ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY

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Matthew M. LaVail · John D. Ash
Robert E. Anderson · Joe G. Hollyfield
Christian Grimm
Editors

Retinal Degenerative Diseases

Springer

Editors
Matthew M. LaVail
Anatomy and Ophthalmology
University of California
San Francisco School of Medicine
San Francisco, CA, USA
matthew.lavail@ucsf.edu

Robert E. Anderson
Ophthalmology and Cell Biology
Dean A. McGee Eye Institute
University of Oklahoma
Health Sciences Center
Oklahoma City, OK, USA
Robert-Anderson@ouhsc.edu

Christian Grimm
Experimental Ophthalmology
University of Zurich
Zurich, Switzerland
cgrimm@opht.uzh.ch

John D. Ash
Ophthalmology
College of Medicine
University of Oklahoma
Health Sciences Center
Oklahoma City, OK, USA
John-Ash@ouhsc.edu

Joe G. Hollyfield
Ophthalmology
Cole Eye Institute at the Cleveland Clinic
Cleveland, OH, USA
hollyfj@ccf.org

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Michael Danciger, Ph.D.
1943–2010

Michael Danciger was a pioneer in the identification of modifier genes of complex genetic disorders that lead to blindness. The impact of his research and collaborations continues to be felt and expanded. Equally important was his delightful personal quality of leaving an indelible mark on everyone he met and with whom he worked. Michael was a long-time supporter of the RD Symposia. He is missed, and we are honored to dedicate this proceedings volume to him.

Robert B. Barlow, Jr., Ph.D.
1939–2009

Robert “Bob” Barlow was an extremely energetic man who contributed to many areas of research in vision and neurobiological science and held many responsible positions that influenced numerous investigators. He was a long-time supporter of the RD Symposia, as was his wife, Pat, pictured here together at the RD2004 meeting in Australia. He is missed, and we are honored to dedicate this proceedings volume to him.

Preface

The International Symposia on Retinal Degeneration have been held in conjunction with the biennial International Congress of Eye Research (ICER) since 1984. These RD Symposia have allowed basic and clinician scientists from around the world to convene and present their new research findings. They have been organized to allow sufficient time for discussions and one-on-one interactions in a relaxed atmosphere, where international friendships and collaborations can be fostered.

The XIV International Symposium on Retinal Degeneration (also known as RD2010) was held from July 13–17, 2010 at the Fairmont Tremblant Hotel in the resort village of Mont-Tremblant, Quebec, Canada. The meeting brought together 232 basic and clinician scientists, retinal specialists in ophthalmology, and trainees in the field from all parts of the world. In the course of the meeting, 38 platform and 134 poster presentations were given, and a majority of these are presented in this proceedings volume. New discoveries and state-of-the art findings from most research areas in the field of retinal degenerations were presented. This was the largest of all of the RD Symposia, with the greatest number of attendees and presentations.

The RD2010 meeting was highlighted by three special plenary lectures. The first was given by *Elise Héon*, MD, of the University of Toronto Hospital for Sick Children, Toronto, Ontario, Canada. Dr. Heon discussed "What Bardet-Biedl Syndrome teaches us about ciliopathies." The second was given by *Gregory Hageman*, PhD, of the John Moran Eye Center, University of Utah, Salt Lake City, UT. Dr. Hageman described a "New era in the understanding of age-related macular degeneration." The third plenary lecture was given by *Jayakrishna Ambati*, MD, of the University of Kentucky, Lexington, KY. Dr. Ambati discussed "Age-related macular degeneration and the other double helix."

We thank the outstanding management and staff of the beautiful Fairmont Tremblant Hotel for all of their assistance in making this an exceptionally smooth-running conference and a truly memorable experience for all of the attendees. These included, in particular, *Isabelle Gilbert*, *Émilie Normandeau,* and *Patrick Skelly*. We also thank *Jonathan Marier* of AVW-TELAV for providing audio/visual equipment and services that resulted in a flawless flow of platform presentations. Lastly, we thank *Steven LeFort* and, particularly, *Marie-Chantal Thibault* of JPdL Tremblant

for their planning and implementing transportation of most of the attendees to and from Montreal and the meeting venue in Mont-Tremblant, as well as all aspects of the end-of-meeting Gala at the beautiful "Summit" overlooking Mont-Tremblant and the majestic Laurentian mountains, with a truly Canadian meal, music, and festivities.

The Symposium received international financial support from a number of organizations. We are particularly pleased to thank The Foundation Fighting Blindness, Columbia, Maryland, for its continuing support of this and all previous biennial Symposia, without which we could not have held these important meetings. In addition, for the fifth time, the National Eye Institute of the National Institutes of Health contributed in a major way to the meeting. In the past, funds from these two organizations allowed us to provide 25–35 Travel Awards to young investigators and trainees working in the field of retinal degenerations. However, the response to the Travel Awards program was extraordinary, with 94 applicants, many more than in the past. For this reason, we sought additional support for the Travel Awards program. The Foundation Fighting Blindness-Canada/Institute of Genetics was a major contributor, and for the first time, we turned to industry sponsors and received generous contributions from Novartis Pharma AG, Alcon, Genentech, Inspire Pharmaceuticals, Pfizer, Inc., Genzyme and Bioptigen, Inc. In total, we were able to fund 42 Travel Awards, the largest number ever for these Symposia. Many of the contributing foundations and industry sponsors sent one to several members of their organization to attend the meeting. Their participation and comments in the scientific sessions were instructive to many, offering new perspectives to some of the problems being discussed.

There were two additional "firsts" for the RD Symposia at RD2010. For the first time, there was a commercial exhibitor at the Symposium, Bioptigen, which demonstrated its Spectral Domain Ophthalmic Imaging System (OCT) for small laboratory animals; this was highly instructive for many of the attendees. Second, the world-famous Tremblant International Blues Festival (17th Edition) was held during the RD2010 meeting, immediately adjacent to the Fairmont Tremblant venue. With almost continuous free performances every evening of the Symposium, many groups of attendees enjoyed these together after dinner.

We also acknowledge the diligent and outstanding efforts of Ms. *Holly Whiteside*, who carried out most of the administrative aspects of the RD2010 Symposium, designed and maintained the meeting website, and participated in the production of this volume. Holly is the Administrative Manager of Dr. Anderson's laboratory at the University of Oklahoma Health Sciences Center, and she has become the permanent Coordinator for the Retinal Degeneration Symposia. Her dedicated efforts with the Symposia since RD2000 have provided continuity not available previously, and we are deeply indebted to her. Also, Dr. *Michael Matthes* in Dr. LaVail's laboratory played a major role in all aspects in the production of this volume, along with the assistance of Ms. *Kelly Ahern*, in Dr. LaVail's laboratory.

Recognizing the need to bring younger individuals into the organizational structure of the RD Symposia, at the RD2008 meeting, we invited Drs. *John Ash* and *Christian Grimm* to become members of the organizing committee. Thus, instead of

the rotating head organizer working mostly with Holly Whiteside to organize the meeting and prepare the proceedings volume, Dr. Ash assumed equal responsibility with Dr. LaVail for both efforts for RD2010. Dr. *Anderson* continued in his role as financial administrator for each of the Symposia, working through the Dean McGee Eye Institute, which generously provides the financial responsibility for the meetings and the mechanism for registration of participants. We were pleased to announce at the Gala at RD2010 that our third new member of the organizing committee is Dr. *Cathy Bowes Rickman*. Dr. Grimm will work closely with Dr. Hollyfield for the RD2012 meeting to be held in Germany.

Finally, we honor the memory of two colleagues who died during the preparation of the RD2010 meeting in 2009 and 2010. *Michael Danciger* was a great friend to most who attend our RD meetings. *Robert Barlow*, likewise, was a long-time attendee of the RD Symposia. Both were outstanding scientists and are missed. We dedicate this volume to Michael and Bob.

San Francisco, CA, USA — Matthew M. LaVail
Oklahoma City, OK, USA — John D. Ash
Oklahoma City, OK, USA — Robert E. Anderson
Cleveland, OH, USA — Joe G. Hollyfield
Zurich, Switzerland — Christian Grimm

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Part I
AMD: Basic Mechanisms, Inflammation and Immunity

Chapter 5
Complement Activation in Retinal Degeneration

Matt Rutar, Riccardo Natoli, Jan Provis, and Krisztina Valter

Keywords Retina • Retinal degeneration • Inflammation • Complement system • Light damage • Hyperoxia • P23H

5.1 Introduction

The complement system is a component of the innate arm of the immune response which provides a rapid host defence against a range of immunological challenges. Through a cascade of enzymatic cleavages culminating chiefly in the deposition of complement component 3 (C3) on activating surfaces, the activity of complement enhances the ability of the host to initiate defence against infection, and clear immune complexes, apoptotic cells and other noxious substances (Gasque 2004).

M. Rutar (✉) • K. Valter
Departments of Research School of Biology, ARC Centre of Excellence in Vision Science, The Australian National University, RN Robertson Bldg, Sullivan's Creek Rd, Canberra, ACT 0200, Australia

ARC Centre of Excellence in Vision Science, The Australian National University, Canberra, ACT 0200, Australia
e-mail: matt.rutar@rsbs.anu.edu.au

R. Natoli
ANU Medical School, The Australian National University, Canberra, ACT 0200, Australia

J. Provis
Departments of Research School of Biology, ARC Centre of Excellence in Vision Science, The Australian National University, RN Robertson Bldg, Sullivan's Creek Rd, Canberra, ACT 0200, Australia

ARC Centre of Excellence in Vision Science, The Australian National University, Canberra, ACT 0200, Australia

ANU Medical School, The Australian National University, Canberra, ACT 0200, Australia

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If activated in an exaggerated and inappropriate manner, however, complement also has the capacity to destroy host tissue. Indeed, pathogenic complement activation is directly implicated in neuronal damage following intracerebral haemorrhage (Yang et al. 2006), and post-ischemic cerebral injury (Figueroa et al. 2005). Recently, complement activation has been found to play a role in age-related macular degeneration (AMD), a retinal disease affecting central vision and a leading cause of blindness in the western world. Gene association studies have identified a number of polymorphisms in complement genes, including CFH (Klein et al. 2005), CFB (Jakobsdottir et al. 2008), C2 (Jakobsdottir et al. 2008) and C3 (Yates et al. 2007; Despriet et al. 2009), which are strongly associated with the incidence of AMD.

We aimed to investigate whether a common role of complement in retinal dystrophy exists, by comparing the expression of the central component C3 in conjunction with cell death in a range of mechanistically distinct degenerative models. These include acute degeneration induced through either excessive light or hyperoxia, and chronic degeneration using the retinitis pigmentosa-mimicking P23H-3 rodent strain.

5.2 Methods

In the light damage model, young adult albino Sprague Dawley (SD) rats were exposed to a light intensity of 1,000 lx for a period of up to 24 h, after which some animals were kept in dim light (5 lx) to recover. At specific time points during (1, 3, 6, 12, 17 and 24 h) and after exposure (3, and 7 days), animals were euthanized and retinas extracted for analysis of C3 mRNA expression, and for counts of apoptotic cells in the outer nuclear layer (ONL) using the TUNEL technique. In the hyperoxia model, adult C57 mice were subjected to 75% oxygen for up to 14 days. Animals were euthanized after 3, 7 and 14 days, whereby retinas were dissected for the analysis of C3 mRNA expression, and TUNEL. In the P23H model, animals from line 3 of this transgenic strain were born and reared until postnatal day 50–130. Age-matched, non-degenerative SD rodents served as the control tissue. At this time, animals were euthanized and retinas extracted for analysis of C3 mRNA expression and TUNEL. C3 mRNA expression levels were determined by quantitative PCR (qPCR), where the assessment of the relative fold change was determined using the $\Delta\Delta C^q$, with GAPDH serving as the reference gene.

5.3 Results

In light-induced degeneration, qPCR results show that expression levels of complement C3 in the retina (Fig. 5.1) increased significantly over the course of 24 h bright light and continued to increase robustly into the post-exposure period to reach a peak differential expression of 1,038% compared to dim-reared animals. Photoreceptor death (TUNEL) dramatically increased by 24 h bright light exposure,

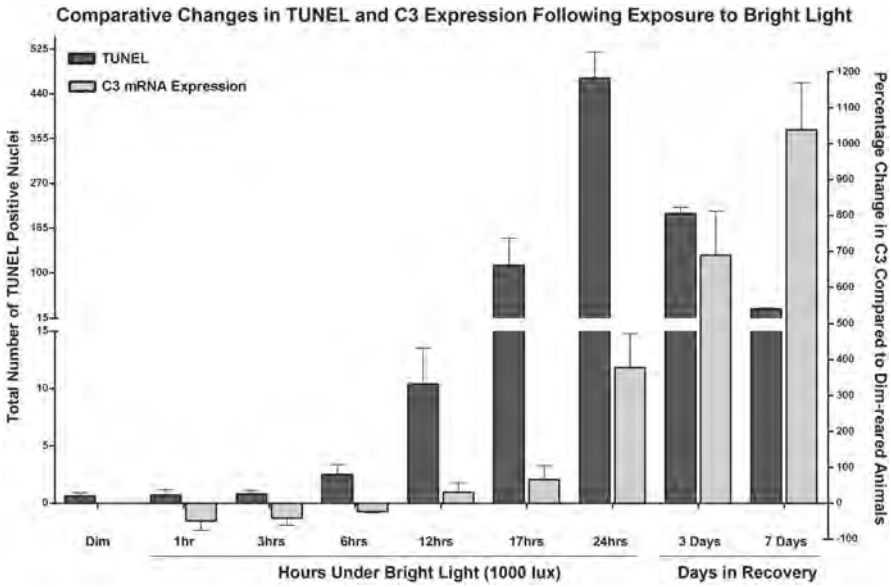

Fig. 5.1 C3 mRNA expression and TUNEL frequency during and following exposure to 1000 lx light. C3 expression during light damage showed a marked up-regulation by 24 h of exposure, which increased further in the ensuing post-exposure period. The number of TUNEL positive nuclei increased dramatically after 24 h of exposure, coinciding with the increase in C3 expression. In the post exposure period, cell death tapered off rapidly though persisted at a relatively low level after 7 days

consistent with the onset of C3 upregulation over the same period (Fig. 5.1). During exposure to hyperoxia, the expression of C3 did not increase after 3 and 7 days (Fig. 5.2). By 14 days, however, C3 expression increased to 180% compared to control animals. The number of TUNEL-positive photoreceptors increased moderately after 7 days hyperoxia, followed by a dramatic increase by 14 days (Fig. 5.2); coinciding with the upregulation of C3. In the retinas of P23H rats, an increase of 80% in C3 expression compared to SD rats was observed, while photoreceptor death was found to be higher in the P23H strain than the SD (Fig. 5.3).

5.4 Discussion

Our results indicate that while the degenerative stimuli in these models differ, an increased expression of C3 mRNA – which fuels the complement cascade – occurs in close association with substantial increases in photoreceptor cell death. Correspondingly, we provide evidence for a common pathway in retinal degeneration involving the activation of complement in response to the induction of photoreceptor injury and death. Our data are consistent with the implication of the

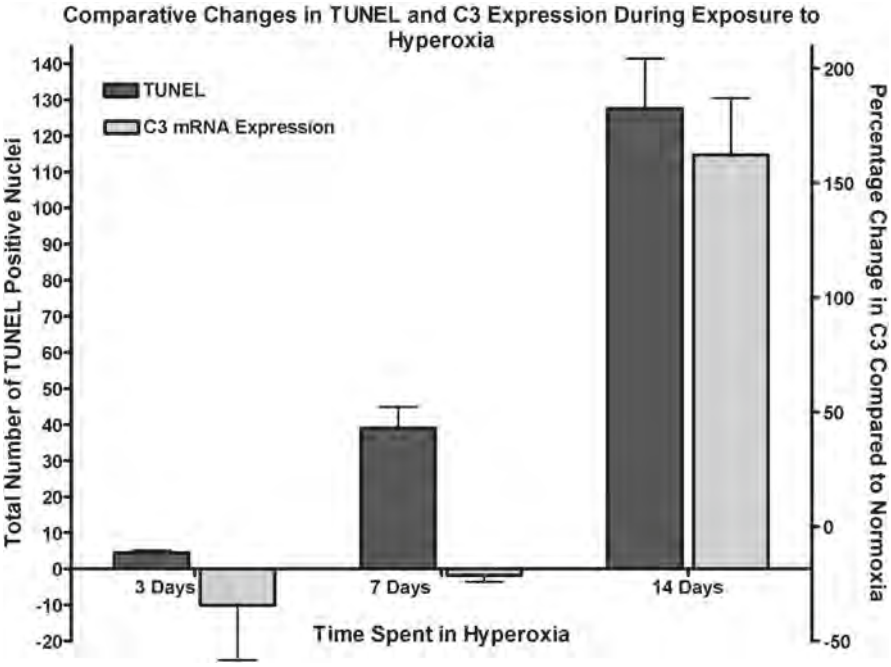

Fig. 5.2 C3 mRNA expression and TUNEL frequency in retinas during exposure to hyperoxia. No increase in C3 expression was evident during the early phases of hyperoxia, although this progressed to a substantial up-regulation by 14 days. A steady increase in TUNEL positive cells was apparent over the 14-day time course, reaching a peak at 14 days hyperoxia

complement system in the clearance of noxious substances, such as apoptotic cells (Gasque 2004; Trouw et al. 2008); which suggests that complement activation is geared toward a beneficial role in the maintenance of retinal homeostasis. This is at odds, however, with the association of complement with the incidence of AMD (Anderson et al. 2010). One explanation is that while complement may be activated to necessitate the efficient clearance of noxious substances, its exaggerated activation in the injured retina – aided by an abundance of apoptotic cells and other activating surfaces – induces further retinal degeneration through propagating an inappropriate inflammatory response (Walport 2001). Indeed, a recent investigation by Rohrer and colleagues has shown that complement activation exacerbates photoreceptor death in light-induced retinal degeneration (Rohrer et al. 2007). While direct evidence for complement-induced pathology in hyperoxia and the degenerative P23H strain are currently lacking, increased synthesis in C3 expression in both models following degeneration – consistent with light damage – supports the existence of a common detrimental role of complement activation in retinal degeneration. Consequently, anticomplement strategies may have a broad therapeutic potential in the treatment of various forms of retinal degeneration.

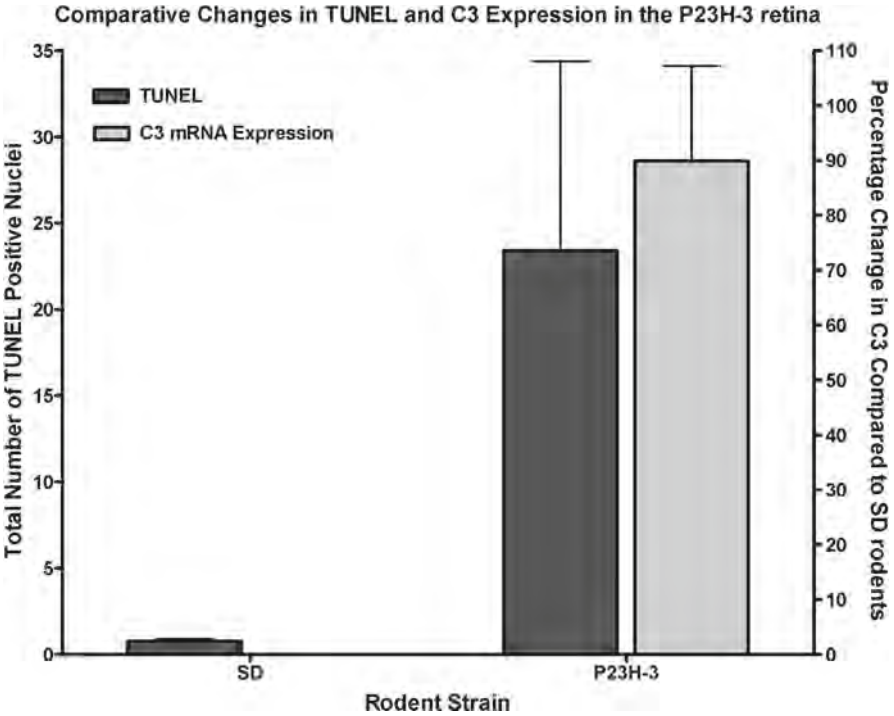

Fig. 5.3 C3 mRNA expression and TUNEL frequency in retinas from SD and degenerative P23H-3 rodents. C3 expression was elevated in retinas from P23H-3 animals compared to those of the SD strain. TUNEL analysis indicated an increase photoreceptor apoptosis in the P23H-3 retina compared to the SD – as expected for this degenerative strain

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