Antibacterial Effects of Previously Infected or Inflamed Vitreous

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The authors investigated the ability of vitreous harvested from eyes previously infected with *Staphylococcus epidermidis* or inflamed with heat-killed cells of the same organism to support subsequent in vitro bacterial growth. Growth of *S. epidermidis* and *S. aureus* was not supported by previously infected or previously inflamed vitreous, but *Pseudomonas aeruginosa* grew in all samples. These findings suggest induction of an antistaphylococcal substance by infection or inflammation of rabbit vitreous by *S. epidermidis*. Invest Ophthalmol 31:2342-2344, 1990

During studies to create a standardized model of *Staphylococcus epidermidis* endophthalmitis, we observed that the bacterial population in the vitreous reached a maximum by 24 hr after intraocular inoculation and then spontaneously declined to sterility after 72 hr. A similar finding was noted by Smith et al in a small number of control, untreated eyes with experimental *S. epidermidis* endophthalmitis. Other studies show a spontaneous inhibition of bacterial growth after inoculations of *Klebsiella oxytoca*, *K. pneumoniae*, and *Pseudomonas aeruginosa* in experimental gram-negative endophthalmitis. These findings suggest that there may be an antibacterial effect in the vitreous after bacterial infections.

To examine this phenomenon further, we evaluated the ability of previously infected vitreous to support further bacterial growth in vitro. In addition, we created sterile inflammation in the vitreous to assess whether prior inflammation alone can suppress subsequent bacterial growth in vitro. We compared the ability to support in vitro bacterial growth of rabbit vitreous samples harvested from: (1) control (uninfected/uninflamed) aphakic eyes, (2) aphakic eyes which had become sterile after intravitreal infection with *S. epidermidis*, and (3) aphakic eyes with sterile inflammation secondary to an intraocular injection of 10⁹ heat-killed *S. epidermidis* cells.

Materials and Methods

Aphakia was created in 42 eyes of albino rabbits by pars plana lensectomy and fragmenting of the lens using a 20-gauge ultrasonic needle. Inflammation was allowed to resolve for 3–4 weeks postoperatively. All animals were maintained and used in conformance with the ARVO Resolution on the Use of Animals in Research.

Microbiologic Methods

*S. epidermidis* (American Tissue Culture Collection, [ATCC] 155) was grown overnight at 37°C on Trypticase soy agar (BBL Microbiology System, Cockeysville, MD). Isolated colonies were used to inoculate 20 ml of Trypticase soy broth (TSB) (Difco Laboratories, Detroit, MI), pH 7.3, that was then incubated for 15 hr at 37°C. Cells were harvested by centrifugation, washed twice, and suspended in sterile 0.9% NaCl. Concentrations were adjusted to give the desired number of bacteria in 0.1 ml by spectrophotometric comparisons. For preparations of nonviable *S. epidermidis* cells, the desired number of bacteria suspended in 0.9% NaCl were heat-killed by incubation for 30 min in a 60°C water bath. Nonviability was confirmed with four plates of 100-μl samples inoculated onto Trypticase soy agar. *S. aureus* (ATCC 12600) and *P. aeruginosa* (ATCC 15442) for use in in vitro inoculations were prepared similarly.

Intravitreal Injection of Organisms

After the animal was anesthetized, a 30-gauge needle was inserted through the pars plana 2 mm from the limbus and advanced under direct observation into the midvitreous cavity where the bacterial inoculation of 0.1 ml was made.
In 16 eyes, infection was established by injection of 0.1 ml of balanced salt solution (BSS) containing 10⁶ S. epidermidis organisms. In a second group (15 eyes) inflammation was induced by injection of 0.1 ml BSS containing 10⁹ heat-killed S. epidermidis organisms. A third group (control) of 13 eyes received no intravitreal injection.

Vitreous Collection and In Vitro Inoculation

Animals were subsequently anesthetized and killed by intracardiac injection of Beuthanasia (Sherine Corp., Kenilworth, NJ) on days 4–7 after intravitreal injection. After death, the vitreous was immediately removed through the pars plana with a 26-gauge needle from all groups. Then 100 μl was removed from each collected vitreous sample for culturing to insure sterility; the remaining vitreous of each sample was then inoculated with either S. epidermidis (ATCC 155, 10⁷ organisms/ml of vitreous), S. aureus (ATCC 1566, 10⁴ organisms/ml of vitreous), or P. aeruginosa (ATCC 15442, 10⁷ organisms/ml of vitreous) and incubated at 37°C.

Bacterial Count Estimation

Bacterial growth after in vitro inoculation was assessed using tube-dilution procedures at 0 and 24 hr. At each time interval, 100 μl of undiluted vitreous was removed for a series of tenfold dilutions in TSB (pH 7.3). After 24-hr incubation at 37°C, each dilution was examined for bacterial growth.

Results

Vitreous from control eyes yielded continued viable recovery of bacteria 25 hr after in vitro inoculations of S. epidermidis (all five samples), S. aureus (all six samples), and P. aeruginosa (both samples) (Table 1).

In the experimental groups, immediately after in vitro bacterial inoculation (time 0) viable organisms could be recovered from all samples. At 24 hr, however, S. epidermidis could not be recovered from the vitreous of either previously inflamed eyes or previously infected eyes. Similarly, S. aureus was not recovered from the vitreous of eyes with sterile inflammation, and only one of five samples of vitreous from eyes previously infected was positive. By contrast, at 24 hr significant growth of P. aeruginosa was detected in all samples of previously inflamed vitreous (five samples) and previously infected vitreous (five samples) (Table 1).

Discussion

The vitreous from the normal phakic rabbit eyes supports bacterial growth in vitro. In this study, we observed continued bacterial growth of S. aureus, S. epidermidis, and P. aeruginosa after in vitro inoculations of bacteria into vitreous from aphakic rabbit eyes.

This ability of the vitreous to support bacterial growth was altered by induction of sterile inflammation due to heat-killed S. epidermidis and by infection of vitreous with S. epidermidis. After intravitreal inoculation with viable S. epidermidis, the vitreous becomes spontaneously sterile. The sterile vitreous from eyes with previous infection and from eyes with strictly sterile inflammation no longer supported in vitro bacterial growth of S. epidermidis and S. aureus. By contrast, P. aeruginosa, a gram-negative organism, grew well in all three types of vitreous.

The exact mechanism for this antimicrobial effect in the vitreous is not known. Davey et al measured changes in pH, PO₂, and concentrations of substances (including glucose, protein, phosphate, calcium, iron, and magnesium) that might influence bacterial growth in the vitreous during untreated experimental gram-negative bacterial endophthalmitis. The results were compared with measurements from a subcutaneous site of infection which was not characterized by a spontaneous inhibition of bacterial growth over the studied time. They concluded that the spontaneous decline in bacterial population in the vitreous during gram-negative bacterial endophthalmitis could not be attributed in changes in pH, PO₂, or concentration of any of the nutrients or growth factors assessed in their study. Our findings suggest a similar conclusion in the spontaneous sterilization in experimental S. epidermidis endophthalmitis. Vitreous from eyes with sterile inflammation had not supported active bacterial metabolism, and yet, it did not support sustained bacterial growth of staphylococcal species after in vitro inoculation. This implies that nutrient changes in the vitreous are not a likely explanation for this phenomenon. Further support for this conclusion is the ability of a gram-negative organism to multiply in vitreous previously infected or inflamed by a gram-positive organism. Similarly, production of inhibi-

Table 1. Culture positive results at 24 hr

<table>
<thead>
<tr>
<th>Inoculated organisms</th>
<th>Condition of vitreous</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>5/5</td>
</tr>
<tr>
<td>S. aureus</td>
<td>6/6</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>2/2</td>
</tr>
</tbody>
</table>

Values are number of samples.
tory substance by the infecting bacteria itself is unlikely to play a role since the antibacterial effect could be produced with heat-killed cells.

Consequently, it is likely that some component of the host response or other, as yet, undetected change in the vitreous due to the intraocular presence of either living or dead *S. epidermidis* is responsible for the bactericidal effect of the vitreous. Since gram-negative bacteria grew well in such vitreous, the bactericidal effect may be due to an elaboration of some inhibitory factor produced by the host which specifically inhibits staphylococcal species. Examples of possible inhibitors include proteins such as lysozomal enzymes and fatty acids. Isolated fatty acids present in abscess contents have been shown to have a bactericidal action on *S. aureus* in vitro and in vivo. Furthermore, since gram-negative bacterial infections themselves are spontaneously inhibited, a separate factor or factors possibly may be induced by gram-negative infections. Further characterization and attempted isolation of these substances is warranted.

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

**References**