Spontaneous Sterilization in Experimental Staphylococcus epidermidis Endophthalmitis

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We created a standardized model of endophthalmitis in the aphakic rabbit eye using a laboratory strain of Staphylococcus epidermidis of known characteristics (ATCC 155). Eyes were injected with the following number of organisms: 170, 3760, 8750, 170,000 and 460,000. Serial quantitative cultures, clinical grading of infection and histopathologic studies were performed on days 1, 2, 3, 7 and 14. Bacteria appeared to multiply rapidly during the first 24 hr with peak recovery at 8 to 24 hr. Fewer bacteria were cultured on the third day after injection, and positive cultures were rare after the third day. Inflammatory scores were initially higher with each increased number of injected bacteria and tended to increase for the first 3 to 5 days. Invest Ophthalmol Vis Sci 31:181-186, 1990

Coagulase-negative staphylococci are a group of gram-positive cocci that are common flora on the skin and in the conjunctival sac. They are frequently identified on clinical culture reports as Staphylococcus epidermidis, although this is only one of the various species included within the group. In recent years, Staphylococcus epidermidis has been increasingly reported as a cause of postoperative and traumatic endophthalmitis, now accounting for 30 to 60% of clinical series.1-4

Several laboratory investigations of experimental staphylococcal endophthalmitis have focused on treatment aspects.5-11 Many variables in bacterial concentration, strain virulence and clinical grading exist in these studies. We have, therefore, created a standardized model of the disease in the aphakic rabbit eye using a strain of known virulence. Quantitative sequential cultures, a quantitative clinical grading system and histopathological correlation are used. This model provides new information about the pathophysiology of the disease and provides a standardized infection that may be exploited in studies of various treatment approaches.

Materials and Methods

Surgery

Aphakia initially was created in all eyes by pars plana lensectomy. Anesthesia was induced by intramuscular injection of a 50/50 mixture of ketamine hydrochloride 100 mg/ml with xylazine 20 mg/ml. Pupillary dilatation was achieved with two drops of cyclopentolate 1% and phenylephrine 10%. A 1 cc retrobulbar injection of 0.5% xylocaine also was given. A lid speculum was inserted, and two small conjunctival flaps on the nasal and temporal side of the limbus were incised. A 20-gauge needle was used to create a sclerotomy and enter the lens 3 mm from the limbus on the nasal and temporal sides. Perfusion of BSS was introduced by a hand-held cannula in one sclerotomy, and fragmentation of the lens was accomplished with a 20-gauge ultrasonic needle. The anterior capsule was left intact in most eyes. Wounds were closed with the 8/0 silk sutures, and a subconjunctival injection of 0.2% of dexamethasone solution was given. Eyes were allowed to quiet for 2 to 3 weeks postoperatively. All animals were maintained and used in conformance with the ARVO Resolution on the Use of Animals in Research.

Microbiological Methods

Staphylococcus epidermidis ATCC 155 was grown overnight at 37°C on Trypticase soy agar (BBL Microbiology System, Cockeysville, MD). Isolated colonies were used to inoculate 200 ml of Trypticase soy broth (Difco Laboratories, Detroit, MI), pH 7.3 that was then incubated for 15 hr at 37°C. Bacteria were harvested by centrifugation, washed two times and suspended in sterile 0.9% NaCl in distilled water to various concentrations. Concentrations were adjusted to give the desired number of bacteria in 0.1 ml by spectrophotometric comparisons. Final concentrations were verified from pour plates of 100 μl dilution samples in Trypticase soy agar.

Vitreous Injection of Organisms

Animals were anesthetized by intramuscular injection as before. The retina was examined to exclude
retinal detachment. The globe was fixated by grasping the superior rectus muscle with a forcep and a 30-gauge needle inserted through the pars plana 2 mm from the limbus. Under direct observation, the needle was advanced into the mid-vitreous cavity where the injection was made.

Five groups of animals were injected with the following size inocula: Group 1, $1.7 \times 10^2$ (28 eyes); Group 2, $3.76 \times 10^3$ (10 eyes); Group 3, $8.75 \times 10^3$ (26 eyes); Group 4, $1.7 \times 10^5$ (14 eyes); Group 5, $4.6 \times 10^5$ (16 eyes). Six eyes for special study were injected with 5800 organisms.

### Clinical Examination

Examinations of the eyes with a slit lamp and indirect ophthalmoscope when necessary were made in all groups on days 1, 2, 3 and 7. Observations were also made on day 4 in Group 5, and day 5 in Groups 3 and 5. All animals were graded for inflammatory signs using the scheme in Table 1. Gradings were in most instances performed by two examiners who discussed each eye and arrived at a score for each tissue; this was done to increase the consistency and the reproducibility of the grade. A clinical score was obtained by adding the scores arithmetically. When posterior structures could not be seen, absence of a grade for them would produce a lower score for more severely affected eyes. To compensate for this, a modifier of 15 was added for eyes in which the iris, vitreous cells, vitreous flare and retina could not be seen. If all of these structures were seen and graded most severe, ie, 4, a total of 16 points would be the maximum possible additional score. If anterior chamber flare and cells could not be assessed because of corneal opacity, the additional lost points were compensated for by adding a modifier of 20. All scores were then averaged to determine the mean score. Group 5 was initially scored using a different system and is therefore not included in Table 2 or the Figures. On day 1, clinical grading was done in the following number of eyes: I, 25; II, 10; III, 26; IV, 14; V, 16. Progressively fewer numbers were graded each subsequent day since animals were sacrificed after culture and for histology.

### Quantitative Cultures

In Groups 1 through 5, animals were anesthetized and 0.5 cc vitreous was removed through the pars plana with a 22-gauge needle. Each eye was cultured only once, and the animal was then sacrificed; this prevented the culture intervention from changing subsequent inflammatory scores. Cultures were obtained on days 1, 2, 3 and 7 in all groups. Cultures were also taken in Group 3 on days 5 and 14, in Group 4 at 8 hr, on days 4 and 5, and in Group 5 on days 4, 5 and 14. Each data point represents at least two eyes, except for day 3 in Group 2.

In six eyes of three rabbits, cultures were obtained at 4, 8, 12 and 24 hr after injection of 5800 organisms. After inducing general anesthesia, these cultures all were obtained by removing 0.1 cc of vitreous with a 25-gauge needle through the pars plana. In two eyes of one of these rabbits, cultures were obtained 5 min after injection to confirm the ability to recover the expected number of living organisms. To follow the course of infection in a single rabbit, cultures were obtained on alternate days from the right and left eyes of one rabbit in Group 3 after the injection of 8750 organisms, sampling on days 1, 2, 3, 5, 7 and 14.

Ten-fold serial dilutions of each sample were car-

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**Table 1. Quantitative clinical grading scale (basis for assignment of points in quantitative clinical grading)**

<table>
<thead>
<tr>
<th>Tissue response</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I. Cornea</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Transparency</td>
<td>Clear</td>
<td>Mild</td>
<td>Mod. (Iris visible)</td>
<td>Severe (Bare iris detail)</td>
<td>Opaque (no iris view)</td>
</tr>
<tr>
<td>b. Vessels</td>
<td>None</td>
<td>&lt;1 mm</td>
<td>1-3 mm from limbus</td>
<td>3-4 mm from limbus</td>
<td>Central involvement</td>
</tr>
<tr>
<td>c. Abscess</td>
<td>None</td>
<td>&lt;1 mm</td>
<td>1-2 mm</td>
<td>3-4 mm</td>
<td>≥5 mm</td>
</tr>
<tr>
<td><strong>II. Anterior chamber</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Protein flare</td>
<td>None</td>
<td>Trace</td>
<td>Mild</td>
<td>Mod.</td>
<td>Severe</td>
</tr>
<tr>
<td>b. Inflammatory cells</td>
<td>None</td>
<td>Trace</td>
<td>Mild</td>
<td>Mod.</td>
<td>Severe</td>
</tr>
<tr>
<td>c. Fibrin/hypopyon</td>
<td>None</td>
<td>Mild strands</td>
<td>Mod. strands &lt;15% hypopyon</td>
<td>≥50%</td>
<td>8-ball</td>
</tr>
<tr>
<td>d. Hemorrhage</td>
<td>None</td>
<td>Mild</td>
<td>Mod.</td>
<td>Severe</td>
<td>Neovas.</td>
</tr>
<tr>
<td><strong>III. Iris</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Blood vessels</td>
<td>None</td>
<td>Mild</td>
<td>Mod.</td>
<td>Severe</td>
<td></td>
</tr>
<tr>
<td>IV. Vitreous</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Protein flare</td>
<td>None</td>
<td>Trace</td>
<td>Mild</td>
<td>Mod.</td>
<td>Severe</td>
</tr>
<tr>
<td>b. Opacities</td>
<td>None</td>
<td>Cells</td>
<td>Multiple clumps</td>
<td>Red reflex</td>
<td>Total</td>
</tr>
<tr>
<td>V. Retinal detachment</td>
<td>None</td>
<td>25%</td>
<td>50%</td>
<td>75%</td>
<td>Total</td>
</tr>
<tr>
<td>VI. Optical media</td>
<td>Clear</td>
<td>View of vessel outlines only</td>
<td>Sharp red reflex</td>
<td>Dull red reflex</td>
<td>Totally opaque</td>
</tr>
</tbody>
</table>

* Modifiers: if cornea opaque, add 20; if AC opaque, add 15.
ried out in physiological saline for all specimens described above. One hundred-microliter aliquots of each dilution were placed in a Petri dish and Trypti-case soy agar was added. Plates were swirled, allowed to solidify and incubated at 37°C for 48 hr. Colony forming units (CFU) were then counted and recorded. Results of cultures are expressed in colony forming units per milliliter (CFU/ml).

### Histopathology

At each time period when cultures were performed, eyes were also enucleated for histopathological examination. General anesthesia was induced as before, and the animal was sacrificed by intracardiac injection of 2 cc Benthanasia solution. The conjunctiva was dissected at the limbus and the extraocular muscles were cut free. The optic nerve was severed and the globe fixed in either Trump’s solution and stored in the refrigerator at 4°C or in formaldehyde 10%. After fixation, the eyes were embedded in Epon 812, and routine sections were cut. The tissues were stained with hematoxylin-eosin for routine light microscopy. The following factors were graded on a 1-4 scale: anterior chamber reaction, corneal and limbal reaction, ciliary body cellular infiltrate, vitreous cavity reaction, choroidal engorgement and cellular infiltrate, retinal detachment or receptor damage. 

### Results

#### Inflammatory Scores

At 24 hr after inoculation of bacteria, all eyes showed signs of clinical infection. Inflammatory scores were progressively higher with each increment of greater inoculum size (Table 2, Fig. 1). The average scores were: Group 1, 8.5; Group 2, 17.2; Group 3, 19.6; Group 4, 26.9. Inflammatory signs in Group 1 were grade 2 to 3 for anterior chamber cells and flare, and grade 1 to 2 for cells and flare in the vitreous. In Groups 4 and 5, there was grade 1 to 2 for corneal edema and fibrin response and hypopyon in the anterior chamber so severe that view of the iris, vitreous and retina was obscured. Although scored on different systems, the findings in Group 4 and 5 were essentially identical. Groups 2 and 3 presented intermediate degrees of severity between these extremes. There was greater variability of findings when lesser numbers of bacteria were injected.

On the second and third days, there was increased inflammation in all groups. The increases were more apparent in Groups 1 through 3 since close to maximal inflammation was noted in Groups 4 and 5 on the first day. Grading in Group 3 demonstrated continued gradual increases in inflammatory scores through day 6. Groups 3 and 5 were graded on day 14 and showed a slight decline in scores from day 7.

#### Quantitative Cultures

Six eyes were injected with 5800 organisms; when sampled 5 min later, $1.2 \times 10^3$ CFU/ml and $1.3 \times 10^3$ CFU/ml were recovered, confirming the accuracy of the method. These two eyes showed a decline in the number of bacteria at 4 hr followed by a rapid growth.

![Fig 1. Mean inflammatory scores plotted on a daily basis.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933150/ on 11/15/2018)
phase, which peaked at 8 hr and declined at 48 hr. In two eyes, growth peaked at 8 hr and declined rapidly, while in the final two, peak recovery was at 4 hr. Peak concentrations were between $10^5$ and $10^7$ CFU/ml in all eyes. All eyes showed a decline from 8 hr values, and by 48 hr, all counts were in the range of $1.5 \times 10^3$ CFU/ml or less; three of six cultures were negative (Fig. 2). In Group 4, values showed a similar peak of organisms between $10^5$ and $10^7$ CFU/ml at 8 hr with lesser concentrations found later (Table 3).

Examining the results on day 1 (Table 3) reveals that in all groups the concentrations were higher than on day 2. Higher values were identified in Groups 4 and 5 where initial inocula were higher. By day 3, values in all groups had fallen to the range of $5 \times 10^2$ CFU/ml or less in all eyes except 1 in Group 5; six of 15 cultures were negative in all groups on day 3. Only two of 12 cultures were positive on days 4 and 5. Only one of 12 cultures was still minimally positive on day 7, and no cultures were positive on day 14. Inflammatory scores showed an increase at the time bacterial counts demonstrated a decline (Fig. 3).

**Histopathology**

On the first day after injection of 170 organisms, eyes demonstrated mild to moderate infiltration with polymorphonuclear leukocytes (PMNs) at the corneal limbus and ciliary body. The vitreous cavity and anterior chamber showed a mild degree of PMN infiltration and accumulation of proteinaceous material and fibrin. These changes did not show a marked increase on days 2, 3 or 7. When 3750 and 8750 organisms were injected, the initial inflammation on days 1 and 2 was generally significantly more severe than after injection of 170 organisms; inflammatory scores were generally twice as great. There were greater numbers of PMNs and increased protein and fibrin in the anterior chamber and vitreous. Increased PMN infiltration was apparent in the iris, ciliary body and corneoscleral limbus. Exudative retinal detachment was present in one eye on day 1; patchy necrosis of outer segments was identified in several eyes by day 3. The degrees of inflammatory cell infiltrate in iris, ciliary body, vitreous and anterior chamber, and of fibrin and protein in the anterior chamber and vitreous cavity remained approximately the same on days 3 through 7, although a tendency toward increased numbers of mononuclear cells was observed. By day 14, fewer cells were present in the various ocular structures, and overall, inflammatory signs were reduced.

After injection of 170,000 organisms, the overall inflammatory reaction was almost twice as great on the first 2 days after injection than after 5000 to

<table>
<thead>
<tr>
<th>Groups</th>
<th>Injected no. of organisms</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>170</td>
<td>$5.3 \times 10^5$</td>
<td>$1.6 \times 10^5$</td>
<td>$7 \times 10^3$</td>
<td>$0 (3)$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3760</td>
<td>$2.6 \times 10^2$</td>
<td>$1.3 \times 10^6$</td>
<td>$5 \times 10^4$</td>
<td>$0 (3)$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>8750</td>
<td>$1.7 \times 10^2$</td>
<td>$8.1 \times 10^6$</td>
<td>$4 \times 10^3$</td>
<td>$0 (3)$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>170,000</td>
<td>$9.5 \times 10^2$</td>
<td>$3.1 \times 10^6$</td>
<td>$1.1 \times 10^3$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>460,000</td>
<td>$2.7 \times 10^2$</td>
<td>$0$</td>
<td>$30$</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
10,000 organisms on the first 2 days. The inflammatory scores then decreased somewhat in the grading periods of days 3, 4, 5 and 7, although some eyes clinically in this group showed worsening clinical inflammatory signs.

Individual inflammatory scores from pathology grading showed good agreement with the clinical inflammatory scores. The average pathology grading did not show a significant increase in inflammatory score on days 3 through 7; each time period contained two to four pathology samples while the clinical grading was of as many as 26 eyes on day 1 with declining numbers on subsequent days as animals were sacrificed.

**Discussion**

We created a model of staphylococcal endophthalmitis by injecting a standardized laboratory strain of the organism into the aphakic eye of the rabbit. We chose this strain because it is well characterized, representative of the species and readily available. A small number of organisms is capable of causing typical clinical endophthalmitis. When a small number of organisms is injected, however, the disease is mild, slowly progressive, and variable in severity from one eye to the next. This variation may result partially from the difficulty of the precise quantitation of small numbers of organisms. In Group 2, all animals were injected from the same preparation of organisms in a single experiment. The variation in clinical response between rabbits despite injection of the same number of organisms suggests a role for host factors in this variation. Disease in animals with small numbers of organisms appeared to stabilize by itself and to resolve without therapy.

With injection of 3760 and 8750 organisms, more severe disease was produced consistently in all animals. The inflammatory changes progressed on a daily basis, tending to stabilize by day 7. Throughout the course of the disease, inflammatory scores were approximately twice as great in these eyes injected with higher numbers of bacteria compared to injections of 170 organisms. Injection of more than 100,000 organisms consistently produced moderately severe clinical inflammation with little tendency to spontaneous resolution.

Culture results from repeated sampling of single eyes and from single samples from a sequence of eyes indicate that organisms multiply for approximately the first 48 hr at variable rates. Peak numbers of organisms were reached in the first 24 hr, often by 8 hr after inoculation. The peak observed values were from $10^3$ to $10^7$ and appeared to be independent of the size of initial injection from $5.8 \times 10^3$ through $4.6 \times 10^5$ organisms. The number of bacteria recovered at 24 hr, however, appeared significantly greater with greater initial inoculum size. The numbers of organisms then declined spontaneously, and organisms were rarely recovered from cultures taken 120 hr or longer after initial inoculation of living bacteria.

Initial inflammatory signs were greater both clinically and histopathologically with increased inoculum size. Both indices demonstrated that inflammation continued to increase throughout the first 3 to 6 days, despite the fact that the number of bacteria began to decline after the first or second day. Significant inflammation was present in eyes that were sterile.

These findings suggest that some component of the inflammatory process or that toxic products produced by dead and dying bacteria are capable of sustaining and increasing the inflammatory changes. Dead bacteria themselves are probably not the major contributor to this process since we have previously shown that $10^6$ heat-killed bacteria produce little inflammatory change (unpublished data). In experimental *Klebsiella oxytoca* endophthalmitis, Meyers-Elliott et al. found the number of organisms to peak at 24 hr, declining to a minimal number recovered at 72 hr, while none were recovered beyond this time. Inflammatory signs increased even after the eyes became sterile.

Davey et al. studied endophthalmitis produced by injections of *Klebsiella pneumonia* or *Pseudomonas aeruginosa* into the vitreous cavity of phakic rabbit eyes. There was a rapid multiplication of organisms for the first 48 hr with a leveling off and subsequent decline of the number of viable organisms after this time. Although they suggested that this might be a...
phenomenon of gram-negative endophthalmitis, our results demonstrate that *S. epidermidis* not only declines in number, but that the vitreous cavity is sterile in almost all eyes tested after 72 hr. A similar finding was noted in a small number of eyes in a *S. epidermidis* by Smith et al in control eyes in a study of intravitreal vancomycin. These studies suggest that the natural course of infection in the rabbit eye is to show a rapid early growth of bacteria with a subsequent spontaneous decline. In the *S. epidermidis* and in *Klebsiella oxytoca* models, the bacteria die out entirely, while the other gram-negative organisms studied to date have the capability to sustain growth at lower levels.

The mechanisms of decline in organisms and spontaneous sterilization of the vitreous cavity in the rabbit experimental endophthalmitis remain unclear, but most likely represent a change in the environment within the closed space of the vitreous cavity. Davey et al noted a fall in the number of bacteria in the vitreous as compared to a subcutaneous croton oil patch after an initial period of rapid growth at both sites. They demonstrated that levels of oxygen, glucose, nitrogen, phosphate, calcium, iron and magnesium were not different at the two sites and that a difference in nutrients did not explain the decline in vitreous bacteria. They suggested that accumulation of an antibacterial substance was likely. We propose that accumulation of inhibiting proteins, white cells, or a fundamental change in the vitreous character itself may account for the spontaneous sterilization we observed.

The phenomenon of spontaneous sterilization suggests care in the interpretation of experimental drug studies. Sterilization of the vitreous cavity often has been used as an endpoint in laboratory studies of the efficacy of intravitreal antibodies with or without vitrectomy. The spontaneous decline of the number of organisms in untreated eyes suggests that this endpoint is valid only when eyes are scrupulously compared to untreated controls. Since sterility may occur spontaneously in experimental disease, the endpoint of treatment studies should be physical and functional salvage of the eye and not simple killing of microorganisms.

These findings have several clinical implications. The rabbit eye seems capable of recovering from infection with an inoculum of small size. Although inflammatory signs were clearly apparent after injection of 170 organisms and culture results showed that bacteria had replicated, the concentrations on day 1 were low and the eyes recovered spontaneously.

Second, the findings of spontaneous sterilization of the vitreous cavity without treatment suggest an explanation of some cases of culture-negative postoperative endophthalmitis. In large clinical series of endophthalmitis, 28 to 44% of cases of apparent clinical infection are culture-negative and, in general, have a good prognosis. One possible explanation is that some of these eyes initially were infected but became spontaneously sterile, particularly if the initial inoculum was small. Inflammation increases despite the declining number of bacteria, leading to diagnostic vitreous taps and therapeutic intervention. These cultures are possibly negative because the bacteria already have died while the inflammatory process continues.

Finally, it is clear that significant inflammation continues in the absence of bacterial replication. This suggests that control of the inflammatory responses may be as important as killing microorganisms in the treatment of endophthalmitis since inflammation continues after bacteria are dead.

**Key words:** endophthalmitis, infection, inflammation, *S. epidermidis*

**References**