The Complement System and Host Defense against Pseudomonas Endophthalmitis

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The authors examined the role of the complement system in host defense against Pseudomonas aeruginosa endophthalmitis. Guinea pigs received intravitreal injections of P. aeruginosa, and comparisons were made between bacterial counts from the vitreous of control guinea pigs and experimental guinea pigs that underwent systemic decomplementation with cobra venom factor. In group 1 (intravitreal injection of 42 organisms), bacterial counts were significantly higher in the vitreous of decomplemented guinea pigs versus control guinea pigs at days 1, 2, and 3 after intravitreal injection but not at day 7 when complement levels returned to normal. In group 2 (intravitreal injection of 102 organisms), bacterial counts were significantly higher in the vitreous of experimental guinea pigs versus control guinea pigs only at day 1 with no statistically significant differences thereafter. In group 3 (intravitreal injection of 150 bacteria), there were no significant differences in bacterial counts in the vitreous of experimental versus control guinea pigs. Our results in group 1 suggest that partially decomplemented guinea pigs show impaired host defense to P. aeruginosa and that this defense is restored as complement levels return to normal. Intravitreal injection of higher numbers of P. aeruginosa as in a group 3 overwhelms and obscures any beneficial effect of the complement system on host defense. Invest Ophthalmol Vis Sci 26:1262-1266, 1985

The complement system is a fundamental element of our normal host defense against infection. The fate of patients with genetic deficiencies of specific complement components underscores the importance of this system: C3 deficiency is associated with frequent, severe bacterial infections; deficiencies of C6, C7 and C8 are associated with a striking susceptibility to Neisseria infections; C5 deficiency or dysfunction is associated with pyogenic infections.

Hemolytic complement has been shown to be present in human tears, cornea, and aqueous humor. Moreover, aqueous humor from patients with anterior uveitis has been shown to contain activated complement (C3a).

There have been few studies of the complement system in host defense of the eye against bacteria. Using systemic decomplementation with cobra venom factor (CVF), Cleveland and associates suggested that complement may be important in corneal defense against Pseudomonas aeruginosa by showing that decomplemented mice had corneal ulcers that were more severe than mice with normal complement levels and that decomplemented mice were unable to clear Pseudomonas ocular infection unlike mice with normal complement levels.

To the best of our knowledge, the role of complement in host defense against bacterial endophthalmitis has not been examined. In the present study, guinea pigs received intravitreal injections of P. aeruginosa, and comparisons were made between bacterial counts from the vitreous of control guinea pigs and experimental guinea pigs that underwent systemic decomplementation with CVF.

Materials and Methods

P. aeruginosa (American Type Culture Collection #27853; Rockville, MD) were plated on sheep blood agar and incubated for 24 hr at 37°C. One colony was harvested, placed in 10 ml of trypticase soy broth, and incubated at 37°C for 24 hr. Subsequently, 100 μl of this suspension was inoculated in another 10 ml of trypticase soy broth and incubated for 24 hr at 37°C. This serial passage was repeated daily until stable growth characteristics were ensured. Serial 10-fold dilutions (from 10^-2 to 10^-8) of the stable P. aeruginosa suspension were carried out in sterile normal saline. A 100-μl aliquot of each dilution was plated in duplicate on sheep blood agar using a platinum loop. Colony counts were obtained 24 hr later and averaged to determine the appropriate dilutions for intravitreal injection.
Hartley strain guinea pigs were used in this study and were cared for and treated in accordance with the ARVO Resolution on the Use of Animals in Research. A total of 168 guinea pigs weighing 300-400 g were numbered and bled by cardiac puncture in order to obtain a 5-ml blood sample from each animal. Sera were obtained by centrifuging the blood samples at 2,000 rpm for 10 min at 4°C and were stored at −70°C prior to hemolytic complement titers. The experimental group consisted of 85 animals that were systematically decomplemented by intraperitoneal injection of 250 U/kg body weight of CVF (Cordis Laboratories; Miami, FL) at 0 hr and 100 U/kg at 8, 24, and 32 hr based on methods described by Cochrane and associates. The control group consisted of 83 animals that received intraperitoneal injections of an equal volume of sterile normal saline at 0, 8, 24, and 32 hr.

Immediately following the intraperitoneal injections at 32 hr, animals were given inhalation anesthesia with methoxyflurane (Pitman-Moore; Washington Crossing, NJ) and topical ocular anesthesia with proparacaine 0.5%. Intravitreal injections were then performed on both eyes of decomplemented and control guinea pigs. Using a 0.25-ml syringe and a 30-gauge needle, a 50-μl volume of P. aeruginosa suspension was injected into the pars plana into the vitreous of guinea pig eyes under direct visualization. Additionally, 50 μl of the same inocula were plated in triplicate on sheep blood agar and incubated at 37°C. Colony counts at 24 hr confirmed the actual number of organisms injected. Group 1 consisted of 25 control and 27 experimental guinea pigs that received an intravitreal injection of 42 ± 6 organisms; group 2 consisted of 40 control and 38 experimental guinea pigs that received 102 ± 12 organisms; and group 3 consisted of 25 control and 27 experimental guinea pigs that received 42 ± 6 organisms.

Vitreous aspirates were homogenized and 100 μl serially diluted (10⁻³ to 10⁻⁷) in sterile normal saline. A volume of 100 μl of each dilution of each vitreous sample was plated in duplicate on sheep blood agar and incubated at 37°C for 24 hr. Colony counts were then obtained permitting direct quantification of the number of viable organisms per milliliter in the vitreous. The mean colony count of right and left eyes of each guinea pig in the experimental and control group was obtained. In each group, the logarithms of the mean colony counts of experimental eyes were compared with those of control eyes using the Student’s t-test at days 1, 2, 3, and 7.

Assays of total hemolytic complement were performed on sera obtained from animals prior to injection of CVF or saline and at the time of death. The assays employed commercially available microtiter plates and reagents obtained from Cordis Laboratories (Miami, Florida). The reaction mixtures were based on methods described by Nelson and associates. For each assay, 1.0 ml of sheep erythrocytes (SRBCs) sensitized with rabbit 7S antibodies to sheep erythrocytes (EA) at a concentration of 1 × 10⁹ cells per ml in glucose-gelatin veronal buffer with 0.0015 M Ca²⁺ and 0.0005 M Mg²⁺ (G₁-GVB⁺²) was washed with 20 ml of gelatin veronal buffer (GVB⁺²) and centrifuged at 1800 rpm for 10 min at 4°C. The supernatant was removed and GVB⁺² was added to yield a uniform suspension of 1 × 10⁸ cells per ml. For each microtiter plate assay, serial dilutions of a 50-μl serum sample ranging from 1:2 to 1:2048 were prepared using GVB⁺². A volume of 50 μl of GVB⁺² and 25 μl of SRBCs with EA was added to each dilution. Two controls were employed: a spontaneous lysis control with wells containing 75 μl of GVB⁺² and 25 μl of SRBC-EA, and a 50% lysis control with wells containing 75 μl of GVB⁺² and 25 μl of a 1:2 dilution of SRBC-EA. Plates were incubated on a Cordix Micromix (Cordis Laboratories; Miami, FL) at 37°C for 60 min. The plates were then centrifuged at 1000 rpm for 6 min at 0°C. The titer of total complement in each sample was determined by locating the well which had an erythrocyte button equal in size to the button in the 50% lysis control. If the endpoint was between two wells, the corresponding dilution was interpolated. The reciprocal of the 50% endpoint dilution gave the titer of total complement in CH₅₀ U/ml. Serum from each guinea pig was assayed in duplicate so that the average of two values was used. Complement titers of experimental and control guinea pigs were compared prior to intravitreal injection of CVF or normal saline and at 1, 2, 3, and 7 days after intravitreal injection using the Student’s t-test. At each time point, both experimental and control sera were assayed in a masked fashion.
Table 1. Total complement titers (CH$_{50}$U/ml) in control vs decomplemented guinea pigs

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Number of bacteria injected</th>
<th>Day after intravitreal injection</th>
<th>Mean total complement titer ± SD</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control guinea pigs</td>
<td>Decomplemented guinea pigs</td>
</tr>
<tr>
<td>1</td>
<td>42</td>
<td>1</td>
<td>402 ± 251</td>
<td>40 ± 25</td>
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<tr>
<td></td>
<td></td>
<td>2</td>
<td>233 ± 203</td>
<td>29 ± 18</td>
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<td></td>
<td></td>
<td>3</td>
<td>973 ± 776</td>
<td>184 ± 188</td>
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<td></td>
<td></td>
<td>7</td>
<td>683 ± 296</td>
<td>1365 ± 59</td>
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<tr>
<td>2</td>
<td>102</td>
<td>1</td>
<td>50 ± 21</td>
<td>14 ± 4</td>
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<td></td>
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<td>2</td>
<td>37 ± 19</td>
<td>20 ± 11</td>
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<td>3</td>
<td>56 ± 40</td>
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<td></td>
<td></td>
<td>7</td>
<td>102 ± 71</td>
<td>171 ± 19</td>
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<tr>
<td>3</td>
<td>150</td>
<td>1</td>
<td>53 ± 33</td>
<td>10 ± 7</td>
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<td></td>
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<td>3</td>
<td>208 ± 121</td>
<td>26 ± 19</td>
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<tr>
<td></td>
<td></td>
<td>7</td>
<td>341 ± 193</td>
<td>320 ± 212</td>
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</tbody>
</table>

by the same technician using the same cellular intermediates and reagents.

Results

Gross observations after intravitreal injections of *P. aeruginosa* disclosed no apparent differences in eyes of control guinea pigs and experimental guinea pigs in all three groups. One day after intravitreal injection, guinea pigs in all groups showed conjunctival hyperemia and edema. Corneal edema was present, but hypopyon formation was only occasionally noted. The liquid vitreous had a cloudy appearance. On the second day after intravitreal injection, severe corneal edema and hypopyon formation were noted in all eyes. The vitreous had a yellow-brown color. Three days after intravitreal injection, the corneas were nearly opaque, and the iris and pupil were barely visible. Approximately 50% of the anterior chamber was filled with hypopyon. The vitreous disclosed purulent material. Seven days after intravitreal injection, the corneas were opaque with vascularization approaching the central cornea. Ring infiltrates were noted in some corneas. The anterior chamber was filled with hypopyon, and the vitreous cavity was filled with purulent material.

The results of complement titers in control and experimental guinea pigs are shown in Table 1. Prior to intraperitoneal injection of CVF or normal saline, there was no statistically significant difference in complement titers between experimental and control guinea pigs. One, two, and three days after intravitreal injection, complement titers were lower in experimental guinea pigs than control guinea pigs and the differences were statistically significant in all three groups (Table 1). The effect of decomplementation was lost 7 days after intravitreal injection. In fact, there appears to be a rebound in complement titers in experimental guinea pigs at this time, but the differences were not statistically significant.

In group 1, the bacterial counts were consistently higher in the vitreous of decomplemented guinea pigs compared to control guinea pigs at days 1, 2, 3, and 7 after intravitreal injection, but the differences were statistically significant only at days 1, 2, and 3 (Table 2). Seven days after intravitreal injection, there was no statistically significant difference in complement levels or bacterial counts between control and experimental guinea pigs. In group 2, the bacterial counts in the vitreous of experimental guinea pig eyes were significantly higher than the bacterial counts of the control guinea pig eyes only at day 1 with no statistically significant differences between counts at days 2, 3, and 7 (Table 2). In group 3, the bacterial counts in the vitreous of experimental guinea pig eyes were higher than controls at day 1, but the difference was only of borderline significance (Table 2). The differences were not statistically significant on days 3 and 7.

Histopathologic examination of representative guinea pig globes from experimental and control animals in all three groups disclosed neutrophil infiltration of the corneal stroma, iris, ciliary body, retina, and choroid one day after intravitreal injection. On the second day after intravitreal injection, massive numbers of neutrophils were found in the corneal stroma, iris and ciliary body. The anterior chamber showed accumulations of neutrophils in a fibrin network. More neutrophils were present in the choroid and retina which showed disruption of the cellular layers. On the third day after intravitreal injection, massive numbers of neutrophils were seen in the corneal stroma, anterior chamber, iris and ciliary body. The retina showed extensive disruption and necrosis. One week after intravitreal injection, the corneal stroma, anterior chamber, iris, ciliary body,
nullify its effect. Complementation could be maintained for approximately 5 days. Prolonged decomplementation is not possible because antibodies to CVF rapidly develop due to the statistically significant differences in control guinea pigs at days 1, 2 and 3 with no discernible differences between experimental and control guinea pigs in neutrophil infiltration at any day.

### Discussion

In the present study, comparisons of total complement levels 1, 2 and 3 days after intravitreal injection showed statistically significant differences in control and experimental guinea pigs in all three groups. The magnitude of decomplementation in our study was consistent with that of previous studies. Differences in total complement titers between control guinea pigs in the three different groups reflect differences in cellular intermediates and reagents that were used at different times. However, at each time point both experimental and control sera were assayed in a masked fashion by the same technician using the same reagents ensuring that fluctuations in cellular intermediates and reagents were not factors contributing to the statistically significant differences in complement titers between control and experimental guinea pigs. Seven days after intravitreal injection, there were no statistically significant differences in complement titers between control and experimental guinea pigs. This is consistent with previous studies of decomplementation in guinea pigs in which decomposition could be maintained for approximately 5 days. Prolonged decomplementation is not possible because antibodies to CVF rapidly develop and nullify its effect.

In guinea pigs receiving an intravitreal injection of 42 organisms (group 1), bacterial counts in vitreous were higher in experimental guinea pigs than control guinea pigs, and these differences were statistically significant at 1, 2, and 3 days after intravitreal injection when experimental guinea pigs showed partial decomplementation. No difference in bacterial counts was found 7 days after intravitreal injection when complement levels in experimental guinea pigs rebounded to above normal levels. These results in group 1 suggest that partially decomplemented guinea pigs showed impaired host defense to *P. aeruginosa* and that this defense was restored as complement levels returned to normal. The results in groups 2 and 3 suggest that injection of higher numbers of organisms overwhelms the ability of the complement system to exert some protective effect beyond the first day after intravitreal injection.

Clearly, the complement system alone is not able to eradicate *P. aeruginosa* from the vitreous of the guinea pig eye but does exert some protective effect that was detectable in our study. Endotoxin of gram-negative bacteria activates the alternative pathway of complement. Functions of activated complement include chemotaxis of neutrophils (C5a), anaphylatoxin activity (C3a, C5a), cytology of bacteria (C5b-9), immune adherence facilitating phagocytosis (C3b, C4b), and mobilization of neutrophils from bone marrow (C3e). Humans with deficiencies of complement components may be less able to defend against *P. aeruginosa* in the eye than those without complement deficiencies. Congenital complement deficiencies have been found to be more prevalent in patients developing a first episode of meningococcal meningitis, meningococcemia, or meningococcal pericarditis.

The normal vitreous probably has little in the way of host defense mechanisms against infection. We
have found that normal vitreous does not contain hemolytic complement (unpublished data). Neutrophils and serum proteins including complement pour into the infected vitreous from the dilated, permeable vessels in the tissues surrounding it.

Grading the severity of intraocular inflammation by gross observation is subjective and involves substantial variability among observers. The model that we used eliminated the need for subjective grading of clinical responses by obtaining actual bacterial counts in the vitreous. Our model is based on methods described by Davis and Chandler \(^{15}\) and Kupferman and Leibowitz \(^{16}\) 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 that were otherwise not readily apparent by gross clinical observation of the eyes. Using this model, we were able to show that the complement system exerts some protective effect in host defense against endophthalmitis caused by \(P.\) \(aeruginosa\). Bacterial counts are an objective assessment of the severity of endophthalmitis and may be useful in evaluating the effect of different immunologic factors and antibiotics in the therapy of endophthalmitis.

**Key words:** complement, host defense, endophthalmitis, \(Pseudomonas aeruginosa\)

**References**