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Modeling Intraocular Bacterial Infections

Roger A. Astley¹, Phillip S. Coburn¹, Salai Madhumathi Parkunan², and Michelle C. Callegan^{1,4,*}

¹Department of Ophthalmology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, USA

²Department of Microbiology and Immunology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, USA

³Oklahoma Center for Neuroscience, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, USA

⁴Dean McGee Eye Institute, Oklahoma City, Oklahoma, USA

Abstract

Bacterial endophthalmitis is an infection and inflammation of the posterior segment of the eye which can result in significant loss of visual acuity. Even with prompt antibiotic, anti-inflammatory and surgical intervention, vision and even the eye itself may be lost. For the past century, experimental animal models have been used to examine various aspects of the pathogenesis and pathophysiology of bacterial endophthalmitis, to further the development of anti-inflammatory treatment strategies, and to evaluate the pharmacokinetics and efficacies of antibiotics. Experimental models allow independent control of many parameters of infection and facilitate systematic examination of infection outcomes. While no single animal model perfectly reproduces the human pathology of bacterial endophthalmitis, investigators have successfully used these models to understand the infectious process and the host response, and have provided new information regarding therapeutic options for the treatment of bacterial endophthalmitis. This review highlights experimental animal models of endophthalmitis and correlates this information with the clinical setting. The goal is to identify knowledge gaps that may be addressed in future experimental and clinical studies focused on improvements in the therapeutic preservation of vision during and after this disease.

Keywords

Endophthalmitis; Bacteria; Antibiotics; Corticosteroids; Inflammation; Vitrectomy; Therapeutics

*Corresponding author: Department of Ophthalmology DMEI PA-418, 608 Stanton L. Young Blvd., Oklahoma City, OK 73104, USA. Phone: (405) 271-3674, Fax: (405) 271-8128, michelle-callegan@ouhsc.edu.

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1. CLINICAL ASPECTS OF BACTERIAL ENDOPHTHALMITIS

Bacterial endophthalmitis is a potentially blinding intraocular infection and inflammation resulting from the entry of bacteria into the interior of the eye. Endophthalmitis begins as an infection of the posterior segment which may interfere with vision. If treatment is ineffective, the infection can become severe, inflammation can evolve rapidly, the retina can become irreversibly damaged and may detach, and significant vision can be lost. Even with prompt and appropriate treatment, one-third of endophthalmitis cases experience significant visual loss (Sadaka et al., 2012). In the most severe cases, enucleation of the globe may be necessary. Endophthalmitis is divided into two general groups on the basis of the origin of infection. Exogenous endophthalmitis occurs as pathogens are introduced into the eye during ocular surgery (post-operative) or by accidental penetrating trauma to the eye (post-traumatic). Endogenous endophthalmitis occurs when pathogens migrate from an infection elsewhere in the body by way of the bloodstream and enter the interior of the eye by crossing the blood-retina barrier (Jackson et al., 2014).

1.1 Post-Operative Endophthalmitis

Endophthalmitis resulting from ocular surgery (post-operative endophthalmitis) is the most common source of intraocular infection. This category of exogenous endophthalmitis has an incidence of 0.01% to 0.1% of ocular surgeries (Krause et al., 2009). While this rate is low, in the developed world, the frequencies of intraocular procedures such as lens replacements and cataract removal are increasing (Brian and Taylor, 2001; West et al., 2005). The primary infectious complication from cataract surgery is endophthalmitis, with incidences of infection ranging from 0.01-0.3% (Endophthalmitis Study Group, European Society of Cataract and Refractive Surgeons, 2007; Taban et al., 2005). Intravitreal injections are also being performed with greater frequency for the treatment of neovascular disorders and inflammation (Peyman et al., 2009). In the United States, the number of intravitreal injections has increased from 4,215 in 2001 to 2.5 million in 2011, with similar increases reported for Canada and the United Kingdom (Merani and Hunyor, 2015). The rate of injection-related complications has also increased, with endophthalmitis incidences after intravitreal injection reported to be 0.006-0.16% per injection and 0.7-1.3% per treatment period (Diago et al., 2009; Klein et al., 2009; Sampat and Garg 2010; VEGF Inhibition Study in Ocular Neovascularization (V.I.S.I.O.N.) Clinical Trial Group et al., 2006).

Endophthalmitis cases which develop from these various sources of infection differ in the types of infecting organisms, the clinical course of disease, and the visual outcome. Acute post-operative endophthalmitis, which typically develops within six weeks of surgery, is most commonly caused by coagulase-negative staphylococci, such as *Staphylococcus epidermidis*. These organisms are relatively avirulent (i.e. do not produce toxins and/or are not invasive), but endophthalmitis should always be viewed as a serious development. Most of these cases have favorable treatment outcomes (Bhagat et al., 2011). Eighty-four percent of post-operative endophthalmitis cases resulted in at least 20/100 visual acuity, while approximately half of the cases resulted in 20/40 visual acuity (Josephberg, 2006). Chronic post-operative endophthalmitis can develop six weeks or more after surgery. Chronic cases of post-operative endophthalmitis are commonly caused by *Propionibacterium acnes*. The

treatment of infections with this organism is sometimes difficult because of misdiagnosis and frequent recurrent inflammation (Winward et al., 1993). Despite the difficulties in treating chronic endophthalmitis, final visual outcomes can be 20/40 or better in 50% of patients (Clark et al., 1999), likely because of the low virulence of the infecting organism. Filtering bleb surgery-related endophthalmitis develops an average of 25 months following surgery and is commonly caused by *Staphylococcus aureus*, *S. epidermidis*, *Streptococcus* species, or *Haemophilus influenzae* (Waheed et al., 1998). Thirty-five percent of patients with bleb-associated endophthalmitis have no light perception 12 months after treatment (Busbee et al., 2004), as these infections are typically caused by more virulent pathogens.

1.2 Post-Traumatic Endophthalmitis

Post-traumatic endophthalmitis accounts for 25%-30% of the cases of exogenous endophthalmitis (Azad et al., 2003; Bhagat et al., 2011). Final visual outcomes depend on the type of injury, the virulence of the pathogen, and the interval between symptoms and treatment (Bhagat et al., 2011; Bhoomibunchoo et al., 2013; Lieb et al., 2003; Parrish and O'Day, 1987). Bacteria are the most common cause of post-traumatic endophthalmitis, but fungi can also be isolated, *Candida* species being the most common isolate (Bhagat et al., 2011). In their analysis of six studies containing 91 eyes that developed post-traumatic endophthalmitis, Parrish and O'Day (1987) reported that in 24 eyes (26%) infected with *S. epidermidis*, 50% of these eyes had a final visual outcome of 20/400 and no eyes were enucleated. In stark contrast, *Bacillus* isolated from seventeen eyes (18.7%), nine of these eyes were enucleated, and one had a final visual acuity of 20/400. Seven eyes (7.7%) harbored Gram-negative bacteria, with three enucleations and the other four with final visual acuities of 20/200 or better. Parrish and O'Day (1987) commented that with better antibiotics and vitrectomy, traumatic endophthalmitis had become a more manageable disease, "However, the overall prognosis for useful vision remains poor." Bhagat et al. (2011), in their more recent review of the current treatments of traumatic bacterial endophthalmitis, observed that "The visual prognosis in traumatized eyes with endophthalmitis is extremely poor." The combination of traumatic damage to the eye and infection by virulent pathogens likely contributes to the poor outcome of many of these cases.

1.3 Endogenous Endophthalmitis

Endogenous bacterial endophthalmitis (EBE) is relatively rare, comprising 2-10% of all cases of bacterial endophthalmitis (Ness et al., 2007). Fungi can also cause endogenous endophthalmitis, with *Candida* sp. and *Aspergillus* sp. being the most common isolates (Demant and Easterbrook, 1977; Khan et al., 2007; Shah et al., 2008). In East Asia, the most commonly isolated bacteria are Gram-negative, 90% of which are *Klebsiella* spp. (Wong et al., 2000). The most common sources of *Klebsiella* in EBE cases are hepatobiliary infections (67%) and pulmonary and urinary tract infections, although the primary site of infection is not always identified. Diabetes has been identified as a major risk factor for EBE (Nishida et al., 2015; Wong et al., 2000). In Europe, *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *S. aureus* were the most commonly reported isolates (Jackson et al., 2014). In North America, the most frequently isolated bacteria were *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and Group B streptococci (Jackson, et al., 2014). EBE caused by *Streptococcus*

and *Staphylococcus* are more often associated with systemic infections such as endocarditis, meningitis, and abscesses, and common risk factors include intravenous drug abuse, diabetes, malignancies and cancer (Ness et al., 2007). Jackson et al. (2014) reported that the primary focus of infection was not identified in 64% of patients. EBE is frequently misdiagnosed as non-infectious uveitis (Jackson et al., 2014). Jackson et al., in their recent review of the EBE literature since 1986, reported that 31% of EBE patients had a final visual acuity of 20/200 or better, 44% had a final visual acuity worse than 20/200, 24% required enucleation or evisceration of the infected eye, and 4% died (Jackson et al., 2014). The authors stated that according to the literature, there have been improvements in the outcome of EBE over the last decade, but the loss of vision and number of enucleations in these cases is still not satisfactory (Jackson et al., 2014).

1.4 Modeling Endophthalmitis

Endophthalmitis is a blinding disease. As the reports above suggest, there has been some progress in improving the prognosis of EBE, but good visual prognoses following severe post-traumatic infections have not improved to an appreciable extent. Experimental data which can be used to guide the treatment of post-traumatic endophthalmitis is limited, and treatments are typically based on retrospective case studies (Bhagat et al., 2011). There are no universal therapeutic regimens recommended for treatment of these infections, regardless of their origin or infecting organisms. Experimental data dealing with EBE is especially lacking, likely due to the difficulty of developing animal models of EBE. For endophthalmitis, experimental animal models of infection provide a useful tool for understanding the underlying mechanisms of disease based on the source of infection and the type of pathogen involved. Although progress has been made using information from clinical studies, experimental models can more quickly and reproducibly test therapies and provide data that can be translated to improvements in standard of care, resulting in better visual outcome for patients. The goals of this review are to highlight experimental animal models of bacterial endophthalmitis, to correlate experimental findings with the clinical setting, and to identify knowledge gaps that may be addressed in future experimental and clinical studies focused on improvements in the therapeutic preservation of vision during and after endophthalmitis.

We note above that a significant number of endophthalmitis cases are caused by *Candida*, especially in EBE cases in immunocompromised individuals. As of this writing, more than forty references are available regarding the therapeutics in rabbit models of *Candida* endophthalmitis. We refer the reader to the following references for more information on experimental animal models of *Candida* endophthalmitis (Papadimitriou et al., 1986; Omuta et al., 2007; Malecase F et al., 1988; Demant and Easterbrook, 1977).

2. EXPERIMENTAL MODELS: THERAPEUTICS AND VIRULENCE

2.1 Primates

To date, only a single study has reported analysis of bacterial endophthalmitis in primates. Bayer et al. (1985) analyzed the importance of the posterior capsule in the risk for developing *S. aureus* endophthalmitis. At the time, there was a concern that the rising

frequency of extracapsular cataract surgeries would result in an increase in the incidence of bacterial endophthalmitis. Adult rhesus and cynomolgus primates were subjected to extracapsular extraction in both eyes, with one eye subjected to a posterior capsulectomy and the posterior capsule of the other eye left intact. In a similar experiment, the posterior capsule of one eye was left intact, but the fellow eye received a posterior capsulectomy and an implanted intraocular lens. Groups were challenged with 10^4 colony forming units (CFU)/0.05 mL *S. aureus*. In both experiments, eyes with posterior capsulectomies developed *S. aureus* endophthalmitis, while eyes with intact capsules did not. These results demonstrated the importance of an intact posterior capsule in maintaining a barrier from spread of organisms from the anterior segment into the posterior segment. This was an important and clinically relevant finding in terms of the importance of retaining the posterior capsule to reducing the risk of endophthalmitis.

2.2 Rabbits

The rabbit model of experimental endophthalmitis has been used to study treatment and surgical efficacies and the interactions of host and bacterial factors during intraocular infection and inflammation. Rabbit eyes are much closer in size to human eyes than they are to rat or mouse eyes, allowing intravitreal injections of organisms to be performed more easily and without the need for special equipment. However, the lack of defined genetic knockout strains for experimentation and the insufficient number of immunological reagents for studying the immune response in rabbits limit their usefulness as a model for dissecting the inflammatory response during disease. By injecting precise numbers of organisms directly into the posterior segment or into the anterior chamber, researchers are able to mimic penetrating injury or accidental inoculation during a surgical procedure. In rabbits and other mammals, intravitreal injection of bacteria across the pars plana typically utilizes an inocula whose growth and infection course mirror that of human cases. Pathogenic organisms are injected at low numbers of colony forming units (10-1000 CFU), while avirulent organisms are injected at greater numbers (10^5 CFU or greater). At these inocula, bacteria are able to adapt to the intraocular environment and initiate infection, even in the face of an incoming inflammatory response. Highly reproducible experimental models of bacterial endophthalmitis that model human disease have been established. Aspects of experimental endophthalmitis that have been studied in rabbits include bacterial growth and intraocular tissue distribution, gross ocular inflammation and pathologic changes, and retinal function.

2.2.1 Therapeutics—Picot first utilized the rabbit eye as a model for endophthalmitis in 1898, when he injected “microorganisms” into the anterior chamber (Picot, 1898). Von Sallman used this model to examine the usefulness of topical penicillin in the treatment of *S. pneumoniae* endophthalmitis (1943) and pioneered the technique of intravitreal injection of antibiotics for treating *S. aureus* endophthalmitis (1944a, 1944b). Maylath and Leopold (1955) used rabbits to develop a method for sampling ocular fluids to identify pathogens and determine antibiotic sensitivity. Tucker and Forster (1972) used the rabbit model to examine the usefulness of anterior chamber paracentesis to isolate organisms for diagnosing the cause of endophthalmitis. At that time, many ophthalmic surgeons felt this technique was “fruitless in delineating the responsible agent” (Tucker and Forster, 1972).

In 1975, Peyman et al. reported the non-toxic therapeutic dosages and clearance curves for fourteen antibiotics (such as amikacin, clindamycin, gentamicin and vancomycin), the antifungal agent amphotericin B, and dexamethasone, which were tested in an intravitreal injection model in rabbits. These antibiotics were also tested against endophthalmitis caused by *S. aureus*, *P. aeruginosa*, *Escherichia coli*, and *Proteus vulgaris*. The authors reported that intravitreal injection of antibiotics was safe and effective, provided that the drugs were used at the proper concentration with the proper injection method, and suggested that gentamicin offered “the best chance of a one dose, intravitreal treatment” for endophthalmitis (Peyman et al., 1975). However, the intravitreal injection of antibiotics for the treatment of bacterial endophthalmitis was considered too experimental for clinical use until the publication of the *Consensus* by Baum, Peyman, and Barza in 1982 (Baum et al., 1982).

Forster (1992) introduced an experimental post-operative endophthalmitis model using rabbits which had undergone extracapsular lens extractions with the posterior capsule left intact. This aphakic model provided reproducible histological and microbiological data following infection caused by *S. epidermidis*, *S. aureus*, *Enterococcus faecalis*, or *Bacillus cereus*. These comprehensive studies suggested that the *in vitro* behavior of these organisms may not accurately predict the microbiological outcome of experimental infections, and that the use of intravitreal vancomycin (1 mg) did not alter the outcome of infection. Alfaro et al. (1996a) and Wiskur et al. (2008b) used similar rabbit models to examine post-traumatic *B. cereus* endophthalmitis and treatment with intravitreal fluoroquinolones. These rabbit models closely mimicked human disease, and intravitreal fluoroquinolones prevented development of the disease if given within a few hours of infection. Alfaro et al. (1996b) also reported that in a rabbit model with surgically induced scleral injury, systemic ciprofloxacin (30 mg) reached vitreous levels in excess of the minimum inhibitory concentrations for common ocular pathogens in traumatized rabbit eyes. These earlier studies in the rabbit paved the way for pathogenesis and therapeutic efficacy studies utilizing more in-depth analysis of retinal function and inflammatory changes during evolving infection.

A different model of intraocular infection initiated by injection of bacteria into the anterior chamber was developed to test the penetration of topically administered antibiotics and prevention of intraocular infection (Kowalski et al., 2004). This model was used to test the efficacies of a number of antibiotics (including moxifloxacin, ofloxacin, levofloxacin, polymyxin B/trimethoprim, chloramphenicol, gentamicin, and povidone-iodine) in preventing *S. aureus* endophthalmitis (Kowalski et al., 2008; Kowalski et al., 2012). In general, moxifloxacin and ofloxacin were more successful in preventing *S. aureus* endophthalmitis compared to the other antibiotics tested. Although the inoculum used in this model was high (10^5 CFU) compared to inocula used in intravitreal injection models of *S. aureus* endophthalmitis, this model may mimic accidental anterior chamber contamination during ocular surgery and is useful for testing the ability of antibiotics administered at the ocular surface in eliminating these organisms.

2.2.2 Bacterial Virulence—The experimental rabbit model has been used to determine the contribution of various bacterial factors to the pathology of bacterial endophthalmitis. The majority of studies on virulence factors utilize bacterial mutants which are deficient in

one or more secreted proteins or exterior surface-bound components. A comparative study examined the rates of intraocular growth, tissue localization, and contributions of cell walls and secreted products to the pathogenesis of endophthalmitis resulting from intravitreal injections of *B. cereus*, *E. faecalis*, and *S. aureus* (Callegan et al., 1999a). After assessing the eyes at various time points, it was discovered that *B. cereus* grew more rapidly and caused greater inflammation at a faster rate in the vitreous than did *S. aureus* and *E. faecalis*. Uniquely, *B. cereus* migrated to the anterior segment during infection, while *S. aureus* and *E. faecalis* did not. While injection of metabolically inactive bacteria or cell wall sacculi of these pathogens did not negatively affect retinal function, these preparations did stimulate significant inflammation (Callegan et al., 1999a). These comparative studies demonstrated that infection with different bacterial pathogens can result in varying infection courses, which is important to consider when devising a therapeutic strategy.

2.2.2.1. Bacillus: *B. cereus* is one of the most destructive pathogens for the eye due to its rapid intraocular growth rate, migration within the eye, and plethora of toxins synthesized during infection. Beecher et al. (1995) examined the role of *B. cereus* toxins in endophthalmitis by intravitreally injecting purified hemolysin BL (HBL), a tripartite dermonecrotic toxin which causes vascular permeability, and crude exotoxin into rabbit eyes. Both HBL and the crude exotoxin produced intraocular inflammation characteristic of that caused by *B. cereus*, suggesting a role for HBL and other toxins in *B. cereus* endophthalmitis. However, after testing an isogenic mutant of *B. cereus* deficient in HBL, Callegan et al. (1999b) reported that HBL alone did not play a significant role in pathogenesis in this endophthalmitis model. Following injection of purified HBL, phosphatidylcholine-specific phospholipase C (PC-PLC), or collagenase, Beecher et al. (2000) reported that these toxins were important in *B. cereus* endophthalmitis. Further studies using isogenic mutants of the *B. cereus* toxins phosphatidylinositol-specific phospholipase C (PI-PLC) or PC-PLC, Callegan et al. (2002a) discounted an individual role for these toxins in experimental endophthalmitis. However, a cohort of toxins under control of the *plcR* global regulator (which include HBL, PI-PLC, and PC-PLC) were found to be involved in the pathogenesis of *B. cereus* and *Bacillus thuringiensis* endophthalmitis, as *plcR*-deficient mutants which did not synthesize these toxins lacked significant intraocular virulence (Callegan et al., 2003a). These studies suggested that targeting the global regulation of toxin production may hamper *B. cereus* virulence in the eye and illustrated the value of using genetic knockouts rather than injecting toxins for the study of their role in endophthalmitis.

To date, *Bacillus* has been the only organism shown to migrate throughout intraocular tissues during experimental endophthalmitis (Callegan et al., 1999a). To determine whether migration throughout the eye contributed to virulence, isogenic motile and nonmotile *Bacillus* were tested in the rabbit model. Mutant nonmotile *B. thuringiensis* were significantly less virulent than wild-type motile *B. thuringiensis* (Callegan et al., 2005). Loss of the swarming phenotype in *B. cereus* had only a modest effect on retinal function or on the overall course of endophthalmitis, but did result in less severe anterior segment disease (Callegan et al., 2006). Other motile ocular pathogens such as *Pseudomonas* or *E. coli* may also carry this virulence trait in endophthalmitis, but this has not been determined. These

studies suggested that targeting the motility phenotype may reduce the intraocular virulence of *B. cereus*.

2.2.2.2. Staphylococcus aureus: The experimental rabbit model has been used to analyze the intraocular virulence of *S. aureus*. Booth et al. (1995) reported that eyes intravitreally injected with a mutant lacking a functional accessory gene regulator (*agr*) were attenuated in the onset of retinal damage and clinical symptoms of endophthalmitis. Comparative studies testing a *S. aureus* mutant lacking a functional staphylococcal accessory regulator (*sar*) did not show a reduction in retinal response significantly different from that of infection with the parental strain. However, eyes infected with a double mutant deficient in *agr* and *sar* retained 73% of their B-wave amplitude at post-infection day five, indicating a synergistic effect between these two global regulators of toxin production (Booth et al., 1997). Other studies with *S. aureus* have reported that α -toxin and β -toxin, but not γ -toxin, play important roles in the pathogenesis of endophthalmitis (Callegan et al., 2002b). Inactivation of these three staphylococcal toxins resulted in a cumulative positive effect on retention of retinal function (Callegan et al., 2002b). Intravitreal injections of metabolically inactive *S. aureus* and purified *S. aureus* cell wall sacculi resulted in significant posterior and anterior segment inflammation, but did not affect retinal function (Callegan et al., 1999a). Metabolically inactive *S. aureus* were more inflammogenic than purified sacculi, indicating that cell wall-associated structures were necessary for significant intraocular inflammation (Callegan et al., 1999a).

2.2.2.3. Enterococci and Streptococci: Experimental rabbit models have been used to analyze the virulence of enterococci and streptococci, two pathogens associated with post-operative endophthalmitis. Stevens et al. (1992) demonstrated that intravitreal injection of an *E. faecalis* harboring a hemolysin-encoding plasmid resulted in a 98% loss of retinal function and no red reflex by post-operative day three, while infection with the isogenic plasmid-free strain caused a 77% loss of retinal function but a retained red reflex during the same time course. Jett et al. (1992) examined the course of experimental *E. faecalis* endophthalmitis in the rabbit and demonstrated that the biocomponent cytolysin Cyl played an important role in the course of the disease. When a cytolytic strain was tested, toxicity to the neural retina was not reduced by antibiotic and/or anti-inflammatory treatment. Using the same model, Mylonakis et al. (2002) demonstrated that deletion of *fsrB*, a gene required for a functioning Fsr quorum-sensing system, resulted in reduced endophthalmitis virulence. Engelbert et al. (2004a) reported that *E. faecalis* serine protease and gelatinase were involved in the progression of the disease. Callegan et al. (1999a) used the *E. faecalis* model to study the role of bacterial cell walls in inflammation. Injection of metabolically inactive *E. faecalis* or cell wall sacculi into the posterior segment caused significant inflammation in a manner similar to that of *S. aureus* cell wall sacculi.

Sanders et al. (2011) demonstrated the virulence potential of the *S. pneumoniae* capsule by injecting an isogenic unencapsulated strain or its wild type parental strain into rabbit eyes. Bacterial capsules cloak organisms from the immune response and, as such, are anti-phagocytic. Significantly higher slit lamp examination scores, reduced retinal function, and increased neutrophil infiltration into the vitreous of rabbits injected with the encapsulated

wild type strain compared with that of the nonencapsulated mutant strain indicated a role for the capsule in the pathogenesis of *S. pneumoniae* endophthalmitis. Strains of *S. pneumoniae* with high production of the toxin pneumolysin produced more severe endophthalmitis in rabbits than strains which were low pneumolysin producers (Sanders et al., 2008). Analysis of differential *S. pneumoniae* gene expression of endophthalmitis discovered increased expression of 112 genes. Of interest were neuraminidase A, neuraminidase B, and serine protease. No significant attenuation of virulence of neuraminidase-deficient mutants was found, but a deficiency in serine protease resulted in increased adherence of *S. pneumoniae* to host cells (Thornton et al., 2015).

2.2.2.4. Targeting Toxins: The value of the rabbit studies described earlier lies in the identification of toxins and other virulence factors important to intraocular virulence. Currently, no therapeutic strategies involve toxin blockade or neutralization. Two similar studies (Han, 2004; Perkins et al., 2004) tested whether human IgG could change the outcome of experimental *S. aureus* endophthalmitis. Han (2004) reported that 0.5, 2.5, 10 or 30 mg/mL human IgG intravitreally injected into rabbit eyes, simultaneously with culture supernatant, resulted in less severe disease. Perkins et al. (2004) reported that the effects of intravitreally injected concentrated *S. aureus* culture supernatant were attenuated by immediately injecting pooled human immunoglobulin at the site of infection. The mechanisms underlying this effect were not reported. Based on the report of pneumolysin as an important virulence determinant of *S. pneumoniae* endophthalmitis in rats (Ng et al., 1997; Ng et al., 2002), Sanders et al. (2010) used the rabbit model to demonstrate the efficacy of immunizing with pneumolysin in protecting the retina from damage during *S. pneumoniae* endophthalmitis. Although these neutralization strategies are not currently used in therapy, these studies further support the importance of bacterial toxins as potential targets for the treatment of endophthalmitis.

2.3 Swine

Experimental models of endophthalmitis in swine were used primarily for analysis of antibiotic efficacy. The swine model was used by Alfaro et al. (1997) to examine the consequence of trauma on the penetration of intravenous ciprofloxacin into the eye. As in the rabbit model (Alfaro et al., 1996b), surgical trauma increased ciprofloxacin concentrations in the vitreous and the concentrations exceeded the minimum inhibitory concentrations for common ocular pathogens (Alfaro et al., 1996b). Alfaro et al. (1997) used Yorkshire pigs to study the effectiveness of ceftazidime, amikacin, and imipenem for post-traumatic *Pseudomonas* endophthalmitis. The animals received surgically induced scleral injury and were injected with 22,000 CFU of *P. aeruginosa*. Animals receiving intravitreal amikacin or imipenem had significantly reduced posterior segment inflammation compared to untreated control animals. Schech et al. (1997) used the swine traumatic ocular injury model to determine the penetration of systemic gentamicin and ceftazidime. Ceftazidime reached vitreous concentrations 33 times the minimum inhibitory concentration for *Haemophilus* and twice that of *Pseudomonas*. However, gentamicin did not achieve therapeutic concentrations in the traumatized eyes. In the context of animal models of endophthalmitis, the swine model appears to have been the least developed or used.

2.4 Guinea Pigs

The experimental guinea pig model of endophthalmitis was one of the first models used to investigate the immune response to antigen challenge and infection. Silverstein and Zimmerman (1959) examined delayed hypersensitivity endophthalmitis in guinea pigs by intraperitoneally injecting egg albumin, bovine serum albumin, or the bacillus of Calmette and Guérin (BCG) tuberculin agent, and challenging with intravitreal injections of the same antigens. At fifteen minutes post-challenge, dilation of the limbal vessels and a haziness of the vitreous occurred, and at 60 minutes polymorphonuclear cells (PMN) entered the peripheral cornea. At 24 hours post-challenge, injected eyes developed severe corneal inflammation, chemosis, and a cloudy vitreous containing fibrin strands and cells.

Aizuss et al. (1985) used the guinea pig model to examine the role of complement in host defense against bacterial endophthalmitis. Guinea pigs were decompemented using cobra venom factor, followed by intravitreal injection of 42, 102, or 150 CFU of *P. aeruginosa*. At one, two, and three days post-injection, there were greater numbers of bacteria in eyes injected with 42 *P. aeruginosa*, but by day seven there were no differences between experimental or control eyes, as the complement levels returned to normal. Eyes injected with 102 *P. aeruginosa* had significantly greater CFU on day one post-injection, while eyes injected with 150 bacteria had similar bacterial counts on all days. The authors concluded that complement played a role in intraocular defense, but higher bacterial loads obscured the beneficial effects. Giese et al. (1994) examined the role of complement in ocular immunity by injecting 50 viable *S. aureus* or 7000 viable *S. epidermidis* into the vitreous of cobra venom factor-decompemented guinea pigs. Bacterial loads of both organisms were increased over a three-day period in decompemented eyes, but by day seven, there were no differences in bacterial counts as complement returned to baseline levels. These results further suggested a role for complement in intraocular immunity during the earlier stages of endophthalmitis. Later work conducted in complement component C3-deficient knockout mice did not support these findings, as discussed in section 2.6.1. This illustrates not only the challenge of modeling infections in animals, but also demonstrates how the use of more sophisticated genetic models can change the perceptions of previous data.

Although the guinea pig model was sufficient for cause-and-effect studies of the immune response to intraocular infection, the generation of rat and mouse models, the wide variety of rodent immunological agents, and the development of genetically-modified mice deficient in different components of the immune system led to a decline in use of the guinea pig as a model for endophthalmitis.

2.5 Rats

Various aspects of *Staphylococcus* endophthalmitis have been examined using rats. Ravindranath et al. (1997) reported that in experimental *S. epidermidis* endophthalmitis, natural antibodies against cell wall components glycerol teichoic acid and ribitol teichoic acid in vitreous may contribute to neutrophil-mediated opsonophagocytosis and clearance of bacteria. In experimental *S. aureus* endophthalmitis, Giese et al. (1998) observed that levels of tumor necrosis factor- α (TNF α), interleukin-1 β (IL1 β), cytokine-induced neutrophil chemoattractant (CINC), and interferon- γ (IFN γ) peaked at 24 hours post-infection.

Petropoulos et al. (2006) reported that increased vitreous levels of TNF α , IL-1 β and IFN γ correlated with the evolving clinical presentation in experimental *S. epidermidis* endophthalmitis. Giese et al. (2000) reported that cellular adhesins E-selectin and ICAM-1 were more intensely expressed in iris, ciliary body, choroid, and retina in response to the ribitol teichoic acid and peptidoglycan of *S. aureus* than in tissues of control eyes. Pharmakakis et al. (2008) observed that intravitreal injection of *S. epidermidis* caused increases in Bax and Fas expression in the rat retina peaking 24 hours and a subsequent increase in the number of apoptotic cells at 72 hours postinjection. The use of neutrophil-depleting antibody in the rat model impeded the onset of intraocular inflammation, but also prevented *S. aureus* clearance (Giese et al., 2003). The use of the rat in endophthalmitis research has declined, likely because of the availability of genetically-altered mouse strains.

2.6 Mice

The mouse model of experimental endophthalmitis shares many of the advantages of the rabbit model of endophthalmitis. For the most part, the numbers of organisms injected into mouse eyes are the same as those injected into rabbit eyes (albeit in much lower volumes), resulting in similar courses of infection. The parameters of infection can be studied qualitatively and quantitatively by examining bacterial growth, ocular inflammation, and retinal function. Semi-quantitation of ocular inflammation can be examined by histopathology and slit-lamp biomicroscopy. Further analysis of inflammation is achieved by quantifying myeloperoxidase in infiltrating inflammatory cells or by flow cytometry of specific cell types, and quantifying inflammatory mediators by real-time PCR and/or ELISA. The value of the mouse model of endophthalmitis is in the multitude of genetic knockout mouse strains available. By comparing the courses of infection between wild type strains and genetically-deficient strains of mice, the role of various immune factors and cells can be examined. However, because the vitreous volume of the mouse eye is approximately 7 μ L (Yu and Cringle, 2006), only a limited amount of material can be injected, and specialized equipment and techniques are needed to achieve reproducible infection.

2.6.1 Complement and Fas ligand—The power of knockout mouse strains to elucidate the role of specific immune factors was illustrated by the study of Engelbert and Gilmore (2005) on the contribution of complement and Fas ligand (FasL) to the innate immune response to *S. aureus* endophthalmitis. Previous work in cobra venom factor-decomplemented guinea pigs implied a role for complement in clearing experimental *S. aureus* endophthalmitis (Aizuss et al., 1985; Giese et al., 1994). Experimental *S. aureus* endophthalmitis in complement component C3-deficient knockout mice followed a similar infection course as did infection in the wild type background strain, indicating that complement did not contribute to the immune response to *S. aureus* endophthalmitis. However, *S. aureus* endophthalmitis in FasL-deficient knockout mice followed a much more aggressive course than infection in wild type background mice which were able to clear the infection (Engelbert and Gilmore, 2005). Sugi et al. (2013) also reported that FasL was required for resolution of inflammation during experimental *S. aureus* endophthalmitis, but not for clearance of the bacteria.

2.6.2 Innate Immunity and the Cellular Response—Pathogenic organisms and their products are recognized by families of innate immune receptors, including Toll-like receptors (TLRs), and cytosolic RIG-I-like receptors (RLRs) and NOD-like receptors (NLRs), which initiate an acute immune response against the pathogen. Kumar et al. (2010) used the mouse model of *S. aureus* endophthalmitis to study the role of TLR2 in infection. Intraocular bacterial loads were decreased following pretreatment of mice with the TLR2 ligand Pam3Cys compared to that of untreated controls. In the *B. cereus* model of endophthalmitis, infected eyes of TLR2 knockout mice had decreased concentrations of proinflammatory cytokines and reduced infiltration of inflammatory cells into the posterior segment, resulting in reduced intraocular inflammation and limited destruction of retinal architecture as compared to wild type controls (Novosad et al., 2011). These studies indicated that TLR2 was important in the innate ocular response to these two Gram-positive pathogens. TLR5 was discounted as an important innate immune receptor in inflammation during *B. cereus* endophthalmitis. Despite the presence of flagella (a TLR5 ligand) on the surface of *B. cereus*, expected TLR5-flagellin interactions were not significant, and intraocular inflammation in TLR5-deficient mice infected with *B. cereus* was similar to that of wild type mice infected with *B. cereus* (Parkunan et al., 2014). Parkunan et al. (2015) reported that mice deficient in MyD88, the intracellular adapter molecule for several TLRs, had significantly delayed intraocular inflammation than wild type mouse eyes infected with *B. cereus*. Similar findings were reported for *S. aureus* in MyD88-deficient mice (Talreja et al., 2015). Surprisingly, deficiencies in Toll/IL-1 receptor domain-containing adapter-inducing interferon β (TRIF, the intracellular adaptor molecule for TLR4) and in TLR4 itself also resulted in significantly less intraocular inflammation during experimental *B. cereus* endophthalmitis, suggesting that MyD88-independent pathways were involved in the intraocular response to *B. cereus* and that *B. cereus* may harbor ligands for TLR4 which instigate intraocular inflammation (Parkunan et al., 2015). The importance of TLR4 in experimental *K. pneumoniae* endophthalmitis was demonstrated using TLR4-deficient mice (Hunt et al., 2014). The data on the course of infection in the *K. pneumoniae* endophthalmitis mouse model (Wiskur et al., 2008a) were cited by Ishii et al. as providing guidance in the successful treatment of a patient with *K. pneumoniae* endophthalmitis (Ishii et al., 2011), emphasizing the clinical value of these experimental models.

Because TLR-deficient mice eventually mounted an inflammatory response to *B. cereus* and *S. aureus*, other innate immune receptors may contribute to inflammation in their absence. One of these, the nucleotide-binding oligomerization domain-containing protein 2 (NOD2), recognizes muramyl dipeptide which is a component of the cell walls of Gram-positive and Gram-negative organisms. In the experimental endophthalmitis model, however, NOD2-deficient mice had a similar inflammatory response and retinal function decline during *B. cereus* infection as did wild type mice (Figure 1). Our findings of IL-1 β upregulation in *B. cereus* infected eyes led us to posit whether inflammasomes were involved in the acute response to intraocular bacteria. The caspase-1/nucleotide-binding domain, leucine-rich repeat protein 3 (NLRP3) inflammasome complex was reported to contribute to IL-1 β synthesis in the eye during endotoxin-induced uveitis (Rosenzweig, et al., 2012). In the experimental endophthalmitis model, however, NLRP3-deficient mice had a similar inflammatory response and retinal function decline during *B. cereus* endophthalmitis as did

wild type mice (Figure 1). While these results suggest that these pathways may not be involved in the intraocular responses to *B. cereus* infection, it does not rule out their involvement in endophthalmitis caused by other pathogens whose infection courses evolve more slowly.

Ramadan et al. (2008) reported a role for TNF α , a potent proinflammatory cytokine, in *B. cereus* endophthalmitis. In eyes of TNF α deficient mice, *B. cereus* replicated more quickly, retinal function declined more rapidly, and a lower number of PMN were recruited into the posterior segment compared with infections in the control mice (Ramadan et al., 2008). Whiston et al. (2008) also reported on the importance of α B-crystallin heat shock proteins in protection during endophthalmitis. The role of this chaperone-like protein in inhibition of apoptosis and retinal damage during experimental *S. aureus* endophthalmitis was demonstrated using mice deficient in α B-crystallin.

Polymorphonuclear neutrophils (PMN) have been identified as the earliest cell type to invade the posterior segment and retina during endophthalmitis caused by a number of species (Booth et al., 1995; Hunt et al., 2014; Jett et al., 1992; Ramadan et al., 2006; Sanders et al., 2010; Talreja et al., 2014). PMN infiltration coincided with permeability of the blood-ocular barrier which, in mice, occurred as early as four hours post-*Bacillus* infection (Moyer et al., 2009). PMN phagocytose and kill organisms in response to many different types of infections. The inflammatory response in the eye can often control bacterial growth of relatively avirulent pathogens, especially if the inoculum is low. The underlying reason for the inability of inflammatory cells to control the growth of more virulent pathogens in the eye has not been investigated, but may be due to either rapid growth which overwhelms the immune response or synthesized toxins which render these cells incapable of anti-bacterial activity. Whatever the case, transgenic and knockout mice deficient in several components of the immune system will be valuable in dissecting the complexities of the immune response to intraocular infection and identifying important host factors that can be targeted in anti-inflammation therapy.

2.6.3 Bacterial Surfaces—The outer envelope of the bacterium is the first entity to encounter the host immune response. Interest in bacterial peptidoglycan and lipopolysaccharide (LPS) as intraocular inflammogens has been studied primarily in the context of non-infectious uveitis in mouse models, utilizing many of the techniques used in the endophthalmitis model to analyze inflammation (Willermain et al., 2012). A comprehensive review of innate and adaptive immunity in non-infectious uveitis is available (Willermain et al., 2012). Components of the Gram-positive cell wall, such as teichoic acid and peptidoglycan, have been shown to induce an intraocular inflammatory response, to be essential for growth in vitreous humor, and to be essential to establish *S. aureus* endophthalmitis (Suzuki et al., 2011; Kumar and Kumar, 2015). Bacterial capsules were shown to be important in the virulence of *S. pneumoniae* endophthalmitis in rabbits (Sanders et al., 2011). Wiskur et al. (2008a) demonstrated that the hypermucoviscous (HMV) phenotype of *Klebsiella pneumoniae* was important to endophthalmitis pathogenesis in mice. Encapsulated *K. pneumoniae* which exhibit the HMV phenotype caused more severe endophthalmitis in mice than did non-HMV isolates. Hunt et al. (2011a, 2014) confirmed

these findings by demonstrating the decreased virulence of a non-HMV *magA*-deficient isogenic mutant of *K. pneumoniae* in the mouse model of endophthalmitis.

2.6.4. Bacterial Virulence—As reported above, the majority of studies on the importance of virulence factors in bacterial endophthalmitis were conducted in rabbit models. More recently, Sadaka et al. (2014) examined *S. aureus* metabolism in ocular fluids and the role of the bacterial transcription regulator CodY on virulence and metabolic genes in growth and virulence potential in an anterior chamber infection model. Metabolic pathways such as the sialic acid, ascorbate (vitamin C), and pseudouridine metabolism pathways were identified as potentially important for growth in ocular fluids. *S. aureus* virulence factors, such as toxic shock syndrome toxin, the Sec3 enterotoxin, and phenol soluble modulins were upregulated during growth in ocular fluids, suggesting their potential importance in intraocular infections. In the *S. aureus* anterior chamber infection model, an absence of CodY repression of target genes resulted in greater ocular inflammation and destruction of the retina than that caused by *S. aureus* containing functional CodY repression. These results were the first to associate metabolic signals with virulence in bacterial endophthalmitis, suggesting that bacterial metabolic repression may be a viable target in pre-empting infection. Kumar and Kumar (2015) reported that injection of purified staphylococcal protein A and α -toxin caused significant reductions in retinal function and dose-dependent increases in IL-1 β , TNF α , IL-6, MIP2 and CXCL1. As mentioned earlier, injection of *B. cereus* toxins into rabbit eyes caused retinal necrosis (Beecher et al. 1995), but the genetic absence of singular toxins did not alter infection virulence (Callegan et al. 1999b; Callegan et al., 2002a). In the case of *S. aureus*, the genetic absence of α -toxin significantly attenuated virulence in rabbits (Callegan et al. 2002b). Although the intravitreal injection of purified toxins and other factors resulted in endophthalmitis-like detrimental changes, the physiological relevance of injecting purified toxins into the eye (or other organs) should be interpreted with caution because the actual concentrations of specific bacterial toxins in the eye during the course of endophthalmitis have not been reported.

2.6.5 Endogenous Endophthalmitis—As mentioned earlier, information regarding the pathogenic mechanisms underlying endogenous bacterial endophthalmitis (EBE) is very limited. We developed the first mouse model of EBE which mimics an infection originating in the bloodstream that migrates across blood-ocular barriers and into the eye (Coburn et al., 2012). Because diabetes underlies a large number of EBE cases, diabetic mice were used to establish the EBE model. C57BL/6J mice were rendered diabetic using streptozotocin for one, three, or five months to simulate increases in vascular permeability observed in human diabetic patients. Age-matched diabetic and nondiabetic mice were intravenously injected with 10^8 CFU of *K. pneumoniae*, the most common Gram-negative cause of EBE. No cases of endophthalmitis were detected in one-month diabetic mice. In the three-month diabetic mice, there was a 23.8% incidence of endophthalmitis. In the five-month diabetic mice, the incidence of endophthalmitis was 27%. Infection incidences correlated with retinal vascular permeability. These results indicated that for *K. pneumoniae*, retinal vascular permeability facilitated EBE (Coburn et al., 2012). Streptozotocin-induced diabetic mice were also used to determine whether retinal vascular permeability facilitated EBE caused by *S. aureus*. In contrast to infections with *K. pneumoniae*, *S. aureus* caused EBE in both control and

diabetic mice with impermeable retinal vasculature, suggesting that *S. aureus* could induce EBE on its own (Coburn et al., 2015). *S. aureus* secretes a number of cytotoxins which could damage cellular barriers, and *S. aureus* infection resulted in disruption of an *in vitro* retinal pigment epithelial barrier (Coburn et al., 2015). Other pathogens such as *B. cereus* and *E. coli* frequently cause EBE, and characterization of these models is in progress. The use of bioluminescent organisms in these models facilitates longitudinal analysis of experimental EBE (Figure 2), with biophotonic imaging of organism migration into the eye as a means of detection without having to harvest tissue at various time points. These models of EBE will be used to analyze mechanisms involved in pathogenesis as well as the testing of therapeutics to better treat the disease.

2.7 Summary

Animals have been used to model bacterial endophthalmitis, beginning with Picot in 1898, Rabbits have been widely used to study treatments, surgical techniques, and bacterial growth and virulence. Other animals, such as primates, swine, guinea pigs, and rats have also been used, but their use has been limited by the lack of immunological reagents and genetically manipulated strains. The power of the mouse model of infectious endophthalmitis is in the multitude of knockout mouse strains available to researchers and in the availability of diverse immunological reagents. Mouse models have allowed researchers to study endophthalmitis in a more quantitative manner than was possible using other species. Important to the development of improved therapeutics is an understanding of the timing and severity of intraocular changes occurring as these infections progress. As illustrated in Figure 3, experimental animal models which mimic human infection can provide this information, but the knowledge gained from these models must be balanced with the understanding that no animal can completely model the human eye. There are differences in the general structure of the rat and mouse eye compared to the human eye (Remtulla and Hallett, 1985, Slijkerman, et al., 2015), and the inflammatory responses of rodents are known to differ from that of humans (Gaidt et al., 2016; Seok et al., 2013; Slijkerman et al., 2015). Combining this data with the use of non-invasive clinical procedures (electroretinography, funduscopy, optical coherence tomography, confocal microscopy, and optokinetic visual acuity testing) will provide a broader and more comprehensive picture of the mechanisms of function loss and inflammation which can be used to drive therapeutic decisions.

3. EXPERIMENTAL MODELS: BENCH TO BEDSIDE

3.1 Systemic Antibiotics

Following experiments in the rabbit endophthalmitis model, von Sallmann and Meyer (1944a) suggested that systemic antibiotics should be used for the treatment of bacterial endophthalmitis. However, the antibiotics available at the time (penicillin and streptomycin) did not penetrate into the eye from the bloodstream in therapeutically beneficial concentrations (Leopold, 1945; Leopold and Nichols, 1946; von Sallmann, 1947). In 1952, Leopold reviewed experimental rabbit endophthalmitis studies and concluded that antibiotics could not penetrate into the vitreous, but still recommended the use of prophylactic systemic antibiotics because of potential “spill-over into the intraocular fluids” during surgery. In

1987, Parrish and O'Day recommended systemic antibiotic prophylaxis for the treatment of traumatic endophthalmitis even though "...we are unaware of any experimental evidence that supports the efficacy of systemic antibiotics in prophylaxis of endophthalmitis...." Some antibiotics have been shown experimentally to reach therapeutic concentrations in the vitreous of rabbits in the model of penetrating eye trauma: intravenous cefazolin (Alfaro et al., 1992), intravenous ciprofloxacin (Alfaro et al., 1996b), combined topical and oral ciprofloxacin (Ozturk et al., 1999), combined topical and oral ofloxacin (Ozturk et al., 2000), and intravenous imipenem (Engelbert et al., 2003). Although some antibiotics do reach therapeutic concentrations in the vitreous, it is a widely held opinion that the use of systemic antibiotics alone is not an effective strategy for the treatment of exogenous or endogenous bacterial endophthalmitis.

The Endophthalmitis Vitrectomy Study (EVS) reported no difference in final visual acuity or vitreous clarity with the use of supplemental intravenous ceftazidime and amikacin (Endophthalmitis Vitrectomy Study Group, 1995; Forster, 1995). However, those antibiotics may not have been the optimal drugs to use for systemic delivery into the eye. Ceftazidime has limited effects against *S. aureus* and *S. epidermidis* (Phillips et al., 1981), and amikacin does not dependably reach therapeutic concentrations in the vitreous (Kasbeer et al., 1975). In order to experimentally correlate clinical findings in the use of systemic antibiotics in the treatment of bacterial endophthalmitis, Engelbert et al. (2004b) compared intravitreal vancomycin and amikacin and intravenous imipenem in the treatment of experimental *S. aureus* endophthalmitis in the rabbit. Intravenous imipenem was previously shown to have excellent penetration into the vitreous and was effective against experimental *S. aureus* endophthalmitis (Engelbert et al., 2003). When administered at 24 hours post-infection, a single intravitreal injection of vancomycin and amikacin sterilized the vitreous and preserved the retina and intraocular structures. Intravenous imipenem combined with intravitreal antibiotics was no more effective than intravitreal antibiotics alone. Intravenous imipenem alone was no more effective than saline controls at preventing destruction of the retina or preventing infiltration of the choroid, retina, and vitreous by inflammatory cells, and only five of nine eyes were culture negative. The authors suggested that the lack of effectiveness of intravenous imipenem may be due to the delay reaching therapeutic levels in the vitreous (Engelbert et al., 2004b).

While Practices and Trends Survey of the American Society of Retina Specialists reported that 41% of respondents used systemic antibiotics "always or at least in selected cases" (Kuhn and Gini, 2005), experimental evidence supported the EVS conclusion that there was no therapeutic benefit to supplementing intravitreal antibiotics with intravenous antibiotics for exogenous endophthalmitis. Experimental evidence indicates that the delay in intravenous antibiotics reaching therapeutic concentrations may result in loss of visual acuity when this method of treatment is used alone for exogenous endophthalmitis, and the use of systemic antibiotics in addition to intravitreal antibiotics did not improve patient outcome for exogenous endophthalmitis. On the other hand, endogenous endophthalmitis is an indication of systemic infection and should be treated as such. For EBE patients, systemic antibiotics with intravitreal antibiotics are recommended (Jackson et al., 2014).

3.2 Intravitreal Antibiotics

As discussed earlier, von Sallmann and Meyer were the first to test systemic antibiotics for the treatment of bacterial endophthalmitis in the rabbit (1944a). However, poor results led to the testing of the intravitreal injection technique with penicillin as a treatment for bacterial endophthalmitis (von Sallmann et al., 1944b). The authors admitted that intravitreal injection was a radical step to take, but current therapies, "...fever treatment and shock therapy...did not as a rule prevent the loss of function in the diseased globes" (von Sallmann et al., 1944b). Although later experiments in rabbits showed that the technique had promise, clinical trials using intravitreal penicillin to treat endophthalmitis were disappointing, which led to this method being abandoned in the 1950s (Peyman et al., 1975). Vast improvements in intravitreal injection safety and drug efficacy have overcome those early fears, and intravitreal injection of antibiotics and other drugs is commonplace in the treatment of ocular diseases. For endophthalmitis, the general consensus is that administration of sufficient quantities of antibiotics into the vitreous at the site of infection is the most effective means of controlling intraocular bacterial growth (Bhagat et al., 2011; Busbee et al., 2006; Forster, 1995; Gentile et al., 2014; Lemley and Han, 2007). By directly injecting antibiotics into the vitreous humor, a therapeutic concentration can be more quickly achieved than by administering drugs via other routes such as systemic, topical, or subconjunctival (Baum et al., 1982; Peyman et al., 2009). Many antibiotics are safe and effective for intravitreal use at therapeutic concentrations (Bhagat et al., 2011; Lopez-Cabezas et al., 2010; Peyman et al., 1975). This is useful when the infectious agent is known. Because the infectious agent is typically not known upon patient presentation, initial treatment for bacterial endophthalmitis is usually empirical and broad-spectrum antibiotics are preferred.

3.2.1. Vancomycin and Ceftazidime—Current recommendations for initial therapy of endophthalmitis include the use of intravitreal vancomycin hydrochloride (1 mg/0.1 mL) and ceftazidime (2.25 mg/0.1 mL) (Bhagat et al., 2011; Bhoomibunchoo et al., 2013; Kuhn and Gini, 2005; Mittra and Mieler, 1999; Roth and Flynn, 1997), antibiotics which inhibit bacterial cell wall synthesis. Vancomycin provides good coverage against Gram-positive bacteria such as *Staphylococcus*, *Streptococcus*, *Enterococcus*, and *Bacillus* species, and has been shown in the rabbit model to be safe and effective with a single dose (Callegan et al., 2011; Forster, 1992; Park et al., 1999; Smith et al., 1997; Wiskur et al., 2008b; Wiskur et al., 2009). However, vancomycin is still considered a last line of defense against Gram-positive bacteria, because it is often one of the few antibiotics to which multidrug-resistant pathogens are susceptible (Benz et al., 2004). Vancomycin is ineffective against Gram-negative bacteria, so ceftazidime is used for coverage of these organisms. Gentile et al. (2014) reported that among endophthalmitis isolates collected from 1987 to 2011, 99.7% of Gram-positive isolates were susceptible to vancomycin and 91.5% of Gram-negative isolates were susceptible to ceftazidime. The development of vancomycin resistance in staphylococci and enterococci (Relham et al., 2015; Sadaka et al., 2012, Sadaka et al., 2015) and ceftazidime resistance in Enterobacteriaceae and *P. aeruginosa* (Pfaller et al., 2007) warrant the careful use of these antibiotics. *Acinetobacter* spp. is a rare cause of endophthalmitis (Roy et al., 2013), but these cases almost always have a poor anatomical and visual outcome.

Acinetobacter spp. are of increasing clinical concern due to their multidrug resistance profiles and ability to form antibiotic-resistant biofilms (Talreja et al., 2014).

3.2.2 Fluoroquinolones—Fourth-generation fluoroquinolones, such as gatifloxacin and moxifloxacin, are members of the C-8-methoxyfluoroquinolone group of antibiotics which inhibit DNA synthesis, and are the most common antibiotics used for the treatment of ocular infections. Fourth-generation fluoroquinolones have structural modifications that lessen the risk of resistance, have broad-spectrum activity against Gram-negative and Gram-positive bacteria, and can penetrate ocular tissues (Scoper, 2008). Newer fluoroquinolones, such as besifloxacin, have extended coverage against fluoroquinolone- and other multidrug-resistant staphylococci (Miller et al., 2013). Gatifloxacin has been reported to be as effective as vancomycin in the treatment of experimental *S. epidermidis* (Lee et al., 2008) and *B. cereus* endophthalmitis (Wiskur et al., 2008b). Moxifloxacin was also as effective as vancomycin in the treatment of experimental *S. aureus* endophthalmitis (Ermis et al., 2007). Moxifloxacin was effective in treating experimental *B. cereus* (Sakalar et al., 2011) and *S. epidermidis* (Ermis et al., 2005) endophthalmitis, and was beneficial when used prophylactically prior to intraocular infection with *E. faecalis* (Tasaka et al., 2013) and *S. aureus* (Kowalski et al., 2004).

Fluoroquinolone-resistant ocular isolates have been reported. Ciprofloxacin and levofloxacin resistance in *S. pneumoniae* (Eliopoulos, 2004; Low, 2004) and resistance to fourth-generation quinolones in *H. influenzae* (Nazir et al., 2004) have been reported. Gentile et al. (2014) reported that 71.0 % of Gram-positive endophthalmitis isolates were susceptible to ciprofloxacin, 65.2% were susceptible to levofloxacin, 78.4% were susceptible to gatifloxacin and 75.3% were susceptible to moxifloxacin. Gatifloxacin and moxifloxacin resistance in coagulase-negative staphylococci from eyes treated prophylactically (Deramo et al., 2006; Jensen et al., 2008; Moshirfar et al., 2007) or eyes with active infection have been reported (Miller et al., 2006; Schimel et al., 2012), as has the emergence of 8-methoxyfluoroquinolone resistance in *S. epidermidis* endophthalmitis isolates (Bispo et al., 2013). Increasing resistance of *E. faecalis* endophthalmitis isolates to fluoroquinolones and other antibiotics has also been recently reported (Kuriyan et al., 2014). Further emergence of resistance to these antibiotics will severely limit their use against ocular infections.

3.2.3 Aminoglycosides—Aminoglycosides such as gentamicin (Peyman et al., 1975) or tobramycin (Bennett and Peyman, 1974) have been injected into the vitreous for the treatment of experimental endophthalmitis caused by Gram-negative bacteria. Gentamicin caused retinal toxicity at concentrations of 80-600 μ g/mL (Conway et al., 1986 and Conway et al., 1989). However, Graham and Peyman (1974) reported that gentamicin was safe and effective at 40-50 μ g/mL, and this concentration has been tested in various rabbit models to treat endophthalmitis (Cottingham and Forster, 1976; Jett et al., 1995; Peyman et al., 1975). Gentamicin should be used cautiously because it has a narrow therapeutic range, and toxic reactions have been reported even when low doses were used and steps were taken to avoid dilution errors (Campochiaro and Lim, 1994). Aminoglycoside resistance in Gram-negative bacteria has been widely reported (Kotra et al., 2000), with resistant ocular isolates of *E. coli*, *K. pneumoniae*, and *P. aeruginosa* commonly encountered (Avent, et al., 2011; Kotra et

al., 2000; Mingeot-Leclercq et al., 1999). Gentile et al. (2014) reported a trend toward decreasing resistance to aminoglycosides in bacterial endophthalmitis isolates collected over the past 20 years.

3.2.4. Bacteriolytic Agents—Antibiotic resistance is an evolving threat to the successful treatment of all types of infections, including intraocular infections. Lytic enzymes which directly target and kill bacteria have been tested in experimental models of endophthalmitis. The staphylolytic endopeptidase staphylolysin, produced by *P. aeruginosa*, was effective in treating experimental methicillin resistant *S. aureus* endophthalmitis in the rat (Barequet et al., 2009). A single injection of staphylolysin given six hours post-infection reduced mean CFU counts threefold compared to controls. Two injections at six and 30 hours post-infection reduced the mean CFU counts by two orders of magnitude compared with untreated controls. Lysostaphin, a metalloendopeptidase which cleaves peptidoglycan pentaglycine crosslinks in staphylococcal cell walls, was effective in treating experimental coagulase-negative staphylococcal and *S. aureus* endophthalmitis (Balzli et al, 2010; McCormick et al., 2006; Dajcs et al., 2001).

A chimeric bacteriophage endolysin (a peptidoglycan hydrolase) was also effective in treating experimental *S. aureus* endophthalmitis. A single intravitreal injection of Ply187 significantly attenuated the development of *S. aureus* endophthalmitis in mouse eyes (Kumar et al., 2014). The staphylolysin and bacteriophage endolysin are highly specific to the *S. aureus* cell wall and as such, are only effective against this genus and would not affect other organisms which comprise the normal flora, which is a therapeutic advantage. Most bacterial species which cause ocular infections have at least one bacteriophage and associated phage lysin that could be developed and tested for ocular use (Fischetti 2010; Fischetti, 2011; Pastagia et al., 2013).

3.2.5 Summary—Intravitreal antibiotics are an essential component of an effective therapeutic strategy for sight-threatening cases of bacterial endophthalmitis. Injection of antibiotics allows a therapeutic level of antibiotic to be quickly established within the eye. Current recommendations for initial therapy of bacterial endophthalmitis include intravitreal injection of vancomycin hydrochloride (1 mg/0.1 mL) and ceftazidime (2.25 mg/0.1 mL). Other antibiotics, such as fluoroquinolones and aminoglycosides have been shown in experimental models to be effective for the treatment of bacterial endophthalmitis. The development of antibiotic resistance is a factor that should always be considered in the choice of antibiotic treatment.

3.3 Intravitreal Corticosteroids

Corticosteroids are synthetic glucocorticoids that decrease inflammation by targeting a number of inflammatory pathways, including interference of TLR signaling pathways and repression of the expression of proinflammatory cytokine genes (Coutinho and Chapman, 2011; Moynagh, 2003). The use of corticosteroids in treating endophthalmitis is not without controversy, with many strongly held opinions as to their effectiveness as intraocular anti-inflammatory agents. Since the inflammatory response caused by bacteria in the vitreous can continue for five to seven days after the organisms have been killed by antibiotics (Meredith,

1990), using corticosteroids with antibiotics is a logical step to control inflammation and prevent tissue damage by the host inflammatory response. Also, when inflammation is limited to the intraocular space, the use of intravitreal corticosteroids is justified and avoids the unpleasant side effects associated with systemic corticosteroid use (Bhagat et al., 2011). Despite this, there is no consensus as to whether intravitreal corticosteroids are of great benefit in the treatment of bacterial endophthalmitis (Bui and Carvounis, 2014).

3.3.1 Experimental Studies—Graham and Peyman (1975) reported that dexamethasone was safely injected into rabbit eyes at concentrations up to 400 μ g, and when used with gentamicin, reduced the inflammatory response to *P. aeruginosa* endophthalmitis. This strategy was effective if treatment was started by five hours post-infection. Using the rabbit model of *P. aeruginosa* endophthalmitis, Kim et al. (1996) reported that eyes treated with dexamethasone and ciprofloxacin at six hours post-infection provided no benefit over eyes treated with ciprofloxacin alone. When treatment was delayed to twelve hours post-infection, dexamethasone and ciprofloxacin treated eyes were culture positive while the ciprofloxacin treated eyes were culture negative. Meredith et al. (1990) reported that either systemic or intravitreal corticosteroids with antibiotics resulted in significantly reduced inflammation compared to treatment with cefazolin alone in the self-limiting *S. epidermidis* endophthalmitis rabbit model. However, when the experiment was repeated using *S. aureus*, systemic dexamethasone administration did not reduce inflammation compared to the untreated controls, and intravitreal dexamethasone resulted in increased inflammation and retinal necrosis (Meredith et al., 1996). In contrast, Yoshizumi et al. (1998) reported that in experimental *S. aureus* endophthalmitis, significantly greater ERG function and less inflammation occurred following treatment at 36 hours postinfection with dexamethasone and vancomycin compared to treatment with vancomycin alone. However, Ermis et al. reported that intravitreal dexamethasone and moxifloxacin administered at 24 hours post-infection did not reduce inflammation in experimental *S. epidermidis* endophthalmitis (2005) or *S. aureus* endophthalmitis (2007). Bucher et al. (2005) and Hosseini, et al. (2009) reported that the treatment of experimental *S. epidermidis* in rabbits using intravitreal triamcinolone acetonide and vancomycin resulted in attenuated clinical signs of inflammation and reduced tissue destruction compared to treatment with vancomycin alone.

Jett et al. (1995) intravitreally infected rabbits with non-cytolytic *E. faecalis* and reported that eyes treated with ampicillin, gentamicin, and dexamethasone experienced no significant reduction in retinal function, while eyes that received antibiotics alone showed a “precipitous decline in ERG responsiveness,” at five days post-infection. In contrast, when rabbit eyes were infected with cytolytic *E. faecalis*, there was a significant loss of retinal function by 24 hours post-infection in all treatment groups, regardless of whether dexamethasone was present or not (Jett et al., 1995). In contrast, Park et al. (1995) reported that rabbit eyes infected with *S. pneumoniae* had significantly less inflammation following treatment with intravitreal dexamethasone and vancomycin compared to treatment with vancomycin alone. Intravitreal dexamethasone was not able to attenuate intraocular inflammation caused by injections of sterile *B. cereus* cell culture supernatants containing multiple toxins (Pollack et al., 2004).

Wiskur et al. (2008b) also reported no therapeutic benefit in including intravitreal dexamethasone in the treatment of experimental *B. cereus* endophthalmitis in the rabbit, and at the six-hour time point, dexamethasone treatment was associated with a decrease in the intravitreal vancomycin concentration. Conversely, Sakalar et al. (2011) reported that intravitreal injection of dexamethasone and moxifloxacin resulted in less inflammation in experimental *B. cereus* endophthalmitis than use of the antibiotic alone. Smith et al. (1997) also reported a reduction in vancomycin concentrations in the dexamethasone treated rabbit eyes in a methicillin resistant *S. epidermidis* model, but did note that intravitreal dexamethasone and vancomycin treatment resulted in less inflammation than use of vancomycin alone. Intravitreal prednisolone was also tested by Wiskur et al. (2009) in a rabbit *B. cereus* endophthalmitis model with vancomycin or gatifloxacin, and no therapeutic benefit was reported.

3.3.2 Summary—The results from these experimental animal studies, summarized in Table 1, suggest that intravitreal corticosteroids may improve the clinical outcome of endophthalmitis caused by less pathogenic organisms, such as *S. epidermidis*, but these drugs may be of less benefit when used in the treatment of more aggressive pathogens such as *B. cereus*, *P. aeruginosa*, or *E. faecalis*. However, the lack of a standard course of therapy, the variety of pathogens used, and use of different combinations of antibiotics and steroids make any interpretation of these data challenging. The clinical reports on corticosteroid use for endophthalmitis varies widely as well, with as many studies reporting on the benefits of corticosteroid use (Albrecht et al., 2011; Das et al., 1999; Gan et al., 2005; Kuhn and Gini, 2005; Lopez-Cabezas et al., 2010) as there are reports that these drugs offer little or no therapeutic benefit (Bui and Carvounis, 2014; Das et al., 1999; Gaudio, 2004; Hall et al., 2008; Shah et al., 2000). Experimental studies using animals deficient in components of inflammation have demonstrated the importance of immune factors not targeted by corticosteroids. Therefore, clinical and experimental drugs which target other factors should be tested with antibiotics in these models to determine their adjunctive efficacy in limiting inflammation during endophthalmitis.

3.4 Vitrectomy

Vitrectomy is the surgical removal of intravitreal components in an effort to clear the visual axis and promote healing of intraocular tissues. The rationale for the therapeutic vitrectomy likens the procedure to draining an abscess (Bhagat et al., 2011; Forster, 1995; Peyman et al., 1975). In an infected eye, the infected vitreous contains living and dead bacteria, bacterial endotoxins and exotoxins, immune cells, and a witches' brew of inflammatory cytokines and chemokines and other toxic mediators which may damage delicate and nonregenerative retinal tissue (Kuhn and Gini, 2005). If these harmful factors are not removed, the retina will continue to be exposed to toxins and inflammatory products well after the posterior segment is sterilized by antibiotics (Callegan et al., 2002b, 2007; Meredith, 1999).

3.4.1 Experimental Studies—Peyman et al. (1975) used the rabbit model of *P. aeruginosa* endophthalmitis to evaluate the effectiveness of vitrectomy. Using a vitreous infusion solution of gentamicin sulfate (8 µg/mL) in normal saline, the authors reported that

vitrectomy with gentamicin was no better than treatment with intravitreal gentamicin (48 μ L/0.1 mL) alone. Cottingham and Forster (1976) reported that vitrectomy alone was not an effective treatment for *S. epidermidis* or *S. aureus* endophthalmitis in rabbits. Also, vitrectomy with gentamicin was no more effective than treatment with intravitreal gentamicin alone for treating *S. epidermidis* endophthalmitis. However, after 24 hours post-infection, vitrectomy with gentamicin was more effective than gentamicin alone for the treatment of *S. aureus* endophthalmitis. Talley et al. (1987) reported that vitrectomy with cefazolin and gentamicin resulted in significant vitreal clarity compared to use of antibiotics alone in the *S. aureus* endophthalmitis model. Forster (1992) tested vitrectomy with infused vancomycin in rabbit models of *E. faecalis*, *S. aureus*, *S. epidermidis*, and *B. cereus* endophthalmitis and reported that "...vancomycin with or without vitrectomy did not significantly alter the overall inflammatory response to these four endophthalmitis isolates." Callegan et al. (2011) tested the efficacy of vitrectomy and vancomycin in the rabbit model of *B. cereus* endophthalmitis and reported a therapeutic benefit only if the vitrectomy was performed at four hours post-infection, during the earliest stages of infection when inflammation was minimal. For experimental *B. cereus* endophthalmitis, vitrectomy with infused vancomycin performed at five or six hours post-infection had no better visual outcomes than treatment with intravitreal vancomycin alone.

3.4.2 Summary—The Endophthalmitis Vitrectomy Study (EVS) found no benefit from vitrectomy for the treatment of bacterial endophthalmitis, except in cases where visual acuity had worsened to light perception (Endophthalmitis Vitrectomy Study Group, 1995; Forster, 1995). Studies done in rabbits (summarized in Table 2) have had mixed results, with some reporting a benefit and others reporting no benefit for vitrectomy in the treatment of experimental bacterial endophthalmitis. The situation is complicated because of the lack of uniformity in the experimental protocols, the lack of quantitative measurements, and the variety of organisms used to evaluate the usefulness of this procedure. Because current vitrectomy technology offers increased safety and control over what was available to the EVS at the time, it has been suggested that the efficacy of vitrectomy in endophthalmitis be considered again (Kuhn and Gini, 2005).

4. CONCLUSIONS AND FUTURE DIRECTIONS

This review has examined the various animal models used to study bacterial endophthalmitis and discussed their advantages and disadvantages in modeling this blinding disease. Medical research relies on testing hypotheses and treatments in animal models because of the obvious ethical concerns in human testing. Some might suggest using cell culture models, but for eye research and endophthalmitis in particular, cell culture models are, at best, a crude facsimile of any part of the eye. Cell culture models cannot mimic the complexity of interactions between cells contributing to the biochemical process of vision or of immune cells recruited into the eye in response to infection.

The rabbit model of bacterial endophthalmitis has served the field well in the development of many current ocular techniques and treatments. However, to progress beyond saving the eye to saving vision, future studies will need to examine the dynamic host/pathogen interactions which evolve during and after the course of infection. The mouse model of

bacterial endophthalmitis, with its wealth of genetically-modified strains, will prove invaluable in resolving our understanding of the complicated interplay of host/pathogen responses. Also, by implementing retinal function and visual acuity testing, genomic and proteomic analysis, and high quality imaging, measurements of vision and the host response will become more quantitative and translatable to human infections. Novel biological therapeutics, such as siRNA, CRISPR/Cas9 gene editing, chemical inhibitors of components of the inflammatory response, monoclonal antibodies (Rodrigues et al., 2009), microspheres (Herrero-Vanrell et al., 2014), and nanoparticles (Conley and Naash, 2010; Diebold and Calonge, 2010) may be tested in these animal models, providing innovative therapeutic strategies and greater insight into the infectious and inflammatory aspects of endophthalmitis.

A hypothetical model of endophthalmitis pathogenesis based on experimental and clinical findings is depicted in Figure 4. Using experimental models, treatments such as intravitreal antibiotics have been investigated and improved, and bacterial endophthalmitis has become a disease that can often be treated by a single injection of antibiotics. However, the visual outcome of patients with severe endophthalmitis has not improved with these advances (Jackson et al., 2014; Josephberg, 2006, Ng et al., 2005), and the use and benefits of adjunct therapies, such as vitrectomy and corticosteroids, remains mired in controversy. A large body of current treatment practices is based upon case reports, retrospective reviews, and prospective clinical studies. These reports and studies are extremely valuable to the development of treatments for bacterial endophthalmitis. However, endophthalmitis rates are low, and clinical studies cannot control for the significant differences of each individual patient scenario. Experimental models of ocular infection and inflammation can provide statistically significant subject numbers and the controlled environment needed to resolve a number of therapeutic scenarios and facilitate improvements in current treatment modalities.

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REFERENCES

- Aizuss DH, Mondino BJ, Sumner HL, Dethlefs BA. The complement system and host defense against *Pseudomonas* endophthalmitis. *Invest. Ophthalmol. Vis. Sci.* 1985; 26:1262–1266. [PubMed: 4030253]
- Albrecht E, Richards JC, Pollock T, Cook C, Myers L. Adjunctive use of intravitreal dexamethasone in presumed bacterial endophthalmitis; a randomized trial. *British J. Ophthalmol.* 2011; 95:1385–1388.

- Alfaro D, Roth D, Liggett P. Systemic antibiotic prophylaxis in penetrating ocular injuries. An experimental study. *Retina*. 1992; 17:139–145. [PubMed: 9143043]
- Alfaro D, Davis J, Kim S, Bia F, Bogard J, Briggs J, Liggett P. Experimental *Bacillus cereus* post-traumatic endophthalmitis and treatment with ciprofloxacin. *Br. J. Ophthalmol*. 1996a; 80:755–758. [PubMed: 8949723]
- Alfaro DV, Hudson SJ, Rafanan MM, Moss ST, Levy SD. The effect of trauma on the ocular penetration of intravenous ciprofloxacin. *Am. J. Ophthalmol*. 1996b; 122:678–679. [PubMed: 8909207]
- Alfaro D, Hudson S, Kasowski E, Barton C, Brucker A, Lopez J, Beverly D, King L. Experimental pseudomonal posttraumatic endophthalmitis in a swine model. Treatment with ceftazidime, amikacin and imipenem. *Retina*. 1997; 17:139–45. [PubMed: 9143043]
- Avent ML, Roger BA, Cheng AC, Paterson DL. Current use of aminoglycosides: indications, pharmacokinetics and monitoring for toxicity. *Inter. Med. J*. 2011; 41:441–449.
- Azad R, Ravi K, Talwar D, Kumar N. Pars plana vitrectomy with or without silicone oil endotamponade in post-traumatic endophthalmitis. *Graefes Arch. Clin. Exp. Ophthalmol*. 2003; 241:478–483. [PubMed: 12756578]
- Balzli CL, McCormick CC, Caballero AR, O'Callaghan RJ. Sustained anti-staphylococcal effect of lysostaphin in the rabbit aqueous humor. *Curr. Eye Res*. 2010; 35:480–486. [PubMed: 20465441]
- Barequet IS, Habet-Wilner Z, Mann O, Safran M, Ohman DE, Kessler E, Rosner M. Evaluation of *Pseudomonas aeruginosa* staphylolysin (LasA protease) in the treatment of methicillin-resistant *Staphylococcus aureus* endophthalmitis in a rat model. *Graefes Arch. Clin. Exp. Ophthalmol*. 2009; 247:913–917. [PubMed: 19280208]
- Bayer TL, O'Donnell FE, Goncalves V, Singh R. Role of the posterior capsule in the prevention of postoperative bacterial endophthalmitis: experimental primate studies and clinical implications. *Br. J. Ophthalmol*. 1985; 69:841–846. [PubMed: 3877524]
- Baum J, Peyman GA, Barza M. Intravitreal administration of antibiotic in the treatment of bacterial endophthalmitis III. Consensus. *Surv. Ophthalmol*. 1982; 26:204–206. [PubMed: 6978542]
- Beecher DJ, Pulido JS, Barney NP, Wong ACL. Extracellular virulence factors in *Bacillus cereus* endophthalmitis: methods and implication of involvement of hemolysin BL. *Infect. Immun*. 1995; 63:632–639. [PubMed: 7822032]
- Beecher D, Olsen T, Somers E, Wong A. Evidence for contribution of tripartite hemolysin BL, phosphatidylcholine-preferring phospholipase C and collagenase to virulence of *Bacillus cereus* endophthalmitis. *Infect. Immun*. 2000; 68:5269–5276. [PubMed: 10948154]
- Bennett TO, Peyman GA. Use of tobramycin in eradicating experimental bacterial endophthalmitis. *Albrecht Von Graefes Arch. Klin. Exp. Ophthalmol*. 1974; 191:93–107.
- Benz MS, Scott IU, Flynn HW Jr, Unonius N, Miller D. Endophthalmitis isolates and antibiotic sensitivities: A 6-year review of culture-proven cases. *Am. J. Ophthalmol*. 2004; 137:38–42. [PubMed: 14700642]
- Bhagat N, Nagori S, Zarbin M. Post-traumatic infectious endophthalmitis. *Surv. Ophthalmol*. 2011; 56:214–51. [PubMed: 21397289]
- Bhoomibunchoo C, Ratanapakorn T, Sinawat S, Sanguansak T, Moontawee K, Yospaiboon Y. Infectious endophthalmitis: review of 420 cases. *Clin. Ophthalmol*. 2013; 7:247–252. [PubMed: 23403798]
- Bispo PJM, Alfonso HW, Miller D. Emerging 8-methoxyfluoroquinolone resistance among methicillin-susceptible *Staphylococcus epidermidis* isolates recovered from patients with endophthalmitis. *J. Clin. Microbiol*. 2013; 51:2959–2963. [PubMed: 23824766]
- Booth MC, Atkuri R, Nanda S, Iandolo J, Gilmore MS. Accessory gene regulator controls *Staphylococcus* virulence in endophthalmitis. *Invest. Ophthalmol. Vis. Sci*. 1995; 36:1828–1836. [PubMed: 7635657]
- Booth MC, Cheung AL, Hatter KL, Jett BD, Callegan MC, Gilmore MS. Staphylococcal accessory regular (*sar*) in conjunction with *agr* contributes to *Staphylococcus aureus* virulence in endophthalmitis. *Infect. Immun*. 1997; 65:1550–1556. [PubMed: 9119503]
- Brian G, Taylor H. Cataract blindness-challenges for the 21st century. *Bull. World Health Organ*. 2001; 79:249–256. [PubMed: 11285671]

- Brinton GS, Topping TM, Hyndiuk RA, et al. Post traumatic endophthalmitis. Arch. Ophthalmol. 1984; 102:547–550. [PubMed: 6704010]
- Bucher RS, Hall E, Reed DM, Richards JE, Johnson MW, Zacks DN. Effect of intravitreal triamcinolone acetonide on susceptibility to experimental bacterial endophthalmitis and subsequent response to treatment. Arch. Ophthalmol. 2005; 123:649–653. [PubMed: 15883284]
- Bui DK, Carvounis PE. Evidence for and against intravitreal corticosteroids in addition to intravitreal antibiotics of acute endophthalmitis. Internat. Ophthalmol. Clin. 2014; 54:215–224.
- Busbee BG, Recchia FM, Kaiser R, Nagra P, Rosenblatt B, Pearlman RB. Bleb-associated endophthalmitis: clinical characteristics and visual outcomes. Ophthalmol. 2004; 111:1495–1503.
- Callegan MC, Booth MC, Jett BD, Gilmore MS. Pathogenesis of Gram-positive bacterial endophthalmitis. Infect. Immun. 1999a; 67:3348–3356. [PubMed: 10377112]
- Callegan MC, Jett BD, Hancock LE, Gilmore MS. Role of hemolysin BL in the pathogenesis of extraintestinal *Bacillus cereus* infection assessed in an endophthalmitis model. Infect. Immun. 1999b; 67:3357–3366. [PubMed: 10377113]
- Callegan MC, Cochran D, Cane S, Gilmore MS, Gominet M, Lereclus D. Contribution of membrane-damaging toxins to *Bacillus* endophthalmitis pathogenesis. Infect. Immun. 2002a; 70:5381–5389. [PubMed: 12228262]
- Callegan MC, Engelbert M, Parke D, Jett BD, Gilmore MS. Bacterial endophthalmitis: epidemiology, therapeutics, and bacterium-host interactions. Clin. Microbiol. Rev. 2002b; 15:111–124. [PubMed: 11781270]
- Callegan MS, Kane S, Cochran D, Gilmore MS, Gominet M, Lereclus D. Relationship of *plcR*-regulated factors to *Bacillus* endophthalmitis virulence. Infect. Immun. 2003; 71:3116–3124. [PubMed: 12761089]
- Callegan MC, Kane ST, Cochran DC, Novosad B, Gilmore MS, Gominet M, Lereclus D. *Bacillus* endophthalmitis: roles of bacterial toxins and motility during infection. Invest. Ophthalmol. Vis. Sci. 2005; 46:3233–3238. [PubMed: 16123424]
- Callegan MC, Novosad BD, Ramirez R, Ghelardi E, Senesi S. Role of swarming migration in the pathogenesis of *Bacillus* endophthalmitis. Invest. Ophthalmol. Vis. Sci. 2006; 47:4461–4467. [PubMed: 17003440]
- Callegan MC, Gilmore MS, Gregory M, Ramadan RT, Wiskur BJ, Moyer AL, Hunt JJ, Novosad BD. Bacterial endophthalmitis: therapeutic challenges and host-pathogen interactions. Prog. Retin. Eye Res. 2007; 26:189–203. [PubMed: 17236804]
- Callegan MC, Guess S, Wheatley NR, Woods DC, Griffin G, Wiskur BJ, Leonard R. Efficacy of vitrectomy in improving the outcome of *Bacillus cereus* endophthalmitis. Retina. 2011; 31:1518–1524. [PubMed: 21606892]
- Campochiaro PA, Lim JJ. Aminoglycoside toxicity in the treatment of endophthalmitis. Arch. Ophthalmol. 1994; 112:48–53. [PubMed: 8285892]
- Clark WL, Kaiser PK, Flynn HW, Belfort A, Mill D, Meisler DM. Treatment strategies and visual acuity outcomes in chronic postoperative *Propionibacterium acnes* endophthalmitis. Ophthalmol. 1999; 106:1665–1670.
- Coburn PS, Wiskur BJ, Christy E, Callegan MC. The diabetic ocular environment facilitates the development of endogenous bacterial endophthalmitis. Invest. Ophthalmol. Vis. Sci. 2012; 53:7426–7431. [PubMed: 23036996]
- Coburn PS, Wiskur BJ, Astley RA, Callegan MC. Blood-retinal barrier compromise and endogenous *Staphylococcus aureus* endophthalmitis. Invest. Ophthalmol. Vis. Sci. 2015; 56:7303–7311. [PubMed: 26559476]
- Conley SM, Naash MI. Nanoparticles for retinal gene therapy. Prog. Ret. Eye Res. 2010; 29:376–397.
- Conway BP, Campochiaro PA. Macular infarction after endophthalmitis treated with vitrectomy and intravitreal gentamicin. Arch. Ophthalmol. 1986; 104:367–371. [PubMed: 3485422]
- Conway BP, Tabathaby CA, Campochiaro PA, D'Amico DJ, Hanninen LA, Kenyon KR. Gentamicin toxicity in the primate retina. Arch. Ophthalmol. 1989; 107:107–112. [PubMed: 2910268]
- Cottingham AJ, Forster RK. Vitrectomy in endophthalmitis. Arch. Ophthalmol. 1976; 94:2078–2081. [PubMed: 1087148]

- Coutinho AE, Chapman KE. The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. *Mol. Cell. Endocrinol.* 2011; 335:2–13. [PubMed: 20398732]
- Dajcs JJ, Thibodeaux BA, Hume EB, Zheng X, Sloop GD, O'Callaghan RJ. Lysostaphin is effective in treating methicillin-resistant *Staphylococcus aureus* endophthalmitis in the rabbit. *Curr. Eye Res.* 2001; 22:451–457. [PubMed: 11584345]
- Das T, Jalali S, Gothwal V, Sharma S, Naduvilath T. Intravitreal dexamethasone in exogenous bacterial endophthalmitis: results of a prospective randomized study. *Br. J. Ophthalmol.* 1999; 53:1050–1055. [PubMed: 10460774]
- Demant E, Easterbrook M. An experimental model of Candida endophthalmitis. *Canadian J. Ophthalmol.* 1977; 12:304–307.
- Deramo VA, Lai JC, Fastenberg DM, Udell IJ. Acute endophthalmitis in eyes treated prophylactically with gatifloxacin and moxifloxacin. *Am. J. Ophthalmol.* 2006; 142:721–725. [PubMed: 16989762]
- Diago T, McCannel CA, Bakri SJ, Pulido JS, Edwards AO, Pach JM. Infectious endophthalmitis after intravitreal injection of antiangiogenic agents. *Retina.* 2009; 29:601–605. [PubMed: 19357558]
- Diebold Y, Calonge M. Applications of nanoparticles in ophthalmology. *Prog. Ret. Eye Res.* 2010; 29:596–609.
- Eliopoulos GM. Quinolone resistance mechanisms in pneumococci. *Clin. Infect. Dis.* 2004; 38(Suppl 4):S350–356. [PubMed: 15127369]
- Endophthalmitis Study Group European Society of Cataract & Refractive Surgeons. Prophylaxis of postoperative endophthalmitis following cataract surgery: results of the ESCRS multicenter study and identification of risk factors. *J. Cataract Refract. Surg.* 2007; 33:978–988. [PubMed: 17531690]
- Engelbert M, Mino de Kaspar H, Mette M, Thiel M, Ta CN, Grasbon T, Schulze-Schwering M, Klauss V, Kampik A. Intravenous treatment of experimental *Staphylococcus aureus* endophthalmitis: imipenem verses the combination of ceftazidime and amikacin. *Graefes Arch. Clin. Exp. Ophthalmol.* 2003; 241:1029–1036. [PubMed: 14618340]
- Engelbert M, Mylonakis E, Ausubel F, Calderwood S, Gilmore MS. Contribution of gelatinase, serine protease, and *fliC* to the pathogenesis of *Enterococcus faecalis* endophthalmitis. *Infect. Immun.* 2004a; 72:3628–3633. [PubMed: 15155673]
- Engelbert M, Mino de Kaspar H, Thiel M, Grasbon T, Ta CN, Schulze-Schwering M, Klauss V, Kampik A. Intravitreal vancomycin and amikacin versus intravenous imipenem in the treatment of experimental *Staphylococcus aureus* endophthalmitis. *Graefes Arch. Clin. Exp. Ophthalmol.* 2004b; 242:313–320. [PubMed: 14986007]
- Engelbert M, Gilmore MS. Fas ligand but not complement is critical for control of experimental *Staphylococcus aureus* endophthalmitis. *Invest. Ophthalmol. Vis. Sci.* 2005; 46:2479–2486. [PubMed: 15980239]
- Ermis SS, Cetinkaya Z, Kiyici H, Ozturk F. Treatment of *Staphylococcus Epidermidis* endophthalmitis with intravitreal moxifloxacin in a rabbit model. *Tohoku J. Exp. Med.* 2005; 205:223–229. [PubMed: 15718814]
- Ermis SS, Cetinkaya Z, Kiyici H, Inan UU, Ozturk F. Effects of intravitreal moxifloxacin and dexamethasone in experimental *Staphylococcus aureus* endophthalmitis. *Curr. Eye Res.* 2007; 32:337–344. [PubMed: 17453955]
- Fischetti VA. Bacteriophage endolysins: a novel anti-infective to control Gram-positive pathogens. *Int. J. Med. Microbiol.* 2010; 300:357–362. [PubMed: 20452280]
- Fischetti VA. Exploiting what phage have evolved to control gram-positive pathogens. *Bacteriophage.* 2011; 1:188–194. [PubMed: 23050211]
- Forster RK. Experimental postoperative endophthalmitis. *Tr. Am. Ophthalmol. Soc.* 1992; 90:505–559.
- Forster R. Endophthalmitis vitrectomy study. *Arch. Ophthalmol.* 1995; 113:1555–1557. [PubMed: 7487626]
- Gaidt MM, Ebert TS, Chauhan D, Schmidt T, Schmid-Burgk JL, Rapino F, Robertson AAB, Cooper MA, Graf T, Hornung V. Human Monocytes engage an alternative inflammasome pathway. *Immunity.* 2016; 44:1–14. [PubMed: 26789912]

- Gan IM, Ugahary LC, van Dissel JT, Feron E, Peperkamp E, Veckeneer M, Mulder PG, Platenkamp GJ, van Meurs JC. Intravitreal dexamethasone as adjuvant in the treatment of postoperative endophthalmitis: a prospective randomized trial. *Graefes Arch. Clin. Exp. Ophthalmol.* 2005; 243:1200–1205. [PubMed: 16235062]
- Gaudio P. A review of evidence guiding the use of corticosteroids in the treatment of intraocular inflammation. *Ocul. Immunol. Inflamm.* 2004; 12:169–192. [PubMed: 15385194]
- Gentile RC, Shukla S, Shah M, Ritterband DC, Engelbert M, Davis A, Hu D-N. Microbiological spectrum and antibiotic sensitivity in endophthalmitis. *Ophthalmol.* 2014; 121:1634–1642.
- Giese M, Mondino B, Glasgow B, Sumner H, Adamu S, Halabi H, Chou H. Complement system and host defense against staphylococcal endophthalmitis. *Invest. Ophthalmol. Vis. Sci.* 1994; 35:1026–1032. [PubMed: 8125713]
- Giese MJ, Sumner HL, Berliner JA, Mondino BJ. Cytokine expression in a rat model of *Staphylococcus aureus* endophthalmitis. *Invest. Ophthalmol. Vis. Sci.* 1998; 39:2785–2790. [PubMed: 9856792]
- Giese MJ, Shum DC, Rayner SA, Mondino BJ, Berliner JA. Adhesion molecule expression in a rat model of *Staphylococcus aureus* endophthalmitis. *Invest. Ophthalmol. Vis. Sci.* 2000; 41:145–153. [PubMed: 10634614]
- Giese MJ, Rayner SA, Fardin B, Sumner HL, Rozengurt N, Mondino BJ, Gordon LK. Mitigation of neutrophil infiltration in a rat model of early *Staphylococcus aureus* endophthalmitis. *Invest. Ophthalmol. Vis. Sci.* 2003; 44:3077–3082. [PubMed: 12824254]
- Graham RO, Peyman GA. Intravitreal injection of dexamethasone: treatment of experimentally induced endophthalmitis. *Arch. Ophthalmol.* 1974; 92:149–154. [PubMed: 4211214]
- Hall EF, Scott GR, Musch DC, Zacks DN. Adjunctive intravitreal dexamethasone in the treatment of acute endophthalmitis following cataract surgery. *J. Clin. Ophthalmol.* 2008; 2:139–145.
- Han DP. Intravitreal human immune globulin in a rabbit model of *Staphylococcus aureus* toxin-mediated endophthalmitis: a potential adjunct in the treatment of endophthalmitis. *Trans. Am. Ophthalmol. Soc.* 2004; 102:305–320. [PubMed: 15747765]
- Herrero-Vanrell R, Bravo-Osuna I, Andres-Guerrero V, Vicario-de-la-Torre M, Molina-Martinez IT. The potential of using biodegradable microspheres in retinal diseases and other intraocular pathologies. *Prog. Ret. Eye Res.* 2014; 42:27–43.
- Hosseini H, Mehryar M, Eghtedari M, Ahmadi F, Nowroozizadeh S. Effect of intravitreal triamcinolone acetate in the treatment of experimental *Staphylococcus epidermidis* endophthalmitis. *Curr. Eye Res.* 2009; 34:145–151. [PubMed: 19219686]
- Hunt JJ, Wang J, Callegan MC. Contribution of mucoviscosity associated gene A (*magA*) to virulence in experimental *Klebsiella pneumoniae* endophthalmitis. *Invest. Ophthalmol. Vis. Sci.* 2011a; 52:6860–6866. [PubMed: 21791595]
- Hunt, JJ.; Callegan, MC. The pathogenesis of infectious endophthalmitis. In: Duane, TD.; Tasman, W.; Jaeger, EA., editors. *Duane's Ophthalmology*. Lippincott Williams & Wilkins; Philadelphia: 2011b. eBook, Chapter 48
- Hunt JJ, Astley R, Wheatley N, Wang J-T, Callegan MC. TLR4 contributes to the host response to *Klebsiella* intraocular infection. *Curr. Eye Res.* 2014; 39:790–802. [PubMed: 24588082]
- Ishii K, Hiraoka T, Kaji Y, Sakata N, Motoyama Y, Oshika T. Successful treatment of endogenous *Klebsiella pneumoniae* endophthalmitis: a case report. *Int. Ophthalmol.* 2011; 31:29–31. [PubMed: 20661624]
- Jackson TL, Paraskevopoulos T, Georgalas I. Systematic review of 342 cases of endogenous bacterial endophthalmitis. *Surv. Ophthalmol.* 2014; 59:627–635. [PubMed: 25113611]
- Jensen MK, Fiscella RG, Moshirfar M, Mooney B. Third- and fourth-generation fluoroquinolones: retrospective comparison of endophthalmitis after cataract surgery performed over 10 years. *J. Cataract Refract. Surg.* 2008; 34:1460–1467. [PubMed: 18721704]
- Jett BD, Jensen H, Nordquist R, Gilmore MS. Contribution of the pAD1-encoded cytolysin to the severity of experimental *Enterococcus faecalis* endophthalmitis. *Infect Immun.* 1992; 60:244–2452.

- Jett BD, Jensen HB, Atkuri RV, Gilmore MS. Evaluation of therapeutic measures for treating endophthalmitis caused by isogenic toxin-producing and toxin-nonproducing *Enterococcus faecalis* strains. *Invest Ophthalmol Vis Sci*. 1995; 36:9–15. [PubMed: 7822163]
- Josephberg RG. Endophthalmitis: the latest in current management. *Retina*. 2006; 26:S47–S50. [PubMed: 16832300]
- Kasbeer RT, Peyman GA, May DR, Homer PI. Penetration of amikacin into the aphakic eye. *Graefes Arch. Klin. Exp. Ophthalmol*. 1975; 196:85–94.
- Khan FA, Slain D, Khakoo RA. Candida endophthalmitis: Focus on current and future antifungal treatment options. *Pharmacotherapy*. 2007; 27:1711–1721.
- Kim IT, Chung KH, Koo BS. Efficacy of ciprofloxacin and dexamethasone in experimental *Pseudomonas* endophthalmitis. *Korean J. Ophthalmol*. 1996; 10:8–17. [PubMed: 8755196]
- Klein KS, Walsh MK, Hassan TS, Halperin LS, Castellarian AA, Roth D, Driscoll S, Prenner JL. Endophthalmitis after anti-VEGF injections. *Ophthalmology*. 2009; 116:1225.e1. [PubMed: 19486799]
- Krause L, Bechrakis NE, Heimann H, Kildal D, Foerster MH. Incidence and outcome of endophthalmitis over a 13-year period. *Can. J. Ophthalmol*. 2009; 44:88–94. [PubMed: 19169320]
- Kotra LP, Haddad J, Mobashery S. Aminoglycosides: perspectives on mechanisms of action and resistance and strategies to counter resistance. *Antimicrob. Agents Ch*. 2000; 44:3249–3256.
- Kowalski RP, Romanowski EG, Mah FS, Sasaki H, Fukuda M, Gordon YJ. A comparison of moxifloxacin and levofloxacin topical prophylaxis fluoroquinolone-resistant *Staphylococcus aureus* rabbit model. *Jpn. J. Ophthalmol*. 2008; 52:211–216. [PubMed: 18661272]
- Kowalski RP, Romanowski EG, Mah FS, Yates KA, Gordon YJ. Topical prophylaxis with moxifloxacin prevents endophthalmitis in a rabbit model. *Am. J. Ophthalmol*. 2004; 138:33–37. [PubMed: 15234279]
- Kowalski RP, Romanowski EG, Shanks RM, Mah FS. The comparison of fluoroquinolones to nonfluoroquinolone antibacterial agents for the prevention of endophthalmitis in a rabbit model. *J. Ocul. Pharmacol. Ther*. 2012; 28:604–608. [PubMed: 22925112]
- Kuhn F, Gini G. Ten years after...are findings of the Endophthalmitis Vitrectomy Study still relevant today? *Graefe's Arch. Clin. Exp. Ophthalmol*. 2005; 243:1197–1199.
- Kumar A, Singh C, Glybina I, Mahmoud T, Yu F. Toll-like receptor 2 ligand-induced protection against bacterial endophthalmitis. *J. Infect. Dis*. 2010; 201:255–263. [PubMed: 19995266]
- Kumar A, Kumar A. Role of *Staphylococcus aureus* virulence factors in inducing inflammation and vascular permeability in a mouse model of bacterial endophthalmitis. *PLoS ONE*. 2015; 10:1–17.
- Kuriyan AE, Sridhar J, Flynn HW Jr, Smiddy WE, Albin TA, Berrocal AM, Forster RK, Belin PJ, Miller D. Endophthalmitis caused by *Enterococcus faecalis*: clinical features, antibiotic sensitivities, and outcomes. *Am. J. Ophthalmol*. 2014; 158:1018–1023. [PubMed: 25089354]
- Lee SY, Ryu EH, Moon SK, Kim HJ, Woo S, Cho M, Koh HJ. Efficacy of intravitreal gatifloxacin in experimental *Staphylococcus epidermidis* endophthalmitis. *J. Korean Ophthalmol. Soc*. 2008; 49:651–660.
- Lemley CA, Han DP. Endophthalmitis: a review of current evaluation and management. *Retina*. 2007; 27:662–680. [PubMed: 17621174]
- Leopold IH. Intravitreal penetration of penicillin and penicillin therapy of infections of the vitreous. *Arch. Ophthalmol*. 1945; 33:211–216.
- Leopold IH, Nichols A. Intraocular penetration of streptomycin following systemic and local administration. *Arch. Ophthalmol*. 1946; 35:33–38.
- Leopold IH. Surgery of ocular trauma. *Arch. Ophthalmol*. 1952; 48:738–748.
- Lieb DF, Scott IU, Flynn HW Jr, Miller D, Feuer WJ. Open globe injuries with positive intraocular cultures: factors influencing final visual acuity outcomes. *Ophthalmol*. 2003; 110:1560–1566.
- Lopez-Cabezas C, Soy Muner D, Roca Massa M, Mensa Pueyo JM. Antibiotics in endophthalmitis: microbiological and pharmacokinetic considerations. *Curr. Clin. Pharm*. 2010; 5:47–54.
- Low DE. Quinolone resistance among pneumococci: therapeutic and diagnostic implications. *Clin. Infect. Dis*. 2004; 38(Suppl 4):S357–362. [PubMed: 15127370]

- Malecaze F, Bessieres MH, Bec P, Fleutiaux S, Mathis A, Seguela JP. Immunological analysis of the aqueous humour in candida endophthalmitis. I: Experimental study. *Br. J. Ophthalmol.* 1988; 72:309–312. [PubMed: 3259895]
- Maylath FR, Leopold IH. Study of experimental intraocular Infection. *Am. J. Ophthalmol.* 1955; 40:86–101. [PubMed: 14388099]
- McCormick CC, Dajcs JJ, Reed JM, Marquart ME, O’Callaghan RJ. The effectiveness of lysostaphin therapy for experimental coagulase-negative *Staphylococcus* endophthalmitis. *Curr. Eye Res.* 2006; 31:225–230. [PubMed: 16531279]
- Merani R, Hunyor AP. Endophthalmitis following intravitreal anti-vascular endothelial growth factor (VEGF) injection; a comprehensive review. *Int. J. Retin. Vit.* 2015; 1:1–19.
- Meredith TA, Aguilar HE, Miller MJ, Gardner SK, Trabelsi A, Wilson LA. Comparative treatment of experimental *Staphylococcus epidermidis* endophthalmitis. *Arch. Ophthalmol.* 1990; 108:857–860. [PubMed: 2350288]
- Meredith TA, Aguilar HE, Drews C, Sawant A, Gardner S, Wilson LA, Grossniklaus HE. Intraocular dexamethasone produces a harmful effect on treatment of experimental *Staphylococcus aureus* endophthalmitis. *Tr. Am. Ophthalmol. Soc.* 1996; 94:241–257.
- Meredith T. Posttraumatic endophthalmitis. *Arch. Ophthalmol.* 1999; 117:520–521. [PubMed: 10206582]
- Miller D, Flynn PM, Scott IU, Alfonso EC, Flynn HW Jr. In vitro fluoroquinolone resistance in staphylococcal endophthalmitis isolates. *Arch. Ophthalmol.* 2006; 124:479–483. [PubMed: 16606872]
- Miller D, Chang JS, Flynn HW, Alfonso EC. Comparative *in vitro* susceptibility of besifloxacin and seven comparators against ciprofloxacin- and methicillin-susceptible/nonsusceptible staphylococci. *J. Ocul. Pharmacol. Ther.* 2013; 29:339–344. [PubMed: 23289847]
- Mingeot-Leclercq MP, Glupczynski Y, Tulkens PM. Aminoglycosides: activity and resistance. *Antimicrob. Agents Ch.* 1999; 43:727–737.
- Mitra RA, Mieler WF. Controversies in the management of open-globe injuries involving the posterior segment. *Surv. Ophthalmol.* 1999; 44:215–225. [PubMed: 10588440]
- Moshirfar M, Feiz V, Vitale AT, Wegelin JA, Basavanthappa S, Wolsey DH. Endophthalmitis after uncomplicated cataract surgery with the use of fourth-generation fluoroquinolones. *Ophthalmol.* 2007; 114:686–691.
- Moyer AL, Ramadan RT, Novosad BD, Astley R, Callegan MC. *Bacillus cereus*-induced permeability of the blood-ocular barrier during experimental endophthalmitis. *Invest. Ophthalmol. Vis. Sci.* 2009; 50:3783–3793. [PubMed: 19264886]
- Moynagh PN. Toll-like receptor signaling pathways as key targets for mediating the anti-inflammatory and immunosuppressive effects of glucocorticoids. *J. Endocrinol.* 2003; 179:139–144. [PubMed: 14596665]
- Mylonakis E, Engelbert M, Qin X, Sifri CD, Murray BE, Ausubel FM, Gilmore MS, Calderwood SB. The *Enterococcus faecalis* *fsrB* gene, a key component of the *fsr* quorum-sensing system, is associated with virulence in the rabbit endophthalmitis model. *Infect. Imm.* 2002; 70:4678–4681.
- Nazir J, Urban C, Mariano N, Burns J, Tommasulo B, Rosenberg C, Segal-Maurer S, Rahal JJ. Quinolone-resistance *Haemophilus influenzae* in a long-term care facility: clinical and molecular epidemiology. *Clin. Infect. Dis.* 2004; 38:1564–1569. [PubMed: 15156444]
- Ness T, Pelz K, Hansen LL. Endogenous endophthalmitis: microorganisms, disposition and prognosis. *Acta Ophthalmol. Scand.* 2007; 85:852–856. [PubMed: 17725616]
- Ng EW, Samiy N, Rubins JB, Cousins FV, Ruoff KL, Baker AS, D’Amico DJ. Implication of pneumolysin as a virulence factor in *Streptococcus pneumoniae* endophthalmitis. *Retina.* 1997; 17:521–529. [PubMed: 9428015]
- Ng EWM, Costa JR, Samiy N, Ruoff KL, Connolly E, Cousins FV, D’Amico DJ. Contribution of pneumolysin and autolysin to the pathogenesis of experimental pneumococcal endophthalmitis. *Retina.* 2002; 22:622–632. [PubMed: 12441729]
- Ng JQ, Morlet N, Perman JW, Constable IJ, McAllister IL, Kennedy CJ, Isaacs T, Semmens JB. Management and outcomes of postoperative endophthalmitis since the endophthalmitis

- vitreotomy study; the Endophthalmitis Population Study of Western Australia (EPSWA)'s fifth report. *Ophthalmol.* 2005; 112:1199–1206.
- Novosad BD, Astley RA, Callegan MC. Role of Toll-Like Receptor (TLR) 2 in Experimental *Bacillus cereus* Endophthalmitis. *PLoS One.* 2011; 6:1–7.
- Nishida T, Ishida K, Niwa Y, Kawakami H, Mochizuki K, Ohkusu K. An eleven-year retrospective study of endogenous bacterial endophthalmitis. *J. Ophthalmol.* 2015; 2015:1–11.
- Omura J, Uchida K, Yamaguchi H, Shibuya K. Histopathological study on experimental endophthalmitis induced by bloodstream infection with *Candida albicans*. *Jpn. J. Infect. Dis.* 2007; 60:33–39. [PubMed: 17314423]
- Ozturk F, Kortunay S, Kurk E, Ilker SS, Basci NE, Bozkurt A. Penetration of topical and oral ciprofloxacin into the aqueous and vitreous humor in inflamed eyes. *Retina.* 1999; 19:218–222. [PubMed: 10380027]
- Ozturk F, Kurt E, Inan UU, Kortunay S, Ilker SS, Basci NE, Bozkurt A. Penetration of topical and oral ofloxacin into the aqueous and vitreous humor of inflamed rabbit eyes. *Internat. J. Pharma.* 2000; 204:91–95.
- Papadimitriou JM, Ashman RB. The pathogenesis of acute systemic candidiasis in a susceptible inbred mouse strain. *J. Pathol.* 1986; 150:257–265. [PubMed: 3806283]
- Park SS, Samiy N, Ruoff K, D'Amico DJ, Baker AS. Effect of intravitreal dexamethasone in treatment of pneumococcal endophthalmitis in rabbits. *Arch. Ophthalmol.* 1995; 113:1324–1329. [PubMed: 7575268]
- Park SS, Vallar RV, Hong CH, von Guten S, Ruoff K, D'Amico J. Intravitreal dexamethasone effect on intravitreal vancomycin elimination in endophthalmitis. *Arch. Ophthalmol.* 1999; 117:1058–1062. [PubMed: 10448749]
- Parkunan SM, Astley R, Callegan MC. Role of TLR5 and flagella in *Bacillus* intraocular infection. *PLoS ONE.* 2014; 9:1–9.
- Parkunan SM, Randall CB, Coburn PS, Astley RA, Staats RL, Callegan MC. Unexpected roles for Toll-like receptor 4 and TRIF in intraocular infection with Gram-positive bacteria. *Infect. Immun.* 2015; 83:3926–3936. [PubMed: 26195555]
- Parrish CM, O'Day DM. Traumatic endophthalmitis. *Internat. Ophthalmol. Clinics.* 1987; 27:112–119.
- Pastagia M, Schuch R, Fischetti VA, Huang DB. Lysins: the arrival of pathogen-directed anti-infectives. *J. Med. Microbiol.* 2013; 62:1506–1516. [PubMed: 23813275]
- Perkins SL, Han DP, Burke JM, Schlievert PM, Wirostko WJ, Tarasewicz DG, Skumatz CMB. Intravitreally injected human immunoglobulin attenuates the effects of *Staphylococcus aureus* culture supernatant in a rabbit model of toxin-mediated endophthalmitis. *Arch. Ophthalmol.* 2004; 122:1499–1506. [PubMed: 15477462]
- Petropoulos IK, Vantzous CV, Lamari FN, Karamanos NK, Anastassiou ED, Pharmakakis NM. Expression of TNF- α , IL-1 β , and IFN- γ in *Staphylococcus epidermidis* slime-positive experimental endophthalmitis is closely related to clinical inflammatory scores. *Graefe's Arch. Clin. Exp. Ophthalmol.* 2006; 244:1322–1328. [PubMed: 16544114]
- Peyman GA, Vastine DW, Misels HI. The experimental and clinical use of intravitreal antibiotics to treat bacterial and fungal endophthalmitis. *Documenta Ophthalmol.* 1975; 39:183–201.
- Peyman GA, Lad EM, Moshfeghi DM. Intravitreal injection of therapeutic agents. *Retina.* 2009; 29:875–912. [PubMed: 19584648]
- Pfaller MA, Sader HS, Fritsche TR, Jones RN. Antimicrobial activity of cefepime tested against ceftazidime-resistant Gram-negative clinical strains from North American hospitals: report from the SENTRY Antimicrobial Surveillance Program (1998-2004). *Diag. Microbiol. Inf. Dis.* 2007; 57:63–68.
- Pharmakakis NM, Petropoulos IK, Georgakopoulos CD, Vantzou CV, Anastassiou ED, Mavropoulos A, Zolota V. Apoptotic mechanisms within the retina in *Staphylococcus epidermidis* experimental endophthalmitis. *Graefes Arch. Clin. Exp. Ophthalmol.* 2008; 247:667–674. [PubMed: 19039600]
- Phillips I, Warren C, Shannon K, King A, Hanslo D. Ceftazidime: *in vitro* antibacterial activity and susceptibility to β -lactamases compared with that of cefotaxime, moxalactam and other β -lactam antibiotics. *J. Antimicrob. Chemother.* 1981; 8:23–31. [PubMed: 19802967]

- Picot V. Recherches experimentales sur l'inoculation de micro-organismes dans la chambre anterieure de l'oeil du lapin. Archives d'Ophthalmologie. 1898; 18:341–358.
- Pollack JS, Beecher DJ, Pulido JS, Wong ACL. Failure of intravitreal dexamethasone to diminish inflammation or retinal toxicity in an experimental model of *Bacillus cereus* endophthalmitis. Curr. Eye Res. 2004; 29:253–259. [PubMed: 15590470]
- Ravindranath RMH, Hasan SA, Mondino BJ. Immunopathologic features of *Staphylococcus epidermidis*-induced endophthalmitis in the rat. Curr. Eye Res. 1997; 16:1036–1043. [PubMed: 9330856]
- Ramadan RT, Ramirez R, Novosad BD, Callegan MC. Acute inflammation and loss of retinal architecture and function during experimental *Bacillus* endophthalmitis. Curr. Eye Res. 2006; 31:955–965. [PubMed: 17114121]
- Ramadan RT, Moyer AL, Callegan MC. A role for Tumor Necrosis Factor- α in experimental *Bacillus cereus* endophthalmitis pathogenesis. Invest. Ophthalmol. Vis. Sci. 2008; 49:4482–4489. [PubMed: 18586878]
- Relhan N, Albini TA, Pathengay A, Kuriyan AE, Miller D, Flynn HW. Endophthalmitis caused by Gram-positive organisms with reduced vancomycin susceptibility; literature review and options for treatment. Br. J. Ophthalmol. Dec. 201523
- Remtulla S, Hallett PE. A schematic eye for the mouse, and comparisons with the rat. Vision Res. 1985; 25:21–31. [PubMed: 3984214]
- Rodrigues EB, Farah ME, Maia M, Penha FM, Regatieri C, Melo GB, Pinheiro MM, Zanetti CR. Therapeutic monoclonal antibodies in ophthalmology. Prog. Ret. Eye Res. 2009; 28:117–144.
- Roth DB, Flynn HW Jr. Antibiotic selection in the treatment of endophthalmitis: the significance of drug combinations and synergy. Surv. Ophthalmol. 1997; 41:395–401. [PubMed: 9163836]
- Roy R, Panigrahi P, Malathi J, Pal SS, Nandi K, Patil A, Nigam E, Arora V. Endophthalmitis caused by *Acinetobacter baumannii*: a case series. Eye. 2013; 27:450–452. [PubMed: 23306728]
- Rosenzweig HL, Woods A, Clowers JS, Planck SR, Rosenbaum JT. The NLRP3 inflammasome is active but not essential in endotoxin-induced uveitis. Inflamm. Res. 2012; 61:225–231. [PubMed: 22119862]
- Sadaka A, Durand ML, Gilmore MS. Bacterial endophthalmitis in the age of outpatient intravitreal therapies and cataract surgeries: host-microbe interactions in intraocular infection. Prog. Ret. Eye Res. 2012; 31:316–331.
- Sadaka A, Palmer K, Suzuki T, Gilmore MS. In vitro and in vivo models of *Staphylococcus aureus* endophthalmitis implicate specific nutrients in ocular infection. PLoS One. 2014; 9:1–14.
- Sadaka A, Durand ML, Gilmore MS. *Staphylococcus aureus* and its bearing on ophthalmic disease. Ocul. Immunol. Inflamm. 2015; 17:1–11. [PubMed: 26679534]
- Sakalar YB, Ozekinci S, Celen MK. Treatment of experimental *Bacillus cereus* endophthalmitis using intravitreal moxifloxacin with or without dexamethasone. J. Ocular. Phar. Ther. 2011; 27:593–598.
- Sampat KM, Garg SJ. Complications of intravitreal injections. Curr. Op. Ophthalmol. 2010; 21:178–183.
- Sanders ME, Norcross EW, Moore QC, Onwubiko C, King LB, Fratkin J, Marquart ME. A comparison of pneumolysin activity and concentration *in vitro* and *in vivo* in a rabbit endophthalmitis model. Clin. Ophthalmol. 2008; 2:793–800. [PubMed: 19668433]
- Sanders ME, Norcross EW, Moore QC III, Fratkin J, Thompson H, Marquart ME. Immunization with pneumolysin protects against both retinal and global damage caused by *Streptococcus pneumoniae* endophthalmitis. J. Ocular. Pharm. Ther. 2010; 26:571–577.
- Sanders ME, Norcross EW, Robertson ZM, Moore QC III, Fratkin J, Marquart ME. The *Streptococcus pneumoniae* capsule is required for full virulence in pneumococcal endophthalmitis. Invest. Ophthalmol. Vis. Sci. 2011; 52:865–872. [PubMed: 21051708]
- Schech JM, Alfaro DV, Laughlin RM, Sanford EG, Briggs J, Dalgetty M. Intravenous gentamicin and ceftazidime in penetrating ocular trauma: a swine model. Retina. 1997; 17:28–32. [PubMed: 9051839]

- Schimel AM, Miller D, Flynn HW Jr. Evolving fluoroquinolone resistance among coagulase-negative *Staphylococcus* isolates causing endophthalmitis. *Arch. Ophthalmol.* 2012; 130:1617. [PubMed: 23229711]
- Scoper SV. Review of third- and fourth-generation fluoroquinolones in ophthalmology: in-vitro and in-vivo efficacy. *Adv. Ther.* 2008; 25:979–994. [PubMed: 18836691]
- Seok J, Warren HS, Cuenca AG, Mindrinos MN, Baker HV, Xu W, et al. Genomic responses in mouse models poorly mimic human inflammatory diseases. *Proc. Natl. Acad. Sci.* 2013; 110:3507–3512. [PubMed: 23401516]
- Shah CP, McKey J, Spirn MJ. Ocular Candidiasis: a review. *Br. J. Ophthalmol.* 2008; 98:466–468. [PubMed: 18369061]
- Shah GK, Stein JD, Sharma S, Sivalingam A, Benson WE, Regillo CD, Brown GC, Tasman W. visual outcomes following the use of intravitreal steroids in the treatment of postoperative endophthalmitis. *Ophthalmology.* 2000; 107:486–489. [PubMed: 10711885]
- Silverstein AM, Zimmerman LE. Immunogenic endophthalmitis produced in the guinea pig by different pathogenetic mechanisms. *Am. J. Ophthalmol.* 1959; 48:435–447. [PubMed: 14446781]
- Slijkerman RWN, Song F, Astuti GDN, Huynen MA, van Wijk E, Stieger K, Collin RWJ. The pros and cons of vertebrate animal models for functional and therapeutic research on inherited retinal dystrophies. *Prog. Ret. Eye Res.* 2015; 48:137–159.
- Smith MA, Sorenson JA, D'Aversa G, Mandelbaum S, Udell I, Harrison W. Treatment of experimental methicillin resistant *Staphylococcus epidermidis* endophthalmitis with intravitreal vancomycin and intravitreal dexamethasone. *J. Inf. Dis.* 1997; 175:462–466. [PubMed: 9203674]
- Stevens SX, Jensen HG, Jett BD, Gilmore MS. A hemolysin-encoding plasmid contributes to bacterial virulence in experimental *Enterococcus faecalis* endophthalmitis. *Invest. Ophthalmol. Vis. Sci.* 1992; 33:1650–1656. [PubMed: 1559764]
- Sugi N, Whiston EA, Ksander BR, Gregory MS. Increased resistance to *Staphylococcus aureus* endophthalmitis in BALB/c mice: Fas ligand is required for resolution of inflammation but not for bacterial clearance. *Infect. Immun.* 2013; 81:2217–2225. [PubMed: 23569113]
- Suzuki T, Campbell J, Swoboda JG, Walker S, Gilmore MS. Role of wall teichoic acids in *Staphylococcus aureus* endophthalmitis. *Invest. Ophthalmol. Vis. Sci.* 2011; 52:3187–3192. [PubMed: 21345983]
- Taban M, Behrens A, Newcomb RL, Nobe MY, Saedi G, Sweet PM, McDonnell PJ. Acute endophthalmitis following cataract surgery: a systemic review of the literature. *Arch. Ophthalmol.* 2005; 123:613–620. [PubMed: 15883279]
- Talley AR, D'Amico DJ, Talamo JH, Casey VJ, Kenyon KR. The role of vitrectomy in the treatment of postoperative bacterial endophthalmitis: an experimental study. *Arch. Ophthalmol.* 1987; 105:1699–1702. [PubMed: 3500702]
- Talreja D, Kaye KS, Yu FX, Salia SK, Kumar A. Pathogenicity of ocular isolates of *Acinetobacter baumannii* in a mouse model of bacterial endophthalmitis. *Invest. Ophthalmol. Vis. Sci.* 2014; 55:2392–2402. [PubMed: 24644055]
- Talreja D, Singh PK, Kumar A. In vivo role of TLR2 and MyD88 signaling in eliciting innate immune responses in staphylococcal endophthalmitis. *Invest. Ophthalmol. Vis. Sci.* 2015; 56:1719–1732. [PubMed: 25678692]
- Tucker DN, Forster RK. Experimental bacterial endophthalmitis. *Arch. Ophthalmol.* 1972; 88:647–649. [PubMed: 4628564]
- Tasaka Y, Suzuki T, Kawasaki S, Uda T, Mito T, Uno T, Ohashi Y. Moxifloxacin as postoperative prophylaxis for *Enterococcus faecalis*-induced endophthalmitis after cataract surgery in aphakic rabbits. *J. Ocul. Pharmacol. Ther.* 2013; 29:403–409. [PubMed: 23249386]
- Thronton JA, Tullos NA, Sanders ME, Ridout G, Wang Y-D, Taylor SD, McDaniel LS, Marquart ME. Differential bacterial gene expression during experimental pneumococcal endophthalmitis. *Ophthalmic Res.* 2015; 53:149–161. [PubMed: 25791614]
- VEGF Inhibition Study in Ocular Neovascularization (V.I.S.I.O.N.) Clinical Trial Group. D'Amico DJ, Masonson HN, Patel M, Adamis AP, Cunningham ET Jr. Guyer DR, Katz B. Pegaptanib sodium for neovascular age-related macular degeneration: two-year safety results of the two prospective, multicenter, controlled clinical trials. *Ophthalmol.* 2006; 113:992–1001.

- Von Sallmann L. Penicillin and sulfadiazine in the treatment of experimental intraocular infection with *Pneumococcus*. Arch. Ophthalmol. 1943; 30:426–436.
- Von Sallmann L, Meyer K. Penetration of penicillin into the eye. Arch. Ophthalmol. 1944a; 31:1–7.
- Von Sallmann L, Meyer K, Di Grandi J. Experimental study on penicillin treatment of ectogenous infection of vitreous. Arch. Ophthalmol. 1944b; 32:179–189.
- Von Sallmann L. Controversial points in ocular penicillin therapy. Trans. Am. Ophthalmol. Soc. 1947; 45:570–636. [PubMed: 16693463]
- Waheed S, Ritterband DC, Greenfield DS, Liebmann JM, Seedor JA, Ritch R. New patterns of infecting organisms in late bleb-related endophthalmitis: a ten year review. Eye. 1998; 12:910–915. [PubMed: 10325984]
- West E, Behrens A, McDonnell P, Tielsch J, Schein O. The incidence of endophthalmitis after cataract surgery among the US Medicare population increased between 1994 and 2001. Ophthalmol. 2005; 112:1388–1394.
- Whiston E, Sugi N, Kamradt M, Sack C, Heirmer S, Engelbert M, Wawrousek E, Gilmore MS, Ksander B, Gregory M. α B-crystallin protects retinal tissue during *Staphylococcus aureus*-induced endophthalmitis. Infect. Immun. 2008; 76:1781–1790. [PubMed: 18227158]
- Willermain F, Rosenbaum JT, Bodaghi B, Rosenzweig HL, Childers S, Behrend T, Wildner G, Dick AD. Interplay between innate and adaptive immunity in the development of non-infectious uveitis. Prog. Retin. Eye Res. 2012; 31:182–194. [PubMed: 22120610]
- Winward KE, Pflugfelder SC, Flynn HW Jr, Roussel TJ, Davis JL. Postoperative Propionibacterium endophthalmitis. treatment strategies and long-term results. Ophthalmol. 1993; 100:447–451.
- Wiskur BJ, Hunt JJ, Callegan MC. Hypermucoviscosity as a virulence factor in experimental *Klebsiella pneumoniae* endophthalmitis. Invest. Ophthalmol. Vis. Sci. 2008a; 49:4931–4938. [PubMed: 18586871]
- Wiskur BJ, Robinson ML, Farrand AJ, Novosad BD, Callegan MC. Toward improving therapeutic regimens for *Bacillus* endophthalmitis. Invest. Ophthalmol. Vis. Sci. 2008b; 49:1480–1487. [PubMed: 18385066]
- Wiskur BJ, Woods DC, Wheatley NR, Callegan MC. Update on improvements for therapy of severe bacterial endophthalmitis. Internat. Sym. Ocular Pharm. Therap (8th). 2009:25–37.
- Wong J-S, Chan T-K, Lee H-M, Chee S-P. An East Asian experience and a reappraisal of a severe ocular affliction. Ophthalmol. 2000; 107:1483–1491.
- Yoshizumi MO, Lee GC, Equi RA, Kim IT, Pitschekian-Halabi H, Adamu SA, Mondino BJ. Timing of dexamethasone treatment in experimental *Staphylococcus aureus* endophthalmitis. Retina. 1998; 18:130–135. [PubMed: 9564693]
- Yu D, Cringle S. Oxygen distribution in the mouse retina. Invest. Ophthalmol. Vis. Sci. 2006; 47:1109–1112. [PubMed: 16505048]

ARTICLE HIGHLIGHTS

- Endophthalmitis is an infection/inflammation of the eye that can result in blindness.
- Endophthalmitis models mimic the behavior of the infecting organism in human eyes.
- Endophthalmitis models mimic the host response to infection in human eyes.
- These models are used to analyze pathogenesis and treatment efficacy during infections.
- These models provide a controlled environment which can be analyzed for statistical significance.

Figure 1. Contribution of NOD2 and NLRP3 to the pathogenesis of experimental *B. cereus* endophthalmitis

Eyes of C57BL/6J, NOD2-deficient, and NLRP3-deficient mice were intravitreally injected with *B. cereus* and infections were assessed by quantifying viable bacteria [A], retinal function [B], myeloperoxidase and proinflammatory mediators IL-6 and CXCL1/KC [C], and by histology [D], as previously described (Parkunan et al., 2014; Parkunan et al, 2015). All eyes were assessed and harvested at 0 and 8 hours postinfection. Mouse eyes were injected with approximately 100 CFU of *B. cereus* per eye [A]. At 0 hours, retinal function was at 100%, myeloperoxidase and proinflammatory mediator levels were below the limit of detection, and no inflammation was observed in all infected eyes, as previously described (data not shown, refer to Parkunan et al., [2014 and 2015] for description of infected eyes at 0 hours). At 8 hours postinfection, the ocular *B. cereus* burden [A], the A-wave and B-wave reductions [B], the ocular myeloperoxidase, IL-6, and CXCL1/KC concentrations [C], and the overall retinal damage and intraocular inflammation [D] was similar in eyes of infected C57BL/6J, NOD2-deficient, and NLRP3-deficient eyes ($P>0.05$, mutant vs. C57BL/6J; mean $\pm$ SEM for N = 5 eyes per group; Mann-Whitney U test, GraphPad Prism 6 (La Jolla, Ca.).

Figure 2. Real-time biophotonic imaging of experimental endogenous *Klebsiella pneumoniae* endophthalmitis

An isolate of *K. pneumoniae* was electroporated with pQF50::lux containing *luxCDABE*, resulting in the bioluminescent strain KLP06lux. [A] Animals were imaged using the Kodak Imaging Station 4000 following intraocular injection of 10^4 CFU KLP06lux to identify the minimum threshold of detectable organisms using this system. The “no light” image was enhanced using blue-for-white color replacement in CorelPhotopaint v9.0. The “merge” image was created by overlaying the “no light” image over the “white light” image and rendering the black transparent so KLP06lux in the eye is visualized in blue. [B] Animals were imaged using the IVIS Lumina system following tail vein injection of 10^7 CFU KLP06lux. One of three animals was infected, consistent with the 30% endogenous endophthalmitis rate in this model (Coburn et al. 2012).

Figure 3. Comparison of experimental endophthalmitis models in mice and rabbits

To initiate infection, bacteria representing common endophthalmitis pathogens were intravitreally injected into rabbit or mouse eyes, as described above. Infected eyes were harvested and processed for hematoxylin and eosin histology. For the Gram-positive organisms (*Bacillus cereus*, *Enterococcus faecalis*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*), time points represent severe inflammation in infected eyes of one or both mammalian species. For Gram-negative organisms (*Escherichia coli* and *Klebsiella pneumoniae*), 15 hours post-infection represents a time of significant ocular inflammation in mice, but ocular inflammation in rabbit eyes was not as severe. The *S. pneumoniae* rabbit histology section was kindly provided by Dr. Mary Marquart (University of Mississippi Medical Center, Jackson MS). The *K. pneumoniae* mouse histology section, ©Association for Research in Vision and Ophthalmology [Wiskur et al., 2008a].

Figure 4. Hypothetical model of endophthalmitis pathogenesis

Once organisms enter the posterior segment of the eye, most begin to replicate. Avirulent organisms, even at high inocula, can be cleared by the inflammatory response, with little or no damage to intraocular structures or loss in vision. Conversely, virulent organisms replicate and secrete one or more toxins which can damage the retina and incite intraocular inflammation which may also cause bystander damage to the retina. With virulent pathogens, this course can occur even with low inocula. Despite treatment, infections with virulent pathogens often result in vision loss. Adapted from Hunt and Callegan (2011b).

Table 1

The efficacies of antibiotics with corticosteroids in reducing inflammation in experimental models of bacterial endophthalmitis.

Pathogen	Corticosteroid	Antibiotic	Reduced Inflammation?	Reference
<i>B. cereus</i>	Dexamethasone	Vancomycin	No	Wiskur et al., 2008b
<i>B. cereus</i>	Prednisolone	Vancomycin or Gatifloxacin	No	Wiskur et al., 2009
<i>B. cereus</i>	Dexamethasone	Moxifloxacin	Yes	Sakalar et al., 2011
<i>E. faecalis</i> (noncytolytic)	Dexamethasone	Ampicillin and Gentamicin	Yes	Jett et al., 1995
<i>E. faecalis</i> (cytolytic)	Dexamethasone	Ampicillin and Gentamicin	No	Jett et al., 1995
<i>P. aeruginosa</i>	Dexamethasone	Gentamicin	Yes	Graham and Peyman 1974
<i>S. aureus</i>	Dexamethasone (systemic)	Cefazolin	No	Meredith et al., 1996
<i>S. aureus</i>	Dexamethasone	Cefazolin	Increased Inflammation	Meredith et al., 1996
<i>S. aureus</i>	Dexamethasone	Moxifloxacin	No	Ermis et al., 2006
<i>S. epidermidis</i>	Prednisolone (systemic) and/or Dexamethasone	Cefazolin	Yes	Meredith et al., 1996
<i>S. epidermidis</i>	Dexamethasone	Vancomycin	Yes	Smith et al, 1997
<i>S. epidermidis</i>	Dexamethasone	Moxifloxacin	No	Ermis et al., 2005
<i>S. pneumoniae</i>	Dexamethasone	Vancomycin	Yes	Park et al., 1995

Drugs were administered via the intraocular route unless otherwise specified.

Table 2

The efficacies of vitrectomy with antibiotics in treating experimental models of bacterial endophthalmitis.

Pathogen	Vitrectomy with Antibiotic	Outcome	Reference
<i>B. cereus</i>	Vancomycin	No improvement in final visual acuity	Forster 1992
<i>B. cereus</i>	Vancomycin	Greater retinal function with earlier treatment	Callegan et al., 2011
<i>E. faecalis</i>	Vancomycin	No improvement in final visual acuity	Forster 1992
<i>P. aeruginosa</i>	Gentamicin	No improvement in outcome	Peyman et al., 1975
<i>S. aureus</i>	None	No reduction in bacterial growth	Cottingham and Forster 1976
<i>S. aureus</i>	Gentamicin	Increase in culture-negative eyes	Cottingham and Forster 1976
<i>S. aureus</i>	Cefazolin	Improved vitreal clarity	Talley et al., 1992
<i>S. aureus</i>	Vancomycin	No improvement in final visual acuity	Forster 1992
<i>S. epidermidis</i>	None	No reduction in bacterial growth	Cottingham and Forster 1976
<i>S. epidermidis</i>	Gentamicin	No reduction in bacterial growth	Cottingham and Forster 1976
<i>S. epidermidis</i>	Vancomycin	No improvement in final visual acuity	Forster 1992