Complement System and Host Defense Against Staphylococcal Endophthalmitis

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Purpose. The authors studied the role of the complement system in host defense against Staphylococcus epidermidis and S. aureus endophthalmitis.

Methods. Guinea pigs in the S. epidermidis model received an intravitreal injection of 7000 viable organisms, and guinea pigs in the S. aureus model received 50 viable organisms. The experimental animals in each model were decomplemented with intraperitoneal (IP) injections of cobra venom factor, whereas the control animals received IP injections of normal saline. Mean log bacterial counts in the vitreous and mean serum complement titers were compared in the experimental and control animals in each model on days 1, 2, 3, and 7.

Results. In the S. epidermidis model, mean log bacterial counts in the vitreous were significantly higher in the experimental group than the control group on days 1 and 2 (P < 0.01) and on day 3 (P < 0.05). Mean serum complement titers were significantly lower in the experimental group at all days (P < 0.01). In the S. aureus model, mean log bacterial counts in the vitreous were significantly higher in the experimental group than the control group on day 2 (P < 0.05) and day 3 (P < 0.01). Mean serum complement titers were significantly lower in the experimental group on days 1, 2, and 3 (P < 0.01), but not on day 7.

Conclusion. These results suggest that decomplemented guinea pigs show impaired host defense to S. epidermidis and S. aureus endophthalmitis and that this defense is restored as complement levels approach normal. Invest Ophthalmol Vis Sci. 1994;35:1026-1032.

Bacterial endophthalmitis is a devastating disease that frequently results in severe visual loss despite early, aggressive therapy. Staphylococcus epidermidis and S. aureus are the most frequent causes of postoperative bacterial endophthalmitis.¹⁻³ In one study, S. epidermidis accounted for 38% and S. aureus accounted for 21% of all culture-positive cases in the postoperative setting.¹ Compared with S. epidermidis, S. aureus endophthalmitis usually pursues a much more virulent course that is frequently associated with a devastating loss of vision. Little is known about the immune response to staphylococcal endophthalmitis or, for that matter, to any type of bacterial endophthalmitis. Rationa

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vated complement fragments are stable at extreme ranges of temperature and pH and cause vasodilation, increased vascular permeability, smooth muscle contraction, and release of histamine from mast cells or basophils and lysosomal proteases from neutrophils. C5a also is chemotactic for neutrophils and macrophages. All these functions of complement may be operative in host defense against invading microorganisms. The fate of patients with genetic deficiencies of specific complement components underscores the importance of this system: C3 deficiencies are associated with frequent, severe bacterial infections; C5 deficiency or dysfunction is associated with pyogenic infections and deficiencies of C6, C7, and C8 are associated with a striking susceptibility to Neisserial infections.

Few studies have examined the role of complement in host defense against bacterial infections of the eye. Cleveland and associates suggested that complement may play a role in corneal defense against *Pseudomonas aeruginosa*, a gram-negative bacteria. They showed that decomplemented mice developed corneal ulcers that were more severe than in mice with normal complement levels and that the decomplemented mice were unable to clear *P. aeruginosa* ocular infection, unlike mice with normal complement levels. Aizuss and associates showed that complement provides a protective effect against *P. aeruginosa* endophthalmitis in guinea pigs. Moreover, patients with endophthalmitis show elevated levels of activated complement in aqueous and vitreous. To the best of our knowledge, the role of complement in host defense against endophthalmitis caused by gram-positive bacteria has not been examined. The goal of this study was to determine if complement provides a protective effect in *S. epidermidis* and *S. aureus* endophthalmitis in guinea pigs. In each model of endophthalmitis, the experimental group was decomplemented with cobra venom factor (CVF) and the control group did not undergo decomplementation. Both groups received intravitreal injections of *S. epidermidis* or *S. aureus*. Comparisons were made between bacterial counts and complement titers in the experimental and control group of each model.

**MATERIALS AND METHODS**

**Bacteria**

We used a strain of coagulase-negative *S. epidermidis* (ATCC 12228; American Type Culture Collection, Rockville, MD) that was grown on blood agar plates (BAP) at 37°C for 24 hours. We used a strain of *S. aureus* that was isolated from a human corneal ulcer, belonged to phage-type 95 and was coagulase-positive, β-hemolytic, pigmented, and gentamicin-sulfate resistant. *S. aureus* was maintained in sheep red blood cells at −70°C and was incubated on a gentamicin-containing BAP at 37°C for 24 hours. Discrete colonies of each bacterial species were subcultured into sterile tryptic soy broth for incubation at 37°C for 24 hours. The bacterial culture was centrifuged at 15,000 rpm for 15 minutes. The pellet was resuspended and washed with sterile normal saline (NS). Using a spectrophotometer (Spectronic 21, Bausch and Lomb, Rochester, NY) at an absorbance of 530 nm, we adjusted the viable bacterial count to approximately $1.5 \times 10^8$ colony-forming units (CFU)/ml, which corresponded to an optical density of 0.16 to 0.21. The bacterial suspension was adjusted by serial dilution in NS to give a final concentration of approximately 7000 CFU *S. epidermidis*/50 μl and 50 CFU *S. aureus*/50 μl for intravitreal injection. These inoculum sizes consistently produced endophthalmitis in pilot studies. The final bacterial concentration was confirmed by plating 0.1 ml of the suspension on BAP in duplicate and incubating the plates at 37°C for 48 hours.

**Animals**

Hartley strain guinea pigs were used in this study and were maintained in accordance with the ARVO Resolution on the Use of Animals in Research. A total of 112 guinea pigs (56 in the *S. epidermidis* model and 56 in the *S. aureus* model) weighing 300 to 400 g each were used.

**Decomplementation**

In each model, the experimental group consisted of 28 animals that were systemically decomplemented by intraperitoneal (IP) injection of 250 U/kg body weight of CVF (Diamedx; Miami, FL) at 0 hours and 100 U/kg body weight of CVF at 8, 24, and 28 hours based on methods described by Cochran and associates and used in our previous study of *P. aeruginosa* endophthalmitis. Each control group consisted of 28 animals that received IP injections of an equal volume of NS at 0, 8, 24 and 28 hours.

**Bacterial Injections**

After the IP injections at 28 hours, all animals were given inhalation anesthesia with methoxyflurane (Pitman-Moore; Mundelein, IL) and topical ocular anesthesia with 0.5% proparacaine (Alcon, Humacao, Puerto Rico). Paracentesis of 50 μl of aqueous humor was performed before intravitreal injections using a 1 ml tuberculin syringe and a 30-gauge needle to limit intraocular pressure increases and minimize extrusion of inoculum. Using a tuberculin syringe with a 30-gauge needle, a 50 μl volume of the *S. epidermidis* or *S. aureus* suspension was injected through the superotemporal pars plana (3.0 mm posterior to the limbus) under direct visualization into the vitreous of the right eye.
eyes of 56 guinea pigs. The left eyes were not inoculated. Additionally, 50 μl of the S. epidermidis or S. aureus suspension were plated in duplicate on BAP and incubated at 37°C. Colony counts at 48 hours confirmed the actual number of S. epidermidis or S. aureus injected.

**Clinical Observations and Bacterial Counts**

In each model, seven experimental and seven control animals were examined and sacrificed at 1, 2, 3, and 7 days after the intravitreal injections. Before death, the clinical ocular inflammatory response was graded in a masked fashion by gross examination, based on a scoring system for the severity of endophthalmitis developed by Peyman and colleagues (Table I). Mean clinical scores for decomplemented and control groups were calculated, and statistical analysis was done using unpaired t-tests. Guinea pigs were anesthetized with methoxyflurane, and blood samples were obtained by cardiac puncture from each animal, allowed to clot at room temperature, and centrifuged at 2,000 rpm for 10 minutes at 4°C. The sera were stored at −70°C before the hemolytic complement assays. The right eye of each sacrificed animal was enucleated and vitreous was aspirated using a 1 ml tuberculin syringe and an 18-gauge needle. Vitreous aspirates were homogenized, and a volume of 100 μl was serially diluted (10^{-1} to 10^{-6}) in NS. One hundred microliters of each dilution from each vitreous sample was plated in duplicate on BAP and incubated at 37°C for 48 hours. Bacterial colony counts for each dilution were obtained to quantify the number of CFU/ml in the vitreous. The mean log colony counts of the experimental eyes were compared with the mean log colony counts of the control eyes using ANOVA with post hoc t tests at days 1, 2, 3, and 7.

**Histopathology**

An additional group of guinea pigs was prepared for histopathologic evaluation of each model after the procedures described above. One control and one experimental animal from each model was sacrificed on days 1, 2, 3, and 7 and the right globe was enucleated and placed in 10% neutral buffered formalin for 2 days, rinsed in distilled water, and placed in 50% ethanol. After 5 days, eyes were processed in graded alcohols, embedded in paraffin, sectioned into 4 μm sagittal segments, and stained with hematoxylin and eosin. Complete horizontal sections of the eyes were selected for microscopic examination. We assessed the extent of the inflammation by estimating the relative number of each cellular component of the infiltrate at 400× magnification.

**Complement Titters**

Levels of total hemolytic complement were assayed in a masked fashion on sera obtained from animals before injection with CVF or NS and on sera at each time point after intravitreal injections. The assay kits were purchased from Diamedix (Miami, FL). The reaction mixtures were based on methods described by Nelson and associates. For each assay, 1.0 ml of sheep red blood cells sensitized with rabbit 7S antibodies to sheep erythrocytes (EA) at a concentration of 1 X 10^9 cells/ml in glucose-gelatin veronal buffer with 0.00015 M Ca^{2+}, and 0.0005 M Mg^{2+} (G1-GVB^{2+}) was washed with 20 ml of gelatin veronal buffer (GVB^{2+}) by centrifugation at 1,800 rpm for 10 minutes at 4°C. The supernatant was removed and GVB^{2+} was added to yield a uniform suspension of 1 X 10^8 cells/ml. For each test tube assay, dilutions of a 0.2 ml serum sample ranging from 1:20 to 1:3400 were prepared using GVB^{2+}. Two controls were used: a spontaneous lysis control (consisting of 0.2 ml of EA7S and 0.8 ml of GVB^{2+}) and a 100% lysis control (consisting of 0.2 ml of EA7S and water) described below. A 0.8 ml volume from each serum dilution was added to 0.2 ml of uniformly suspended EA7S in GVB^{2+}. Tubes were incubated at 37°C for 60 minutes in a shaking water bath to prevent the cells from settling. After 50 minutes incubation, 2.8 ml of distilled water was added to the 0.2 ml of EA7S in the 100% lysis tube, which was then replaced in the shaking water bath. After 60 minutes, all tubes were removed and 2.0 ml of cold NS was added to each tube except the 100% lysis tube. All tubes were immediately centrifuged at 1,800 rpm for 10 minutes at 4°C. The supernatants were poured off into properly labeled tubes, and the absorbance of the supernatant was measured.

**TABLE 1. Grading Scale of Severity of Endophthalmitis**

<table>
<thead>
<tr>
<th>Conjunctiva</th>
<th>0 = Normal/absent</th>
<th>1 = Mild edema</th>
<th>2 = Edema, mild hyperemia, slight exudate</th>
<th>3 = Edema, marked hyperemia, heavy exudate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornea</td>
<td>0 = clear</td>
<td>1 = Focal edema</td>
<td>2 = Diffuse edema</td>
<td>3 = Opaque</td>
</tr>
<tr>
<td>Iris</td>
<td>0 = Normal/absent</td>
<td>1 = Mild hyperemia</td>
<td>2 = Marked hyperemia</td>
<td>3 = Marked hyperemia, synechiae, irregular pupil</td>
</tr>
<tr>
<td>Vitreous</td>
<td>0 = Clear</td>
<td>1 = Areas of vitreous haze, some fundus details visible, good red reflex</td>
<td>2 = Moderate vitreous haze, no fundus details visible, partial red reflex</td>
<td>3 = No red reflex</td>
</tr>
</tbody>
</table>

For each assay, 1.0 ml of sheep red blood cells sensitized with rabbit 7S antibodies to sheep erythrocytes (EA) at a concentration of 1 X 10^9 cells/ml in glucose-gelatin veronal buffer with 0.00015 M Ca^{2+}, and 0.0005 M Mg^{2+} (G1-GVB^{2+}) was washed with 20 ml of gelatin veronal buffer (GVB^{2+}) by centrifugation at 1,800 rpm for 10 minutes at 4°C. The supernatant was removed and GVB^{2+} was added to yield a uniform suspension of 1 X 10^8 cells/ml. For each test tube assay, dilutions of a 0.2 ml serum sample ranging from 1:20 to 1:3400 were prepared using GVB^{2+}. Two controls were used: a spontaneous lysis control (consisting of 0.2 ml of EA7S and 0.8 ml of GVB^{2+}) and a 100% lysis control (consisting of 0.2 ml of EA7S and water) described below. A 0.8 ml volume from each serum dilution was added to 0.2 ml of uniformly suspended EA7S in GVB^{2+}. Tubes were incubated at 37°C for 60 minutes in a shaking water bath to prevent the cells from settling. After 50 minutes incubation, 2.8 ml of distilled water was added to the 0.2 ml of EA7S in the 100% lysis tube, which was then replaced in the shaking water bath. After 60 minutes, all tubes were removed and 2.0 ml of cold NS was added to each tube except the 100% lysis tube. All tubes were immediately centrifuged at 1,800 rpm for 10 minutes at 4°C. The supernatants were poured off into properly labeled tubes, and the absorbance of the supernatant was measured.
supernatants was measured at 415 nm in a spectrophotometer.

The hemolytic titer was calculated as follows: The absorbance of the spontaneous lysis control was subtracted from the absorbance of the 100% lysis control and from the absorbance of each serum dilution to give the corrected absorbance. The corrected absorbance for each serum dilution was divided by the absorbance of the 100% lysis control to obtain the degree of lysis (y) for each serum dilution. From a table provided by Diamedix, y/1-y values were obtained. This y/1-y value was plotted against the volume of undiluted serum for each dilution on a 2 X 3-cy-cle log-log paper. When y/1-y equaled 1, this represented 50% lysis and corresponded to 1 CH50 unit. The number of CH50 units in the undiluted serum (whole hemolytic complement titer) was calculated by dividing 1 ml by the volume that represented 1 CH50 unit. The mean titers of the experimental and control guinea pigs were compared at 1, 2, 3, and 7 days after intravitreal injection using ANOVA with post hoc t-tests.

**RESULTS**

**Clinical Observations**

In the *S. epidermidis* study (Table 2), day 1 observations of decomplemented guinea pigs demonstrated absent to mild inflammation in cornea and iris and a mild to moderate inflammatory response in conjunctiva and vitreous. In control animals, absent to mild reactions were elicited in cornea, iris and vitreous, whereas conjunctiva revealed generally mild edema and hyperemia. On day 2, decomplemented guinea pigs showed predominantly moderate to severe inflammation in conjunctiva, and vitreous and a mild to severe response in the cornea and iris. The control group exhibited mild responses in all four categories. Day 3 examinations of decomplemented animals revealed a mild to

**TABLE 2. Clinical Scores in Decomplemented (D) and Control (C) Eyes After Intravitreal Injection of *Staphylococcus epidemidis* or *S. aureus***

<table>
<thead>
<tr>
<th>Day After Intravitreal Injection</th>
<th>Conjunctiva</th>
<th>Cornea</th>
<th>Iris</th>
<th>Vitreous</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. epidermidis</td>
<td>S. aureus</td>
<td>S. epidermidis</td>
<td>S. aureus</td>
</tr>
<tr>
<td>D</td>
<td>C</td>
<td>D</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Mean</td>
<td>0.57</td>
<td>0.14</td>
<td>0.57</td>
<td>0.86</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Mean</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
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<tr>
<td>2</td>
<td>1</td>
<td>2</td>
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<tr>
<td>3</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Mean</td>
<td>1.37</td>
<td>2.33</td>
<td>1.33</td>
<td>2.33</td>
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<tr>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
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<tr>
<td>3</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Mean</td>
<td>1.50</td>
<td>2.57</td>
<td>1.50</td>
<td>2.57</td>
</tr>
</tbody>
</table>

*P < 0.05 for difference between decomplemented and control groups.*
severe inflammation in conjunctiva and cornea, and generally severe reactions in iris and vitreous. The control guinea pigs displayed absent to mild responses in conjunctiva, cornea, and iris, and generally a mild vitreal reaction. For day 7, decomplemented guinea pigs manifested mild to severe inflammation in conjunctiva, cornea and iris. The vitreal responses ranged from a moderate haze to severe opacification. In control animals, conjunctiva, cornea, and iris generally registered absent to mild reactions whereas absent to severe responses appeared in vitreous.

In the *S. aureus* study (Table 2), day 1 evaluations of the decomplemented group disclosed absent to mild responses in conjunctiva, cornea, and iris with mild to moderate vitreal inflammation. For the control guinea pigs, absent to mild reactions were exhibited in all four categories. On day 2, decomplemented animals showed mild to severe inflammation in cornea and iris, moderate to severe reactions in conjunctiva, and only severe responses in vitreous. The control group elicited mild to moderate reactions in all four categories. Day 3 observations for decomplemented guinea pigs revealed predominantly moderate to severe inflammation in all categories. The day 3 control group findings were mild to moderate in conjunctiva, cornea and iris, whereas vitreous demonstrated moderate to severe responses. For day 7, decomplemented guinea pigs displayed moderate to severe inflammatory reactions in all four categories. The control animals generally expressed mild to moderate responses in all categories except vitreous, which showed moderate to severe inflammation.

**Bacterial Culture Results**

Positive vitreal cultures were found in all of the decomplemented and control guinea pigs in both the *S. epidermidis* and *S. aureus* models at all time points. Bacterial counts were higher in the vitreous of the decomplemented animals at all time points. The *S. epidermidis* experimental group was significantly higher than the control group at days 1 and 2 (*P* < 0.01) and day 3 (*P* < 0.05) (Fig. 1). The *S. aureus* experimental group was significantly higher than the control group at days 2 (*P* < 0.05) and 3 (*P* < 0.01) (Fig. 2).

**Histopathologic Results**

The primary cellular response to staphylococcal endophthalmitis was mediated by neutrophils. Histopathologic examination of selected guinea pig eyes from the decomplemented and control animals of the *S. epidermidis* and *S. aureus* models revealed neutrophilic and lymphocytic infiltration of the corneal stroma, ciliary body, retina, and choroid. In the *S. epidermidis* and *S. aureus* models no differences in the number or type of cells were evident in the decomplemented and control groups.

**Complement Titers**

The results of the total hemolytic complement assays in control and decomplemented guinea pigs are summarized in Figure 3. Before IP injection of CVF or NS, there was no statistically significant difference in complement titers between decomplemented and control guinea pigs. Mean serum complement titers were significantly lower in decomplemented than control groups in both the *S. epidermidis* and *S. aureus* models on days 1, 2, and 3 (*P* < 0.01). On day 7, the *S. epidermidis* decomplemented group was still significantly
Total Complement Titer in Control vs Decomplemented Guinea Pigs

FIGURE 3. Complement titers during course of *S. epidermidis* and *S. aureus* endophthalmitis. *P* < 0.01 at days 1, 2, and 3 between decomplemented and control groups for each model. *P* < 0.01 at day 7 in the *S. epidermidis* model and *P* > 0.05 in the *S. aureus* model.

No significant differences in bacterial counts were found at day 7 in both models because the complement levels were returning to normal. These results suggest that decomplemented guinea pigs show impaired host defense against *S. epidermidis* and *S. aureus* endophthalmitis. Clinically, guinea pigs in the decomplemented groups demonstrated a more severe inflammatory response than those in the control groups. The inflammation in the decomplemented animals may reflect the increased numbers of bacteria proliferating in the vitreous and the inflammatory reaction related to this independent of the complement system. Grading the severity of intraocular infection by gross observation is subjective and involves substantial variability among observers. The model we used in this and a previous study reduces the need for reliance on subjective grading of clinical responses by obtaining actual bacterial counts in the vitreous. Our model has been adapted from methods described by Davis and Chandler and Kupferman and Leibowitz, who evaluated the effect of antibiotics by measuring the number of bacteria surviving in the cornea. Obtaining bacterial counts in the vitreous allowed us to detect differences between experimental and control guinea pigs. Using this model, we were able to show that the complement system participates in host defense against staphylococcal endophthalmitis.

Guinea pigs were systemically decomplemented with CVF, and the magnitude of decomplementation was consistent with that reported by Cochrane and associates. Experimental and control sera at each time point were assayed in a masked fashion using the same reagents to ensure that fluctuations in cellular intermediates and reagents were not factors contributing to the statistically significant differences in titers between the decomplemented and control guinea pigs.

Seven days after intravitreal injection, complement titers in decomplemented guinea pigs were returning to normal. This is consistent with the findings of Cochrane and associates, who showed that decomplementation in guinea pigs could be maintained for approximately 5 days. Antibodies to CVF develop and nullify its effect so that prolonged decomplementation cannot be maintained.

The normal vitreous probably has little in the way of host defense mechanisms against infection. Neutrophils and serum proteins including antibodies and complement probably enter the infected vitreous from the dilated, permeable vessels in the inflamed retina. Complement components have been shown to be present in human aqueous and vitreous humor. Mondino and associates have shown that activated complement was present in the aqueous and vitreous of patients with bacterial endophthalmitis and that the highest levels were found in this disease entity. Activated complement induces chemotaxis of polymorphonuclear leukocytes, increases vascular dilation and permeability, enhances immune adherence facilitating phagocytosis and causes cytolysis of bacteria by the membrane attack complex (C5 to C9).

Gram-positive organisms activate both the classical and alternative complement pathways. Components of the staphylococcal cell wall such as teichoic acid, peptidoglycan, and protein A activate the classical pathway in the presence of antibody specific to them. In addition, teichoic acid, peptidoglycan and protein A all activate the alternative complement pathway without the need for specific antibody.

In rabbits, *S. epidermidis* and *S. aureus* endophthalmitis is associated with a humoral but not a delayed hypersensitivity response to these organisms. Both these models of endophthalmitis are associated with progressive increases in vitreous IgG and IgA antibody levels to staphylococcal antigens. Rapid and extensive cellular damage and optimal phagocytosis of bacterial organisms are dependent on the presence of antibody and activation of the classical complement pathway.
Key Words
endophthalmitis, *S. epidermidis*, *S. aureus*, complement, host defense

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References