Chronic Anaerobic Bacterial Endophthalmitis in Pseudophakic Rabbit Eyes

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Experimental anaerobic bacterial endophthalmitis was produced in pseudophakic and aphakic rabbits by using anterior chamber inoculation of $2.5 \times 10^6$ Propionibacterium acnes organisms. Clinical inflammation was more intense and prolonged in operated eyes with an intraocular lens in place. The presence of an intraocular lens favors the development of chronic low-grade $P. \text{acnes}$-related inflammation. Invest Ophthalmol Vis Sci 28:259–263, 1987

Anaerobic bacterial infection (eg, due to Propionibacterium acnes) is a potential complication associated with the implantation of any prosthetic device. Cases of $P. \text{acnes}$ infection associated with implanted heart valves, artificial joints, and cerebrospinal fluid (CSF) shunts have been reported. Such $P. \text{acnes}$ infections are typically delayed in onset and may have a chronic, indolent course. In the ophthalmic literature, Forster has reported two cases of $P. \text{acnes}$ endophthalmitis following intraocular lens (IOL) implantation. Both presented as delayed, smoldering clinical inflammation. To determine the role of the IOL prosthesis in the development and course of anaerobic bacterial endophthalmitis, we studied a rabbit model of aphakic and pseudophakic $P. \text{acnes}$ endophthalmitis.

Materials and Methods

Animals

New Zealand albino rabbits (2.8 to 3.0 kg) were used for all experiments. They were housed in facilities approved by the American Association of Laboratory Animal Science. All animal eyes were found to be normal by slit-lamp biomicroscopy prior to entry into the study. Our investigations using animals conformed to the ARVO Resolution on the Use of Animals in Research.

Experimental Design

A total of 26 male rabbits were entered into control or experimental groups. Experimental rabbits undergoing standard extracapsular lens extractions with or without IOL implantation had subsequent injection of $P. \text{acnes}$ organisms into the anterior chamber (inoculum of $2.5 \times 10^6 \ P. \text{acnes}$). One group (five rabbits) with pseudophakic eyes and another group (three rabbits) with aphakic eyes were injected 1–3 days after surgery. Two additional experimental groups (4 rabbits with pseudophakic eyes and five rabbits with aphakic eyes) were injected 2–3 wk after surgery; in these nine rabbits, postsurgical inflammation had resolved, and drug therapy had been discontinued for 1 wk prior to inoculation. Control groups (nine rabbits) followed the same injection schedule but received sterile saline in place of the bacterial inoculum. Severity and duration of $P. \text{acnes}$ endophthalmitis were compared in aphakic and pseudophakic rabbit eyes and to saline-injected pseudophakic and aphakic eyes (controls). Postsurgical inflammation and other variables were considered in all analyses.

Surgical Techniques

Rabbits underwent unilateral extracapsular lens extraction (ECCE) alone or ECCE with polymethylmethacrylate posterior chamber IOL implantation. Preoperative dilation was achieved using 10% phenylephrine hydrochloride and 1% tropicamide. Anesthesia was induced by an intramuscular injection of xylazine hydrochloride (3 mg/kg) and ketamine hydrochloride (15 mg/kg); topical anesthesia (0.5% proparacaine hydrochloride) was also employed. An anterior chamber maintainer was obliquely inserted at the 6-o’clock limbal position. Balanced salt solution with 5 units of heparin per ml (to prevent fibrin clotting of the rabbit aqueous) was used as irri-
gating fluid. A vitreoretinal blade was used to enter the anterior chamber at the 10-o’clock limbal position. A “Christmas tree” anterior capsulotomy was performed with a bent 25-gauge needle attached to an irrigating system. After extension of the corneoscleral incision to 3 mm, fragmentation of the lens nucleus was accomplished using the Cavitron-Kelman 7007 Phacoemulsification unit (Coopervision Systems; Irvine, CA). Cortex was removed using the irrigation-aspiration handpiece. The posterior capsule was kept intact. For IOL implantation, the incision was extended to 6.5 mm, and the IOL was inserted with forceps and positioned behind the iris with a lens manipulator. The wounds were closed with 8-0 silk sutures. Eyes to be inoculated after resolution of surgical inflammation were treated with a single subconjunctival injection of 2 mg dexamethasone sodium phosphate plus 20 mg gentamicin sulfate followed by daily 0.05% dexamethasone sodium phosphate ointment to facilitate resolution of inflammation.

Preparation of Anaerobic Bacteria

A clinical isolate of *P. acnes* from the CSF of a patient with an infected CSF shunt prosthesis was frozen at -70°C in skim milk. The isolate was thawed and subcultured anaerobically on Brucella 5% sheep blood agar twice prior to inoculum preparation. A 24-hr plate culture was transferred to an anaerobic chamber, and colonies from it were used as the inoculum source. This eliminated the possibility of nonspecific reactions in the eyes due to proteinaceous and other material from anaerobic broth media. Colonies were suspended in sterile, pyrogen-free, nonbacteriostatic saline (Travenol Laboratories, Inc., Deerfield, IL) to a turbidity corresponding to a Number 1 MacFarland standard (approximately 10⁸ colony-forming units per ml). Aliquots of serial dilutions made in the sterile saline were used for the determination of viable counts (triplicate plates using the rotar pipet method) and intraocular injection. The aliquots were placed in Hungate-style screw cap tubes containing an oxygen-free atmosphere and glass beads (to facilitate mixing) prior to injection.

Inoculation of Anaerobic Bacteria

Anesthetized animals underwent anterior chamber inoculations of 2.5 X 10⁶ organisms in 0.1 ml sterile saline or 0.1 ml sterile saline (controls) through the limbus. Preliminary studies showed that 10⁷*P. acnes* produced severe endophthalmitis in phakic, aphakic, and pseudophakic rabbit eyes. The lower count inoculum size was used in an attempt to produce chronic, low-grade endophthalmitis. Two tuberculin syringes with 30-gauge needles were introduced simultaneously into the anterior chamber. One-tenth milliliter of aqueous humor was aspirated (to prevent loss of inoculum due to increased pressure) followed by injection of inoculum from the second syringe. Remaining inoculum was placed on blood agar for aerobic incubation, chocolate agar for incubation under 10% CO₂, and supplemented fluid thioglycolate medium to confirm inoculation of *P. acnes* only. The anterior chamber, rather than the vitreous, was used as the site for injection of organisms because it is the probable route of infection in human endophthalmitis following ECCE with IOL implantation.

Quantitation of Clinical Inflammation

Animals were examined by slit-lamp biomicroscopy for severity of inflammation on days 3, 4, 5, 7 and then weekly after inoculation. Using a scoring scale for endophthalmitis, a summary score was calculated to quantitate the inflammation observed in the infected eyes at each examination day (Table 1). Clinical signs most diagnostic for endophthalmitis were weighted more heavily in the determination of the summary score.

Microbial Recovery and Identification

Rabbits were euthanized with sodium pentobarbital (6g/ml) prior to aqueous and vitreous humor aspirations. Anterior chamber paracentesis of 0.1 ml of fluid was performed through the limbus using a 22-gauge needle and tuberculin syringe; vitreous aspirates were obtained through the pars plana using an 18-gauge needle and tuberculin syringe. Each aspirate was inoculated directly into fluid thioglycolate medium supplemented with vitamin K₁, hemin, and Tween 80. The broth medium was incubated at 35°C and in-

### Table 1. Scoring scale for *P. acnes* endophthalmitis

<table>
<thead>
<tr>
<th>Clinical sign</th>
<th>Grade</th>
<th>Multiplication factor</th>
<th>Score§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conjunctival injection</td>
<td>0-4+</td>
<td>1</td>
<td>0-4</td>
</tr>
<tr>
<td>Chemosis</td>
<td>0-4+</td>
<td>1</td>
<td>0-4</td>
</tr>
<tr>
<td>Corneal edema</td>
<td>0-4+</td>
<td>2</td>
<td>0-8</td>
</tr>
<tr>
<td>Aqueous cells + flare*</td>
<td>0-4+</td>
<td>2</td>
<td>0-8</td>
</tr>
<tr>
<td>Iris hyperemia</td>
<td>0-4+</td>
<td>2</td>
<td>0-8</td>
</tr>
<tr>
<td>Hypopyon†</td>
<td>0-4+</td>
<td>4</td>
<td>0-16</td>
</tr>
<tr>
<td>Fibrin deposits†</td>
<td>0, 2+, 4+</td>
<td>4</td>
<td>0, 8, 16</td>
</tr>
<tr>
<td>(anterior chamber)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitreous haze</td>
<td>0-4+</td>
<td>8</td>
<td>0-32</td>
</tr>
</tbody>
</table>

* Grades of aqueous cells and flare were summed and then divided by 2.
† Quantitation of hypopyon (0 = none, 1+ = <10%, 2+ = 10-25%, 3+ = 25-50%, 4+ = >50%)
‡ Quantitation of fibrin deposit (0 = none, 2+ = small clot in pupillary opening, 4+ = large clot)
§ "Summary score" is a summation of individual scores above, and was assigned to the clinically graded signs of endophthalmitis as outlined above. We have weighted intraocular features such as hypopyon, fibrin deposits, and vitreous haze more heavily because of their association with endophthalmitis.
spected daily for up to 1 wk. When the medium became turbid, or at days 3 and 7, the broth culture was transferred into an anaerobic chamber, vortexed, and subcultured to a blood agar plate (for anaerobic incubation) and a chocolate agar plate (for incubation under 10% CO₂). Anaerobic isolates were identified using biochemical tests and gas liquid chromatography as previously described.10

Statistical Analysis

The clinical inflammation summary score means were calculated for each animal group at each examination day. Using ordinary least squares techniques, the mean summary scores were regressed against (1) implant: an indicator variable equal to 1 if the eye had an IOL; (2) days: the number of days post-inoculation; (3) day 11: an indicator variable equal to 1 if the observation was made more than 11 days since the inoculation; (4) days * day 11: an interaction term equal to the product of days and day 11; and (5) constant: the constant term. The effect of the IOL on inflammation is measured by the coefficient on implant; the effect of time on inflammation is a combination of the coefficients for days, day 11, and days * day 11.

Results

The computer-generated plots of summary score versus days post-inoculation graphically demonstrated the differences in inflammatory response between the pseudophakic and aphakic eyes inoculated with P. acnes (Figs. 1,2). Inflammation reached a maximum by day 3 after inoculation, followed by a rapid decline that slowed by day 11. The height of the curve for pseudophakic eyes was always greater than that for aphakic eyes. Clinically, both groups of experimental pseudophakic eyes exhibited a greater incidence of hypopyon and anterior chamber fibrin deposits than did both groups of experimental aphakic eyes. By day 11, inflammation due to surgery, paracentesis, and saline, had resolved (control eyes). From day 12 to day 24 after inoculation, the inflammation in all groups of aphakic and pseudophakic eyes declined at a slower rate. On day 24, inflammation in the aphakic eyes had almost completely resolved; however, the pseudophakic eyes continued to demonstrate marked inflammation. We randomly selected two rabbits from each of the two P. acnes pseudophakic groups for long-term clinical follow-up. These eyes were followed to 46 days postinoculation, at which time they continued to exhibit low-grade inflammation with a clinical summary score equal to or greater than that determined for the experimental aphakic eyes on day 24.

Statistically, the regressions demonstrated that the inflammation was significantly greater (P < 0.001) in eyes with IOLs independent of the time postsurgery at which the eyes were inoculated with P. acnes (Table 2).

For all control groups, inflammation (due to either surgery, paracentesis, or saline injection) was markedly

Fig. 1. Graphic comparison of P. acnes-induced endophthalmitis in pseudophakic and aphakic rabbit eyes. Anterior chambers were injected with 2.5 X 10⁶ organisms during the acute (<72 hr) postoperative inflammatory period. A summary score reflecting the clinical grading of inflammation was calculated for each rabbit on each examination day. The mean summary score for each group was plotted versus time. A computer-generated plot based on the predicted values of the regressions was used to fit the curve. Note that the pseudophakic eyes (circles) exhibit a more intense inflammation than do the aphakic eyes (squares).

Fig. 2. Graphic comparison of P. acnes-induced endophthalmitis in pseudophakic and aphakic rabbit eyes. Anterior chambers were injected with 2.5 X 10⁶ organisms 1 wk following resolution of surgical inflammation. A summary score reflecting the clinical grading of inflammation was calculated for each rabbit on each examination day. The mean summary score for each group was plotted versus time. A computer-generated plot based on the predicted values of the regressions was used to fit the curve. Note that the pseudophakic eyes (circles) exhibit a more intense inflammation than do the aphakic eyes (squares).
Table 2. Regression results: coefficients and T-values

<table>
<thead>
<tr>
<th>Variables</th>
<th>P. acnes-inoculated</th>
<th>P. acnes-inoculated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>supernormal</td>
<td>uninflamed</td>
</tr>
<tr>
<td></td>
<td>pseudophakic eyes</td>
<td>pseudophakic eyes</td>
</tr>
<tr>
<td>Implant</td>
<td>3.81* (8.24)†</td>
<td>4.64 (5.92)</td>
</tr>
<tr>
<td>Days</td>
<td>-1.97 (-6.42)</td>
<td>-2.1 (-5.73)</td>
</tr>
<tr>
<td>Day 11</td>
<td>-20.25 (-15.49)</td>
<td>-14.53 (-6.42)</td>
</tr>
<tr>
<td>Days*day 11</td>
<td>1.84 (8.56)</td>
<td>1.89 (5.09)</td>
</tr>
<tr>
<td>Constant</td>
<td>24.63 (22.76)</td>
<td>20.88 (11.19)</td>
</tr>
<tr>
<td>Number of</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>observations</td>
<td>R-square</td>
<td>0.990</td>
</tr>
</tbody>
</table>

* The first of two numbers given for each entry is the coefficient.
† The second number given is the r-value.

less (summary score peak < 7 on day 3) than that seen in the experimental groups and was completely resolved by 11 days.

In all cases, immediate culture of the remaining oculum confirmed that P. acnes was the only organism injected. Recovery of P. acnes was obtained from only 2 of the 26 intraocular aspirates taken at day 24 after inoculation.

Discussion

Previous experimental evidence supports the clinical observations that a foreign body renders tissue more susceptible to infection.12,13 In our rabbit model of anaerobic bacterial endophthalmitis, operated eyes with an IOL prosthesis demonstrated a greater intensity of P. acnes-related ocular inflammation than did the aphakic eyes (no IOL) in both the acute and chronic phases of disease. During the acute phase, however, other postoperative and postinoculation inflammatory components may contribute to the peak and rapid decline of inflammation demonstrated by the summary score curves. Although these components (eg, effects of saline, paracentesis, intraocular surgery) may add to the severity of inflammation observed acutely, we have demonstrated that they contribute only for a maximum of 11 days (control eyes). Thus, after day 11, the magnitude of differences in inflammation observed between aphakic and pseudophakic eyes with P. acnes can be attributed to the presence of the IOL.

P. acnes was chosen as the pathogen in our studies because it is an anaerobic organism of low virulence commonly found on normal human conjunctiva.14 It is a strong inflammatory stimulus that can activate complement15,16 and trigger the release of polymorphonuclear leukocyte (PMN) chemotactic factors17 and hydrolytic enzymes.18 However, a recent study has shown that the organism is not rapidly killed by PMNs, lysozyme, chymotrypsin, H2O2, sensitized human serum, PMN granule lysate, or PMN and monocyte cell lysates, and is only variably killed by monocytes.19 P. acnes may therefore be a cause of persistent postoperative inflammation in the eye as it is elsewhere.

Several possibilities exist to further explain this observation that experimental P. acnes endophthalmitis is more intense and prolonged in eyes with an IOL prostheses. The IOL may act as a nidus for infection or electrostatic forces may attract bacteria to the IOL resulting in greater numbers of organisms retained in pseudophakic eyes. The iris and ciliary body in eyes undergoing IOL implantation may be more traumatized, thus altering the blood-aqueous barrier more in the pseudophakic than in the aphakic eyes. The altered blood-aqueous barrier may take as long as 10½ months for complete repair in rabbits.20 This prolonged and/or increased alteration of the blood-aqueous barrier may result in a greater and/or longer inflammatory response to P. acnes in eyes with IOL implants. This, coupled with the inability of PMNs and variable ability of monocytes to kill P. acnes,19 may account for the persistence of intraocular inflammation. Although our results did not confirm an increase in P. acnes survival, the low recovery rate of P. acnes may reflect suboptimal culture technique or may be related to sampling late in the course of illness.

To our knowledge, this is the first animal model of pseudophakic, anaerobic bacterial endophthalmitis described in the literature. We are aware of only two prior reports of experimental anaerobic endophthalmitis;21,22 both studies used phakic animal eyes. Further research is necessary to determine why eyes with IOL implants develop more intense and prolonged P. acnes endophthalmitis. The role of this organism in cases of prolonged postoperative inflammation in pseudophakic eyes needs to be determined.

Key words: endophthalmitis, anaerobic bacteria, pseudophakia, rabbits

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References


