Complement Deposition and Microglial Activation in the Outer Retina in Light-Induced Retinopathy: Inhibition by a 5-HT$_{1A}$ Agonist

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**Purpose.** Increasing evidence supports a role for complement in the pathogenesis of age-related macular degeneration (AMD). This study evaluated retinal microglia, T-lymphocytes, and complement deposition in a light-induced retinopathy model. The effect of a serotonin (5-hydroxytryptamine, 5-HT$_{1A}$) agonist on these processes was investigated.

**Methods.** Rats were dark adapted for 24 hours before a 6-hour blue light exposure. Some animals were predosed subcutaneously with AL-8309A. Retinas were evaluated at different times after light exposure. Paraflin sections were stained with antibody for a microglial marker (Iba1), a T-lymphocyte marker (CD3), and complement components C1q, C3, factor B, factor H, and membrane attack complex (MAC).

**Results.** Light exposure resulted in substantial photoreceptor and RPE loss. Robust microglia activation and migration to the outer retina occurred rapidly. Substantial T-lymphocyte recruitment did not occur. Complement alternative pathway was strongly activated, resulting in the deposition of C3, factor B, factor H, and MAC in the area of photic lesions. Dosing with AL-8309A prevented retinal lesions and decreased microglia activation/recruitment and complement deposition in the outer retina.

**Conclusions.** In blue light exposed retinas, microglia were activated and migrated toward the outer retina, whereas a T-lymphocyte response was minimal. The innate immune system was markedly activated, with substantial complement deposition in the outer retina after light exposure. This complement deposition was prevented by AL-8309A. This model may be useful in the evaluation of complement inhibitors and other neuroprotectants intended for ocular use. AL-8309 is under evaluation in the clinic and may be useful in the treatment of AMD. AL-8309A prevented retinal lesions and decreased microglia activation/recruitment and complement deposition in the outer retina.

**BIOCHEMICAL, GENETIC, AND CLINICAL LINES OF EVIDENCE HAVE CONVERGED TO INDICATE A ROLE FOR THE ALTERNATIVE COMPLEMENT PATHWAY IN THE PATHOGENESIS OF AGE-RELATED MACULAR DEGENERATION (AMD).** Complement components including C3 (and activated fragments), membrane attack complex (MAC), and factor H, are present in drusen as measured immunochromatically, and some have been verified by mass spectrometry. Genetic variants of complement factor H, and other complement components have been associated with altered risks for AMD. Genetic variants of complement factor H have been associated with an increased risk for AMD in several studies, and a meta-analysis further documents that each allele contributes to the increased odds of AMD (except in some Asian populations). Patients with AMD also have signs of systemic complement activation, exhibiting significantly higher serum levels of factor B, C3a, C5a, SC5b-9, C3d, and Ba.

Similar convergent lines of evidence also implicate oxidative stress in the pathogenesis of AMD. have shown that oxidatively modified proteins are more abundant in the eyes and plasma of AMD patients than of controls. Campo-chiaro et al. have shown that oxidation products of lipids, nucleic acids, and proteins are abundantly present in the eyes of patients with geographic atrophy, a form of advanced AMD. The ARED study demonstrated that progression to advanced neovascular AMD was attenuated by a dietary supplement containing antioxidants, zinc, or both. Cigarette smoke contains high levels of oxidants, and smoking is one of the strongest risk factors for AMD.

In animal models in which the retina is exposed to oxidative stress by genetic or environmental manipulations, pathologic conditions similar to AMD develop. For example, exposure of rodents to photo-oxidative stress (bright light exposure) results in an outer retinal degeneration exhibiting all features of late atrophic AMD, including loss of photoreceptors and RPE. This damage can be prevented by pretreatments with antioxidants. Genetic ablation of the cytoplasmic anti-oxidant enzyme SOD1 leads to an age-dependent development of drusen, RPE dysfunction, and choroidal neovascularization in mice, all hallmarks of AMD. Decreasing the expression of the related mitochondrial enzyme SOD2 in the RPE of mice leads to several features of early AMD, including increased quantities of A2E and iso-A2E, visual pigment breakdown products that accumulate in aged RPE, increased RPE autofluorescence, CEP adduct formation, Bruch’s membrane thickening, and degeneration of photoreceptors and RPE cells.

How might oxidative stress and the complement system interact in the pathogenesis of AMD? Several possibilities for how oxidative stress may lead to complement activation have been proposed. In vitro experiments have shown that factor H, an inhibitory modulator of complement expressed by RPE cells, is downregulated by peroxide treatment of cells. Photoactivation of RPE cells fed bisretinoids leads to activation of the alternative complement pathway and to deposition of complement on the RPE surface.
mice with CEP-adducted proteins leads to complement deposition on RPE cells and RPE cell damage.32

We routinely use a rodent light-induced retinopathy model to search for outer retinal neuroprotectants. In this study, we examined light-damaged retinas to determine whether activation of the complement system occurs subsequent to a severe light-induced oxidative stress. Our results indicate that robust activation of the alternative complement system occurs locally in the retina, with precise localization to the sites of phototoxic injury.30,31,33,34,35 Traumatic brain injury,36,37 and excitotoxicity.38,39

**METHODS**

**Animals, Drugs, and Light Treatments**

All animals were handled in compliance with the ARVO Statement for the Use of Animals in Ophthalmic Research and under the supervision of the Alcon Animal Care and Use Committee. Adult (300–450 g) Sprague-Dawley rats (Charles River, Wilmington, MA) were dark adapted for 24 hours before induction of photochemical lesions. Phototoxic–induced lesions were generated by exposing animals to blue light (λ = 450 nm, 3.1 mW/cm²; fluorescent lamps F40/BB; Philips, Andover, MA) for 6 hours. Rats were single housed in clear polycarbonate cages with minimal bedding to prevent burrowing. Movement of rats within the cage was unrestricted during this light exposure. Animals were allowed to recover for the indicated period in darkness before their eyes were harvested for histochemical analysis. Control animals were maintained under broad-band fluorescent (Cool White, 45 fc; Sylvania, Danvers, MA) cyclic light (12 hours light/12 hours dark). Control rats were not exposed to blue light, nor were they treated with drug or vehicle. Food and water were available ad libitum to all experimental groups.

Drug-treated animals received subcutaneous injections of AL-8309A (10 mg/kg) once daily starting 2 days before light exposure, once immediately before light exposure, and once the day after light exposure. AL-8309A (citrate salt) was provided by Dainippon Sumitomo (Osaka, Japan) and was dissolved in a sterile 0.9% sodium chloride vehicle. AL-8309A is a potent agonist of the serotonin 5-HT1A receptor (EC50 = 9.6 nM, Emax = 136%). The reported affinity of AL-8309A to other serotonergic, adrenergic and dopaminergic receptors is 2 to 3 orders of magnitude less than at the 5-HT1A receptor.

**Histology**

Eyes from rats exposed to blue light for 6 hours were obtained under deep anesthesia. Ocular tissues were evaluated immediately after light exposure (0 hour) or after 1-, 2-, and 7-day postexposure periods in the dark (n = 8–14 rats per group). Orientation of the eye was maintained by placement of a marker suture at the 12-o’clock position before ocular enucleation. The cornea and lens were then removed, and posterior poles were fixed by immersion in a mixture of paraformaldehyde (2%) and glutaraldehyde (2%) in (0.1 M) phosphate buffer (pH 7.4). Tissues were washed, dehydrated in an ascending ethanol series, and embedded in JB-4 plastic resin. Thick sections (1–1.5 μm) were cut, stained (Multiple Stain; Polysciences, Warrington, PA), and analyzed using a quantitative computer image analysis system attached to the microscope.

Retinal pigment epithelium (RPE), outer nuclear layer (ONL) and inner nuclear layer (INL) thickness, and inner segment plus outer segment (IOS) length were measured to assess changes in retinal morphology resulting from this blue light exposure. Measurements were made at 200-μm intervals from the optic nerve head at three nasal and three temporal retinal locations along the horizontal meridian. For each eye, at least four sections were randomly selected for evaluation, including one section through the optic nerve and three superior retina positions. Analysis of variance (ANOVA) was performed to determine whether differences in retinal thickness were significant (P < 0.05). Dunnett’s test was used to perform all pairwise multiple comparisons when ANOVA detected significant differences.

**Immunohistochemistry**

Retinas were fixed in alcoholic zinc/4% paraformaldehyde, processed, and embedded in paraffin. Sections (4 μm) were stained with hematoxylin and eosin, a macrophage/microglial marker (ionized calcium binding adaptor molecule 1 [Iba1]), or a T-lymphocyte marker (CD3) to evaluate the retinal inflammatory reaction to the blue light insult. Briefly, sections were incubated with primary antibody (Table 1) in the blocking buffer overnight, then detected with biotinylated donkey anti-rabbit (or goat or chick) IgG (1:400, 2.5 μg/mL) for 30 minutes and streptavidin–HRP conjugate (Dako K0397 1:1000, 1 μg/mL) for 30 minutes. Labeling was developed with 3,3′-diaminobenzidine (K3468; DAKO, Carpinteria, CA) for 5 minutes, which produces a permanent brown reaction signal. Cell nuclei were counterstained with Gill’s hematoxylin. For indirect immunofluorescence detection, biotinylated secondary antibody was followed by incubation with streptavidin Al- exa Fluor 594 conjugate (1:400, 5 μg/mL) for 60 minutes and a DAPI nuclear counterstain for 15 minutes. Additional antibodies used (suppliers and dilutions) are provided in Table 1. Images were captured with a fluorescence microscope (DM6000B; Leica, Wetzlar, Germany) with a digital camera system. Images are from a similar retinal location located in the superior midperiphery retina.

Counts of microglia and T cells in the whole retina were made along the full-length of each retinal section ( ora serrata to ora serrata) as a measure of activation (increased number of microglia) and number per outer retina (stained cells in the ONL, IOS, and subretinal space) as a measure of recruitment (microglia or lymphocyte cell distribution shift) by a masked observer. For each eye, five sections were randomly selected to evaluate retinal areas similar to those described in the histology section, which included sections through the optic nerve and the superior retina. For each section, counts were made from the nasal to temporal ora serrata. The number of eyes with deposition of complement C1q, C3, factor B, factor H, and MAC were compared between experimental groups. Measurements were made in similar posterior retinal locations as described.

**RESULTS**

**Light-Induced Histopathology**

Typical pathomorphologic changes were observed in the retinas of light-exposed rats (Fig. 1A). Control retinas had a uni-
form appearance with intact, evenly spaced RPE and well-aligned, compact photoreceptor outer segments, and all photoreceptor cell bodies exhibited uniformly stained chromatin occupying the entire soma. At varying times after light exposure, normal RPE thickness ($X \pm 0.1 \mu m$ [SEM]), ONL thickness ($X = 34 \mu m \pm 0.8 \mu m$ [SEM]), or photoreceptor segment length ($X = 25 \mu m \pm 0.5 \mu m$ [SEM]) measured in control rats was reduced in rats exposed to blue light (Fig. 1B). After this light exposure, damage occurred primarily to the superior-temporal retina. Blue light exposure resulted immediately in vacuolization in the RPE, pyknotic cells with condensed chromatin in the ONL, and swelling of the ONL and photoreceptor inner segments (Fig. 1A). By 24 hours after light exposure, at least 40% of the cells in the ONL were pyknotic, necrotic, or both; photoreceptor outer segments were shortened and disorganized, and RPE thickness was more variable. Microglia (based on Iba1 staining of paraffin-embedded tissue (see Fig. 2) are seen above the RPE. Two days after light exposure and continuing to the seventh day, there is loss of photoreceptors and thinning of the ONL, IOS thinning and disorganization, degeneration and loss of the RPE, and increased numbers of microglia in the subretinal space. (B) Twenty-four hours after light exposure, significant shortening of the IOS length was measured (*$P < 0.05$). Significant thinning of the ONL was measured 7 days after light exposure. Scale bar, 20 $\mu m$.

Microglial and T-Lymphocyte Response to Light Exposure

Microglia in the control rat retina were few and quiescent ($X = 36 \pm 2$ [SEM] cells per section). They had a dendritic morphology with fine processes. They were exclusively localized to the inner retina, in the inner plexiform layer (IPL) and the ganglion cell layer (GCL) (Fig. 2A) often adjacent to blood vessels, and were never observed in the ONL, OPL, IOS, or RPE. This distribution was in agreement with previous reports.

The number, distribution, and morphology of the retinal microglia changed rapidly with blue light exposure. Immediately after light exposure, the number of microglia increased 1.5-fold, and these cells became motile and amoeboid and streamed toward the outer retina (Figs. 2B, 2F). Cells were present in the INL and had a vertical orientation, suggesting motion toward the outer retina. Of the total number of microglia counted in the retina, 13% were already present in the ONL and among the outer segments (Fig. 2G). The total number of microglia increased. One day after light exposure, 44% of the significant INL lesions were detected in rats receiving 6-hour blue light exposure at any postexposure times evaluated.

FIGURE 1. Temporal changes in retinal morphology after blue light exposure. (A) Albino rats were exposed to blue light for 6 hours, and ocular tissues were evaluated immediately after light exposure (0 hr) or after 1, 2, and 7 day postexposure periods in the dark. (Control) Normal retinal morphology. (0 hr) Immediately after blue light exposure, the ONL and photoreceptor inner segments were swollen, and pyknotic cells with condensed chromatin were observed in the ONL (down arrowhead) along with vacuolization in the RPE. One day after light exposure, at least 40% of the cells in the ONL were pyknotic, necrotic, or both, photoreceptor outer segments were shortened and disorganized, and RPE thickness was more variable. Microglia (based on Iba1 staining of paraffin-embedded tissue (see Fig. 2) are seen above the RPE. (down arrowhead, 48 hrs and 1 wk) Two days after light exposure and continuing to the seventh day, there is loss of photoreceptors and thinning of the ONL, IOS thinning and disorganization, degeneration and loss of the RPE, and increased numbers of microglia in the subretinal space. (B) Twenty-four hours after light exposure, significant shortening of the IOS length was measured (*$P < 0.05$). Significant thinning of the ONL was measured 7 days after light exposure. Scale bar, 20 $\mu m$.  

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microglia was found in the ONL, among the outer segments, and in the RPE (Figs. 2C, 2F, 2G). On the second day after light exposure, the number of cells in the retina increased by 5-fold, and, in the outer retina, reached a peak and contained 60% of the total number of cells (Fig. 2D). The number of microglia decreased by day 7 after light exposure but remained greatly elevated (3-fold) over control numbers, and 38% of these cells remained in the outer retina (Fig. 2E). Longer times after blue light exposure were not explored in this study.

This profound effect of photo-oxidative stress on retinal microglia prompted a study of T lymphocytes. Almost no T lymphocytes were observed in the control rat retina (Figs. 3A, 3D, 3E). Although there appears to be a large fold increase of T cells at 2 days after light (Figs. 3B, 3D, 3E), the absolute numbers are low compared with the microglia response. This observation is in marked contradistinction to uveitic models in which abundant T-cell invasion of the retina is seen. Therefore, as a positive control, we intravitreally injected a uveitogenic immunogen (GFP-containing nanoparticles) and saw robust T-cell recruitment to the retina, indicating that the methods used to detect T cells were valid (Fig. 3C).

**Complement Deposition in Response to Light**

C3 is the central protein of the complement cascade, the convergent point of the classical and alternative pathways. It also is the first effector protein of complement. Using an antibody to C3, no immunostaining was observed in control retina (Fig. 4A) or in retina immediately after light exposure. However, 1 day later, the outer retina was strongly stained for C3 (Fig. 4B). C3 was present at the ONL, the outer segments, and the RPE in all retinas evaluated. Staining was apparent on day 2 in all retinas and was somewhat diminished by day 7 after light, a time when the outer retina is considerably thinned (Figs. 4C, 4D).

To determine which upstream and downstream elements of the complement system were involved in the innate immune system’s response to light, immunostaining for several additional complement proteins was performed on control retinas and retinas harvested at different times after light exposure. Substantial labeling of all tested factors from the alternative pathway was observed, with peak labeling at day 2 in all light-exposed retinas evaluated. Representative images of retinas immunostained for complement components at day 2 after light exposure are presented in Figure 5B. C1q, an early active fragment of the classical pathway, was not present in the control retina or in the light-exposed retina. C3 deposition was confirmed, with clear labeling in the outer neural retina and the RPE. Factor B, which on cleavage assembles with C3b to become part of C3 convertase, colocalizes with C3 in the outer retina, including the ONL and the IOS. Factor H, an inhibitory modulator, colocalized with C3 and factor B, as did...
MAC. In summary, after a light-induced photo-oxidative insult, robust deposition of all alternative, but not classical, complement pathway components measured was observed precisely at the site of degeneration (i.e., the somas and processes of photoreceptors and the RPE).

**Effect of a Serotonin 5-HT1A Agonist**

We have previously shown that AL-8309A is a potent 5-HT1A agonist that protects the retina from this severe photo-oxidative stress.33 When experimental animals were treated with the 5-HT1A agonist AL-8309A (three subcutaneous injections of 10 mg/kg over 2 days before and immediately before light exposure and one injection the day after exposure), substantial inhibition of all light-induced immune measures was observed (Fig. 5C). Photo-oxidative–induced microglial activation and recruitment to the outer retina were substantially and significantly inhibited (Fig. 6). In AL-8309A–treated rats, the number of microglia in the whole retina was significantly reduced by more than 2-fold, and the number of microglia in the outer retina was significantly reduced by almost 10-fold compared to rats dosed with a saline vehicle (Figs. 6A, 6B). The small numbers of T-lymphocytes that entered the retina in response to the light exposure was also inhibited. T-lymphocytes were significantly decreased by 7-fold in the whole retina and 2-fold in the outer retina of AL-8309A–treated rats compared with vehicle-treated rats (Figs. 6C, 6D). The deposition of alternative complement components C3, factor B, factor H, and MAC was almost completely inhibited as well (Table 3, Fig. 5C). Retinal lesions or robust immunolabeling were not observed in control rats not exposed to blue light or rats treated with AL-8309A and exposed to blue light for 6 hours. In vehicle-dosed rats, light-induced retinal lesions were observed in 80% of the light-exposed rats, and all retinas with these lesions demonstrated significant staining in the lesion area.

**DISCUSSION**

Although an acute and severe model, light-induced retinopathy has been described as “…the only available model exhibiting all the hallmarks of AMD including choroidal ablation, RPE ablation, photoreceptor cell death and retinal decimation”.

Blue light–damaged rat retinas also exhibit CEP-adduct forma-
of C3, CFB, and CFH was observed by 24 h after light exposure and were detected in all eyes immediately after light exposure. Deposition harvest and analyzed immunohistochemically immediately at the end of light exposure (0d) or after 1, 2, or 7 days.

Numbers provide fractions of retinas exhibiting robust immunolabeling for the indicated complement component. Retinal lesions were detected in all eyes immediately after light exposure. Deposition of C3, CFB, and CFH was observed by 24 h after light exposure and MAC deposition was observed 48 h after light exposure.

### Table 2. Time Course of Complement Deposition in Light-Damaged Retinas

<table>
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<th>Treatment</th>
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<th>Factor H</th>
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<tr>
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</table>

d, days; h, hours; H&E, hematoxylin and eosin.

Rats were treated with blue light for 6 hours (L6h). Eyes were harvested and analyzed immunohistochemically immediately at the end of light exposure (0d) or after 1, 2, or 7 days.

Numbers provide fractions of retinas exhibiting robust immunolabeling for the indicated complement component. Retinal lesions were detected in all eyes immediately after light exposure. Deposition of C3, CFB, and CFH was observed by 24 h after light exposure and MAC deposition was observed 48 h after light exposure.

The present results indicated that two more features of advanced-stage atrophic human AMD were also present in blue light–induced retinopathy (albeit exaggerated in temporal development and intensity): the presence of complement components2 and of microglia31 in the outer retina.

The data presented here demonstrate that a robust activation of innate immunity occurs in the outer retina in response to a severe photo-oxidative insult. Many questions arise concerning how this occurs, its importance for concomitant retinal degeneration, and its potential relationship to human disease.

Microglial activation occurred rapidly in response to blue light exposure. By the end of the light-exposure period, resident inner-retinal microglia appeared to be streaming into the outer retina. Presumably signaling molecules, such as cytokines and chemokines, mediated this migration, but the identity and cellular source of these molecules remain to be established. The number of Iba1-positive cells increased rapidly, peaking at day 2 after light exposure. The origin of these cells was also unclear and in dispute. Did the resident cells proliferate, or was there an invasion by blood-borne macrophages? If the latter, did they originate from the choriocapillaris, inner retina, or both? Ni et al.42 evaluated retinal microglia activation and migration in a blue light damage model and concluded that potential systemic macrophage/microglia infiltrating the outer retina did not occur until 7 days after light exposure, and these cells constituted <1.2% of the total number of microglia in the ONL. No such cells were observed in normal retinas or at 1 and 3 days after light exposure, when the number of activated retinal microglia peaked. Ng and Streilein43 also suggested that in photic injury, microglia migrated from the inner to the outer retina. Differentiating systemic from resident microglia is a difficult question; additional studies are ongoing to address this issue.

Complement deposition occurred later. None was observed immediately after light exposure, and even 24 hours later it had not peaked. Different components appeared with different time courses: core and modulatory components of the C3 convertase (C3, factor B, and factor H) appeared before there was any immunohistochemically detectable terminal MAC (Table 2). What was the source of the complement? There was abundant complement in serum, and it is possible that light damage to the RPE caused a disruption of the blood retinal barrier at that site. Alternatively, there was evidence that macrophages, neural retina, and RPE cells could produce complement. This question may be experimentally approached using in situ hybridization of probes specific for mRNAs coding for complement components.

Our results disagree with those of a previous study,44 which concluded that light damage does not lead to complement deposition in the retina.30 There were several differences between the studies that may account for this difference. We suspect the most likely reason for this discrepancy was that in the study by Montalvo et al.,44 the retinas were processed for immunohistochemical staining immediately after exposure to light. The present results indicate that 1 to 2 days are necessary before robust complement staining is observed in light-damaged retinas. The previous study used Balb/c mice, whereas we used Sprague-Dawley rats. We have not yet examined mice, and perhaps complement deposition does not occur in that species, but we have observed complement deposits in both rabbit and cynomolgus monkey retinas damaged by light (unpublished data, 2009). The other difference is that Montalvo et al.44 used 24-hour exposure to bright white light, and we used 6-hour exposure to blue light. The intensity and spectral quality of the light used to induce damage may be critical factors. A key question that the present study does not address is the role of innate immunity in the pathogenic mechanism of retinal degeneration induced by light. It has been thought that excess light induces the production of reactive oxygen species, leading to tissue damage and apoptotic or necrotic photoreceptor and RPE cell death. Is the microglial activation and complement deposition observed here merely a secondary phenomenon, the recruitment of the sanitation crew to remove the damaged and dying cells? Or are the microglia inciting an inflammatory reaction that induces degeneration? Are the oxidation products formed by light leading to complement upregulation and deposition that then lead to tissue damage? A study by Rohrer et al.45 is pertinent. Mice genetically manipulated to lack the alternative complement component factor D were substantially more resistant to light damage, as assessed with both structural and functional measures, than wild-type controls. These data indicate that complement does participate causally, if partially, in the pathogenesis of light damage. Additional studies using pharmacologic and genetic interventions could be informative. Does a highly efficacious and appropriately bioavailable complement inhibitor protect the retina from light damage? Does genetic elimination of a central component of complement affect the degeneration?

The serotonin 5-HT1A agonist AL-8309A has been shown to potently prevent functional and structural damage to the retina.55 This retinoprotective activity is rapidly activated and persists for up to 48 hours, and this drug is efficacious when administered by a topical ocular route of administration in this blue light–induced retinopathy model.55 The mechanism for this is incompletely understood, but we have shown that both ARPE-19 cells and primary cultures of human RPE cells are protected from oxidative injury by pretreatment with AL-8309 or other 5HT1a agonists (Rhoades KL et al. JOVS 2009;50:ARVO E-Abstract 6777). Treatment of ARPE-19 cells with AL-8309A leads to increased ERK 1/2 phosphorylation and upregulation of antioxidant and antiapoptotic proteins, including SOD-1, SOD-2, Bcl-2, and Bcl-XL. AL-8309 treatment leads to similar changes in vivo (unpublished results, 2008). These data are consistent with results from other CNS systems: 5-HT1A agonists have been shown to activate the mitogen-activated protein kinase (MAPK/ERK) signaling pathway,46,47 leading to increased expression of SOD and catalase,48,49 components of the antioxidant defense system, antiapoptotic proteins (e.g., Bcl-2 and Bcl-XL),50,51 and inhibitors of apoptosis proteins (e.g., XIAP).46,49,51 5-HT1A receptor activation of ERK can also lead to the inhibition of caspase 3.51
It was of interest to determine whether AL-8309A affects the microglial activation and complement deposition in the outer retina that occurs in the blue light model. The present results demonstrate that AL-8309A provided near complete inhibition of both microglia activation/recruitment and complement deposition. This agent is not known to be an immunosuppressant or a complement inhibitor, and preliminary tests have not shown these activities (unpublished data, 2007). Evidence cited here suggests that 5-HT₁A agonist treatment leads to the upregulation of endogenous antioxidative and...
antistress proteins in the RPE and the neural retina. Therefore, we hypothesize that cellular defenses are bolstered and that, in the face of a severe oxidative insult, retinal cells become less damaged and send out fewer distress signals responsible for the activation of innate immunity. The fact that microglial recruitment, the earliest example of immune activation we have seen, is greatly attenuated, suggests that AL-8309A acts at a very early step and is consistent with this hypothesis.

How oxidative stress and innate immunity interact in the development of AMD is unclear. The present results indicate that oxidative stress can be a trigger for the activation of microglia and the complement system, in vivo, in the retina. Similar mechanisms may be operative in human disease. Bolstering retinal cellular defenses with a 5-HT1A agonist limits the damage and subsequent immune activation caused by oxidative stress. This same action may similarly ameliorate the progression of AMD in humans. A clinical trial (Geographic Atrophy Treatment Evaluation [GATE], ClinicalTrials.gov) evaluating the safety and efficacy of topically administered AL-8309B (HCl salt) for the treatment of advanced nonexudative AMD (geographic atrophy) is ongoing.

References


