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

development of therapeutic strategies.

Over the past few years, genetic engineering techniques have generated a number of animal models of AMD in mice, rats, rabbits, pigs, and dogs (16). However in mammals, a well-defined fovea is found only in primates (humans and monkeys), and a search for a monkey line affected with macular degeneration has been persistent for a long time.

A monkey with macular degeneration was first described by Stafford et al in 1974. They reported that 6.6 % of the elderly monkeys they examined showed pigmentary disorders and drusen-like spots (17). El-Mofty et al reported that the incidence of maculopathy was 50% in a colony of rhesus monkeys at the Caribbean Primate Research Center of the University of Puerto Rico (18). At the Tsukuba Primate Research Center (Tsukuba City, Japan), Suzuki et al found a single cynomolgus monkey (*Macaca fascicularis*) in 1986 with a large number of small drusen around the macular region (Fig. 4) (19-21). This single affected monkey has multiplied to a large pedigree of more than 65 affect and 210 unaffected monkeys. Drusen were observed in the macular region as early as one year after birth, and the numbers increased and

spread toward the peripheral retina throughout life. No histological abnormalities have been found in the retina, retinal vessels, or choroidal vasculatures of the eyes with drusen. Immunohistochemical and proteomic analyses of the drusen from these monkeys showed that the drusen were very similar to those in other monkeys with aged macular degeneration sporadically found in older monkeys and also with human drusen (22, 23). These observations by Umeda et al have shown that the Tsukuba monkeys produce drusen that are biochemically similar to those in human AMD patients, but the development of the drusen occurs at an accelerated rate of over 25 times. Currently, 240 loci of the cynomolgus monkey are being investigated to try to identify the disease causing gene and to understand the biological pathways leading to complement activation.

The eyes of monkey are structurally similar to human eyes which make them extremely valuable for macular degeneration studies. However, there are limitations in using this species over other laboratory animals. Monkeys have a relatively longer life span, have a longer gestation period, have a lower birth numbers resulting in a slower rate of expanding the pedigree, more difficult to genetically manipulate, and the cost of

maintenance is high. In the other laboratory animals, the differences in the eye structure, lack of a fovea, and a low cone/rod ratio compared to humans have been considered to be a disadvantage for using them as AMD models. However, they are easier to manipulate genetically and easier and less expensive to maintain. This has made the development of a mouse model of AMD very attractive, and a number of mouse AMD models have been reported recently.

The mouse model described by Ambati et al is deficient either in monocyte chemoattractant protein-1 or its cognate C-C chemokine receptor-2. These mice were found to develop the cardinal features of AMD including accumulation of lipofuscin in drusen beneath the RPE, photoreceptor atrophy, and CNV (24). An impairment of macrophage recruitment allowed the accumulation of C5a and IgG, which leads to the production of vascular endothelial growth factor by the RPE cells and the development of CNVs.

Another mouse model that has three known AMD risk factors: age, high fat cholesterol rich diet, and expression of human apolipoprotein E (apoE2, apoE3, apoE4) has been developed (25). ApoE4-deficient mice are severely affected showing diffuse

subretinal pigment epithelial deposits, drusen, thickened Bruch's membrane, and atrophy, hypopigmentation, and hyperpigmentation of the RPE.

Oxidative stress has long been linked to the pathogenesis of AMD. Imamura et al reported a Cu, Zn-superoxide dismutase (SOD1)-deficient mice that had features typical of AMD in human. Senescent Sod1 (-/-) mice had drusen, thickened Bruch's membrane, and choroidal neovascularization (26). The number of drusen increased with age and also after exposure of young Sod1 (-/-) mice to excess light. The retinal pigment epithelial cells of Sod1 (-/-) mice showed oxidative damage, and their beta-catenin-mediated cellular integrity was disrupted. These findings suggested that oxidative stress may affect the junctional proteins necessary for the barrier integrity of the RPE. These observations strongly suggested that oxidative stress may play a major role in AMD.

The complement components, C3a and C5a, are present in drusen, and were observed in Bruch's membrane of a laser-induced CNV mice model. Neutralization of C3a or C5a by antibody or by blockade of their receptors by a complement inhibitor significantly reduced the CNV. These observations revealed a role for immunological

mechanisms for the angiogenesis and provided evidence for future therapeutic strategies for AMD.

Although the pathology of AMD is pronounced in the macula area, it is not confined to this region. Characteristics of human AMD such as thickening of Bruch's membrane, accumulation of drusen, and CNV have been observed in mouse models. Nevertheless, the primate model will still be the choice for AMD studies, especially at the stage when new therapeutic methods are tested and evaluated for the first time. However, it would be wise and more productive to study both primate and mouse models in AMD research. This will be necessary to learn the mechanisms underlying the disease and to identify clinical and molecular markers for the early stages of AMD. The findings from these studies will provide critical information needed to develop therapies for AMD.

3. Glaucoma

3.1. Overview of Glaucoma

Glaucoma is a heterogeneous group of complex neurodegenerative disorders

that is characterized by the constriction of the visual field, death of retinal ganglion cells (RGCs), and a pathognomonic deformation of the optic nerve head (ONH) known as glaucomatous cupping. Glaucomas are classified into three main types: open-angle, closed-angle, and congenital glaucoma. Each of these types is subdivided into primary and secondary types.

3.2. Epidemiology and Genetics of Glaucoma

Primary open-angle glaucoma (POAG) is the most common form of glaucoma, and occurs in about 4.5 million people worldwide and accounts for 12% of all global blindness. By the year 2020, over 11 million people have been predicted to be blind from primary glaucoma (28). POAG is often, but not always, associated with elevated intraocular pressure (IOP), which is one of the main risk factors in glaucoma. However, about a third of all patients with POAG develop the disease without an IOP elevation, and in these patients, the IOP is continuously below 21 mmHg. This form of POAG is called normal tension or low tension glaucoma (NTG). A reduction of the IOP, even in cases of NTG, is the main, clinically-proven, treatment for glaucoma.

3.3. Animal Models for Glaucoma

Among the different animal models of glaucoma, the monkey models are superior because of the anatomical similarity of the monkey eyes to human eyes and the phylogenetic similarities of these two species. At the same time, monkeys are extremely expensive and experiments on them require a highly skilled team of investigators.

Most of the existing animal models of POAG, including the monkey models, are based on the elevation of the IOP. An elevation of the IOP develops from an imbalance between aqueous humor production and outflow. Aqueous humor, a fluid produced by the ciliary body of the eye, drains out of the eye and into the blood circulatory system. The eye's outflow system consists of a series of endothelial cell–lined structures which include the trabecular meshwork (TM), Schlemm's canal (SC) which serves as a collector vessel, and the episcleral venous system (Fig. 6). In most glaucoma models, the IOP is elevated as a result of a reduction or blockage of the aqueous humor outflow. In monkeys, an elevation of IOP is commonly induced by laser photocoagulation of the

TM (29-30). Several days after the laser treatment, the IOP increases and this elevation may last for more than a year, although more than one laser session is usually required to achieve a sustained elevation of the IOP. The IOP in treated eyes is usually between 25 and 60 mmHg.

Other methods to elevate the IOP in monkey eyes include the anterior chamber injection of; ghost red cells (31), latex microspheres (32), cross-linked polyacrylamide gels (33), and enzymes (34). Topical steroids have also been shown to elevate the IOP (35). These latter treatments produce less consistent elevations than laser photocoagulation (36).

Monkey glaucoma models have been shown to have changes in the optic disk, optic nerve, RGC, and nerve fiber layers similar to those observed in glaucomatous human eyes. Apoptosis was shown to be the cause of the RGCs death in a monkey photocoagulation model (37), and apoptosis was later confirmed to be the cause in other animal models and in humans with glaucoma. Monkey glaucoma models have also been successfully used to study changes in the retinal gene expression pattern 30 days after laser photocoagulation of the TM (38), and to test the effectiveness of new

classes of hypotensive drugs (36).

Rodent became the animal of choice to when a large number of animals were required, e.g., when examining the mechanism of RGC degeneration and neuroprotection. Several rat models of elevated pressure-induced optic nerve damage have been developed during the last decade, and they have been used to study changes in the retina and the optic nerve. Rats are easy to handle and the relatively large size of their eyes allows multiple, awake measurements of the IOP with commercially available equipment (39). This latter is important because it is well-documented that general anesthesia induces a rapid decrease in the IOP. Although there are certain differences in the structure of the rat and human eyes, all of the eye structures affected in glaucomatous human eyes exist in the rat eye.

In rat models, the IOP elevation is achieved by; injection of concentrated saline solution into the episcleral veins (40), laser photocoagulation of the trabecular meshwork (TM) after an injection of Indian ink into the anterior chamber (41), laser photocoagulation of the TM (42), and laser cauterization of episcleral veins (43). All of these methods that lead to an elevation of the IOP require special training of the

investigators.

Successful treatment of the eye leads to a rapid elevation of the IOP, although the level of elevation varies from eye to eye. Saline injection generally produces a wide range of IOP elevation from a very minimal rise to a two-fold increase over the IOP in control eyes. The elevation of the IOP generally lasts for several weeks, and a second laser treatment is often required in the photocoagulation method to maintain an elevated IOP for more than 3 weeks.

A chronic elevation of the IOP in rats leads to apoptosis of the RGCs, degeneration of the optic nerve fibers, and remodeling of the ONH similar to those observed in human glaucomatous eyes (40;44-45). Rat models of glaucoma have been used to study the effects of elevated IOP on the electroretinogram (46), neuroprotective drugs (47), and molecular changes in the retina and optic nerve using the candidate gene approach and array hybridization (48).

A mutant rat strain was reported to have unilateral or bilateral enlargement of the eyes with an IOP ranging from 25 to 45 mmHg. In this strain of rat, cupping of the optic nerve head was detected by funduscopic examination, and the cupping was more

pronounced in older animals. The number of RGCs also declined with age (49). Unfortunately, this strain was obtained from the Royal College of Surgeons colony that has a mutation in the receptor tyrosine kinase gene, leading to degeneration of the photoreceptors. Therefore, this strain can hardly be considered a good glaucoma model.

The construction of mouse models of glaucoma has lagged behind rat glaucoma models for a long time despite the advantages of mice over rats and other mammalian species for cost-effective genetic manipulations, availability of a wide spectrum of methods, and the existence of many genetically-modified strains. However, it should be remembered that mouse and human eyes have certain important differences including the arterial blood supply to the optic nerve head and the absence of a lamina cribrosa (50). The lamina cribrosa, a collagenous scaffold supporting the optic nerve, plays a critical role in the damage/protection of the human optic nerve.

One of the main difficulties working with mice is that their eyes are much smaller than the eyes of humans and rats, and new methods had to be developed to measure the IOP in mice. To date, several invasive and non-invasive methods of IOP measurements have been developed for mice. The first method remains one of the

most reliable and accurate method and does not depends upon the mechanical properties of the cornea. It involves the insertion of glass microneedle connected to a pressure transducer into anterior chamber of the eye. Using this method, it was shown that common mouse strains have different IOP between 10 and 20 mmHg (51). Other methods of IOP measurements in mice were later developed including non-invasive techniques. Non-invasive methods allow multiple IOP measurements to be completed in a short period of time, but the results of these measurements may depend upon mechanical properties of the cornea. To obtain reliable IOP readings, any of the described techniques requires training.

Transgenic and gene-targeted knockout approaches have been used to develop several mouse models of glaucoma. The main advantage of these models is that the animals with the mutated gene give more uniform elevation of the IOP and similar damage to the retina and optic nerve than those with surgically-induced elevated IOPs. A large number of animals can be produced, and once a mutant mouse line is obtained, no special training is needed to produce more affected mice.

Several lines of transgenic mice have been developed that contained BAC DNAs

with a mouse Tyr423His point mutation and Tyr437His point mutation of the human Myocilin (Myoc) genes. The Tyr437His mutation in the Myoc gene leads to severe glaucoma in humans, and the mouse Tyr423His mutation corresponds to this human mutation. Expression of mutated mouse Myoc in the ocular drainage structures led to moderate (about 2 mmHg during the day and 4 mmHg at night) elevation of the IOP and progressive degenerative changes in the peripheral RGC layer and optic nerve that resembled glaucomatous changes in human eyes (52). In one-year-old animals, the peripheral retina of transgenic mice had approximately 20% fewer RGCs than in the peripheral retina of control littermates.

Transgenic mice with a targeted mutation in the gene for the a1 subunit of collagen type I have also been constructed. This mutation blocks the cleavage of collagen by matrix metalloproteinase-1. Transgenic mice expressing mutated collagen had elevated IOPs. The difference in the IOP between control and transgenic mice gradually increased to a maximum of 4.8 mmHg at 36 weeks. Because these mice had progressive optic nerve axon loss with normal organization of the drainage structures, it has been suggested that these mice may be used as a mouse model of POAG (53).

Recent data have demonstrated that transgenic mice expressing mutated optineurin under the control of the ubiquitous ROSA26 promoter develop optic nerve cupping and death of the RGCs without elevation of the IOP (54). These transgenic mice may represent the first animal model of NTG.

The surgical methods used to produce rat glaucoma models have also been used in mice. However, performing surgery on the mouse eye is even more challenging than on rat eyes because of the difference in size. A significant elevation of the IOP was found in the eyes of C57BL/6J mice that had an injection of indocyanine green dye into the anterior chamber and diode laser treatment of the TM and episcleral vein region (55). At 10 days after the surgery, the mean IOP in the operated eyes was 33.6 ± 1.5 mmHg versus 15.2 ± 0.6 mmHg in the control eyes. However, the IOP returned to normal 60 days after the surgery. Histological examination of the treated eyes 65 day after the surgery revealed anterior synechia, decrease in the number of RGCs, thinning of all retinal layers, and damage to the optic nerve structures without evidence of prominent cupping (55). A reduction in the function of the outer retinal layers, confirmed by ERG studies, may indicate that this model produces more extensive changes in the

retina compared to the glaucoma in humans.

Similar to above model, an elevation of IOP was induced by argon laser photocoagulation of the episcleral and limbal veins in C57BL/6J mouse eyes (56) or by cauterization of three episcleral veins in CD1 mouse eyes (57). During the first 4 weeks following laser treatment, the mean IOP in the treated eyes was about 1.5 times higher than in control eyes. The number of RGCs had decreased by 22.4 ± 7.5% of that in the controls at 4 weeks after treatment. Most of the TUNEL positive apoptotic cells were detected in the peripheral retina (56).

Cauterization of the episcleral veins led to a maximum IOP elevation within 2-9 days, and the IOP decreased progressively thereafter to reach more or less normal values after 24-33 days. There was a 20% decrease in the number of RGCs two weeks after the surgery (57).

The DBA/2J strain has high IOP and has become a popular mouse model to study secondary angle-closure glaucoma. This mouse strain has mutations in two genes, *Tyrp1* and *Gpnmb* (58). DBA/2J mice develop pigment dispersion, iris transillumination, iris atrophy, and anterior synechia. At the age of 9 months, the IOP was elevated in

most mice and the elevation was accompanied by the death of the RGCs, optic nerve atrophy, and optic nerve cupping. Although no group of the RGCs was especially vulnerable or resistant to degeneration, fan-shaped sectors of dead or surviving RGC radiated from the ONH (59). It has been suggested that axon damage at the ONH might be a primary lesion in this model (59).

Several important observations were made from the studies on the DBA/2J model. It was shown that the pro-apoptotic protein BAX is required for the survival of RGCs but not for RGC axon degeneration suggesting that BAX may be a candidate human glaucoma susceptibility gene (60). Unexpectedly, high-dose of γ-irradiation accompanied with syngenic bone marrow transfer protected the RGCs in DBA/2J mice (61). Similar to the results obtained with rat and monkey models, genes involved in the glial activation and immune response were activated in DBA/2J retina as shown by array hybridization (62).

Complement component, 1Q, was up-regulated in the retina of several animal models of glaucoma as it is in human glaucoma with the timing suggesting that complement activation plays a significant role in the pathogenesis of glaucoma (63).

Taken together, these findings confirm that animal models might be used to look for a molecular mechanism involved in glaucoma in humans.

The modulation of the activity of genes involved in the development of the anterior segment of the eye may lead to relatively rare developmental glaucomas that account for less than 1% of all glaucoma cases. Several genes have been implicated in congenital glaucoma and anterior segment disgenesis. They include *Cyp1b1, Foxc1, Foxc2, Pitx2, Lmx1b,* and *Pax6*. Several lines of mice with defects in these genes have been studied with glaucoma in mind (see (64) for review). For example, mutation in the *CYP1B1* gene (Cytochrome P450, family 1, subfamily b, polypeptide 1) may lead to primary congenital glaucoma (PCG) in humans. Although Cyp1b1 knockout mice did not develop elevated IOP, they had ocular abnormalities similar to the defects in humans with PCG, viz., small or absent Schlemm's canal, defects in the TM, and attachment of the iris to the TM and anterior synechia.

Mutations in the *FOXC1* gene, that encodes a transcription factor with a forkhead-winged-helix DNA binding domain, cause a range of eye abnormalities associated with glaucoma, e.g., iris hypoplasia, Axenfeld and Rieger anomaly, and

Rieger syndrome. Foxc1$^{-/-}$ mice die at birth, while Foxc1$^{+/-}$ animals are viable but have defects in the eye drainage structures without changes in IOP. Similar eye defects were observed in Foxc2$^{+/-}$ mice. It has been suggested that *Foxc1*$^{+/-}$ and *Foxc2*$^{+/-}$ mice are useful models for studying anterior segment development and anomalies, and may allow the identification of genes that interact with *Foxc1* and *Foxc2* (or *FKHL7* and *FKHL14*) to produce a phenotype with elevated IOP and glaucoma.

Other animals, including rabbit, pig, and bovine have also been used to develop animal models of glaucoma but none of them is widely used for different reasons. Zebrafish became a powerful model for advanced genetic studies in vertebrates, especially in the case of complex diseases, and was proposed as a model for identification of modifier genes for glaucoma (65).

In summary, animal models of glaucoma, including the most widely used rodent and monkey models, have already provided interesting new information about mechanisms of glaucoma in humans. However, it should be remembered that even in monkey models the time course of changes in the glaucomatous eyes may be significantly accelerated compared to that in human glaucomatous eyes, and all

discussed models are indeed just models of glaucoma in humans. Results obtained with these models should not be automatically applied to human glaucoma and should be confirmed by testing in humans whenever possible. It has become clear that reaction to the same insult, e.g., elevated IOP, may be somewhat different in different animal models. Glaucoma studies in animals may help us identify the molecular mechanisms involved in the development of glaucoma in each particular model. By comparing these mechanisms, it may be possible to find some common mechanism that might be involved in glaucoma formation in humans. This will be extremely valuable for the development of new therapeutic approaches for glaucoma treatment and prevention in humans.