Experimental Eye Research 87 (2008) 89–95

Contents lists available at ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

Disruption of the complement cascade delays retinal ganglion cell death following retinal ischemia-reperfusion

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ARTICLE INFO

Article history:
Received 10 October 2007
Accepted in revised form 23 April 2008
Available online 30 April 2008

Keywords:
retinal ganglion cell
ischemia
complement
animal model

ABSTRACT

Recent reports have indicated that components of the complement cascade are synthesized during the degeneration of retinal ganglion cells (RGC) in glaucoma. While complement deposition in the retina may simply serve to aid phagocytosis of damaged RGC, activation of the complement cascade can also contribute to neuronal loss in neurodegenerative diseases. This study was designed to determine if disruption of the complement cascade affects RGC survival in a murine model of retinal ischemia-reperfusion (I/R) injury. We induced retinal ischemia in the eyes of normal mice and mice with a targeted disruption of the complement component 3 (C3) gene. Tissue was harvested 7 and 21 days after induction of I/R and retinal complement synthesis was determined by quantitative PCR and immunohistochemical methods. RGC death and associated axon loss was evaluated through histological examination of the optic nerve and retina. Our data show that retinal I/R induces the expression and deposition of complement components. C3 deficient mice clearly exhibited reduced optic nerve damage and substantial preservation of RGC 1 week after I/R when compared to normal animals ($p = 0.005$). Three weeks after the ischemic event C3 deficient mice retained more RGC cell bodies although the degree of optic nerve damage was similar between both groups. These findings demonstrate that inhibition of the complement cascade delays optic nerve axonal and RGC degeneration in retinal I/R. It appears that injured RGC are targeted and actively destroyed through complement mediated processes. These results may have implications for the pathophysiology and clinical management of ischemic retinal conditions.

1. Introduction

The degeneration of retinal ganglion cells (RGC) occurs in response to optic nerve damage induced by glaucoma, nerve crush or transection as well as retinal ischemia-reperfusion (I/R) injury. Retinal I/R may occur as a complication of a number of ocular diseases, including anterior ischemic optic neuropathy or central retinal artery occlusion. The molecular events leading to RGC degeneration in retinal I/R remain insufficiently understood although a number of mechanisms have been implicated, including glutamate excitotoxicity, free radical generation, nitric oxide release, retinal acidosis, and disturbances in intracellular calcium homeostasis (reviewed in Osborne et al., 2004).

Neurodegenerative processes are often accompanied by the deposition and local synthesis of components of the complement cascade (Barnum, 2002; Laufer et al., 2001). Complement activation in response to central nervous system injury appears to have both beneficial as well as harmful consequences and presumably limits the scope of local inflammatory responses by facilitating rapid removal of dying cells and cellular debris (Gasque et al., 2002). On the other hand, inhibition of the complement cascade through the removal of specific complement components reduces neuronal damage in hypoxic–ischemic brain injury (Cowell et al., 2003; Mocco et al., 2006; Ten et al., 2005). These findings suggest that complement activation can also contribute to the death of neurons under certain conditions.

We previously reported that ocular hypertension and glaucoma are correlated with retinal expression of complement components 1q (C1q) and 3 (C3) and that both molecules are deposited in association with RGC and the nerve fiber layer (Kuehn et al., 2006). Similar findings have been reported by other investigators using a variety of animal models as well as human glaucomatous donor eyes (Ahmed et al., 2004; Miyahara et al., 2003; Stasi et al., 2006; Steele et al., 2006; Yang et al., 2007). The presence of complement components in glaucomatous tissue may represent a relatively non-specific response to neuronal injury, functioning primarily in

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doi:10.1016/j.exer.2008.04.012

the clearance of cellular debris from RGC undergoing apoptosis. In addition, as we reported previously, the cytolytic terminal complement complex (TCC) can also be detected by immunohistochemical methods in the glaucomatous retina (Kuehn et al., 2006). Compared to astrocytes, neurons express low levels of complement inhibitors and are accordingly quite sensitive to TCC mediated lysis (Singhrao et al., 1999). Thus it is possible that complement activation may actively precipitate RGC death through TCC mediated lysis.

C3 occupies a pivotal role in the activation of the complement cascade. Activation along all three complement pathways converges on C3 which is then cleaved to produce C3a and C3b. C3b is not only required for the eventual formation of the TCC but also for the cleavage of C5 into C5b and C5a. Both C3a and C5a are anaphylatoxins with the potential to elicit glial cell activation in the retina and optic nerve head, another mechanism that has been suggested to contribute to RGC death in glaucoma (reviewed in Kuehn et al., 2005).

We recently observed that complement components are also expressed in the ischemic retina. In order to elucidate the function of complement in the pathobiology of RGC death in vivo we induced I/R in the retinae of mice with a targeted disruption of the *C3* gene and in normal, C3 sufficient, mice (hereafter referred to as C3D and C3S, respectively). Our data show that the *C1qa* and *C3* genes are transcribed and that both proteins accumulate in the post-ischemic retina. Moreover, C3 deficient mice exhibit delayed RGC degeneration when compared to normal animals, suggesting that complement mediated processes are actively involved in the degeneration of RGC.

2. Methods

2.1. Animals

Mice carrying a targeted disruption of the complement component 3 gene (*C3*) were obtained from The Jackson Laboratories (Bar Harbor, ME), strain B6.129S4-C3^{tm1Crr}/J (C3D). C57BL/6J mice served as controls (C3S). All animals were between 3 and 4 months of age at the onset of the study and both male and female mice were used. All animals were treated in accordance with the statements of the Association for Research in Vision and Ophthalmology (ARVO) and guidelines of the University of Iowa Animal Care and Use Committee.

2.2. Induction of ischemia

Animals were anesthetized using 1.8% isoflurane and 100% oxygen, and body heat was maintained through a heating pad. A 30-gauge needle connected to a reservoir containing phosphate buffered saline was inserted through the cornea into the anterior chamber of the eye. The saline reservoir was elevated to yield an intraocular pressure of 104 mmHg, sufficient to completely prevent circulation through the retinal vasculature. Elevated intraocular pressure was confirmed by blanching of the iris and retina and ischemia was maintained for 45 min in the left eye only; right eyes did not receive cannulation and served as controls. Additional controls included wild type mice ($N = 5$) receiving cannulation without elevation above physiological intraocular pressure (approximately 11 mmHg).

Mice were euthanized either 1 or 3 weeks after the ischemic insult ($N = 15$ per time point and genotype).

2.3. Immunohistochemistry

Eyes were fixed in 4% paraformaldehyde for 2–4 h immediately after death and infiltrated with increasing concentrations of sucrose (Barthel and Raymond, 1990). Tissue was then embedded in optimum cutting temperature (OCT) medium, frozen and sectioned to a thickness of 7 μm. Goat polyclonal antibodies raised against human C1q and C3 (both used at 1:100, CalBiochem, San Diego, CA) were used to detect the site of complement deposition. Antibody binding was visualized by incubation with anti goat-IgG secondary antibodies conjugated to Alexa Fluor 488 (1:200). Gamma synuclein was detected using rabbit anti human polyclonal antibodies (Abcam, Cambridge, MA) at 1:100 dilution and visualized using Alexa Fluor 546 labeled secondary antibodies. To facilitate orientation all sections were counterstained with the nuclear dye DAPI (InVitrogen, Carlsbad, CA). Images were obtained using an Olympus BX41 microscope (Olympus, Melville, NY) equipped with a SPOT RT camera (Diagnostic Instruments, Stering Heights, MI).

2.4. Quantitative PCR

Real-time PCR was carried out as described previously (Mullins et al., 2006). Eyes were preserved in RNAlater (Ambion, Austin, TX) and stored at −80 °C until the neural retina from the entire eye was harvested. RNA from individual eyes was isolated using the RNeasy mini kit (Qiagen, Valencia, CA) and subjected to on-column DNase digestion (Qiagen). RNA from each eye (250 ng total) was reverse transcribed in a random primed reaction using SuperscriptIII reverse transcriptase (Gibco, Grand Island, NY). Real time PCR was carried out on an ABI model 7700 sequence analyzer using QuantiTect SybrGreen PCR master mix (Qiagen). Quantities were compared against a standard curve derived from mouse liver cDNA and all samples were normalized to the level of 18S RNA. All reactions were carried out in duplicate. Negative controls included reactions without cDNA. PCR primers were designed to span at least one intron to minimize the influence of potentially remaining traces of genomic DNA. The primers used are: C1qa-forward 5′-TGG ACA GTG GCT GAA GAT GT-3′; C1qa-reverse 5′-ATT GCC TTT CAC GCC CTT CAG T-3′, C3-forward 5′-GCC AGC TCC CCA TTA GCT CT-3′, C3-reverse 5′-CTC TTC GCT CTC CAG CCG TA-3′, 18S rRNA-forward 5′-CGT TGA ACC CCA TTC GTG AT-3′, 18S RNA reverse 5′-TGG GAA TTC CTC GTT CAT GG-3′. Differences in gene expression levels were evaluated using a two-tailed Student's *t*-test. $N = 5$ mice per time point and genotype.

2.5. Assessment of optic nerve damage

Optic nerves were dissected and prepared using standard electron microscopy methods as described previously (Kuehn et al., 2006). One micrometer thick sections were stained with 1% paraphenylenediamine (PPD) to visualize degenerating neurons (Sadun et al., 1983) and photomicrographs were collected at a magnification of 200×. Optic nerve damage was categorized into one of four grades using an evaluation method previously used by us and similar to those of the other investigators (Chauhan et al., 2006; Jia et al., 2000; Kuehn et al., 2006; Libby et al., 2005). Grade 1 designates healthy nerves, only a very small number (<50) of darkly stained (damaged) axons are detected across the entire optic nerve. Grade 2 designates minor damage; stained axons are readily discernable but represent no more than 10% of all axons. Grade 3 designates moderate damage, 11–40% of axons stain darkly, some axon loss or slight gliosis may be evident. Grade 4 designates severe nerve damage. The majority of axons are damaged with obvious axonal loss, and swollen axons and/or glial cell proliferation can be detected. An example for each grade is given in Fig. 1.

The degree of damage was evaluated independently and in a masked fashion by three investigators and optic nerves were classified according to the median score. Differences were statistically evaluated using the Mann–Whitney rank-sum test. *p*-values

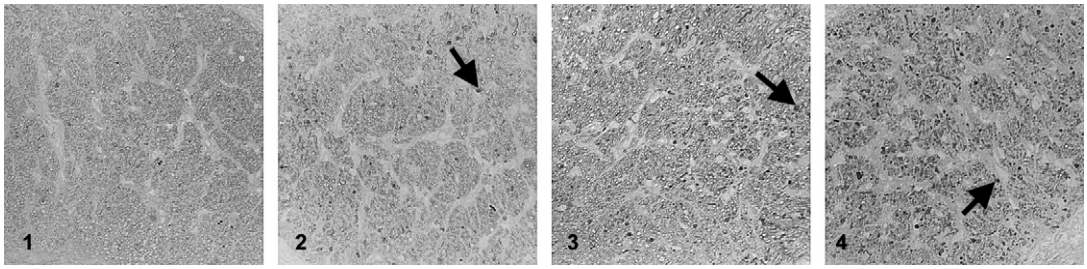

Fig. 1. PPD stained mouse optic nerve sections illustrating the grading scheme used. Optic nerve damage was evaluated based upon the number of PPD stained axons (arrows) and the degree of gliosis. 1, no damage; 2, minor damage; 3, moderate damage; 4, severe damage.

below 0.05 were considered statistically significant. $N = 15$ mice per time point and genotype.

2.6. Determination of retinal RGC

Quantitation of surviving RGCs was performed using gamma synuclein labeling to identify RGCs. Whole mount preparations of retinas were incubated with anti gamma synuclein antibodies as described above, except that 0.5% Tween 20 was included in the incubation buffer. From each animal both the ischemic and control eyes were analyzed, and five non-overlapping images of the midperipheral region were captured at 400× magnification using a LaserSharp2000 confocal scanning microscope (BioRad, Hercules, CA).

The resultant series of images were analyzed using ImageJ software with the Cell Counter plug-in (Abramoff et al., 2004). From each image an area of 150 × 150 μm was analyzed. Percent survival rates were calculated for each individual using RGC densities of the treated and the contralateral control eye. Differences between groups of animals were evaluated using Student's *t*-test. $N = 5$ mice per time point and genotype.

3. Results

In order to investigate the role of the complement system in the pathophysiology of retinal I/R, we compared the degree of axonal damage induced by transient retinal I/R in normal and *C3* knock out mice. Histochemical analysis prior to the initiation of the study demonstrated that absence of the *C3* gene does not lead to abnormal ocular development or obvious morphological aberrations in *C3* knockout mice when compared to wild-type animals (data not shown). Similarly, no differences in the appearance of the optic nerve are noted.

Retinal I/R results in retinal deposition of C1q and C3 detectable by immunohistochemistry. C1q deposition patterns are similar in C3S and C3D mice and are primarily associated with the retinal ganglion cell layer (GCL) and nerve fiber layer (NFL) (Fig. 2), although faint labeling is also apparent in the inner plexiform and nuclear layers. C3 labeling in C3S mice was also associated primarily with these retinal structures, although sparse labeling of individual cells in the inner nuclear layer could occasionally be observed as well. These binding patterns are obvious 7 days after induction of I/R but are observed less frequently 3 weeks after induction. The detected patterns of deposition are similar to those observed previously in rats with ocular hypertension and human glaucoma patients (Kuehn et al., 2006).

The deposition of complement components is accompanied by a concomitant increase in the amount of mRNA coding for C1qa and C3. Although expression levels vary considerably between individuals, quantitative PCR analysis reveals increased expression of C1qa in ischemic eyes of both C3D and C3S animals throughout the entire study period (Fig. 3A). One week after I/R C1qa expression levels are significantly elevated compared to the untreated contralateral eyes (C3D $p = 0.045$ and C3S $p = 0.031$). Three weeks after I/R C1qa mRNA levels continue to be elevated in comparison to the control eyes (C3D $p = 0.050$ and C3S $p = 0.036$) albeit at overall reduced expression levels. Both C3D and C3S mice exhibit roughly equivalent levels of *C1qa* expression. While average C1qa mRNA levels in C3D appear to be higher than in C3S mice, the differences are not statistically significant ($p = 0.29$ and 0.51, respectively, 7 and 21 days after I/R). Likewise, although average *C1qa* expression appears to decline between 7 and 21 days after I/R, statistical significance is not reached ($p = 0.16$ for C3S and 0.07 for C3D).

Robust *C3* expression in C3S mice is evident in experimental eyes both 7 and 21 days after I/R induction and does not significantly decline ($p = 0.74$). Using the assay employed here *C3* expression is detectable only at very low levels in the control eyes of C3S mice (Fig. 3B). C3 mRNA can also be detected at low levels in this strain of C3D mice (data not shown), however due to the engineered disruption of the gene a functional protein is not formed (Wessels et al., 1995). Taken together, these findings demonstrate that complement activation and local synthesis of at least

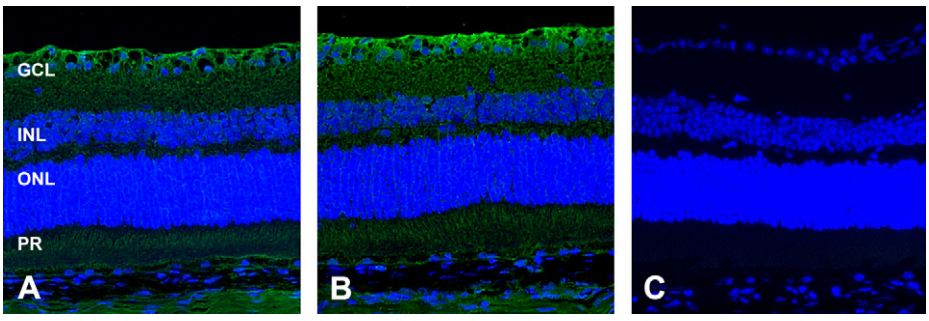

Fig. 2. Immunohistochemical detection of C1q (A) and C3 (B) in the post-ischemic retina of a C3S mouse. Seven days after induction of I/R, complement immunoreactivity can be observed primarily associated with the retinal nerve fiber layer and the ganglion cell layer. C, negative control; secondary antibody only. GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer; PR, photoreceptor cells.

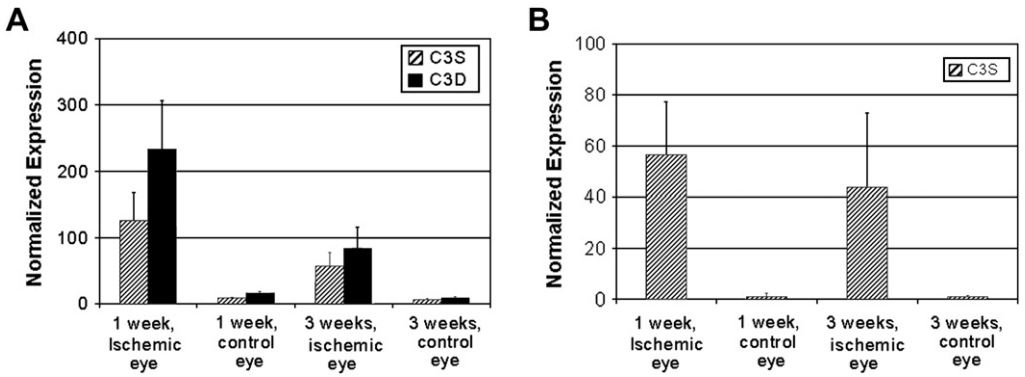

Fig. 3. Detection of retinal *C1qa* (A) and *C3* (B) transcription by quantitative PCR. Robust expression of *C1q* was observed both 1 and 3 weeks after I/R. C3 expression was observed in C3S mice at approximately equal levels at 1 and 3 weeks post-ischemia although the detected levels varied widely between individual animals. Expression of both genes in the contralateral control eyes was negligible in all animals (error bars = standard error).

a subset of complement components are features of RGC degeneration as a result of ischemic retinal injury.

As expected, transient retinal ischemia results in degenerative changes of the optic nerve. Histological evaluation of the optic nerve following staining with PPD is a semiquantitative and rapid method to estimate the degree of axonal damage and optic nerve gliosis. The appearance of the optic nerve is well correlated to the degree of RGC loss (Bernstein et al., 2007). Optic nerve sections are evaluated and assigned to one of several grades of damage. In this study a scheme involving four grades was employed. Using this method, optic nerves from ischemic and contralateral control eyes of 30 C3D and 30 C3S mice were analyzed. In C3S animals, prominent optic nerve damage is apparent 1 week after I/R; more than half of all optic nerves display either moderate or severe damage (average damage grade = 2.7) (Fig. 4A). In concurrence with findings by other investigators (Lafuente et al., 2002) we observed only minimal additional axonal loss between 1 and 3 weeks in these control animals (average damage = 2.6, C3S 1 week vs. 3 weeks $p = 0.79$). In contrast, C3D mice display a marked reduction in axonal degeneration, exhibiting only minor levels of optic nerve damage (average = 1.7) 1 week after the induction (Fig. 4B). Comparison of the damage in the ischemic eyes between C3S and C3D animals after 1 week reveals this decrease to be statistically significant ($p = 0.005$).

While axon loss in C3S animals does not progress noticeably between 1 and 3 weeks after I/R, optic nerves of C3D do continue to degenerate during this period (C3D 1 week vs. 3 weeks $p = 0.042$). Three weeks after the insult, optic nerve damage in the study eyes of C3D mice is markedly increased (average = 2.5) and almost as pronounced as that observed in C3S mice ($p = 0.78$). The optic nerves of the contralateral control eyes display only a minor amount of PPD staining. The observed level does not increase between 1 and 3 weeks and the differences between C3S and C3D animals are minor and not statistically significant ($p = 0.37$ and 0.75, respectively) (Fig. 4C). The appearance of these optic nerves is similar to that observed in cannulated mouse eyes not subjected to I/R (data not shown).

In order to ascertain that optic nerve degeneration is accompanied by loss of retinal RGC we determined the number of surviving RGC through direct counts. Ischemic and contralateral control retinas were examined after labeling of RGC with gamma-synuclein antibodies (Soto et al., 2008). In the ganglion cell layer gamma synuclein is expressed at high levels specifically by RGC but it is also synthesized at low levels by individual cells of the inner nuclear layer (Fig. 5A). Using confocal microscopy signals from these two populations can be easily discriminated in flat mounted retinal preparations, allowing accurate quantitation of RGC density (Fig. 5B).

Data obtained demonstrated that 1 week after I/R only 39% of RGC survive in C3S mice (Fig. 6). In contrast, over 95% of RGC are present in C3D mice ($p = 0.005$). RGC density did not decline further in normal mice. C3D animals continue to lose RGC but even 3 weeks after ischemia a significantly higher fraction (69%) of RGC remain when compared to C3S mice (41%) ($p = 0.03$).

Taken together, data indicate that while disruption of the complement cascade does decelerate optic nerve degeneration, and presumably RGC death, it does not ultimately result in enhanced RGC survival.

4. Discussion

Activation of the complement system in neurodegenerative diseases and cerebrovascular disorders is a common phenomenon and has been reported to occur in Alzheimer's and Parkinson's

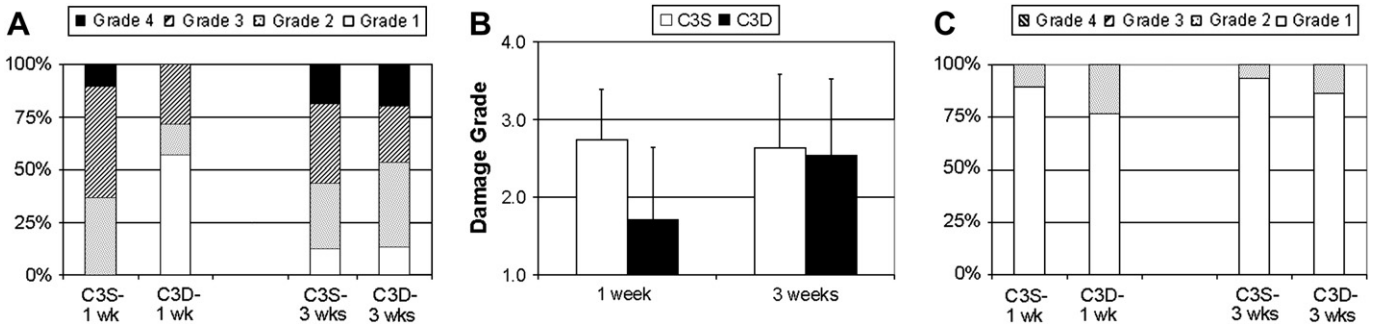

Fig. 4. Axonal damage in the optic nerves of ischemic (A,B) and contralateral control (C) eyes. (A) One week after I/R C3D mice exhibit less axonal damage than C3S animals. However, in contrast to C3S mice, axonal degeneration continues in C3D mice and 3 weeks after induction of I/R both groups display similar levels of damage (B). Contralateral control eyes displayed only minor or no damage in all animals at both time points (C).

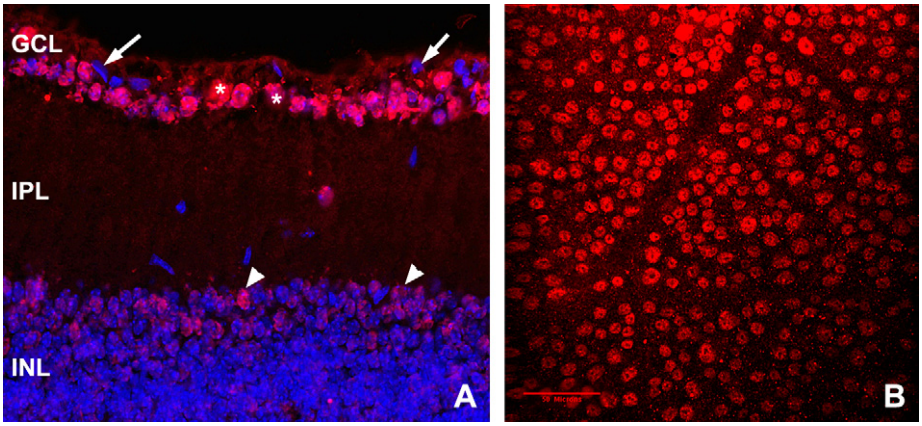

Fig. 5. (A) Immunohistochemical detection of gamma synuclein (red) and nuclei (blue) in a sagittal section of a healthy murine retina. RGC (asterisks) are strongly labeled with this marker. Additional cells exist in the ganglion cell layer that do not react with this antibody (arrows). Weak labeling by a subpopulation of cells in the inner nuclear layer can also be observed (arrowheads). (B) Whole mounted mouse retina incubated with anti synuclein gamma antibodies.

disease as well as in I/R injury in the brain (Kulkarni et al., 2004; Rus et al., 2006). In human eyes with glaucoma and in animal models of ocular hypertension complement components are also locally synthesized and deposited in the nerve fiber and ganglion cell layers (Ahmed et al., 2004; Kuehn et al., 2006; Miyahara et al., 2003; Stasi et al., 2006). Here we describe that complement synthesis and deposition is also induced by retinal I/R and that disruption of the complement cascade delays RGC degeneration.

The data reported herein provide a first insight into the role of complement in the pathophysiology of retinal I/R. Our findings demonstrate that in normal mice axonal damage and RGC loss occurs rapidly within the first week after the ischemic event. Damage is essentially complete in most animals 1 week after ischemia and little or no additional RGC loss is observed during the following 2 weeks. These findings are in agreement with those reported by Lafuente et al. who demonstrated rapid loss of RGC within days after an ischemic insult followed by a period of more gradual axon loss (Lafuente et al., 2002). In contrast, mice lacking C3 exhibit a marked reduction in the degree of optic nerve damage 1 week after the ischemic event. However, subsequent rates of axon loss in C3D mice appear to be higher than those in normal animals, and 3 weeks after the ischemic event both C3S and C3D animals exhibit an approximately equal degree of optic nerve damage.

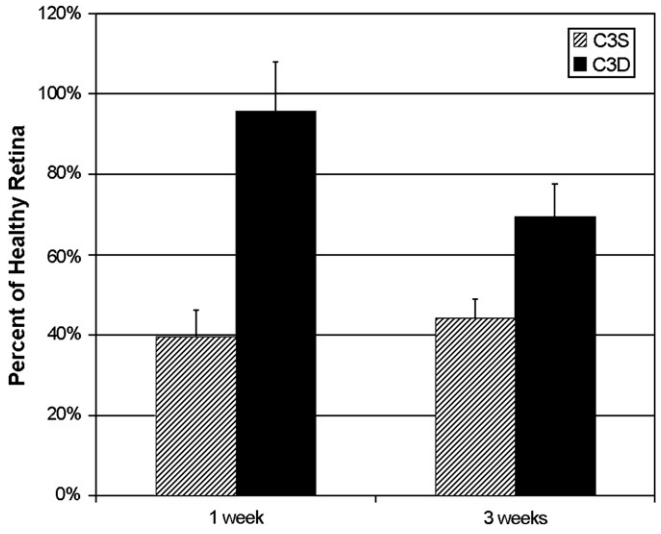

Fig. 6. Morphometric analysis of RGC density in both C3 deficient (C3D) and sufficient (C3S) mice. One week after induction of I/R almost all cells remain in C3D mice whereas C3S mice have lost approximately 60% of all RGC. In C3S no additional loss was observed 3 weeks after I/R. Values represent the proportion of RGC density between the control and ischemic eye of each individual (error bars = standard error).

Similarly, C3S mice experienced all RGC soma loss during the first week after I/R whereas C3D mice lost almost no RGC during the same period. RGC loss in C3D mice did occur between 1 and 3 weeks after ischemia, but at the end of this study mice with a disrupted complement cascade retained higher numbers of RGC cell bodies than C3S mice. This finding seems to conflict with the fact that at 3 weeks post-I/R optic nerve damage is similar between both groups of mice. However, recent data by Soto et al. (2008) suggest that RGC disconnected from their targets may persist in the retina of DBA2/J mice. Alternatively, our data suggest that axon loss precedes degeneration of the RGC soma in C3D mice consequently it is conceivable that C3D mice will eventually have proportions of surviving RGC similar to those of normal mice.

Our finding that removal of C3 delays, and perhaps to some degree prevents, the development of optic nerve and RGC damage suggests three conclusions. First, complement activation is not the principal cause of RGC death after I/R. Clearly other, primary, events appear to damage RGC leading to eventual axonal loss and cell death. Second, C1q typically requires a cell surface bound binding partner for activation of the complement cascade. This indicates the existence of early and specific markers of RGC distress that are recognized by complement. Third, axonal degeneration and RGC death occur more rapidly in the presence of C3. This indicates that the role of complement is not limited to the removal of cell debris of RGC undergoing apoptosis. Rather it appears that complement actively promotes and accelerates the degradation of damaged RGC.

Complement activation may result in accelerated RGC degeneration by several cellular mechanisms. Our earlier findings that the membrane attack complex is formed in association with RGC in ocular hypertension and glaucoma suggests the possibility that injured RGC are killed directly through complement mediated lysis. Deposition of complement in the ganglion cell layer presumably affects primarily RGC since neuronal cells posses only limited capacities to control complement activation (Singhrao et al., 1999). Alternatively it is possible that complement activation and the concomitant generation of the anaphylatoxins C3a and C5a initiates or modulates the reactive glial response in the ischemic retina (Kaur et al., 2006; Wang et al., 2002; Woldemussie et al., 2004). In normal animals these molecules are released during the complement cascade, but in C3D mice C3a is not expected to be produced although a recent report suggests that C5a can also be created through a C3 independent pathway (Huber-Lang et al., 2006). Both C3a or C5a receptors are present on resident cells of the inner retina

(Vogt et al., 2006) and C3a receptor expression appears to be enhanced on microglia in the ischemic retina (Kaur et al., 2006). Consequently it is possible that anaphylatoxin release contributes to the accumulation of glia derived cytotoxic mediators such as TNFα or nitric oxide (Neufeld et al., 2002; Tezel and Wax, 2000). In addition, anaphylatoxin release may result in increased vascular permeability and the chemotactic recruitment of macrophages – two phenomena which have been reported to occur after retinal I/R (Neufeld et al., 2002; Zhang et al., 2005; Zheng et al., 2007).

Another interesting aspect of this study is the eventual death of RGC in the absence of C3. Perhaps the most straightforward explanation might be that RGC are damaged by a primary ischemic insult to a degree that precludes sustained survival and are destined to degenerate, albeit at a slower rate than in the presence of a fully functional complement cascade. However, it is also conceivable that the extended presence of injured axons and RGC in C3D mice elicits an abnormal response not present in C3S animals that results in continued RGC loss through a secondary mechanism. Such responses might include enhanced reactive gliosis due to prolonged inflammatory stimuli or the formation of autoantibodies to RGC. Both mechanisms have been suggested to take place in the retina and brain (Kim et al., 2004; Storini et al., 2005; Wax et al., 2002). Finally, other physiological differences exist between C3D and normal mice, for example C3D animals exhibit decreased vascular permeability and abnormalities in the humoral immune response (Fischer et al., 1996; Williams et al., 1999). Consequently it is conceivable that pathobiological mechanisms other than the ones discussed above are responsible for the observed differences in RGC degeneration.

Given the importance of RGC for vision, the question arises what physiological advantage is gained from the destruction of RGC axons by complement. Over the past few years evidence has accumulated that one of the most important functions of complement is, apart from defense against invading pathogens, the support of phagocytosis of cellular debris (Sahu and Lambris, 2001; Taylor et al., 2000). Genetic complement deficiencies resulting in the failure to carry this process out efficiently can lead to the formation of autoantibodies and the development of lupus (Bohlson et al., 2007; Carroll, 1998; Mitchell et al., 2002). Thus rapid removal of damaged axons reduces the likelihood of the development of a humoral immune response to retinal antigens. Furthermore, active destruction of damaged RGC limits the duration and extent of tissue inflammation. This may be particularly important in the retina where even a minor degree of inflammation can lead to decreased vision. As such, it is likely that the main reasons for retinal synthesis of complement after I/R are to shorten the period of active inflammation and to prevent the induction of an inappropriate adaptive immune response.

Acknowledgements

The authors would like to thank Dr Michael Anderson for his help in the evaluation of the optic nerves, Dr Robert Mullins for help with immunohistochemical methods and Paul Abrams, Jared Vincenti and Adam Hedberg-Buenz for technical assistance. This work was supported by an unrestricted grant from Research to Prevent Blindness to the UI Department of Ophthalmology, an Unrestricted Glaucoma Educational Grant from Allergan (CYK), and the Charles D. Phelps Memorial Endowment (BJ and AVD).

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