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Complement Regulatory Activity of Normal Human Intraocular Fluid Is Mediated by MCP, DAF, and CD59

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Abstract

Purpose

To identify the molecules in normal human intraocular fluid (aqueous humor and vitreous) that inhibit the functional activity of the complement system.

Methods

Aqueous humor and vitreous were obtained from patients with noninflammatory ocular disease at the time of surgery. Samples were incubated with normal human serum (NHS), and the mixture assayed for inhibition of the classical and alternative complement pathways using standard CH_{50} and AH_{50} hemolytic assays, respectively. Both aqueous humor and vitreous were fractionated by microconcentrators and size exclusion column chromatography. The inhibitory molecules were identified

by immunoblotting as well as by studying the effect of depletion of membrane cofactor protein (MCP), decay-accelerating factor (DAF), and CD59 on inhibitory activity.

Results

Both aqueous humor and vitreous inhibited the activity of the classical pathway (CH_{50}). Microcentrifugation revealed the major inhibitory activity resided in the fraction with an $M_r \geq 3$ kDa. Chromatography on an S-100-HR column demonstrated that the most potent inhibition was associated with the high-molecular-weight fractions (≥ 19.5 kDa). In contrast to unfractionated aqueous and vitreous, fractions with an $M_r \geq 3$ kDa also had an inhibitory effect on the alternative pathway activity (AH_{50}). The complement regulatory activity in normal human intraocular fluid was partially blocked by monoclonal antibodies against MCP, DAF, and CD59. Immunoblot analysis confirmed the presence of these three molecules in normal intraocular fluid.

Conclusions

Our results demonstrate that normal human intraocular fluid (aqueous humor and vitreous) contains complement inhibitory factors. Furthermore, the high-molecular-weight factors appear to be the soluble forms of MCP, DAF, and CD59.

The complement system is one of the major immunosurveillance mechanisms in humans.[1–3] Activation of complement is an important component in the inflammatory response to injury and leads to the production of biologically active molecules.[1–3] Therefore, complement activation is strictly controlled by several complement regulatory proteins to limit damage to host tissue. Human tissue and organs are protected from the autologous complement system through the activity of membrane regulatory proteins such as decay acceleration factor (DAF, CD55), membrane cofactor protein (MCP, CD46), membrane inhibitor of reactive lysis (MIRL, CD59), and membrane attack complex inhibitory protein (MIP).[4–10] Soluble forms of MCP, DAF, CD59, and MIP have been reported in human serum and other biological fluids.[11–23] The origin of these soluble proteins is uncertain; they

may be the products of proteolysis of membrane-bound proteins, alternative splicing of mRNA, or posttranslational modification.[11–23]

The human eye is an immunologically privileged site with an important role assigned to immunosuppressive factors present in normal intraocular fluids.[24,25] It is largely isolated from circulating cells and proteins of the immune system, such as plasma complement. The presence of a functionally active complement system in the eye has been the subject of controversy and the role of complement in the host defense of ocular tissue is not well understood. Several reports in the literature have identified the classical and alternative complement pathways in the cornea, aqueous humor, and tears.[18,26–35]

During an inflammatory reaction, the eye is potentially threatened by homologous complement attack, and unregulated complement activation would be detrimental to ocular tissue. With the use of immunohistochemical technique we[36] and others[37,38] have demonstrated the presence of three membrane-bound complement regulatory proteins—MCP, DAF, and CD59—that are differentially expressed in the normal human eye. This differential expression is of interest because it contrasts with the rather uniform expression of these molecules on peripheral blood and epithelial cells.[5–10] Identification of MCP, DAF, and CD59 in the cornea and inner structures of the human eye suggests that a regulatory system exists to protect these cells from destruction by complement activation. In the absence of these proteins, C3 convertase of both the classical and alternative pathways as well as membrane attack complex (MAC) would be deposited on ocular cells, leading to tissue damage. Thus, control of complement activation during an inflammatory response is important to prevent inadvertent damage to the eye.

In the present study we examined normal human aqueous and vitreous fluids for their ability to inhibit the functional activity of the complement system. We then identified the complement regulatory factors present in these fluids.

Materials and Methods

Reagents

Sterile sheep/rabbit whole blood preserved in Alsevers solution and

polyclonal rabbit anti-sheep erythrocytes (hemolysin) were purchased from Rockland (Gilbertsville, PA). Sephacryl S-100 HR, Ponceau-S, and Bradfdord reagent were obtained from Sigma Chemical Company (St. Louis, MO), and Micron-3 microconcentrator was from Amicon Inc. (Beverly, MA).

Antibodies

Monoclonal antibodies to human MCP (GB24, mouse IgG1) and DAF (IA10, mouse IgG2a) were kindly provided by John P. Atkinson, Department of Internal Medicine, Washington University School of Medicine, St. Louis, MO. DAF monoclonal antibody (1H4, mouse IgG1) and mouse anti-human CD59 (10G10) were a generous gift from Douglas M. Lublin, Department of Pathology, Washington University School of Medicine, St. Louis, MO. Neutralizing antibodies to human CD59 (MEM 43), MCP monoclonal antibody J4-48 (mouse IgG1), and DAF monoclonal antibody BRIC 110 (mouse IgG1) were from Accurate Chemical and Scientific Corp. (Westburg, NY). One mouse monoclonal antibody to human MCP, E4.3 (IgG2a) was purchased from Pharmingen (San Diego, CA). Control monoclonal antibodies, MOPC-21 (mouse IgG1) and UPC-10 (mouse IgG, 2a) were purchased from Sigma Chemical Company.

Patient Population

Specimens of aqueous humor (n = 49) and vitreous (n = 49) were obtained from patients with noninflammatory ocular diseases, such as macular pucker (n = 7), age-related macular degeneration (n = 34), and macular hole (n = 8). This patient population had no intraocular inflammation or abnormalities of the iris or retinal vasculature, which would result in increased vascular permeability. They underwent surgery for the underlying disease, and specimens were obtained at the start of intraocular surgery. The presence of a normal lens or pseudophakia ensured separation of the aqueous humor in the anterior chamber from the vitreous in the vitreous cavity. Samples (100–200 μl of aqueous humor and 200 to 300 μl of vitreous) were obtained undiluted and used immediately or stored at −80°C.

All human studies were performed following the guidelines of the Declaration of Helsinki and were approved by our institutional review board. Informed written consent was obtained from all patients before inclusion in the study.

Complement Inhibitory Activity

Aqueous humor and vitreous samples obtained from normal human patients were incubated with normal human serum (NHS) at 37°C for 2 hours. The mixture (50 μl NHS + 25 μl aqueous/vitreous; final concentration ~33.3%) was assayed for inhibition of the classical and alternative complement pathways using the standard CH_{50} and AH_{50} hemolytic assays. In these assays NHS obtained from normal healthy subjects was used as the source of complement and was stored in aliquots at −80°C. NHS was also treated with fractions obtained after microcentrifugation and gel filtration column, as described above. Total complement activity in aqueous and vitreous alone was also determined.

CH_{50} Assay

CH_{50} assay was used following the method described in the literature[39,40] and utilizes sheep erythrocytes (SRBC) as target cells. Briefly, a suspension containing 1×10^9 SRBC/ml was prepared in the GVB^{2+} buffer (gelatin/Veronal-buffered saline with Ca^{2+} and Mg^{2+}), pH 7.35. Hemolysin (rabbit anti-sheep antiserum) was titrated to determine the optimal dilution to sensitize SRBC. Diluted hemolysin (1:800) mixed with an equal volume of SRBC (1×10^9 SRBC/ml), and the whole was incubated at 37°C for 15 minutes. This resulted in 5×10^8/ml antibody-coated erythrocytes (EA). EA (100 μl) were incubated with 100 μl of five serial twofold dilutions (1:20, 1:40, 1:80, 1:160, and 1:320) of the NHS or similar dilution of the mixture of NHS and the test sample at 37°C for 1 hour. Test sample is defined as unfractionated aqueous/vitreous, filtrate, and retain obtained after microconcentration as well as peaks 1, 2, and 3 obtained after size exclusion column. NHS incubated with GVB^{2+} buffer was used as the control. Background control was obtained by incubating EA with buffer alone (serum was not added), and total lysis (100% hemolysis) was determined by adding distilled water to EA. The reaction was stopped using 1.2 ml of ice-cold 0.15 M NaCl, the mixture was spun to pellet the unlysed cells, and the optical density of the supernatant was determined spectrophotometrically (412 nm). The percentage of hemolysis was determined relative to the 100% lysis control. Background control did not exceed 10% of total lysis. The results of the assay were calculated as described previously.[39,40] Complement activity was quantitated by determining the serum dilution required to lyse 50% of

cells in the assay mixture. The results were expressed as the reciprocal of this dilution in CH_{50} units/ml of serum.

AH_{50} Assay

AH_{50} assay was carried out using the standard methods described in the literature,[39,41] which depend on lysis of unsensitized rabbit erythrocytes (Erab) by human serum by activation of the alternative pathway. Activation of the calcium-dependent classical pathway was prevented by addition of the calcium chelator ethylene glycol tetraacetic acid (EGTA) to the assay buffer, and magnesium, necessary for both pathways, was added to the buffer. Briefly, a cell suspension of rabbit RBC (2×10^8 cell/ml) was prepared in the GVB-Mg^{2+}-EGTA buffer. A serial 1.5-fold dilution (1:4, 1:6, 1:9, 1:13.5, and 1:20.25) of NHS or similar dilution of the mixture of NHS and the test sample was prepared in GVB-Mg^{2+}-EGTA buffer, and 100 µl of each serum dilution was added to 50 µl of standardized Erab. NHS incubated with GVB-Mg^{2+}-EGTA buffer was used as the control. The mixture was then incubated at 60 minutes at 37°C in a shaking water bath to keep cells in suspension, and 1.2 ml of ice-cold NaCl (0.15 M) was used to stop the reaction. The tubes were spun at 1250*g*, at 4°C, for 10 minutes to pellet the cells, and the optical density of the supernatant was determined spectrophotometrically (412 nm). Background control had 100 µl GVB-Mg^{2+}-EGTA buffer, and 50 µl Erab and did not exceed 10% of the total lysis. In the total lysis control tube 100 µl of distilled water was added to 50 µl Erab suspension, and the percentage of hemolysis was determined relative to 100% lysis control. The results of the assay were calculated as described previously.[39,41] Complement activity was quantitated by determining the serum dilution required to lyse 50% of cells in the assay mixture. The results were expressed as the reciprocal of this dilution in AH_{50} units/ml of serum.

Molecular Size Determination

Microcentrifugation

Three samples of human aqueous and vitreous (100 µl each) were pooled separately. Pooled aqueous and vitreous samples were spun separately at 7500*g*, at 4°C for 2 hours, using Micron-3 microconcentrators (molecular weight cutoff, 3 kDa). Filtrate and retain were reconstituted to the original sample volume using appropriate GVB buffer and were tested for the complement inhibitory activity in the CH_{50} and AH_{50} assays as described

above. The buffer, centrifuged as described above was used as the control for filtrate, whereas noncentrifuged buffer was used as control for retain. This experiment was repeated three times.

Size Exclusion Chromatography

Ten aqueous humor (100 µl each) and vitreous (100 µl each) samples were pooled separately, and total protein concentration was determined using Bradford reagent. Bradford reagent was used according to the manufacturer's specifications. One milliliter of pooled aqueous/vitreous sample (total protein 2.0 mg) was fractionated by size exclusion column chromatography using Sephacryl S-100-HR (fractionation range, 1–100 kDa). The column (1.0 × 30.0 cm; bed volume, 18.0 ml) was equilibrated with phosphate-buffered saline (PBS) containing 0.05% CHAPS, and the samples were treated with CHAPS (0.05%) before loading onto the column. The column was eluted with PBS containing 0.05% CHAPS, using a flow rate of 3.0 ml/h. The fractions were collected (100 × 2 ml), the optical densities were measured at 280 nm. This experiment was repeated four times. The elution profile of vitreous and aqueous humor demonstrated the presence of three major peaks. The fractions corresponding to these peaks were pooled into three composite fractions, concentrated to the original volume, and tested individually for anticomplementary activity using CH_{50} and AH_{50} assays as described above. In two separate experiments molecular weight markers (7.5–215 kDa) obtained from Bio-Rad (Hercules, CA) were also applied to the Sephacryl S-100–HR column (as described above), and the elution profile was obtained.

Effect of Neutralizing Antibodies

The effect of neutralizing antibodies to human MCP (GB24; mouse IgG1), DAF (1H4; mouse IgG1), and CD59 (MEM 43; mouse IgG2a) on complement inhibitory activity was studied by modification of a previously described method.[15] Briefly, 5 µl (200 µg/ml) of each antibody was treated independently with 25 µl of the test sample (aqueous humor or vitreous peak 1) at 4°C for 30 minutes. After 30 minutes, 25 µl of the sample was removed and was incubated with 50 µl of NHS at room temperature for 1 hour. The mixture was then subjected to CH_{50} assay as described above. Controls for MCP and DAF included incubation of the test sample with equivalent concentration of MOPC-21 (mouse IgG1), whereas UPC-10 was used as a

control for CD59 antibodies. Additional controls consisted of incubation of the test sample with 5 μl GVB^{2+} buffer (gelatin/Veronal-buffered saline with Ca^{2+} and Mg^{2+}). This experiment was repeated four times.

SDS-PAGE and Immunoblotting

Aqueous humor and vitreous peak 1 were used in Western blot analysis, and immunoblotting was performed using the enhanced chemiluminescence Western blotting detection system ECL + Plus (Amersham Pharmacia Biotech, Arlington Heights, IL) according to manufacturer's recommendations. Normal human urine was used as the positive control. Briefly, electrophoresis was performed on 10% or 14% SDS-PAGE slab gel under nonreducing conditions, and the separated proteins were transferred to PVDF membranes. The transfer efficiency was monitored by staining the membrane with Ponceau-S. Unbound protein sites on the blots were blocked with 5% nonfat dry milk. The blot was then reacted with different dilutions of pooled monoclonal anti-human MCP (GB24, J4 to 48, E4.3), anti-DAF (IA10, 1H4, BRIC110), or anti-CD59 (10G10, MEM43) separately overnight at 4°C. Control blots were reacted with equivalent concentration of pooled nonrelevant monoclonal antibodies (MOPC-21, UPC-10). The blots were washed to remove unbound primary antibody and bound antibodies were visualized by using goat anti-mouse IgG that has been conjugated with horseradish peroxidase (HRP). The secondary antibody was diluted according to the manufacturer's instructions. The membrane was then treated with the chemiluminescent substrate, and the blot was then exposed to x-ray film to detect chemiluminescent signal.

Statistical Analysis

Statistical analysis of the test results was performed using the Student's *t*-test, and $P < 0.05$ was considered statistically significant.

Results

Functional Inhibition of the Complement System by Human Intraocular Fluid

The anti-complementary activity of human intraocular fluid was studied using

two sensitive hemolytic assays, namely CH_{50} and AH_{50}. NHS was treated with aqueous and/or vitreous (final concentration, ~33%), and the mixture was assayed for inhibition of the complement system using these assays. NHS treated with appropriate GVB buffer was used as the control. The results are summarized in Tables 1 and 2. All aqueous humor and vitreous samples used in our study inhibited the complement-mediated lysis of sensitized SRBCs by NHS compared with controls. These differences were statistically significant (Table 1). In contrast, none of the samples studied had an inhibitory effect on the lysis of RaRBCs by NHS via the alternative pathway (Table 2). Furthermore, aqueous humor and vitreous alone were not able to lyse sensitized SRBCs in a CH_{50} assay or RaRBCs in an AH_{50} assay (data not shown).

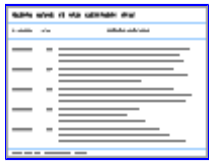

Table 1
Effect of Human Aqueous Humor and Vitreous on the Classical Pathway Hemolytic Activity

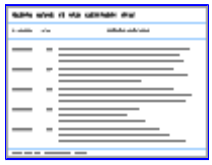

Table 2
Effect of Human Aqueous Humor and Vitreous on the Alternative Pathway Hemolytic Activity

Size of the Complement Inhibitory Factor

The molecular size of the complement inhibitory factor(s) was determined by microcentrifugation and size exclusion chromatography. Pooled aqueous humor and vitreous were separately subjected to microconcentration as described in Materials and Methods. After microcentrifugation, the low-molecular-weight fraction (< 3 kDa) contained some inhibitory activity compared with the control, which was not statistically significant (Fig. 1). However, the upper unfiltered fraction of both aqueous humor and vitreous had a significant ($P < 0.05$) inhibitory effect on the CH_{50} value compared with the buffer control (Fig. 1). This upper unfiltered fraction (≥ 3 kDa) also inhibited the activity of the alternative pathway in the AH_{50} assay (data not shown). However, the inhibition of CH_{50} was more than the AH_{50} inhibition. These results suggested that the size of the inhibitory factors was ≥ 3 kDa.

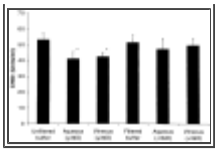

Figure 1
Size determination of complement inhibitory activity of normal human intraocular fluid. Using Micron-3 microconcentrators, pooled aqueous humor (n = 3) and vitreous (n = 3) were separately divided into two fractions and the effect of both fractions (≥ (more ...)

This was confirmed by size exclusion chromatography using Sephacryl-S-100 HR. Chromatography of pooled vitreous on an S-100-HR column revealed the presence of three peaks (Fig. 2); a similar elution pattern was obtained with pooled aqueous samples (data not shown). Peaks 1 (M_r ≥ 19.5 kDa), 2(M_r < 19.5 kDa to > 7.5 kDa), and 3 (M_r < 7.5 kDa) were tested individually for their ability to inhibit the classical and alternative complement pathways. All three peaks were found to have an inhibitory effect on the CH_{50} value (Table 3). Although the total protein content of peaks 2 and 3 was greater than peak 1 (Fig. 2), the most potent inhibition of CH_{50} was associated with peak 1 (Table 3). Aqueous and vitreous peaks 1, 2, and 3 had an inhibitory effect on the AH_{50} value also (not shown); however, CH_{50} inhibition was greater than AH_{50} inhibition and was used in our subsequent experiments to monitor the inhibitory activity of human intraocular fluid.

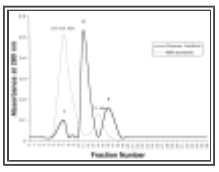

Figure 2
Elution profiles of human vitreous (—) and molecular weight standards (- - -) from a Sephacryl-S-100 HR column with PBS containing 0.05% CHAPS as the mobile phase. Pooled vitreous (10 samples) was fractionated by size exclusion chromatography, (more ...)

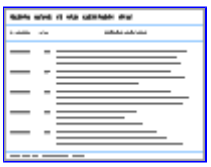

Table 3
Inhibition of the Classical Pathway Hemolytic Activity by Fractions Obtained after Size Exclusion Chromatography

Characterization of Complement Inhibitory Factors

Our previously published studies demonstrated the presence of MCP, DAF, and CD59 on many ocular cells.[36] Furthermore, the soluble form of these complement regulatory molecules had previously been demonstrated by others in several body fluids.[11–23] Consequently, we performed studies to investigate the effect of neutralizing monoclonal antibodies to human MCP, DAF, and CD59 on the complement inhibitory activity of normal human

intraocular fluid. Because vitreous and aqueous peaks 1 represented the high-molecular-weight (≥ 19.5 kDa) fraction (Fig. 2) and most of the inhibition of CH_{50} was associated with them, they were studied to identify the inhibitory molecule(s). Figure 3 shows the effect of these antibodies on the complement inhibitory activity of vitreous peak 1. NHS treated with vitreous peak 1 preincubated with antibodies to MCP or DAF was more lytic (had a higher CH_{50}) and resulted in partial restoration (~30%) of the decreased lysis noted with vitreous peak 1, treated with an equivalent concentration of MOPC-21, an irrelevant monoclonal antibody (Fig. 3A). Similar experiments with anti-CD59 antibody preincubation resulted in decreased (~23%) complement-inhibiting activity compared with an irrelevant monoclonal antibody control (UPC-10; Fig. 3B). These differences were statistically significant ($P < 0.05$); similar results were obtained with aqueous peak 1 (data not shown).

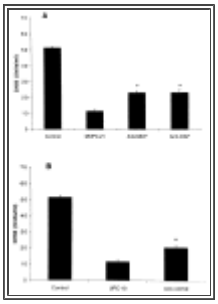

Figure 3
Effect of anti-MCP, anti-DAF (**A**), and anti-CD59 (**B**) antibodies on the complement inhibitory activity. Vitreous peak 1 was treated with monoclonal neutralizing antibodies to MCP, DAF, and CD59 or an irrelevant monoclonal antibody of the same isotype (MOPC-21 (more ...)

Immunoblotting studies were performed to confirm that MCP, DAF, and CD59 are present in aqueous humor and vitreous. Aqueous and/or vitreous peaks 1 were electrophoresed on polyacrylamide gel and blotted to PVDF membranes. Normal human urine was used as the positive control, and pooled monoclonal antibodies were used. Representative immunoblotting profiles of MCP, DAF, and CD59 in aqueous humor peak 1 are shown in Figure 4. Under nonreducing conditions pooled anti-MCP antibodies specifically identified a protein band with an approximate molecular mass of 48 kDa (Fig. 4A), whereas pooled anti-DAF antibodies recognized a 55-kDa protein (Fig. 4B). CD59 was identified in aqueous peak 1 as a protein of 20 to 22 kDa that comigrated with urine CD59 (Fig. 4C). Control blot (Fig. 4D) incubated with pooled irrelevant monoclonal antibodies (MOPC-21, UPC-10) did not show any reactivity. Similar results were obtained with vitreous peak 1 (data not shown).

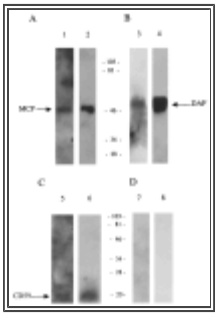

Figure 4
Immunoblotting analysis of sMCP, sDAF, and sCD59 in normal human intraocular fluid. Aqueous humor peak 1 (*lanes 1, 3, 5,* and *7*) was subjected to SDS-PAGE under nonreducing conditions, and transblotted onto a PVDF membrane. 10% SDS-PAGE was run for MCP (more ...)

Discussion

t is well established that the eye is an immunologically privileged site. One explanation for the inhibition of delayed type hypersensitivity, which characterizes immune privilege, is that severe inflammation will not only protect the organ from invasion by a pathogenic organism but has the potential to cause irreversible visual damage. Most studies of the mechanisms of immune regulation do not focus on the complement system. In this article we examined the complement regulatory activity of normal human intraocular fluid (aqueous humor and vitreous) using two sensitive hemolytic assays, namely, CH_{50} and AH_{50}. CH_{50} is a simple quantitative functional assay used to determine the functional status of the classical pathway because it depends on sequential activation of the classical pathway components (C1–C9) to lyse sheep erythrocytes sensitized with rabbit anti-sheep erythrocyte antibodies.[39,40] The AH_{50} assay is similar to CH_{50}, but it measures total alternative pathway hemolytic activity and depends on lysis of unsensitized rabbit erythrocytes by human serum by activation of the alternative complement pathway.[39,41]

Because activation of the complement system within the eye has the potential to destroy important intraocular structures, we studied both normal human aqueous humor and vitreous for factors, which might inhibit the complement cascade. We observed that both fluids significantly inhibited the classical pathway of complement activation. However, inhibition of the alternative complement pathway was observed only when the aqueous and vitreous samples were fractionated by microcentrifugation or size exclusion chromatography.

We started to characterize these complement inhibitory factors on the basis of

molecular size. Microcentrifugation studies suggested that the size of the complement inhibitory factors was ≥ 3 kDa. This was confirmed by the chromatography of whole pooled aqueous humor and vitreous, which also demonstrated that the major complement inhibitory activity was localized to the high-molecular-weight fractions (peak 1, fractions 5–8). Identification of these factors was established by immunoblot studies, which demonstrated the presence of MCP, DAF, and CD59 in the high-molecular-weight fraction. Confirmation of these results was obtained by reversal of the complement inhibitory activity of aqueous humor and vitreous by the addition of monoclonal neutralizing antibodies to MCP, DAF, and CD59. The nature of the inhibitory molecules present in peaks 2 and 3 was not examined in the present study. In summary, our data suggest that the inhibitory property of human intraocular fluid is associated with the presence of soluble forms of MCP, DAF, and CD59. Because we were only able to partially reverse the complement inhibitory activity by neutralizing antibodies to MCP, DAF, and CD59, it is reasonable to expect that other soluble complement regulatory proteins will be demonstrated in normal intraocular fluid.

Previous reports have demonstrated the inhibition of the classical complement pathway by aqueous humor and vitreous from both the guinea pig[42] and humans.[43] Complement regulatory factors, such as C1 inhibitor and factor I have been identified in normal intraocular fluid.[35] Recently, Goslings et al.[44] reported the presence of a small-molecular-weight factor (< 1.3 kDa) in normal rabbit aqueous humor that inhibits the classical complement pathway at the level of C1q. Our study clearly identifies other complement regulatory factors. The differences in our results could be attributable to the differences in the sample processing and fractionation. In addition, Goslings et al.[44] studied the effect of rabbit aqueous humor on the heterologous human complement system, and complement regulatory proteins have been reported to show species selectivity.[1–4] Similar conflicting observations were reported with human seminal plasma. In 1984, Price and coworkers[45] demonstrated that the complement regulatory activity in human seminal plasma was associated with a factor(s) with a molecular weight < 3.5 kDa. However, Chowdhury et al.[15] subsequently showed that human seminal plasma contained a higher molecular weight complement inhibitory factor (> l0 kDa) that significantly reduced the activity of the alternative as well as classic complement pathways. These complement inhibitory factors

were identified as MCP and DAF.

Soluble forms of membrane-bound complement regulatory proteins have been reported in various body fluids.[11–23] Soluble membrane cofactor protein (sMCP) has been detected at low concentrations in plasma, tears, and seminal fluid.[12] In our study we demonstrated that both aqueous and vitreous as well as urine contained sMCP as a single protein with a molecular weight of ~48 kDa. This contrasts with broad two band profile reported for membrane-bound MCP on blood cells and various cell lines.[7,8] Seya et al.[14] reported, with results similar to ours, that seminal plasma sMCP is also a single-band protein but with a molecular weight of ~60 kDa. In contrast, MCP purified from plasma and serum consisted of three bands of 29, 47, and 56 kDa using SDS-PAGE/immunoblotting.[13] In this study the 47- and 56-kDa soluble forms were demonstrated to possess cofactor activity for factor I mediated cleavage of C3b.

DAF has been reported in human plasma, saliva, synovial fluid, cerebral fluid, urine and tears as well as the aqueous humor and vitreous.[15–18] However, the molecular weight of sDAF appears to vary in different body fluids ranging from 55 to 100 kDa, thus indicating that sDAF may be heterogeneous in comparison to membrane-bound DAF.[5,6] In 1990, Lass et al.[37] demonstrated that human tears contain two DAF forms, one with an apparent molecular weight of 72 kDa (resembling membrane DAF) and a second previously undescribed form with an apparent M_r of 100 kDa. Soluble DAF has been demonstrated to preserve complement regulatory activity and to limit spontaneous complement activation in the fluid phase.[17]

CD59 has been found as a single diffuse protein (20–22 kDa) in cell-free seminal plasma, retroplacental sera, umbilical cord sera, amniotic fluid, colostrum/milk, sera from blood in children up to 12 months of age, cerebrospinal fluid, urine, and saliva.[19–23,46,47] These soluble forms of CD59 have been shown to retain inhibitory activity for the membrane attack complex.[20,47] In our study, sCD59 was also identified as a single, broad band of 20 to 22 kDa on immunoblot analysis. Membrane-bound CD59 has been reported to give a single broad band in the same molecular weight range on SDS-PAGE/immunoblotting.[9]

In conclusion, we believe that the complement inhibitory activity

demonstrated by normal human intraocular fluid (both aqueous humor and vitreous) is expressed by the complement regulatory proteins MCP, DAF, and CD59. Although in the present study a mechanism for the generation of the soluble forms of MCP, DAF, and CD59 was not investigated, our results show that these molecules are functionally active. Thus, we have identified at least two important mechanisms by which complement activation within the human eye can be regulated:

1. Membrane bound MCP, DAF, and CD59[36];
2. Soluble forms of these proteins in normal intraocular fluids.

We postulate that the complement system serves as a primary defense mechanism of the eye against pathogenic infections. This surveillance function of complement is finely regulated by soluble and membrane-bound complement regulatory proteins so that there is destruction of the putative pathogen without induction of uveitis and vision loss. Interference with complement regulation leads to enhanced complement activation and intraocular inflammation (anterior uveitis) in Lewis rats.[48]

Soluble complement regulatory proteins may have important clinical implications. They may be therapeutically useful in suppression of antibody mediated intraocular inflammation and the prevention of ocular tissue damage. Soluble complement regulators have been used previously to inhibit inflammation in other model systems.[49,50] The role of complement and complement regulatory proteins in ocular immune privilege is currently being explored in our laboratory.

Footnotes

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