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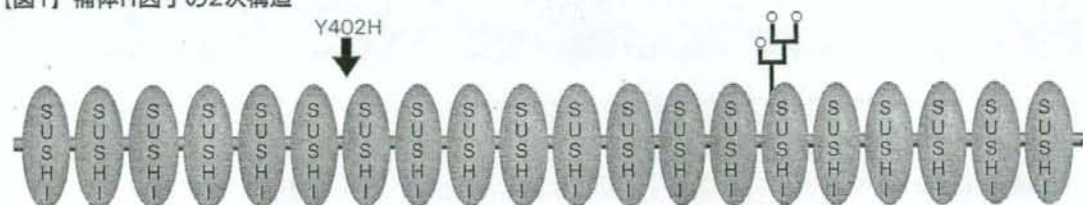
- ・加齢黄斑変性と相関性のある遺伝子にはABCA4, ApoE, Fibulin5などがある
- ・加齢黄斑変性のリスク遺伝子は11の染色体の13の遺伝子座位が散在する
- ・補体H因子の遺伝子多型と加齢黄斑変性の高い相関性が最近発表されたが日本人では認められなかった
- ・感覚器センターでは加齢黄斑変性の症例登録システムと血液収集を開始した

加齢黄斑変性 (age-related macular degeneration ; AMD) は多因子疾患と考えられており、遺伝的な背景に環境因子が加わって初めて発症すると考えられている¹⁾。そのため単一遺伝子の変異によって発症する黄斑ジストロフィのような発達の著しい研究とは異なり、AMDの遺伝子解析は思うように進んでいない。これまでの研究からAMDの原因の約25%が遺伝的要因と推測されているが、その遺伝因子も単一ではなく複数存在することが示唆されている²⁾。これまでに発見された黄斑ジストロフィの原因遺伝子のなかからAMDとの相関性が報告されている遺伝子としてはStargardt病の原因遺伝子であるABCA4³⁾やApoE⁴⁾、そしてFibulin5⁵⁾があるが、その関係を疑う研究者も多い。

近年、ヒトゲノムプロジェクトによる全染色体の塩基配列が決定され、平均で1千塩基に1つ発見される1塩基配列の違い(例：アデニン(A)がチミン(T)と置き換わる)、いわゆる遺伝子多型(single nucleotide polymorphism ; SNP) が注目されてきた。ゲノム上に散在するSNPを組み合わせて、これまで未知遺伝子の探索に利用されてきた連鎖解析マーカーと同様に利用することが可能になってきたからである⁶⁾。これらの連鎖解析技術を用いたAMDのリスク遺伝子座位(リスク遺伝子が存在する染色体上の領域)が最近報告された。その結果、11の染色体(1, 2, 4, 5, 9, 10, 12, 15, 16, 18, 20)の13の座位にAMDのリスク遺伝子が存在することが明らかとなり、これらのすべてあるいはいくつかの遺伝子多型と環境因子が組み合わさって発症すると推測されている^{7,8)}。

補体H因子に関する報告

【図1】 補体H因子の2次構造



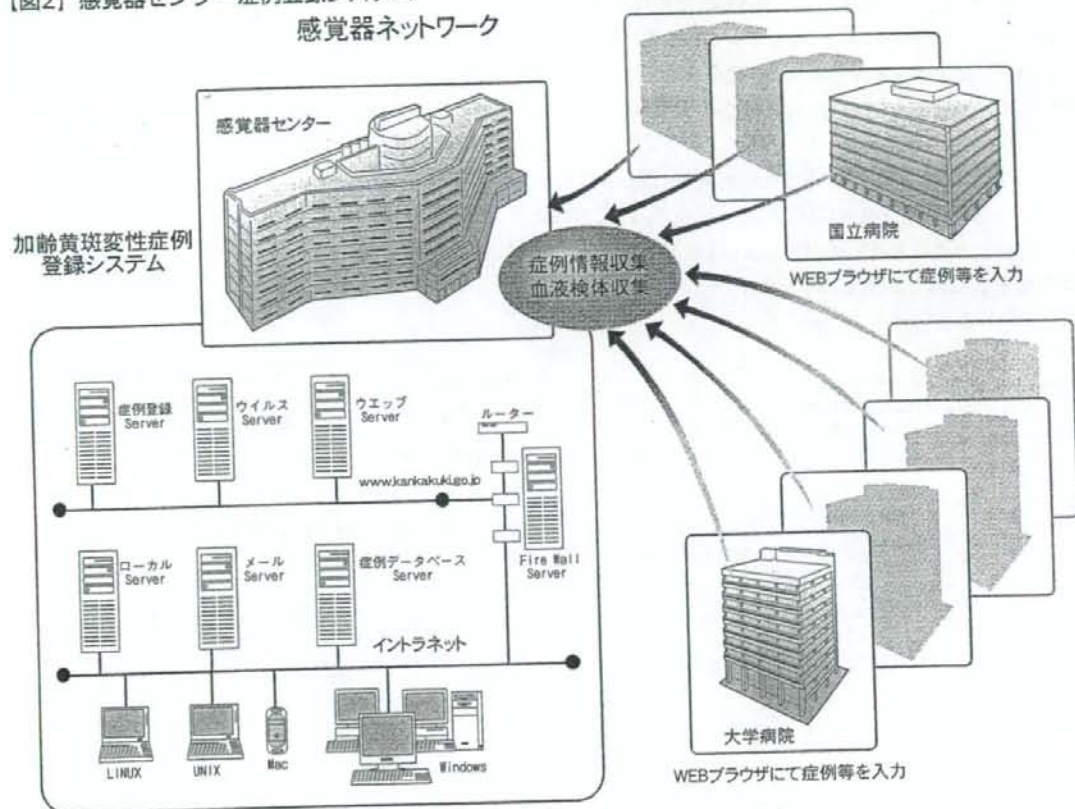
AMDとの相関が報告された補体H因子はSUSHIドメインが20回反復される細長い構造をしている。矢印は遺伝子多型Y402Hが発見された第7SUSHIドメインの位置を示す。

今年4月にScience誌に3つの論文が連続で報告され^{9,10,11}、新聞などでも記事として取り上げられた。さらにその直後にその内容に類似する2つの論文が別の科学誌にも報告されている^{12,13}。5つの異なるグループがほぼ同時に発表したこの研究内容とは、染色体1番のAMDリスク遺伝子の同定の報告であった。この遺伝子は自然免疫システムの古典経路と2次経路からなる補体活性経路に対してこれを抑制する補体H因子(complement factor H)である【図1】。5つの論文は402番のアミノ酸がヒスチジンからトリプトファンに変異する多型がAMDと強く連鎖することを報告した。しかしこの多型が患者および健常者に現れる頻度については5つの論文で数字が異なっている。Hainesらの論文¹⁰ではH402Tは健常者(185人)で46%、患者(495人)では96%の頻度で現れると書かれているが、Zarepariらの論文では健常者(275人)で34%、患者(616人)で61%と大きく数字が異なる。さらに著者らが独自に日本人だけを対象に行った調査では、健常者(89人)で5%、患者(96人)で8%とさらに大きく異なることが明らかとなった。これほど大きく数字に隔たりがある理由は今後の国際的な研究によって解明されるであろう。2005年6月14日に米国国立眼研究所(National Eye Institute: NEI)で補体H因子に関するシンポジウムが開かれ、この研究に携わる代表的な研究者が集まってこれまでの研究経過と今後の方向性が話し合われた。この模様はインターネット上で同時配信され、録画映像もウェブサイト(<http://videocast.nih.gov>)で見ることができる¹⁴。

ドルーゼンの研究

【図2】 感覚器センター症例登録システム

感覚器ネットワーク



感覚器センターがAMDの情報と血液検体を収集するために構築した感覚器ネットワークシステム。全国の大学病院や国立病院機構病院がネットワークに参加している。

AMDは発症初期に網膜色素上皮細胞とBruch膜の間にドルーゼンといわれる蛋白質や細胞断片からなる複合体の蓄積が観察される。AMDがアルツハイマー、糸球体腎炎そして粥状動脈硬化症など局所的な補体活性化と炎症反応による沈着物を特徴とする疾患に類似すると考えたHagemanとAndersonらの研究グループは、免疫染色法という方法で患者の網膜切片を使ってこれを証明した。Hollyfieldらも質量分析計を使って直接ドルーゼンの組成成分を分析したところ、前者と同様な蛋白質が含まれていることを明らかにした。どのようなきっかけで炎症反応が起こるのか、ドルーゼンは網膜やその周辺にどのような悪影響を及ぼすのか、そしてドルーゼンの蓄積を防ぐことがAMDを未然に防ぐ方法なのか、今後数年間の研究によってこの回答が得られる可能性が高い。AMDの最大の環境危険因子として喫煙があるが、喫煙によって補体H因子の量が減少することが報告されている。すなわち、喫煙者は補体の活性化を抑制する能力が低いことを意味する。著者らの研究室ではサルを使って補体の活性化を網膜色素上皮細胞下で誘導し、人工的にドルーゼンの蓄積を促す実験に取り組んでいる。

AMDの研究はこのように遺伝学と病理学の2本柱がうまく協調して進行しているが、遺伝子解析技術の進歩によって遺伝的多因子の同定がさらに加速されると考えられる。今回発見された補体H因子は11の染色体に散在する13の遺伝子の1つであり、今後同様な遺伝子が次々と発見され、検証されると考えられる。日本での今後の課題として、日本人AMD患者の遺伝情報が欠落していることである。これまでに福岡県久山町でAMDの疫学調査などが行われてきたが、遺伝学的解析には至っていない。今回の遺伝子多型についても日本人では有意な差が観察できなかったことから、この疾患に対する日本人と欧米人の遺伝的素因は異なっていると考えられ、米国主導の研究結果をそのまま日本人に当てることが難しい。感覚器センターでは加齢黄斑変性DNAバンクを設立して、全国の大学および国立病院機構の病院から患者DNAをプールして独自に日本人のための大規模な遺伝子解析を開始した【図2】。

ヒトが得る情報の9割は感覚器(視聴覚)を通じて入ってくると考えられており、世界最速で高齢化が進行する国民のquality of life(QOL)を維持するためにも高齢化に伴って発症するAMDに対する国家レベルの対策が求められている。

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(岩田 岳)

COMPLEMENT ACTIVATION OF DRUSEN IN PRIMATE MODEL (*Macaca fascicularis*) FOR AGE- RELATED MACULAR DEGENERATION

Takeshi Iwata

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

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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 factors play a 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 a 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, Zernant, 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.

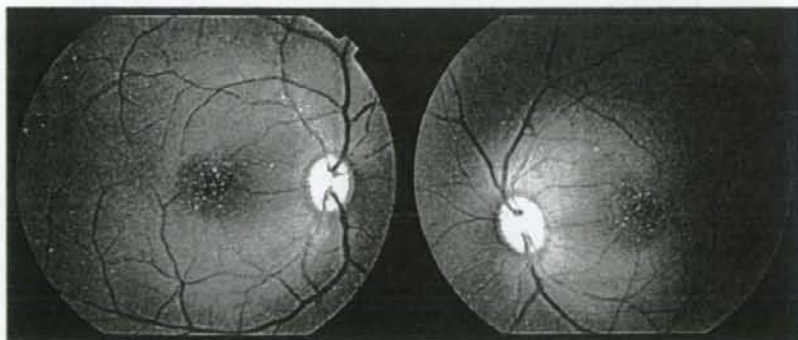


Figure 1. Fundus photograph of both eyes of Suzuki Monkey showing accumulation of drusen (white spot) around the macular region.

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 (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.

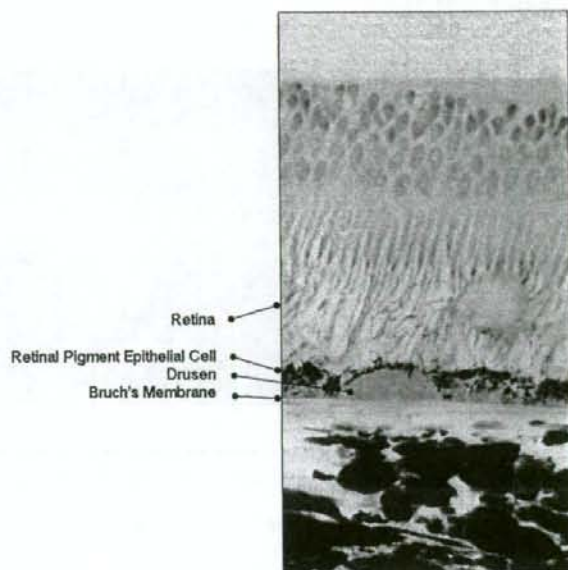


Figure 2. Retinal histological section of affected Suzuki Monkey showing the accumulation of drusen between the retinal pigment epithelium and Bruch's membrane.

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.

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.

7. ACKNOWLEDGEMENT

This work was supported by the research grant from the Ministry of Health, Labour and Welfare of Japan.

8. REFERENCES

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