Evaluation of Susceptibility and Innate Immune Response in C57BL/6 and BALB/C Mice During *Candida albicans* Endophthalmitis

Bruce G. Rottmann,[1] Pawan Kumar Singh,[1] Sneha Singh,[1] Sanjay G. Revankar,[2] Pranatharthi H. Chandrasekar,[2] and Ashok Kumar[1,3]

[1]Department of Ophthalmology, Visual, and Anatomical Sciences, Wayne State University School of Medicine, Detroit, Michigan, United States
[2]Division of Infectious Disease, Department of Internal Medicine, Wayne State University School of Medicine, Detroit, Michigan, United States
[3]Department of Biochemistry, Microbiology and Immunology, Wayne State University School of Medicine, Detroit, Michigan, United States

PURPOSE. Candida remains the leading cause of fungal endophthalmitis. However, the pathobiology and innate immune responses in this disease are not well characterized. Here, we developed two murine models of candida endophthalmitis and evaluated their disease susceptibility and differential immune response.

METHODS. Endophthalmitis was induced in C57BL/6 (B6) and BALB/c mice by intravitreal injection of *Candida albicans* (CA). Disease progression was monitored by slit-lamp examination and clinical scoring, followed by retinal function assessment using electroretinography (ERG). Enucleated eyes were used to estimate fungal burden and retinal tissue damage by hematoxylin and eosin and TUNEL staining. The level of inflammatory mediators were determined by quantitative Polymerase Chain Reaction (qPCR) and enzyme-linked immunosorbent assay, whereas neutrophil infiltration was assessed by flow cytometry and immunostaining.

RESULTS. Intravitreal injection of CA at 6500 colony-forming units resulted in sustained (non-resolving) ocular inflammation in both B6 and BALB/c mice as evidenced by increased levels of inflammatory cytokines (tumor necrosis factor–α, interleukin-1β, and interleukin-6) and chemokine (CXCL2/MIP-2). In both mouse strains, fungal burden peaked at 24 to 48 hours post-infection (hpi) and decreased by 72 to 96 hpi. CA-infected eyes exhibited increased polymorphonuclear neutrophils (PMN) infiltration and retinal tissue damage. Overall retinal function declined rapidly, with a significant reduction in ERG response at 12 hpi and near-total loss by 24 hpi. Differential analyses revealed increased pathology in BALB/c versus B6 mice.

CONCLUSIONS. C. albicans was able to cause endophthalmitis in mice. Although BALB/c mice were found to be more susceptible to CA endophthalmitis, both BALB/c and B6 models could be used to study fungal endophthalmitis and test therapeutic modalities.

Keywords: fungal endophthalmitis, *Candida albicans*, mouse model, retina, inflammation, ERG

Endophthalmitis is a vision-threatening complication that arises as a result of microbial infection in the anterior or posterior chamber of the eye causing inflammation, retinal damage eventually leading to loss of vision, and blindness.[1–3] Exogenous endophthalmitis occurs when microbes on the ocular surface, or from an external source, are introduced into the eye, whereas endogenous endophthalmitis arises from the hematogenous seeding of an infectious pathogen. Although bacterial endophthalmitis is far more common than fungal endophthalmitis,[4,5] fungal infections still pose a serious challenge to ophthalmologists due to lack of differential diagnosis and limited treatment options.[6] The incidence of fungal endophthalmitis varies greatly and depends on several factors such as the use of total parenteral nutrition, broad-spectrum antibiotics, recent abdominal surgery, neutropenia, glucocorticoid therapy in inpatients, and both intravenous drug use (IVDU) and recent central venous catheters, which includes peripherally inserted central catheters (PICC lines) in outpatients.[7] In the United States and Europe, fungal endophthalmitis is less common, whereas in India and China, it can account for 10% to 20% of all cases of endophthalmitis.[8–10] In terms of fungal endophthalmitis etiology, *Aspergillus spp.* is the most common cause of exogenous endophthalmitis, whereas *Candida spp.* primarily cause endogenous endophthalmitis,[11,12] with *C. albicans* being the most prevalent species.[11–16]

C. albicans is ubiquitously present and being a part of normal human gut microbiota, it is considered as a commensal organism.[17–19] It is a dimorphic fungus that can grow in both yeast (single-cell) and pseudohyphal (branching/filamentous) forms.[18,19] *Candida spp.*, specifically *C. albicans*, accounts for more than 70% of systemic fungal infections and is the fourth most common cause of nosocomial bloodstream infections.[20] The incidence of Candida endophthalmitis has been reported to range from 0.1% to 1.6%, and the rates of total ocular involvement range from 2.7% to 37%.[13–15,21–24] Ocular candidiasis is a potentially severe complication that can lead to visual field defects or blindness if appropriate therapy is delayed.[25–27] Although *C. albicans* can cause disease in immunocompetent individuals with risk factors, it is considered as a potential threat to anyone who is immunocompromised.[17]

Ocular candidiasis occurs in approximately 10% to 25% of Candida infection cases.[7,14,15] However, more invasive *C. albicans* infections, including candidemia, can occur in the presence of predisposing risk factors.[20] Invasive candidiasis is known to infect the kidneys, heart, brain, female genital tract, and oral cavity.[13,28] Although relatively less common, *C. albicans* can cause post-operative endophthalmitis due to colonization on intraocular lenses or contamination of irrigating solutions or ventilation systems.[7,29,30] Exogenous candida endophthalmitis has also been reported following penetrating keratoplasty.[31,32] Clinically, ocular candidiasis can be classified as chorioretinitis with a lesion restricted to the choroid and retina, or, as endophthalmitis, which involves the vitreous body[26,33–35] characterized by "fluffy balls" within the vitreous, chorioretinitis, hypopyon, perivasculitis, optic neuritis, and chorioretinal lesions.[36,37]

Although much is known about the epidemiology of fungal endophthalmitis, such as its incidence/prevalence among different populations and associated risk factors, the disease pathobiology is not well understood. Prior studies have mainly used rabbit models of candida endophthalmitis[29,38,39] to gauge the efficacy of antifungal drugs (i.e., drug, dose, time-course, route of administration). However, these studies do not examine the disease process, i.e., the initiation, progression, and eventual termination of the host immune response. Therefore, the development of appropriate experimental models will allow us to gain a better understanding of the pathogenesis of this blinding eye disease. In this study, we evaluated the susceptibility of C57BL/6 and BALB/c, two commonly used mouse strains, to *C. albicans* endophthalmitis and assessed the disease pathobiology and innate immune response.

MATERIALS AND METHODS

Mice and Ethics Statement

C57BL/6 (B6) and BALB/c mice (female, 6–8 weeks old) were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). Mice were housed in a restricted access Division of Laboratory Animal Resources (DLAR) facility within the Kresge Eye Institute and cared for by the DLAR staff. Mice were maintained on a 12-hour/12-hour light/dark cycle, were fed LabDiet rodent chow (LabDiet; Pico Laboratory, St. Louis, MO, USA), and were given water ad libitum. The animals were treated in compliance with the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmologic and Vision Research. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Wayne State University.

Induction of Candida Endophthalmitis

Endophthalmitis was induced in mice by intravitreal injection of *Candida albicans* (CA), strain SC5317. The right eye of each mouse was infected with indicated dosages (colony-forming units [CFU]/eye) of CA, whereas the contralateral eyes injected with phosphate-buffered saline solution (PBS) served as control. Following infection, clinical investigation of each eye was performed periodically using slit-lamp examination. The extent of ocular pathology was graded by the assignment of clinical scores, ranging from 0 to 4, using criteria described previously.[40–42] At the desired time points post-infection, the mice were euthanized, and ocular tissue was harvested for further analysis.

Fungal Burden Estimation

The fungal burden in the infected eyes was determined using the standard plate count method. Briefly, at the desired time points post-infection, mice were euthanized, and eyes were enucleated; they were then homogenized in sterile PBS in a tissue lyser (QIAGEN, Valencia, CA, USA) using stainless steel beads. The eye homogenates were serially diluted in sterile PBS and plated on tryptic soy agar (TSA) plates. Following overnight incubation at 37°C the CA colonies were counted, and the results were expressed as the mean of CFU/eye $\pm$ standard deviation (SD).

Cytokine/Chemokine ELISA

The level of intraocular inflammatory cytokines and chemokines were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits. Twenty micrograms of total protein from eye lysates were used for cytokine/chemokines measurement by ELISA. ELISA for cytokines tumor necrosis factor–α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), and chemokine CXCL2/MIP-2 was performed as per the manufacturer's recommendations (R&D Systems, Minneapolis, MN, USA). The data were presented as mean cytokines/chemokines concentrations (pg/mg of eye lysates) $\pm$ SD.

RNA Extraction and Real-Time PCR Analysis

Total RNA was extracted from the retinal tissue using TRIzol reagent as per manufacturer's instruction (Invitrogen, Carlsbad, CA, USA). cDNA was synthesized from 1.0 µg total RNA using a Maxima First Strand cDNA synthesis kit (ThermoFisher Scientific, Rockford, IL, USA), per the manufacturer's instructions. The quantitative Polymerase Chain Reaction (qPCR) was performed for pro-inflammatory cytokine (*Tnf-α, Il-1β,* and *Il-6*) and chemokine (*Cxcl2/Mip-2*) genes using the StepOnePlus (Applied Biosystems, Grand Island, NY, USA). TaqMan primers and probes (Prime Time mini-qPCR assay) were purchased from Integrated DNA Technologies (IDT, Coralville, IA, USA). Gene expression was quantified by the comparative $\Delta\Delta C_T$ method. The level of gene expression in the test samples was normalized to that of GAPDH (an endogenous reference).

Histological Analysis and Immunofluorescence Staining

Histological analysis by hematoxylin and eosin (H&E) staining was used to examine the extent of retinal tissue damage following CA infection. At the designated time points post-infection, mice were euthanized, and whole eyes were enucleated. Eyes were fixed in 4% formaldehyde and sent for processing. Embedding, sectioning, and H&E staining was performed by Excalibur Pathology, Inc. (Oklahoma City, OK, USA). Keyence microscope (Keyence, Itasca, IL, USA) was used to capture high-resolution images of the histological slides to examine retinal detachment, retinal folding, and tissue infiltration.

Paraffin-embedded sections were used for immunostaining. The eye sections were deparaffinized by immersing slides into o-xylene three times, for five minutes each. The slides were dehydrated and rehydrated by treatment with ethanol gradient (75%, 95%, and 100%) for five minutes each followed by immersion into PBS. To access retinal cell death, terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining was performed on retinal sections using ApopTag fluorescein in situ apoptosis detection kit (Millipore, Billerica, MA, USA) as per the manufacturer's instructions. For neutrophil (PMN) infiltration, sections were immunostained with Fluorescein isothiocyanate (FITC)-conjugated anti-Ly6G antibody (BD Biosciences, Franklin Lakes, NJ, USA). After rinsing thrice with PBS, slides were mounted with Vectashield anti-fade mounting medium containing DAPI (Vector Laboratories, Burlingame, CA, USA) and observed under an Eclipse 90i fluorescence microscope (Nikon, Melville, NY, USA).

Electroretinography (ERG) Analysis

Scotopic electroretinography (ERG) was used to assess retinal function. ERG was performed before CA infection to establish a baseline reading for all eyes, and then at desired time points post CA infection to assess the effect of infection on retinal physiology as described previously.[3,43,44] Briefly, dark-adapted mice were anesthetized using ketamine/xylazine, and pupils were dilated using a 2.5% phenylephrine and 1.0% tropicamide ophthalmic solution. Mouse body temperatures were maintained at 37°C using a heating pad during the procedure. Indifferent, silver-embedded thread eye electrodes (Ocuscience LLC, Kansas City, MO, USA) were used to record the response. Reference needle electrodes (stainless steel subdermal electrodes) were placed in both cheeks, and a ground needle electrode was placed in the tail. The response was acquired by an ERG machine (OcuscienceLLC, Kansas City, MO, USA) and analyzed using ERGVIEW 4.882. Ganzfield light stimulus was used to present ten 10-ms flashes, with light intensities increasing from 0.0001 to 100 cd-s/m². The amplitude of both a- and b-waves was recorded to measure overall retinal ERG response.

Flow Cytometry Analysis

Flow cytometry was used to determine the infiltration of PMNs as described previously.[6,41,42] Briefly, following euthanasia, retinas were isolated from CA infected and control mice at desired time points. To obtain a sufficient number of cells, two retinas were pooled for each time point and immediately digested with Accumax (Millipore) for 10

minutes at 37°C then triturated by passing through a 23-gauge needle/syringe to make a single-cell suspension. The cell suspension was filtered through a 40-µm cell strainer (BD Falcon, San Jose, CA, USA) to remove tissue debris. Cells were rinsed with 0.5% bovine serum albumin (BSA) in PBS and then incubated with Fc Block (BD Biosciences) in the dark for 30 minutes. After blocking, the cells were rinsed with 0.5% BSA and incubated with phycoerythrin (PE)-Cy5-conjugated CD45 and Ly6G-FITC antibodies (BD Biosciences) in the dark for 30 minutes. Single antibody stained cells were used for compensation controls. After two washes, cells were acquired using BD Accuri C6 Flow Cytometer (BD Biosciences) and analyzed using Accuri C6 software.

Statistical Analysis

All data are presented as the mean ± standard deviation (SD) unless otherwise indicated. GraphPad Prism V8 (GraphPad Software, La Jolla, CA, USA) was used for statistical analysis. Student *t*-test or one-way analysis of variance (ANOVA) was used for comparisons followed by Dunnett's' post hoc test wherever applicable. A *P*-value <0.05 was considered statistically significant.

RESULTS

C. albicans Induced Inflammatory Response in Mouse Eyes

To develop a mouse model of candida endophthalmitis, we started this investigation by titrating the infective dose of CA that can induce sustained (non-resolving) inflammation in the eyes, a hallmark of endophthalmitis. We intravitreally injected five different dosages of *C. albicans* (500, 1000, 2500, 6500, and 13000 CFU/eye in 2 µL volume) in B6 mice. Contralateral eyes were injected with 2 µL PBS and served as a control. The disease pathology was evaluated using non-terminal and terminal procedures as described in the timeline shown in Figure 1A. Twenty-four hours post-infection, enucleated eyes were lysed in PBS and processed for fungal burden enumeration by plate count as well as to measure the level of inflammatory cytokines using ELISA. Our results show that CA displays an inoculum-dependent growth in the B6 mouse eyes (Fig. 1B). Similarly, CA induces a dose-dependent inflammatory response in the eyes as indicated by increased production of TNF-α, IL-1β, and IL-6 cytokines in eyes challenged with different dosage of CA (Fig. 1C). Since 6500 and 13000 CFUs showed a comparable inflammatory response, we decided to use 6500 CFU for the induction of endophthalmitis for the remainder of the study.

BALB/c Mice Exhibited Intense Corneal Haze/Opacity and Fungal Burden

Since we observed the induction of an inflammatory response in B6 mouse eyes, which is indicative of CA endophthalmitis, we sought to elucidate the disease pathobiology in depth and evaluate the effects of genetic background on disease susceptibility. We used B6 and BALB/c mice, the two most commonly used mouse strains for immunological studies.[45] Moreover, prior studies have shown differential immune and pathological responses during ocular infection in these mice strains.[46–48] Our results show that intravitreal injection of CA induced a time-
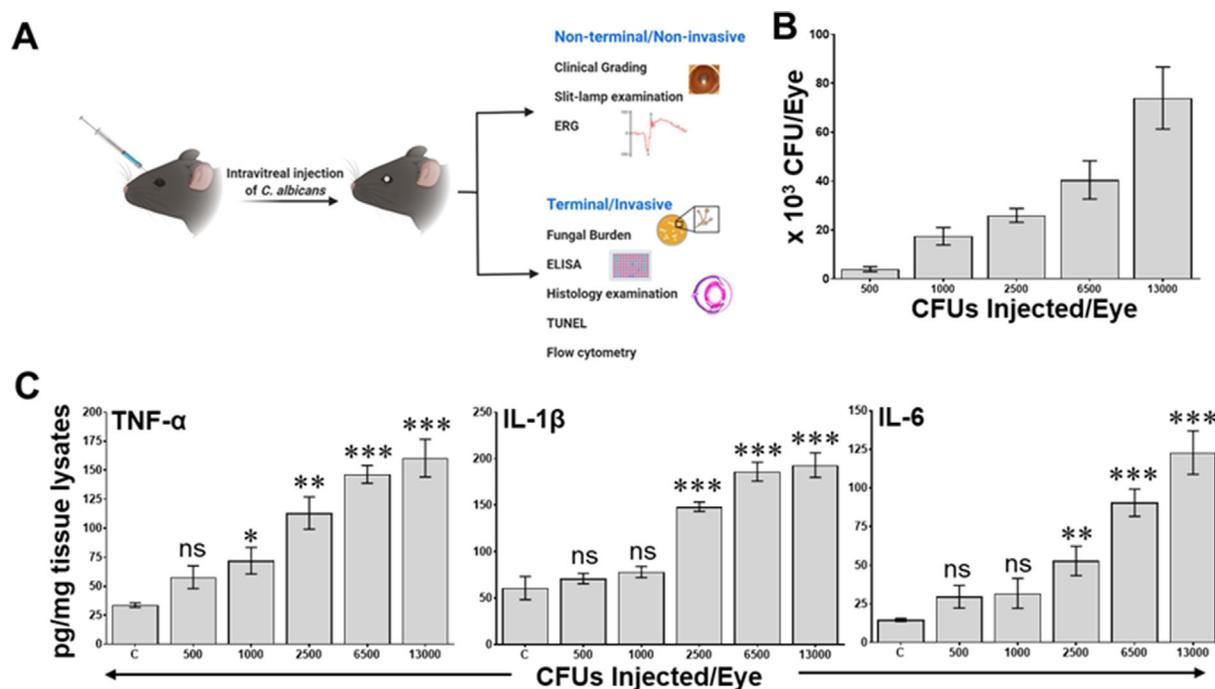
FIGURE 1. *C. albicans* proliferate in mouse eyes and induce an inflammatory response. The eyes of B6 mouse ($n = 6$) were injected with different doses (500, 1000, 2500, 6500, 13000 CFUs/eye) of *C. albicans* (*CA*) strain SC5314 **(A)**. Eyes with PBS injection were used as control (*C*). At 24 hours post-infection eye lysates were subjected to fungal burden estimation by serial dilution and plate count **(B)**. The eye lysates were subjected to ELISA for proinflammatory cytokines (*TNF-α, IL-1β*, and *IL-6*) quantification **(C)**. ns, not significant. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; one-way ANOVA (C vs. CA infection).

dependent increase in corneal haze, anterior chamber opacity, and hypopyon in the eyes of both of these mice strains in comparison to their respective controls. However, differential analyses revealed that BALB/c mice had comparatively higher amount of haze, opacity, and hypopyon in contrast to B6 mice at the indicated time points (Fig. 2A). We also graded the disease pathology using a five-point scale (0 to 4, indicating less to increasing pathology), as described previously.[49,50] We observed a time-dependent increase in clinical score in infected eyes, and the overall clinical scores were higher in BALB/c in comparison to B6 mice (Fig. 2B).

To determine whether there is any strain-specific difference in intraocular fungal growth in these mice, the total fungal burden was estimated. Our time-course data revealed that in both B6 and BALB/c mice, the overall CA growth was stable at 6 and 12 hpi, peaked at 24 to 48 hpi, and declined by 72 to 96 hpi (Fig. 2C). However, in comparison to B6 mice, CFU count was significantly higher in BALB/c mice at their respective time points. Even though the viable fungal count declined at 72 and 96 hpi in both mouse strains, the clinical scores were significantly higher at these time points, indicating persistent intraocular inflammation.

B6 Mouse Eyes had More Inflammatory Mediators During CA Endophthalmitis.

We observed differences in CA induced pathology in B6 versus BALB/c mouse eyes. Therefore, we assessed the retinal innate immune response by measuring the levels of proinflammatory cytokines/chemokines in these mice following CA infection at transcripts, as well as protein levels using qPCR and ELISA, respectively. Our qPCR data showed induced expression of inflammatory cytokines (*Tnf-α, Il-1β,*

and *Il-6*) and chemokine (*Cxcl2/Mip-2*) transcripts following CA infection in both B6 and BALB/c mouse retina with respect to their uninfected control eyes (Fig. 3A). This also coincides with their increased protein levels as quantitated by ELISA (Fig. 3B). The time course analyses revealed that overall mRNA and protein levels of inflammatory mediators increased at 6 to 12 hpi, peaked at 24 to 48 hpi, and slightly decreased by 72 to 96hpi. The differential analyses of BALB/c and B6 mice revealed that the levels of these proinflammatory cytokines were comparatively higher in the B6 mice as compared to BALB/c mice at their respective time points. These results indicate that B6 mice induce a stronger retinal innate immune response as compared to BALB/c mice against CA infection, which may provide protection or delay the disease progression in these mice.

C. albicans Caused More Retinal Tissue Damage and Cell Death in BALB/c Mice

Eyes with infectious endophthalmitis are prone to suffer irreversible retinal tissue damage because of increased microbial growth and persistent host-evoked inflammation.[4,42,51] Here, we performed a histological analysis to assess the extent of CA induced retinal tissue damage in the infected eye of B6 and BALB/c mice. Our data showed that CA caused significant retinal tissue damage in both mouse strains, as evidenced by the disorganization of retinal architecture, retinal detachment, and folding, as well as fibrin deposition and heavy cellular infiltrates in the vitreous cavity. Moreover, the pathological changes were time dependent, with retinal tissue destruction starting at 24 hpi and gradually increasing up to 96 hpi (Fig. 4A). Comparatively, BALB/c mice exhibited more retinal tissue damage than B6 mice, especially at
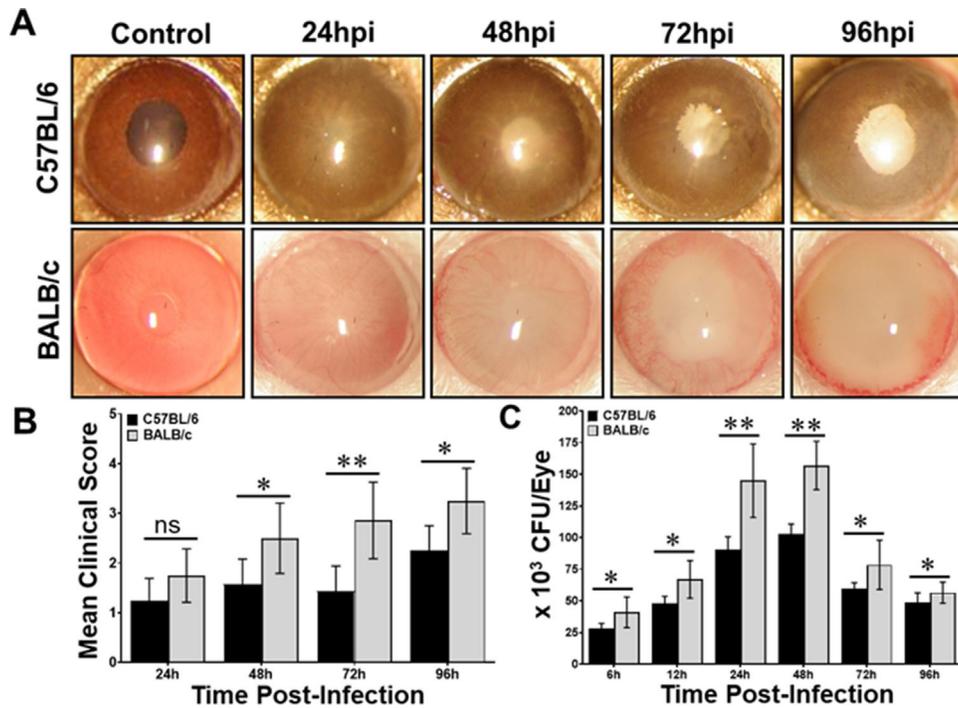
FIGURE 2. *C. albicans* induces endophthalmitis in C57BL/6 and BALB/c mice. C57BL/6 and BALB/c mice eyes (n = 6–8) were infected with *C. albicans* SC5314 (6500 CFU/eye) by intravitreal injection. Eyes with PBS injection at 96 hours post-infection (hpi) were used as control (*C*). Representative micrograph showing CA-induced corneal haze/opacity in the eye **(A)**. The disease progression was quantitated on a five-point scoring system (score of 0 indicating a healthy eye and scores of 1–4 indicating increasing severity) **(B)**. At indicated time points, the whole eye lysates were used for estimation of the intraocular fungal burden by serial dilution and plate count method and represented as CFU/eye **(C)**. ns, not significant; $^{*}P < 0.05$; $^{**}P < 0.01$; Student's *t*-test (B6 vs. BALB/c).
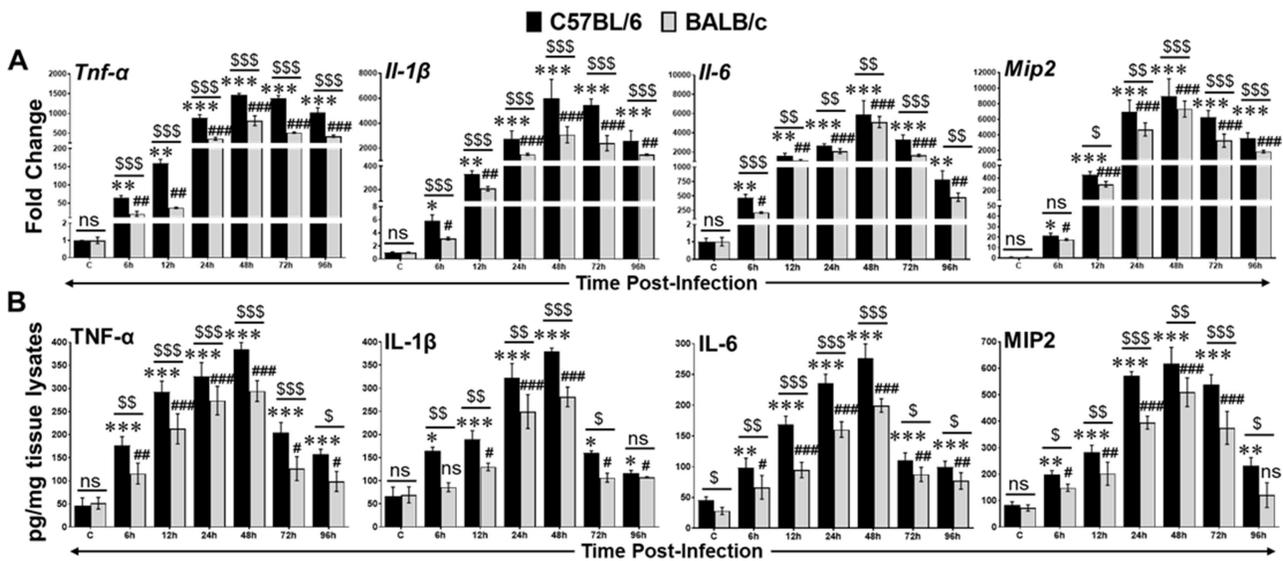

FIGURE 3. C57BL/6 and BALB/c mice exhibit differential inflammatory response during *Candida* endophthalmitis. B6 and BALB/C mouse eyes (n = 6–8) were infected by intravitreal injection of 6500 CFU/eye of *C. albicans* for indicated time points. Eyes with PBS injection harvested at 96 hours post-infection were used as control (*C*). At designated time points, neural retina was harvested and subjected to qPCR analysis for inflammatory mediators, *Tnf-α, Il-6, Il-1β,* and *Mip-2* **(A)**. In another set of experiments, whole eye lysates were subjected to ELISA to quantify the protein levels of inflammatory cytokines **(B)**. ns, not significant; $^{*,\ \#}P < 0.05$; $^{**,\ \#\#}P < 0.01$; $^{***,\ \#\#\#}P < 0.001$; one-way ANOVA (C vs. CA infected); $^{\$}P < 0.05$; $^{\$\$}P < 0.01$; $^{\$\$\$}P < 0.001$; Student's *t*-test (B6 vs. BALB/c).

early time points. However, at 72 hpi or later time points, the damage was too severe in both mouse strains to observe any noticeable distinctions.

To further assess the CA induced retinal cell death, we performed TUNEL staining. Our data showed that CA infection resulted in a time-dependent induction of retinal cell
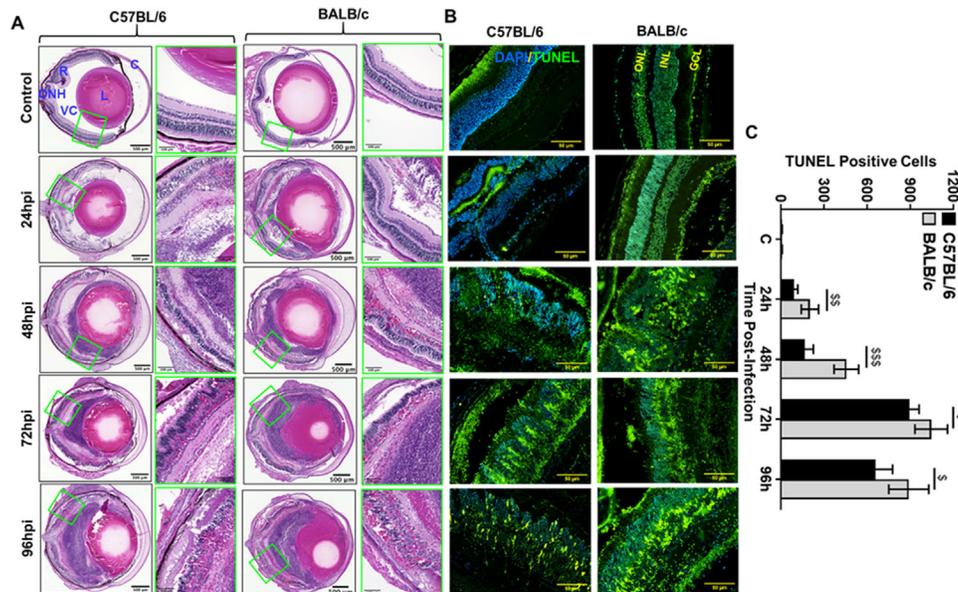
FIGURE 4. *C. albicans* infection causes retinal damage and cell death in the eyes. B6 and BALB/c mouse eyes (n = 6–8) were infected by intravitreal injection of *C. albicans* SC5345 (6500 CFU/eye). Eyes with PBS injection harvested at 96 hours post-infection (hpi) were used as control. At indicated time points post-infection eyes were enucleated, fixed, and stained with H&E **(A)**. Retinal sections were subject to TUNEL staining to evaluate retinal cell death **(B)**. The *green* cells represent TUNEL-positive cells whereas the *blue* (DAPI positive) cells are nuclei. The average TUNEL positive cells were counted and presented as a *bar graph* **(C)**. L, lens; C, cornea; VC, vitreous chamber; R, retina; ONH, optic nerve head; ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer. $^{\$}P < 0.05$; $^{\$\$}P < 0.01$; $^{\$\$\$}P < 0.001$; Student's *t*-test (B6 vs. BALB/c).

death, indicated by increased TUNEL positivity with time (Figs. 4B, 4C). Only a few TUNEL-positive cells were visible at 24 hpi, whereas by 96 hpi TUNEL positivity was all across the retinal layers. In contrast, PBS-injected control eyes did not show any TUNEL-positive cells. The comparative analysis shows a relatively higher number of TUNEL-positive cells in BALB/c than B6 mice at their respective timepoints.

PMN Infiltration was Higher in B6 Mice as Compared to BALB/c

Neutrophils are the first innate immune cells to infiltrate the eye during bacterial or fungal endophthalmitis and provide host defense.[6,41,52–54] However, uncontrolled infiltration of PMNs can cause host-induced irreversible damage to the retina. We performed flow cytometry analysis to quantitate CA-induced PMN infiltration in both B6 and BALB/c mice. To this end, our results indicated that CA infection caused a time-dependent increase in PMN (CD45 and Ly6G, double-positive cells) infiltration in the eyes of both of these mouse strains with respect to their control (Fig. 5A). Comparative analysis revealed that B6 mouse retina had significantly higher PMN infiltration as compared with BALB/c mice (Fig. 5B). The comparative analyses revealed that B6 mice showed a time-dependent increase in PMN infiltration, whereas in BALB/c mice PMNs increased initially up to 48 hpi, but slightly declined thereafter. In addition to flow cytometry, the infiltration of PMNs was also confirmed by immunostaining of retinal sections with the anti-Ly6G-FITC antibody. Similar to flow cytometry data, the immunostaining assay confirmed a time-dependent increase in CA-induced PMN infiltration in these mice strains as revealed by increased Ly6G positivity in infected eyes (Figs. 5C, 5D). These results correlated with the increased early produc-

tion of inflammatory cytokines and chemokines, which ultimately delayed the disease progression in the B6 mice.

Retinal Function Declined Rapidly in Both B6 and BALB/c Mice During CA Endophthalmitis

The aforementioned results indicate the differences in disease pathology and retinal innate immune response in BALB/c versus B6 mice. BALB/c mice exhibited increased corneal haze, fungal burden, and histopathological damage as compared with B6 mice, whereas B6 mice exhibited higher cytokine and PMN infiltration with a slight delay in disease progression as compared with BALB/c mice. To assess the overall role of CA-induced pathology on visual function, we performed scotopic ERG. In both B6 and BALB/c mice, retinal function declined rapidly following CA infection, evident by a significant decrease in a- and b-wave amplitudes by 12 hpi and a near-complete loss of visual function by 24 hpi. In contrast, the control eye injection with PBS showed a minor decline in a- and b-wave amplitudes at the 12-hour time point but fully recovered with time; this decline could be due to a needle-induced injury/wound used for PBS injection. Despite several pathological differences in B6 versus BALB/c mice, we did not observe any significant difference in ERG response in these mouse strains. These results indicate that eye being an immune-privileged organ, the retinal function is highly prone to pathogen/inflammation-mediated damage, and even small pathological changes can lead to a total loss of visual function without therapeutic intervention.

DISCUSSION

C. albicans remains the leading cause of chorioretinitis and endophthalmitis during candidemia, which can lead to
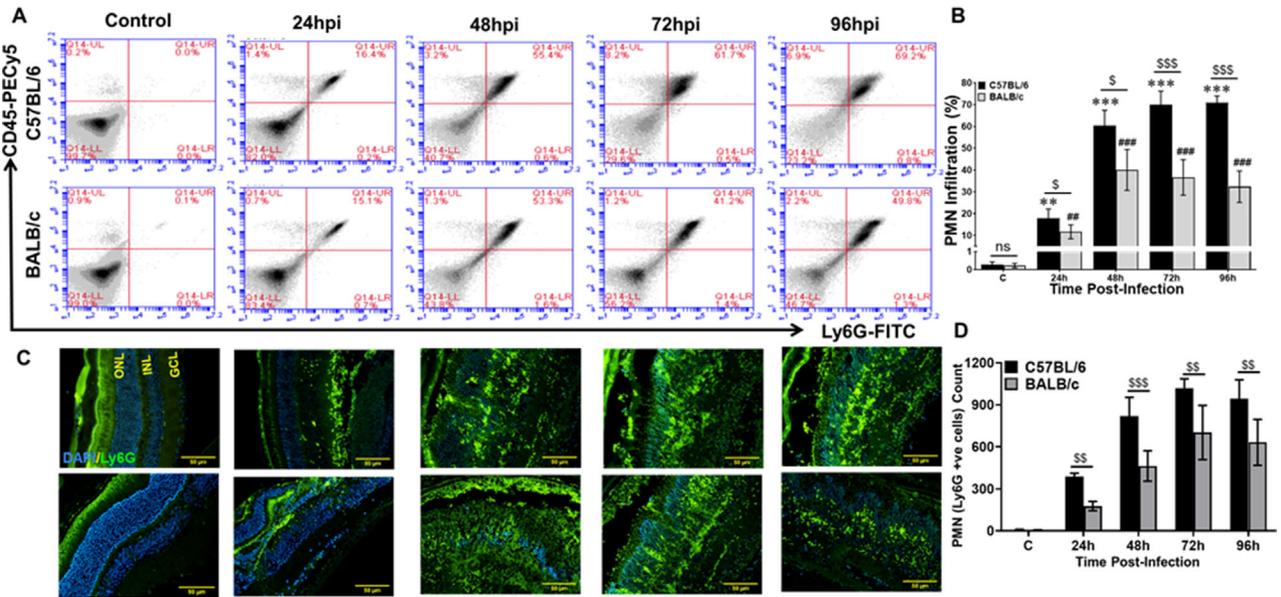
FIGURE 5. *C. albicans* induces PMN infiltration in C57BL/6 and BALB/c mouse eyes. B6 and BALB/C mouse eyes (n = 6–8) were infected by intravitreal injection of *C. albicans* SC5345 (6500 CFU/eye). Eyes with PBS injection harvested at 96 hours post-infection were used as control (*C*). At indicated time points retina were harvested, and single-cell suspensions were stained with anti-CD45-PECy5 and anti-Ly6G-FITC antibodies. The representative *dot plots* indicate CA induced retinal PMN (CD45-Ly6G double-positive) infiltration **(A)**. The *bar graph* represents the quantification of *dot plots*. **(B)**. The retinal sections were immunostained with anti-Ly6G-FITC antibodies and representative images showing *green* positive cells as PMNs **(C)**. The average PMNs (Ly6G-positive cells) were counted and presented as a *bar graph* **(D)**. ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer. **,##P < 0.01; ***,###P < 0.001; one-way ANOVA (C vs. CA infected), $P < 0.05; $$P < 0.01; $$$P < 0.001; Student's *t*-test (B6 vs. BALB/c).
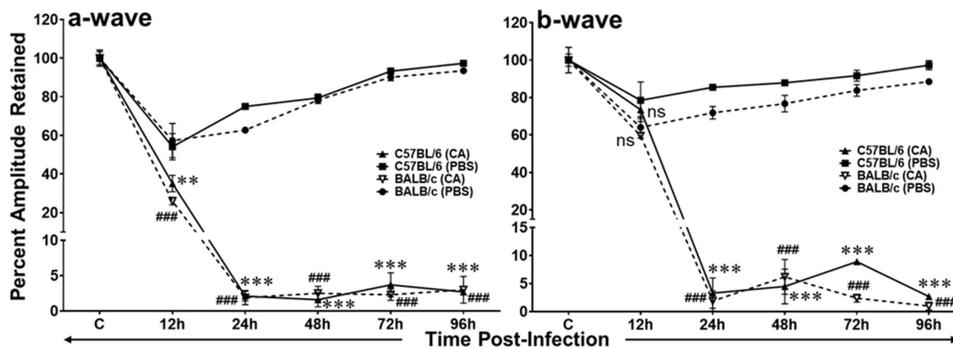

FIGURE 6. Retinal function declines in *C. albicans* infected eyes. B6 and BALB/C mouse eyes (n = 6–8) were infected by intravitreal injection of *C. albicans* SC5345 (6500 CFU/eye). Eyes with PBS injection at 96 hours post-infection were used as controls. Scotopic electroretinogram (ERG) analysis was performed at indicated time points. The *line plot* represents the percent a- and b-wave amplitude retained at indicated time points with respect to control eyes set at 100%. *ns*, not significant; **P < 0.01; ***,###P < 0.001; Student's *t*-test.

significant vision loss.[37] However, the pathobiology of CA endophthalmitis is not well studied, in part due to a lack of experimental models. Therefore, the current study aims to develop mouse models of CA endophthalmitis and to evaluate mouse strain/genetic diversity specific pathological and immunological changes in the disease. Thus, using B6 and BALB/c mouse strains, we demonstrated that CA proliferates in mouse eyes and elicits marked inflammatory response. We also discovered strain-specific differences in retinal innate immune response, disease progression, and ocular pathology, with BALB/c mice more susceptible to CA endophthalmitis than B6 mice. The disease pathology observed in both mouse models correlated with previous

findings in rabbit model[55] and human patients.[56] To the best of our knowledge, this is the first study to elucidate the innate immune responses and pathogenesis of CA endophthalmitis in murine models (Fig. 7).

At this time, relatively little is known about the complex host-pathogen interactions that take place in fungal endophthalmitis. In the rabbit models of candida endophthalmitis, induced by either systemic or the intravitreal route of infection, slight focal edema, hyperemia, irregular pupil, and absence of red reflex is observed along with progressive inflammation and increasing fungal burden in the vitreous chamber at logarithmic scale.[57] In our mouse models, a significant inflammatory response was elicited after CA
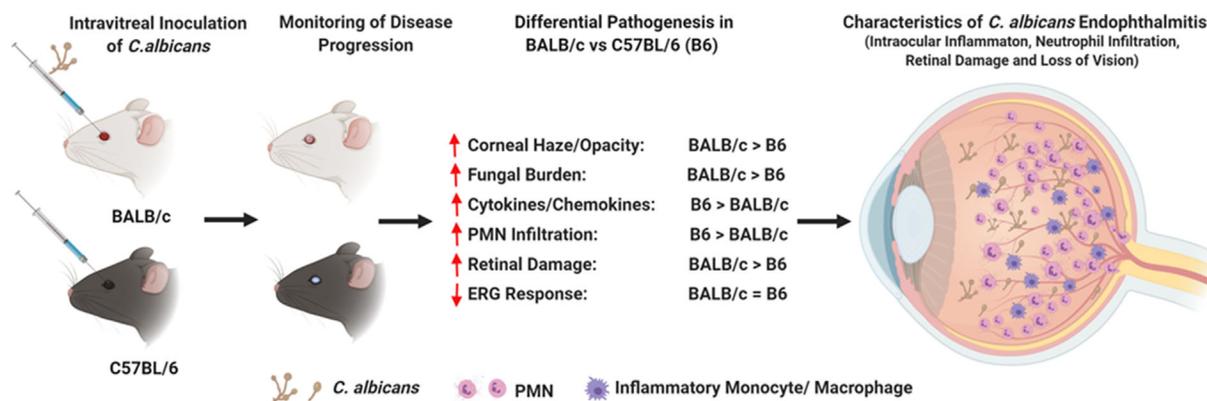
FIGURE 7. Pathobiology of *C. albicans* endophthalmitis. *C. albicans* by intravitreal inoculation can proliferate in the eyes, this results in the massive infiltration of inflammatory immune cells (e.g., PMNs) and the expression of proinflammatory cytokine/chemokines. The increased intraocular fungal burden and host-induced inflammatory response can lead to retinal tissue destruction resulting into loss of visual function and blindness. CA caused differential pathogenic response in BALB/c versus C57BL/6 with BALB/c mice exhibiting increased suscepbtibility to ocular tissue damage.

infection, along with a time-dependent increase in corneal haze and opacity. Because the eyes of BALB/c mice are devoid of pigment in both the iris and the retinal pigment epithelium, the degree of corneal haze was much easier to visualize as compared to B6 mice. Regardless, both mouse models exhibited an increase in ocular pathology and fungal burden which peaked at 48 hpi and declined thereafter. The comparative analysis revealed that the intraocular fungal burden was significantly higher in BALB/c than B6 mice, indicating a more favorable milieu for fungal growth in BALB/c mouse eyes. In contrast, B6 mice had significantly higher levels of inflammatory cytokines/chemokines than BALB/c mice. As inflammatory mediators are known to recruit innate immune cells, such as neutrophils, to eliminate invading pathogens, their increased levels in B6 mice might correlate with the reduced fungal burden. Interestingly, in our models, the pro-inflammatory mediators were induced as early as 6 hpi with a time-dependent increase, whereas in the systemic model of ocular candidiasis, the induction of IL-1β and TNF-α is not observed untill three days post-infection.[58] These observed differences could be due to the exogenous versus endogenous route of CA inoculation of the eye.

At one end host-evoked inflammatory response aids in combating infectious endophthalmitis, while on another hand, persistent inflammation and infiltration of immune cells can lead to host-induced damage which eventually result in disruption of the normal visual axis and vision loss.[6,56] In our mouse models, we observed an upregulated cytokine/chemokine response, as well as an increase in cellular infiltration in the vitreous cavity, accompanied by retinal folding/detachment and significant damage to retinal architecture. Infiltrating PMNs are considered as the first line of defense during infectious endophthalmitis and are one of the main cell types involved in the initiation of an inflammatory response. During ocular infections, residential retinal innate immune cells, such as the microglia[59] and the Müller glia,[60] secrete neutrophil chemotactic factors that attract PMNs to the site of infection. We observed that CA infection leads to a significant increase in the infiltration of PMNs into the retina. Our comparative analyses revealed that B6 mice had increased PMN infiltration than BALB/c which coincided with higher levels of neutrophil chemoattractant, CXCL2/MIP2, in B6 versus BALB/c mice. It is well

known that mouse genetic background plays an important role in orchestrating immune responses with studies showing that B6 mice generate Th1-response whereas BALB/c mice favor Th2-response.[61–63] The effect of inflammatory cytokines on Th1/Th2 skewing has been shown to play a protective or detrimental role against pathogens.[64] For example, TNFα has been shown to promote macrophage activation in Th1 environment leading to protection, while, in the presence of Th1+Th2 response it causes necrosis during Mycobacterial infection.[65] Similarly, different dosages of *Leishmania major* have been shown to skew the Th2 versus Th1 response in Th2-dominant BALB/c mice leading to its susceptibility or resistance.[66] These studies indicate that the type of inflammatory cytokines and infectious dosage of pathogens modulate the Th1/Th2 response leading to susceptibility and resistance toward infections. Similarly, BALB/c mice have been reported to fail in facilitating bacterial killing in comparison to C57BL/6 mice in a murine model of septic peritonitis, which is attributed to their differential cytokine response.[62] Our data showing lower fungal burden and delayed disease progression in B6 mice in contrast to BALB/c mice, could be attributed to increased PMN infiltration and production of inflammatory mediators. Therefore, these strain-specific differences in the microenvironment may alter the Th1/Th2 milieu and ultimately susceptibility to the infection in CA endophthalmitis. Reduced neutrophil chemotaxis and infiltration have been shown to contribute in the disease progression in various infection models.[67–69] In one of our recent studies, we also demonstrated that depletion of PMNs increases mice susceptibility to *Aspergillus endophthalmitis*, which results in severe ocular pathology.[70] Inflammation and infiltrating immune cells work as a double-edged sword: they help in controlling infection but can also contribute to damaging tissue.[49,61,71,72] The visual function of the retina is highly susceptible to the host-induced inflammatory damage. In our current study, we observed a significant decline in ERG response with Candida infection by 12 hpi and nearly a total loss of visual function just in 24 hours of infection. Although we observed differences in disease pathology in BALB/c and B6 mice, the overall loss of visual function was similar in these mice.

In this study, we examined the effects of *C. albicans* endophthalmitis in two commonly used laboratory mouse

strains, C57BL/6 and BALB/c. We chose these two mouse strains because, (1) both of these strains are well known and readily available for use in most laboratories, and (2) studies have suggested that the inherent genetic differences between these two mouse strains may play an important role in the pathology of various ocular diseases.[46,48] Studies have shown that the Th1-dominant C57BL/6 mouse is highly susceptible to corneal destruction by *Pseudomonas aeruginosa*, whereas the Th2-dominant BALB/c mouse is resistant to the disease.[63,73,74] Similarly, a study by Sugi et al.[61] demonstrated that C57BL/6 mice, which express the FasL.1 allotype, are highly susceptible to *Staphylococcus aureus* endophthalmitis, while BALB/c mice, which express the FasL.2 allotype, are less susceptible to the disease. In both of these studies, this difference is attributed to the lower intensity of the inflammatory response seen in BALB/c mice during bacterial infection. It is well known that the variability in the inflammatory response seen between these two mouse strains is primarily due to the Th1-vs.-Th2 dichotomy. However, in contrast to bacterial endophthalmitis, we observed that BALB/c mice are more susceptible to fungal (candida) endophthalmitis in comparison to B6 mice. Although there are no studies available testing the susceptibility of mouse strains to ocular fungal infections, Xin et al.[75] evaluated the susceptibility of A/J, BALB/c and B6 mice to systemic Candida infection. In this study, systemic (intravenous) inoculation of 5×10^5 CFU of CA resulted in the death of BALB/c mice within one to two weeks, whereas the B6 mice survived for two to three weeks, even with a higher infective dose of 1×10^6 CFU.[75] These findings led to the conclusion that BALB/c mice are more susceptible to CA infection as compared with B6 mice. These discoveries also indicate differences in the susceptibilities of mouse strains in bacterial versus fungal endophthalmitis. In this study, we investigated the pathobiology of exogenous CA endophthalmitis; it would be interesting to evaluate the susceptibility during endogenous endophthalmitis. Indeed, we are establishing a murine model of endogenous candida endophthalmitis and investigating the mechanism of breach of the blood-retinal barrier by CA to enter into the eye.

In conclusion, our study is the first to demonstrate the pathobiology of CA endophthalmitis in the murine models and the differential susceptibility of B6 and BALB/c mice. While we observed a disparity in immune response (e.g., cytokines/chemokine expression and PMN infiltration) and pathological changes (histological damage and cell death) in B6 versus BALB/c mouse eyes, the overall disease outcome, i.e., loss of retinal function, was similar. These models can be used as cost-efficient, time-effective, reproducible models to understand host immune response during CA-induced endophthalmitis and other important fungal pathogens. With the increased use of immunosuppressive agents, a key risk factor for ocular candidiasis, it is now more important than ever that we understand the nature of this disease and develop new and better methods to diagnose and treat candida endophthalmitis.

Disclosure: **B.G. Rottmann**, None; **P.K. Singh**, None; **S. Singh**, None; **S.G. Revankar**, None; **P.H. Chandrasekar**, None; **A. Kumar**, None

References

1. Durand ML. Endophthalmitis. *Clin Microbiol Infect*. 2013;19:227–234.
2. Vaziri K, Schwartz SG, Kishor K, Flynn HW, Jr. Endophthalmitis: state of the art. *Clin Ophthalmol*. 2015;9:95–108.
3. Guest JM, Singh PK, Revankar SG, Chandrasekar PH, Kumar A. Isavuconazole for treatment of experimental fungal endophthalmitis caused by *Aspergillus fumigatus*. *Antimicrob Agents Chemother*. 2018;62:e01537–e01618.
4. Miller FC, Coburn PS, Huzzatul MM, LaGrow AL, Livingston E, Callegan MC. Targets of immunomodulation in bacterial endophthalmitis. *Progr Retinal Eye Res*. 2019;73:100763.
5. Pandey RK, Yu FS, Kumar A. Targeting toll-like receptor signaling as a novel approach to prevent ocular infectious diseases. *Indian J Med Res*. 2013;138:609–619.
6. Gupta N, Singh PK, Revankar SG, Chandrasekar PH, Kumar A. Pathobiology of Aspergillus Fumigatus Endophthalmitis in Immunocompetent and Immunocompromised Mice. *Microorganisms*. 2019;7:297.
7. Durand ML. Bacterial and Fungal Endophthalmitis. *Clin Microbiol Rev*. 2017;30:597–613.
8. Chakrabarti A, Shivaprakash MR, Singh R, et al. Fungal endophthalmitis: fourteen years' experience from a center in India. *Retina*. 2008;28:1400–1407.
9. Dave VP, Pappuru RR, Pathengay A, et al. Aspergillus Endophthalmitis: Clinical Presentations and Factors Determining Outcomes. *Asia Pac J Ophthalmol (Phila)*. 2020;9:9–13.
10. Joseph J, Sontam B, Guda SJM, et al. Trends in microbiological spectrum of endophthalmitis at a single tertiary care ophthalmic hospital in India: a review of 25 years. *Eye*. 2019;33:1090–1095.
11. Klotz SA, Penn CC, Negvesky GJ, Butrus SI. Fungal and parasitic infections of the eye. *Clin Microbiol Rev*. 2000;13:662–685.
12. Kalkanci A, Ozdek S. Ocular fungal infections. *Current Eye Research*. 2011;36:179–189.
13. Son HJ, Kim MJ, Lee S, et al. Risk factors and outcomes of patients with ocular involvement of candidemia. *PLoS One*. 2019;14:e0222356.
14. Oude Lashof AM, Rothova A, Sobel JD, et al. Ocular manifestations of candidemia. *Clin Infect Dis*. 2011;53:262–268.
15. Donahue SP, Greven CM, Zuravleff JJ, et al. Intraocular candidiasis in patients with candidemia. Clinical implications derived from a prospective multicenter study. *Ophthalmology*. 1994;101:1302–1309.
16. Kato H, Yoshimura Y, Suido Y, et al. Prevalence of, and risk factors for, hematogenous fungal endophthalmitis in patients with Candida bloodstream infection. *Infection*. 2018;46:635–640.
17. Yapar N. Epidemiology and risk factors for invasive candidiasis. *Ther Clin Risk Manag*. 2014;10:95–105.
18. Mayer FL, Wilson D, Hube B. *Candida albicans* pathogenicity mechanisms. *Virulence*. 2013;4:119–128.
19. Duhring S, Germerodt S, Skerka C, Zipfel PF, Dandekar T, Schuster S. Host-pathogen interactions between the human innate immune system and Candida albicans-understanding and modeling defense and evasion strategies. *Front Microbiol*. 2015;6:625.

Investigative Ophthalmology & Visual Science

20. Kim J, Sudbery P. *Candida albicans*, a major human fungal pathogen. *J Microbiol*. 2011;49:171–177.

21. Munoz P, Vena A, Padilla B, et al. No evidence of increased ocular involvement in candidemic patients initially treated with echinocandins. *Diagn Microbiol Infect Dis*. 2017;88:141–144.

22. Rodriguez-Adrian LJ, King RT, Tamayo-Derat LG, Miller JW, Garcia CA, Rex JH. Retinal lesions as clues to disseminated bacterial and candidal infections: frequency, natural history, and etiology. *Medicine (Baltimore)*. 2003;82:187–202.

23. Brooks RG. Prospective study of *Candida endophthalmitis* in hospitalized patients with candidemia. *Arch Intern Med*. 1989;149:2226–2228.

24. Parke DW, 2nd, Jones DB, Gentry LO. Endogenous endophthalmitis among patients with candidemia. *Ophthalmology*. 1982;89:789–796.

25. Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. *Clin Infect Dis*. 2004;39:309–317.

26. Pappas PG, Kauffman CA, Andes DR, et al. Clinical Practice Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society of America. *Clin Infect Dis*. 2016;62:e1–e50.

27. Verfaillie C, Weisdorf D, Haake R, Hostetter M, Ramsay NK, McGlave P. Candida infections in bone marrow transplant recipients. *Bone Marrow Transplant*. 1991;8:177–184.

28. Kullberg BJ, Arendrup MC. Invasive Candidiasis. *N Engl J Med*. 2015;373:1445–1456.

29. Arikan S, Ozdek S, Aktas Z, Dincel A, Hasanreisoglu B. Vitreous and aqueous penetration of intravitreally and orally administered moxifloxacin in an experimental rabbit model of fungal endophthalmitis. *J Pharm Pharmacol*. 2013;65:659–664.

30. Kim DY, Moon HI, Joe SG, Kim JG, Yoon YH, Lee JY. Recent clinical manifestation and prognosis of fungal endophthalmitis: a 7-year experience at a tertiary referral center in Korea. *J Korean Med Sci*. 2015;30:960–964.

31. Chen JY, Jones MN, Srinivasan S, et al. Endophthalmitis after penetrating keratoplasty. *Ophthalmology*. 2015;122:25–30.

32. Wilhelmus KR, Hassan SS. The prognostic role of donor corneoscleral rim cultures in corneal transplantation. *Ophthalmology*. 2007;114:440–445.

33. Zhuang H, Ding X, Gao F, et al. Optical coherence tomography features of retinal lesions in Chinese patients with endogenous *Candida endophthalmitis*. *BMC Ophthalmol*. 2020;20:52.

34. Ueda T, Takesue Y, Tokimatsu I, et al. The incidence of endophthalmitis or macular involvement and the necessity of a routine ophthalmic examination in patients with candidemia. *PLOS ONE*. 2019;14:e0216956.

35. Zhuang H, Ding X, Gao F, et al. Optical coherence tomography features of retinal lesions in Chinese patients with endogenous *Candida endophthalmitis*. *BMC Ophthalmology*. 2020;20:52.

36. Vilela RC, Vilela L, Vilela P, et al. Etiological agents of fungal endophthalmitis: diagnosis and management. *Int Ophthalmol*. 2014;34:707–721.

37. Pappas PG, Kauffman CA, Andes DR, et al. Clinical practice guideline for the management of candidiasis: 2016 update by the Infectious Diseases Society of America. *Clin Infect Dis*. 2016;62:e1–e50.

38. Deren YT, Ozdek S, Kalkanci A, Akyurek N, Hasanreisoglu B. Comparison of antifungal efficacies of moxifloxacin, liposomal amphotericin B, and combination treatment in experimental *Candida albicans* endophthalmitis in rabbits. *Can J Microbiol*. 2010;56:1–7.

39. Gupta SK, Dhingra N, Velpandian T, Jaiswal J. Efficacy of fluconazole and liposome entrapped fluconazole for *C. albicans* induced experimental mycotic endophthalmitis in rabbit eyes. *Acta Ophthalmologica Scandinavica*. 2000;78:448–450.

40. Whiston EA, Sugi N, Kamradt MC, et al. alphaB-crystallin protects retinal tissue during Staphylococcus aureus-induced endophthalmitis. *Infect Immun*. 2008;76:1781–1790.

41. 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.

42. Talreja D, Kaye KS, F-s Yu, Walia 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.

43. Singh PK, Donovan DM, Kumar A. Intravitreal injection of the chimeric phage endolysin Ply187 protects mice from *Staphylococcus aureus* endophthalmitis. *Antimicrob Agents Chemother*. 2014;58:4621–4629.

44. Francis R, Singh PK, Singh S, Giri S, Kumar A. Glycolytic inhibitor 2-deoxyglucose suppresses inflammatory response in innate immune cells and experimental staphylococcal endophthalmitis. *Exp Eye Res*. 2020;197:108079.

45. Sellers RS, Clifford CB, Treuting PM, Brayton C. Immunological variation between inbred laboratory mouse strains: points to consider in phenotyping genetically immunomodified mice. *Veterinary Pathology*. 2011;49:32–43.

46. Wu M, McClellan SA, Barrett RP, Hazlett LD. β-Defensin-2 Promotes Resistance against Infection with *P. aeruginosa*. *J Immunol*. 2009;182:1609–1616.

47. Zhou Z, Barrett RP, McClellan SA, et al. Substance P delays apoptosis, enhancing keratitis after *Pseudomonas aeruginosa* infection. *Invest Ophthalmol Vis Sci*. 2008;49:4458–4467.

48. Shi H, Ebrahim AS, Berger EA. A contrast in pathogenic responses between C57BL/6J and BALB/cJ mice using a model of retinal injury. *Am J Pathol*. 2018;188:2717–2728.

49. 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.

50. Kumar A, Singh CN, Glybina IV, Mahmoud TH, Yu FS. Toll-like receptor 2 ligand-induced protection against bacterial endophthalmitis. *J Infect Dis*. 2010;201:255–263.

51. 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:e0128423–e0128423.

52. Guest JM, Singh PK, Revankar SG, Chandrasekar PH, Kumar A. Isavuconazole for treatment of experimental fungal endophthalmitis caused by *Aspergillus fumigatus*. *Antimicrob Agents Chemother*. 2018;62:e01537–e01518.

53. Francis R, Singh PK, Singh S, Giri S, Kumar A. Glycolytic inhibitor 2-deoxyglucose suppresses inflammatory response in innate immune cells and experimental staphylococcal endophthalmitis. *Exp Eye Res*. 2020;197:108079.

54. Mursalin MH, Coburn PS, Livingston E, et al. S-layer impacts the virulence of bacillus in endophthalmitis. *Invest Ophthalmol Vis Sci*. 2019;60:3727–3739.

55. Omuta 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.

56. Durand ML. Endophthalmitis. *Clin Microbiol Infect*. 2013;19:227–234.

57. Livermore JL, Felton TW, Abbott J, et al. Pharma-cokinetics and pharmacodynamics of anidulafungin for experimental *Candida endophthalmitis*: insights into the utility of echinocandins for treatment of a potentially sight-threatening infection. *Antimicrob Agents Chemother*. 2013;57:281–288.

58. Abe M, Kinjo Y, Ueno K, et al. Differences in Ocular Complications Between Candida albicans and Non-albicans Candida Infection Analyzed by Epidemiology and a Mouse Ocular Candidiasis Model. *Front Microbiol*. 2018;9:2477.

59. Kochan T, Singla A, Tosi J, Kumar A. Toll-like receptor 2 ligand pretreatment attenuates retinal microglial inflammatory response but enhances phagocytic activity toward Staphylococcus aureus. *Infect Immun*. 2012;80:2076–2088.

60. Shamsuddin N, Kumar A. TLR2 mediates the innate response of retinal Muller glia to *Staphylococcus aureus*. *J Immunol*. 2011;186:7089–7097.

61. 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.

62. Watanabe H, Numata K, Ito T, Takagi K, Matsukawa A. Innate immune response in Th1- and Th2-dominant mouse strains. *Shock*. 2004;22:460–466.

63. Hazlett LD, McClellan S, Kwon B, Barrett R. Increased severity of *Pseudomonas aeruginosa* corneal infection in strains of mice designated as Th1 versus Th2 responsive. *Invest Ophthalmol Vis Sci*. 2000;41:805–810.

64. Bretscher P. On analyzing how the Th1/Th2 phenotype of an immune response is determined: classical observations must not be ignored. *Front Immunol*. 2019;10:1234.

65. Hernandez-Pando R, Rook GA. The role of TNF-alpha in T-cell-mediated inflammation depends on the Th1/Th2 cytokine balance. *Immunology*. 1994;82:591–595.

66. Bretscher PA, Wei G, Menon JN, Bielefeldt-Ohmann H. Establishment of stable, cell-mediated immunity that makes "susceptible" mice resistant to Leishmania major. *Science*. 1992;257:539–542.

67. Brubaker AL, Rendon JL, Ramirez L, Choudhry MA, Kovacs EJ. Reduced neutrophil chemotaxis and infiltration contributes to delayed resolution of cutaneous wound infection with advanced age. *J Immunol*. 2013;190:1746–1757.

68. Bubeck SS, Cantwell AM, Dube PH. Delayed inflammatory response to primary pneumonic plague occurs in both outbred and inbred mice. *Infect Immun*. 2007;75:697–705.

69. Fournier BM, Parkos CA. The role of neutrophils during intestinal inflammation. *Mucosal Immunol*. 2012;5:354–366.

70. Gupta N, Singh PK, Revankar SG, Chandrasekar PH, Kumar A. Pathobiology of Aspergillus Fumigatus Endophthalmitis in Immunocompetent and Immunocompromised Mice. *Microorganisms*.; 2019;7(9):297.

71. Talreja D, Kaye KS, F-s Yu, Walia SK, Kumar A. Pathogenicity of ocular isolates of Acinetobacter baumannii in a mouse model of bacterial endophthalmitis. *Investigative Ophthalmology & Visual Science*. 2014:2392–2402.

72. Livingston ET, Mursalin MH, Callegan MC. A Pyrrhic Victory: The PMN Response to Ocular Bacterial Infections. *Microorganisms*. 2019;7(11):537.

73. McClellan SA, Huang X, Barrett RP, van Rooijen N, Hazlett LD. Macrophages restrict Pseudomonas aeruginosa growth, regulate polymorphonuclear neutrophil influx, and balance pro- and anti-inflammatory cytokines in BALB/c mice. *J Immunol*. 2003;170:5219–5227.

74. Hazlett LD. Corneal response to *Pseudomonas aeruginosa* infection. *Prog Retin Eye Res*. 2004;23:1–30.

75. Xin H, Mohiuddin F, Tran J, Adams A, Eberle K. Experimental mouse models of disseminated *Candida auris* infection. *mSphere*. 2019;4:e00339–19.