Staphylococcus aureus is an important cause of severe bacterial endophthalmitis. Both immunoglobulin (Ig) G and A antibody titers to ribitol teichoic acid (RTA), the major antigenic determinant of the S. aureus cell wall, were measured by an enzyme-linked immunosorbent assay in serum, tears, aqueous, and vitreous on days 3, 7, 10, 14, 21, and 30 after intravitreal injection of viable S. aureus in rabbits. Clinical examination showed vitreous opacification in all rabbits from days 7-30. Histopathologic examination showed acute inflammation on day 3 and chronic inflammation on days 7-30 in the conjunctiva, cornea, iris, ciliary body, and trabecular meshwork. The vitreous cavity contained neutrophils and necrotic cells on all days. Retinal necrosis was present on days 14-30. Lymphoid follicles with plasma cells were identified in the conjunctiva, ciliary body, and choroid. The vitreous of experimental eyes showed increasing numbers of bacteria from days 3-14, followed by a decrease in numbers on day 21 and absence of viable bacteria on day 30. Increases in IgG antibody levels to RTA were first detected in serum where they were higher than in tears, aqueous, and vitreous until day 14. Vitreous IgG antibody levels to RTA in experimental eyes exceeded all other samples on day 14 and progressively increased thereafter; the other samples declined. The IgA antibody levels were increased in tears on day 14 and in the vitreous of experimental eyes on days 14, 21, and 30. Vitreous IgG antibody levels to RTA were substantially higher than vitreous IgA antibody levels. An inverse correlation was found between vitreous IgG antibody levels and positive vitreous cultures, suggesting that the humoral immune response may be important in the spontaneous sterilization of the vitreous in this model. Invest Ophthalmol Vis Sci 32:1523-1533, 1991

Staphylococcus aureus is one of the most common causes of postoperative infectious endophthalmitis. The cell wall of S. aureus contains three major antigenic components: protein A, peptidoglycan, and ribitol teichoic acid (RTA). These assays have been useful in diagnosing and assessing the response to therapy in patients with staphylococcal diseases including bacteremia with metastatic abscesses, endocarditis with negative blood cultures because of prior antibiotic therapy, and deep-tissue infections that are inaccessible to culturing. Moreover, serum antibody levels to RTA correlate with the development of corneal phlyctenules, catarrhal infiltrates, and blepharitis in rabbits immunized with S. aureus antigens and challenged with viable S. aureus.

Little is known about the ocular immune response to bacterial endophthalmitis in general and S. aureus endophthalmitis in particular. To study the humoral immune response to S. aureus endophthalmitis, we measured antibody levels to RTA in serum, tears, aqueous, and vitreous using an enzyme-linked immunosorbent assay (ELISA) at various times after intravitreal injection of S. aureus in a rabbit model. Antibody levels to RTA were correlated with clinical signs of inflammation, vitreous culture results, and gross and histopathologic findings.

Materials and Methods

Organism

We used a strain of S. aureus isolated from a human corneal ulcer. The strain belonged to phage type 95 and was coagulase positive, B-hemolytic, pigmented, and gentamicin sulfate resistant. S. aureus, maintained in sheep red blood cells, was incubated on a gentamicin-containing blood agar plate (BAP) at 37°C for 18 hr and transferred to sterile tryptic soy broth for incubation at 37°C for 24 hr. The suspension was then washed with sterile normal saline. A spectrophotometric optical density of 0.16-0.21 at 530 nm corresponded to a viable bacterial count of

From the Ocular Inflammatory Disease Center, Jules Stein Eye Institute, UCLA School of Medicine, Los Angeles, California.
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approximately $1.5 \times 10^8$ colony forming units (CFU)/ml. This suspension of 
*S. aureus* was adjusted by serial dilution in sterile normal saline to yield a final 
concentration of approximately 150 CFU/0.1 ml for intravitreal injection. This inoculum size was found 
in preliminary studies to result consistently in endophthalmitis. Final bacterial concentrations were 
confirmed by plating 0.1 ml of the suspension onto gentamicin-containing BAP in triplicate and incubating 
the plates at 37°C for 48 hr.

Sample Collection

Thirty-six New Zealand albino female outbred rabbits, weighing 2–3 kg, were used in this study: 30 experimental and 6 control rabbits. Phakic rabbits were used to minimize anatomic disturbances in the globe. All investigations described in this manuscript conformed to the ARVO Resolution on the Use of Animals in Research. Before all sample collections, the animals were sedated with an intramuscular injection of 50mg of chlorpromazine hydrochloride. Preimmune (defined as before the intravitreal injection of 
*S. aureus*) samples of serum and tears were collected from all rabbits. Arterial blood was drawn from rabbit ears and then centrifuged at 2000 rpm for 15 min to separate the serum from the clot. Approximately 30–35 µl of tears were collected from the tear film meniscus of each eye of each rabbit by capillary attraction into 5–µl Accupettes (Dade Diagnostics, Aguada, Puerto Rico) as previously described. All sample collections were done in the morning at approximately the same time of day. Samples of serum, tears, aqueous, and vitreous were not pooled for analysis.

Inoculation

General anesthesia was induced before intraocular injections by marginal ear vein injection of 3 ml of 
Equi-Thesin (chloral hydrate 42.5 mg/ml and sodium pentobarbital 10.5 mg/ml; UCLA Pharmaceutical, 
Los Angeles, CA), given 30 min after sedation with intramuscular chlorpromazine. After induction of 
general anesthesia, two drops of proparacaine 0.5% solution (Alcaine; Alcon, Humacao, Puerto Rico) 
were applied to the eye.

Paracentesis of 0.1 ml of aqueous humor was done 1 mm anterior to the limbus using a 30-gauge needle 
on a tuberculin syringe immediately before intravitreal injection to limit intraocular pressure increases. 
Aqueous humor collected in this manner served as the preimmune aqueous samples. The right eye of the 
experimental rabbits received intravitreal injections of 0.1 ml of the bacterial suspension using a similar syringe and needle. The needle was inserted approximately 2 mm posterior to the corneoscleral limbus 
through the superonasal pars plana. Under direct ophthalmoscopic visualization, the needle was positioned 
behind the lens in the midvitreous body with the bevel directed anteriorly. The left eye of each experimental rabbit served as the unoinoculated control eye.

Control rabbits were used to ensure that rabbits boarded in our animal facility did not develop rising 
antibody titers to RTA during the study. These rabbits received intravitreal injections consisting of 0.1 
ml of sterile normal saline into their right eyes as described. Vitreous humor from the uninjected left eye of the control rabbits served as normal, undisturbed vitreous.

Rabbits were randomly assigned to one of six groups consisting of five experimental and one control animal per group. One group was killed on postinoculation days 3, 7, 10, 14, 21, and 30. The rabbits to be killed were graded clinically by gross external examination and direct ophthalmoscopy based on a scoring system for severity of endophthalmitis adapted from Peyman and colleagues (Table 1). Thirty minutes after chlorpromazine sedation, blood and 30–35 µl of tears from each eye were collected. The rabbits were then killed by a marginal ear vein injection of 10–15 ml of Equi-Thesin. Aqueous humor aspirates (0.2–0.3 ml) were collected from each eye as described. Vitreous humor aspirates (0.2–0.3 ml) were also obtained from each eye using a 22-gauge needle on a 10-ml syringe inserted through the pars plana. Rarely, aspirates were contaminated by blood. These were discarded, and repeat aspirates from a different site were obtained.

| Table 1. Grading scale of severity of endophthalmitis* |
|---------------|---------------|---------------|---------------|---------------|
| **Conjunctiva** | 0 = Normal | 1 = Mild edema | 2 = Edema, mild hyperemia, slight exudate | 3 = Edema, marked hyperemia, heavy exudate |
| **Cornea** | 0 = Clear | 1 = Focal edema | 2 = Diffuse edema | 3 = Opaque |
| **Iris** | 0 = Normal | 1 = Mild hyperemia | 2 = Marked hyperemia | 3 = Marked hyperemia, synechiae, irregular pupil |
| **Vitreous** | 0 = Clear | 1 = Areas of vitreous haze, some fundus detail visible, good red reflex | 2 = Moderate vitreous haze, no fundus detail visible, partial red reflex | 3 = No red reflex |

* Adapted from Peyman and colleagues.
Aqueous and vitreous samples were separated into equal volumes and placed into two separate microcentrifuge tubes (Fisher, Pittsburgh, PA). Vancomycin hydrochloride (50 µl of a 50 mg/ml solution; Lederle, Carolina, Puerto Rico) was added to one sample to be designated for ELISA. The other sample without vancomycin hydrochloride was inoculated onto a gentamicin-containing BAP. The remainder of the sample was frozen at -70°C. Culture plates were incubated at 37°C for 48 hr. Cultures positive for bacterial growth were quantified. Serial dilutions of positive vitreous cultures were done, and 100-µl aliquots were streaked onto trypticase soy agar plates and incubated at 37°C for 24 hr. Colony counts were then determined.

Both eyes were enucleated from each rabbit, placed in 10% buffered formalin for 96 hr, and then stored in 80 ml of a 50% ethanolic solution before sectioning in coronal and sagittal planes. Representative experimental and control globes from each time point were embedded in paraffin, sectioned into 4-µm segments, and stained with hematoxylin and eosin and Gram stain for histopathologic evaluation.

Preparation of RTA

The preparation of *S. aureus* RTA followed methods described previously. *S. aureus* was grown in 100 l of tryptic soy broth containing 4 µg/ml of gentamicin in a 250-l fermenter (Fermentation Design, New Brunswick, NJ) at 37°C until the maximum logarithmic phase of growth was reached at approximately 6 hr. The bacterial cells were disrupted by several passages through a French Pressure Cell Press (American Instrument, Silver Springs, MD) at 20,000 pounds per square inch. The unbroken cells were separated from the broken cells by low-speed centrifugation (2000 rpm for 15 min). The broken cells were removed and centrifuged again at 15,000 rpm for 20 min. The pellet containing the broken cells was treated with 200 µg/ml DNase and 200 µg/ml RNase (Sigma, St. Louis, MO) to remove nucleic acids and 0.5 mg/ml trypsin (Sigma) to remove proteins including protein A. The cell wall fraction was purified further with 2% sodium dodecyl sulfate to remove cell membranes, washed several times in sterile distilled water, and lyophilized.

The RTA was extracted from the cell wall preparation with 10% (w/v) aqueous trichloroacetic acid at 4°C three times overnight on a shaker. The three supernatants were pooled and extracted three times with a double volume of absolute ethyl ether each time. The aqueous phase containing RTA was precipitated with five volumes of 95% ethanol for 72 hr at 4°C. The RTA precipitate was passed through three changes of acetone, dried, and stored at 20°C in a desiccator. Chemical analysis of RTA by precolumn derivatization disclosed the presence of alanine, glycine, and N-acetylgalactosamine, the components of RTA. Other components of *S. aureus* cell wall were absent or present in only trace amounts. Antibody titers of selected serum samples were similar using our RTA and that provided to us by Dr. Roman Dziarski, PhD (Indiana University, Gary, IN; preparation described by Dziarski and associates). An ELISA was used to measure antibodies to RTA in rabbit serum, tears, aqueous, and vitreous. Purified RTA was dissolved in phosphate-buffered saline (PBS, pH 7.4) containing 10% carbonate coating buffer (pH 9.6) at a concentration of 10 µg/ml. Each well of the flat-bottom microtiter plates (NUNC Immunoplates; Irvine, Santa Ana, CA) was coated with 100 µl of the RTA preparation. The plates were incubated at room temperature for 12 hr. The plates were washed four times with PBS-Tween 20 (pH 7.4) (American Qualex, La Mirada, CA). Eight microliters of serum, tear, aqueous, or vitreous was added to wells in the plates, and serial dilutions were made in PBS from 1:25 to 1:51,200. All samples were tested in duplicate. The plates were incubated at 37°C for 1 hr and then were washed four times with PBS-Tween 20. To measure immunoglobulin (Ig) G antibody levels to RTA, 100 µl of 1:12,000 goat anti-rabbit IgG, heavy and light chain specific, conjugated with peroxidase (American Qualex) was added to each well. To measure IgA antibody levels to RTA, 100 µl of 1:6,000 sheep anti-rabbit IgA, against secretory component and the alpha chain, conjugated with peroxidase (Cooper, Malvern, PA) was added to each well. Both of these antisera gave a single precipitation line when reacted against rabbit serum in immunoelectrophoresis. The plates were incubated at 37°C for 1 hr and then were washed five times with distilled water to remove any unbound antibody. After this, a volume of 100 µl of o-phenylenediamine containing 1% ureaperoxide in 1 M citrate buffer (pH 4.75) was added to each well. The plates were incubated in darkness at room temperature for 20 min. A Titertek Multiskan (Flow, McLean, VA) was used to measure absorbance in each well at a wavelength of 414 nm. Wells containing serially diluted lysozyme- or peroxidase-labeled antiserum without rabbit serum, tears, aqueous, or vitreous consistently produced absorbance values of less than 0.08. Absorbance readings greater than or equal to 0.1 were considered positive; ELISA titers were expressed as the reciprocal of the dilution of serum, tears, aqueous, or vitreous having...
an absorbance reading of 0.1. Positive and negative controls were tested on each plate to ensure consistency of results.

### Statistical Analysis

The ELISA titers in serum, tears, aqueous, and vitreous were converted to logarithm (log) values. The log values were used because titers obtained by dilution assays represent a sequential doubling of values. Variance in each group at each time point was stabilized on a log scale, and the distribution of the original titer values was well approximated by a log normal distribution. Antibody levels in serum, tears, and aqueous obtained at each point after immunization were compared with antibody titers of normal vitreous humor obtained from the uninoculated eyes of control rabbits using a student t-test ± standard error of the mean. Statistical significance was accepted for $P < 0.05$.

#### Clinical Scores

On day 3, four of five rabbits showed conjunctival hyperemia, edema, and scant exudates (Table 2). At this time the corneas in most rabbits were clear, and anterior chamber findings consisted only of mild iris hyperemia. Vitreous findings ranged from clear with intact fundus detail to loss of the red reflex in one rabbit. Three of five rabbits had some areas of vitreous opacification detectable. By day 7, all rabbits had marked conjunctival hyperemia with moderate to heavy exudate. The irises showed vessel engorgement accompanied by pupillary irregularity. All rabbits had loss of the red reflex. These clinical changes persisted throughout the duration of the study in all experimental rabbits. Only two rabbits had corneal clouding (one on day 3 and the other on day 14). None of the rabbits had hypopyon. On day 30, three of the five rabbits had less severe inflammation of the conjunctiva and iris, but all five had opacification of the vitreous sufficient to obscure the red reflex.

#### Cultures

All vitreous aspirates from the right eyes of experimental rabbits were positive for *S. aureus* on days 3, 7, and 14. On day 10, one of five vitreous aspirates was negative for bacterial growth. This animal had clinical signs of endophthalmitis and the highest serum, aqueous, and vitreous antibody titers of this group. On day 21, two of five vitreous aspirates were negative for bacterial growth; on day 30, all vitreous aspirates were negative (Table 2).

Quantitation of bacterial growth showed increasing numbers of CFU/ml from days 3–14. On day 21, samples obtained at each point after immunization were compared with antibody titers of normal vitreous humor obtained from the unoinoculated eyes of control rabbits using a student t-test ± standard error of the mean. Statistical significance was accepted for $P < 0.05$.

### Table 2. Clinical scores and culture results

<table>
<thead>
<tr>
<th>Days</th>
<th>Conjunctiva</th>
<th>Cornea</th>
<th>Iris</th>
<th>Vitreous culture OD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 3 rabbits</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Day 7 rabbits</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Day 10 rabbits</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Day 14 rabbits</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Day 21 rabbits</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Day 30 rabbits</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

* Denotes control rabbit in each group.

### Table 3. Quantitative vitreous culture results from experimental eyes injected with *Staphylococcus aureus*

<table>
<thead>
<tr>
<th>Days postinoculation</th>
<th>Median colony forming units/ml (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>$2.0 \times 10^4 (9.4 \times 10^2 - 6.2 \times 10^6)$</td>
</tr>
<tr>
<td>7</td>
<td>$1.5 \times 10^7 (5.0 \times 10^5 - 2.9 \times 10^9)$</td>
</tr>
<tr>
<td>10</td>
<td>$4.0 \times 10^6 (4.0 - 7.5 \times 10^9)$</td>
</tr>
<tr>
<td>14</td>
<td>$1.2 \times 10^9 (1.8 \times 10^7 - 3.5 \times 10^9)$</td>
</tr>
<tr>
<td>21</td>
<td>$1.4 \times 10^9 (0 - 9.5 \times 10^9)$</td>
</tr>
<tr>
<td>30</td>
<td>0</td>
</tr>
</tbody>
</table>
there was a decrease in CFU/ml, and all cultures were negative on day 30 (Table 3).

Vitreous samples from the uninoculated left eyes of experimental rabbits, and both eyes of control rabbits (ie, right eye inoculated with sterile normal saline and uninoculated left eye) were negative for bacterial growth at every time. Aqueous samples from both eyes of experimental and control rabbits were negative at every time.

**Gross Examination and Histopathology**

Gross examination disclosed the presence of white exudate in the vitreous cavity on all days. This exudate was maximal on days 14 and 21.

On day 3, histopathologic examination showed acute inflammation, characterized by dense neutrophilic infiltration, predominating in the substantia propria of the limbus, trabecular meshwork, anterior and posterior lens surfaces, ciliary body, vitreous, and choroid. Rare neutrophils were seen in the sclera, iris, and retina. The choroid was edematous, and retinal detachments with underlying relatively acellular subretinal fluid were present.

On day 7, there was a diffuse increase in inflammation with a greater number of lymphocytes noted in the conjunctival and uveal tissues. The trabecular meshwork was filled with neutrophils and lymphocytes. Iris blood vessels were dilated and contained moderate numbers of neutrophils and lymphocytes. The lens was surrounded by exudate, and posterior synechiae were noted. The vitreous cavity was filled with necrotic neutrophils. These were also present in the subretinal fluid and were interspersed throughout the retina. Choroidal effusions were increased from those seen on day 3.

On days 10 and 14, there was chronic inflammation of the conjunctiva and uvea composed of infiltrates of numerous lymphocytes and plasma cells (Fig. 1). Peripheral vascularization of the cornea was noted. A cyclitic membrane comprised of spindle cells contiguous with the ciliary body epithelium ex-

![Fig. 1. Day 14: Light micrograph of rabbit anterior segment demonstrating massive infiltration of the ciliary body (CB) by lymphocytes and plasma cells. Insert illustrates abundance of plasma cells (arrows) within the ciliary body. C = cornea, I = iris (original magnification X40; insert X400).](image)
tended around the lens. The vitreous contained necrotic neutrophils, and the retina showed severe cellular necrosis. There were moderate to severe choroidal effusions.

On day 21, there was substantial infiltration of the ciliary body and choroid with plasma cells, histiocytes, and lymphoid follicles that persisted on day 30 (Fig. 2). There were numerous lymphocytes and plasma cells in the trabecular meshwork. The corneal vascularization, cyclitic membranes, vitreous and retinal necrosis, and choroidal effusions all persisted.

On day 30, only rare cells were seen in the trabecular meshwork of all eyes, otherwise the histopathologic findings were similar to those on day 21. On day 30, the three eyes with less severe disease by clinical determination had scattered neutrophils and fibrin in the vitreous, but the retina was preserved without inflammatory cell infiltration.

Gram stain revealed numerous intracellular and extracellular gram-positive cocci in the vitreous, retina, and uvea on days 3-14. Gram-positive forms seen on days 21 and 30 were not clearly identifiable as
cocci due to obfuscation by the extensive cellular necrosis also present in these sections. No gram-positive forms were observed in the anterior chamber at any time.

**Antibody Titers**

When compared with preimmune values, serum IgG antibody levels to RTA were increased significantly at all times in the experimental animals (Fig. 3). Tear IgG antibody levels to RTA in the right eye of experimental rabbits were significantly increased on days 10, 14, 21, and 30. Aqueous IgG antibody levels to RTA in the right eye, not detectable in preimmune samples, were significantly increased on days 7, 10, 14, 21, and 30. In the experimental rabbits, IgG antibody levels to RTA were maximal in the serum, the tears, and aqueous of the right eye on day 14. Afterward, all three progressively decreased on days 21 and 30. At all times, mean IgG antibody titers to RTA were greater in serum than in tears or aqueous. Vitreous IgG antibody levels to RTA in the right eye, also not detectable in normal vitreous samples, were significantly increased on days 7, 10, 14, 21, and 30. Mean vitreous IgG antibody titers to RTA surpassed those of serum, tears, and aqueous on day 14 and progressively increased on days 21 and 30. The mean vitreous IgG antibody titer to RTA on day 30 was eight times greater than the mean serum titer at the same time. Moreover, the mean vitreous titer of IgG antibody to RTA on day 30 was three times the maximal mean serum titer that was found on day 14.

Tear IgG antibody titers to RTA in experimental rabbits were not significantly different between the inoculated right eyes and the fellow, uninoculated left eyes at any time. Aqueous IgG antibody to RTA was observed only rarely in low levels in the left eye of experimental animals. Vitreous IgG antibody to RTA was never found in the left eyes of experimental rabbits.

Serum IgA antibody levels to RTA were not detected in experimental rabbits at any time in this study (Fig. 4). Tear IgA antibody levels to RTA in the right eye were significantly increased on day 14 only. Aqueous IgA antibody levels to RTA in the right eye were detectable in some animals in very low levels on days 10, 14, and 30, but they were not significantly different from preimmune values. Vitreous IgA antibody levels to RTA were significantly increased on days 14 and 21 and approached significance on day 30 ($P = 0.057$). Mean vitreous IgA antibody titers to RTA at their peak (day 21) were 25 times less than mean IgG antibody titers measured at the same time.

As was the case for tear IgG antibody titers, there was no significant difference in tear IgA antibody titers to RTA between the inoculated right and uninoculated left eyes of experimental rabbits at any time. Aqueous and vitreous IgA antibodies to RTA were never detected in the left eye of experimental animals.

Control rabbits did not show any change in titers of

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**Fig. 3.** Mean IgG antibody titers to ribitol teichoic acid (±SEM) on days 3 through 30 following intravitreal injection of *S. aureus.* For graphical representation of data, the mean of the log values for each sample at each time point was converted to the corresponding titer.
IgG or IgA antibody to RTA in serum, tears, aqueous, or vitreous at any time.

A comparison of quantitative vitreous culture results and vitreous IgG antibody levels to RTA revealed an inverse correlation from days 3–30 (Fig. 5).

Discussion

Endophthalmitis is a devastating disease that frequently results in visual loss despite early, aggressive therapy. S. epidermidis and S. aureus are the most

Fig. 4. Mean IgA antibody titers to ribitol teichoic acid (±SEM) on days 3 through 30 following intravitreal injection of S. aureus. For graphical representation of data, the mean of the log values for each sample at each time point was converted to the corresponding titer.

Fig. 5. Comparison between quantitative culture results (expressed as mean logio ± SEM) and the vitreous IgG antibody level to ribitol teichoic acid (expressed as the mean log2 ± SEM) on days 3 through 30 following intravitreal injection of S. aureus. Comparisons were performed graphically on a log scale to demonstrate the general trends between CFU and vitreous IgG antibody titers while maintaining a linear scale for the ordinate. N = 5 for each time point.
frequent causes of postoperative infectious endophthalmitis.12 In contemporary reports, \textit{S. aureus} accounts for approximately 20\% of all culture-positive cases in the postoperative setting.13–15 Compared with \textit{S. epidermidis}, \textit{S. aureus} endophthalmitis usually pursues a much more virulent course16,17 and is frequently associated with severe visual loss.14,15,17

To the best of our knowledge, the humoral response during bacterial endophthalmitis has never been described. Previous studies evaluating the immune response in other types of infectious endophthalmitis are few. In a rabbit model of endogenous candidal endophthalmitis, Malecaze and colleagues18 showed increases in serum and aqueous IgG to Candida within 10 days and concluded that antibody production probably occurs within the eye. The clinical studies of Mathis and colleagues19 support the diagnostic utility of measuring intraocular antibody levels by detecting increased aqueous antibody levels to \textit{Candida albicans} in five of six patients with suspected \textit{C. albicans} endophthalmitis. \textit{Toxocara canis} endophthalmitis has been diagnosed by the detection of specific antibody in the aqueous20 and vitreous.21 Biglan and colleagues21 observed that all patients had vitreous antibody titers that were equal to or greater than serum antibody titers, suggesting that intraocular antibody production or concentration probably occurred.

Previous experimental investigations have characterized the immune response to antigens injected into the vitreous. Antigen injected intravitreally has been shown to leak rapidly into the circulation.22 Shimada and Silverstein23 suggested that the initial effect of intravitreal antigen injection is specific antibody production in the spleen or other lymphoid tissues. Once activated, specific immunocytes then travel hematogenously to the eye where they interact with antigen, mediate the inflammatory response, and initiate intraocular antibody production. Hall and Prickman24 and Smith et al25 observed systemic antibody production before uveal antibody production after intravitreal injection of antigen. All these models23–25 describe the ocular immune response to a specific antigen rather than to a viable, replicating organism.

In this study, we describe the humoral immune response associated with \textit{S. aureus} endophthalmitis. As seen after intravitreal injection of antigen in other studies,23–25 the initial IgG antibody response in \textit{S. aureus} endophthalmitis was detected in the serum where the levels were higher than tears, aqueous, and vitreous until day 14. Our finding of serum antibody to RTA on day 3 supports the theory that antigen processing and antibody formation initially occurs at extraocular sites after leakage of the antigen from the vitreous. Moreover, the sensitivity of our ELISA assay probably enhanced our ability to detect serum antibody at this early time. A systemic antibody response measured by agglutinin titers was seen as early as 4 days after the intraocular injection of typhoid vaccine before specific antibody was detected intraocularly.26

Tear IgG antibody levels to RTA probably result from local production by plasma cells in the lacrimal gland. Since RTA-specific IgG antibodies were present in serum, transudation of IgG from inflamed conjunctival vessels in the inoculated eye is another possible source of IgG in tears.27 However, tear IgG antibody levels were similar in the fellow, uninoculated eyes which were not inflamed. This finding suggests that conjunctival transudation is not a major contributor to tear IgG antibody levels in this model. Tear IgA antibody levels to RTA also probably result from the local production by IgA-producing plasma cells in the lacrimal gland.28 Franklin and McGee29 demonstrated the unique properties of the lacrimal gland epithelium that allow it to selectively "capture" committed IgA-producing B-lymphocytes. Leakage from conjunctival vessels cannot explain the presence of IgA in tears, since IgA antibody levels were not detected in serum.

Possible sources of aqueous antibody levels to RTA include leakage from serum, diffusion from vitreous, or local production by plasma cells in the ciliary body and iris. Leakage of serum IgG antibody into the aqueous after disruption of the blood–aqueous barrier by inflammation is supported by the finding that aqueous IgG antibody levels rose and fell with serum and were always less than those in the serum. Serum was not a source of aqueous IgA antibody because IgA antibody was never detected in serum. Diffusion of antibody from the vitreous is another possible source of aqueous IgG and IgA antibody levels because vitreous antibody levels were always higher than those in the aqueous. It is unlikely, however, that aqueous IgG antibody to RTA was primarily the result of diffusion from the vitreous because aqueous levels decreased on days 21 and 30, and vitreous levels progressively increased. Anterior segment production of IgG and IgA may occur from plasma cells which were found in the iris and ciliary body of rabbits in our study.

Vitreous antibody levels to RTA could originate from leakage from serum or intraocular production by choroidal plasma cells. Serum IgG may contribute to vitreous IgG antibody levels after disruption of the blood–retina barrier due to inflammation. If serum were the only source of IgG antibody in vitreous, then the rising vitreous antibody levels on days 21 and 30 could only be explained by concentration of antibody because serum levels were falling at these times. Serum could not be a source of vitreous IgA antibody because IgA was not detected in serum, suggesting
that vitreous IgA is probably produced in the eye. Choroidal plasma cells, evident by day 10 and arranged in follicles on days 21 and 30, may produce IgG and IgA antibody and contribute to vitreous antibody levels. Intraocular production of IgG antibody could also explain the rising vitreous antibody levels on days 21 and 30 when serum IgG levels were falling.

On day 21, the mean vitreous IgG antibody level was more than 25 times greater than the mean vitreous IgA antibody level which was maximal at this time. Shimada et al.23 also found that antigen-specific intraocular IgG antibody production comprised more than 90% of all Ig classes present after the intravitreal injection of antigen. In our study, increases in intraocular antibody were also predominantly of the IgG class. This may be important from the standpoint of host defenses; IgG is a better opsonin for bacterial phagocytosis than IgA.30

Bacteria were absent in the anterior chamber by Gram stain and aqueous culture results at all times. The absence of bacterial growth in aqueous samples might be related to the anatomic barrier provided by the intact lens–iris diaphragm. The anterior chamber also appears to be more effective in eliminating bacteria than the vitreous, perhaps because of the rapid turnover of aqueous.22 This finding is also observed in human studies where negative anterior chamber aspirates are frequently encountered despite positive vitreous aspirates and severe clinical disease.12

Bacterial cultures were negative in all vitreous samples on day 30. This suggests that the gram-positive forms observed in sections from day 30 probably represent either dead bacteria or necrotic cellular and bacterial debris. Spontaneous sterilization of the vitreous during endophthalmitis has been previously described.31-33 Spontaneous resolution of active infection may result from nutrient depletion in the vitreous body and macrophages, especially in the presence of complement activated through the classic pathway. The complement system plays an important role in the host immune response to infection. Functions of activated complement include chemotaxis of polymorphonuclear leukocytes, increased vascular dilation and permeability, immune adherence facilitating phagocytosis, and cytolyis of bacteria by the membrane attack complex of complement.27 Elevated levels of C3a and C4a have been demonstrated in the aqueous35 and vitreous36 of humans during bacterial endophthalmitis. Moreover, the complement system has been shown to exert a protective effect against pseudomonal endophthalmitis in guinea pigs.37

We observed an inverse correlation between vitreous IgG antibody levels and vitreous cultures that were positive for S. aureus (Fig. 5). On day 3, all vitreous cultures were positive, and vitreous antibody levels were barely detectable. On day 30 vitreous antibody levels were maximal, and all vitreous cultures were negative. Some eyes in this study had less severe disease by clinical, gross, and histopathologic evaluation. In general, these animals also had the highest intraocular antibody titers in their groups and negative vitreous cultures. These findings suggest, but do not prove, that intraocular antibody levels may exert a protective effect in this model.

Key words: immune response, Staphylococcus aureus, endophthalmitis, ribitol teichoic acid, enzyme-linked immunosorbent assay

References