Differential Expression of the Complement Regulatory Proteins in the Human Eye

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Purpose. The presence of complement activation products in the human eye during infection or inflammation has been well described. During complement activation the host must be protected from attack against self tissue; this is achieved by three membrane-bound complement regulatory proteins: membrane cofactor protein (MCP, CD46), decay accelerating factor (DAF, CD55), and membrane attack complex inhibiting protein (CD59). This study was undertaken to analyze the expression of these proteins in the normal human eye.

Methods. Tissues were sectioned by cryostat and both polyclonal and monoclonal antibodies to MCP, DAF, and CD59 were used. Control stains were performed with nonrelevant antibodies of the same immunoglobulin subclass and normal rabbit serum as well as by omission of the primary and secondary antibodies.

Results. All three proteins were found to be differentially expressed in the human eye. With anti-MCP, strong staining of the corneal epithelium and weak staining of the corneal keratocytes in stroma and photoreceptor cells was observed. Staining with anti-DAF was very strong in the corneal epithelium and the ciliary body and moderate in the corneal stroma (keratocytes) and iris. In contrast, anti-CD59 stained very strongly in the corneal epithelium, corneal stroma (keratocytes), iris, choroid, and all layers of the retina, and moderately in the ciliary body.

Conclusions. Identification of MCP, DAF, and CD59 in the human eye provides evidence that a regulatory system exists to protect these cells from destruction by complement-activating events. It remains to be determined if other more specialized functions exist for these proteins, especially in the case of CD59 because of its extensive expression in the retina. Invest Ophthalmol Vis Sci. 1993; 34:3579-3584.

The complement system is an important component of humoral immune defenses. It provides a recognition and effector pathway, which promotes the inflammatory response, assists in the processing of immune complexes, and directly alters the membranes of microorganisms. The activation of the complement system triggers a sequence of biologic reactions in which one component activates the next in a cascade fashion. A critical step in the complement reaction sequence in both the classical and alternative pathway is the formation of the C3 convertases. These proteases activate the third (C3) complement component resulting in the generation and deposition of the major opsonic fragments C3b and C4b on the cell membrane. In addition, the convertase promotes the assembly of the membrane attack complex. The host must be protected from the inadvertent activation of complement on its own tissue. Thus, it is essential that the formation and function of the convertases be carefully regulated so that the opsonic activity of C3b and the cytolytic activity of the membrane attack complex are directed against foreign not self tissue.

Because C3b and membrane attack complex attach to self tissue they must be regulated to prevent amplification. Decay accelerating activity and cofactor activity are two important mechanisms that downregulate the complement cascade at the critical step of C3 activation. They are achieved by two membrane-bound regulatory proteins, namely decay accelerating...
factor (DAF;CD55) and membrane cofactor protein (MCP, CD46). DAF, a glycolipid anchored 70 kD glycoprotein, prevents the activation of homologous complement by inhibiting the assembly and promoting the decay of the C3 and C5 convertases of both the classical and alternative pathways. However, DAF does not irreversibly inactivate C3b or C4b and, after interaction with DAF, these molecules still possess cytolytic activity. MCP, a widely distributed C3b/C4b binding protein of 55 to 70 kD, serves as a cofactor for the plasma serine protease factor I, which irreversibly inactivates the hemolytic potential of C3b or C4b. The resulting cleavage products cannot serve as substrates for the convertases. Thus, MCP and DAF operate in a complementary fashion to protect host cells from complement-mediated damage. Regulation of the formation and activity of the membrane attack complex is mediated by CD59. CD59, a 20 kD protein also with a glycolipid anchor, exerts its inhibiting effect at the stage of C8 and/or C9 binding.

Many but not all components of the classical and alternative pathways have been shown to be present in the aqueous humor, tears, and cornea of normal persons. Ocular trauma, infection, or inflammation can result in a breakdown of the blood–aqueous or blood–retinal barriers, leading to elevated levels of many complement components and possibly activation of the complement system. The activation products, C3b and C4b, have been demonstrated to be present in the aqueous humor of eyes with anterior uveitis. Also, complement activation is believed to play an important role in ulceration of the human cornea induced by gram-negative bacteria. Cleveland and associates demonstrated that decommitted mice had more severe corneal ulcers caused by Pseudomonas aeruginosa than mice with normal complement levels. A number of investigators have reported the deposition of immunoglobulins and C3 in the conjunctival basement membrane of patients with ocular cicatricial pemphigoid, suggesting a pathogenic role for the complement system. In experimental allergic uveitis caused by retinal S-antigen, complement activation has been demonstrated to be important for inflammation. In this model, complement depletion by cobra venom factor has been shown to decrease the inflammatory response. Various serum proteins that regulate the activation of the complement system such as C1 inhibitor and C5b inactivator (Factor I) have been reported to be present in the aqueous humor and cornea. DAF has also been shown to be present in various ocular tissues, including tears, aqueous humor, and vitreous humor.

Because the three membrane proteins, MCP, DAF, and CD59, play an important role in regulating complement activation on host cells, we analyzed the normal human eye for their expression. Comparison of the staining intensities of the various ocular structures indicates that these complement-regulatory proteins are differentially expressed on eye tissue.

### MATERIALS AND METHODS

#### Tissues

Twelve normal human eyes were obtained from the Mid-America Eye Bank in St. Louis within 2 to 4 hours of donor’s death. The age range of the donors was 40 to 83 years, with an average age of 56 years. All tissues were placed in OCT compound (Miles Indianapolis, IN), snap-frozen, and stored in sealed vials at —80°C until sectioned by cryostat.

#### Antibodies

One mouse monoclonal antibody, 1A10, and one rabbit polyclonal antibody, DL 6.2, was used to identify DAF. GB24, a mouse monoclonal antibody, and one rabbit polyclonal antibody was used to localize MCP. For CD59, two monoclonal antibodies, IF5 and A35, and one rabbit polyclonal antibody was used. The following dilutions of antibodies were used for immunocytochemical staining: monoclonal antibodies, 1:200 and rabbit polyclonal antibodies 1:400.

#### Immunohistochemical Studies

Tissues were sectioned by cryostat, air dried overnight (18 hours), fixed in cold acetone for 10 minutes and rehydrated in phosphate-buffered saline, pH 7.2. In some experiments after air drying and washing with phosphate-buffered saline, the fixed sections were immersed in a 0.3% hydrogen peroxide/methanol solution for 20 minutes to inactivate endogenous peroxidase and then rinsed well in phosphate-buffered saline. All of the monoclonal and polyclonal antibodies to complement-regulatory proteins (MCP, DAF, and CD59) were tested in each experiment to avoid interexperiment variations. Immunocytochemical stains for the monoclonal antibodies were performed using an anti-mouse immunoglobulin G immunoperoxidase staining kit from Vector Laboratories (Burlingame, CA) according to the manufacturer’s directions. For the rabbit antibodies, a biotinylated anti-rabbit immunoglobulin G reagent from Vector Laboratories was used. All the sections were incubated with HRP-labeled avidin-biotin complex (ABC kit PK6100, Vector Laboratories), washed with phosphate-buffered saline and then immersed in a sodium acetate solution containing the chromogen aminoethylcarbazol and hydrogen peroxide for approximately 10 minutes. The slides were then washed in tap water, counterstained for 10 minutes with Mayer’s haematoxylin, washed thoroughly in cold tap water, and coverslipped with an aqueous mounting media for viewing by light micros-
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antibodies of the same immunoglobulin subclass and

RESULTS

weak staining was observed in the corneal stroma (Fig. 2A) and MCP was not identified on the iris (Fig. 3A), ciliary body or choroid (not shown).

Membrane Cofactor Protein (MCP;CD46)

The corneal epithelium was very strongly stained while weak staining was observed in the corneal stroma (Fig. 1A). An identical staining pattern was observed with the monoclonal and polyclonal anti-MCP antibodies. There was no staining in Bowman’s membrane or the corneal endothelium (not shown). No difference in the staining pattern was observed when the limbus was compared to the central cornea. Weak staining was observed in the photoreceptor cells with anti-MCP antibodies (Fig. 2A) and MCP was not identified on the iris (Fig. 3A), ciliary body or choroid (not shown).

Decay Accelerating Factor (DAF;CD55)

Staining patterns in the eye were identical using both the monoclonal and polyclonal antibodies. DAF was compared to the corneal epithelium. However, in contrast to our

Table 1 summarizes the results.

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DAF was identified in the inner plexiform layer, inner nuclear layer, outer plexiform layer, outer nuclear layer, and rods and cones (Fig. 2C).

Table 1. Expression of Membrane Cofactor Protein (MCP), Decay Accelerating Factor (DAF), and Membrane Attack Complex-Inhibiting Protein (CD59) in the Normal Human Eye

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<tr>
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<th>MCP</th>
<th>DAF</th>
<th>CD59</th>
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<tbody>
<tr>
<td>Cornea</td>
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<tr>
<td>Epithelium</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
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<tr>
<td>Bowman’s membrane</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Stroma</td>
<td>+</td>
<td>++</td>
<td>+++</td>
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<tr>
<td>Endothelium</td>
<td>+/0</td>
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<tr>
<td>Ciliary body</td>
<td>0</td>
<td>+++</td>
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<tr>
<td>Iris</td>
<td>0</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Choroid</td>
<td>0</td>
<td>0</td>
<td>+++</td>
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<tr>
<td>Retina</td>
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<tr>
<td>Ganglion cell layer</td>
<td>0</td>
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<td>Inner plexiform layer</td>
<td>0</td>
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<td>Inner nuclear layer</td>
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Comparative staining intensities were graded as extremely intense (+++) to negative (0).

With the use of immunohistochemical techniques, we observed the differential expression of MCP, DAF, and CD59 in the normal human retina. All of these proteins are very strongly expressed on the corneal epithelium and the limbus, as well as on the central cornea. MCP, DAF, and CD59 are all expressed on the corneal keratocytes in the stroma; however, the expression of CD59 on these cells is more than DAF followed by MCP. DAF and CD59 are expressed on the ciliary body and iris, whereas only DAF is present on the choroid and retina. This differential expression of these three proteins is of interest and contrasts with their rather uniform expression on peripheral blood cells and epithelial cells.

DISCUSSION

To the best of our knowledge, this is the first report describing the differential expression of MCP and CD59 in the human eye. Lass et al. recently studied the expression of DAF. Using immunohistochemical staining of the human eye they also localized DAF to the corneal epithelium. However, in contrast to our observations they found low levels of staining in the corneal endothelium and within the inner layers of retina. We observed moderate expression of DAF in
FIGURE 1. Complement-regulatory proteins in the cornea. The human cornea was examined immunohistochemically for MCP (A), DAF (B) and CD59 (C) using polyclonal antibodies (described in Materials and Methods). MCP, DAF, and CD59 were strongly expressed on the corneal epithelium. Corneal keratocytes in the stroma stained weakly with anti-MCP (A), moderately with anti-DAF (B) and intensely with anti-CD59 (C). No staining was observed in the control sections (D). Original magnification × 40.

FIGURE 2. Complement regulatory proteins of the retina. Staining of the retina with anti-MCP (A), anti-DAF (B) and anti-CD59 (C) using the polyclonal antibodies. Immunohistochemical staining shows strong expression of CD59 in various layers of the retina, and weak staining of photoreceptor cells by antibodies against MCP. No staining was observed with anti-DAF (B) and in the control sections (D). Original magnification × 40.
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FIGURE 3. Immunohistochemical analyses of complement-regulatory proteins in the human iris. Sections were stained with polyclonal antibodies against MCP (A), DAF (B), and CD59 (C). Anti-DAF (B) and anti-CD59 (C) staining of the iris is evident. No staining was observed with anti-MCP (A) as well as in the control sections (D). Original magnification X 40. Insert B shows a higher magnification (X 100).

the iris, which was negative in the report by Lass et al. These differences could be attributable to the fact that we have used one monoclonal antibody (IA10) and one rabbit polyclonal antibody to localize DAF whereas Lass and coworkers used pooled anti-DAF monoclonal antibodies (IA10, IIH6, and VIIIA7).

Several studies have shown the presence of functionally active complement proteins in the cornea, aqueous humor, and tears. Moreover, the external surface of the cornea (the corneal epithelium) is constantly being exposed to a number of potential pathogens, whereas the inner structures of the eye may be exposed during transient episodes of pathogen dissemination via the blood stream. Many of these pathogens have been shown to activate the complement system, which could lead to complement-mediated damage to ocular tissues. The identification of MCP, DAF, and CD59 in the cornea and inner structures of the eye suggests the existence of a complement-regulating system that protects these cells from destruction by complement-activating events. In the absence of these proteins, C3 convertase of both the classical and alternative pathway could be deposited on the ocular cells. This may result in increased C3b deposition. The activated complement cascade may also cause release of histamine from mast cells and basophils, with a resultant increase in capillary permeability and contraction of smooth muscle. The chemotactic activity of C5a and C5a could attract polymorphonuclear leukocytes into the eye, with the release of superoxide anions and other oxidant species that could damage intraocular tissues.

To investigate if a deficiency or abnormality in the expression of MCP, DAF, and CD59 may play a role in ocular surface disease, we plan to systematically examine corneal tissue from patients with different diseases such as pseudophakic bullous keratopathy, herpes zoster keratitis, and keratitis, rheumatoid corneal melt, and primary Sjogren’s syndrome.

Key Words
complement system, membrane cofactor protein, decay accelerating factor, CD59, eye

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References