Graefe's Arch Clin Exp Ophthalmol
(2004) 242:710–716

REVIEW

DOI 10.1007/s00417-004-0976-x

Felix Roth
Almut Bindewald
Frank G. Holz

Keypathophysiologic pathways in age-related macular disease

Received: 7 May 2004
Accepted: 21 June 2004
Published online: 10 August 2004

F. Roth · A. Bindewald · F. G. Holz (✉)
Department of Ophthalmology,
University of Bonn,
Ernst-Abbestrasse 2, 53127 Bonn, Germany
e-mail: frank.holz@ukb.uni-bonn.de
Tel.: +49-228-2875647
Fax: +49-228-2875603

Abstract *Purpose:* To review current knowledge of key pathogenetic pathways in age-related macular disease (AMD). *Methods:* Experimental evidence and clinical observations are reviewed. *Results:* A number of common downstream pathophysiologic pathways appear to be relevant in AMD manifestations irrespective of primary heterogeneous etiologies. These include sequelae of oxidative damage, retinal pigment epithelium (RPE) cell dysfunction with accumulation of lipofuscin and impairment of lysosomal functions, deposition of subsequently incompletely degraded material at the basal RPE cell side and alterations in Bruch's membrane extracellular matrix, immunologic responses to extracellular material (drusen) with subsequent growth of drusen, induction of choroidal neovascularization as a result of imbalance between anti-angiogenetic and proangiogenetic factors as well as cell death (geographic atrophy) without prior neovascular events. *Conclusions:* Understanding is expanding regarding the sequence of events that lead to early and late lesions in AMD. Therapeutic approaches that focus on the molecular mechanisms are more likely to succeed than currently available treatment options as exemplified by the management of choroidal neovascularisations.

Introduction

Age-related macular disease (AMD) is a complex, multifactorial disease and now the most common cause for legal blindness in industrialized countries [11, 36]. Prevalence will further increase given demographic developments in ageing populations. Current prophylactic and therapeutic options are limited. A better knowledge of the pathogenesis appears mandatory to develop efficient therapies in order to prevent central visual loss from AMD. In the human eye various age-related changes occur in the outer retina, the retinal pigment epithelium (RPE), Bruch's membrane and the choroid. Several of these changes, which are influenced by a variety of initiating, promoting or inhibiting genetically and exogenous factors, are thought to contribute to the pathogenesis of AMD. The understanding of underlying molecular mechanisms has been expanding over the recent years and is reviewed herein. Novel insights have already led to new targets for intervention.

Oxidative damage

Several experimental and clinical findings indicate that oxidative mechanisms contribute to the disease process. Because of the high oxygen demand, life-long exposure to light and the presence of polyunsaturated fatty acids the retina is particularly prone to photo-oxidative damage [7] with subsequent formation of free radicals and peroxidation of adequate substrates. These mechanisms may become more important with age as there is a reduction in the local antioxidative enzymes in the RPE and a decrease in macular pigment density, which serves as a filter for short-wavelength light and with its two constituents, lutein and zeaxanthin, as an antioxidant. With increasing

cumulative oxidative stress peroxidation of biomolecules including lipids could result in the formation of undegradable higher molecular polymers which, in turn, lead to accumulation of material in the lysosomal compartment of RPE cells, so-called lipofuscin (LF) granules. Lipofuscin may sensitize the RPE to light with subsequent aggravation of oxidative damage and contribute to impairment of pigment epithelial functions. The correlation between RPE lipofuscin accumulation and distribution of drusen and the fact that race, light iris colour and age are known risk factors for AMD further support the hypothesis of oxidative mechanisms in the pathogenesis of AMD. It has been argued that enhancing of the concentration of antioxidants in the outer retina could have a protecting effect against the oxidative damage. Interestingly, recent results from the Age-Related Eye Disease Study (AREDS) indeed demonstrate a prophylactic effect with regard to the conversion from early to late-stage AMD of high doses of antioxidants (vitamin C, vitamin E, beta-carotin) [1].

Lipofuscin formation and interaction with cellular function

Release of incompletely degraded material at the basal RPE cell side as an initial event implicates malfunctioning lysosomal function with age. An essential function of postmitotic RPE is the phagocytosis of constantly shed photoreceptor outer segment disks and degradation with subsequent release of completely degraded material at the basal cell side where it is normally cleared by the choriocapillaris. With age lipofuscin accumulates in the lysosomal compartment [12, 15, 21, 23, 49, 72]. Although the mechanisms of lipofuscinogenesis are incompletely understood, there is strong evidence that oxidative damage plays an important role, with antioxidant deficiency or prooxidant conditions being of importance [4, 7, 14, 61]. Electron microscopic studies have shown a stepwise conversion of lysosomal structures to LF granules. Apparently, once formed the RPE cell has no means to either degrade or transport LF material/granules into the extracellular space via exocytosis. Subsequently, these granules are trapped in the cytoplasm and may occupy large amounts of the cytoplasmic volume. Although lysosomal hydrolytic enzymes are capable of degrading most cellular macromolecules, aggregation of molecules and posttranslational modifications generates biomolecules that are no longer degradable by human enzymes.

Recent analyses of compounds in isolated human LF granules revealed various molecules with *toxic properties* including malondialdehyde (MDA), 4-hydroxynonenal (HNE) and advanced glycation endproducts (AGE) [67] as well as a Schiff base reaction product, N-retinylidene-N-retinylethanolamine (A2-E) [18]. A2-E represents a major fluorophore of LF in the RPE [18, 19]. Molecular mechanisms have been identified how A2-E interferes with normal lysosomal function upon reaching critical levels [9, 40, 65]. A2-E induces a striking inhibition of lysosomal degradation in human RPE cells [19] by inhibition of the ATP dependent lysosomal proton pump [9]. Furthermore, A2-E has phototoxic and detergent properties and is capable of inducing disintegration of various organelle membranes upon reaching a critical concentration [64–66]. In search for further potentially toxic compounds of LF besides A2-E, RPE human LF granules have been isolated and purified and a proteome-analysis has been performed. Interestingly, a broad spectrum of LF proteins showed specific posttranslational modifications including MDA, HNE and AGE [67]. This underscores the potential contribution of oxidative damage in lipofuscin biogenesis.

Further evidence for a pathophysiologic role of LF includes a similar topographic distribution of LF and drusen, accelerated accumulation of LF in monogenetic macular dystrophies such as Best or Stargardt disease [2, 17, 53, 70] and a striking deposition of A2-E in RPE cells in ABCR knockout mice with strong dependence on light exposure. Recently, additional animal models with RPE LF accumulation have been established, including P-8 strain senescence accelerated mice, cathepsin-D-deficient transgenic mice, canine ceroid lipofuscinoses, and rds –/– mice [13, 47, 48, 54]. Interestingly, besides LF accumulation some of these models also display other AMD-like phenotypic changes including sub-RPE deposits [13]. These models will be helpful to further understand pathogenetic mechanisms and to identify targets for intervention in order to prevent deleterious effects of excessive LF accumulations.

Information on LF accumulation in the RPE has been largely obtained from in vitro studies using fluorescence microscopy techniques [16, 23, 24, 71, 72] and in vivo from fundus spectro-photometric investigations [15]. More recently, with the advent of scanning laser ophthalmoscopy, it is now possible to record fundus autofluorescence (FAF) images and to study topographic variations in vivo when using appropriate excitation wavelengths and barrier filters [8, 51, 52, 62, 69]. The technique was initially introduced by Rückmann and coworkers [62]. We recently introduced another novel confocal scanning laser ophthalmoscope (cSLO, Heidelberg Retina Angiograph, HRA) for FAF imaging which directly generates digital images [8, 10, 39, 46]. Delori and co-workers have shown that the autofluorescence signal derives from RPE LF [15]. Using the cSLO technique we demonstrated impairment of neurosensory retinal function and subsequent development of atrophy and visual loss confined to areas with prior excessive LF accumulation in eyes with AMD. Therefore, these clinical findings are in accordance with the above hypotheses based on experimental results implicating LF accumulation in cellular dysfunction and macular degeneration.

FAF imaging has also led to new, refined phenotypic classifications of AMD manifestations which may be helpful to identify genetic factors.

Bruch's membrane, composition and biogenesis of drusen

Bruch's membrane between the RPE and the choroid shows various morphological and biochemical alterations with age [22]. These include changes in elastic and collagenous fibres, diffuse thickening and accumulation of material, which are thought to result in reduced elasticity and increased resistance to diffusion. The earliest clinically visible abnormality in AMD is the accumulation of drusen in the extracellular in inner aspects of Bruch's membrane. Biochemically the deposited material is a complex composition of lipids, glycoproteins and proteins [38, 59]. Especially the lipoidal compounds increase exponentially beyond 40 years of age, and the accumulation in the macular area is higher than in peripheral Bruch's membrane [68]. The proportion of cholesterol esters of the lipid component—the major lipid species are phospholipids and fatty acids rather than cholesterol and cholesterol esters—argue for an intracellular and, therefore, pigment epithelium, as opposed to the extracellular, choroidal origin of the material [37]. Especially diffuse thickening and progressive deposition of material in Bruch's membrane may result in a barrier to normal metabolic exchange between the RPE and the choriocapillaris [25, 55]. The reduction of the hydraulic conductivity of Bruch's membrane may hamper the movement of water towards the choroid, thus causing it to accumulate in the sub-RPE space and leading to RPE detachment. All late-stage manifestations associated with severe visual loss in AMD are considered as processes reactive to these initial alterations in Bruch's membrane and the RPE.

Role of immune response

Recent evidence suggests a role for local inflammation with complement activation and immune complex deposition in eyes with AMD [3, 6, 44, 56]. Cellular debris that is derived from compromised RPE cells becomes sequestered between the RPE basal lamina and Bruch's membrane. Failure to eliminate the entrapped material generates a *local pro-inflammatory signal* that is sufficient to trigger subsequent events including local upregulation of cytokines, acute phase reactants, and other pro-inflammatory mediators, activation of the complement cascade, invasion of incipient drusen by processes of dendritic cells from the choroid and the induction of an immune response to exposed antigens in the sub-RPE space. Thus, extracellular debris and the chronic inflammatory stimulus constitute a "nucleation" site for drusen growth, which is supported by the findings that drusen contain a variety of inflammation-related proteins including CRP, vitronectin, α1-antichymotrypsin, amyloid P component and fibrinogen [6]. A number of these proteins appear to be produced locally by distressed RPE cells rather than circulating in the blood [5, 33, 58]. The identification and localisation of multiple complement activators (nuclear fragments, membrane bound vesicles, lipofuscin, cholesterol and microfibrillar debris) [20, 42] as well as terminal complement compounds in drusen support the conclusion that drusen compounds act as a trigger of the complement cascade, a basic physiological reaction to foreign cells, dead and sick cells or cell fragments.

Interestingly, these inflammatory responses appear to be analogous to processes observed in other age-related diseases such as Alzheimer's disease or atherosclerosis, where accumulation of extracellular deposits elicits a local chronic low-grade inflammatory response [5, 31, 32, 44, 45, 56]. Furthermore the presence of IgG and complement C3 and C5 intermediates is strongly suggestive of the presence of immune complexes, and is consistent with the presence of circulating autoantibodies in patients with AMD [30, 60]. The possible contribution of the immune system is further underscored by findings in patients with membranoproliferative glomerulonephritis type II. Here, complement activation and immune complex deposition cause glomerular injury, and the ocular phenotype is characterized by drusen resembling those in AMD in ultrastructure and composition (including C5 and IgG deposition) [57], as well as secondary CNV [50].

A mouse model deficient either in monocyte chemoattractant Ccl-2 or its cognate receptor Ccr-2 has recently been shown to develop cardinal features of AMD including accumulation of RPE-lipofuscin and A2-E, drusen, atrophy and choroidal neovascularisation [3]. This intriguing model implicates macrophage dysfunction in the pathogenesis of AMD. The extracellular debris between RPE and Bruch's membrane contains IgG and C5a (activated complement compound C5), which mediate choroidal macrophage infiltration. In Ccl-2 –/– and Ccr-2 –/– mice and in human eyes with AMD impaired macrophage recruitment may allow the accumulation of C5a and IgG, usually degraded by the macrophages. This accumulation may induce vascular endothelial growth factor (VEGF) production by RPE cells and thus possibly mediating the development of CNV [3].

Finally, drusen appear to be targets of invasion by processes of *dendritic cells* [33]. As part of the inflammatory response, dendritic cells originating from the choroid may extend through Bruch's membrane into the extracellular space below the RPE and have a primary role in antigen capture and presentation to T-lymphocytes. In rodents, choroidal dendritic cells have been implicated in experimental models of ocular inflamma-

tion [26, 27, 43], but the exact role and timing of an immune response directed against one or more autoantigens in the sub-RPE space and its relationship to drusen biogenesis remains to be elucidated.

Choroidal neovascularization

CNV represents the most common cause for severe visual loss in AMD. As soon as new vessels originating from the choriocapillaris grow through Bruch's membrane they expand in horizontal direction either between the inner Bruch's membrane and the RPE cell layer ("occult" CNV by angiographic characterstics) or in a plane further anterior through the RPE cell layer ("classic" CNV). Subsequent dysfunction of the neurosensory retina results from various mechanisms including hyperpermeability and haemorrhages. Over time there is a reduction in the vascular component of the membrane with a concurrent increase in the proportion of fibrous tissue. These neovascular processes in AMD are thought to result from a local imbalance of growth factors inducing growth of blood vessels inwards from the choroid [73]. Maintenance of the physiological architecture of the choriocapillaris requires a well-regulated balance between various factors derived from the retinal pigment epithelial basolateral plasma membrane domain including pigment epithelial derived factor (PEDF), VEGF and angiopoetins 1 and 2. VEGF, overexpressed by stimuli including hypoxia, induces the formation of new blood vessels, while PEDF serves as an inhibiting factor. The effect of PEDF is apparent in the vitreous and the cornea, which do not contain blood vessels. In addition to VEGF and PEDF, recent results indicate that angiopoetins 1 and 2 with their receptor Tie-2 play a part in the formation of CNV [34]. Ang 1 functions as an agonist and promotes vascular integrity and maturation, inhibits apoptosis and reduces permeability. Ang 2 is an antagonist of Ang 1 and promotes VEGF-induced angiogenesis. The receptor Tie-2 as well as Ang 1 and 2 have been shown to be present in choroidal neovascular tissue [34]. There are also receptors present that are specific for active endothelium in CNV membranes including a_v-integrins and TGF-beta receptor CD 105.

Interestingly, with age there is a reduction in the amount of PEDF in the vitreous [35], while there is an increased level of VEGF in humans with AMD and CNV [28]. Thus the concept has been brought forward that the normal non-vascular nature of Bruch's membrane is due to the suppression of inward growth of choroidal blood vessels by the RPE. The stimulus to change in growth factor production by RPE is unknown, but it is assumed that it may be due to lack of metabolic supply from plasma due to reduced diffusion of material through the thickened Bruch's membrane, or to reduced oxygen supply consequent upon changes in the choroidal capillaries [55]. Recent studies suggest immune responses as a contributing cause for CNV development. A chronic low-grade inflammatory process in the sub-RPE space, as outlined above, may induce local damage to Bruch's membrane which would allow new vessels to breach the sieve-like extracellular matrix. In addition, RPE overexpression of VEGF, stimulated by complement components and IgG combined with fragmentation of the Bruch's membrane, provides an environment permissive for CNV [3].

A similar mechanism could account for retinal angiomatous proliferations (RAP) [74]. This particular phenotype may occur in the setting of early AMD and involves invasion of the outer retina by retinal blood vessels, which may precede or occur simultaneously with CNV. Just as growth factors may prevent vascularisation of Bruch's membrane under physiologic conditions they may also account for the avascular nature of the outer retina. In the first instance this would involve growth factors secreted through the basolateral plasma membrane domain of the RPE cell and in the second through the apical domain. It is therefore speculated that RAP may be due to a change in the balance of growth factors arising from the apical domain rather than the basal lateral plasma membrane domain.

Geographic atrophy

Geographic atrophy represents the second most common cause for severe visual loss in AMD and has been interpreted as the natural evolution of the disease if neovascular events do not occur prior to immediate cell death. In geographic atrophy there is a loss of the outer neurosensory retina, the RPE and the choriocapillaris and the outer plexiform layer of the retina becomes adherent with Bruch's membrane [29]. Typically, the atrophic areas have well-defined borders with increased pigmentation at the margin. The atrophy initially tends to develop in the perifoveal area, while the fovea may be spared until later during the clinical course remeniscent of other bull's eye maculopathies. This pattern reflects the density of rod photoreceptor cells. Primary dysfunction and cell death of RPE cells is thought to occur initially followed by collateral loss of neighbouring photoreceptor cells and the choriocapillaris. Recent findings with FAF imaging using a confocal scanning laser ophthalmoscopy implicated excessive accumulation in RPE cells in the evolution of geographic atrophy [41]. Hereby, an excitation wavelength of 488 nm is generated by an argon laser or an optically pumped solid state laser [39, 46, 63]. Emission is recorded above 500 nm by inserting a barrier filter. The evidence that the signal originates from lipofuscin in the RPE is derived from the work of Delori and co-workers [15]. Areas with increased FAF signal and, thus, excessive RPE lipofuscin precede the development of new atrophic areas

and the enlargement of preexisting atrophy [41]. Hereby, a high interindividual variation in the phenotypic patterns of abnormal fundus autofluorescence has been noted with an impact on the progression of disease [10].

Furthermore in patients with unilateral visual loss from AMD, abnormal fundus autofluorescence in the fellow eye is associated with an increased risk for the development of GA. If the presence of A2-E or increasing lipofuscin is important to genesis of geographic atrophy, targets to slow down accumulation of lipofuscin in RPE cells need to be identified for potential future prophylactic and therapeutic interventions.

Outlook

New insights have emerged in recent years that lead to a better understanding of the key pathogenetic pathways associated in various manifestations of AMD. Better knowledge of molecular mechanisms is a prerequisite for the development of new strategies for more successful therapeutic interventions than those available to date.

References

1. Age-Related Eye Disease Study Research Group (The AREDS Study Group) (2001) A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. Arch Ophthalmol 119:1417–1436
2. Allikmets R, Shroyer NF, Singh N, Seddon JM, Lewis RA, Bernstein PS, Pfeiffer A, Zabriskie NA, Li Y, Hutchinson A, Dean M, Lupski JR, Leppert M (1997) Mutation of the Stargardt disease gene (ABCR) in age-related macular degeneration. Science 277:1805–1807
3. Ambati J, Anand A, Fernandez S, Sakurai E, Lynn BC, Kuziel WA, Rollins BJ, Ambati BK (2003) An animal model of age-related macular degeneration in senescent Ccl-2- or Ccr-2-deficient mice. Nat Med 11:1390–1397
4. Anderson RE, Kretzer FL, Rapp LM (1994) Free radicals and ocular disease. Adv Exp Med Biol 366:73–86
5. Anderson DH, Ozaki S, Nealon M (2001) Local cellular sources of apolipoprotein E in the human retina and retinal pigmented epithelium: implications for the process of drusen formation. Am J Ophthalmol 131:767–781
6. Anderson DH, Mullins RF, Hagemann GS, Johnson LV (2002) A role for local inflammation in the formation of drusen in the aging eye. Am J Ophthalmol 134:411–431
7. Beatty S, Koh H, Phil M, Henson D, Boulton M (2000) The role of oxidative stress in the pathogenesis of age-related macular degeneration. Surv Ophthalmol 45:115–134
8. Bellmann C, Holz FG, Schapp O, Volcker HE, Otto TP (1997) Topography of fundus autofluorescence with a new confocal scanning laser ophthalmoscope. Ophthalmologe 94:385–391
9. Bergmann M, Schutt F, Holz FG, Kopitz J (2004) Inhibition of the ATP-driven proton pump in RPE lysosomes by the major lipofuscin fluorophore A2-E may contribute to the pathogenesis of age-related macular degeneration. FASEB J 18:562–564
10. Bindewald A, Jorzik JJ, Loesch A, Schutt F, Holz FG (2004) Visualization of retinal pigment epithelial cell in vivo using digital high-resolution confocal scanning laser opthalmoscopy. Am J Opthalmol 137:556–558
11. Bird AC (1996) Age related macular disease. Br J Ophthalmol 80:1–2
12. Boulton M, Marshall J (1986) Effects of increasing numbers of phagocytic inclusions on human retinal pigment epithelial cells in culture: a model for aging. Br J Ophthalmol 70:808–815
13. Brankov M, Zhang D, Robert T, Rakoczy EP (2003) Cathepsin D mutations associated with retinal dystrophy in transgenic mice. ARVO #1702
14. Davies S, Elliott MH, Floor E, Truscott TG, Zareba M, Sarna T, Shamsi FA, Boulton ME (2001) Photocytotoxicity of lipofuscin in human retinal pigment epithelial cells. Free Radic Biol Med 15:256–265
15. Delori FC, Dorey CK, Staurenghi G, Arend O, Goger DG, Weiter JJ (1995) In vivo fluorescence of the ocular fundus exhibits RPE lipofuscin characteristics. Invest Ophthalmol Vis Sci 36:718–729
16. Dorey CK, Wu G, Ebenstein D, Garsd A, Weiter JJ (1989) Cell loss in the aging retina: relationship to lipofuscin accumulation and macular degeneration. Invest Ophthalmol Vis Sci 30:1691–1699
17. Eagle RC, Lucier AC, Bernadino VB, Janoff M (1980) Retinal pigment epithelial abnormalities in fundus flavimaculatus; a light and electron microscopical study. Ophthalmology 87:1189–2000
18. Eldred GE (1993) Age pigment structure. Nature 364:396
19. Eldred GE, Lasky MR (1993) Retinal age-pigments generated by self-assembling lysosomotropic detergents. Nature 361:724–726
20. Farboud B, Aotaki-Keen A, Miyata T, Hjelmelund LM, Handa JT (1999) Development of a polyclonal antibody with broad epitope specificity for advanced glycation endproducts and localization of these epitopes in Bruch's membrane of the aging eye. Mol Vis 5:11
21. Feeney L (1978) Lipofuscin and melanin of human retinal pigment epithelium. Invest Ophthalmol Vis Sci 17:583–600
22. Feeney-Burns L, Ellersieck MR (1985) Age-related changes in the ultrastructure of Bruch's membrane. Am J Ophthalmol 100:686
23. Feeney-Burns L, Berman ER, Rothman H (1980) Lipofuscin of human retinal pigment epithelium. Am J Ophthalmol 90:783–791
24. Feeney-Burns L, Hilderbrand ES, Eldridge S (1984) Aging human RPE: morphometric analysis of macular, equatorial, and peripheral cells. Invest Ophthalmol Vis Sci 25:195–200
25. Fisher RF (1987) The influence of age on some ocular basement membranes. Eye 1:184
26. Forrester JV, McMenamin PG, Liversidge J, Lumsden L (1993) Dendritic cells and "dendritic" macrophages in the uveal tract. Adv Exp Med Biol 329:599–604
27. Forrester JV, McMenamin PG, Holthouse I, Lumsden L, Liversidge J (1994) Localization and characterization of major histocompatibility complex class II-positive cells in the posterior segment of the eye: implications for induction of autoimmune uveoretinitis. Invest Ophthalmol Vis Sci 35:64–77

28. Frank RN, Amin RH, Eliott D, Puklin JE, Abrams GW (1996) Basic fibroblast growth factor and vascular endothelial growth factor are present in epiretinal and choroidal neovascular membranes. Am J Ophthalmol 122:393
29. Green WR, Enger C (1993) Age-related macular degeneration histopathologic studies. The 1992 Lorenz E. Zimmerman Lecture. Ophthalmology 100:1519–1535
30. Gurne Dh, Tso MO, Edward DP, Ripps H (1991) Antiretinal antibodies in serum of patients with age-related macular degeneration. Ophthalmology 98:602–607
31. Hageman GS, Mullins RF, Clark WG, Johnson LV, Anderson DH (1995) Drusen share molecular constituents common to atherosclerotic, elastotic and amyloid deposits. Invest Ophthalmol Vis Sci 36:432
32. Hageman GS, Mullins RF, Russell SR, Johnson LV, Anderson DH (1999) Vitronectin is a constituent of ocular drusen and the vitronectin gene is expressed in human retinal pigment epithelial cells. FASEB J 13:477–484
33. Hagemann GS, Luthert PJ, Victor Chong NH, Johnson LV, Anderson DH, Mullins RF (2001) An integrated hypothesis that considers drusen as biomarkers of immune mediated processes at the RPE-Bruch's membrane interface in aging and age related macular degeneration. Prog Retin Eye Res 20:705–732
34. Hangai M, Murata T, Miyawaki N (2001) Angiopoietin-1 upregulation by vascular endothelial growth factor in human retinal pigment epithelial cells. Invest Ophthalmol Vis Sci 42:1617
35. Holekamp (2001) Deficiency of antiangiogenic pigment epithelial-derived factor in the vitreous of patients with wet age-related macular degeneration. Retina Society Annual Meeting, Chicago
36. Holz FG, Pauleikhoff D, Spaide RF, Bird AC (2004) Age-related macular degenration. Springer, Berlin Heidelberg New York
37. Holz FG, Dorey CK, Sheraidah G, Bird AC (1994) Lipofuscin fluorescence, Bruch's membrane fluorescence and lipid deposits in aging donor eyes. Invest Ophthalmol Vis Sci 35:1501
38. Holz FG, Sheraidah G, Pauleikhoff D, Bird AC (1994) Analysis of lipid deposits extracted from human macular and peripheral Bruch's membrane. Arch Ophthalmol 112:402
39. Holz FG, Bellmann C, Margaritidis M, Schütt F, Otto TP, Völcker HE (1999) Patterns of increased in vivo fundus autofluorescence in the junctional zone of geographic atrophy of the retinal pigment epithelium associated with age-related macular degeneration. Graefes Arch Clin Exp Ophthalmol 237:145–152
40. Holz FG, Schuett F, Kopitz J, Eldred GE, Kruse FE, Völcker HE, Cantz M (1999) Inhibition of lysosomal degradative functions in RPE cells by a retinoid component of lipofuscin. Invest Ophthalmol Vis Sci 40:737–743
41. Holz FG, Bellmann C, Staudt S, Schutt F, Volcker HE (2001) Fundus autofluorescence and development of geographic atrophy in age-related macular degeneration. Invest Ophthalmol Vis Sci 42:1051
42. Ishibashi T, Murata T, Hangai M (1998) Advanced glycation end products in age related macular degeneration. Arch Ophthalmol 116:1629–1632
43. Jiang HR, Lumsden L, Forrester JV (1999) Macrophages and dendritic cells in IRBPinduced experimental autoimmune uveoretinits in B10RIII mice. Invest Ophthalmol Vis Sci 40:3177–3185
44. Johnson LV, Ozaki S, Staples MK, Erickson PA, Anderson DH (2000) A potential role for immune complex pathogenesis in drusen formation. Exp Eye Res 70:441–449
45. Johnson LV, Leiter WP, Staples MK, Anderson DH (2001) Evidence that drusen formation is a byproduct of complement-mediated attack on retinal pigment epithelium. Invest Ophthalmol Vis Sci 42:944
46. Jorzik JJ, Bindewald A, Dithmar S, Holz FG Digital simultaneous fluorescein and indocyanine green angiography, fundus autofluorescence and redfree imaging using a novel solid state laser-based confocal scanning laser ophthalmoscope (submitted)
47. Katz ML, Siakotos AN (1995) Canine hereditary ceroid-lipofuscinosis: evidence for a defect in the carnitine biosynthetic pathway. Am J Med Genet 57:266–271
48. Kedzierski W, Nusinowitz S, Birch D, Clarke G, McInnes RR, Bok D, Travis GH (2001) Deficiency of rds/peripherin causes photoreceptor death in mouse models of digenic and dominant retinitis pigmentosa. Proc Natl Acad Sci USA 98:7718–7723
49. Kennedy CJ, Rakoczy PE, Constable IJ (1995) Lipofuscin of the retinal pigment epithelium: a review. Eye 9:763–771
50. Leys A (1990) Subretinal neovascular membranes associated with chronic membranoproliferative glomerulonephritis type II. Graefes Arch Clin Exp Ophthalmol 228:499–504
51. Lois N, Halfyard AS, Bunce C, Bird AC, Fitzke FW (1999) Reproducibility of fundus autofluorescence measurements obtained using a confocal scanning laser ophthalmoscope. Br J Ophthalmol 83:276–279
52. Lois N, Halfyard AS, Bird AC, Fitzke FW (2000) Quantitative evaluation of fundus autofluorescence imaged "in vivo" in eyes with retinal disease. Br J Ophthalmol 84:741–745
53. Lopez PF, Maumenee IH, de la Cruz Z, Green WR (1990) Autosomal-dominant fundus flavimaculatus: clinicopathologic correlation. Ophthalmology 97:798–809
54. Majji AB, Cao J, Chang KY, Hayashi A, Aggarwal S, Grebe RR, De Juan E Jr (2000) Age-related retinal pigment epithelium and Bruch's membrane degeneration in senescence-accelerated mouse. Invest Ophthalmol Vis Sci 41:3936–3942
55. Moore DJ, Hussain AA, Marshall J (1995) Age-related variation in the hydraulic conductivity of Bruch's membrane. Invest Ophthalmol Vis Sci 36:1290–1297
56. Mullins RF, Russell SR, Anderson DH, Hagemann GS (2000) Drusen associated with aging and age-related macular degeneration contain proteins common to extracellular deposits associated with atherosclerosis, elastosis, amyloidosis, and dense deposit disease. FASEB J 14:835–846
57. Mullins RF, Aptsiauri N, Hageman GS (2001) Structure and composition of dusen associated with glomerulonephritis: implications for the role of complement activation in drusen biogenesis. Eye 15:390–395
58. Ong DE, Davis JT, O'Day WT, Bok D (1994) Synthesis and secretion of retinal binding protein and transthyretin by cultured retinal pigment epithelium. Biochemistry 33:1835–1842
59. Pauleikhoff D, Harper CA, Marshall J, Bird AC (1990) Aging changes in Bruch's membrane. A histochemical and morphologic study. Ophthalmology 97:171
60. Penfold PL, Provis JM, Furby JH, Gatenby PA, Billson FA (1990) Autoantibodies to retinal astrocytes associated with age-related macular degeneration. Graefes Arch Clin Exp Ophthalmol 228:270–274
61. Rozanowska M, Jarvis-Evans J, Korytowski W, Boulton M, Burke JM, Sarna T (1995) Blue light induced reactivity of retinal age pigment: in vitro generation of oxygen reactive species. J Biol Chem 270:18825–18830
62. von Rückmann A, Fitzke FW, Bird AC (1995) Distribution of fundus autofluorescence with a scanning laser ophthalmoscope. Br J Ophthalmol 79:407

63. von Rückmann A, Fitzke FW, Bird AC (1997) Fundus autofluorescence in age-related macular disease imaged with a laser scanning ophthalmoscope. Invest Ophthalmol Vis Sci 38:478–486
64. Schutt F, Davies S, Kopitz J, Holz FG, Boulton ME (2001) Photodamage to human RPE cells by A2-E, a retinoid component of lipofuscin. Invest Ophthalmol Vis Sci 41:2303–2308
65. Schutt F, Bergmann M, Holz FG, Kopitz J (2002) Isolation of intact lysosomes from human RPE cells and effects of A2-E on the integrity of the lysosomal and other cellular membranes. Graefes Arch Clin Exp Ophthalmol 240:983–988
66. Schutt F, Ueberle B, Schnolzer M, Holz FG, Kopitz J (2002) Proteome analysis of lipofuscin in human retinal pigment epithelial cells. FEBS Lett 528:217–221
67. Schutt F, Bergmann M, Holz FG, Kopitz J (2003) Proteins modified by malondialdehyde, 4-hydroxynonenal, or advanced glycation end products in lipofuscin of human retinal pigment epithelium Invest Ophthalmol Vis Sci 44:3663–3668
68. Sheraidah G, Steinmetz R, Maguire J, Pauleikhoff D, Marshall J, Bird AC (1993) Correlation between lipids extracted from Bruch's membrane and age. Ophthalmology 100:47
69. Solbach U, Keilhauer C, Knabben H, Wolf S (1997) Imaging of retinal autofluorescence in patients with age-related macular degeneration. Retina 17:385–389
70. Weingeist TA, Kobrin JL, Watz KE (1982) Histopathology of Best's macular dystrophy. Arch Ophthalmol 100:1108–1114
71. Weiter JJ, Delori FC, Wing GL, Fitch KA (1986) Retinal pigment epithelial lipofuscin and melanin and choroidal melanin in human eyes. Invest Ophthalmol Vis Sci 27:145–152
72. Wing GL, Blanchard GC, Weiter JJ (1977) The topography and age relationship of lipofuscin concentration in the retinal pigment epithelium. Invest Ophthalmol Vis Sci 17:601–607
73. Witmer AN, Vrensen GF, Van Noorden CJ, Schlingemann RO (2003) Vascular endothelial growth factors and angiogenesis in eye disease. Prog Retin Eye Res 22:1–29
74. Yannuzzi LA, Negrao S, Iida T (2001) Retinal angiomatous proliferation in age-related macular degeneration. Retina 21:416–434