Pathogenesis of *Staphylococcus* in the Rabbit Anterior Chamber

Dalia O. Girgis,1 Julian M. Reed,1 Kathryn S. Monds,1 Joseph J. Dajcs,1,2 Mary E. Marquart,1 Brett A. Thibodeaux,1 and Richard J. O’Callaghan1,3

**Purpose.** To investigate the host defense against *Staphylococcus* in the rabbit anterior chamber.

**Methods.** The bactericidal activity of rabbit aqueous humor was investigated in vitro. Rabbit anterior chambers were injected with viable *Staphylococcus aureus* or *Staphylococcus epidermidis* (1,000 or 500,000 colony-forming units [CFU]), killed bacteria, culture supernatants of either organism, or purified *S. aureus* α-toxin. CFU as well as phospholipase (PLA2) and myeloperoxidase (MPO) activities of aqueous humor were determined up to 25 hours postinfection (PI).

**Results.** The number of viable *S. aureus* or *S. epidermidis* was significantly reduced when incubated with aqueous humor for 30 minutes (*P* ≤ 0.0001). Rabbits challenged with either *S. aureus* or *S. epidermidis* demonstrated a significant reduction in CFU in aqueous humor by 1 hour PI (*P* ≤ 0.0044). Eyes infected with either *S. aureus* or *S. epidermidis* demonstrated a significant increase in MPO activity beginning at 1 hour PI (*P* ≤ 0.0455), but only *S. aureus* caused an increase in PLA2 activity at 20 and 25 hours PI (*P* ≤ 0.0002). No significant increases in PLA2 activity were observed after injection of killed bacteria into the aqueous humor at any time point; however, injection of *S. aureus* supernatant or α-toxin into the anterior chamber significantly increased PLA2 activity (*P* ≤ 0.0210). Injection of α-toxin also resulted in significant increases in MPO activity beginning at 10 hours after injection (*P* ≤ 0.001).

**Conclusions.** This study demonstrates that aqueous humor has a potent host defense capability and that *S. aureus*, but not *S. epidermidis*, triggers a PLA2 response in the rabbit anterior chamber that appears to be due to α-toxin. (Invest Ophthalmol Vis Sci. 2005;46:1371–1378) DOI:10.1167/iovs.04-08888

Bacterial endophthalmitis is a potentially devastating complication of intraocular surgery that can lead to poor visual outcomes or possibly to blindness.1 With every intraocular surgery, there is a risk of introducing microorganisms into the eye, resulting in endophthalmitis.2–4 Postoperative endophthalmitis occurs most frequently after cataract surgery, with the overall incidence of post–cataract-surgery endophthalmitis in the United States at approximately 0.1%.1,5–7 The incidence after other types of intraocular surgery has been reported to range between 0.05% and 0.58%,1,2,5–7. In the United States, coagulase-negative staphylococci are the most common causes of post–cataract-surgery endophthalmitis (60%–70% of cases), followed by *S. aureus*.1,3,8–9 Symptoms of endophthalmitis typically appear 24 to 48 hours after surgery.8 Generally, eyes infected with more virulent bacteria (i.e., *S. aureus*) have a rapid onset of endophthalmitis.8

The predominant etiologic agents of acute postoperative endophthalmitis are generally microorganisms of the eyelid margin and tear film.3 Staphylococci comprise the majority of the normal flora of the eye lid margins and can gain access to the anterior chamber by contamination of intraocular lenses and instruments as well as through entry of fluid into the operative field.9 Studies have shown that bacteria routinely enter the anterior chamber during cataract extraction and remain there at the end of surgery in a significant proportion of patients, with contamination rates ranging from 29% to 45%.10–12 However, the high contamination rate and relatively low rate of endophthalmitis suggest that ocular host defense mechanisms are capable of clearing the contaminating bacteria after cataract surgery.11–13

Recent studies have demonstrated the bactericidal activity against *S. aureus* of group IIA PLA2 in tears.14–16 Phospholipase A2 comprises a group of lipolytic enzymes that release fatty acids, typically arachidonic acid, from the sn-2 position of membrane phospholipids for production of important lipid inflammatory mediators such as eicosanoids and platelet-activating factor (PAF).17 PLA2 can be isolated from a variety of cells and fluids, including polymorphonuclear leukocytes (PMNs), macrophages, tear film, and aqueous humor.18–19 Saari et al.15 have demonstrated that the concentration of PLA2 in normal aqueous humor is substantially lower than that found in the tear film. Furthermore, Diamond et al.20 have reported the presence of a peptide in rabbit aqueous humor that may play a role in the bactericidal properties of aqueous humor against pathogens such as *S. aureus* and *Pseudomonas aeruginosa*.

In previous studies, we have demonstrated that PLA2 activity increases substantially in the aqueous humor and tear film during *S. aureus* keratitis.15 This increase in PLA2 activity in infected rabbit eyes probably reflects a host response to changes in the ocular environment.15 Other studies have demonstrated that PLA2 activity increases in the eyes of patients with blepharitis21 and keratoconjunctivitis sicca.22 Whereas PLA2 activity against *Staphylococcus* has been investigated in the tear film14,15 as well as in models of *Staphylococcus* keratitis,23,24 no such studies have been conducted in the aqueous humor. As a result, the purpose of this study was to investigate PLA2 activity in rabbit aqueous humor as a host defense against *Staphylococcus*.
Bacterial Strains

*S. aureus* strain 8325-4, a well-characterized laboratory strain with a positive phenotype for toxin production (α, β, γ, and δ-toxins), was used in this study and has been described for use in the rabbit intrastromal injection and topical inoculation models of keratitis.24–27 Tested also was *S. epidermidis* strain ATCC 14990 (nasal isolate; American Type Culture Collection, Manassas, VA), a strain used in other *S. epidermidis* pathogenesis studies, including ocular studies of infection.28–30 The *S. aureus* strain, but not the *S. epidermidis* strain, was hemolytic on rabbit or sheep blood agar incubated at 37°C.

Rabbits

New Zealand White rabbits used in these studies were maintained in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Rabbits were housed on a 12-hour light-dark cycle, changing to darkness at 6 PM and to light at 6 AM. In all experiments, six eyes were used per group, and, due to the short time course of infection, both eyes of each rabbit were used.

Rabbit Anterior Chamber Injections

Overnight cultures of either *S. aureus* strain 8325-4 or *S. epidermidis* strain 14990 were diluted 1:100 in sterile tryptic soy broth (TSB; Difco Laboratories, Detroit, MI) and incubated at 37°C. Bacteria were grown to an absorbance of 0.3 to 0.42 at 650 nm, and cultures were serially diluted in sterile TSB to approximately 1 × 10^7 CFU/mL (1000 CFU inocula) or 5 × 10^7 CFU/mL (500,000 CFU inocula).

For injection of bacteria into the anterior chamber, rabbits were anesthetized by subcutaneous injection of a 1:5 mixture of 100 mg/mL xylazine (Rompun; Miles Laboratories, Shawnee Mission, KS) and 100 mg/mL ketamine HCl (Ketaset; Bristol Laboratories, Syracuse, NY). One drop of proparacaine HCl (0.5%; Falcon Pharmaceuticals, Fort Worth, TX) was administered to each eye before injection. A 30-gauge needle attached to a syringe (Hamilton Co., Reno, NV) was inserted through the cornea into the anterior chamber of anesthetized rabbits, to inject each eye with 10 μL of either *S. aureus* or *S. epidermidis* (approximately 1,000 CFU or 500,000 CFU). Serial dilutions of bacterial cultures were made in sterile TSB, and 0.1-mL aliquots were plated onto tryptic soy agar (TSA) in triplicate to confirm the number of CFU injected. At various times (1, 5, 10, 15, 20, and 25 hours) postinfection (PI), rabbits were killed by intravenous injection with sodium pentobarbital solution (100 mg/mL; Sigma-Aldrich, St. Louis, MO). Immediately before death, a 26-gauge needle on a 1-mL syringe was inserted through the cornea into the anterior chamber of anesthetized rabbits, and an aliquot (0.1 mL) of aqueous humor was removed. Because of the small sample size of aqueous humor per eye, undiluted aqueous humor samples could not be cultured. Aqueous humor samples were serially diluted in sterile phosphate buffered solution (PBS), aliquots (0.1 mL) were inoculated onto TSA plates, and the plates were incubated at 37°C for 24 hours. Colonies were counted, and CFU per anterior chamber were expressed as base 10 logarithms.

In Vitro Bactericidal Effects of Rabbit Aqueous Humor

Aliquots of normal aqueous humor were collected from the anterior chambers of anesthetized rabbits as just described, pooled, and stored at −70°C.

Log phase *S. aureus* strain 8325-4 and *S. epidermidis* strain 14990 were grown in a modified M9 synthetic medium, as previously described.14 Bacteria of either strain (75–125 CFU in 20 μL) were mixed with rabbit aqueous humor in volumes of 225 or 300 μL, and the mixtures were incubated at 35°C for 30 or 60 minutes. After incubation, aliquots were cultured on TSA in triplicate. Controls included bacteria in PBS or bacteria in heat-inactivated aqueous humor (heated at 80°C for 5 minutes). The number of viable bacteria was determined after 24 hours of incubation. All assays were performed in triplicate.

Radioactive Labeling of *S. aureus*

*S. aureus* strain 8325-4 was radioactively labeled as previously described.14 Briefly, bacteria were grown overnight in M9 synthetic medium with amino acids, subcultured to log phase with 0.5 μCi/mL of 14C-labeled oleic acid, and then incubated at 37°C for 2.5 hours. After incubation, the bacteria were centrifuged, the supernatant removed, and the bacterial pellet resuspended in TSB and incubated for 30 minutes at 37°C. The bacteria were then washed in PBS with 1% bovine serum albumin (BSA; Sigma-Aldrich), resuspended in PBS, and stored at −70°C.

Phospholipase A2 Activity Determinations

The activity of PLA2 in the aqueous humor was determined using radioactively labeled *S. aureus*, as previously described.15 Briefly, radiolabeled bacteria were incubated with Tris buffer (40 mM Tris-HCl, pH 7.5; control) or aqueous humor (1:5 dilution in Tris-HCl buffer, pH 7.5), with 2 mM CaCl2 added. The mixtures were incubated for 30 minutes at 37°C. Reactions were terminated with the addition of ice-cold BSA and samples were centrifuged to pellet the bacteria. An aliquot of supernatant was used to quantify, by liquid scintillation counting, the products of hydrolysis as counts per minute (CPM). All assays were performed in triplicate.

Myeloperoxidase Activity Assay

The amount of myeloperoxidase (MPO) activity was quantified in aqueous humor collected from uninfected and infected rabbits, as previously described.15 Briefly, hexadecyltrimethylammonium bromide (CTAB; Sigma-Aldrich) was added to each sample at a final concentration of 0.5% and a MPO microtiter assay, based on an o-dianisidine-based colorimetric reaction, was used. Reactions were incubated at room temperature, and the change in optical density at 450 nm was determined every 2 minutes for 12 minutes. The units of MPO were calculated as previously described for the microtiter plate assay.51,52 One unit of MPO activity was equivalent to approximately 100,000 PMNs. All assays were performed in triplicate.

In Vivo Effects of Culture Supernatant and Cellular Components

Overnight cultures of either *S. aureus* strain 8325-4 or *S. epidermidis* strain 14990 were diluted 1:100 in sterile TSB (10 mL) and grown at 37°C for 24 hours. Bacteria were centrifuged to a pellet and supernatants collected. Supernatants were filter sterilized (0.22 μm) before injection. An aliquot (10 μL) of supernatant (*S. aureus* or *S. epidermidis*) alone, or *S. aureus* culture supernatant combined 1:1 (20 μL total volume) with filter-sterilized normal serum or purified antibody to staphylococcal α-toxin (Sigma-Aldrich) were injected into the anterior chamber as previously described (n = 6 eyes/group).

In addition, overnight cultures of either *S. aureus* strain 8325-4 or *S. epidermidis* strain 14990 were diluted 1:100 in sterile TSB and grown at 37°C. Cultures (10 mL) were grown for 24 hours. The bacteria were then centrifuged to a pellet and resuspended in PBS (10 mL). Resuspended bacteria (*S. aureus* or *S. epidermidis*) were then irradiated (254 nm; EL Series Ultraviolet Hand Lamp; Ultra-Violet Products, Upland, CA). To determine the amount of time required to achieve 100% bacterial killing, the number of surviving bacteria were quantified by culturing aliquots on TSA plates at 1, 5, 10, 15, 30, 45, and 60 minutes after irradiation. After 5 minutes of irradiation, no surviving bacteria were found to be present. For in vivo testing, the bacteria were irradiated for 10 minutes, centrifuged to a pellet, and resuspended in TSB (10 mL). An aliquot (10 μL) of either irradiated *S. aureus* or *S. epidermidis* was injected into the anterior chamber, as described earlier.
α-Toxin

Commercially available α-toxin (Sigma-Aldrich) was purified by isoelectric focusing (Rotofofr, Bio-Rad, Hercules, CA), as previously described.33 Samples of α-toxin (2 mg/mL) were isoelectrically focused against ampholytes (BioLyte 3/10; Bio-Rad) for approximately 4 hours, and fractions were tested for pH and hemolytic activity. Activity of fractions was determined by the lysis of rabbit erythrocytes, and active fractions underwent an additional round of isoelectric focusing. Fractions were analyzed by SDS-PAGE and silver-stained to determine purity of the protein. α-Toxin was inactivated by heating at 60°C for 2 hours, as determined by its inability to lyse rabbit erythrocytes.34

Purified α-toxin (1 μg in 10 μL) was injected into the aqueous humor of rabbits as described earlier, to analyze the toxicity to the anterior chamber. Control experiments included rabbit eyes injected with heat-inactivated α-toxin. PLA₂ and MPO activities were measured from the aqueous humor collected from rabbits (n = 6 eyes/group) at 5, 10, and 20 hours after injection of α-toxin.

Clinical Scores

Eyes examined for pathologic effects of α-toxin were graded on a scale of 0 to 4 (0 being normal), relative to the following parameters: chemosis, injection, corneal edema, corneal infiltrates, and iritis.

Statistical Analysis

The mean and the SEM for CFU/aqueous humor, CFU per bactericidal assay reaction, CPM, clinical scores, and MPO units were determined on computer (SAS, Cary, NC). Statistical analyses were performed with a one-way nested analysis of variance for each group. Protected t-tests were then determined between least square means derived from each variance analysis of each group. P ≤ 0.05 was considered significant.

RESULTS

Staphylococcus Inoculated into the Anterior Chamber

Injection of S. epidermidis (1,000 or 500,000 CFU) into the rabbit anterior chamber resulted in mild to moderate pathologic changes by 25 hours PI (Figs. 1A, 1B); however, injection of S. aureus (1,000 or 500,000 CFU) in the anterior chamber resulted in severe disease, including severe infiltrate and edema, so that infections could not be continued beyond 25 hours PI (Figs. 1C, 1D).

To analyze the interaction of Staphylococcus and aqueous humor in vivo, 1,000 or 500,000 CFU of S. aureus or S. epidermidis was injected directly into the aqueous humor, and viable bacteria in the anterior chamber were quantified up to 25 hours PI. Rabbits injected with either inoculum of S. aureus or S. epidermidis failed to demonstrate increases in bacterial loads; instead, a significant reduction in CFU in aqueous humor appeared as early as 1 hour PI (Fig. 2, P ≤ 0.0044). Eyes challenged with 1000 CFU of either S. aureus or S. epidermidis contained <10 CFU by 25 hours PI, and eyes challenged with 500,000 CFU of either S. aureus or S. epidermidis contained <1,000 CFU at 25 hours PI (Fig. 2).

Bactericidal Activity of Aqueous Humor In Vitro

To analyze the bactericidal properties of aqueous humor in vitro, aqueous humor in volumes of 50 to 300 μL were mixed with 20 μL of M9 medium containing approximately 100 CFU of either S. aureus or S. epidermidis. The number of viable S. aureus or S. epidermidis was significantly reduced when incubated with aqueous humor (225 μL) for 30 minutes (Fig. 3, P ≤ 0.0001). Incubation of S. aureus with 225 μL of aqueous humor for 30 minutes killed essentially all the bacteria (Fig. 3A), and incubation of S. epidermidis with 300 μL of aqueous humor for 60 minutes achieved near sterility (Fig. 3B). Heating of the aqueous humor (80°C for 5 minutes) eliminated the bactericidal activity.

PLA₂ and MPO Activities in Infected Aqueous Humor

PLA₂ activity was studied in normal rabbits or in rabbits into which either S. aureus or S. epidermidis was injected directly into the anterior chamber. Eyes challenged with 1,000 CFU of S. aureus demonstrated a significant increase in PLA₂ activity relative to uninfected eyes at 25 hours PI (Fig. 4A; P = 0.0002), and rabbits challenged with 500,000 CFU of S. aureus demonstrated a significant increase in PLA₂ activity beginning at 20 hours PI (Fig. 4B; P ≤ 0.0001). However, rabbits challenged with either 1,000 or 500,000 CFU of S. epidermidis did not demonstrate a significant increase in PLA₂ activity up to 25 hours PI (Figs. 4C, 4D).

To determine the influx of PMNs into the anterior chamber, we assayed the activity of MPO in the aqueous humor up to 25 hours PI. Rabbit eyes challenged with 1,000 CFU S. aureus demonstrated a significant increase in MPO activity beginning at 20 hours PI (Fig. 5A, P ≤ 0.0056), whereas rabbits challenged with 500,000 CFU S. aureus demonstrated a significant increase in MPO activity as early as 1 hour PI (Fig. 5B, P ≤ 0.0001). Rabbits infected with 1,000 CFU S. epidermidis demonstrated a significant increase in MPO activity at 25 hours PI (Fig. 5C, P = 0.0349), and rabbits infected with 500,000 CFU S. epidermidis demonstrated a significant increase in MPO activity at 20 hours PI (Fig. 5D, P = 0.0455).

Mediator of Increased PLA₂ Activity in Aqueous Humor

Irradiated S. aureus or S. epidermidis free of culture supernatant was injected into rabbit aqueous humor and PLA₂ and MPO activities measured. No significant increases in PLA₂ or
MPO activities were observed at either 20 or 25 hours in the anterior chamber of rabbits in which either irradiated *S. aureus* or *S. epidermidis* were injected (Fig. 6, *P* < 0.0001). Ocular pathologic changes were not observed in these rabbit eyes.

In contrast to injection of killed bacteria, the injection of *S. aureus* culture supernatant resulted in severe inflammation including corneal infiltrate and edema. Significant increases in PLA2 and MPO activities occurred at both 20 and 25 hours (Figs. 6A, 6C; *P* < 0.0001). However, injection of *S. epidermidis* culture supernatant into the rabbit anterior chamber did not cause a significant increase in either PLA2 or MPO activity (Figs. 6B, 6D), and no significant pathologic changes were observed.

Culture supernatant of *S. aureus* was combined (1:1) with either antiserum to *α*-toxin or normal rabbit serum and injected into the anterior chamber. Eyes receiving normal serum and supernatant at 20 hours PI had clinical scores two times higher than eyes injected with antibody to *α*-toxin plus culture supernatant (*P* < 0.0001). At 25 hours PI, clinical scores for eyes injected with supernatant plus normal serum were three times higher than eyes injected with supernatant plus antibody to *α*-toxin (*P* < 0.0001). To investigate further the effects of *S. aureus* products on PLA2 activity and PMN influx, purified *S. aureus* *α*-toxin was injected into the rabbit anterior chamber. Injection of active *α*-toxin (1 μg) resulted in a significant increase in PLA2 activity by 20 hours (Fig. 7; *P* < 0.0001), and a significant increase in MPO activity was observed as early as 10 hours (Fig. 8; *P* < 0.0001).

The ocular changes induced by injection of active *α*-toxin (1 μg) into the rabbit anterior chamber appeared as early as 5 hours after injection and included blanching of the iris and formation of a mild corneal infiltrate (Fig. 9B). By 10 hours, moderate corneal infiltrates had formed, and erythema of the conjunctiva was observed (Fig. 9C). By 20 hours after injection of *α*-toxin, edema and erythema of the conjunctiva as well as dense corneal infiltrates were observed (Fig. 9D).

Injection of heat-inactivated *α*-toxin into the anterior chamber did not cause any ocular changes in the anterior chamber and did not increase PLA2 or MPO activities in the aqueous humor at any time point (Figs. 7, 8, 9).

**DISCUSSION**

The findings of this study demonstrate that the rabbit anterior chamber rapidly eradicated a substantial number of staphylococci. The findings also demonstrate that the presence of *S. aureus*, but not *S. epidermidis*, in the anterior chamber in-
duced significant increases in the activity of PLA₂, which could further contribute to the antibacterial environment of the anterior chamber. These findings support clinical observations of high contamination rates of the anterior chamber at the conclusion of intraocular surgery, yet low rates of clinical infection. Furthermore, the toxicity of \textit{S. aureus} correlates with the severe reactions observed in this form of endophthalmitis.

The killing of \textit{Staphylococcus} in the rabbit aqueous humor occurs rapidly for both \textit{S. aureus} and \textit{S. epidermidis}. The defense of the eye against an inoculum of 500,000 log phase staphylococci suspended in a nutritive medium was striking. The killing of bacteria in vitro by aliquots of rabbit aqueous humor indicates that the reduction of CFU in vivo was a result of rapid bacterial killing. The bacterial killing achieved in all rabbits was potent, although a rabbit-to-rabbit variation in the number of the residual bacteria in the anterior chamber could reflect differences among individual rabbits in bacterial killing. Bacterial killing occurred despite the fact that the PLA₂ activity in the normal aqueous humor is relatively low compared with that of the tear film.\textsuperscript{19} Furthermore, \textit{S. epidermidis} were killed efficiently without inducing a PLA₂ response after injection of viable bacteria into the anterior chamber. These findings support the concept that a reactant other than PLA₂ mediates substantial bacterial killing in the anterior chamber.\textsuperscript{20} The rabbit aqueous humor has been reported to contain a peptide that could mediate a bactericidal effect against \textit{Staphylococcus}.\textsuperscript{20}

Factors that influence the progression of contaminating bacteria to endophthalmitis may include bacterial virulence, inoculum size, and the integrity of the posterior lens capsule.\textsuperscript{11} The importance of bacterial pathogenesis is reflected by the observation that injection of \textit{S. aureus} produced a severe reaction that was apparently mediated by α-toxin. This finding is consistent with the observation that \textit{S. aureus} endophthalmitis is commonly associated with an early onset and a poor visual outcome,\textsuperscript{2,3,55–59} whereas patients diagnosed with \textit{S. epidermidis} endophthalmitis often have a delayed onset and a better visual result.\textsuperscript{7,36,40,41}
In these studies, *S. aureus* elicited a substantial increase in PLA₂ activity in the aqueous humor, but *S. epidermidis* produced almost no inflammatory reactions. The observation that killed *S. aureus* cells induced almost no disease in the anterior chamber, whereas *S. aureus* culture supernatants or purified α-toxin were extremely inflammatory support the clinical findings of more severe disease. The findings of this study also indicate that the inflammation elicited by *S. aureus* α-toxin occurred in conjunction with an increase in PLA₂ activity in rabbit aqueous humor. The results of the present study further support the importance of α-toxin in endophthalmitis as reported by Booth et al. 142 The virulence factors of *S. epidermidis* mediating endophthalmitis have not yet been identified. 43

PLA₂ has been implicated in diverse inflammatory states 44,45 and may contribute to the inflammatory response associated with endophthalmitis. PLA₂ action liberates free arachidonic acid, which serves as a precursor for the production of proinflammatory mediators such as prostaglandins and leukotrienes. 46,47 The loss of vision in endophthalmitis is associated with a massive inflammatory response in the posterior pole leading to retinal destruction and detachment. 43 The inadvertent entry of *S. aureus* into the anterior chamber in the ocular surgery setting may elicit a strong PLA₂ response, which indirectly could contribute to overall host damage and poor disease resolution. The fact that *S. epidermidis* did not elicit a strong PLA₂ response could in part explain the more favorable outcome in such cases of endophthalmitis.

**FIGURE 6.** PLA₂ and MPO activities in aqueous humor injected with *S. aureus*- or *S. epidermidis*-irradiated cells or supernatant. *S. aureus* strain 8525-4 or *S. epidermidis* strain 14990 supernatant was injected into rabbit aqueous humor (n = 6 eyes/group). Irradiated bacteria (*S. aureus* or *S. epidermidis*) were also injected into rabbit aqueous humor (n = 6 eyes/group). TSB was injected into the anterior chamber of control rabbits (n = 6 eyes/group). PLA₂ activity was measured at 20 and 25 hours and expressed as CPM. MPO activity was measured, with 1 unit of MPO activity equivalent to approximately 100,000 PMNs. Data are expressed as the mean ± SEM. †Significant difference.

**FIGURE 7.** PLA₂ activity in aqueous humor injected with *S. aureus* α-toxin. Purified α-toxin (1 μg) was injected into rabbit aqueous humor (n = 6 eyes/group). Heat-inactivated (HI) α-toxin was injected into aqueous humor of control rabbits (n = 6 eyes/group). PLA₂ activity was measured at 5, 10, and 20 hours and expressed as CPM. Data are expressed as the mean ± SEM. †Significant difference.

**FIGURE 8.** MPO activity in aqueous humor injected with *S. aureus* α-toxin. Purified α-toxin (1 μg) was injected into rabbit aqueous humor (n = 6 eyes/group). Heat-inactivated (HI) α-toxin was injected into aqueous humor of control rabbits (n = 6 eyes/group). MPO activity was measured at 5, 10, and 20 hours. One unit of MPO activity was equivalent to approximately 100,000 PMNs. Data are expressed as the mean ± SEM. †Significant difference.
The findings of this study suggest that an understanding of how specific bacterial products, such as *S. aureus* α-toxin, trigger an aggressive inflammatory response may be important in limiting the visual loss associated with endophthalmitis. Furthermore, additional studies are needed to determine how the defense mechanisms of the aqueous humor function and how they could be maintained throughout surgical procedures and possibly augmented in protecting the eye from infection that can result in endophthalmitis.

**References**


