Documenta Ophthalmologica **106:** 25–29, 2003.

Why study rod cell death in retinal degenerations and how?

C.E. Remé, C. Grimm, F. Hafezi, H.-P. Iseli & A. Wenzel
Research Unit, Laboratory of Retinal Cell Biology, Department of Ophthalmology, University Hospital Zürich, Zürich, Switzerland

Key words: apoptosis, degeneration, light-damage, makula, retina, rod

Abstract

Age-related macular degeneration (AMD) is a main causes of severe visual impairment in the elderly in industrialized countries. The pathogenesis of this complex diseases is largely unknown, even though clinical characteristics and histopathology are well described. Because several aging changes are identical to those observed in AMD, there appears to exist an unknown switch mechanism from normal ageing to disease. Recent anatomical studies using elegant innovative techniques reveal that there is a 30% rod loss in normal ageing, which is increased in early AMD. Those and other observations by Curcio and co-workers indicate that early rod loss is an important denominator of AMD (Curcio CA. Eye 2001; 15:376). As in retinitis pigmentosa (RP), rods appear to die by apoptosis. Thus it seems mandatory to study the regulation of rod cell death in animal models to unravel possible mechanisms of rod loss in AMD. Our laboratory investigates signal transduction pathways and gene regulation of rod death in our model of light-induced apoptosis. The transcription factor AP1 is essential, whereas other classical pro- and antiapoptotic genes appear to be less important in our model system. Caspase-1 gene expression is distinctly upregulated after light exposure and there are several factors which completely protect against light-induced cell death, such as the anesthetic halothane, dexamethasone and the absence of bleachable rhodopsin during light exposure. A fast rhodopsin regeneration rate increased damage susceptibility. Our data indicate that rhodopsin is essential for the initiation of light-induced rod loss. Following photon absorption, there may be the generation of photochemically active molecules wich then induce the apoptotic death cascade.

Introduction

Ophthalmologists are well aware of age-related macular degeneration (AMD) as the leading cause of severe visual impairment in the elderly in industrialized nations. However, nowbody 'knows' the pathogenesis and only few curative strategies exist for selected cases. There is circumstantial evidence that suggests disease-promoting factors [2–4] and therefore provides directions where and how to search [5]. Why is that?

AMD is a complex disease affecting photoreceptors and pigment epithelium as well as Bruchs membrane and choriocapillaris. There are several clinical and pathohistological features such as drusen and lipofuscin accumulation, which occur in affected people [6, 7] but also in those never developing an overt macular degeneration. Thus, there appears to be a continuum from ageing changes to disease indicating that the pathogenesis may result from an intricate interplay between genetic and environmental factors, which do not interact similarly in normal aging. The events that control the 'switch' from aging to disease are unknown.

The elegant morphological analyses of the ageing and diseased human retina by Curcio [1] leads the way towards an approach to study AMD. Her data along with sensitive psychophysical tests may eventually allow early diagnosis and, hopefully, treatment of this disease complex. The macular extends over 6 mm in the central fundus (21.5° visual angle), the fovea over 0.8 mm (2.75° visual angle). In the macula, the rod/cone ratio is 9:1, with the highest rod density occuring in an ellipsoid ring at 3–5 mm excentricity in the parafovea, therefore, the parafovea is rod dominated. The only rod free zone of the macula lies in the central 0.35 mm or 1.25° visual field [8]. These and the following data may indicate, that AMD is not

a disease that primarily affects cones or the pigment epithelium, respectively.

Detailed anatomical analysis in human autopsy eyes using new graphical and statistical techniques revealed the striking observation that there is a 30% rod loss in the central 28° visual field in the normal ageing retina, whereas cone numbers remain remarkably constant [9]. In AMD, however, this loss is distinctly more pronounced with rod densities further reduced by additional 30–40% compared to controls [10]. Early rod loss was observed in geographic atrophy as well as exudative AMD. Recent functional studies elucidated greatest visual deficits in the same area where the highest rod loss is seen. In ageing and AMD, rod sensitivity is found to decline and dark adaptation time is prolonged, however, in correlation with anatomy, more pronounced in AMD [11, 12]. Whereas in ageing changes rod loss and functional disturbances prevail, the pathology of AMD comprises severe disturbances of the pigment epithelium and Bruchs membrane with gradual distortions and misshaping of cones up to ensuing cone cell death. However, central cones may be spared for considerable time periods which may explain the relatively good vision in some patients despite funduscopic signs of degeneration [10], a fact well known to clinicians.

Thus, in ageing and AMD, early rod loss is a predominant feature, which in AMD is followed by alterations of PE and Bruchs membrane and gradual death of cones. Thus it cannot be entirely excludet that one of the primary defects may reside in photoreceptors rather than the PE. For example, there may be an accumulation of undigestible or toxic compounds in disk membranes which would enter the PE with disk shedding and accelerate the accumulation of phagolysosomes and subsequently lipofuscin with a reduction of vital functions and eventually PE cell death. Similar to rod loss in retinitis pigmentosa (RP), rods in AMD die by apoptosis. Whereas RP comprises a multitude of forms on a genetic basis with more than 100 genes already known, AMD represents a disease complex which is likely to be caused by genetic as well as environmental factors. However, recent investigations have elucidated that in some animal models mirroring human RP, light as an environmental component can accelerate the disease progression [13]. According to epidemiological studies in humans, this may be true for AMD as well [14].

Why are rods dying and how is rod cell death related to the characteristic features of aging and AMD? To understand initial events in AMD it appears necessary to investigate in detail the regulation of rod cell death. Similarly one may ask, why are rods dying in RP, which events from gene mutation to cellular dysfunction can initiate the apoptotic death cascade? Understanding of such mechanisms may allow early therapeutic interventions which might help patients to retain useful vision longer than untreated patients or with the presently available therapies which attempt to remedy already manifest disease.

Therefore, our laboratory focusses on mechanisms of apoptotic rod cell death. We developed the model of light-induced apoptosis in retina and PE, which resembles in many respects the morphological and functional features of human retinal degenerations. The advantage of our system is that a synchronized burst of cell death is created and controlled studies on a molecular and cellular level are possible, such as dose–responses, spectral dependence, strain differences, protective measures and genetic regulation [15, 16].

Chromophore and spectrum for light-induced apoptosis

Supporting earlier evidence that rhodopsin is the main chromophore for mediating light damage [17], we could demonstrate in a knock-out mouse model which cannot (re)generate a functional rod visual pigment [18] that retinas lacking rhodopsin are completely protected against light damage [19]. Investigations using different mouse strains extended earlier observations of the existence of strain differences in light damage susceptibility [20] and identified the RPE65 protein as one of the main factors for this phenomenon. *Rpe65* sequence variations [21] influenced levels of RPE65 and kinetics of rhodopsin regeneration after bleaching. Fast regeneration resulted in a high susceptibility to light damage, whereas slow regeneration rendered retinas more resistant to light exposure [22]. The importance of the rhodopsin metabolism was further supported by the observation that halothane-mediated inhibition of the visual cycle prevented damage after exposure to white light [23, 24]. Light damage by blue light exposure, however, was independent of a functional visual cycle, since blue light was capable to regenerate rhodopsin by a photochemical process, photoreversal of bleaching [25], *in vivo* [26]. Photoreversal provides unbleached rhodopsin molecules for the absorption of a large number of photons within short time periods sufficient for the in-

duction of photoreceptor apoptosis [27]. However, in the total absence of rhodopsin, blue light did not induce light damage demonstrating that both, white light and blue light depend on rhodopsin to induce damage and probably use similar signaling pathways [27].

An essential protein in rhodopsin regeneration and therefore in light damage is RPE65 [18]. So far it was believed that mutations in *Rpe65* lead to loss of rod function in humans and mice. Using a double knockout strategy that combined either a functional cone knockout (*CNG3a*$^{-/-}$) with *Rpe65*$^{-/-}$ or a functional rod knockout (*Rho*$^{-/-}$) with *Rpe65*$^{-/-}$ we showed that loss of RPE65 disrupts cone function and reduces rod sensitivity by three log units [28]. This shows that in *Rpe65*$^{-/-}$ animals both, the cone and the rod systems are affected and that cones too depend on the Rpe65 protein for visual pigment (re)generation.

Intracellular signalling cascades leading to rod apoptosis

Activation of AP-1 is a common feature observed during light induced photoreceptor apoptosis [29]. Lack of c-Fos, one of the main components of induced AP-1 complexes protects retinas against acute light damage [30]. We tested whether the pharmacological inhibition of AP-1 activity would prevent apoptosis by intraperitoneal injections of single doses of dexamethasone (DEX). DEX activated retinal glucocorticoid receptor which was translocated to nuclei and inhibited AP-1 activity. This treatment protected against light damage and showed that AP-1 activity is essential for the intracellular signaling in light damage [31].

AP-1 complexes consist of two proteins of the Fos- (c-Fos, FosB, Fra-1, Fra-2) and Jun (c-Jun, JunD, JunB) family of proteins, respectively. Some AP-1 members like c-Fos and c-Jun have transactivation domains required for transcriptional activation of Ap-1 target genes. Other proteins like Fra-1 do not have such a domain. Although lack of c-Fos prevents light-induced apoptosis, it can be replaced by Fra-1 if expressed under the control of c-Fos regulatory elements [32]. This strongly suggests that the transactivation capacity of c-Fos is not required for the execution of apoptosis after light exposure. Similarly to c-Fos, c-Jun has a strong transactivation domain at its N-terminus. However, this domain needs to be phosphorylated for full activity. Mice expressing a nonphosphorylatable mutant of the c-Jun protein (JunAA) are phenotypically normal and retinas are not protected against light damage. In agreement with this, we did not observe increased activity of N-terminal phosphorylation of c-Jun protein in wild-type mice by Jun N-terminal kinases (JNK) [33]. These data propose that AP-1 might not be required for transactivation but rather for transrepression of target genes.

Further studies indicated that gene expression of *caspase-1* is stronlgy induced upon light exposure suggesting a prominent role of caspase-1 in the apoptotic process during the degeneration of the retina after light insult [34]. In contrast to this observation, other 'classical' pro- or antiapoptotic genes did not show distinct responses after light exposure.

Phototransduction, to be or not to be?

Which mechanisms operate after the absorption of photons by rhodopsin? Is phototransduction involved in mediating light damage in the retina? In our system which uses short exposures to relatively bright white light or blue light (403 nm), phototransduction does not seem to be involved, because transducin alpha knock out mice (T$\alpha^{-/-}$) are not protected (manuscript in preparation). However, long-term exposures to relatively low white light illuminances does not injure mice lacking tranducin activation (M. Simon, personal communication), indicating that different light regimens may use different pathways of damage induction.The rate of rhodopsin regeneration is normal in T$\alpha^{-/-}$ mice, therefore, protection is not achieved by a reduced availability of unbleached rhodopsin during light exposure. By contrast, short wavelenght blue light does inflict severe lesions in Tko mice, suggesting again that rhodopsin-initiated lesions can occur, but they are not mediated by regular phototransduction processes. These and other observations suggest to us that 'white light is blue light' in that the blue components in white light may be responsible for light damage, at least under high illuminance conditions.

Thus we conclude that lesions inflicted by both high, and low light levels and by blue light are mediated by rhodopsin. Phototransduction pathways may be involved at low intensities, whereas photochemical processes are likely to initiate harmful reactions at high light levels. This implies for the human eye that the retina and photoreceptors, especially rods, can potentially suffer from light damage induced by high, as well as low intensities on an acute but also chronic basis. Toxic photoproducts may be created

within ROS and may represent a significant part of the 'undigestible' materials accumulating in the PE phagolysosomal system and contributing to the potentially harmful effects of lipofuscin. Several recent studies have demonstrated mechanisms of how lipofuscin can impair PE function and, furthermore, can act as a photosensitizing agent [35, 36].

These and other data indicate that to study rod cell death may unravel important initial steps in the pathogenesis of AMD. Upstream of the ensuing death cascade it will be significant to find and characterize toxic photoproducts arising after photon capture by rhodopsin. A major task for the future will be to approach the 'switch' that occurs in normal ageing into disease, leading to the deleterious loss of central visual functions.

Acknowledgements

This summary paper is dedicated to our collegue Günter Niemeyer, with whom we shared many fruitful discussions and collaborations over the years. Our sincere thanks are addressed to Prof. Dr. Dr. Theo Seiler for generous support of and interest in our work. We thank Dora Greuter, Gabi Hoegger and Coni Imsand for excellent technical assistance, which often goes far beyond the usual tasks of expert laboratory technicians, and Ulrich Busse for carefully handling the administrative jobs in our laboratory. Our laboratory is supported by the Swiss National Science Foundation, the German National Research Council, the Velux Foundation, Glarus, Switzerland, the Fonds for Scientific Research, University of Zürich, Switzerland, the EMDO and Hartmann Mueller-Foundation Zürich, Switzerland, the Theorodore Ott Fonds, Zürich, Switzerland.

References

1. Curcio CA. Photoreceptor topography in ageing and age-related maculopathy. Eye 2001; 15(Pt 3): 376–83.
2. Young RW. Pathophysiology of age-related macular degeneration. Surv Ophthalmol 1987; 31(5): 291–306.
3. Pauleikhoff D, Holz FG. Die altersabhängige Makuladegeneration. Ophthalmology 1996; 93: 299–315.
4. Eldred GE. Lipofuscin fluorophore inhibits lysosomal protein degradation and may cause early stages of macular degeneration. Gerontology 1995; 41(Suppl 2): 15–28.
5. Bird AC. What is the future of research in age-related macular disease? Arch Ophthalmol. 1997; 115(10): 1311–3.
6. Bird AC, Bressler NM, Bressler SB, et al. An international classification and grading system for age-related maculopathy and age-related macular degeneration. The International ARM Epidemiological Study Group. Surv Ophthalmol 1995; 39(5): 367–74.
7. Kliffen M, van der Schaft TL, Mooy CM, de Jong PT. Morphologic changes in age-related maculopathy. Microsc Res Tech 1997; 36(2): 106–22.
8. Curcio CA, Sloan KR, Kalina RE, Hendrickson AE. Human photoreceptor topography. J Comp Neurol 1990; 292(4): 497–523.
9. Curcio CA, Millican CL, Allen KA, Kalina RE. Aging of the human photoreceptor mosaic: evidence for selective vulnerability of rods in central retina. Invest Ophthalmol Vis Sci 1993; 34(12): 3278–96.
10. Curcio CA, Medeiros NE, Millican CL. Photoreceptor loss in age-related macular degeneration. Invest Ophthalmol Vis Sci 1996; 37(7): 1236–49.
11. Curcio CA, Owsley C, Jackson GR. Spare the rods, save the cones in aging and age-related maculopathy. Invest Ophthalmol Vis Sci 2000; 41(8): 2015–8.
12. Owsley C, Jackson GR, White M, Feist R, Edwards D. Delays in rod-mediated dark adaptation in early age-related maculopathy. Ophthalmology 2001; 108: 1196–202.
13. Naash ML, Peachey NS, Li ZY, et al. Light-induced acceleration of photoreceptor degeneration in transgenic mice expressing mutant rhodopsin. Invest Ophthalmol Vis Sci 1996; 37(5): 775–82.
14. Cruickshanks KJ, Klein R, Klein BE, Nondahl DM. Sunlight and the 5-year incidence of early age-related maculopathy: the beaver dam eye study. Arch Ophthalmol 2001; 119(2): 246–50.
15. Reme CE, Grimm C, Hafezi F, Marti A, Wenzel A. Apoptotic cell death in retinal degenerations. Prog Retin Eye Res 1998; 17(4): 443–64.
16. Reme CE, Grimm C, Hafezi F, Wenzel A, Williams TP. Apoptosis in the retina: the silent death of vision. News Physiol Sci 2000; 15: 120–4.
17. Williams TP, Howell WL. Action spectrum of retinal light-damage in albino rats. Invest Ophthalmol Vis Sci 1983; 24(3): 285–7.
18. Redmond TM, Yu S, Lee E, et al. Rpe65 is necessary for production of 11-cis-vitamin A in the retinal visual cycle. Nat Genet 1998; 20(4)): 344–51.
19. Grimm C, Wenzel A, Hafezi F, et al. Protection of Rpe65-deficient mice identifies rhodopsin as a mediator of light-induced retinal degeneration. Nat Genet 2000; 25(1): 63–6.
20. LaVail MM, Gorrin GM, Repaci MA. Strain differences in sensitivity to light-induced photoreceptor degeneration in albino mice. Curr Eye Res 1987; 6(6): 825–34.
21. Danciger M, Matthes MT, Yasamura D, et al. A QTL on distal chromosome 3 that influences the severity of light-induced damage to mouse photoreceptors. Mamm Genome 2000; 11(6): 422–7.
22. Wenzel A, Reme CE, Williams TP, Hafezi F, Grimm C. The Rpe65 Leu450Met variation increases retinal resistance against light-induced degeneration by slowing rhodopsin regeneration. J Neurosci 2001; 21(1): 53–8.
23. Keller C, Grimm C, Wenzel A, Hafezi F, Reme C. Protective effect of halothane anesthesia on retinal light damage: inhibition of metabolic rhodopsin regeneration. Invest Ophthalmol Vis Sci 2001; 42(2): 476–80.
24. Sieving PA, Chaudhry P, Kondo M, et al. Inhibition of the visual cycle in vivo by 13-cis retinoic acid protects from light damage and provides a mechanism for night blindness in iso-

tretinoin therapy. Proc Natl Acad Sci U S A. 2001; 98(4): 1835–40.
25. Williams TP. Photoreversal of rhodopsin bleaching. J Gen Physiol 1964; 47: 679–89. 11
26. Grimm C, Reme CE, Rol PO, Williams TP. Blue Light's effects on rhodopsin: photoreversal of bleaching in living rat eyes. Invest Ophthalmol Vis Sci 2000; 41(12): 3984–90.
27. Grimm C, Wenzel A, Williams T, et al. Rhodopsin-mediated blue-light damage to the rat retina: effect of photoreversal of bleaching. Invest Ophthalmol Vis Sci 2001; 42(2): 497–505.
28. Seeliger MW, Grimm C, Stahlberg F, et al. New views on RPE65 deficiency: the rod system is the source of vision in a mouse model of Leber congenital amaurosis. Nat Genet 2001; 29(1): 70–4.
29. Hafezi F, Marti A, Grimm C, Wenzel A, Remé CE. Differential DNA binding activities of the transcription factors AP-1 and Oct-1 during light-induced apoptosis of photoreceptors. Vis Res 1999; 39(15): 2511–8.
30. Hafezi F, Steinbach JP, Marti A, et al. The absence of c-fos prevents light-induced apoptotic cell death of photoreceptors in retinal degeneration in vivo. Nat Med 1997; 3(3): 346–9.
31. Wenzel A, Grimm C, Seeliger MW, et al. Prevention of photoreceptor apoptosis by activation of the glucocorticoid receptor. Invest Ophthalmol Vis Sci 2001; 42(7): 1653–9.
32. Fleischmann A, Hafezi F, Elliott C, et al. Fra-1 replaces c-Fos-dependent functions in mice. Genes Dev 2000; 14(21): 2695–700.
33. Grimm C, Wenzel A, Behrens A, et al. AP-1 mediated retinal photoreceptor apoptosis is independent of N-terminal phosphorylation of c-Jun. Cell Death Differ 2001; 8(8): 859–67.
34. Grimm C, Wenzel A, Hafezi F, Reme CE. Gene expression in the mouse retina: the effect of damaging light. Mol Vis 2000; 6: 252–60.
35. Suter M, Reme C, Grimm C, et al. Age-related macular degeneration. The lipofusion component *N*-retinyl-*N*-retinylidene ethanolamine detaches proapoptotic proteins from mitochondria and induces apoptosis in mammalian retinal pigment epithelial cells. J Biol Chem 2000; 275(50): 39625–30.
36. Schutt F, Davies S, Kopitz J, Holz FG, Boulton ME. Photodamage to human RPE cells by A2-E, a retinoid component of lipofuscin. Invest Ophthalmol Vis Sci 2000; 41(8): 2303–08.

Address for correspondence: C.E. Remé, Research Unit, Laboratory of Retinal Cell Biology, Department of Ophthalmology, University Hospital Zürich, Zürich, Switzerland