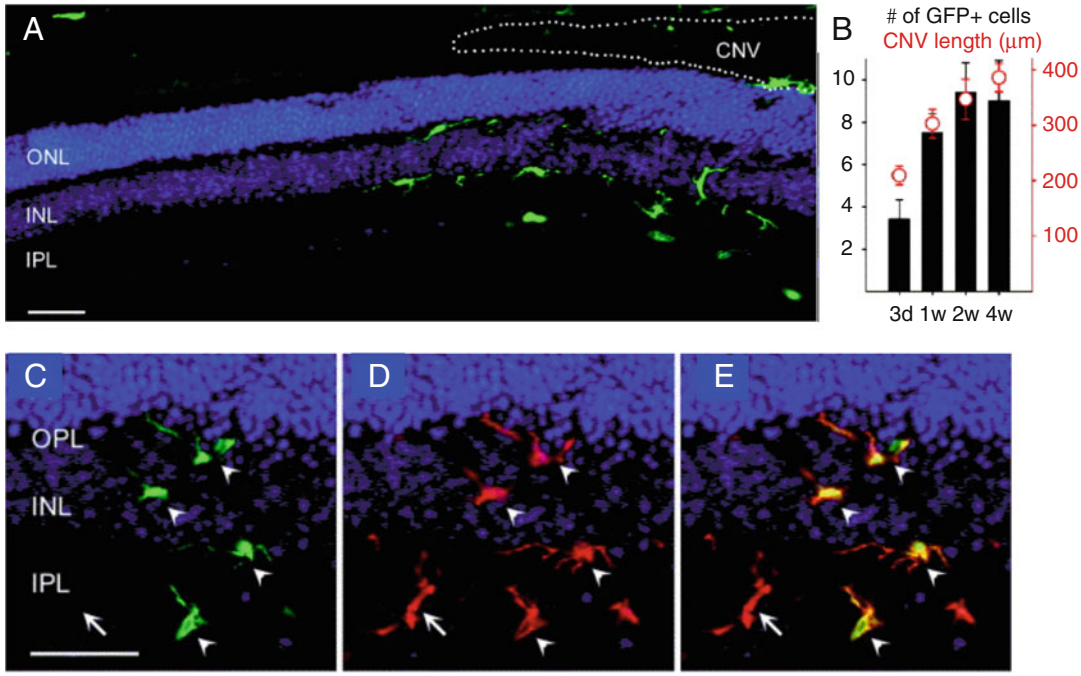

Fig. 6.7 (**a**) Bone marrow-derived cells, labeled green fluorescent protein (GFP), infiltrate the neurosensory retina overlying choroidal neovascularization (CNV) but are absent in adjacent retinal regions outside of the CNV. (**b**) The frequency of GFP+ bone-marrow derived cells directly correlates with the lateral extension of CNV and increases over time following laser induction, plateauing at 2 weeks. (**c–e**) GFP-labeled cells are macrophages. In these representative images (4 weeks after CNV) of (C) GFP-labeling and (**d**) F4/80 macrophage label, all GFP-labeled cells were F4/80 immunoreactive (*arrowheads*), appearing yellow in (**e**). A single resident microglial cell (arrow; not GFP-labeled) could also be seen. ONL, outer nuclear layer; INL, inner nuclear layer; IPL, inner plexiform layer; CH, choroid. Scale Bars = 50 μm. Adapted with permission from Caicedo A, et al., Exp Eye Research 2005; 81:38-47. Copyright Elsevier Ltd. 2005

phase of monocytes that give rise to reparative macrophages are subsequently recruited to the incipient CNV [52–54]. On full transformation, these reparative macrophages secrete either predominantly angiogenic factors (e.g., VEGF, PDGF, and Ang-1) to mediate capillary angiogenesis, the formation and growth of capillary CNV; or profibrotic and vascular factors (e.g., TGF-β, osteopontin, IGF-1, FGF, CTGF) to mediate neovascular remodeling, formation and growth of arteriolarized CNV with perivascular fibrosis via recruitment and activation of mesenchymal precursors to become vascular smooth muscle cells and myofibroblasts. The specific determinants of whether reparative macrophages exhibit either proangiogenic or profibrotic effector function are unknown, but based on available evidence, risk factors such as age, cigarette smoke exposure, low-grade exposures to microbial toxins (e.g., PAMPs), and latent macrophage infection with CMV [105, 108, 224–226] all shift reparative macrophages to profibrotic, neovascular remodeling effector functions [53, 240]. Thus, both subsets contribute to CNV biology, but CNV lesions experience a dynamic, biphasic recruitment of macrophages, first with an early recruitment of inflammatory macrophages promoting induction and initial development of CNV, and subsequently with a second delayed but more sustained recruitment of reparative macrophages that mediate

continued CNV formation and growth. In total, the effects of macrophages on CNV pathobiology is driven not only by this biphasic recruitment of macrophage subsets, but also by the relative numbers of primed monocytes in the blood (giving rise to each subset) at the time of recruitment [241, 242]. Thus, under this paradigm, therapeutic strategies directed against inflammatory macrophages could be effective to prevent progression to NVAMD, while strategies directed against reparative macrophages could be effective to limit CNV growth, and specifically to limit the biology of neovascular remodeling.

6.3.1.3 NLRP3 Inflammasome

The NLRP3 inflammasome is a multimeric complex of cellular cytosolic proteins that assembles in response to specific exogenous and endogenous danger signals, such as pathogen-associated molecular patterns (PAMPs), which include microbial-associated toxins (e.g., LPS, zymosan, viral antigens) and danger associated molecular patterns (DAMPs), which include protein or nucleic acid components (e.g., cytoplasmic DNA, noncoding RNA transcripts) released or exposed following cellular injury [139]. Assembly and activation of the NLRP3 inflammasome thus integrates the cellular response to various injury stimuli, activating caspase-1 via proteolytic cleavage, which in turn cleaves precursors of IL-18 and IL-1β to active cytokines that are secreted and mediate pro-inflammatory signaling [139]. The NLRP3 inflammasome was classically identified and characterized in innate immune cells including macrophages and microglia but has been more recently described and characterized in specialized epithelial cells, including RPE cells. While NLRP3 activation can serve as an important host defense mechanism, dysregulation of inflammasome activation is now thought to contribute to a variety of chronic diseases, including diabetes, neurodegenerative diseases such as Alzheimer's disease, and AMD [139].

Several studies have demonstrated that NLRP3 activation in RPE cells may serve as a final common pathway in response to various injury stimuli linked to AMD and that activation of the NLRP3 inflammasome and production of IL-18 and IL-1β may mediate RPE cellular injury and eventually cell death (by way of pyroptosis, a specialized form of inflammatory programmed cell death), leading to GA [243–247]. Initial work in this area evaluated cytotoxicity associated with the noncoding RNA motif known as Alu repeat and found that deficiency of DICER1 at the RPE led to Alu accumulation and RPE cell death, with corroborative features present in histopathology of human dry AMD [248]. Subsequent investigations found that Alu cytotoxicity and atrophic disease was mediated by activation of the NLRP3 inflammasome and production of IL-18 at the RPE [245]. Moreover, Alu RNA activation of the NLRP3 inflammasome at the RPE was found to be independent of TLR but dependent on P2X7 [249], and P2X7-mediated NLPR3 inflammasome activation and RPE atrophy was readily inhibited by nucleoside reverse transcriptase inhibitors (NRTIs) [250]. Additional work has determined that NLRP3 inflammasome activation is generalizable to other potential triggers of AMD disease, including iron toxicity, complement, reactive oxygen species, and lipid dysregulation [243–247, 251]. Collectively, these findings support inhibition of the NLRP3 inflammasome, IL-18, IL-1β, and potentially downstream effector events as a therapeutic strategy to prevent or slow GA in dry AMD. On the other hand, several other studies suggest that NLRP3 inflammasome expression in the setting of AMD is not found at the RPE but is instead restricted to macrophages and microglia [252–254], and that activation of the NLRP3 inflammasome in macrophages by stimuli such as complement components as well as subsequent IL-18 production actually serve a protective role, limiting severity of experimental CNV [253]. These opposing conclusions highlight that further study is needed to characterize the specific roles of NLRP3 inflammasome activation in different cell types and at various stages

of AMD disease before effective therapeutic strategies can be developed for AMD.

6.3.2 Complement

Histopathological studies have demonstrated that complement, along with other components of the immune system are abundant in drusen, the hallmark of dry AMD [255, 256] (Fig. 6.8) and at the border of GA in advanced dry AMD [257]. In addition, reduced levels of negative regulators of complement have been found in RPE overlying drusen and GA [258, 259]. In addition to histopathological studies, polymorphisms in numerous complement genes have been associated with AMD. The first discovered, and strongest genetic association is the 402H polymorphism in complement factor H (CFH) [4–7]. Subsequently, genome wide association screens (GWAS) have identified multiple complement components including complement component 2 (C2), complement factor B (CFB), complement component 3 (C3), complement component 9 (C9), complement factor I (CFI), and vitronectin (VTX) [260–265]. While these studies have strongly associated complement polymorphisms with AMD, the mechanistic role of complement in the pathogenesis of both dry and neovascular AMD is still being characterized.

Recently, rare and ultra-rare variants in complement proteins have been linked to both development and progression of AMD and have lent support to a functional role for complement dysregulation in AMD pathogenesis. For example, ultra-rare variants of CFH have been linked to autosomal dominant early onset drusen, which is considered to be a severe inherited form of dry AMD [266]. Rare variants of CFH and C3b which are associated with AMD affect the region of contact between CFH and C3b suggesting that in some cases defects in C3b inhibition by CFH result in increased activation of the alternative pathway leading to development of dry AMD [267–269]. Other rare variants in both CFH and CFI have been associated with reduced C3b degradation in serum-based assays [270]. Finally, rare AMD-associated variants in CFI, a major negative regulator of complement, have been associated with reduced serum levels of CFI while rare variants in C9 have been associated with increased serum levels of C9; both of which may predispose to increased complement activity [270]. Taken together, these studies suggest that genetic variants which favor increased complement activity predispose people toward development of AMD. Recently, the increase in clinical trials aimed at prevention of the development of GA or CNV (late AMD) has led to considerable interest in identifying patients at risk for rapid progression as these patients would most benefit from therapy and are better suited for clinical trials. In addition to clinical markers for rapid progression, genetic approaches have been applied. For example, a bivariate GWAS which took into account both AMD status as well as time to progression to advanced AMD defined as CNV or GA identified four previously discovered risk alleles as risk factors for progression to advanced AMD including regions coding for CFH, C3 and C2-CFB-SKIV2L suggesting complement as a risk factor for both development and progression of AMD [271].

Given the suggestion that increased complement activity is a risk factor for AMD, numerous studies on in vitro and in vivo models of AMD have been used to further dissect the molecular mechanisms by which complement may cause or worsen AMD. From these studies, several nonmutually exclusive theories regarding the role of complement in pathogenesis of AMD have emerged. These include direct cellular toxicity to RPE and/or photoreceptors, injury to choroidal endothelium resulting in localized hypoperfusion, upregulation of pro-angiogenic proteins within RPE leading to CNV, and recruitment of macrophages which play important roles in both dry and neovascular AMD (see sections on Macrophages in Dry AMD and Macrophages in NVAMD). In addition, noncanonical roles for CFH have been described which challenge the traditional paradigm of complement biology in AMD. As discussed below, perturbations in a single complement factor can mediate more than one of the above mechanisms and can impact both dry and neovascular AMD.

Effector proteins from the complement cascade are found in drusen and may play a role in

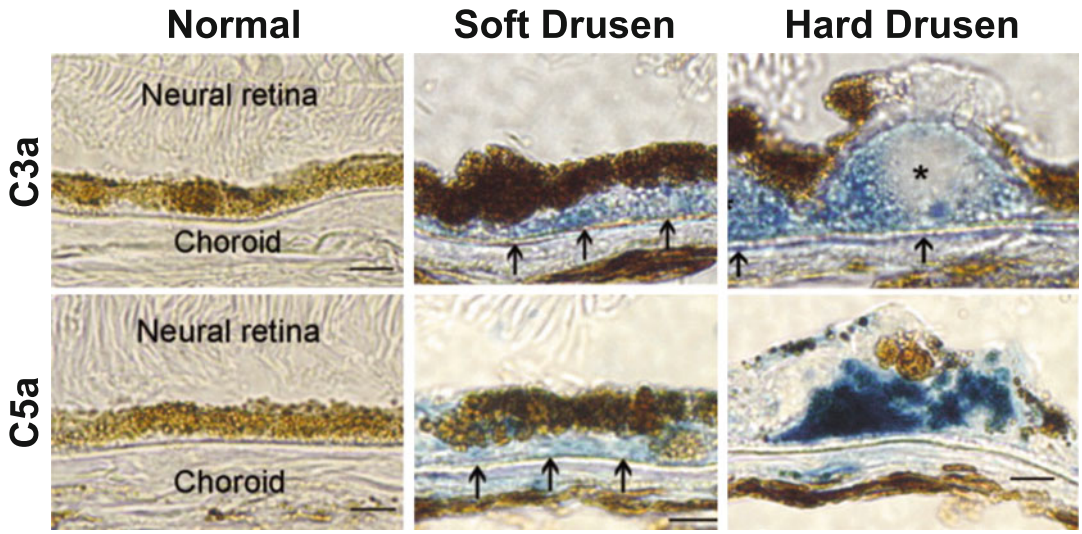

Fig. 6.8 Histopathology of drusen and complement in dry AMD. C3a (top panels) and C5a (bottom panels) are present in soft or hard drusen in histopathological specimens from eyes with dry AMD but not in healthy eyes. C3a and C5a are stained in blue; RPE pigment is seen in brown. Scale bars =10 μm. Adapted with permission from Nozaki, M., et al., Proc Natl Acad Sci USA 2006; 104:2328-33. Copyright National Academy of Sciences, U.S.A. 2006

the development of advanced AMD. For example, both C5a and C3a are found in drusen in human tissues [6, 255, 256] (Fig. 6.8). Both proteins are capable of upregulating VEGF expression in cultured RPE and in mouse RPE-choroid complexes in vivo. C3a and C5a are also upregulated at sites of laser induced CNV in mice. Genetic or pharmacologic blockade of C3a, C5a or their respective receptors results in reduction in experimental CNV lesion size [255]. In addition, both C3 and C5 are localized to photoreceptor outer segments (POS) located at the border of GA in human histological samples. Isolated POS were capable of activating complement in vitro and C5a was found to mediate macrophage recruitment resulting in photoreceptor cell death in a mouse model of toxin induced retinal degeneration. In this model, genetic deletion of C3 and C5 resulted in reduced photoreceptor cell death [257]. Taken together, these data suggest multiple roles for C3 and C5, particularly in the pathogenesis of advanced AMD.

In addition to increased complement effector proteins, reduced levels of endogenous negative regulators of complement have also been noted in histopathological specimens from eyes with AMD. Perturbations of these proteins result in development of RPE dysfunction, thickened Bruch's membrane, macrophage infiltration and more severe CNV in mice. For example, CD59 was found to be decreased in regions of drusen or GA in human pathology specimens [259]. Mice deficient in CD59a (the ortholog to human CD59), a membrane-bound inhibitor of MAC develop subretinal deposits composed of mononuclear phagocytes, with the subretinal myeloid cells appearing comparable to subretinal macrophages observed in human specimens with dry AMD [146, 259]. CD59 is also downregulated at sites of laser-induced CNV in mice and deficiency in CD59 results in increased lesion size which was associated with increased MAC assembly at the lesion site [272]. Interestingly, an RPE-choroid targeted fusion protein of CD59 resulted in decreased lesion size and reduced MAC assembly [272]. CD46, another membrane bound, negative regulator of MAC which is expressed in RPE and neural retina is also reduced in human AMD histopathology

specimens [259]. Mice deficient in CD46 were found to develop a dry AMD phenotype including thickening of Bruch's membrane, RPE vacuolization, multinuclear RPE cells [273]. These mice also demonstrate increased VEGF expression within RPE-choroid and larger lesions in the laser induced CNV model [273]. Finally, pentraxin-3 (PTX3), which can function as a negative regulator of complement activity is decreased in RPE overlying drusen in human samples. PTX3 is upregulated by oxidative stress in cultured RPE and deficiency in PTX3 caused enhanced activation of complement as well as the NLRP3 inflammasome in response to oxidative stress which resulted in enhanced macrophage recruitment to the choroid [258].

Complement-mediated injury to the choriocapillaris endothelial cells resulting in decreased vascular density and subsequent RPE dysfunction has also been proposed as a driver of AMD. Histopathological studies demonstrate that there is a generalized decrease in choriocapillaris vascular density in AMD cases versus controls and further shows reduced choriocapillaris vascular density beneath drusen compared to adjacent regions [274]. This suggests that alterations in the morphology of choriocapillaris are associated with dry AMD. In vitro studies of cultured choriocapillaris endothelium exposed to complement containing human serum demonstrate MAC-mediated cell lysis of some cells and upregulated angiogenesis associated genes including VEGF, MMP-3 and MMP-9 in surviving cells. This observation ties complement to both choriocapillaris loss as well as development of CNV in AMD [275].

Polymorphisms in CFH represent one of the most common genetic associations with AMD. While rare variants which result in defected downregulation of complement activity have been described, the H402 polymorphism has not been found to disrupt these functions. However, noncanonical roles for CFH which are impacted by this polymorphism have been described. CFH was found to be a major serum binding partner for malondialdehyde (MDA) adducts, which are produced by oxidative stress. CFH binding to MDA decreased macrophage phagocytosis of MDA adducts and decreased inflammatory signaling in vivo. However, this function was impaired in H402 CFH [276]. CFH has also been shown to compete with lipoproteins for binding sites within Bruch's membrane; deficiency in CFH results in increased sub-RPE deposits in a mouse model of dry AMD [277].

6.3.3 Adaptive Immunity

Traditionally, adaptive immunity has not been considered as a major contributor to either dry or neovascular AMD. This is in part due to limited histopathologic evidence of lymphocytes in AMD specimens and because the retina and choroid lack an identifiable or characterized lymphatic system for support of a traditional afferent arc of the adaptive immune response. However, as will be discussed, emerging clinical evidence, as well as several preclinical models, suggests plausible albeit limited roles for T cells and for autoimmunity in subset of AMD disease.

6.3.3.1 T Cells and Associations with AMD

While histopathologic evidence of lymphocytes in AMD has been limited, it has not been entirely lacking. Penfold, et al., initially described the presence of lymphocytes in eyes of patients with GA and NVAMD and suggested that lymphocytes may play a role in both diseases by promoting RPE atrophy and alteration in Bruch's membrane, respectively [76, 89]. CD8+ T cells have been observed by fluorescence microscopy in the choroid of frozen sections of eyes of AMD patients with GA [278] and NVAMD [279]. It remains uncertain whether T cells observed in association with GA or within CNV are responding to specific antigens or have been recruited as part of bystander activation. Since MHC class II expression has been found on RPE cells [280] and may be enhanced on microglia or infiltrating macrophages in aging and AMD [281, 282], antigen-presentation to infiltrating T cells is plausible; on the other hand, there is evidence to support antigen-independent activation of cytotoxic CD8+ T

cells in experimental mouse models of RPE injury [283]. In patients, levels of the chemokines CXCL10 and CXCL11, both chemotactic for CXCR3+ T cells, were found at increased levels in the RPE/choroid in patients with NVAMD and GA, respectively, while both chemokines were found to be elevated in the plasma of patients with AMD, as compared to normal controls [284]. In another study, increased percentages of CD56+ and CD28- memory T cells in the blood were associated with increased risk of developing AMD [285], with the risk increasing further for those patients also having at least one CFH H402 allele [285], and it was also found that lower percentages of CD8+ $CXCR3^{high}$ T cells and CD4 + CD69 + CXCR3+ T cells were present in NVAMD as compared to controls [286]. Meanwhile, the percentage of circulating CD4+ T cells was increased in patients with NVAMD with subretinal fibrosis, as compared to patients without subretinal fibrosis [287]. On the other hand, in the murine laser-induced CNV model, IL-4, a T helper cell type 2 (Th2) derived cytokine, conditions resident microglia and recruited macrophages to produce a soluble form of the VEGF Receptor 1 (also known as sFlt1) and subsequently suppress capillary angiogenesis [288]. Collectively, these data suggest the possibility that some T cell subsets may play a role in AMD pathobiology, and that others may play a protective role against the development of various aspects of AMD disease.

6.3.3.2 IL-17 and T_h 17 T cells

IL-17 is a pro-inflammatory cytokine that signals through the IL-17 receptor (IL-17R) system, activating signaling pathways and inducing production of other proinflammatory cytokines (e.g., TNF-α, IL-6) and chemokines (e.g., IL-8, MCP-1) that promote the recruitment of monocytes, neutrophils, and other T cells. IL-17 can also directly mediate effector biology on target cells, including endothelial cells, epithelial cells, and neurons [289]. The predominant source of IL-17 is a subset of CD4+ T cells known as T helper 17 (T_h17) cells, though it can also be produced by other types of immune cells. Increased IL-17 expression has been found in close approximation with loci of GA in the setting of AMD, as compared to normal age-matched controls, and IL-17 localized predominantly to CD3+ cells, a marker of T cells as well as to IBA-1+ macrophages/microglia [290]. IL-17 and another T_h17 cytokine IL-22 have been found to be elevated in the plasma of AMD patients as compared to non-AMD controls [291, 292], and that this elevation may be in part mediated by stimulation of CD4+ T cells by the activated complement component C5a [292]. It has also been demonstrated that hypomethylation of the promoter for IL17RC, a component of the IL-17R complex, in AMD patients as compared to controls, in association with elevated expression of IL-17RC mRNA and protein in peripheral blood as well as in the affected retina and choroid, suggesting a role for both local and systemic alterations in IL-17 signaling in AMD [291]. However, another study analyzed the methylation status of the IL-17RC promoter using multiple analytical methods and found no significant difference in the methylation status between patients with AMD and age-matched controls in two independent cohorts, so the role of altered IL-17R in AMD remains uncertain [293]. There is also debate about the potential role of IL-17 in NVAMD. One study found that IL-17, derived from γδ-T cells and THY1+ innate lymphoid cells (ILCs) and not T_h17 cells, promoted experimental CNV growth in a VEGF independent manner [294]. Other studies offer controverting evidence, indicating that IL-17 alone does not impact vessel growth but can indirectly influence neovascularization by modulating the effects of other angiogenic factors (e.g., VEGF, bFGF, HGF) [295]. Thus, while there is considerable evidence to suggest a potential association of IL-17 with. AMD, its precise role with advanced dry AMD with GA and NVAMD remains to be elucidated [296].

6.3.3.3 Autoimmunity and AMD

Several reports have identified immunoglobulins and immune complexes in association with drusen [256, 297–299], which may reflect one or more aspects of aberrantly activated adaptive immunity including molecular mimicry, antigen-trapping, desequestration, or formation of neo-antigens. There is considerable emerging

evidence that autoimmunity, specifically autoantibody-mediated disease, may play a role in AMD. Penfold and colleagues initially described autoantibodies to retinal astrocytes in association with AMD [300], suggesting a role for antiretinal autoantibodies in a subset of AMD disease. Numerous studies since have demonstrated, by various techniques, especially immunohistochemistry, elevated levels of antiretinal autoantibodies in the peripheral circulation of AMD patients as compared to controls [289]. Multiple different types of autoantibodies have been described, including autoantibodies to glial fibrillary protein (GFAP), which is expressed by Muller cells and astrocytes, recoverin, which is expressed by photoreceptors, and α-enolase, which is found in retinal ganglion cells and inner nuclear layer. Additional autoantibodies have been found to be elevated in the sera of AMD patients, including antibodies to phosphatidyl serine (PS), JO-1, U1-snRNP-68, elastin, cytochrome C, sc-100, and collagen III, all of which were specifically associated with NVAMD/CNV and not dry AMD, while autoantibodies to fibronectins were specifically associated with dry AMD [289]. Autoantibodies to various components of drusen, extracellular matrix, and Bruch's membrane have also been described in AMD patients, indicating that aberrant activation of antibody-mediated adaptive immunity could be mediated by desequestration of RPE and retinal cellular antigens which are aberrantly deposited in the subRPE space in AMD. Alternatively, components of drusen and extracellular deposits, such as oxidized lipoproteins, can become neo-antigens to activate an immune response arc, leading to formation of autoantibodies [289, 301]. As has been found in other diseases such as atherosclerosis, scavenging macrophages may then ingest neo-antigens and become antigen-presenting cells at the site of drusen formation or RPE cell injury, serving to re-stimulate recruited T cells and thereby activating the effector phase of the immune response.

A variation on neo-antigen concept is the development of adjuncts or adducts of RPE and retinal proteins, which are also recognized by autoantibodies. Oxidation of docosahexaenoate-(DHA-) containing lipids generates carboxyethylpyrrole (CEP) protein adducts. CEP-adducted proteins have been found at higher levels in drusen and in blood from AMD patients as compared to normal controls [302–306]. Additionally, mean titers of anti-CEP autoantibody are higher in AMD patients as compared to normal controls [307]. These findings are bolstered by work in mice, where immunization with CEP-adducted mouse serum albumin generated development of autoantibodies directed against CEP and subsequently led to the development of RPE deposits and photoreceptor degeneration [308]. CEP-induced immune response in this model is thought to be via activation of T lymphocytes by inflammatory macrophages, leading to increased expression of interferon-γ (IFN-γ) and IL-17 [308]. Collectively, these findings across human histopathology, studies of AMD sera, and preclinical mouse models provide strong evidence to indicate that factors associated with AMD, specifically oxidative injury, can generate protein adducts that serve as neo-antigens, and the development of autoantibodies against these adducted proteins could potentially contribute to AMD disease progression.

Population-based analyses suggest a wide array of retinal autoantibodies may be present in sera of AMD patients, and antigenic targets include proteins that are implicated in autophagy, immunomodulation, protection from oxidative stress, and apoptosis [309], indicating that aberrant activation of antibody-mediated immune mechanisms could potentially amplify a host of cellular dysfunctions that have been independently associated with AMD, both for disease onset and progression. However, it remains uncertain whether the development of such autoantibodies are isolated to mediating AMD disease or if presence of these autoantibodies reflects a more generalized dysfunction of the immune system with the RPE and retina being an opportunistic target of this more generalized dysfunction [310, 311]. Moreover, the presence of retinal autoantibodies are not specific for disease, as they are frequently found in normal subjects without any sign of AMD or retinal

disease [312]. Presently, there is a lack of direct evidence demonstrating that the presence of retinal autoantibodies is a risk factor either for the onset of early AMD or the development of more advanced AMD disease, such as GA or CNV. However, the current associative data provides a compelling starting point for further investigation into the potential link between autoimmunity and AMD.

6.3.4 Infection and AMD

Interactions between the immune system and infectious pathogens such as bacteria and viruses could also modulate AMD disease pathobiology via alterations in innate and adaptive immune effector mechanisms. Accordingly, several hypotheses have been put forth to suggest an infectious etiology of AMD.

6.3.4.1 Direct infection at AMD locus of disease

One possibility is that the virus or bacteria may directly infiltrate and infect the locus of disease at the RPE, Bruch's membrane, and choriocapillaris. Based on the hypothesis that certain bacterial or viral pathogens may produce chronic infection of vascular endothelial cells or the vascular interstitium leading to vascular disease, several studies have explored such a link. *Chlamydia pneumoniae*, an obligate intracellular bacterium, was identified as a novel risk factor in cardiovascular diseases, and numerous studies identified *C. pneumoniae* as a potential contributor to atherosclerosis. Chronic infection of vascular endothelial cells may upregulate cell surface molecules that recruit macrophages or alter responses to injury. For instance, *C. pneumoniae* endothelial infection can enhance endotoxin binding to LDL particles that might induce various inflammatory cascades at the site of uptake [313]. Additionally, chlamydial heat shock proteins (HSPs) can directly stimulate macrophages and other cellular amplification systems [314]. On this basis, several studies explored whether *C. pneumoniae* may also have relevance to AMD. One study discovered evidence of *C. pneumoniae* in CNV tissue from patients with NVAMD, both by immunohistochemistry (IHC) and polymerase chain reaction (PCR), in contrast to no detectable pathogen in non-AMD tissue specimens [315]. The authors of this study also found that exposure to *C. pneumoniae* induced VEGF production in cultured monocyte-derived macrophages and induced secretion of IL-8 and MCP-1 by cultured RPE cells. Meanwhile, a serological association was found between AMD and presence of antibodies to *C. pneumoniae* [316]. While this study raised the intriguing possibility that direct infection may contribute to AMD pathobiology, there is limited evidence for other pathogens detected within CNV and limited additional evidence that specifically C. pneumoniae infection is a generalizable phenomenon in NVAMD [103, 104]. Another potential mechanism by which pathogens may contribute to disease is by release of PAMPs or microbial toxins, which may directly interact with pattern recognition receptors (PRRs) expressed at the RPE surface. For example, TLR2 is highly localized to the apical surface of the RPE in both human and mouse eyes. Activation of TLR2 signaling, evident as nuclear localization of NF-kB, was present in RPE of human eyes with AMD, while in mice, local ocular inhibition of TLR2 reduced experimental CNV in both laser-induced CNV and spontaneous CNV models [20].

6.3.4.2 Molecular mimicry

Another possibility is molecular mimicry, wherein the foreign pathogen expresses antigens that are closely related to self-antigens present at the RPE and/or retina. For instance, antigen-specific immune responses directed against bacterial heat-shock protein (HSPs) (e.g., *C. pneumoniae* HSPs) may cross-react with host proteins expressed in the retina [317]. Additionally, immune responses to bacterial or viral antigens trapped in tissues after occult infection, may also stimulate antigen-specific immunity, or autoimmunity by cross-reactive molecular mimicry [318]. Alternatively, T cells may be recruited by innate responses and become activated by antigen-independent bystander mechanisms.

6.3.4.3 Total pathogen burden

A distinct concept is total pathogen burden, which hypothesizes that systemic immune alterations observed in association with AMD occur as a result of cumulative infections with multiple pathogens over the course of life [289]. In this hypothesis, immune alterations are not due to one single pathogen or infection, but multiple pathogens carry more and cumulative risk—for example, this idea has been put forth to explain elevated levels of C-reactive protein (CRP) in cardiovascular disease [319]. Accordingly, it has been observed that patients with elevated serum antibody titers against all three of cytomegalovirus (CMV), *C. pneumoniae*, and *Helicobacter pylori* had increased risk of NVAMD (over presence of dry AMD) [108]. The concept of total pathogen burden can be understood through priming effects on the innate immune system. Chronic exposure to microbial components can prime, or partially activate, monocytes in spleen, lymph node, bone marrow, or other sites of exposure or surveillance, altering the expression of certain cytokines and mediators and committing exposed cells to specific effector functions. These functions then become fully manifest upon recruitment to the disease locus (i.e., RPE and retina) and transformation into fully activated macrophages. Indeed, the presence of periodontal disease, which is a known primer of circulating monocytes, has been independently associated with AMD, even after controlling for other more established risk factors [320]. Importantly, immune mechanisms of AMD disease are not mutually exclusive. CEP adducts, which are thought to play a role in autoimmune mechanisms of AMD as noted above, have also been found to potentiate TLR2 and TLR1 signaling in macrophages, and could synergize with total pathogen burden to prime monocyte and macrophage effector function in the setting of AMD [321].

6.3.4.4 Latent infection of immune cells

Cytomegalovirus (CMV) is a common virus that infects people of all ages. While primary infection causes little to no symptoms in most people, it is frequently followed by establishment of persistent or latent infection. In assessing CMV IgG titers among dry AMD patients, NVAMD patients, and controls without AMD, there was a significant association of high CMV titer with presence of NVAMD, as compared to dry AMD and controls, suggesting that CMV could contribute to the development of CNV [108]. Possible mechanisms for this biology include latent infection of monocytes and macrophages, as latent infection occurs in bone marrow hematopoietic progenitor cells destined to develop into monocytes [322]. As these infected cells mature, macrophage pro-inflammatory genes become transactivated by CMV immediate-early gene products that are expressed during latency [322–325]. These activated infected monocytes may produce higher levels of inflammatory mediators upon recruitment to the site of RPE/Bruch's membrane injury, triggering CNV formation. Indeed, in mice, latent infection of macrophages with murine CMV (MCMV) was associated with increased growth and severity of experimental CNV in the laser-induced model [105]. In this model, latent MCMV infection was not detected in the choroid and RPE indicating that the effects of CMV in formation of CNV are not due to local latent infection within ocular tissues. This represents a mechanism distinct from atherosclerosis, where the potential mechanisms for the link between CMV and atherosclerosis center around local infection of the diseased blood vessel center around enhanced scavenging of LDL particles by endothelial cells infected with CMV [326–328].

6.4 Conclusions: Understanding the Role of Immune Mechanisms in AMD

In this chapter, we have reviewed innate immunity, adaptive immunity, and immune amplification especially the complement system, and we have explored how each of these may contribute to AMD.

We embrace the "response to injury" paradigm for AMD pathobiology as a means to understand and integrate how immune mechanisms

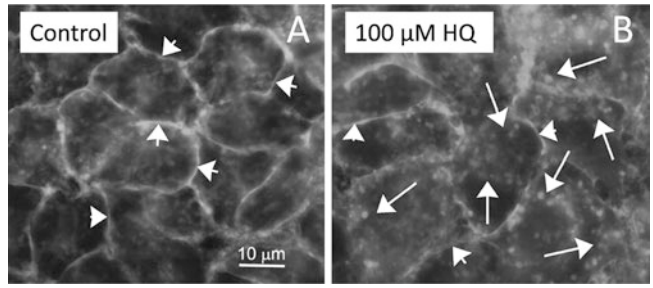

Fig. 6.9 Images of cultured retinal pigment epithelium cells exhibiting extensive cell membrane blebbing following sublethal oxidative injury after exposure to 100 µM hydroquinone (HQ), as compared to control uninjured cells without blebbing (arrows, cell membrane). Scale bar = 10 µm. Adapted with permission from Marin-Castano, ME., et al., Invest Ophthalmol Vis Sci 2006; 47:4098-4112. Copyright Association for Vision and Research in Ophthalmology 2006

contribute to AMD [150]. In this paradigm, various stimuli, including oxidants, lipofuscin cytotoxicity, immune cell-derived mediators, blue light exposure, or systemic factors such as hyperlipidemia, oxidized lipoproteins, and hormonal changes (i.e., increased angiotensin or aldosterone) [150, 329], can mediate nonlethal injury for various cell types, especially, RPE, photoreceptors, and choriocapillaris endothelium. For example, RPE cells can react to nonlethal injury with many responses relevant to deposit formation in AMD, including blebbing of cell membrane (Fig. 6.9) [330], cytosol and organelles (but without activation of programmed cell death or nuclear fragmentation) after oxidant-mediated injury of the cell membrane, which can serve as a starting point for subRPE deposit formation. In this setting, immune mechanisms may serve as a trigger of nonlethal injury. Alternatively, blebbing might activate an immune response by desequestration of intracellular antigens to provide a target for antigen-specific immunity, or blebs might provide a substrate for nonspecific activation of complement, triggering an immune response that can interfere with healthy repair [331, 332]. Importantly, response to injury may be critical not only for disease onset but also for disease progression [150], in the setting of CNV induction and conversion to neovascular AMD or with onset and progression of GA.

In AMD, interaction of various immune mechanisms with nonimmune cells and factors generates exaggerated or abnormal reparative responses to chronic, recurrent injurious stimuli, producing the cardinal pathological features of disease. Developing a precise understanding of how each of these immune mechanisms contributes to AMD pathobiology will be essential to enable the development and validation of clinically useful biomarkers to subtype and stratify AMD patients by specific disease mechanism and to facilitate the development of immune-targeted therapies for patients with AMD.

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