Cytokine Expression in a Rat Model of Staphylococcus aureus Endophthalmitis

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PURPOSE. To examine the ability of viable Staphylococcus aureus to induce the production of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, cytokine-induced neutrophil chemoattractant (CINC), and interferon (IFN)-γ after intraocular injection.

METHODS. Experimental rat eyes were injected with a 25-μl volume of approximately 80 colony-forming units of viable S. aureus; control eyes received sterile saline. Eyes were graded daily for signs of clinical inflammation and were removed 6, 24, 48, and 72 hours after injection. One group was prepared for histologic analysis, and vitreous was removed from the other group for cytokine analysis, using standard enzyme-linked immunosorbent assay procedures.

RESULTS. TNF-α, IL-1β, CINC, and IFN-γ were detected in experimental vitreous samples at increased levels that peaked at 24 hours. TNF-α, IL-1β, and CINC declined at 48 hours, but IFN-γ remained elevated. At 72 hours, levels returned to baseline. Statistically significant elevations of TNF-α, IL-1β, and CINC were detected in experimental samples at 24, but not at 6 and 48 hours compared with levels in saline control samples (P < 0.03). A statistically significant increase in IFN-γ was detected at 24 and 48 hours compared with control levels (P < 0.05). In experimental animals, clinical inflammation and inflammatory cells peaked at 24 hours, persisted at 48 hours, and began to decline thereafter. Neutrophils were the predominant inflammatory cell detected at 24 (72.3% of cells) and 48 (60.1%) hours. By 72 hours, the total number of inflammatory cells had decreased by 75.0%, and the cellular infiltrate had changed so that neutrophils equaled monocytes-macrophages.

CONCLUSIONS. S. aureus induced the expression of TNF-α, IL-1β, CINC, and IFN-γ. The time course of these cytokine levels could account for the clinical inflammatory responses and the entry and decline of vitreous cells in this model of bacterial endophthalmitis. (Invest Ophthalmol Vis Sci. 1998;39:2785–2790)

Staphylococcus aureus is a common cause of postoperative endophthalmitis1 and comprises 9.9% of the bacterial isolates reported in the Endophthalmitis Vitrectomy Study.2 Endophthalmitis produced by S. aureus, a Gram-positive bacterium, is associated with early onset and a poor visual outcome.1,3 Direct tissue damage by the bacteria and the host inflammatory response are responsible for the tissue destruction associated with endophthalmitis. Cytokines are important host mediators of the inflammatory-immune response, and activation of these responses in the immediate postinfection period is critical in eliminating infectious agents and in decreasing tissue damage. Whole fixed and heat-killed staphylococci and bacterial components (e.g., protein A, lipoteichoic acid, peptidoglycan, and α-toxin) from a variety of Gram-positive bacteria, including S. aureus, have been shown to induce the in vitro production of interleukin (IL)-1, -4, -6, and -8; tumor necrosis factor (TNF)-α, and interferon (IFN)-γ from mononuclear cells, lymphocytes, endothelial cells, and neutrophils.4,5 Therefore, these mediators may be involved in the early phases of endophthalmitis. A principal goal of these studies was to gain knowledge about the local, early host immune response to S. aureus infections of the eye. In particular, we examined the ability of viable S. aureus to induce the production of vitreous cytokines in an established rat model of endophthalmitis.
Cytokines can be generally grouped into four categories or phases of inflammatory activity: recognition, recruitment, removal, and repair. The principal cytokines produced early and rapidly in response to infection and involved in recognition are TNF-α and IL-1. Tumor necrosis factor has been implicated in the pathogenesis of experimental and clinical uveitis, corneal neovascularization, proliferative vitreoretinopathy, and central nervous system demyelination of the type found in optic neuritis. Interleukin-1 seems to be involved in retinal inflammatory diseases and has been implicated in the recruitment of leukocytes into the retina and in the disruption of the ocular vascular barriers. In addition, IL-1 activity has been detected in the vitreous of patients with diabetic retinopathy. Cytokines associated with the recruitment phase of the inflammatory response (called chemokines) are responsible for mobilization of leukocytes into sites of inflammation. Cytokine-induced neutrophil chemoattractant (CINC) is a rat chemokine with functions equivalent to those of human IL-8 that has been identified in the aqueous humor of rats with endotoxin-induced uveitis. CINC production can be induced by TNF and IL-1, which also induce IL-8 production. Cytokines associated with the removal phase comprise a group of proteins that activate and induce the differentiation of other cells that enhance the clearance of antigens or infectious agents. These proteins include IFN-γ and IL-2. Interferon-γ may also function as a proinflammatory cytokine and has been detected in ocular tissues from patients with sympathetic ophthalmia or uveitis and in the vitreous of patients with acquired immune deficiency syndrome who have vitritis and retinitis.

Few investigators have evaluated the intraocular cytokine response to Gram-positive bacteria. Successful treatment of bacterial endophthalmitis requires not only the elimination of the infecting organism and its components, but also control of the host immune response. In the present study, we have expanded our investigations in experimental endophthalmitis by showing the presence of TNF-α, IL-1β, CINC, and IFN-γ in the vitreous of eyes injected with viable S. aureus.

**MATERIALS AND METHODS**

**Bacterial Strains and Components**

In this study, a prototypic strain of S. aureus was used to induce endophthalmitis; it was a gift from Ambrose Cheung, Rockefeller University, New York, New York. This strain maintains its hemolytic pattern when propagated on sheep erythrocyte agar, and produces α-, β- and δ-hemolysins; lipases; and fibronectin-binding protein. The isolate used in these studies was maintained in sheep red blood at −70°C. When ready for use, bacteria were plated onto rabbit or sheep blood agar plates. An isolated colony was subcultured into sterile tryptic soy broth. Bacteria were centrifuged and washed with lipopolysaccharide-free 0.85% sterile normal saline (NS). Dilutions of bacteria were made with NS, according to a previously described method.

**Intravitreal Injections**

Inbred female Lewis rats (Harlan Sprague-Dawley, San Diego, CA) raised in a pathogen-free environment were used in this study. Studies were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Injections were performed according to a previously described protocol. Right eyes of experimental and control rats received 25-μl intravitreal injections containing 80 colony-forming units per milliliter and NS, respectively. Twenty-five microliters of the same S. aureus suspension was plated onto five blood agar plates to confirm the approximate number of colony-forming units of S. aureus injected.

**Clinical Inflammatory Scores**

Eyes of all rats were examined daily with a slit lamp biomicroscope and direct ophthalmoscope and before death at 6, 24, 48, and 72 hours after treatment. Clinical inflammation was graded according to a scoring system developed by Peyman et al. and Chan et al.

**Cytokine Detection**

Vitreous was removed from eyes of 134 rats at 6, 24, 48, and 72 hours after injection of NS or viable S. aureus. Sera were obtained according to a previously described protocol. Right eyes were removed and immediately frozen on dry ice. Frozen globes were dissected posterior to the iris, the lens was lifted out, and the vitreous was removed and placed in conical tubes on ice. Vitreous samples from three eyes were pooled for experimental and control groups. Pooled samples were spun at 16,000g for 30 minutes at 4°C. The supernatant was removed and stored at −70°C until assayed for cytokines.

Samples were evaluated using commercially purchased standard sandwich enzyme-linked immunosorbent assays (ELISAs; Biosource, Camarillo, CA) for the detection of TNF-α (sensitivity 4 pg/ml), IL-1β (sensitivity 3 pg/ml), and IFN-γ (sensitivity 13 pg/ml). A vitreous ELISA was developed to detect CINC and modified from a previously described protocol (sensitivity 50 pg/ml).

**Histopathology**

Thirty-nine rats were killed at 6, 24, 48, and 72 hours and their eyes prepared according to standard methods for histologic examination. Eyes of all rats were examined daily with a slit lamp biomicroscope and direct ophthalmoscope and before death at 6, 24, 48, and 72 hours after treatment. Clinical inflammation was graded according to a scoring system developed by Peyman et al. and Chan et al.

**FIGURE 1**. Diagram defining the 4 fields in the vitreous chamber used to quantitate inflammatory cells. Anterior chamber: 1, 2, 3, 4.
analysis. To quantify infiltrating cells, we counted cells in the vitreous in three random 5-μm sections per eye. One section was located superior to the optic nerve, the second contained the optic nerve, and the third was inferior to the optic nerve. Thus, we focused on the areas where cells enter into the eye. Neutrophils, lymphocytes, and monocytes-macrophages were counted at 400X in four defined high-powered fields in the vitreous chamber, using an eyepiece measuring grid. Cells from 12 fields (4 per section) per eye were totaled and used to calculate the percentage of each cell type per eye (Fig. 1). None of the eyes used in the analysis contained large vitreous or subretinal hemorrhages or showed crystalline lens damage.

Statistics
We compared mean clinical scores by mixed-model repeated measures analysis of variance (ANOVA). This model correctly took into consideration the within and between animal variability. The Fisher–Tukey least significant difference criterion was used to compute post hoc t tests in this model. We used a nonparametric Kruskal–Wallis one-way analysis of variance with post hoc P computed by Fisher–Tukey's method for multiple comparisons to compare mean total cell number, mean percentage of inflammatory cells, and mean cytokine concentration. Tabulated data are presented as mean ± SEM. Significance was determined at P < 0.05.

RESULTS

Clinical Inflammation
Six hours after injection, 14 (93.3%) of 15 rats in the experimental group and 15 (83.3%) of 18 rats in the control group did not show signs of inflammation (Fig. 2). Mild iris hyperemia in the remaining rats of both groups accounted for the low inflammatory scores.

After 24 hours, 41 (91.1%) of 45 animals in the experimental group showed signs of endophthalmitis. The conjunctiva and cornea remained normal in this group; the most marked inflammatory changes were noted in the iris and the anterior and vitreous chambers. Mildly to markedly dilated iris vessels (mean, 1.40 ± 0.11) were seen on ophthalmoscopic examination. Experimental animals also showed a grade 1 to 2 anterior chamber response (mean, 1.10 ± 0.12) manifested by anterior chamber cells and fibrin along with posterior synechia. Thirty-four (75.6%) of 45 eyes contained a vitreous exudate. A statistically significant increase in the inflammatory response was present in the experimental rats in comparison with that in the control rats (P < 0.0001).

At 48 hours, the clinical inflammatory scores in experimental eyes had slightly declined. There was a decrease in the anterior chamber response (mean, 0.76 ± 0.12) but the iris findings (mean, 1.40 ± 0.13), and vitreous exudates remained approximately the same. A statistically significant difference in inflammation between experimental and control animals was still present (P < 0.0001).

At 72 hours, a marked decline in clinical inflammatory findings in experimental (P < 0.0001) and control groups from the value at 48 hours was noted. In the experimental group, most of the decrease in inflammatory scores was accounted for by a decline in the anterior chamber response. Only 1 (6.7%) of 15 animals showed anterior chamber inflammation. Vitreous exudates were still present in 11 (73.3%) of 15 rats, but a reduction in size was noted. The inflammatory response in the experimental group was still significantly greater than that seen in the control group (P < 0.0001).

Cytokines
At 6 hours, TNF-α was detected in experimental and saline control vitreous samples (Fig. 3A), but not in vitreous samples from un.injected eyes. At 24 hours, a greater than 10-fold increase in TNF-α was detected in the experimental group compared with that in the control group (P < 0.03). This represented a greater than fourfold increase compared with the 6-hour level and a greater than 600-fold increase compared with the uninjected level. At 48 hours, the TNF-α level declined from the 24-hour peak by 81.8% in the experimental group but still was significantly elevated when compared with that in the control group (P < 0.01). When experimental and control samples were compared at 72 hours, no significant difference in the TNF-α levels was observed. No TNF-α was detected in the sera of experimental and control rats at any of the time points.

Interleukin-1β was found in all experimental vitreous samples at 6, 24, 48, and 72 hours (Fig. 3B). At 24 hours, experimental levels of IL-1β were more than 4000 times higher than control levels (P < 0.03). The level of IL-1β in the experimental group peaked at 24 hours, decreased by approximately 78.5% at 48 hours, and continued to decrease at 72 hours. Interleukin-1β was below detectable limits in saline-injected eyes and was not detected in sera of experimental and control animals at any time point.

CINC was detected in experimental vitreous samples at 6, 24, 48, and 72 hours, whereas lower levels were detected in saline-injected control samples (<912.0 pg/ml). No CINC was
detected in uninjected vitreous samples (Fig. 3C). At 6 hours, a greater than 2500-fold increase was detected in comparison with levels in uninjected samples, whereas a 2.75-fold increase was recorded compared with the level in control samples. At 24 hours, a statistically significant increase was seen in experimental samples compared with the level in control samples ($P < 0.02$). The level of CINC peaked at 24 hours, decreased by 80.0% at 48 hours, and approached control levels by 72 hours. No concentrations of CINC were detected in the sera of experimental or control rats at any of the time points.

Interferon-γ was found in experimental vitreous samples at 24, 48, and 72 hours (Fig. 3D). Experimental vitreous IFN-γ levels were significantly greater at 24 ($P < 0.03$) and 48 ($P < 0.01$) hours when compared with those in control vitreous samples. The level of IFN-γ peaked at 24 hours, decreased slightly at 48 hours, and returned to baseline at 72 hours. A statistically significant decrease in level was noted between the 48-hour and 72-hour time points ($P < 0.01$). No IFN-γ was detected in the vitreous of saline-injected eyes or in the sera of S. aureus-injected animals at any of the time points.

**Histopathology**

Essentially, no inflammatory cells were observed in the vitreous of experimental eyes at 6 hours and in control eyes at all time points. At 24 hours, the mean total cellular response (31 ± 5) in the vitreous chamber of experimental eyes significantly increased from the 6-hour time point ($P < 0.005$; Fig. 4). The primary infiltrating cells were neutrophils (72.3%) followed by monocytes-macrophages (12.7%) and lymphocytes (2.3%). The clinically visible vitreous exudate in experimental eyes was primarily composed of neutrophils. At 48 hours, a decrease in total cell number (25 ± 2) was noted compared with that at the 24-hour time point in the experimental group. At this time, neutrophils comprised the primary cell population (60.1%) in experimental vitreous. The monocyte and lymphocyte populations increased as a percentage of total cells from the 24-hour time point. At 72 hours, the total number of inflammatory cells decreased (19 ± 3) from that recorded at the 48-hour time point. At this time, a 1.5-fold decrease in the neutrophil percentage was noted, whereas the monocyte-macrophage and lymphocyte populations increased as a percentage of total cells. No plasma cells were detected at any of the time points.

Inflammatory cells were seen around the ciliary body and tracking posteriorly into the vitreous chamber and around the optic nerve head. Most experimental eyes and a few control eyes showed breakdown of the blood-retinal barrier manifested as a proteinaceous exudate seen in the anterior or
were similar to the inflammatory scores in the animals used for histologic analysis at 6, 24, 48, and 72 hours. The percentage of inflammatory cells do not add up to 100.0% because some cells were unidentifiable.

**DISCUSSION**

*S. aureus* is a virulent pathogen that can produce endophthalmitis with poor visual outcome. Few investigators have evaluated the host intraocular immune response to *S. aureus* and its components. Previous studies have shown that *S. aureus* and its components can induce in vitro production of various cytokines. Injection of purified TNF and IL-1 into rat and rabbit vitreous can elicit ocular inflammatory responses. However, none of these studies reflect the intraocular responses to viable bacteria in endophthalmitis. To clarify the early inflammatory response during *S. aureus* endophthalmitis, our study was focused on cytokine involvement elicited by intraocular injection of live bacteria.

Our results show that intravitreal injection of viable *S. aureus* induced the production of vitreous TNF-α, IL-1β, CINC, and IFN-γ and clinical signs of endophthalmitis. In the experimental group, cytokine levels peaked at 24 hours as did values in clinical inflammatory findings and total vitreous cell numbers. The elevated levels of vitreous TNF-α, IL-1β, and CINC at 6 hours and the lack of systemic levels in experimental samples suggest that their production is local in response to bacteria and/or components. Potential sources include vitreous hyalocytes, endothelial cells of retinal, iris, and ciliary body vessels; and resident macrophages. At 6 hours, a small but insignificant increase in TNF-α and CINC was noted in NS control vitreous samples. This may have been produced as a result of injection trauma. The increase in TNF-α, IL-1β, CINC, and IFN-γ at 24 hours may be related to the constant inflammatory stimulus present in the vitreous as a result of bacterial growth and release of cell wall and secreted components. At this time, inflammatory cells that have entered the eye may augment the cytokine response. *S. aureus* has been shown to be a more potent inducer of TNF-α production from granulocytes and mononuclear leukocytes than has lipopolysaccharide. In addition, macrophages have been shown to produce TNF-α after *S. aureus* exposure. The initial source of IFN-γ in the vitreous at 24 hours may be from natural killer cells that have been shown to produce IFN-γ in a T-cell-independent manner after stimulation with *S. aureus*. Breakdown of the blood-retinal barrier seen at 24 hours could be partially accounted for by TNF-α’s ability to alter tight junctions in endothelial cells inducing leakage of plasma proteins and water.

Eyes infected with *S. aureus* showed primarily a neutrophil response, which was expected. Studies have shown that mice injected with TNF-α produce neutrophil- and monocyte-specific chemokines, and in vitro cell culture studies have shown that heat-killed *S. aureus* induces the expression of IL-6 and IL-8 but not the expression of chemokines specific for other inflammatory cells. In addition, injection of purified CINC into the anterior chamber of Lewis rats results in the emigration of neutrophils into the aqueous humor. Our histologic data are similar to those in other in vivo studies in which intravitreal injections of TNF and IL-1 induced infiltration of neutrophils. The predominant neutrophil response could also be related to an early differential expression of adhesion molecules that favor neutrophil emigration. At 48 hours, TNF-α, IL-1β, and CINC levels declined rapidly from the 24-hour peak, whereas clinical inflammation and IFN-γ levels remained elevated. This prolonged clinical response could be caused by bacterial products (e.g., toxins) and other host inflammatory mediators (e.g., leukotriene B4) that may be present in the vitreous and may be involved in later responses. The cellular response at 48 hours was characterized by a decrease in neutrophils and an increase in lymphocytes and monocytes-macrophages. Their subsequent activation by *S. aureus* products may account for the 48-hour IFN-γ level. This response was followed at 72 hours by a shift in cell type to a population of approximately equal numbers of neutrophils and monocytes-macrophages. This shift may be partially related to neutrophil apoptosis, decreased production of neutrophil chemotactic agents such as CINC, production of other cytokines that change adhesion molecule expression, and production of other chemotactic agents that favor lymphocyte and monocyte-macrophage infiltration. The continued presence of neutrophils may be related to the ability of TNF-α, IL-1β, and IFN-γ to prolong neutrophil survival. The presence of IFN-γ at 48 hours may enhance antibody formation by B lymphocytes and help downregulate some of the inflammatory response, whereas the rapid decline in TNF-α and IL-1β levels, as noted in our model, would also be beneficial to the host. The cytoxic effects of TNF are enhanced in the presence of other cytokines, particularly IFN-γ.

When present together, TNF-α and IL-1β induce a more pronounced inflammatory response than when present separately. Thus, the observed decrease in TNF-α and IL-1β at the time of IFN-γ increase would enable
repair without toxicity. The decrease in TNF-α levels may be related to production of soluble TNF receptors, decreased biosynthesis, a short half-life of TNF mRNA, TNF protein degradation, and the production of antiinflammatory cytokines (e.g., IL-10, IL-12), which decrease TNF-α production in macrophages.\(^{25}\) Constant stimulation of monocytes by bacterial or host products may induce a refractory state inhibiting the production of TNF.\(^{25}\) Other cytokines, for example IL-4 and inactivating substances such as those detected in patients injected with lipopolysaccharide, may produce inhibitory effects on IL-1β production, resulting in a reduced level.\(^{25}\)

In summary, we have identified four inflammatory-immune cytokines in the vitreous of rat eyes infected with \textit{S. aureus}. The ocular inflammatory-immune response to intracellular bacteria is multifactorial. This study provides a more complete understanding of cytokine mediators produced in the eye in the period of early response to \textit{S. aureus} infection. However, important questions remain to be studied. Do additional proinflammatory cytokines other than the ones detected in this study play a role in \textit{S. aureus} endophthalmitis? Are \textit{S. aureus} factors responsible for induction of these cytokines? What is the ocular source of these cytokines? What is the association of these cytokines with pathologic changes in the eye? Our results should be helpful in subsequent studies of the role of inflammatory cytokines in the pathogenesis of endophthalmitis and evaluation of new treatment strategies.

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**References**


