Bacterial endophthalmitis: Therapeutic challenges and host–pathogen interactions

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Abstract

Endophthalmitis is an infection of the posterior segment of the eye that frequently results in loss of vision. This devastating result occurs despite prompt and often aggressive therapeutic and surgical intervention. Over the past decade, research has centered on determining the bacterial and host factors involved in this potentially blinding disease. The initial focus on the bacterial factors responsible for intraocular virulence has recently expanded into analysis the inflammatory response to infection, including the molecular and cellular interactions between the pathogen and host. This review discusses the epidemiology and therapeutic challenges posed by endophthalmitis, as well as recent findings from the analysis of interactions between the host and pathogen. Based on these findings, a model for the pathogenesis of endophthalmitis is presented. A more comprehensive understanding of the molecular and cellular interactions taking place between pathogen and host during endophthalmitis will expose possible therapeutic targets designed to arrest the infection and prevent vision loss.

Keywords: Endophthalmitis; Bacteria; Retina; Vitreous; Infection; Therapy

Contents

1. Introduction .......................................................... 190
   1.1. Post-operative endophthalmitis ........................................ 190
   1.2. Post-traumatic endophthalmitis ...................................... 191
   1.3. Endogenous endophthalmitis ....................................... 191
2. Therapeutic challenges ............................................... 191
3. The contribution of bacterial virulence factors to pathogenicity ........................................ 192
   3.1. Bacillus ......................................................... 192
   3.2. Enterococci .................................................... 193
   3.3. Streptococcus pneumoniae ........................................ 193
   3.4. Staphylococcus ................................................ 194
   3.5. Gram-negative bacteria ........................................... 194
   3.6. Bacterial migration in the eye ................................... 195

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1. Introduction

Bacterial endophthalmitis can occur following the introduction of an infectious agent into the posterior segment of the eye, causing intraocular infection and inflammation. Contamination of the posterior segment with bacteria can occur during a surgical procedure (post-operative), following trauma caused by a penetrating foreign object (post-traumatic), or during metastasis of bacteria into the eye from a distant infection site (endogenous). The clinical presentation of endophthalmitis can vary widely, from a mild and therapeutically responsive inflammation, to complete vision loss or loss of the eye itself, despite the use of aggressive therapy and surgery. The majority of endophthalmitis cases are a complication of intraocular surgery. The isolates involved are usually normal flora of the surface of the eye and surrounding mucosa, such as \textit{Staphylococcus epidermidis}, which are generally not highly virulent. Therapy for endophthalmitis caused by avirulent pathogens is usually successful, often with retention of full visual acuity. Conversely, post-traumatic endophthalmitis is typically caused by environmental pathogens such as \textit{Bacillus cereus}, resulting in severe and potentially blinding infections. Cases of endophthalmitis caused by highly virulent pathogens are often non-responsive to treatment, resulting in significant vision loss.

1.1. Post-operative endophthalmitis

The rates of post-operative endophthalmitis have been low for many years, but recent reports suggested that this type of ocular infection may be on the rise. Fluctuations in the number of cases appear to correlate with the type of intraocular surgery performed (Busbee, 2006). Post-operative endophthalmitis has been reported as a consequence of nearly every type of ocular surgery, but is most common following cataract surgery. A recent report identified an increase in the number of post-operative endophthalmitis cases, from 0.1% in the 1990s, to 0.2% for the period 2000–2003 (West et al., 2005). Among the types of cataract surgeries performed, phacoemulsification accounted for approximately 48% of the post-operative endophthalmitis cases, while 38.5% and 6.6% of cases followed extracapsular or intracapsular extraction, respectively (Ng et al., 2005).

Numerous reports have demonstrated that Gram-positive bacteria cause the vast majority of post-operative endophthalmitis cases. Coagulase-negative staphylococcal isolates are the most common, causing 47–70% of all post-operative endophthalmitis cases. Other species involved include \textit{Staphylococcus aureus}, streptococci, enterococci, and Gram-positive rods such as \textit{Bacillus}. Gram-negative bacteria were isolated from a relatively low number of post-operative endophthalmitis cases (6%). Most intraocular infections resulting from infection with coagulase-negative staphylococci can be treated with antibiotic and anti-inflammatory agents, resulting in restoration of partial or complete vision. However, the more virulent the bacterial strain, the more devastating the visual outcome. Intraocular infections with \textit{S. aureus}, enterococci, \textit{Bacillus}, or Gram-negative strains are often intractable, and blindness or loss of the eye itself is not uncommon (Josephberg, 2006; Ng et al., 2005).

The therapeutic success of treatment of post-operative endophthalmitis depends largely on accurate and prompt diagnosis. Of the Gram-positive endophthalmitis cases cited in the Endophthalmitis Vitrectomy Study (EVS), 84% of cases resulted in at least 20/100 visual acuity, while 50% of these cases resulted in 20/40 visual acuity. Severe cases were caused by more virulent strains. Eyes infected with Gram-negative bacteria, streptococci, and \textit{S. aureus} were more difficult to treat, and only 30% of these eyes attained 20/100 visual acuity. Of the entire EVS patient group, 11% had 5/200 vision and 5% had no light perception (Josephberg, 2006). Since the early 1980s, the preferred route of antibiotic administration changed from the use of subconjunctival and intravenous antibiotics to the immediate injection of antibiotics directly into the vitreous (Baum et al., 1982). However, the visual outcome of patients has not improved significantly as a result of this change (Josephberg, 2006; Ng et al., 2005).
1.2. Post-traumatic endophthalmitis

Although there are not as many cases of post-traumatic endophthalmitis as there are following intraocular surgery, infection rates following penetrating injuries are higher. The rates of endophthalmitis following ocular trauma ranged from 3% to 17%, with an increased likelihood for significant vision loss due to the potential virulence of environmental isolates involved (Jonas et al., 2000; Meredith, 1999; O’Brien and Choi, 1995; Thompson et al., 1993). Staphylococci are the most common causes of post-traumatic endophthalmitis, with B. cereus ranked as the second most common cause. B. cereus is ten times more likely to be isolated from cases of post-traumatic endophthalmitis than from post-operative endophthalmitis cases (Das et al., 2005).

During traumatic injury, the penetrating intraocular foreign body (IOFB) may remain lodged in the eye. Ocular injuries with IOFBs accounted for 18–40% of all penetrating eye injuries. The involvement of IOFBs in penetrating injuries predisposes a risk of developing endophthalmitis (Brinton et al., 1984; Thompson et al., 1993, 1995; Williams et al., 1988). In such cases, the vast majority of IOFBs were typically found in the posterior segment. Of all penetrating eye injuries, 2–16% developed sight-threatening endophthalmitis, the incidence and severity of which were dependent upon whether IOFB removal was immediate or delayed (Essex et al., 2004; Jonas et al., 2000; Lieb et al., 2003; Thompson et al., 1995).

Post-traumatic endophthalmitis is difficult to treat, not only because of the potential virulence of infecting organisms, but also because of the varying time between injury and treatment, the condition of the injury upon presentation, and the age of the patient. All factors considered, immediate therapeutic intervention is critical in order to target all potential organisms and arrest the potentially destructive inflammatory response.

1.3. Endogenous endophthalmitis

Endogenous endophthalmitis occurs when the interior of the eye is seeded with bacteria from a distant site of infection. This form of endophthalmitis occurs most often in immunocompromised individuals, those with prolonged indwelling medical devices, and intravenous drug abusers. Endogenous endophthalmitis is relatively rare, accounting for only 2–8% of all endophthalmitis cases (Jackson et al., 2003; Okada et al., 1994; Romero et al., 1999), and is associated with a poor visual prognosis. The visual outcome of endogenous endophthalmitis has not improved over the past 55 yr, despite the use of improved antibiotics and aggressive surgical intervention. Patients with severe systemic infections may not present with ocular symptoms initially, and therefore the focus on the eye may be limited until a patient complaint of ocular pain or change in vision.

Over 50% of endogenous endophthalmitis cases are caused by fungal organisms, particularly Candida albicans (which causes 75–80% of fungal cases) (Romero et al., 1999). The bacterial causes of endogenous endophthalmitis vary geographically. Gram-negative bacteria cause 32–37% of cases in North America and 70% of cases in East Asia. Among Gram-negative endophthalmitis cases, Klebsiella spp. is the most common etiologic agent. Bacillus spp. and coagulase-negative staphylococci are the most common causes of Gram-positive endogenous endophthalmitis (Jackson et al., 2003). Despite aggressive therapeutic and surgical intervention, these patients typically retain only count fingers vision (Jackson et al., 2003).

Immunocompromise is an important factor in the development of endogenous endophthalmitis. In a recent review, 56% of patients with endogenous bacterial endophthalmitis were also immunocompromised, and diabetes was the most common underlying disease involved (Jackson et al., 2003). The increased risk of infection to diabetics has been well-documented; however, no correlation has been shown between diabetes and post-operative or post-traumatic endophthalmitis. Links between underlying ocular diseases associated with diabetes (i.e. diabetic retinopathy) have not been established. For endogenous endophthalmitis, Type II diabetes is the most common underlying condition, particularly in patients with secondary Klebsiella liver abscesses (Jackson et al., 2003). Intravenous drug use, which over time can also lead to immunocompromise, is the second most common underlying condition associated with endogenous endophthalmitis. Approximately one-third of endogenous endophthalmitis patients reported were under treatment of an immunosuppressive agent (Jackson et al., 2003).

2. Therapeutic challenges

Treatment of bacterial infections and inflammation in the interior of the eye poses a unique dilemma. Anatomic barriers and the delicate nature of the interior of the eye are factors to consider during treatment. Key anatomic barriers that prevent adequate treatment of endophthalmitis are the inner and outer blood–retinal barrier and the blood–aqueous humor barrier, collectively called the blood–ocular barrier (Cunha-Vaz, 1997, 2004). The blood–ocular barrier, similar to the blood–brain barrier, consists of tight junctions between the endothelial cells and basement membrane of retinal capillaries and retinal pericytes. It not only protects the interior of the eye from assault by cells, macromolecules, and drugs, but also prevents the entrance and subsequent activity of most systemic antimicrobial and anti-inflammatory drugs. Intraocular barriers can be bypassed by direct injection of drugs into the vitreous. Photoreceptors and other cells of the retina are exquisitely sensitive to insult, and high doses of antimicrobial agents necessary to sterilize the eye may have toxic effects on the retina, potentially disrupting the biochemical pathways necessary for vision. In addition, damage can occur not only from toxic virulence factors produced by the organism in the eye, but also from bystander damage caused by the influx of inflammatory cells into the posterior
segment. Overall, clinicians must take into account a myriad of unique challenges posed by the blood–ocular barrier, bystander damage of the immune response, and potential damage caused by drugs in order to protect the vision of patients with bacterial endophthalmitis.

The clinical presentation of endophthalmitis can vary widely. The outcome of infection depends on many factors, including the age and immune status of the patient, condition of the eye upon presentation, the infecting organism’s virulence and antibiotic susceptibility profile, and the time between injury/surgery and therapy. However, regardless of the source of infection, clinicians often do not know the identity of the infecting strain and must treat the eye empirically. Vancomycin, aminoglycosides, and cephalosporins are commonly used to treat bacterial endophthalmitis. To be effective, these antibiotics may require direct injection into the vitreous, because the blood–ocular barrier may prevent adequate penetration into the vitreous at levels above the minimal inhibitory concentration for the infecting pathogen when these drugs are administered systemically. Experimental studies have demonstrated that without early treatment with intravitreal antibiotics, vision may be lost (Aguilar et al., 1996; Forster, 1992). The EVS suggested that systemic antibiotic administration following vitrectomy was not an effective adjunct therapy for endophthalmitis because systemic use in combination with intravitreal antibiotics did not improve visual outcome or vitreal clarity (Endophthalmitis Vitrectomy Study Group, 1995).

The most commonly utilized therapeutic combinations for intravitreal injections have included vancomycin (1.0 mg) and amikacin (0.4 mg) or ceftazidime (2.2 mg). Vancomycin has been reported to have 100% effectiveness against the most commonly causative Gram-positive endophthalmitis organisms (Benz et al., 2004; Recchia et al., 2005). Amikacin and ceftazidime have approximately the same success rate against Gram-negative organisms (89%) (Han et al., 1996). However, toxicity to retinal cells has been reported following amikacin use (Campochiaro and Conway, 1991). Ceftazidime has been recommended as the antibiotic of choice for endophthalmitis caused by Gram-negative species (Campochiaro and Lim, 1994; Jackson et al., 2003).

Fluoroquinolone antibiotics, while used frequently as topical agents for corneal infections, are effective against most intraocular pathogens. Fourth generation fluoroquinolones, such as gatifloxacin and moxifloxacin, have been shown to penetrate the blood–ocular barrier, suggesting their potential effectiveness as intraocular drugs (Busbee 2004). Gatifloxacin administered orally prior to pars plana vitrectomy resulted in aqueous and vitreous concentrations that were above the MIC_{90} for the most common endophthalmitis pathogens (Hariprasad et al., 2003). However, topical administration of moxifloxacin and gatifloxacin resulted in vitreous concentrations of each antibiotic that were below the MIC_{90} for common endophthalmitis pathogens (Costello et al., 2006). Compared with other quinolones, moxifloxacin has been reported to penetrate more effectively into intraocular tissues following topical administration (Robertson et al., 2005; Yagi et al., 2006). Concerns remain regarding possible toxic effects of fluoroquinolones and further evaluation of intraocular toxicity is necessary. Unfortunately, fluoroquinolone resistance among ocular pathogens appears to be on the rise, which may impact future therapeutic regimens for this disease (Miller et al., 2006).

Inflammation during infection is necessary for the clearance of organisms, but can result in bystander damage to the interior of the eye. Intraocular inflammation can occur following intravitreal injection of bacteria or their components, such as toxins or cell wall constituents that are shed from the organisms during infection, either during bacterial replication (Callegan et al., 1999a, 2002b; Fox et al., 1984; Kufoy et al., 1990), or following treatment with cell wall-active antibiotics (Callegan et al., 2002b). To minimize ocular inflammation during endophthalmitis, clinicians often use intravitreal injection of dexamethasone in conjunction with antibiotics. The use of steroids in the treatment of endophthalmitis has been controversial and there remains no clear evidence of their benefit for this disease. Experimental models and clinical studies have reported that concomitant administration of dexamethasone with antibiotics was detrimental (Meredith et al., 1996), beneficial (Gan et al., 2005; Liu et al., 2000; Smith et al., 1997; Yildirim et al., 2002) or had no effect (Aguilar et al., 1996; Ermis et al., 2005; Pollack et al., 2004; Shah et al., 2000). Despite the controversy, dexamethasone is frequently used as an adjunct to antibiotics for the treatment of endophthalmitis.

Vitrectomy is often used to debride and remove the nidus of infection during severe cases of endophthalmitis. The vitreous is removed with a miniature hand-held cutting device and replaced with transparent fluid to maintain intraocular pressure and pH necessary for visual function. The EVS reported that in suspected endophthalmitis cases following intraocular surgery, vitrectomy was an effective adjunct to antibiotic therapy, but only for patient who had lost vision to only light perception (Endophthalmitis Vitrectomy Study Group, 1995). In cases of post-traumatic endophthalmitis where the eye contains retained IOFBs, immediate vitrectomy and intravitreal antibiotic injection has been recommended (Abu El-Asrar et al., 1999). For endogenous endophthalmitis, adjunct vitrectomy conferred significant improvements in vision, but any delay in vitrectomy resulted in a comparative loss of visual acuity (Yoon et al., 2003). Most reports agree that vitrectomy should be performed without delay in severe cases of endophthalmitis, especially those involving IOFBs.

3. The contribution of bacterial virulence factors to pathogenicity

3.1. Bacillus

*Bacillus* spp. are a major cause of rapidly blinding cases of post-traumatic and endogenous endophthalmitis. The
majority of patients with Bacillus endophthalmitis lost significant visual function or the eye itself in less than 2–3 days (Dus et al., 2001, 2005; David et al., 1994; Ho et al., 1982; O’Day et al., 1981). B. cereus causes the vast majority of Bacillus endophthalmitis cases. However, endophthalmitis can also be caused by Bacillus thuringiensis, a bacterium commonly used for organic gardening and farming that is genetically and phenotypically similar to B. cereus (Callegan et al., 2006a). For B. cereus and B. thuringiensis, the quorum sensing transcriptional regulator plcR controls the expression of many extracellular virulence factors (Agaisse et al., 1999). In an experimental rabbit model of endophthalmitis, B. cereus and B. thuringiensis were significantly less virulent when plcR was non-functional (Callegan et al., 2003). Wild type Bacillus caused severe intraocular inflammation by 12 h post-infection. Severe intraocular inflammation did not occur until 30 h post-infection in eyes infected with plcR-deficient Bacillus. Wild type Bacillus also caused nearly complete loss of retinal function by 18 h, while plcR-deficient mutants required 36 h to reach >90% reduction in retinal function. Reduced virulence was likely due to reduced toxin expression by plcR-deficient strains. The ability of B. cereus toxins to induce the type of damage seen in endophthalmitis has been demonstrated in a model of sterile endophthalmitis, in which bacterial supernatants from wild type and plcR-deficient B. cereus were examined in mice (Fig. 1). Supernatant from wild type B. cereus caused rapid retinal function loss and more severe inflammation than did supernatant from plcR-deficient B. cereus (R. T. Ramadan, B. D. Novosad, and M. C. Callegan, Abstr. Molec. Pathogen. Infectious Inflammatory Eye Res. Conf., 2005). In terms of individual toxins, those tested to date (hemolysin BL, phosphatidylinositol-specific phospholipase C, and phosphatidylcholine-specific phospholipase C) contributed little to the overall pathogenesis of experimental B. cereus endophthalmitis (Callegan et al., 1999b, 2002a). Taken together, these data highlight the importance of plcR dysfunction to the pathogenesis of Bacillus endophthalmitis and its role as a potential therapeutic target.

3.2. Enterococci

Enterococci are a rarer cause of post-operative endophthalmitis, and it is often associated with filtering bleb surgery. However, expressing fewer virulence traits than B. cereus, it is more amenable to study. The main toxin expressed by strains of E. faecalis, the cytolsin, has been shown to contribute to the severity of infection. Cytolsin is a secreted toxin that can lyse bacteria, erythrocytes, and other mammalian cells. The presence of cytolsin rendered experimental E. faecalis endophthalmitis refractory to antibiotic and anti-inflammatory drug treatment (Jett et al., 1992, 1998). In terms of toxin regulation, the E. faecalis quorum sensing system fsr has been evaluated for its role in endophthalmitis pathogenicity (Mylonakis et al., 2002). The fsr quorum sensing system regulates the production of gelatinase (GelE) and a serine protease (SprE) in a density dependent manner (Qin et al., 2000). When deletions were made in gelE, sprE, or both genes, experimental endophthalmitis was attenuated, with the gelE deletion having the most significant effect. However, these mutations were not able to achieve the same degree of attenuation as an fsr-deficient mutant (Engelbert et al., 2004; Mylonakis et al., 2002). These results suggest the importance of the fsr quorum-sensing system as a potential therapeutic target. In addition to the toxicity of secreted virulence factors, clinical isolates of enterococci possess an antibiotic resistance profile that renders them resistant to most commonly used antibiotics. Enterococci have also been shown to produce biofilms on intraocular lens material (Kobayakawa et al., 2005), further highlighting their potential virulence for the eye.

3.3. Streptococcus pneumoniae

S. pneumoniae has been isolated from post-traumatic and post-operative cases of endophthalmitis. Two potential virulence factors (pneumolysin and autolysin) have
been analyzed for their contribution to endophthalmitis pathogenicity. Pneumolysin is a cytolytic toxin and can activate the classical complement pathway (Paton et al., 1997), while autolysin regulates the stability of the pneumococcal cell wall (Ng et al., 2002). In an experimental rat model of endophthalmitis, infection with a pneumolysin-deficient strain caused less intraocular inflammation than infection with the wild type strain at 24 h post-infection. By 48 h post-infection, however, inflammation was similar in eyes infected with the pneumolysin-deficient and wild type parental strains. In contrast, infection with the autolysin-deficient mutant resulted in less intraocular inflammation than infection with the pneumolysin-deficient mutant and wild type pneumococcus at both time points. These results suggest that autolysin may be an important pneumococcal virulence factor in endophthalmitis, perhaps by functioning in the liberation of inflammogenic pneumococcal cell wall fragments during infection (Ng et al., 2002).

3.4. Staphylococcus

Staphylococci are the predominant organisms recovered from cases of post-traumatic and post-operative endophthalmitis. In addition to the many virulence factors produced by both \textit{S. aureus} and \textit{S. epidermidis}, the increased incidence of resistance to several commonly used antibiotics in clinical isolates threatens to further increase the rate of treatment failures for staphylococcal endophthalmitis. Like enterococci, staphylococci can also readily form biofilms on several types of abiotic surfaces, including intraocular lenses (IOLs), highlighting their potential for contamination and infection of the interior of the eye.

Experimental rat and rabbit models of endophthalmitis have been used to demonstrate the importance of \textit{S. aureus} cytolytic toxins and global regulation of those toxins. In terms of the significance of individual toxins to pathogenicity, \textit{S. aureus} \(a\)-toxin and \(b\)-toxin appear to be important to intraocular virulence. Eyes infected with strains deficient in \(a\)-toxin, \(b\)-toxin, or both toxins were less inflamed and retained greater retinal function than eyes infected with wild type \textit{S. aureus} (Callegan et al., 2002b). The global regulators \textit{agr} and \textit{sar}, which control the density-dependent production of \textit{S. aureus} adhesins and toxins, are critical for the intraocular virulence of \textit{S. aureus}. Infections in eyes inoculated with strains deficient in \textit{agr}, \textit{sar}, or both global regulators were highly attenuated (Booth et al., 1995, 1997; Giese et al., 1999), more so than that observed during infection with staphylococcal strains deficient individual toxins.

The majority of experimental studies regarding the contribution of \textit{S. epidermidis} virulence factors to intraocular infections have analyzed the contribution of adhesins to biofilm formation on lens material. \textit{S. epidermidis} bound to IOLs may be a source of infecting organisms that seed the posterior segment and result in endophthalmitis or chronic inflammation. The ability of \textit{S. epidermidis} to form biofilms on IOL materials contributes not only to colonization, but also to circumvention of an immune response designed to eliminate bacteria, and to limitation in the effectiveness of antibiotics (Baili\'f et al., 2006).

Although \textit{S. epidermidis} produces few toxins, this organism can readily evade the immune system by biofilm formation, a process mediated by surface proteins such as autolysin, polysaccharide intercellular adhesin, fibrinogen-binding proteins, and accumulation-associated protein (Foster, 2005). The role of these specific adhesins in the formation of intraocular biofilms or the pathogenicity of \textit{S. epidermidis} endophthalmitis has yet to be determined.

3.5. Gram-negative bacteria

As stated above, Gram-negative endophthalmitis following surgery or trauma is relatively rare. Although less common than Gram-positive infections, Gram-negative endophthalmitis is of particular concern because these infections are significantly more difficult to treat and visual outcome is typically poor. In western countries, Gram-negative endogenous infections are less common, accounting for approximately 32–37% of endogenous endophthalmitis cases. However, in eastern countries, particularly Taiwan, Gram-negative bacteria were responsible for 70% of endogenous bacterial endophthalmitis cases (Jackson et al., 2003).

\textit{Klebsiella} spp. has surpassed \textit{E. coli} as the most common Gram-negative cause of endogenous bacterial endophthalmitis, accounting for 80–90% of all cases in East Asian countries (Wong et al., 2003). Over half of \textit{K. pneumoniae} endogenous endophthalmitis patients were diabetic, and two-thirds had an underlying liver abscess caused by \textit{Klebsiella}. For \textit{K. pneumoniae}, over 77 serological types have been classified. The two most common serotypes cultured from cases of endogenous \textit{K. pneumoniae} endophthalmitis were the K1 and K2 serotypes (Podschun and Ullmann, 1998). K1 serotypes contain two genes that are associated with a hypermucoviscosity phenotype, \textit{magA} and \textit{rmpA} (Yu et al., 2006). This phenotype is strongly associated with immune system avoidance, bacteremia, and liver abscess formation. It is not known whether hypermucoviscosity or other virulence factors contribute to the persistence of \textit{K. pneumoniae} in the bloodstream and/or its ability to infect the eye, nor whether these factors contribute to inflammation and retinal function loss once \textit{K. pneumoniae} has reached the interior of the eye. Preliminary studies have shown that \textit{K. pneumoniae} can replicate quickly in the vitreous, but cause inflammation and retinal function loss at a rate considerably slower compared to than that observed in experimental \textit{B. cereus} endophthalmitis models (Fig. 2, B. J. Wiskur, B. D. Novosad, R. Ramadan, M. C. Callegan, Abstr. Assoc. Res. Vision Ophthalmol Meet. Abstr. 5071, 2005).
4. The host response to endophthalmitis

4.1. Immune privilege

The eye is an immune privileged site where multiple mechanisms work together to protect the visual axis from destructive inflammation. Immune privilege was first described by Medawar in the 1940s as an anatomical site where foreign tissue grafts survived for prolonged periods of time (Medawar, 1948). Using this criterion, Streilein et al. (2003) identified several immune privileged sites within the eye: the anterior chamber, the vitreous cavity, and the subretinal space. Both tissue allografts and immunogenic tumor cells experience immune privilege within these sites. Direct experiments demonstrating that bacteria experience immune privilege within the eye have not been performed, but several studies imply that this phenomenon occurs. Hoebe et al. (2005) demonstrated that subcutaneous injection of $5 \times 10^5$ CFU of S. aureus was rapidly cleared in C57BL/6 mice within 7 days. By contrast, Engelbert and Gilmore (2005) demonstrated that C57BL/6 mice were unable to clear an intravitreal injection of as few as 5000 CFU of S. aureus, and the bacterial load within the eye increased to $2 \times 10^9$ CFU within 72 h, resulting in its rapid destruction. Taken together, these studies suggested that immune privilege in the eye limits bacterial clearance as well as permitting graft survival and tumor cell proliferation.

A wide array of cell surface (Fas ligand and complement regulatory proteins) and soluble factors (transforming growth factor-β, z-melanocyte stimulating hormone, vasoactive intestinal peptide, calcitonin gene-related peptide, soluble Fas ligand, etc.) are involved in creating the immunosuppressive environment within the eye (Streilein, 2003). One effect of ocular immune privilege is the limitation of inflammation within the eye. For example, intraocular tumors that experience immune privilege fail to
induce inflammation, while rejection of the same tumors from non-privileged sites is accompanied by vigorous inflammation (Chen et al., 1998). The extent to which inflammation is limited in response to bacteria in the posterior segment of the eye is less clear. Bacteria in the vitreous appear to benefit from immune privilege and evidenced by the survival and proliferation of even small inocula. However, most bacteria appear nevertheless to trigger vigorous inflammation. The relationship of immune privilege to innate immunity, specifically as it relates to modulating the host-pathogen dynamic, has only begun to be studied and many questions remain to be answered: (i) How is inflammation in response to bacterial stimuli different between immune privileged and non-privileged sites; (ii) Does intraocular inflammation affect bacterial growth; (iii) How long is immune privilege maintained in the presence of an inflammatory response; and (iv) Is the destruction of ocular tissue mediated primarily by bacteria or inflammation? Answers to these questions may lead to improved treatments for endophthalmitis, enabling a rapid clearance of the invading pathogen while protecting fragile ocular tissues.

4.2. Chronic versus acute inflammation

The inflammatory response triggered by intraocular pathogens can be acute or chronic. Acute inflammation is most commonly associated with more virulent bacterial infections (B. cereus, E. faecalis, streptococci, Gram-negative organisms or S. aureus) and an invariably poor visual outcome (Callegan et al., 2002c). As early as 48 h post-infection both the anterior and posterior segments are involved, resulting in corneal edema, neutrophil infiltrate within the cornea and aqueous humor, vitritis, and retinal periphlebitis (Mandelbaum and Forster, 1996). By contrast, chronic inflammation is associated with less virulent bacterial infections (Propionibacterium acne and S. epidermidis) and a better visual outcome (Mandelbaum and Forster, 1996). While the onset of chronic inflammation is typically delayed and clinically milder, a failure to treat this infection during its early stage can result in loss of vision. In endophthalmitis with acute or chronic inflammation, early diagnosis and eradication of bacteria are key for successful treatment and preservation of vision.

4.3. Sensors of innate immunity

Innate immunity is the first line of defense that coordinates an immediate and rapid immune response to microbial challenge. The first step in the host defense against infection is distinguishing self from non-self and “sensing” an invading microbial pathogen. The innate immune system uses pattern recognition receptors to initiate innate immunity against microbial components, known as pathogen associated molecular patterns (e.g., LPS, peptidoglycan, flagellin, etc.) (Tosi, 2005). These pathogen associated molecular patterns trigger innate immunity through the activation of the complement pathway, and through the Toll-like receptor subfamily of pattern recognition receptors. Toll-like receptors (TLRs) are the human homologs of the toll receptor first discovered in Drosophila melanogaster as a key component of innate immunity (Tosi, 2005). TLRs recognize specific bacterial motifs and are expressed on cells of the innate system such as neutrophils, monocytes, macrophages, dendritic cells, and mast cells. To date, 10 human TLRs have been identified and several are specific for bacteria (Tosi, 2005). Peptidoglycan and lipoteichoic acid from Gram-positive bacteria are ligands for TLR-2, LPS from Gram-negative bacteria is a ligand for TLR-4, and CpG motifs in bacterial DNA are ligands for TLR-9. Recently, it was demonstrated that TLRs, including TLR-2, TLR-4, and TLR-9, are expressed in the retina and/or the retinal pigment epithelial (RPE) cells, and may serve as the first line of defense against invading bacterial pathogens in the posterior segment of the eye (Kumar et al., 2004). Triggering of the TLRs expressed on RPE induces the production of multiple proinflammatory cytokines, chemokines, and adhesion molecules including IFN-β, IL-6, IL-8, MCP-1, and sICAM-1 (Kumar et al., 2004).

4.4. Complement

Components of the bacterial cell wall, such as LPS, can directly activate the complement system via the alternative pathway (Tosi, 2005). The alternative pathway is one of three routes to the activation of complement in response to the presence of pathogens. Activation of the complement system provides a very effective host defense against invading organisms by generating anaphylatoxins that: (i) trigger inflammation, (ii) attract phagocytes to the site of infection via chemotactic factors, (iii) promote the opsinization and lysis of invading bacteria, and (iv) cause vasodilation and increased vascular permeability (Tosi, 2005). While the complement system is effective at eradicating invading pathogens, activation of complement often results in significant bystander tissue damage. Therefore, while active complement components are present within the eye, specifically C3 convertase and membrane attack complex, the activation of complement is kept in check by complement regulatory proteins (Sohn et al., 2000). The complement regulatory proteins CD55, CD46, CD59, and Crry are all expressed in the normal eye and have been shown to tightly regulate the activation of the complement system (Sohn et al., 2000). Early studies using guinea pigs decomplemented with cobra venom factor (CVF) demonstrated that, in complement depleted animals, guinea pigs displayed impaired host defense to S. aureus endophthalmitis (Giese et al., 1994). Similar effects were demonstrated with CVF-treated mice (Engelbert and Gilmore, 2005). However, studies using C3−/− mice that were deficient in the central component of the complement system, the absence of C3 was found to be inconsequential to the outcome of endophthalmitis.
4.5. Fas ligand

Fas ligand is constitutively expressed within the normal eye and plays a critical role in maintaining immune privilege by inducing apoptosis in infiltrating T cells and inhibiting adaptive immunity (Griffith et al., 1995). However, recent studies reveal that the membrane form of FasL can also promote innate mediated inflammation through the activation of neutrophils (Gregory et al., 2002). Moreover, Engelbert and Gilmore (2005) demonstrated that Fas ligand is critical in the clearance of bacterial endophthalmitis, and may play a direct role in recruiting and activating neutrophils. While normal mice readily cleared an infection with 500 CFU of *S. aureus*, mice deficient in Fas ligand were unable to clear the same size inoculum. In the absence of Fas ligand, bacteria grew more rapidly and fewer neutrophils were recruited to the site of infection. These findings are best interpreted using recent studies by Gregory et al. (2002) indicating that Fas ligand plays a critical role in the activation of the early innate immune response within the eye. Membrane bound Fas ligand appears to activate innate immunity, whereas soluble Fas ligand is immunosuppressive. The balance between the various forms of Fas ligand may critical in regulating host inflammation triggered by invading bacterial pathogens. The membrane form of Fas ligand may act to amplify the early innate response, by triggering the infiltrating cells to secrete more proinflammatory cytokines such as IL-1β and MIP-2α, resulting in a robust innate response. Once bacteria are cleared, inflammation may be suppressed by the liberation of Fas ligand from the cell surface.

4.6. Cellular infiltration

Neutrophils are an essential component of innate immunity, and are the primary infiltrating cell type in the early phase of endophthalmitis (Callegan et al., 2002b; Giese et al., 2003; Ramadan et al., 2006). However, the recruitment and activation of neutrophils within the eye represent an important biological dilemma. The generation of toxic reactive oxygen intermediates and other inflammatory mediators by neutrophils may be essential for clearance of bacteria, but may contribute to irreversible bystander tissue damage in the eye. Using an *S. aureus* model of endophthalmitis, Giese et al. (2003) demonstrated that depletion of neutrophils early in the inflammatory response reduced the severity of host inflammation, yet severely hampered the clearance of bacteria.

Robust inflammation is a hallmark of intraocular infection 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 post-infection (Callegan et al., 2005; Ramadan et al., 2006). Further analysis by myeloperoxidase assays and flow cytometry confirmed that the primary infiltrating cell was the PMN (Ramadan et al., 2006). The numbers of CD18+/Gr-1+ PMN were minimal at 4 and 6 h post-infection, but increased significantly thereafter. Histologically, PMN influx into the vitreous near the iris and ciliary body and into the anterior segment increased significantly after 6 h post-infection. The pathways involved in inducing this inflammation, despite immune privilege, remain to be fully elucidated.

4.7. Cytokines, chemokines, and adhesion molecules

The primary function of the innate immune system is to detect invading pathogens and clear them as quickly as possible. Cytokines and chemokines are signaling molecules that play a central role in detecting the invading pathogen, recruiting inflammatory cells, and clearing the infection. Using a rat model of endophthalmitis, Giese et al. (1998) demonstrated that within 6 h of intravitreal inoculation with *S. aureus*, TNF-α, IL-1β, and CINC (rat homolog of IL-8) are detected within the vitreous. These were found to contribute to the break down of the blood retinal barrier, and to the recruitment of leukocytes, in particular neutrophils. The adhesion molecules ICAM-1 and E-selectin are also upregulated early in iris, ciliary body, and retinal vessels, serving to enhance the infiltration of leukocytes to the site of infection (Giese et al., 2000). In contrast, IFN-γ peaks at 24 h post-infection, correlating with an increased infiltration of macrophages and lymphocytes (Giese et al., 1998). During experimental *B. cereus* endophthalmitis, the influx of C18+/Gr-1+ PMN into the posterior segment occurred simultaneously with the increase of TNFα in the eye at approximately 4–6 h post-infection (Ramadan et al., 2006). While the time course of cytokine production has been determined in these studies, the source of these cytokines is currently unknown. Overall, these studies do not address whether these
molecules serve in a cause or effect role in the clinical outcome of endophthalmitis.

5. Changes to the retina in response to infection

5.1. Blood retinal barrier permeability

As discussed previously, the transparency of the visual axis is critical for vision, and this seems to be the driver in the evolution of the active immune suppression mechanisms that result in immune privilege in the eye. In addition to the active release of immunosuppressive molecules into the eye, other factors contribute to immune privilege. Among these are the absence of blood vessels and lymphatic drainage pathways from the internal compartments of the eye (except the uveoscleral pathway), and a paucity of local antigen presenting cells. Although the intersection of the conjunctiva and cornea is one of the most immunologically active sites in the eye, the anterior and posterior chambers, retina, and subretinal space are sequestered from the systemic circulation by the blood ocular barrier limits the incursion of macromolecules into the aqueous, vitreous, and the subretinal spaces. These selective barriers allow nutrients into the neural retina, while simultaneously protecting the neural retina from harmful macromolecules and cells (Magone and Whitcup, 1999; Streilein, 1999). The blood aqueous humor barrier is an epithelial barrier formed by the non-pigmented layer of ciliary epithelium and the posterior iridial epithelium, and the endothelium of iridial vessels. The inner blood retinal barrier is formed by intercellular tight junctions between the endothelium of the retinal vessels, preventing seepage of plasma constituents into the retina. The outer blood retinal barrier is formed by tight junctions between the retinal pigment epithelium (RPE), which manages the blood supply from the choroid to the photoreceptors. The blood ocular barrier limits the incursion of macromolecules into the aqueous, vitreous, and the subretinal spaces. These selective barriers allow nutrients into the neural retina, while simultaneously protecting the neural retina from harmful macromolecules and cells (Magone and Whitcup, 1999; Streilein, 1999).

Multiple causes of blood retinal barrier breakdown have been identified, including infection (Cellini and Baldi, 1991; Pepose et al., 1985), diabetes (Lobo et al., 2000; Schalnus and Orloff, 1995; Yoshida et al., 1993), and intraocular surgery (Men et al., 2003). Associations have been reported between the breakdown of the blood retinal barrier and almost every retinal disease, particularly vascular retinopathies and pigment epitheliopathies (Forrester and Menamin, 1999). Inflammation-mediated damage to the neurosensory retina and RPE may affect the basic photochemical process of vision. Inflammation has been shown to play a key role in damaging both the inner and outer components of the blood retinal barrier (Cunha-Vaz, 1997, 2004). Ocular inflammation stimulates upregulation of cell adhesion molecules, permitting inflammatory cell and macromolecule extravasation into the vitreous and subretinal spaces (Huber et al., 2006; Xu et al., 2003). Inflammatory mediator production in this area likely exacerbates and maintains the inflammatory response, which can result in loss of vision (Koizumi et al., 2003). During experimental autoimmune uveitis, leukocytes have been shown to have an active role in tight junction disruption and blood retinal barrier breakdown during retinal inflammation (Xu et al., 2005).

To date, few studies have examined the role of blood retinal barrier breakdown in the pathogenesis of bacterial endophthalmitis. Endophthalmitis induced by E. coli LPS resulted in a dose-dependent breakdown of the inner components of the blood retinal barrier, as demonstrated by MRI (Metrickin et al., 1995). There was not an active infection in this study, and other elements of inflammation were not analyzed. Significant localization of serum albumin in both the inner and outer blood retinal barriers has been reported in human cases of endophthalmitis (Vinores et al., 1994). As stated earlier, neutrophils enter the posterior segment at about the time that inflammatory cytokines are detected during experimental bacterial endophthalmitis (Giese et al., 1998; Ramadan et al., 2006).

The primary barrier cells of the blood retinal barrier are the RPE, which are polarized cells with basal sides intricately folded and attached to the thin basal lamina, forming the inner layer of Bruch’s membrane (Magone and Whitcup, 1999; Streilein, 1999). RPE cells, in addition to their barrier function, also contribute to retinal physiological homeostasis. In terms of the potential role of RPE dysfunction in blood retinal barrier permeability during endophthalmitis, efforts are focused on analyzing the interactions between RPE and bacteria and bacterial toxins. RPE are sensitive to B. cereus growth and toxin production. Exposure of RPE to wildtype or quorum sensing deficient Bacillus in vitro resulted in lactate dehydrogenase release, regardless of quorum regulated factors (Fig. 3, A. L. Moyer, C. Roach, M. C. Callegan. Assoc. Res. Vision Ophthalmol. Meet. Abstr. 4016, 2004). In vitro, B. cereus infection of RPE monolayers resulted in toxin-dependent degradation of ZO-1 (Fig. 4, A. L. Moyer, B. D. Novosad, M. C. Callegan. Abstr. Assoc. Res. Vision Ophthalmol. Meet. Abstr. 5078, 2005). Since RPE function is dependent on the integrity of intercellular tight junction proteins formed between cells, the dysfunction or degradation of proteins contributing to tight junction formation as a result of infection may lead to a breach of the blood retinal barrier.

5.2. Retinal architectural and function loss

Retinal detachment is one of the most serious complications of endophthalmitis and occurs with an incidence between 4% and 21% (Abu El-Asrar et al., 2000; Azad et al., 2003; Bali et al., 2003; Doft et al., 2000; Endophthalmitis Vitrectomy Study Group, 1995; Lieb et al., 2003). Retinal structural changes during endophthalmitis, in addition to detachment in its entirety, include photoreceptor layer folding and detachment, and complete dissolution of retinal cell layers (Callegan et al., 1999a, b, 2002b). Motile organisms, such as Bacillus, have been
observed within retinal folds by 8 h post-infection (Callegan et al., 1999b, 2002b). Retinal detachment and destruction of the retinal layers has also been observed in endophthalmitis caused by organisms which remain in the midvitreous, such as S. aureus, E. faecalis, or non-motile Bacillus (Callegan et al., 2002b).

The exact mechanisms of retinal function loss, and loss of retinal architecture during endophthalmitis, are not yet understood. During endophthalmitis, bacteria replicate and produce toxins in close proximity to the delicate cells of the retina. The contribution of bacterial toxin production to the loss of retinal function and architecture has recently been analyzed in an experimental model of B. cereus endophthalmitis (Ramadan et al., 2006), focusing primarily on the interaction of Bacillus toxins with the Muller cell. Muller cells are elongated neuroglial cells that extend across the thickness of the retina, from the photoreceptor cell layer to the inner limiting membrane abutting the vitreous. Muller cells provide structural and physiological support to the retina, and contribute to the generation of the retinal response (specifically the b-wave and the slow P3 component of the electroretinogram), by regulating K+ distribution across the retina (Reichenbach and Robinson, 1995; Sarthy et al., 1998; Sarthy and Ripps, 2001). The contribution of Muller cell dysfunction to retinal structural alterations or function loss during endophthalmitis is not yet clear. However, analysis of experimental retinal detachment models suggests that detachment can cause, or be a secondary effect of, Muller cell dysfunction (Francke et al., 2001; Lewis and Fisher, 2003; Sarthy et al., 1998; Willbold and Layer, 1998). In detachment models, Muller cells undergo stress and subsequently upregulate glial fibrillary acidic protein (GFAP), an intermediate filament protein. During the progression of experimental B. cereus endophthalmitis in the mouse, slight increases in GFAP immunostaining were detected at 8 h post-infection, and significant GFAP immunostaining was detected at 16 h post-infection, suggesting that the retina is undergoing stress during infection (Ramadan et al., 2006). Whether this stress is attributable to Muller cells or other GFAP-synthesizing cells, such as astrocytes, is presently being investigated. The Muller cell end plates lie in the inner limiting membrane next to the vitreous, in close proximity to bacterial replication and toxin production. The initial decline in retinal function, especially in the b-wave, occurs in parallel with logarithmic growth of B. cereus and maximal toxin production, suggesting that there may be interactions between Bacillus and/or its toxins and the Muller cell.

6. Hypothetical model of endophthalmitis

Advances in the understanding of molecular and cellular interactions between the host and pathogen during endophthalmitis, make possible the proposal of a model for the pathogenesis of this infection: Following introduction of bacteria into the posterior segment, endophthalmitis follows one of two general paths: (i) infection, mild inflammation, effective treatment, and recovery of vision, or (ii) infection, significant inflammation, ineffective
treatment, and vision loss. In either case, the immune privileged intraocular environment is generally permissive for bacterial replication, which depending on organism results in inflammation. During the growth of virulent organisms, toxin production results in vision loss. Toxins may (1) interact specifically with the retina, potentially causing dysfunction of cells essential for vision and ocular homeostasis (e.g., induce Müller cell dysfunction, which may affect retinal function and architecture); (2) induce RPE dysfunction leading to breakdown of the blood retinal barrier and unimpeded influx of inflammatory cells and mediators into the posterior segment; and or (3) interact with inflammatory cells resulting in upregulation of inflammatory cytokines, resulting in severe inflammation and bystander damage. The ultimate result is disruption of retinal architecture, retinal cell death, and a breach of the barriers that provide an immune modulated environment within the eye.

7. Future directions

Streilein (1996) noted that immune privilege in the eye posed a Faustian dilemma: “To maintain accurate vision, the eye must develop strategies that limit its vulnerability to the blinding consequences of trauma, inflammation, neovascularization, regeneration, and autoimmunity.” Yet, like other parts of the body, the eye must confront from time to time the challenge of infection. Because of the importance of vision to survival, natural selection has led to an optimized balance between threats to the host from compromised vision stemming from systemic inflammation and autoimmunity, and vulnerability to infection. As a result, the host–pathogen dynamic as it occurs in the eye, and as it is influenced by various mechanisms of immune privilege, is not likely to be similar to that which occurs at other anatomical sites. This was most evident by the recent unexpected finding that in the eye, complement does not play a measurable role in the clearance of S. aureus (Engelbert and Gilmore, 2005). To understand the pathogenesis of ocular infection toward the goal of optimizing treatment and salvaging vision, then, it will be necessary to test host–pathogen interactions directly in this organ.

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