Expression and Modulation of RPE Cell Membrane Complement Regulatory Proteins

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PURPOSE. Complement, inflammation, and oxidant injury contribute to age-related macular degeneration (AMD). Membrane complement regulatory proteins (mCRPs) such as CD46, CD55, and CD59, protect host cells from complement attack. The factors that regulate RPE mCRP expression are not well understood. In this study, the authors sought to determine whether cytokines and hydroquinone (HQ) affect mCRP expression in cultured human RPE (hRPE) and cultured mouse RPE (mRPE) cells.

METHODS. Cultured hRPE and mRPE cells were stimulated with cytokines for various times or with HQ for multiple 6-hour periods. mRNA and protein expression of mCRPs in cultured hRPE cells from 10 donors, native hRPE, and mouse eyecups and native mRPE cells were evaluated by real-time RT-PCR, Western blot analysis, and flow cytometry, respectively.

RESULTS. Three mCRPs were expressed in cultured hRPE cells (CD59 > CD46 > CD55). CD46 and CD59 protein were detected in native hRPE cells. CD59 protein levels in cultured hRPE cells were higher than in native hRPE cells. CD46 protein polymorphisms were observed in cultured hRPE cells. Cultured hRPE cell mCRP expression was upregulated by TNF-α, IL-1β, and a repetitive nonlethal dose of HQ. CD59a levels were higher in mouse eyecups than in nonocular tissues. Mouse mCRP mRNA and protein were detected in native mRPE cells. Responsiveness to cytokines in cultured mRPE cells differed from that in cultured hRPE cells.

CONCLUSIONS. Human and mouse RPE cell mCRPs are upregulated by inflammatory cytokines and repetitive nonlethal oxidant exposure in a species-specific manner. Increased cell mCRPs may help to protect RPE cells from complement- and oxidant-mediated injury in diseases such as AMD. (Invest Ophthalmol Vis Sci. 2009;50:3473–3481) DOI:10.1167/iovs.08-3202

The complement cascade plays a central role in the modulation of inflammatory responses. In addition to a variety of soluble regulatory proteins, cells express several key membrane complement regulatory proteins (mCRPs) including membrane cofactor protein (MC; CD46), decay-accelerating factor (DAF; CD55), and membrane inhibitor of reactive lysis (MIRL; CD59) to prevent host tissue bystander damage after complement activation. CD46 and CD55 act early in complement activation to disable the central amplification enzymes C3 and C5 convertases. CD59 functions later in the cascade to prevent membrane attack complex formation by inhibiting the incorporation of C9.

In studies to investigate the in vivo functions of mCRP proteins, corresponding CD46, CD55, and CD59 orthologues have been identified in the mouse. The CD55 and CD59 genes are duplicated in the mouse (daf-1, daf-2, cd59a, cd59b). In addition, a unique rodent transmembrane protein known as Crry (complement-receptor 1-related gene/protein y) has both CD46 and CD55 activities and is considered a functional homologue of human CD46. The Crry−/− mouse is an embryonic lethal phenotype. Although there are several noticeable differences between human and mouse mCRPs, the generation of CD55/59 double-deficient (CD55−/−CD59−/−) mice has provided insight into the function of these proteins in vivo.

Posterior segment mCRP expression and distribution in human eyes has been described. However, two previous reports using immunohistochemical studies differ on the distribution of CD55 and CD59 in the human retina. It is unclear whether freshly isolated native human RPE (hRPE) cells and cultured hRPE cells express CD55 and CD59. The expression and importance of Crry and CD59a have been reported in rodent models associated with posterior diseases such as laser-induced choroidal neovascularization, and adenovirus-delivered human CD59 is reported to prevent cultured mouse RPE (mRPE) cells from human membrane attack complex deposition and vesiculation. Further, the effect of cytokines on mCRP expression has been reported in human orbital fibroblasts and nonocular cells. However, no consistent pattern of response to cytokines has been observed. To our knowledge, the effect of cytokines, such as those found in the RPE microenvironment in AMD, on RPE mCRP expression, has not been investigated.

In the present study, we investigated whether proinflammatory cytokines, which can be generated in an AMD microenvironment, and hydroquinone (HQ), an important oxidant in cigarette smoke, modulate the expression of mCRPs in cultured hRPE cells. We also sought to determine whether mCRP modulation in cultured hRPE cells differs from that in cultured mRPE cells.

MATERIALS AND METHODS

Human Cell Culture and Treatment

Human donor eyes were collected (North Carolina Organ Donor and Eye Bank, Inc., Winston-Salem, NC) from 95 to 345 minutes (174.5 ± 109.3) after death and were cultured within 24 hours of death in accordance with the provisions of the Declaration of Helsinki for research involving human tissue. RPE cells for culture studies were
harvested from eyes as previously described. Cells were grown in Eagle's minimal essential medium (MEM; Invitrogen, Carlsbad, CA) with 10% fetal bovine serum (FBS; Hyclone Laboratories, Logan, UT) and with 100 U/mL penicillin and 100 μg/mL streptomycin sulfate (Invitrogen) at 37°C in a humidified environment containing 5% CO₂. In addition, ARPE-19 cells (continuous RPE cell line; a generous gift from Leonard Hjelmeland, University of California at Davis), and HeLa cells (American Type Culture Collection, Rockville, MD) were also grown in MEM containing 10% FBS. The culture details of human lens epithelial cells, human trabecular meshwork cells, human choroidal endothelial cells, and human uveal melanoma cells including MKT-BR, OCM-1, and 92.1 were as we have previously described. Endothelial cells, and human uveal melanoma cells including MKT-BR, OCM-1, and 92.1 were as we have previously described.

**mRPE Cell Culture and Treatment**

C57BL/6 (wild-type [WT]) mice were purchased from the Jackson Laboratory (Bar Harbor, ME). The guidelines of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research were followed, and the study was approved by the Institutional Animal Care and Use Committee of Duke University. mRPE cells were isolated and cultured based on methods previously described for humans and mice with slight modification. Briefly, intact eyes were washed twice with PBS containing 100 U/mL penicillin and 100 μg/mL streptomycin sulfate (Invitrogen). The eyes were cut posterior to the ora serrata to remove the anterior segments. Eyecups were washed with PBS, and the retina was gently removed. The eyecups were rinsed in chelating agent (Versene; Invitrogen), and the RPE cells were enzymatically dislodged using 0.25% trypsin-EDTA (Invitrogen). Pooled RPE cells of WT mice were maintained in Dulbecco MEM (DMEM) containing 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin sulfate (Invitrogen) at 37°C in a humidified environment containing 5% CO₂. Fourth-passage cells were examined with antibody to cytokeratin 18 (Sigma, St. Louis, MO) by Western blot analysis and were used for the experiments. Experimental treatments were the same as those described for cultured hRPE cells. Recombinant mouse TNF-α (mTNF-α; 22 ng/mL; R&D Systems Inc.) was used as an additional RPE cell inducer. To determine whether mRPE cells responses to cytokine treatment in young mice differ from those in middle-aged mice, we selected mice at different ages. Experiments were repeated three times using mice with different ages (see Fig. 10 legend) and both sexes.

**Real-Time RT-PCR Analysis**

Total RNA was isolated with a kit (RNAeasy Plus Mini Kit; Qiagen Inc., Valencia, CA) according to the manufacturer’s specifications, and real-time quantitative reverse-transcription–polymerase chain reaction (qPCR) was performed as we have previously described. Briefly, duplicate reactions were prepared with 18 μL PCR master mix consisting of 9 μL master mix (iQ SYBR Green Supermix; Bio-Rad, Hercules, CA), 1 μL cDNA template, 1 μL each of gene-specific primer pairs (Table 1), and 6 μL RNase-free water. Reactions were denatured at 95°C for 2 minutes and amplified for 50 cycles at 95°C for 15 seconds, 60°C for 15 seconds, and 72°C for 15 seconds. Real-time quantification of mCRP genes was normalized to the threshold cycle (Cₚ) value of either human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or mouse peptidyl-prolyl cis-trans isomerase A in the corresponding species, where Cₚ equals the PCR cycle number at which the amount of amplified sample product reached 100 relative fluorescence units. Fold difference of mCRP expression, relative to HeLa cells, nontreated cells, or mouse testis expression, was calculated by comparing Cₚ (2^{-ΔΔCp}). A melting curve for all products was obtained immediately after amplification by increasing the temperature in 0.4°C increments from 65°C for 85 cycles of 10 seconds each. The experiments were separately repeated three times with similar results.

**Protein Extraction from Native hRPE Cells**

Eyes were obtained from donors without any known ocular diseases (donor 1, 65-year-old woman; donor 2, 57-year-old man; donor 3, 60-year-old woman). After the neural retina was removed, 0.15 mL mammalian protein extraction reagent (Pierce, Rockford, IL) containing protease inhibitor cocktail (Roche, Indianapolis, IN) was added to the eyecup, and the RPE was gently scraped off the Bruch’s membrane with a rubber policeman. Another 0.05 mL extraction reagent was added to rinse the eyecup and then was collected. Combined lysates were sonicated for Western blot analysis.

**Western Blot Analysis**

Cell extracts were prepared, and Western blot analysis was performed as we have previously described. Samples were run in nonreducing sample buffer, and membranes were incubated overnight at 4°C with the following antibodies purchased from AbD Serotec (Raleigh, NC) in 3% milk: mouse antibody directed against human CD46 (MCA2113, 1:1000), CD55 (MCA1614GA, 1:1000), and rat antibody directed against human CD59 (MCA715, 1:1000). The following antibodies directed against pan-cytokeratin (1:1000), CD55 (MCA1614GA, 1:1000), and rat antibody directed against pan-cytokeratin (1:1000; Sigma), cytokeratin 18 (1:1000; Sigma), GPDH (1:10,000; Chemicon, Temecula, CA), and β-actin (1:6000; Santa Cruz Biotechnology Inc., Santa Cruz, CA). For mouse tissue, mouse antibody against mouse CD59a (7A6), generously provided by B. Paul Morgan (University of Wales College of Medicine, Cardiff, UK), was diluted in 3% milk (1:1000). The blots were then washed three times (20 minutes per wash) in Tris-buffered saline containing 0.1% Tween-20 and were incubated with anti–mouse or anti-rat IgG conjugated with horseradish peroxidase (1:5000 in 3% milk; Jackson ImmunoResearch Laboratories Inc., West Grove, PA) for 60 minutes at room temperature (RT). Immunoreactive bands were visualized using an enhanced chemiluminescence light detection kit (Amersham, Piscataway, NJ).

**Flow Cytometry**

Measurement of mCRP surface proteins by flow cytometry was performed according to the recommended protocol by AbD Serotec (#02, 20056).

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indirect immunofluorescence staining for flow cytometry). Briefly, 100 
µL of cells (1 × 10^6 cells/mL) in 1% bovine serum albumin (BSA)/PBS were 
incubated with CD46, CD55, and CD59 antibodies (AbD Serotec) at the 
recommended dilution (1:10) at RT for 30 minutes, then washed with 
2 mL of 1% BSA/PBS. The cells were centrifuged at 400g for 
5 minutes, and the supernatant was discarded. The cells were incubated 
with fluorescein (FITC)-conjugated rabbit anti–mouse/rat IgG antibody 
(1:50; Jackson ImmunoResearch Laboratories, Inc.) at RT for 30 min-
utes and were washed with 2 mL of 1% BSA/PBS. After centrifuging at 
400g for 5 minutes, the cells were resuspended in 0.2 mL of 0.5% 
paraformaldehyde in PBS for flow cytometry analysis.

**mRNA and Protein Extraction from Mouse Posterior Segment and Native mRPE Cells**

CD55−/−CD59−/− mice were provided by Wen-Chao Song (University of 
Pennsylvania School of Medicine, Philadelphia, PA). The original 
CD55−/− and CD59−/− mice were created in C57BL/6 to a 129J 
background. They had been back-crossed with C57BL/6J mice for nine 
generations. Mice were euthanatized, and the eyes were immediately 
removed with PBS, the RPE cells were scraped from the Bruch's membrane in PBS 
again with PBS, and the retina was gently removed. After washing with 
0.5% paraformaldehyde in PBS for flow cytometry analysis.

Native mRPE cells were isolated based on published methods with 
modifications. RPE cells were obtained from 10 eyes of WT and 
CD55−/−CD59−/− mice, respectively, and were pooled for total RNA 
and protein extraction, respectively. Six eyes and three eyes were 
incubated with 2% dispase for 5 minutes. Eyecups were washed with PBS three times and then incubated with Alexa Fluor 647 or Alexa Fluor 594–conjugated goat anti–rat/mouse IgG antibody (1:500, Invitrogen) for 1 hour at RT. Slides were washed with PBS three times and then incubated with Alexa Fluor 647 or Alexa Fluor 594–conjugated goat anti–rat/mouse IgG antibody (1:500, Invitrogen) for 1 hour at RT. Slides were washed with PBS and incubated with 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI; Sigma) for 3 minutes. Fluorescent stain was observed with a confocal microscope (C90i; Nikon, Garden City, NY) equipped for epifluorescence.

**Immunofluorescent Localization of mCRPs in Mouse Posterior Segment**

After the eyes were enucleated, a corneal incision was created, and the 
eyes were then fixed in freshly prepared 4% paraformaldehyde for 3 
hours. Cornea and lens were removed, and posterior segments were 
fixed in 1% paraformaldehyde overnight. Six-micrometer cryosections 
were fixed with cold acetone for 5 minutes, rehydrated with PBS, and 
blocked with 5% normal goat serum in 1% BSA/PBS at 4°C overnight. Sections were washed with PBS three times and then incubated with Alexa Fluor 647 or Alexa Fluor 594–conjugated goat anti–rat/mouse IgG antibody (1:500, Invitrogen) for 1 hour at RT. Slides were washed with PBS and incubated with 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI; Sigma) for 3 minutes. Fluorescent stain was observed with a confocal microscope (C90i; Nikon, Garden City, NY) equipped for epifluorescence.

**Statistical Analysis**

Data are expressed as mean ± SD. Multiple comparisons were per-
formed on the data (see Figs. 3, 4). ANOVA was performed on these 
data to identify statistically significant differences among groups. Com-
parisons between two groups are shown (see Fig. 5). Accordingly, these 
data were analyzed for statistically significant differences with a 
two-tailed t-test. Analysis of relative gene expression using qPCR was 
performed as we and others have previously described. P < 0.05 
was considered statistically significant.

![Figure 1](https://iovs.arvojournals.org/figure/1A.png)

**Figure 1.** Levels of human mCRPs in cultured ocular cells. (A) RNA 
from cultured hRPE cells, other human ocular cell types, and HeLa cells 
was extracted, reverse transcribed to cDNA, amplified with CD46-, CD55-, 
and CD59-specific primer pairs, and quantified by qPCR. Expression of 
three genes was calculated relative to expression of HeLa cells using 
2^−ΔΔCt. Ages of RPE cell donors 1 to 10 were 7, 31, 61, 33, 48, 61, 17, 58, 
51, and 48 years, respectively. (B) Cultured hRPE cells were stained 
with CD46, CD55, and CD59 primary antibodies and FITC-conjugated rab-
bit anti–mouse or anti–rat secondary antibodies and were resuspended in 
0.5% paraformaldehyde and assayed by flow cytometry.
cells were probed with antibody to CD46 and were reprobed with GAPDH. Donors in lanes 1–5 correspond to donors 1–5 in Figure 1A, and donors in lanes 7–11 correspond to donors 6–10 in Figure 1B. The donor in lane 6 was 50 years old.

RESULTS

Levels of CD46, CD55, and CD59 in hRPE Cells and Ocular Cells

To determine the abundance of CD46, CD55, and CD59 mRNA in cultured hRPE cells and to compare these levels with those in other ocular cell types, qPCR was performed on samples from 10 human donors, with HeLa cells as a positive control. In cultured hRPE cells, CD59 expression was highest, CD46 was intermediate, and CD55 expression was lowest among the three mCRPs tested. Human choroidal endothelial cells expressed relatively high levels of CD55 compared with cultured hRPE cells, and CD59 expression was lower in lens and human trabecular meshwork cells compared with cultured hRPE cells, human choroidal endothelial cells, and ocular melanoma cells (MKT-BR and 92.1; Fig. 1A).

Flow cytometry was performed to determine whether the corresponding mCRP cell surface proteins were expressed on cultured hRPE cells. As shown in Figure 1B, the mCRP cell surface protein expression pattern was similar to the mRNA expression pattern.

To determine whether hRPE mCRP protein levels observed in vitro were mirrored by hRPE cell levels in situ, we next performed Western blot analysis on freshly isolated native hRPE cells. HeLa cells were used as a positive control. Notably, there were doublets of CD46 in one donor and in cultured hRPE cells compared with the single CD46 band seen in the other two donors (Fig. 2A).

To further characterize the CD46 doublets, we ran samples collected from ARPE-19 cells and cultured hRPE cells from 11 human donors—WT (CFHYH402), heterozygous (CFHHY402), or homozygous (CFHHH402)—with respect to the Y402H complement factor H (CFH) variant. Interestingly, strong doublets of CD46 protein were observed in the cultured hRPE cells from donors with the CFHHH402 and CFHYH402 variants (Fig. 2B).

Upregulation of Human mCRP Expression by Cytokines and HQ in Cultured hRPE Cells

To evaluate whether proinflammatory cytokines affect human mCRP mRNA expression, we first looked at CD46 and CD59, which were expressed in native and cultured hRPE cells. TNF-α significantly increased CD59 mRNA expression on days 1, 2, and 3 after stimulation. IL-1β significantly increased CD59 mRNA expression on day 2 and day 3 after stimulation (Fig. 3). Total CD46 and CD59 protein levels were not affected by TNF-α or IL-1β stimulation by Western blot analysis (data not shown).

Total proteins were prepared for Western blot analysis, but this method is not optimal to detect changes in cell surface protein levels. To further determine whether human mCRP surface proteins were affected by cytokines, flow cytometry was used. As shown in Figure 4, TNF-α significantly increased CD59 surface protein expression on days 2 and 3 after stimulation and only significantly increased CD46 on day 3 after treatment. IL-1β significantly increased CD46 and CD59 3 days after stimulation (Fig. 4).

HQ is an important oxidant in cigarette smoke. When cells were exposed to repetitive nonlethal doses of HQ, cell surface CD46, CD55, and CD59 protein levels were significantly increased (Fig. 5).
Expression and Distribution of Mouse mCRP mRNA and Protein in Mouse Posterior Segment and Native mRPE Cells

Although the expression and distribution of mCRPs differ in humans and mice,1,11 mouse models remain a useful tool to investigate the role of these proteins. Before we evaluated mouse mCRPs in specific mouse posterior segment tissues, we first determined mouse mCRPs in whole eyecups. As shown in Figures 6A and 6B, CD59a mRNA and protein were more abundant in eyecups than in testis and kidney in WT mice. CD59a mRNA expression and protein were not detected in the eyecups and nonocular tissues of CD55+/−/CD59−/− mice. As expected, CD59b mRNA was detected only in WT and CD55+/−/CD59−/− testis, but not in eyecups. CD59 was strongly detected in photoreceptors and outer plexiform layers and weakly in RPE layers of WT mice but were not detected in CD55+/−/CD59−/− retinas (Figs. 6C, 6D). Staining was not seen after treatment with phosphatidylinositol-specific phospholipase C, which cleaves the glycosylphosphatidylinositol (GPI)-anchored protein (data not shown).

As shown in Figure 7, GPI-CD55 mRNA and protein were detected only in the WT testis but not in WT eyecups, CD55+/−/CD59−/− eyecup, or CD55+/−/CD59−/− testis. As expected, transmembrane (TM)-CD55 was detected only in WT and CD55+/−/CD59−/− testis.

Cry mRNA was observed in the WT eyecup at higher levels than in WT testis, and at even higher levels in the CD55+/−/CD59−/− eyecup (Fig. 8A). Cry was detected in the ganglion cell, inner plexiform, inner nuclear, outer plexiform, and RPE layers (Figs. 8B, 8C). The kidney was stained with Cry as a positive control for the antibody used on ocular tissues. The staining pattern of Cry in glomeruli and tubules was consistent with that reported in previous publications (not shown).

Based on the preceding set of experiments, CD59a and Cry levels were higher in the WT mouse eyecup than in nonocular tissues. We next determined whether these mCRPs were expressed in freshly isolated native mRPE cells. mRNA expression of rhodopsin (retina marker), RPE65 (RPE marker), and fibroblast specific protein-1 (choroid marker) indicated the relative purity of RPE cells in qPCR (Fig. 9A). As shown in Figure 9, Crry and CD59a mRNA and CD59a protein were expressed in freshly isolated native mRPE cells.

Upregulation of GPI-CD55 mRNA Expression by Cytokines in Cultured mRPE Cells

To investigate whether proinflammatory cytokines affect mouse mCRP expression in cultured mRPE cells and to provide insight into mouse mCRP function in an in vitro system, we stimulated cultured mRPE cells with hTNF-α, hIL-1β, and mTNF-α. GPI-CD55 mRNA expression was upregulated by hTNF-α and hIL-1β in a time-dependent manner. mTNF-α also upregulated GPI-CD55, which peaked at 48 hours and persisted for 72 hours (Fig. 10). In contrast to cytokine regulation of mouse CD55 and human CD59 and CD46, mouse CD59 and Cry expression levels were not altered by cytokine stimulation in cultured mRPE cells (Fig. 10).

Discussion

In this study, we determined the expression and regulation by cytokines and oxidants of human and mouse RPE mCRPs. We have demonstrated for the first time that cultured hRPE CD59 mRNA and cell surface protein expression are higher than
those of CD46 and that CD55 expression is lowest among the three mCRPs tested. CD59 protein expression in cultured hRPE cells was higher than in native isolated hRPE cells. CD46, CD55, and CD59 expression were upregulated by inflammatory cytokines and repetitive nonlethal oxidant HQ in cultured hRPE cells. The distribution and regulation of these mCRPs vary in a species-specific manner.

Human CD59 is a GPI-anchored glycoprotein widely distributed throughout the body. To date, cultured hRPE cell CD59 mRNA and protein expression have not been reported. Previous studies differ on the distribution of CD59 protein in the human retina. One group observed that CD59 is highly expressed in almost every layer of human retina, though the

**Figure 6.** Expression and distribution of CD59 in mouse posterior segment. Total RNA (A) and proteins (B) were extracted from eyecup, testis, and kidney of WT and CD59−/− CD59−/− (KO) mice. mRNA expression (A) and protein levels (B) were determined by qPCR and Western blot analysis, respectively. WT1, WT2, KO1, and KO2 represented different mice. (C, D) Distributions of CD59. Red: CD59 (1:300). Blue: DAPI (nuclei stain). *CD59 stain. Scale bars: 20 μM (C), 10 μM (D). Control, PBS alone without primary antibody. All experiments were repeated three times in different mice with similar results.

**Figure 7.** Expression of CD55 in mouse posterior segment. (A) RNA was extracted from eyecup and testis of WT and CD55−/− CD59−/− (KO) mice, reverse transcribed to cDNA, amplified with daf 1 (GPI-CD55) and daf 2 (TM-CD55) primer pairs, and quantified by qPCR. Experiments were repeated three times in different mice with similar results. (B) Proteins from eyecup and testis of WT and CD55−/− CD59−/− (KO) mice in nonreducing sample buffer were separated by SDS-PAGE, transferred to a nitrocellulose membrane, and probed with antibody to CD55. Experiments were repeated twice in different mice with similar results.

**Figure 8.** Expression and distribution of Crry in mouse posterior segment. (A) mRNA was extracted from eyecup and testis of WT and CD55−/− CD59−/− (KO) mice, reverse transcribed to cDNA, amplified with Crry primer pairs, and quantified by qPCR. Experiments were repeated three times in different mice with similar results. (B) Sections of eyecup from WT mice were obtained. Red: Crry (1:200). Blue: DAPI (nuclei stain). Arrowheads: Crry stain. Control, PBS alone without primary antibody. Scale bars: 10 μM (B), 5 μM (C). Experiments were repeated three times in different mice with similar results.
RPE layer was not shown. Another group indicated that CD59 is expressed throughout the nerve fiber layer and is not detected in the RPE. Interestingly, in our study, CD59 mRNA and protein were expressed in cultured hRPE cells at relatively high levels, whereas CD59 protein was detected at low levels in freshly isolated native hRPE cells from three donors. This result suggests that CD59 mCRP expression in vitro may differ from that in an organism. Given that several different culture systems have been used as AMD models, our data further support the necessity of confirming observations and results obtained from in vitro systems in an in vivo system.

Low CD59 levels have been associated with human disease. For example, it has been suggested that the low abundance of CD59 on neurons may be associated with neurodegeneration in Alzheimer disease. Whether the relatively low level of CD59 in RPE cells observed in situ contributes to RPE loss in diseases such as AMD remains to be investigated. Regardless, the relatively high level of CD59 in cultured hRPE cells observed in this study may protect cultured hRPE cells from complement-mediated attack in an in vitro system.

Human CD46 is a transmembrane glycoprotein with a characteristic doublet (58 – 68 kDa and 48 – 56 kDa) pattern on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in a variety of human tissues and cell lines. Three patterns of CD46 appear in human peripheral blood mononuclear cells: upper band predominant (U) phenotype, 65%; approximately equal distribution (E) of upper and lower bands, 29%; and lower band predominant protein (L) phenotype, 6%. It is thought that these two CD46 isoforms are structurally and functionally similar and are produced by alternative splicing of CD46 mRNA in an area coding for the serine/threonine/proline-rich region or for the cytoplasmic tail. CD46 from human granulocytes appears only as a single broad band at 56 to 80 kDa and has less affinity for complement component C3b. In the present report, we observed strong CD46 doublets in cultured hRPE cells from donors with the CFHHH402 and CFHYH402 variant. Increased risk for AMD has been also observed in patients with the CFHHH402 and CFHYH402 variant. It is unknown whether RPE CD46 affinity for C3b in patients with the CFHYY402 variants is different from that with CFHHH402 and CFHYH402 variants or whether these differences influence the pathogenesis of AMD.

The responsiveness of mCRP to cytokines in cultured hRPE cells differs from that observed in other ocular cells (orbital fibroblast and human uveal melanoma cell lines) and nonocular...
CD46 and CD59 were upregulated by TNF-α and IL-1β in cultured hRPE cells. In contrast, CD46 and CD59 are largely unaffected by cytokines in a variety of cultured human cells. CD46 and CD59 are often low, expression is increased on large- and small-arterioles, and, as we have demonstrated previously, they can upregulate RPE cell survival protein expression. These activities could serve a protective function in diseases such as AMD. On the other hand, cytokines such as TNF-α have been implicated in AMD development, for example by serving as an angiogenic stimulus. The RPE cell microenvironment is complex, and the ultimate influence of inflammatory cytokines on AMD likely depends on the relative balance between protective mechanisms and disease-inducing pathways.

Complement activation has been observed after oxidative stress in vitro and animal models. Most of these studies focused on oxidative stress in ischemia-reperfusion injuries and indicated that the lectin pathway plays an important role in nonocular systems. Other evidence suggests that oxidative stress can influence complement function and levels of complement regulatory proteins. For example, smoking decreases plasma CFH levels, and smoke-modified C3 has diminished binding to CFH. Hydrogen peroxide (H2O2) increases iC3b deposition in human umbilical vein endothelial cells. The upregulation of mCRPs by repetitive nonlethal oxidant exposure may help to protect RPE cells from complement-mediated injury.

Mouse models are useful tools to investigate the function of mCRPs in vivo. Humans and rodents differ in their tissue distribution of mCRPs. Mouse expression of cd59a and cd59b genes are expressed only in the mouse testis, whereas daf-1 and cd59a are expressed broadly. Mouse CD46 expression is restricted to the testis, and Crry is ubiquitously expressed in the mouse.

In this study, we showed that CD59a mRNA and protein are more abundant in the WT eye cup than in nonocular tissues, suggesting that CD59a may play an important role in protecting the mouse posterior segment from complement-mediated injury. The importance of retinal CD59a has been previously reported in the mouse laser-induced choroidal neovascularization model. However, an in vivo role for CD59 in complement-mediated RPE cell injury, for example in a dry AMD model, has yet to be described.

The modulation of cytokines on mCRPs in cultured mRPE cells differs from that in cultured hRPE cells. CD46 and CD59 were upregulated by TNF-α and IL-1β in cultured hRPE, whereas only GPR-CD55 was upregulated by cytokines in cultured mRPE. Cytokine upregulation of CD55 but not CD46 and CD59 has been reported in other cultured human cells and immortalized murine cardiac endothelial cells. Although the expression of CD55 on resting endothelium in situ is often low, expression is increased on large- and small-vessel endothelial cells in vitro. Collectively, the findings indicate that CD55 expression is regulated differently in cells and tissues from different organs in a species-specific manner. Furthermore, CD55 is regulated differently than CD46 and CD59. The data reported here point to an important role for murine, but not human, CD55 as a complement regulator in addition to that reported for Crry. Taken together, our data indicate that CD59 and Crry may protect mRPE cells under noninflammatory conditions and that CD55 may enhance mRPE cytoprotection during subacute and chronic inflammation.

The results from the present study suggest that the upregulation of RPE mCRPs by proinflammatory cytokines and repetitive nonlethal oxidant exposure may help to protect RPE cells from complement-mediated injury in healthy persons and in those with diseases such as AMD. However, the relative importance of these, and other complement regulatory molecules, remains to be determined. Further in vivo studies are under way in our laboratory to gain better understanding of the function of these molecules to protect RPE cells from complement-mediated injury.

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References
