smaller (with diameters of less than 50 μm), and less likely to progress to later stages of the disease. Soft drusen are larger, dark yellowish in color, and more likely to be associated with more advanced stages of the disease. In later stages of AMD, choroidal neovascularization and leakage of serous fluid into the subretinal (occult CNV) or intraretinal (classical CNV) regions leads to cell death and detachment of the RPE. Visual acuity is significantly affected when geographic atrophy of the RPE takes place in the fovea.

Epidemiology of macular degeneration

AMD has a multifactorial (or complex) etiology with contributions from a combination of environmental and genetic factors and a strong age effect. The prevalence of AMD increases dramatically with age, although the prevalence cited in various reports is highly dependent on the definition used for AMD. Overall, AMD increases from less than 1 to 2% at 50 years of age to as high as 15% at 90 years old. It has been suggested that increased skin pigmentation tends to protect from AMD, and this correlates to lower prevalence of AMD in African derived populations than Caucasians in some, but not all, studies. Various other risk factors may predispose to AMD including systemic hypertension and atherosclerosis, as well as cigarette smoking. Both photo-oxidation and inflammation have been suggested as possible pathogenic mechanisms for AMD, although the precise mechanism through which these result in disease has not been delineated.

Genetic factors have been implicated in AMD by epidemiological studies including twin studies and formal segregation analyses (Heiba *et al.*, 1994; Hammond *et al.*, 2002; Seddon, Ajani, and Mitchell, 1997). First degree relatives of individuals with AMD appear to have a two- to four-fold increased incidence of AMD over control individuals without a family history of AMD. Twin studies suggest that concordance for AMD in monozygotic twins is approximately twice that in dizygotic twins. Formal segregation analysis suggests that there is a major gene effect accounting for approximately 60% of AMD with a single major gene accounting for about 55% of AMD risk. Overall, these data suggest that the etiology of AMD has a significant genetic component.

HUMAN STUDIES OF MACULAR DEGENERATION

Mendelian linkage and association studies

In addition to ARMD, several Mendelian forms of macular degenerations have been described. The age of onset, pattern of inheritance, and clinical characteristics of these diseases vary widely. To date, about 17 human Mendelian macular degeneration genes have been mapped (Tuo, Bojanowski, and Chan, 2004). So far genes for nine different forms of human Mendelian macular degenerations have been identified using a positional cloning approach. These genes can be broadly classified into two groups: genes that are expressed in photoreceptors (ELOVL4, RDS/peripherin, RPGR, and ABCA4) and genes expressed in RPE (Bestrophin, EFEMP1, TIMP3, Hemicentin-1, and CTRP5). The genes ELOVL4, RDS, RPGR, and ABCA4 are expressed in both rod and cone photoreceptors. Except for the ELOVL4 gene, mutations in the remaining three genes were shown to be associated with retinitis pigmentosa (RP) in addition to macular degeneration. Mutations in TIMP3, EFEMP1, Hemicentin-1, CTRP5, and Bestrophin have not been implicated in RP. Of the genes involved in causing macular degeneration, all four photoreceptor-expressed genes are associated with an atrophic phenotype, whereas the RPE-expressed genes are associated with subretinal deposits and drusen in the early stages of the disease, which then progresses to neovascularization at later stages. The genes EFEMP1, TIMP3, Hemicentin-1, and CTRP5 share structural homology and are components of the extracellular matrix, and Bestrophin was reported to be a membrane channel. Recently, Fibulin-5, which also belongs to the fibulin family of extracellular proteins and shares homology with the EFEMP1 and CTRP5 proteins, was shown to be associated with AMD.

Linkage and association studies of AMD

Although some families show Mendelian inheritance of AMD, the disease in the general population is inherited in a complex or multifactorial fashion. In attempts to identify the genes that contribute to AMD risk in the population at large, investigators have looked at inheritance of AMD in small families or even pairs of affected siblings. A number of studies have examined families in which more than one member is affected with AMD, to determine whether polymorphic genetic markers at known positions in the human genome are co-inherited with the disease. These genome-wide scans have identified at least 21 linked regions on multiple chromosomes, including most consistently regions on chromosomes 1q, 9q, 10q, 12q, and 16q. However, AMD has not been associated with mutations in genes in any of these regions except complement factor H.

Three genes, ATP binding cassette subfamily A member 4 (ABCA4), apolipoprotein E (APOE), and complement factor H (HF1), have been reported to be associated with susceptibility to AMD in the general population (Tuo, Bojanowski, and Chan, 2004). However, the role of ABCA4 is somewhat controversial, and it probably is responsible for a few percent of AMD cases at most. Involvement of APOE in AMD seems to be more solid, with most studies showing a risk ratio of individuals carrying at least one APOE-ε4 allele reduced to about 40 to 50% of control values, although some studies could not replicate this finding. Recently, a Y402H polymorphism in the complement factor H protein has been shown to be associated with a two- to seven-fold increase in risk for AMD in two studies of unrelated individuals.

The gene encoding complement factor H lies in the chromosome 1q25-31 region implicated in linkage studies of both a large single family and of multiple small families and sibling pairs. One study suggested that this gene might account for as much as 50% of the hereditary tendency of AMD in the general population (Edwards *et al.*, 2005). In addition, the biochemical activities of both APOE and HF1 are consistent with the proposed atherosclerotic and inflammatory associations of AMD and the histological and biochemical analysis of the subretinal deposits. Thus, significant progress is being made in understanding the biological nature of the genes associated with macular degenerations and their roles in the disease. However, despite these advances little is understood about the overall mechanism underlying the disease process.

BIOCHEMISTRY AND PATHOLOGY OF MACULAR DEGENERATION

Histological changes

Among the early hallmarks of AMD are drusen, which are complex deposits of lipids, proteins, glycoproteins, and glycosaminoglycans that accumulate in the extracellular and inner aspects of Bruch's membrane (Anderson *et al.*, 2002). These subretinal deposits, accompanied by a diffuse thickening of Bruch's membrane, have been speculated to form a physical barrier between the RPE and choroid, obstructing the flow of nutrients from choroid to RPE, or possibly resulting in loss of cell-cell contact between RPE and Bruch's membrane and causing degeneration of retinal tissue. The RPE cells are responsible for phagocytosis and degradation of outer segment disks shed by photoreceptors. As they age and undergo oxidative stress, lipofuscin accumulates in the lysosomal compartment and leads to cellular damage and further impaired function. Though the origin of drusen remains controversial, current opinions are beginning to favor the vasculature of the choriocapillaris as a primary source rather than an intracellular source from the RPE. It is possible that the presence of lipofuscin and cellular debris excites an immune reaction and leads to the formation of drusen. This is reflected by the presence of immune components in drusen (Anderson *et al.*, 2002; see later).

Chorioretinal neovascularization (CNV) is the most common cause of vision loss in AMD. New vessels from the choriocapillaris grow through Bruch's membrane and branch horizontally through the RPE cell layer (termed classic CNV) or between the inner Bruch's membrane and RPE (termed occult CNV because it doesn't show up on angiography). Although the impetus for CNV has not been definitively determined, there are suggestions that imbalances in growth factors include pigment epithelial derived factor (PEDF, which inhibits vascular outgrowth) and vascular endothelial growth factor (VEGF, which stimulates vascular growth), possibly as a result of hypoxia and inflammation of the RPE. Even in the absence of CNV, the changes to the RPE Bruch's membrane and the outer plexiform layer of the retina result in scar formation at that level with concomitant damage to the neurosensory outer retina, termed geographic atrophy, which can also result in loss of central vision.

Composition of drusen and its implications

Understanding the composition of drusen provides important clues to the molecular pathology of the disease. In addition to classical immunohistochemical techniques, several advanced proteome analysis tools have begun to provide detailed information about the nature and composition of drusen. Perhaps the most significant of the new findings is that drusen contain protein molecules that mediate inflammatory and immune processes. These include immunoglobulins, components of complement pathway, and modulators for complement activation (e.g., vitronectin, clusterin, membrane cofactor protein, and complement receptor-1), molecules involved in the acute-phase response to inflammation (e.g., amyloid P component, α1-antitrypsin, and apolipoprotein E), major histocompatibility complex class II antigens, and HLA-DR antigens (Crabb *et al.*, 2002). Cellular components also have been identified in drusen, including RPE debris, lipofuscin, and melanin, as well as processes of choroidal dendritic cells, which are felt to contribute to the inflammatory response (Mullins *et al.*, 2000).

In addition to immune components, a number of other proteins occur in drusen, some of them also found in atherosclerotic plaques and other age-related diseases in which protein deposits occur. The most common of these appear to be TIMP-3, clusterin, vitronectin, and serum albumin. Other proteins found in drusen include serum amyloid P component, apolipoprotein E, IgG, Factor X, and some complement proteins (Mullins *et al.*, 2000). A number of proteins are found exclusively or in increased amounts in drusen associated with AMD than in drusen from individuals unaffected by AMD. These include some crystallins, EEFMP1, and amyloid-beta. In addition, the presence of immunoreactive proteins and oxidative modifications of many proteins found in drusen implicate both oxidation and immune functions in the pathogenesis of AMD.

Immune aspects

These findings have led to the suggestion that immune complex-mediated inflammation damages RPE cells, and choroidal dendritic cells are activated and recruited by injured RPE, whereas RPE cells respond to control dendritic cell activation by secreting proteins that modulate the immune response. Shed or phagocytosed cell membranes of injured RPE or dendritic cells are postulated to function as cores for these secreted components to accumulate and form extracellular deposits.

Furthermore, the codistribution of IgG and terminal complement complexes in drusen implicates an immune response directed against retinal antigens, and the immune complex formation might be taking place at the site of drusen formation. This hypothesis is supported by the presence of putative anti-retinal autoantibodies in the sera of patients with ARMD. Anti-retinal autoantibodies previously have been reported in a number of ocular disorders, including retinitis pigmentosa, paraneoplastic retinopathies, and retinal vasculitis (Anderson *et al.*, 2002). In addition, patients with membranoproliferative glomerulonephritis, in which complement activation and immune complex deposition cause glomerular injury, develop drusen deposits resembling those in ARMD in ultrastructure and composition including C5 and IgG. However, the role of antiretinal autoantibodies in the pathogenesis of ARMD has not been examined in detail. It remains unknown whether the initiation of chronic inflammation and subsequent drusen formation requires autoimmune-mediated events as a primary factor. To clarify the role of autoimmunity, immunogenic molecules for circulating antiretinal autoantibodies in patients need to be identified.

Oxidative aspects

Oxidative damage is implicated in the pathogenesis of AMD by both theoretical considerations and experimental data (Roth, Bindewald, and Holz, 2004). The retina has a highly active metabolism with a resultant high oxygen demand, and is exposed to light and polyunsaturated fatty acids, all of which tend to increase its susceptibility to photo-oxidative damage. In a fashion somewhat analogous to that seen in the lens, as the retina ages its antioxidant defenses begin to decline, here including both antioxidant enzymes and antioxidants such as lutein, and macular pigment density. As the RPE age oxidation of lipids and other cellular components result in accumulation of nonmetabolizable material as lipofuscin in the lysosomes, leading to their enlargement and formation of lipofuscin granules. These closely parallel drusen formation in time and distribution in the retina. In addition, epidemiological correlation of AMD with light exposure, age, and light pigmentation as well as the prevention or delay of AMD by antioxidant vitamins in the AREDS trial also support an oxidative role in AMD.

ANIMAL MODELS OF MACULAR DEGENERATION

Overview

Limited access to appropriate biological materials, especially eye samples from affected donors at different stages of the disease, are an absolute necessity to study mechanisms underlying the macular degenerations. Because it is nearly impossible to obtain these human retinal tissues from patients or from normal controls, animal models play a crucial role for investigating the biological pathway of disease development and for testing therapeutic strategies. Because age-related macular degeneration shares phenotypic similarities with monogenic macular degenerations, manipulation of these genes associated with monogenic macular degenerations to develop transgenic mouse models has been popular. Over the past few years, genetic engineering technologies has allowed the generation of a rapidly growing number of animal models for retinal diseases (Chader, 2002). Animal models have been used to investigate potentially protective therapeutic agents to treat photoreceptor degeneration, stem cell technology, or to test somatic gene therapy strategies (Ali *et al.*, 2000). They are also valuable for studying environmental effects like diet or light on the degeneration process. The animals that have been used to evaluate therapeutic strategies involve rodents, rabbits, pigs, and dogs. However, macula is found only in primates and birds; a monkey model with macular degeneration would be extremely valuable as they not only have a defined macula, but they are also evolutionarily close to humans.

Macular degeneration in monkeys was first described by Stafford in 1974 (Stafford, Anness, and Fine, 1984). He reported that 6.6% of elderly monkeys showed pigmentary disorders and/or drusen-like spots. El-Mofty and colleagues reported 50% incidence of maculopathy in a rhesus monkey colony at the Caribbean Primate Research Center of the University of Puerto Rico in 1978. The following report from the center indicated that specific maternal lineages had a statistically significant higher prevalence of drusen. Researchers have described a cynomolgus monkey (*Macaca fascicularis*) colony at the Tsukuba Primate Research Center (Tsukuba city, Japan) with a high incidence of macular degeneration and its pattern of inheritance (Umeda *et al.*, 2005).

Several other naturally occurring animal models have been described for retinal diseases. Rodents, mainly mice, are the most popular animal models as maintenance is less expensive compared to larger animals. However, a low cone:rod ratio and lack of a macula make mice less suitable for studying cone diseases and macular degenerations. Although the pathology in human ARMD is pronounced in the macula area, it is not confined to this central region alone. Abnormal accumulation of drusen and progressive degeneration of the retina, RPE, and underlying choroid characteristics were observed in mouse models generated by candidate gene manipulation or senescence acceleration (Ambati *et al.*, 2003). Choroidal neovascularization also has been described in naturally occurring mouse models. These observations suggest that the lack of a macula in mice may not be a disadvantage when considering the advantages of using the mouse as a model for studying macular degenerations with drusen.

Although monkey models are extremely important for macular degeneration study, there are limitations

using nonhuman primates as animal models, such as longer gestation and life span, slow rate of expanding the pedigree, and cost of maintenance. These limitations can be overcome only by utilizing the mouse model parallel to the monkey model. One such model is a mouse line expressing an inactive form of cathepsin D. The impaired enzymatic activity affects phagocytosis of photoreceptor outer segments in the RPE cells, and the mice demonstrate basal laminar and linear deposits.

Animal model of early and late onset macular degeneration monkey

In 1986, a single cynomolgus monkey (*Macaca fascicularis*) with heavy drusen was found in the Tsukuba Primate Research Center. After 19 years of mating experiments, that single pedigree has grown to having 57 affected and 182 unaffected monkeys. Macular changes are observed as early as two years after birth, with basal laminar deposits first appearing in the macular region and progressing toward the peripheral retina throughout the lifetime (see Figure 68.4). In all the cases examined no abnormalities were found in the optic disc, retinal blood vessels, or choroidal vasculatures. The affected monkeys share phenotypic similarities with the early stages of ARMD, such as drusen and accumulation of lipofuscin. The immunohistochemical and proteome analysis of drusen in these monkeys share significant similarity with composition of age-related macular degeneration monkeys and also with previously reported human drusen composition. The meaning of this observation is that early onset monkeys produce the same drusen as ARMD patients at an accelerated rate of 25 times. Thirteen human candidate gene loci have been excluded by linkage and haplotype analysis. Therefore, the gene associated with macular degeneration in these monkeys is likely to be novel and the genes involved in causing drusen phenotype in humans and monkeys could be either the same or belong to the same biological pathway.

Studies involving early-onset and late-onset macular degeneration monkeys present a unique opportunity to study two independent target points in the biological pathway of retinal tissue that lead to degeneration of the macula at different stages of life. The gene associated with monkey macular degeneration is likely to be a novel gene as we have excluded most of the known macular degeneration loci. Cloning of the monkey macular degeneration gene will allow us to study the biological processes causing degeneration of retina. Due to high conservation between human and macaque genomes, genes associated with macular degeneration in monkeys should possibly play a key role in maintaining the normal function of retina in humans and is likely to be associated with macular degeneration in humans. Although some of the monogenic macular degeneration genes are not associated with ARMD, the phenotype observed in monkeys strongly suggests that this gene may play a role in human ARMD, and this cannot be established until validated by screening patients with ARMD. Understanding the mechanism underlying macular degeneration in these monkeys will enhance our understanding of the disease, identify clinical or molecular markers for early detection, and provide critical information needed to develop therapies for these diseases.

Progressive Open Angle Glaucoma (POAG)

BRIEF OVERVIEW

Epidemiology of POAG

Primary open angle glaucoma is a major cause of blindness throughout the world, affecting between 1 and 2% of individuals over 40 years of age (Klein *et al.*, 1992). The greatest risk factor for the development of POAG is ocular hypertension, to the extent that an elevated intraocular pressure (IOP) is often incorporated into the definition of glaucoma. In addition, the evidence implicating a genetic influence in glaucoma is very strong, and has been borne out in both model-based and model-free linkage studies. Finally, diabetes and myopia have been suggested to be related to development of POAG, but the evidence for this is inconsistent, although it seems likely that high myopia might contribute to development of POAG.

Pathology and physiology of POAG

Although the etiology and even the pathophysiology of glaucoma are still poorly understood, risk factors for glaucoma can be thought of as including both those in the anterior chamber, which tend to increase intraocular

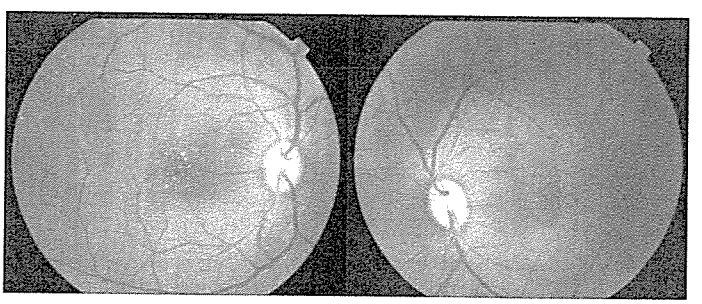

Figure 68.4 Funduscopic view of the retina in Tsukuba primate model of macular degeneration showing drusen and macular changes.

pressure, and those in the retina and optic nerve, which tend to increase susceptibility to damage from elevated or even normal intraocular pressure. Clinically, glaucoma generally is characterized by excavation of the optic disc as seen on funduscopic examination and visual field defects with elevated intraocular pressure included either as a part of the disease or a risk factor. In a simplified schema, one might think of increased resistance of the trabecular meshwork or Schlemm's canal to outflow of the aqueous humor causing an increase in intraocular pressure, which then acts upon sensitive retinal ganglion cells. These cells then degenerate, resulting in both the increased depth and width of the optic cup and the visual field defects. If the increased pressure is acute as it usually is in juvenile onset glaucoma, this process can be painful, but generally POAG is an insidious disease in which the first recognized symptom may be irreversible visual field changes.

Although primary open angle glaucoma (POAG) is characterized by visual field loss corresponding to the excavation of the optic disc (see Figure 68.5), it is usually associated with an elevation of the intraocular pressure (IOP) over 21 mmHg. Although the pathogenesis of glaucomatous optic neuropathy is poorly understood, it is generally accepted that the IOP is a major risk factor. By definition, there is no increase in IOP over 21 mmHg at any time in eyes with normal-tension glaucoma (NTG), although it is difficult to rule out fleeting or previous elevations of IOP. IOP is heavily influenced by the inflow and outflow of aqueous humor, a plasma filtrate actively generated at stroma of ciliary body and filtered across the blood-aqueous barrier. The aqueous flows from the posterior chamber to the anterior chamber via the pupil and is released through two routes, the trabecular route and uveoscleral route. Any disturbance in the flow can cause abnormal IOP leading to a death of retinal ganglion cells (RGC), and damage to the surrounding structure of the optic nerve head where optic nerve fibers leave the eye for visual cortex.

HUMAN STUDIES OF POAG

Linkage studies

At least six loci for autosomal dominant POAG have been mapped through linkage studies, termed GLC1A-F, on chromosomes 1q23, 2cen-q13, 3q21-q24, 8q23, 10p15-p14, and 7q35-q36. A genome-wide scan in multiple small families from an Afro-Caribbean population provided significant evidence for linkage to regions on chromosomes 2q (but separate from the Mendelian POAG locus GLC2B and the infantile glaucoma locus GLC3A on chromosome 2) and 10p. Presumably, these represent loci for glaucoma risk factors common in the general population, as do the loci on chromosomes 2, 14, 17, and 19, identified by examining siblings in an American population of European descent. It is particularly important to note that few of these studies have been confirmed; especially the technically more difficult and laborious studies of POAG in the general population.

Association studies

In addition to the identification of myocilin as a causative gene in glaucoma described earlier, which was carried out by linkage studies primarily in families with juvenile glaucoma and very elevated intraocular pressure, association studies have identified sequence changes in myocilin as a risk factor in a small percentage of POAG cases. Two additional genes have been shown to be involved in glaucoma by demonstrating an association between sequence changes in those genes and glaucoma in population studies. One of these genes is optineurin, for which the strongest associations have been obtained with normal tension glaucoma, but which also might be associated with POAG in some populations. A second is the OPA1 gene, which is known primarily as a cause of optic atrophy, but is also associated with normal tension glaucoma, though not with high tension primary open angle glaucoma in most studies. Association of both these genes with normal tension glaucoma suggests that

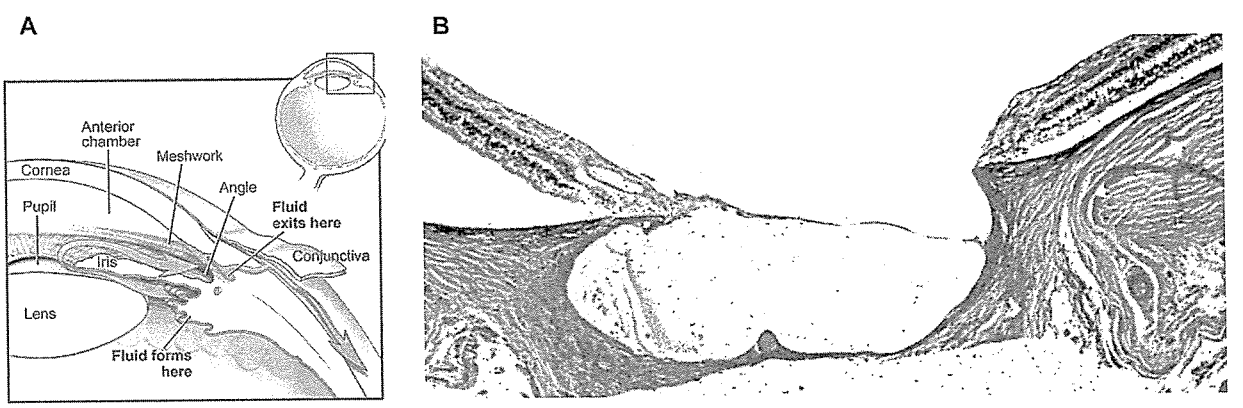

Figure 68.5 A. Diagram depicting the flow of aqueous humor from synthesis in the ciliary body to exit from the anterior chamber through the trabecular meshwork and Schlem's canal. **B.** Histological section showing an excavated optic cup in an individual with glaucoma. Courtesy of Dr. Chi Chao Chan, National Eye Institute, National Institutes of Health, from the collection of Dr. W. R. Green.

there may be some relationship between normal tension glaucoma and optic atrophy, and also emphasizes the importance of genetic changes that sensitize the retina and optic nerve to minor elevations of even normal intraocular pressure.

BIOCHEMISTRY AND PATHOLOGY OF POAG

Histological changes

It is estimated that roughly 20 to 50% of the large retinal ganglion cells (RGC) are lost in POAG. Although the reduction of RGC density occurs equally throughout the retina, visual sensitivity is first lost in areas where the initial RGC density is low, especially in the peripheral regions of the retina. As the disease progresses, atrophy of the nerve fiber layer is usually observed as additional RGC is lost. Typically, vertical collapse of the optic nerve head (ONH), loss of the neural rim at the ONH, rearrangement of central blood vessels, and loss of supporting tissue occur. Scanning electron microscopy of retinas with early stages of glaucoma shows evidence of initial collapse of the anterior lamina cribrosa, primarily in the vertical poles of the optic nerve head. Based on primate studies, optic cups with larger diameters are more susceptible to high ocular pressure and thus to glaucoma.

Role of the trabecular meshwork

Trabecular meshwork (TM) is a lamellated sheet of complex tissue that covers the inner wall of Schlemm's canal. TM has uniquely developed at the angle of primates, filtering the aqueous humor out of the eye. TM consists of two parts: the nonfiltering portion mainly occupied by trabecular cells and the filtering portion. Trabecular cells are highly phagocytic cells removing particles, cell debris, and protein from the aqueous humor. The first glaucoma locus, the *trabecular meshwork inducible glucocorticoid response* (TIGR), also known as myocilin, initially was identified by looking at genes whose transcription is highly induced by steroids in these cells. The filtering portion consists of three tissues: the cribriform layer, the corneoscleral meshwork, and the uveal meshwork. These trabecular beams or strands are intertwiningly connected to each other, forming a complex filtering mesh surrounding Schlemm's canal. The trabecular beams are thickened by accumulation of extracellular materials and decrease of cell density within the corneoscleral and uveal meshwork in aged eyes.

ANIMAL MODELS OF POAG

Overview: Difficulty of modeling the human eye

Limited access to appropriate biological materials, especially eye samples from affected donors at different stages of the POAG, is an impediment to the study of mechanisms underlying the disease. Because of the extreme difficulty in obtaining such diseased eyes from both patients and normal controls, animal models play a crucial role in investigating the biological pathway of disease development and in testing therapeutic strategies.

Different types of animal models for POAG have been found or created to mimic the optic nerve damage to resemble POAG phenotypes in humans. The greatest difficulty in constructing an animal model for POAG lies in the diversity of the anterior structures of the eye among different species (Tripathi and Tripathi, 1972, 1973). These structural differences include different iridocorneal angles or absence of specific quadrants from the TM. Nevertheless, within the limited areas in which interpretation of the data from a specific animal model parallels that in the human, various animals including the cow, dog, cat, horse, rabbit, chicken, and monkey can be used to observe POAG under various experimental conditions.

Animal models of POAG

Various animal models for inducible glaucoma have been reported. Argon laser photocoagulation of the TM in rhesus monkeys results in sustained elevation of IOP and has been used extensively to study early damage to the optic nerve head (May *et al.*, 1997). Corticosteroids such as betamethasone and dexamethasone have been used to treat rabbits, dogs, and cats to develop ocular hypertension (Bonomi *et al.*, 1978). Steroid treatment generally produces progressive glaucoma, but this process is reversed after about two months after cessation of the steroid. Trabecular blockage caused by inflammation after α-chymotrypsin treatment also has been used to produce elevated IOP in rabbit and monkey eyes (Vareilles *et al.*, 1977). Some types of avian species (chicken, quail, and turkey) have been known to develop elevated IOP as a consequence of continuous exposure to light.

Mouse models of glaucoma

Naturally occurring inherited animal glaucoma models are rare. However, extensive classification of IOP in mouse strains and molecular biological techniques to manipulate certain genes to produce transgenic or knockout/knockin mice recently have resulted in the development of a number of animal models with definitely known genetic causes for their disease (Chang *et al.*, 1999). As discussed earlier, four genes, myocilin (MYOC, TIGR), cytochrome P4501B1 (CYP1B1), optineurin (OPTN), and WDR36, currently are associated with glaucoma. OPTN, mutations of which are responsible for 16.7% of families with hereditary human NTG, is homologous to an inhibitory regulatory subunit of the high molecular kinase complex for the phosphorylation of NF-κB. Some of its known functions include inhibition of the tumor necrosis factor-alpha pathway, interaction with transcription factor IIIA, and mediation of the Huntington and Rab8 interaction for regulation of

membrane trafficking and cellular morphogenesis. OPTN is induced by TNF-α and binds to an inhibitor of TNF-α and the adenovirus E3-14.7 kDa protein. To determine the effects of human glaucoma mutations in a transgenic mouse system, mice over-expressing wild type OPTN, OPTN carrying the glaucoma associated mutation E50K, and OPTN with exon5 deleted were constructed. Although wild-type OPTN do not show any abnormalities and the exon 5 deleted construction was found to be lethal prenatally, mice transgenic for the E50K mutant OPTN show steep optic nerve cupping with rearrangement of supporting tissue and blood vessels 18 weeks after birth (see Figure 68.6). The RGC and astrocyte loss observed is similar to the end phase changes seen in human glaucoma patients. Understanding the mechanism underlying normal tension glaucoma in these transgenic mice will enhance our understanding of each step leading to optic nerve cupping and how to prevent it. Based on the success of the mouse model, use of larger animals such as transgenic rabbits or pigs, in which more precise measurement of IOP and trials of surgical procedures suitable for therapy in humans are possible are currently being investigated.

Other glaucoma mouse models have been made through genetic manipulation. Knockout and transgenic mouse models of myocilin were made to answer the question whether elevated expression of the myocilin protein can influence the IOP (Gould *et al.*, 2004). Up to a fifteen-fold increase in myocilin expression failed to result in elevation of the IOP, any abnormality of retinal ganglion cells, or cupping of the optic nerve head. Mice lacking the cytochrome P450 1B1 (CYP1B1) gene were generated on B6 and 129X1/SvJ mouse stains (Libby *et al.*, 2003). Both strains were affected by the CYP1B1 deficiency with focal angle abnormalities, but 129X1/SvJ albino strains lacking tyrosinase were more severely affected, suggesting the presence of tyrosinase as an important developmental molecule.

Figure 68.6 Histological section demonstrating excavation of the optic disc in an 18-week-old E50K mutant OPTN transgenic mouse.

Conclusion

In this chapter we have provided a brief overview of age-related eye diseases and the current state of knowledge and research on three of these. Age-related cataracts, age-related macular degeneration, and progressive open angle glaucoma account for much of the population burden imposed by age-related eye diseases. Although no perfect system to study these diseases exists today, an increasing number of experimental models are being developed. Although none is an exact replica of the clinical disease and should not be applied indiscriminately, each of these can provide useful information on some aspects of the disease in humans. They promise to accelerate the pace of research and provide mechanistic and therapeutic insights into the diseases that threaten sight in our aging population.

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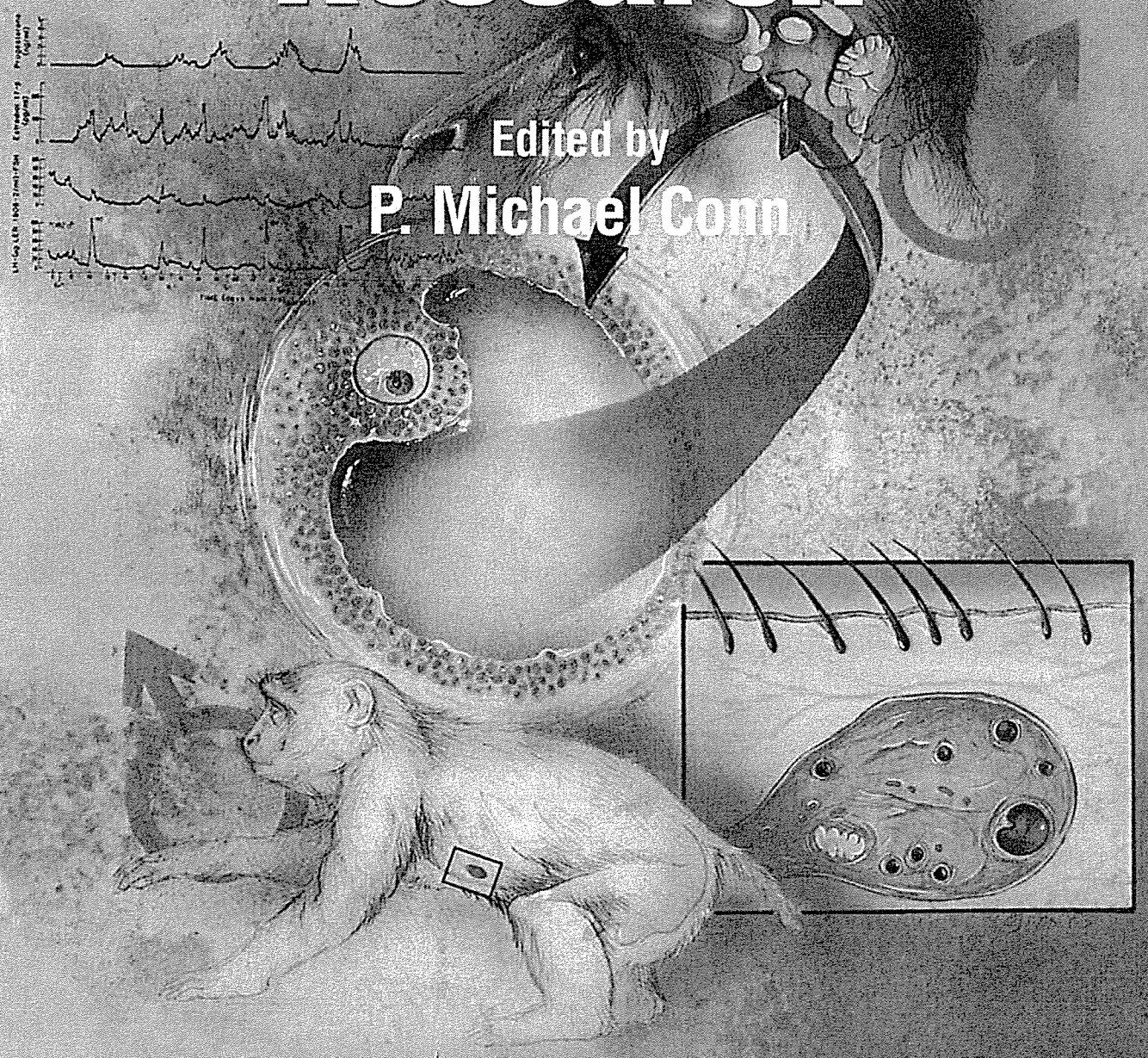

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Source Book of Models for Biomedical Research

Chapter 33: Animal Models for Eye Diseases and Therapeutics

Subtitle: Animal models of Age-Related Macula Degeneration and glaucoma

Takeshi Iwata, Ph.D.[1] and Stanislav Tomarev, Ph.D.[2]

[1]National Institute of Sensory Organs, National Hospital Organization Tokyo Medical Center, 2-5-2 Higashigaoka, Meguro-ku, Tokyo 152-8902 Japan.

TEL/FAX: +81-(3)3411-1026

e-mail: iwatatakeshi@kankakuki.go.jp

[2]National Eye Institute, National Institutes of Health, Bldg. 7, Rm. 103, 7 Memorial Drive, MSC 0704, Bethesda, MD 20892-0704 USA.

TEL: (301)496-8524

FAX: (301)496-8760

e-mail: tomarevs@nei.nih.gov

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1. Visual impairment and importance of animal models for eye diseases

It is believed that more than 80% of the information our brain receives comes from the visual system. Dysfunction of the visual system can alter the normal human life style and significantly lower the quality of life. The causes of visual impairments and blindness vary among ethnic groups and the global regions where they live. There are many causes of visual impairments including diabetic complications, infections, and trauma, however the most prevalent causes of visual impairment are cataracts, glaucoma, and age-related macular degeneration (AMD). According to the World Health Organization, there were more than 161 million visually impaired individuals in 2002, and 124 million of this group had low vision and 37 million were blind (http://www.who.int/mediacentre/factsheets/fs282/en/index.html) (Fig. 1).

Cataract, glaucoma, and AMD are responsible for 69% of blindness globally. Although cataracts are the leading cause of blindness worldwide, recent advances in cataract surgery has significantly reduced the visual impairments caused by cataracts especially in developed countries. Glaucoma, an optic neuropathy, is often associated with elevated intraocular pressure and is responsible for blindness in 6.7 million people

across the world. Glaucoma is more common in individuals of African ancestry, and the incidence of glaucoma increases with age.

The most prevalent eye disease for elderly Europeans and Americans is AMD. This degenerative disease progresses from retinal deposits called drusen to neovascularization and retinal hemorrhages resulting in irreversible loss of central vision.

In spite of the high incidence of AMD and glaucoma, a limited amount of information is available on the underlying pathological mechanisms causing these diseases. Obtaining tissues for any disease is often difficult, and even when obtained, they may not be informative because the tissues are usually collected many hours or even days after death. Because experimental studies of AMD and glaucoma are limited in humans, the availability of animal models is very valuable because they can be used to investigate the molecular mechanisms causing these diseases and to test new therapeutic interventions. Animal models, compared to other experimental methods, e.g., cell and organ cultures or postmortem models, allow the study of different pathological factors and therapeutical treatments under in vivo conditions, i.e., with the visual and other systems of the body intact. Appropriate animal models, e.g., monkey,

mouse, zebrafish, also facilitate the identification of new genes involved in the pathology as well as elucidate the genetic relationships between causative and modifier genes. Equally important, specific genes can be altered in these models. Thus, it is possible to induce mutations in animals, and then search for specific phenotypes, e.g., abnormal intraocular pressure (IOP) and retinal ganglion cell degeneration. Subsequently, the affected genes can be identified by standard genetic procedures.

Many animal models of AMD and glaucoma have been developed in different mammalian and non-mammalian species. None of these models is a perfect reproduction of the human disease, and when choosing the animal model for research, the investigator should evaluate the following: 1) similarity of the visual system of the model to that of humans especially the eye; 2) similarity of the time course of pathological changes in the model and in human eyes; 3) ability to perform genetic manipulations; 4) training required to produce affected animals; 5) size of the eye; 6) availability and difficulties in the methods of analysis; 7) availability of animals; and 8) cost.

2. Age-related macular degeneration (AMD)

2.1. Introduction of AMD

The retina is composed of nine layers of neural and glial cells that are arranged concentrically at the posterior pole of the eye. Incoming light is focused on the central area of the retina called the fovea which is located in the center of the macular area (Fig. 2). In humans, the size of the macula is approximately 6 mm in diameter (Fig.3). The outer (posterior) surface of the retina is covered by a monolayer of retinal pigment epithelial (RPE) cells which forms a diffusion barrier between the neural retina and the choroidal blood supply. The RPE regulates the transport of proteins to the retina, and controls the hydration and ionic composition of the subretinal space. The physiological condition of the RPE is closely associated with the pathogenesis of AMD.

AMD is a blinding disorder characterized by a marked decrease in central vision associated with RPE atrophy with or without choroidal neovascularization (CNV). Many factors including genetic, behavioral, and environmental, are involved in this disease. AMD is characterized by the degeneration of cone photoreceptors in the foveal region of the retina resulting in a decrease of central visual acuity. The progressive impairment of

the retinal pigment epithelial (RPE) cells, and damage to Bruch's membrane and choriocapillaris results in retinal atrophy and photoreceptor dysfunction. In some cases, CNV develops, and the new vessels penetrate Bruch's membrane and pass into the subretinal space.

Two types of AMD are recognized; the non-neovascular type is called the dry-type AMD and includes more than 80% of the cases, and the neovascular type is called the wet-type AMD which is progressive with a higher probability of blindness. The prevalence of AMD differs considerably among the different ethnic groups, but the incidence increases with age in all groups. A lower prevalence of AMD has been reported in individuals of African ancestry than of Anglo-Saxon ancestry. Other risk factors for AMD are cigarette smoking, obesity, hypertension, and atherosclerosis.

2.2. Epidemiology and genetics of AMD

Extensive epidemiological studies have shown a genetic component for AMD. Thus, twin studies have shown a higher concordance for AMD in monozygotic twins than in dizygotic twins (1-3). In addition, first degree relatives of individuals with AMD

have a 2 to 4 fold higher incidence of AMD over individuals without a family history of AMD. Genetic segregation studies have also shown a genetic effect that accounts for approximately 60% of AMD with a single major gene accounting for about 55% of the risk of developing AMD. Overall, the data have suggested that the etiology of AMD has a significant genetic component. Only a small proportion of the families with AMD show Mendelian inheritance, and the majority of the individuals inherit AMD in a complex multi-gene pattern.

There have been a number of attempts to identify the genes which cause AMD. With the help of the haplotype marker project (HapMap Project), genome wide scanning has identified at least 13 loci linked to AMD on different chromosomes (4-6).

Recently, a polymorphism of complement factor H gene (*Y402H*) was shown to be associated with an increased risk for AMD (7-10). These results were confirmed in many of the countries with large Caucasian populations but not in Japan (11,12). This gene is located on chromosome 1q25-31 where one of the candidate loci was identified by linkage studies. Another recent study reported that a haplotype association of tandemly located complement 2 and factor B was protective for AMD (13).

2.3. Pathology and biochemistry of AMD

The early stage of the dry type AMD is characterized by a thickening of Bruch's membrane, aggregation of pigment granules, and increasing numbers of drusen. The thickening of Bruch's membrane obstructs its function as a 'barrier' between the choroid and the RPE that protects the neural retina from the choriocapillary. Drusen are small yellowish-white deposits that are composed of lipids, proteins, glycoproteins, and glycosaminoglycans. They accumulate in the extracellular space and the inner aspects of Bruch's membrane (Fig. 3). Drusen are not directly associated with visual loss but represent a risk factor for both the dry-type and wet-type AMD. The classification of hard and soft drusen is based on their size, shape, and color; hard drusen are yellowish with diameters <50 μm and are found in eyes that are less likely to progress to advanced stages of the disease, while soft drusen are darker yellow and larger in size, and are found in eyes more likely to progress to more advanced stages of AMD. A small percentage of dry-type AMD patients progress to the late stage of the wet-type AMD that is characterized by geographic atrophy or detachment of RPE and the

development of CNV in the macular region. The presence of a CNV is the factor that most damages the neural retina because the newly developed vessels grow from the choriocapillaris through Bruch's membrane and extend laterally through the RPE cell layer (classic CNV) or extend between the inner Bruch's membrane and RPE (occult CNV). In advanced stages of AMD, the CNV and fluid leaked into the subretinal or intraretinal regions leads to cell death and retinal detachment.

Recent analyses of the progression of drusen have provided important clues that help understand the molecular pathology of AMD. Using both immunohistochemistry and proteomic techniques, the materials in drusen were found to be composed of molecules that mediate inflammatory and immune processes (14, 15). These molecules include components of the complement pathway and modulators of complement activation, viz., vitronectin, clusterin, membrane cofactor protein, and complement receptor-1. In addition, molecules triggering inflammation, viz., amyloid P component, a1-antitrypsin, and apolipoprotein E, were identified in drusen. Cellular debris from macrophages, RPE cells, and choroidal dendritic cells has also been identified in drusen. On the other hand, crystallins, EEFMP1, and amyloid-beta have been found at

higher levels in drusen from individuals unaffected by AMD. The presence of immunoreactive proteins and the oxidative modifications of many proteins in drusen imply that both oxidation and immune functions are involved in the pathogenesis of AMD.

All of these findings suggest that complement activation triggers innate immune responses in the subretinal space. The co-distribution of IgG and terminal complement complexes in drusen indicate that immune responses that directly target antigens in retinal cells might also be occurring. Anti-retinal autoantibodies have been reported in a number of ocular disorders, e.g., macular degeneration in an aged monkey model.

2.4. Animal models for AMD

Access to appropriate biological materials from affected donors at different stages of a disease is an absolute necessity for the study of mechanisms underlying the disease process. However, because it is nearly impossible to obtain retinal tissues from patients or controls, the development of animal models becomes crucial for investigating the biological pathways involved in the progression of the disease and for the