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A Role for Tumor Necrosis Factor-Alpha (TNF α) in Experimental *Bacillus cereus* Endophthalmitis Pathogenesis

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Abstract

Purpose—To determine the contribution of tumor necrosis factor-alpha (TNF α)e pathogenesis of experimental *Bacillus cereus* endophthalmitis.

Methods—Experimental *B. cereus* endophthalmitis was induced in wild-type control (B6.129F1) or age-matched homozygous TNF α knockout mice (TNF $\alpha^{-/-}$, B6.129S6-Tnf^{tm1Gkl}/J). At various times postinfection, eyes were analyzed by electroretinography, and harvested for quantitation of bacteria, myeloperoxidase, proinflammatory cytokines and chemokines, and histological analysis.

Results—*B. cereus* replicated more rapidly in eyes of TNF $\alpha^{-/-}$ mice compared to that of B6.129F1 eyes. Retinal function decreased more rapidly in eyes of TNF $\alpha^{-/-}$ mice compared to that of B6.129F1 eyes. Retinal layers were not as structurally intact at 6 and 12 h postinfection in TNF $\alpha^{-/-}$ eyes compared to that of B6.129F1 eyes. Histological analysis suggested less PMN infiltration in the vitreous of TNF $\alpha^{-/-}$ mice than in B6.129F1 mice. B6.129F1 eyes also had greater myeloperoxidase concentrations than did eyes of TNF $\alpha^{-/-}$ mice. In general, concentrations of proinflammatory cytokines and chemokines (IL-1 β , IL-6, and MIP-1 α) were greater in eyes of TNF $\alpha^{-/-}$ mice than in B6.129F1 eyes.

Conclusions—TNF α is important to intraocular pathogen containment by PMN during experimental *B. cereus* endophthalmitis. In the absence of TNF α , less PMN migrated into the eye, facilitating faster bacterial replication and retinal function loss. Although greater concentrations of proinflammatory cytokines were synthesized in the absence of TNF α , the resulting inflammation was less, and an equally devastating course of infection occurred.

Keywords

Bacillus cereus; endophthalmitis; inflammation; mouse; retina

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Commercial Relationships: N for Ramadan, Moyer, and Callegan.

INTRODUCTION

Bacillus cereus is feared as an ocular pathogen because of its ability to blind rapidly during endophthalmitis.^{1–4} *B. cereus* endophthalmitis often results in significant vision loss or loss of globe architecture in 1 to 2 days. Several reports have credited the pathogenesis of *B. cereus* and other severe forms of bacterial endophthalmitis to toxins produced by the offending strain.^{5–11} However, the intraocular inflammatory response can be just as hazardous. Intraocular inflammation during endophthalmitis can be transient, as in the case of infection with avirulent organisms, or can evolve rapidly, as occurs during *B. cereus* endophthalmitis.¹

The primary function of innate immunity is to detect invading pathogens and clear them as quickly as possible. During an acute intraocular infection, a primary and essential component of this response is neutrophil influx. Cellular infiltration in human endophthalmitis cases has been described as vitritis, the presence of a hypopyon, and corneal ring abscess formation. Experimental models have identified polymorphonuclear leukocytes (PMN) as the primary infiltrating cell type during bacterial endophthalmitis.^{12–15} The recruitment and activation of neutrophils within an infected eye is a biological dilemma. PMN infiltration is necessary for bacterial clearance, but the generation of toxic reactive oxygen intermediates and other inflammatory mediators by PMN may result in bystander damage to delicate tissues of the retina. Robust inflammation is a hallmark of endophthalmitis caused by *B. cereus* and other types of virulent bacteria. In experimental *B. cereus* endophthalmitis, inflammatory cells were observed in the posterior chamber in close proximity to the optic nerve head as early as 4 h postinfection.¹³ Further analysis confirmed that the primary infiltrating cell was the PMN. The numbers of CD18⁺/Gr-1⁺ PMN were minimal at 4 and 6 h postinfection, but increased significantly thereafter. The influx of CD18⁺/Gr-1⁺ PMN into the posterior segment occurred simultaneously with the increase of TNF α in the eye at approximately 4–6 h postinfection.¹³ Despite their potential importance, the roles of TNF α and several other cytokines in endophthalmitis remain unexplored.

TNF α is a potent mediator of acute inflammatory reactions via activation of proinflammatory signaling cascades. TNF α is a cytokine secreted by macrophages and neutrophils, and is important in upregulating cell adhesion expression on vascular endothelial cells. TNF α also stimulates mononuclear phagocytes to produce cytokines, such as IL-1, IL-6 and itself.¹⁶ In an experimental rat model of *S. aureus* endophthalmitis, TNF α , IL-1 β , and CINC (rat homologue of CXCL8) were detected in the vitreous within 6 h of intravitreal inoculation.¹⁴ The authors hypothesized that upregulation of proinflammatory cytokines may have contributed to the breakdown of the blood-retina barrier and the recruitment of neutrophils into the eye. Upregulation of TNF α , IL-1 β , and IFN γ ; has also been detected in experimental *S. epidermidis* endophthalmitis.¹⁷ Injection of TNF α into the vitreous of rabbits¹⁸ and rats¹⁹ induced vascular permeability and cellular infiltration. Studies have also demonstrated upregulation of TNF α and other proinflammatory cytokines in experimental autoimmune uveoretinitis.²⁰ No studies have quantified cytokines or chemokines in the human eye during endophthalmitis, but based on experimental studies, it is reasonable to hypothesize that proinflammatory cytokines are key mediators of acute inflammation during this infection.

The inflammatory pathways involved in *B. cereus*-induced intraocular inflammation remain to be fully elucidated. However, such a rapid response strongly suggests that acute phase mediators and immune cells are involved. As stated above, TNF α is upregulated in the eye in parallel with influx of PMN under experimental conditions¹³, but the contribution of TNF α to the pathogenesis of endophthalmitis has not been determined. We hypothesized that TNF α is an important proinflammatory cytokine that contributes to recruitment of PMN into the eye during experimental endophthalmitis. To test this hypothesis, we analyzed infection in wild-type control and homozygous TNF α knockout mice. The results demonstrated that

TNF α was important in bacterial growth control via the acute inflammatory response to *B. cereus* endophthalmitis. In the absence of TNF α , inflammation was muted, resulting in more rapid bacterial replication and retinal function loss. Compensating proinflammatory cytokines and chemokines were synthesized in the eye in the absence of TNF α , resulting in less inflammation, but an equally devastating course of infection.

METHODS

Mice and Infections

Breeding pairs of background mice (B6.129F1) and homozygous TNF $\alpha^{-/-}$ mice (B6.129S6-Tnf $^{tm1Gkl/J}$)²¹ were obtained from Jackson Laboratories (Bar Harbor, ME). C57BL/6J mice were also used for comparisons with B6.129F1 mice for some experiments. Mice were bred and cared for in housing facility conditions according to institutional guidelines and guidelines provided by the Association for Research in Vision and Ophthalmology. Male and female mice from the breeding colonies were used between 6–8 weeks of age, with the appropriate age-matched controls. Polymerase chain reaction (PCR) was performed to confirm the homozygosity of littermates (data not shown).

Mice were infected intravitreally with wildtype *B. cereus* as previously described.¹³ Briefly, mice were anesthetized generally with a ketamine/xylazine cocktail (85 mg/kg body weight, Ketavet [Phoenix Scientific, St. Joseph MO]/14 mg/kg body weight, Rompun [Bayer Corp., Shawnee Mission KS]) and topically with 0.5% proparacaine HCl (Ophthetic, Allergan, Hormigueros, Puerto Rico). Bacteria were injected into the mid-vitreous with a sterile glass capillary needle containing 100 CFU *B. cereus* strain ATCC 14579 in 0.5 μ L brain heart infusion (BHI) medium. At various times postinfection, endophthalmitis was analyzed by biomicroscopy, quantitation of intraocular bacterial growth, proinflammatory cytokines and chemokines, and myeloperoxidase (MPO, to estimate PMN infiltration), and electroretinography, as described below.

Electroretinography

Retinal function was assessed by electroretinography as previously described.¹³ Following injection of *B. cereus*, mice were dark-adapted for at least 6 h. Prior to ERG, mice were then anesthetized with ketamine/xylazine as described above and pupils were dilated with 10% topical phenylephrine (Akorn, Inc., Buffalo Grove IL). Gold-wire electrodes were placed on each cornea and a reference electrode was placed in the mouth. The stimulus used to evoke the response was delivered by a white sphere that mimicked a Ganzfeld. The interval between 2 flashes (10-ms duration) was 60 s to prevent light adaptation. A-wave and B-wave amplitudes were measured from the initiation of the light flash to the trough of the A-wave, and the trough of the A-wave to the peak of the B-wave, respectively. A total of five readings were recorded and averaged. Percentages of retinal function retained compared with controls were calculated as described previously.¹³ Values represent the mean $\pm$ standard error of the mean (SEM) for $N \geq 6$ samples per time point.

Bacterial Growth

Globes were homogenized with 1-mm sterile glass beads (BioSpec Products, Inc., Bartlesville OK) in 400 μ L of PBS. Bacteria were quantified by track plating serial 10-fold dilutions onto BHI agar.^{13,22} Values represent the mean $\pm$ SEM for $N \geq 5$ eyes per time point.

Cytokines and Chemokines

Eyes were analyzed for the presence of representative proinflammatory cytokines and chemokines shown to be upregulated in various experimental models of ocular infection and

inflammation.^{13,14,17,23–27} Globes were removed and homogenized with 1-mm glass beads in a protease inhibitor cocktail (Triton X-100, 0.5 M EDTA, 10 mM sodium orthovanadate [Sigma, St. Louis MO] and Protease Inhibitor [Calbiochem, La Jolla, CA] in PBS, pH 7.4). Supernatants were then analyzed for IL-1 β , MIP-1 α , KC, and IL-6 by ELISA (Quantikine Kits, R&D Systems, Minneapolis MN) according to manufacturers instructions. Concentrations in supernatants of tissue homogenates were compared to that of a standard curve. Values represent the mean $\pm$ SEM for N=6 eyes per time point.

PMN and Myeloperoxidase

To compare the numbers of circulating PMN, whole blood was harvested and PMN were quantified with a hemocytometer. Values represent the mean $\pm$ SEM for N=3 mice per genotype.

To estimate the extent of PMN infiltration into the eye, MPO was quantified.¹³ Mouse eyes were removed, homogenized with 1-mm glass beads in a lysis buffer (200 mM NaCl, 5 mM EDTA, 10 mM Tris, 10% glycine [vol/vol], 1 mM PMSF, 1 μ g/ml leupeptide, 28 μ g/ml aprotinin) and supernatants were analyzed for MPO levels by sandwich ELISA (Mouse MPO ELISA Test Kit, Cell Sciences, Canton MA). Values represent mean $\pm$ SEM for N $\geq$ 6 eyes per timepoint.

Histology

Globes were harvested and eyes were fixed in Perfix and incubated for 24 h. Globes were embedded in paraffin, sectioned, and stained with hematoxylin and eosin stain by standard procedures. Histology sections were scored by a masked observer and graded on a scale of 0 to 4+ in terms of severity.¹³ Sections presented are representative of N=3 eyes per time point.

Anti-TNF α and *B. cereus* Endophthalmitis

A pilot study was undertaken to analyze the potential anti-inflammatory effects of anti-TNF α (infliximab, Remicade®, Centocor Inc.). 50 ng/0.5 μ l of anti-TNF α was injected immediately prior to *B. cereus* infection. MPO concentrations (N=3) were analyzed at 10 h postinfection.

Statistics

Student's t-test was used for statistical comparisons between mouse strains at each time point. Wilcoxon's rank sum test was used for statistical comparison between infection groups. A P $\leq$ value 0.05 was considered significant.

RESULTS

Bacterial Growth

B. cereus grew logarithmically in eyes of B6.129F1 wild-type controls and TNF α ^{-/-} mice, as well as in eyes of C57BL/6J background control mice (Figure 1). The rates of growth in eyes of C57BL/6J and B6.129F1 wild-type controls were similar (P>0.5 at all time points). However, *B. cereus* grew faster in eyes of the TNF α ^{-/-} strain, with greater numbers of viable *B. cereus* recovered per eye at 6, 8, and 12 h postinfection compared to that recovered from B6.129F1 wild-type control eyes (P<0.05). In the absence of TNF α , faster intraocular bacterial replication occurred.

Retinal Function

Retinal function analysis of endophthalmitis in eyes of TNF α ^{-/-} and B6.129F1 wild-type mice is summarized in Figure 2. A- and B-wave amplitudes declined at significantly greater rates in infected eyes of TNF α ^{-/-} mice compared to that observed in eyes of B6.129F1 mice (P<0.01

at 6 and 8 h postinfection). Taken together, retinal function loss in eyes of the $\text{TNF}\alpha^{-/-}$ mice was approximately 3-fold greater at 6 h than that of eyes of wild-type B6.129F1 mice. By 12 h postinfection, retinal function was < 5% in all infected eyes of either mouse strain. In the absence of $\text{TNF}\alpha$, retinal function declined more rapidly, likely due to increased intraocular bacterial replication.

Whole Blood PMN and Intraocular Inflammation

PMN were quantified in whole blood of $\text{TNF}\alpha^{-/-}$ and B6.129F1 wild-type mice. Manual counts detected similar numbers of PMN from the blood of $\text{TNF}\alpha^{-/-}$ mice ($6.92 \pm 0.05 \log_{10}$ PMN/ml) and wild type mice ($6.84 \pm 0.1 \log_{10}$ PMN/ml; $P=0.052$).

Intraocular inflammation was analyzed by quantifying MPO of infiltrating PMN (Figure 3) and proinflammatory cytokines and chemokines (Figure 4) in eyes during endophthalmitis. At 0 h and 4 h postinfection, MPO concentrations were similar in $\text{TNF}\alpha^{-/-}$ and B6.129F1 eyes ($P>0.05$). MPO concentrations of $\text{TNF}\alpha^{-/-}$ and B6.129F1 eyes were greater at 4 h postinfection than at 0 h postinfection ($P<0.01$), with significant increases within each group at each time point thereafter. MPO concentrations were significantly less in eyes of $\text{TNF}\alpha^{-/-}$ mice compared to that of eyes of B6.129F1 mice at 6, 8, and 12 h postinfection ($P<0.05$ at all time points). In the absence of $\text{TNF}\alpha$, PMN influx into the infected eyes of $\text{TNF}\alpha^{-/-}$ mice was less than that of infected wild-type eyes.

In general, proinflammatory cytokine and chemokine concentrations were greater in $\text{TNF}\alpha^{-/-}$ eyes than in wild-type B6.129F1 eyes. In $\text{TNF}\alpha^{-/-}$ eyes, concentrations of KC were greater than in eyes of B6.129F1 at 4, 8, and 12 h postinfection ($P<0.05$ at all time points). Concentrations of MIP-1 α and IL6 were greater in $\text{TNF}\alpha^{-/-}$ eyes than that of B6.129F1 eyes at 8 and 12 h postinfection ($P<0.05$). Concentrations of IL-1 β were greater in $\text{TNF}\alpha^{-/-}$ eyes than in B6.129F1 eyes at 12 h only ($P<0.05$). Upregulation of other proinflammatory cytokines and chemokines in the absence of $\text{TNF}\alpha$ resulted in lower numbers of infiltrating PMN in infected eyes of $\text{TNF}\alpha^{-/-}$ mice.

Histology

Whole eye and retinal histology of infected $\text{TNF}\alpha^{-/-}$ and B6.129F1 wild-type eyes demonstrated evolving endophthalmitis similar to that previously reported in this model¹³ (Figure 5). At 6 h postinfection in B6.129F1 wild-type eyes, the majority of infiltrating PMN observed were located in close proximity to the optic nerve head (histology score ranging from 1+ to 2+). At this time, retinal architecture was slightly disrupted (histology score of 1+). At 12 h postinfection, significant loss of retinal architecture was observed and significant numbers of PMN were seen in the posterior segment, near the ciliary body, and in the anterior chamber (histology scores ranging from 2+ to 3+). At 6 h postinfection in $\text{TNF}\alpha^{-/-}$ mouse eyes, very few PMN were seen in the posterior segment (histology score ranging from 0 to 1+), and retinal architecture was disrupted to a greater degree compared to that of wild-type eyes (histology score of 2+). By 12 h postinfection, retinas of infected eyes of $\text{TNF}\alpha^{-/-}$ mice were disrupted and detached, with great numbers of *B. cereus* accumulating near the retina and optic nerve, and PMN were present in both segments (histology score ranging from 3+ to 4+). In $\text{TNF}\alpha^{-/-}$ mice, retinal disruption evolved more rapidly, but PMN influx was less than that seen in B6.129F1 wild-type mice.

Anti- $\text{TNF}\alpha$ and *B. cereus* Endophthalmitis

At 10 h postinfection, MPO concentrations were decreased by approximately 40% in eyes injected with 50 ng anti- $\text{TNF}\alpha$ alone compared to that of uninjected control eyes.

DISCUSSION

Pathogen recognition and a well-regulated inflammatory response to infection are essential in clearing invading organisms with minimal damage to surrounding tissue. A tightly controlled response is even more critical in the eye, where non-regenerative cells and tissues responsible for vision reside. Experimental models of bacterial endophthalmitis have demonstrated that once a pathogen is introduced into the posterior segment, an acute response occurs, including synthesis of proinflammatory cytokines and influx of PMN into the eye.^{12–14} In the case of virulent pathogens such as *S. aureus* or *B. cereus*, low numbers of bacteria can be cleared effectively by an adequate inflammatory response.^{13,28} Once an inoculum threshold is passed, bacterial growth and toxin production overwhelm the inflammatory response. In an exhaustive attempt to clear the infection, PMN fill the posterior and anterior segments.

Regulation of inflammation is the key to removing the pathogen without harming the eye, but bystander damage from infiltrating cells can occur. For *S. aureus* endophthalmitis, depletion of neutrophils early in the inflammatory response reduced the severity of host inflammation, but severely hampered bacterial clearance, resulting in a more severe infection.¹² In the present study, depression of initial cellular influx in the absence of TNF α resulted in higher bacterial numbers and faster retinal function loss. Explosive inflammation is characteristic of *B. cereus* endophthalmitis. However, due to rapid bacterial growth, migration, and toxin production by *B. cereus* in the eye¹, attempts at infection control by the host are typically futile.

We reported that one of the earliest cytokines detected during experimental *B. cereus* endophthalmitis was TNF α .¹³ TNF α was detected in whole eyes during experimental *B. cereus* endophthalmitis as early as 4 h postinfection, a time when PMN were first observed infiltrating into the posterior segment. During many different types of infections, TNF α initiates a cascade of proinflammatory cytokine synthesis and contributes to increased vascular permeability and upregulation of cell adhesins, effectively recruiting macrophages and neutrophils to the infection site. Because TNF α levels increased in parallel with increasing numbers of intraocular PMN during experimental *B. cereus* endophthalmitis, we sought to determine to what extent TNF α contributed to this initial inflammatory response by comparing infections in wild-type and TNF $\alpha^{-/-}$ mice. Studies of non-ocular infections in TNF $\alpha^{-/-}$ or TNF α -receptor knockout mouse strains have demonstrated the value of TNF α in containment of a wide range of ocular pathogens, including *S. aureus*^{29,30}, *Candida*³¹, and pneumococcus^{32,33}.

To ensure that experimental endophthalmitis was reproducible in mouse strain B6.129F1, the wild-type strain used in these studies, bacterial growth rates and pathology were compared to that of C57BL/6J mice. Ocular TNF α concentrations were similar in these two mouse strains during experimental infection (data not shown). Retinal function loss and MPO levels in B6.129F1 eyes were comparable to that of C57BL/6J eyes, as reported previously.¹³ Intraocular *B. cereus* growth and clinical signs of infection were similar at all time points, further validating the use of this genetic background for these studies.

B. cereus grew more rapidly and to greater numbers in eyes of TNF $\alpha^{-/-}$ mice than in eyes of B6.129F1 wild-type mice. As expected, retinal function declined more rapidly in TNF $\alpha^{-/-}$ mice than in B6.129F1 mice. Histological evidence demonstrated that retinas were damaged and detached to a greater degree in TNF $\alpha^{-/-}$ eyes than in eyes of B6.129F1 mice. These results suggested that in the absence of TNF α bacterial growth was unimpeded, facilitating greater retinal damage and function loss. Greater numbers of bacteria likely resulted in higher concentrations of toxins produced in the eye, resulting in faster retinal damage and loss of function. The importance of toxins to the intraocular virulence of *B. cereus* endophthalmitis has been well-documented.^{1,7–9}

In infected TNF α ^{-/-} mouse eyes, inflammation was muted compared to that of eyes of B6.129F1 mice. PMN migrated in fewer numbers into the eyes in TNF α ^{-/-} mice than in B6.129F1 mice, as demonstrated by MPO assay and histology. Taken together, these data indicated that in the absence of TNF α , fewer PMN migrating into the posterior segment resulted in higher intraocular bacterial loads and subsequently, more significant retinal damage. This result would suggest that TNF α contributed to the early recruitment of PMN into the eye and subsequent pathogen control during endophthalmitis. By virtue of its role in affecting blood-retinal barrier integrity^{34,35}, the absence of TNF α may have resulted in less barrier permeability and migration of fewer PMN into the eye. The absence of TNF α has been demonstrated to decrease tight junction-associated permeability^{36,37} and result in less PMN infiltration³⁶ in experimental models of acute lung inflammation and restraint stress (small intestine analyzed). Manual counts detected similar numbers of PMN from the blood of TNF α ^{-/-} and B6.129F1 mice, so differences in intraocular PMN quantities were not the result of differences in whole blood PMN between the mouse strains. Our PMN numbers in TNF α ^{-/-} mice were similar to those reported by Kuprash *et al.*³⁸ who detected statistically higher whole blood white blood cells and neutrophils in TNF α ^{-/-} mice compared to that of C57BL/6 mice. Since the numbers of PMN were greater at 4 h postinfection in eyes of both mouse strains than that of freshly-infected eyes at 0 h, PMN quantities at 4 h likely represent cells that were recruited to the eye as a result of infection.

In the absence of TNF α , other proinflammatory cytokines and chemokines were synthesized during infection to facilitate recruitment of PMN into the eye. In this study, KC was detected at 4 h postinfection, and higher concentrations of KC were detected in TNF α ^{-/-} eyes than in B6.129F1 eyes. Because KC and MPO levels paralleled one another and increased beginning at 4 h postinfection in a manner similar to that of TNF α in C57BL/6J mice¹³, KC may also be an important recruiting cytokine in the eye during the initial stages of experimental *B. cereus* endophthalmitis. KC and its homologs have been detected in experimental and human ocular infections, including keratitis caused by fungi³⁹, Adenovirus⁴⁰, *Pseudomonas*^{24,41}, and *Staphylococcus*⁴², acute bacterial conjunctivitis⁴³, and in human uveitis cases⁴⁴. Further studies in transgenic mice deficient in KC can confirm the contribution of this cytokine to the pathogenesis of bacterial endophthalmitis.

In terms of proinflammatory cytokine synthesis, IL-6, KC, MIP-1 α , and IL-1 β were synthesized to higher levels in TNF α ^{-/-} mice than in B6.129F1 mice. IL-1 β and IL-6 were below the limits of detection at 4 h postinfection, but were detected at 8 h in eyes of both mouse strains, 4 h later than the initial influx of PMN into the posterior segment. MIP-1 α levels were just above the limit of detection at 4 h postinfection, but levels increased thereafter. IL-1 β levels were significantly higher in TNF α ^{-/-} eyes than in B6.129F1 eyes at 12 h only. Higher concentrations of cytokines/chemokines may be synthesized to compensate for the absence of the most potent recruiting cytokine, TNF α . However, greater cytokine concentrations may not necessarily translate to greater numbers of PMN in the eye, as was demonstrated in this model, particularly if the absence of TNF α resulted in a greater impermeability of the blood-ocular barrier. Studies have demonstrated the production of increased levels of other proinflammatory cytokines/chemokines in the absence of TNF α in experimental models of infection and inflammation.^{29,45–47} Because IL-6, MIP-1 α , and IL-1 β were produced at a later stage of infection when significant numbers of PMN were already present in the eye, these cytokines could be a product of the infiltrating inflammatory cells themselves and perhaps played only a minor role in initial PMN recruitment. However, PMN are not the only potential source of cytokines in the eye during intraocular inflammation. Other resident ocular cells, such as retinal or optic nerve head glia and microglia, may also synthesize cytokines/chemokines. These particular cell types have been shown to synthesize proinflammatory cytokines during various states of infection, inflammation, and retinal stress.^{48–53} The specific cells involved in cytokine and chemokine synthesis during intraocular bacterial infection are yet to be identified.

Using single-gene knockouts in mediators of the host response is sufficient for analyzing a deficiency in one mediator, but this approach has its disadvantages. For example, the same types of cells synthesize IL-1 β and TNF α . These cytokines act on comparable cell types during inflammation and also signal through the NF κ B pathway. Hypothetically, the absence of one cytokine may induce synthesis of other compensating cytokines (in a manner similar to that seen in this study), confounding the role of the original cytokine of interest in infection. Functional redundancy has been reported for TNF α and IL-1 in experimental autoimmune uveitis, where deletion of receptors for both cytokines were more effective in reducing infiltrating cell numbers than deleting each receptor alone.⁵⁴ In the context of experimental *B. cereus* endophthalmitis, IL-1 β was first detected well after TNF α and KC were initially detected and PMN were present the posterior segment, suggesting a minimal role for IL-1 β during the initial stages of inflammation.

Because the absence of TNF α was demonstrated in this infection model to dampen the initial inflammatory response during *B. cereus* endophthalmitis, it was of interest to analyze whether therapy targeting TNF α would effectively attenuate inflammation. Our preliminary data demonstrated the anti-inflammatory potential of anti-TNF α when injected immediately prior to *B. cereus* infection. Infliximab has attenuated intraocular inflammation in experimental models of choroidal neovascularization^{55,56} and endotoxin-induced uveitis⁵⁷, and in human uveitis patients.^{58–62} Infliximab was recently shown to be non-toxic at levels up to 1.7 mg in rabbit eyes.⁶³ These findings suggest the potential for attenuation of inflammation during endophthalmitis by targeting TNF α and perhaps other cytokines, but this sort of therapy would likely be best suited for the initial stages of infection.⁶⁴ Continuing studies will determine the therapeutic potential of cytokine targeting in conjunction with early antibiotic treatment in reducing inflammation during bacterial endophthalmitis.

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Figure 1. Bacterial growth during experimental *B. cereus* endophthalmitis

C57BL/6J, B6.129F1 wild-type, and TNFα^{-/-} mouse eyes were injected with 100 CFU of *B. cereus*. Eyes were harvested, homogenized, and analyzed for bacterial growth. *B. cereus* grew to higher concentrations in infected eyes of TNFα^{-/-} mice than in eyes of wild type or background mice (P<0.05 at all time points). Values represent the mean ± SEM of N ≥ 5 eyes per time point.

Figure 2. Retinal function analysis during *B. cereus* endophthalmitis

B6.129F1 wild-type and $TNF\alpha^{-/-}$ mouse eyes were injected with 100 CFU of *B. cereus*. Retinal function was assessed by electroretinography. At 6 h and 8 h postinfection, the A- and B-wave amplitudes retained were significantly less in $TNF\alpha^{-/-}$ eyes than that of B6.129F1 eyes. By 12 h postinfection, retinal function was nearly abolished in all infected eyes. Values represent the mean $\pm$ SEM of $N \geq 6$ eyes per time point.

Figure 3. Infiltration of PMN into mouse eyes during *B. cereus* endophthalmitis

B6.129F1 wild-type and TNFα^{-/-} mouse eyes were injected with 100 CFU of *B. cereus*. PMN infiltration was analyzed by quantifying myeloperoxidase (MPO) in whole eyes. MPO concentrations were greater in B6.129F1 eyes than in TNFα^{-/-} eyes at 6, 8, and 12 h postinfection (P<0.05), suggesting greater numbers of PMN in wild-type eyes than in TNFα^{-/-} eyes. Values represent the mean ± SEM for N ≥ 6 per group.

Figure 4. Proinflammatory cytokine expression during *B. cereus* endophthalmitis

B6.129F1 wild-type and TNFα^{-/-} mouse eyes were injected with 100 CFU of *B. cereus*. Whole eyes were analyzed for proinflammatory cytokines and chemokines by ELISA. In general, levels of MIP1α, IL-6, KC, and IL-1β were greater in TNFα^{-/-} mouse eyes than in B6.129F1 eyes during infection. Values represent the mean ± SEM of N = 6 eyes per time point.

Figure 5. Whole organ (top) and retinal (bottom) histology of *B. cereus* endophthalmitis
 B6.129F1 wild-type and $TNF\alpha^{-/-}$ mouse eyes were injected with 100 CFU of *B. cereus*. Eyes were harvested and processed for hematoxylin and eosin staining. In B6.129F1 eyes, retinas remained essentially intact but inflammation was significant during infection. In $TNF\alpha^{-/-}$ eyes, retina were disrupted to a significant degree by 6 h postinfection, but inflammation was less than that observed in B6.129F1 eyes. Sections are representative of N = 3 per group. CH, choroid; RPE, retinal pigment epithelium; PCL, photoreceptor layer; ONL, outer nuclear layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer; VIT, vitreous. Magnification, $\times 20$ (top) and $\times 200$ (bottom).