

and proteomic techniques, the materials in drusen were found to be composed of molecules that mediate inflammatory and immune processes (Russell, Mullins, Schneider, and Hageman 2000; Mullins, Russell, Anderson, and Hageman 2000). 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, amyloid P component, α 1-antitrypsin, and apolipoprotein E, were identified in drusen. Cellular debris from macrophages, RPE cells, and choroidal dendritic cells has also been identified in drusen. Additional proteins such as crystallins, EEFMP1, and amyloid-beta have been also found in drusen. 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. 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.

5 Primate Model for AMD

Over the past few years, genetic engineering techniques have generated a number of animal models of AMD in mice, rats, rabbits, pigs, and dogs (Chader 2002). 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 (Stafford, Anness, and Fine 1984). 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 (El-Mofty, Gouras, Eisner, and Balazs 1978). At the Tsukuba Primate Research Center (Tsukuba City, Japan), Suzuki et al found a single cynomolgus monkey (*Macaca fascicularis*) (Suzuki Monkeys) in 1986 with a large number of small drusen around the macular region (Nicolas, Fujiki, Murayama, Suzuki, Mineki, Hayakawa, Yoshikawa, Cho, Kanai 1996; Nicolas, Fujiki, Murayama, Suzuki, Shindo, Hotta, Iwata, Fujimura, Yoshikawa, Cho, Kanai 1996; Suzuki, Terao, and Yoshikawa 2003). This single affected monkey has multiplied to a large pedigree of more than 65 affected 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. However, abnormality in electroretinogram (ERG) were observed in sever case showing dysfunction of the macula.

Immunohistochemical and proteomic analyses of the drusen from these monkeys showed that the drusen were very similar to those in other monkeys with aged

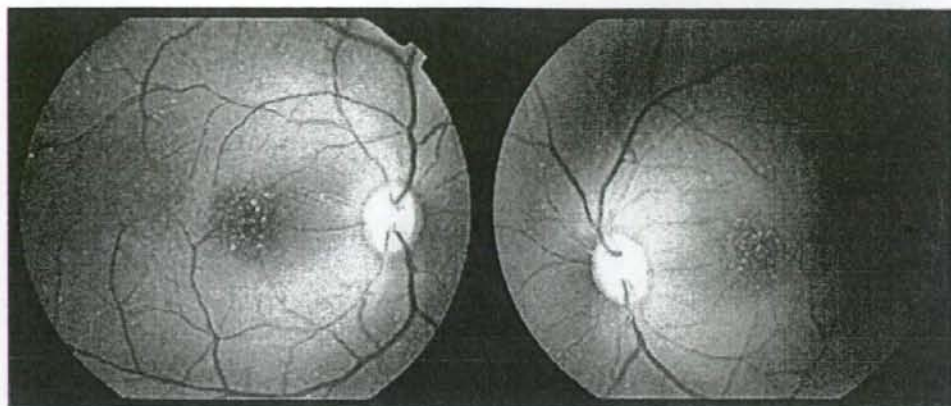


Fig. 1. Fundus photograph of both eyes of Suzuki Monkey showing accumulation of drusen (white spot) around the macular region.

macular degeneration sporadically found in older monkeys and also with human drusen (Umeda, Ayyagari, Allikmets, Suzuki, Karoukis, Ambasadhan, Zernant, Okamoto, Ono, Terao, Mizota, Yoshikawa, Tanaka, and Iwata 2005; Umeda, Suzuki, Okamoto, Ono, Mizota, Terao, Yoshikawa, Tanaka, and Iwata 2005; Ambati, Anand, Fernandez, Sakurai, Lynn, Kuziel, Rollins, and Ambati 2003). These observations have shown that the Suzuki Monkeys produce drusen that are biochemically similar to those in human AMD patients, but the development of the drusen occurs at an accelerated rate. More than 240 loci are being investigated to try to identify the disease causing gene and to understand the biological pathways leading to complement activation. Simultaneously, we have been studying a colony of aged monkeys which develop drusen after 15 years of birth.

Drusen components of these sporadically found affected monkeys were compared with human and Suzuki Monkeys by classical immunohistochemical techniques and by proteome analysis using mass spectrometer. Significant finding was that drusen contained 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, α 1-antitrypsin, and apolipoprotein E), major histocompatibility complex class II antigens, and HLA-DR antigens (Umeda et al. 2005). Cellular components have also 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. In addition to immune components, a number of other proteins were found in drusen. These appear to be vitronectin, clusterin, TIMP-3, serum amyloid P component, apolipoprotein E, IgG, Factor X, crystallins, EEFMP1, and amyloid-beta. The presence of immunoreactive proteins and oxidative modified proteins implicate both oxidation and immune functions in the pathogenesis of AMD.

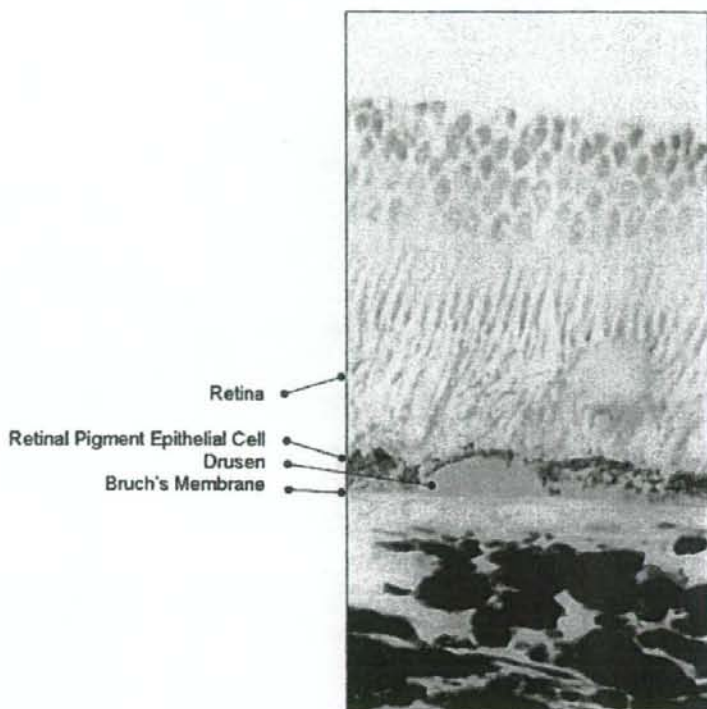


Fig. 2. Retinal histological section of affected Suzuki Monkey showing the accumulation of drusen between the retinal pigment epithelium and Bruch's membrane.

The eyes of monkey are structurally similar to human eyes which make them extremely valuable for AMD 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 expansion of the pedigree, more difficult to genetically manipulate, and the maintenance cost 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.

6 Mouse Model for AMD

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 (Ambati et al. 2003). 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 (Malek, Johnson, Mace, Saloupis, Schmechel, Rickman, Toth, Sullivan, and Bowes Rickman 2005). 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 (Imamura, Noda, Hashizume, Shinoda, Yamaguchi, Uchiyama, Shimizu, Mizushima, Shirasawa, and Tsubota 2006). 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 (Nozaki, Raisler, Sakurai, Sarma, Barnum, Lambris, Chen, Zhang, Ambati, Baffi, and Ambati 2006). 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.

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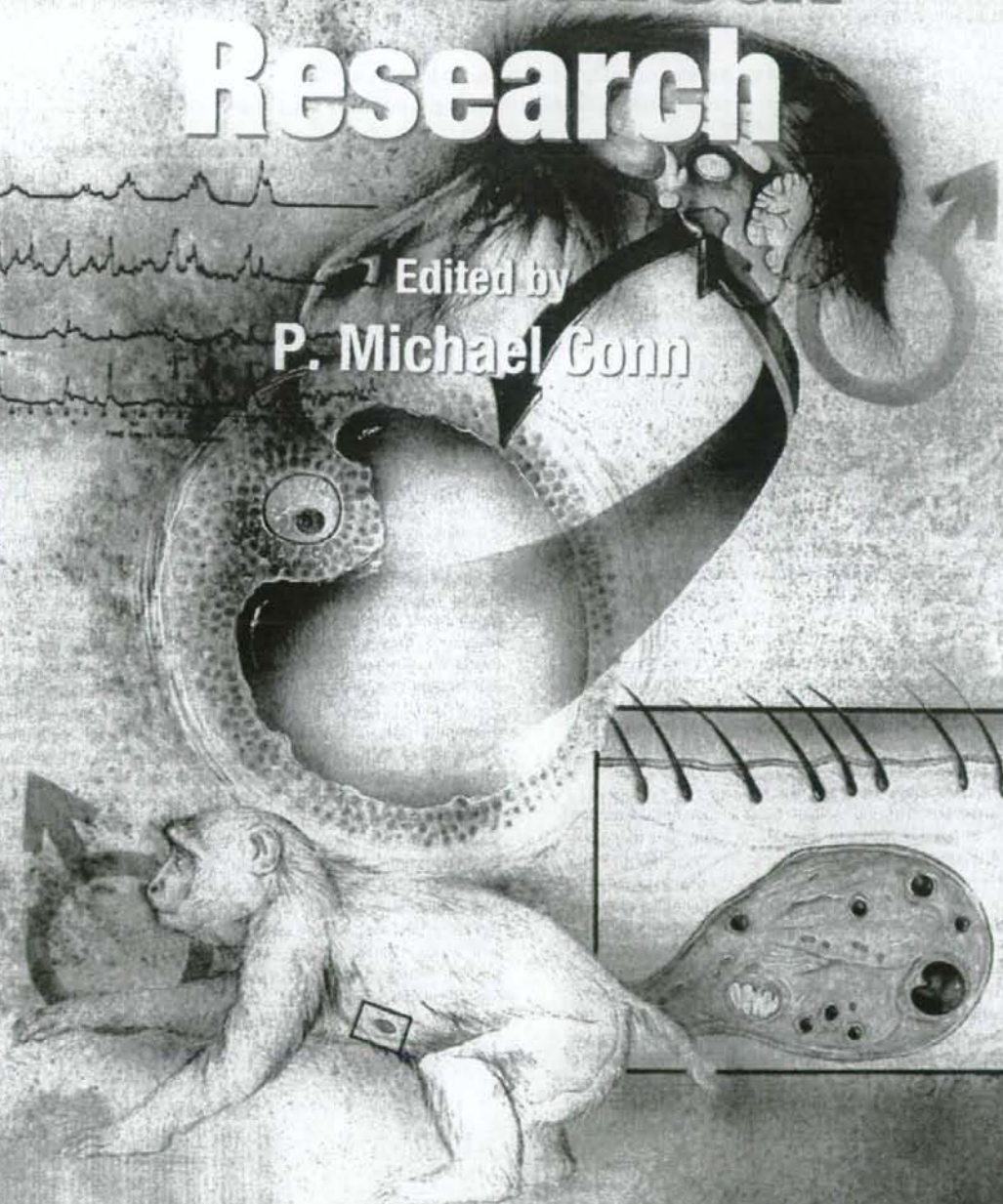
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Sourcebook of Models for Biomedical Research

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Sourcebook of Models for Biomedical Research

Edited by

P. Michael Conn, PhD

Associate Director and Senior Scientist, Oregon National Primate Research Center and

Professor of Physiology and Pharmacology and of Cell and Developmental Biology, Oregon Health and Science University, Beaverton, OR

The collection of systems represented in the *Sourcebook of Models for Biomedical Research* reflect the diversity and utility of models that are used in biomedicine. That utility is based on the consideration that observations made in particular organisms will provide insight into the workings of other, more complex systems. Some models have the advantage that the reproductive, mitotic, developmental or aging cycles are rapid compared with those in humans; others are utilized because individual proteins may be studied in an advantageous way and have human homologs. Other organisms are facile to grow in laboratory settings, lend themselves to convenient analyses, have defined genomes or present especially good human models of human or animal disease. The *Sourcebook of Models for Biomedical Research* is a comprehensive and extensive collection of these important medical parallels. While the entire book is not devoted to the remarkable success of the genomic programs, this work is well represented and indexed within these pages. This volume will be an invaluable resource for pharmaceutical and academic researchers across a wide range of biological fields.

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- Insights on how some organisms that are able to grow in laboratory settings or lend themselves to convenient analyses have defined genomes or present especially good human models of human or animal disease
- Uses a range of tables and figures to make comparisons of models so that observations not available in primary publications can become useful to the reader

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Complement Activation of Drusen in Primate Model (*Macaca fascicularis*) for Age-Related Macular Degeneration

Takeshi Iwata

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

1 Introduction

Dysfunction of the visual system can alter normal human life style and lower quality of life. The most prevalent causes of visual impairment worldwide are cataracts, glaucoma, and age-related macular degeneration (AMD). These eye diseases 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. 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, a limited amount of information is available on the underlying pathological mechanisms causing these diseases. Obtaining tissues from the AMD donors is often difficult, and even when obtained, they are usually collected many hours or even days after death. Because of limitation for human tissue, the availability of animal models is becomes valuable because they can be used to investigate the molecular mechanisms of the disease and to test new therapeutic intervention.

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 macula. In humans, the average size of the macula is only 6 mm in diameter. The outer 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.

2 Introduction 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.

3 Genetics of AMD

Epidemiological studies have shown that genetic factor play critical role for AMD. Twin studies have previously shown a higher concordance for AMD in monozygotic twins than in dizygotic twins (Heiba, Elston, Klein, and Klein 1994; Seddon, Ajani, and Mitchell 1997; Hammond, Webster, Snieder, Bird, Gilbert, and Spector 2002). In addition, first degree relatives of individuals with AMD have 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. Previous 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. 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 (Iyengar, Song, Klein, Klein, Schick, Humphrey, Millard, Liptak, Russo, Jun, Lee, Fijal, and Elston 2004; Schick, Iyengar, Klein, Klein, Reading, Liptak, Millard, Lee, Tomany, Moore, Fijal, and Elston 2003; Majewski, Schultz, Weleber, Schain, Edwards, Matise, Acott, Ott, and Klein 2003). Recently, a polymorphism of complement factor H (CFH) gene (*Y402H*) was shown to be associated with an increased risk for AMD (Klein, Zeiss, Chew, Tsai, Sackler, Haynes, Henning, SanGiovanni, Mane, Mayne, Bracken, Ferris, Ott, Barnstable, and Hoh 2005; Edwards, Ritter, Abel, Manning, Panhuysen, and

Farrer 2005; Haines, Hauser, Schmidt, Scott, Olson, Gallins, Spencer, Kwan, Noureddine, Gilbert, Schnetz-Boutaud, Agarwal, Postel, and Pericak-Vance 2005; Hageman, Anderson, Johnson, Hancox, Taiber, Hardisty, Hageman, Stockman, Borchardt, Gehrs, Smith, Silvestri, Russell, Klaver, Barbazetto, Chang, Yannuzzi, Barile, Merriam, Smith, Olsh, Bergeron, Zernant, Merriam, Gold, Dean, and Allikmets 2005).

These results were confirmed in many of the countries with large Caucasian populations but not in Japan (Okamoto, Umeda, Obazawa, Minami, Noda, Mizota, Honda, Tanaka, Koyama, Takagi, Sakamoto, Saito, Miyake, and Iwata 2006; Gotoh, Yamada, Hiratani, Renault, Kuroiwa, Monet, Toyoda, Chida, Mandai, Otani, Yoshimura, and Matsuda 2006). 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 (Gold, Merriam, Hancox, Taiber, Gehrs, Cramer, Neel, Bergeron, Barile, Smith, AMD Genetics Clinical Study Group, Hageman, Dean, Allikmets 2006). HTRA1, a serine protease 11 was recently discovered to be strongly associated with AMD. Unlike the CFH, our study shows strong association with this gene for Japanese AMD patients (Yang, Camp, Sun, Tong, Gibbs, Cameron, Chen, Zhao, Pearson, Li, Chien, Dewan, Harmon, Bernstein, Shridhar, Zabriskie, Hoh, Howes, and Zhang 2006; Dewan, Liu, Hartman, Zhang, Liu, Zhao, Tam, Chan, Lam, Snyder, Barnstable, Pang, and Hoh 2006).

4 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. 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 $<50 \mu\text{m}$ 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 (Russell, Mullins, Schneider, and Hageman 2000; Mullins, Russell, Anderson, and Hageman 2000). 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, amyloid P component, α 1-antitrypsin, and apolipoprotein E, were identified in drusen. Cellular debris from macrophages, RPE cells, and choroidal dendritic cells has also been identified in drusen. Additional proteins such as crystallins, EEFMP1, and amyloid-beta have been also found in drusen. 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. 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.

5 Primate Model for AMD

Over the past few years, genetic engineering techniques have generated a number of animal models of AMD in mice, rats, rabbits, pigs, and dogs (Chader 2002). 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 (Stafford, Anness, and Fine 1984). 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 (El-Mofty, Gouras, Eisner, and Balazs 1978). At the Tsukuba Primate Research Center (Tsukuba City, Japan), Suzuki et al found a single cynomolgus monkey (*Macaca fascicularis*) (Suzuki Monkeys) in 1986 with a large number of small drusen around the macular region (Nicolas, Fujiki, Murayama, Suzuki, Mineki, Hayakawa, Yoshikawa, Cho, Kanai 1996; Nicolas, Fujiki, Murayama, Suzuki, Shindo, Hotta, Iwata, Fujimura, Yoshikawa, Cho, Kanai 1996; Suzuki, Terao, and Yoshikawa 2003). This single affected monkey has multiplied to a large pedigree of more than 65 affected 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. However, abnormality in electroretinogram (ERG) were observed in sever case showing dysfunction of the macula.

Immunohistochemical and proteomic analyses of the drusen from these monkeys showed that the drusen were very similar to those in other monkeys with aged

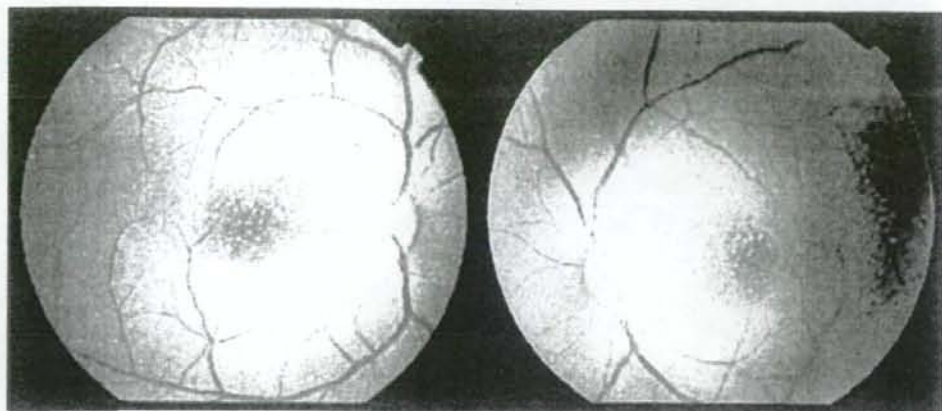


Fig. 1. Fundus photograph of both eyes of Suzuki Monkey showing accumulation of drusen (white spot) around the macular region.

macular degeneration sporadically found in older monkeys and also with human drusen (Umeda, Ayyagari, Allikmets, Suzuki, Karoukis, Ambasadhan, Zernant, Okamoto, Ono, Terao, Mizota, Yoshikawa, Tanaka, and Iwata 2005; Umeda, Suzuki, Okamoto, Ono, Mizota, Terao, Yoshikawa, Tanaka, and Iwata 2005; Ambati, Anand, Fernandez, Sakurai, Lynn, Kuziel, Rollins, and Ambati 2003). These observations have shown that the Suzuki Monkeys produce drusen that are biochemically similar to those in human AMD patients, but the development of the drusen occurs at an accelerated rate. More than 240 loci are being investigated to try to identify the disease causing gene and to understand the biological pathways leading to complement activation. Simultaneously, we have been studying a colony of aged monkeys which develop drusen after 15 years of birth.

Drusen components of these sporadically found affected monkeys were compared with human and Suzuki Monkeys by classical immunohistochemical techniques and by proteome analysis using mass spectrometer. Significant finding was that drusen contained 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, α 1-antitrypsin, and apolipoprotein E), major histocompatibility complex class II antigens, and HLA-DR antigens (Umeda et al. 2005). Cellular components have also 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. In addition to immune components, a number of other proteins were found in drusen. These appear to be vitronectin, clusterin, TIMP-3, serum amyloid P component, apolipoprotein E, IgG, Factor X, crystallins, EEFMP1, and amyloid-beta. The presence of immunoreactive proteins and oxidative modified proteins implicate both oxidation and immune functions in the pathogenesis of AMD.

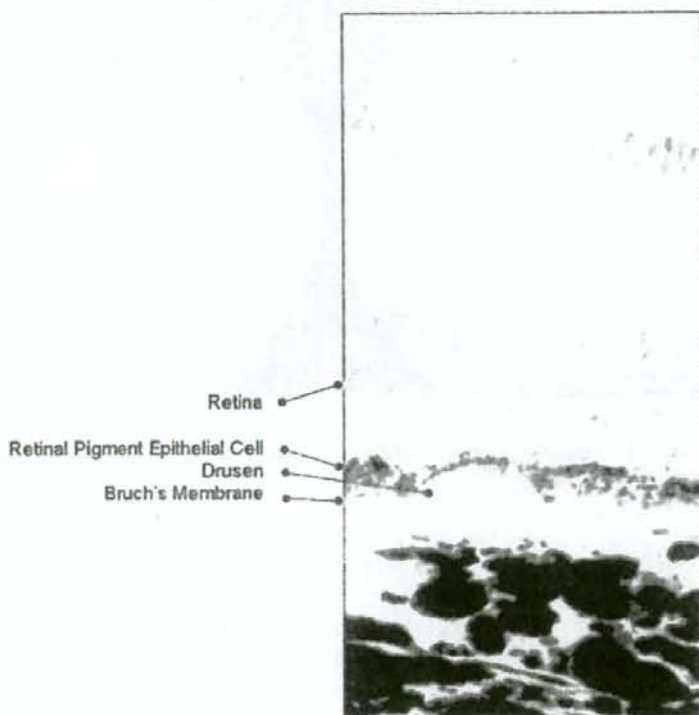


Fig. 2. Retinal histological section of affected Suzuki Monkey showing the accumulation of drusen between the retinal pigment epithelium and Bruch's membrane.

The eyes of monkey are structurally similar to human eyes which make them extremely valuable for AMD 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 expansion of the pedigree, more difficult to genetically manipulate, and the maintenance cost 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.

6 Mouse Model for AMD

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 (Ambati et al. 2003). 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 (Malek, Johnson, Mace, Saloupi, Schmechel, Rickman, Toth, Sullivan, and Bowes Rickman 2005). 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 (Imamura, Noda, Hashizume, Shinoda, Yamaguchi, Uchiyama, Shimizu, Mizushima, Shirasawa, and Tsubota 2006). 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 (Nozaki, Raisler, Sakurai, Sarma, Barnum, Lambris, Chen, Zhang, Ambati, Baffi, and Ambati 2006). 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.

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特集：視覚障害(3)

眼疾患バイオマーカーの探索

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はじめに

近年ゲノム配列や体内のタンパク質を分析して疾患の発症を予測する研究が行われている。この背景には飛躍的な技術革新によるゲノム解析技術や質量分析を用いた微量タンパク質の網羅的解析技術などの進歩がある。これらの技術はすでに多くの疾患で成果を上げており、眼科分野でも応用されている。患者の眼球において、まだ生理機能が保持されている環境では再生医療を、そして環境が破綻している場合には人工網膜等による視力回復が検討されている。本章ではこれらの研究に加え、未発症の患者予備軍を選別し、病気の予防あるいは遅延を目的とした予防医学について、その選別部分について最近の研究を紹介したい。

感覚器症例情報収集システムの構築

眼球内部は透明な組織で構成されていることから容易に組織内部を観察できる一方、生検することがきわめて困難な組織でもある。したがって早期診断に利用できるサンプルは涙液、血液、尿に限られ、必然的に研究対象は白血球のDNAと血漿成分に絞られる。また、手術中に破棄される房水、硝子体の

一部についても倫理委員会の承認と患者の同意があれば研究材料として利用できる。われわれはこれらのDNAや体液から疾患と相関する疾患バイオマーカーの探索を行っている。平成15年、国立病院機構および厚生労働省の支援を受けて、オンラインによる症例情報の収集とDNA・血漿バンクの設立を目的とした「感覚器ネットワーク」を東京医療センター臨床研究センター（感覚器センター）に構築した（図1）。関連施設の国立病院および大学病院から白内障、緑内障、加齢黄斑変性、網膜色素変性の症例情報をウェブ画面上から登録し、同時に検体を受け取る仕組みである。症例情報には個人情報を除く患者の臨床所見が詳細に登録できるようになっており、眼底写真の添付も可能である。遺伝子やタンパク質の解析結果は症例情報と一体化され、個々の患者についてデータベースが構築されることによって、疾患バイオマーカーの探索をより容易にしている。

遺伝子解析による加齢性眼疾患の感受性遺伝子の探索

これまでの研究から加齢性眼疾患の多くは多因子疾患（遺伝、習慣、環境）であると考えられている。

国立病院機構東京医療センター 臨床研究センター 分子細胞生物学研究部
別刷請求先：岩田岳 国立病院機構東京医療センター 臨床研究センター 分子細胞生物学研究部
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Search for Biomarker of Ocular Diseases

Takeshi Iwata

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