Monitoring of Strain-Dependent Responsiveness to TLR Activation in the Mouse Anterior Segment Using SD-OCT

Laura Elizabeth Downie, Matthew James Stainer, and Holly Rose Chinnery

Department of Optometry and Vision Sciences, University of Melbourne, Parkville, Victoria, Australia

Correspondence: Holly Rose Chinnery, Department of Optometry and Vision Sciences, University of Melbourne, Parkville, VIC, Australia 3010; holly.chinnery@unimelb.edu.au
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PURPOSE. To determine whether spectral-domain optical coherence tomography (SD-OCT) can be used to longitudinally monitor inflammation in the mouse anterior segment and to identify any strain-dependent differences in responsiveness to distinct toll-like receptor (TLR) ligands.

METHODS. Corneal inflammation was induced in BALB/c and C57BL/6 mice following central corneal abrasions and topical application of saline, TLR-4 ligand, lipopolysaccharide (LPS), or TLR-9 ligand, CpG-oligodeoxynucleotide (CpG-ODN; CpG). Anterior-segment images were captured using SD-OCT at baseline, 24 hours, and 1 week post treatment. Corneal thickness, stromal haze, and the number of keratic precipitates (KP) and anterior chamber (AC) cells were longitudinally compared to determine differences between mouse strains, time points, and TLR activation.

RESULTS. In both mouse strains, treatment with CpG, but not saline or LPS, resulted in a similar number of KPs and AC cells. In C57BL/6 mice, central corneal thickness (CCT) increased in CpG- and LPS-treated eyes at 24 hours, which normalized by 1 week. In BALB/c mice, a significant increase in CCT occurred in eyes treated with CpG at 1 week. Stromal haze peaked in C57BL/6 eyes treated with LPS- or CPG-treatment at 24 hours; however, BALB/c eyes showed persistent and marked increases in corneal haze compared with baseline at 1 week post treatment.

CONCLUSIONS. Spectral-domain OCT enables high-resolution, longitudinal, in vivo imaging of anterior segment inflammation in mice and revealed novel strain- and time-dependent differences in response to distinct TLR activation in the cornea.

Keywords: SD-OCT, inflammation, cornea, toll-like receptors, imaging

Rodent models of corneal and anterior segment inflammation are commonly used to investigate the mechanisms and immunopathology of ocular disease, including sterile inflammation, uveitis, and microbial keratitis.1–3 Traditionally, examination of cellular and structural changes in the anterior segment in animal studies of corneal/uveal inflammation is performed using histology. Major limitations of these methods include the inability to progressively monitor the pathological processes affecting tissue architecture over time within a single experimental cohort, and the need to use large numbers of animals and several tissue sections to ensure that a representative area of the eye is examined. The potential for the introduction of histologic artifacts including tissue shrinkage, swelling, and distortion, may also complicate interpretation of the immunopathology.

Optical coherence tomography (OCT), which uses optical interferometry to detect reflected, nonscattered, coherent light, was first applied to retinal imaging approximately 20 years ago.4 This technique enables the noninvasive acquisition of high-resolution, cross-sectional images of the eye. Optical coherence tomography has subsequently evolved as a clinically-relevant tool for assessing the posterior and anterior segments during ophthalmic evaluation. Application of OCT technology to the research domain, including both clinical and experimental animal investigations, has enabled novel, in vivo insights into a spectrum of ocular diseases. While OCT has been primarily adopted to evaluate the posterior segment,5 there is a relative paucity of literature relating to its utility for anterior segment assessment, particularly as a research tool for evaluating ocular pathology in experimental animal models of disease.

Corneal and anterior segment inflammation can be experimentally induced by applying toll-like receptor (TLR) ligands to an injured mouse cornea.6-7 Toll-like receptors are pattern recognition receptors that recognize distinct components of microbial pathogens and also endogenous molecules.8 Bacterial lipopolysaccharide (LPS), which is a feature of gram-negative bacteria, is known to activate the host cell surface receptor TLR4.9,10 The human and mouse cornea express TLR4 and can functionally respond to LPS in vitro and in vivo.11,12 Intracellular TLR9 recognizes unmethylated CpG motifs found in high concentrations in bacterial DNA and viral DNA.13 Application of synthetic CpG oligodeoxynucleotide (CpG-ODN; referred to herein as CpG) to a debrided cornea has been demonstrated to partially mimic inflammation that occurs in response to herpes simplex virus infection.14,15

Immunohistochemical analysis of corneal cross-sections at 24 hours post treatment has shown that both LPS and synthetic CpG induce neutrophil and macrophage infiltration of the corneal stroma, increased corneal thickness and stromal haze in C57BL/6 mice.5–7 Few studies of TLR ligand-mediated ocular inflammation have compared the responsiveness of the two mouse strains commonly used in experimental animal studies of ocular disease, namely C57BL/6j and BALB/c. There is evidence for strain-dependent responses to bacterial keratitis,16
with dominant T-helper type-1 (Th1)-responder C57BL/6 mouse strains being more susceptible to corneal perforation than dominant Th2 responder strains, such as BALB/c mice, in an experimental model of Pseudomonas aeruginosa infection.\textsuperscript{16,17} However, it is unclear whether similar strain-dependent differences exist in relation to corneal TLR activation and/or the nature of the time-course of the anterior segment inflammatory response in vivo.

This study investigated whether SD-OCT could be used to accurately monitor temporal changes in the anterior segment inflammatory response in vivo and discern differences in the responsiveness of two different mouse strains to two distinct TLR ligands. Our results demonstrate the merit of using SD-OCT to longitudinally quantify and assess anterior ocular inflammation in an experimental animal model and highlight novel differences in the anterior segment response following equivalent challenge with TLR ligands. In particular, susceptibility to changes in central corneal thickness (CCT) show a strong strain-dependence that is most pronounced with TLR9 receptor activation.

**Materials and Methods**

**Animals and Induction of Corneal Inflammation**

Female wild-type C57BL/6 and BALB/c mice aged 6 to 10 weeks (n = 6–9 per group) were purchased from the Animal Resources Centre (Canning Vale, WA, Australia). Once deeply anesthetized (ketamine/xylasine; 80:10mg/kg; Therapon, Burwood, Victoria, Australia), anterior segment SD-OCT was performed (time 0 hours, see below). Immediately following imaging, the central corneal epithelium (1-mm diameter) was debrided as previously described.\textsuperscript{6} Following debridement, 20 µg of either phosphorothioate Cpg oligonucleotide 1826 (Type B, TLR9 ligand; Invivogen, San Diego, CA, USA), Ultra Pure Escherichia coli LPS (strain K12, TLR4 ligand), or sterile saline was applied topically. The anterior segment was imaged at time 0 hours (baseline), 24 hours, and at 1 week post treatment. Mice were euthanized at 1 week after treatment by lethal injection of sodium pentobarbitone (Therapon). All procedures were approved by the Florey Institute of Neuroscience and Mental Health Animal ethics committee and conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Optical Coherence Tomography**

Once anesthetized, one drop of sterile saline was applied to the eye to prevent corneal drying. Mice were placed on the animal imaging mount and rodent alignment stage (AIM-RAS) attached to the Bioptigen Envisu R2200 VHR SD-OCT (Bioptigen, Inc., Durham, NC, USA). Volumetric 4 × 4 mm rectangular scans (1000 A-scans/100 B-scans), were captured using the 18-mm telecentric lens for quantifying inflammatory cells in the anterior chamber (AC) and keratic precipitates (KPs) and for calculating CCT and peripheral corneal thickness (PCT). Volumetric 1.4 × 1.4 mm scans (1000 A-scans/100 B-scans) of the central cornea were captured for measuring corneal haze. All scans were analyzed using ImageJ software (http://image.nih.gov/ij/; provided in the public domain by the National Institutes of Health, Bethesda, MD, USA).

**SD-OCT Image Analysis**

The methodological approaches to SD-OCT image analyses are summarized in Figure 1, which shows representative anterior segment images from a BALB/c mouse.

**Anterior Segment Cellular Response.** The anterior segment cellular response was manually calculated using 4 × 4 mm frames (Fig. 1A). Keratic precipitates were defined as circumscribed, white punctate spots abutting the corneal endothelium (Fig. 1B, white arrows). Anterior chamber (AC) cells were classified as individual opaque spots with free borders that did not contact the iris, lens, cornea, or trabecular meshwork (Fig. 1C, arrowheads). Lens cells were considered as white, punctate spots positioned on the anterior aspect of the intraocular lens (Fig. 1D, black arrow). The central image frame (‘C0’, Figs. 1E, 1F) was determined by selecting the frame with maximal pupil dilatation. Keratic precipitates and AC cells were counted in each frame either inferior or superior to C0. The ‘anterior segment cellular response’ was defined as the total number of cells (KPs + AC cells + lens cells) quantified within an entire eye. To confirm that KPs were adherent to the endothelium, and not a temporary artifact due to supine positioning during imaging, three mice at experimental endpoint were rotated 180° within the AIM-RAS holder and images were acquired. Cells were counted in the artificial superior and inferior hemifields and compared with original, prone positions.

**Corneal Thickness.** Corneal thickness was measured for the central, superior peripheral, and inferior peripheral regions using 4 × 4 mm B-scan images (Fig. 1E). Thickness (µm) was measured as the distance from epithelium to endothelium at the point where a vertical line was orthogonal to the anterior corneal curvature. For quantitative analyses, CCT (green double-headed arrow) was taken as the mean measure from 11 frames, being C0 and five adjacent superior and inferior image frames (Fig. 1E). Peripheral corneal thickness (yellow double-headed arrow) was quantified separately for the superior and inferior cornea. The frame midway between the superior limbus and C0 was the reference superior image frame (PS0, Figs. 1E, 1G). The frame midway between the inferior limbus and C0 was the reference inferior image frame (PI0, Figs. 1E, 1H). For quantification, superior- and inferior-PCT (blue double-headed arrow) were taken as the mean measure of three frames, being the relevant reference frame (PS0 or PI0) and one adjacent superior and inferior frame (Fig. 1E). Peripheral corneal thickness measurements represent unadjusted thickness values, taken directly from the peripherally-located SD-OCT images (as defined in Figs. 1E, 1F).

**Corneal Haze.** Central corneal stromal haze was quantified using z-stacks created from the 1.4 × 1.4 mm B-scan images (Fig. 1I). First, the central image frame was extracted (as described for the anterior segment cellular response). A z-stack was constructed using ImageJ, through assembling the central frame with four adjacent superior and inferior frames (Fig. 1J). The corneal apex was determined as the point where a vertical line was orthogonal to the anterior corneal curvature. On each z-stack, the pixel intensity profile across a vertical cross-section (from epithelium to endothelium) was measured at the corneal apex and 10 additional lateral locations positioned approximately 10 µm apart (Fig. 1K, black lines), using the *inlines* function in Matlab (vR2012a; Mathworks, Natick, MA, USA). Pixel intensity values across these cross-sections were averaged over the 11 measurements and then normalized to the mean background pixel intensity, to create a two-dimensional representation of mean pixel intensity over the corneal depth (defined as a “haze profile,” Fig. 1L). The corneal haze profile shows characteristic peaks and troughs that allow delineation of the epithelial–stromal interface (Fig. 1L). To quantify central corneal stromal haze, the area under the haze profile curve corresponding to the stroma was divided by the stromal thickness to determine mean pixel intensity for each eye.
Statistical Analyses

Data were analyzed by masked observers and statistical analyses performed using GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA). Two-way, repeated-measures ANOVA followed by Bonferroni post hoc tests were used to determine statistical significance across time and between different treatment groups and mouse strains.

RESULTS

SD-OCT: Anterior Segment Cellular Response

Representative SD-OCT scans of the anterior segment cellular response to saline, LPS, and CpG at both 24 hours and 1 week post treatment are shown for C57BL/6 (Fig. 2) and BALB/c mice (Fig. 3). In both strains, the cellular response was more pronounced for CpG than LPS treatment at 24 hours (C57BL/6: Figs. 2A–C; BALB/c: Figs. 3A–C) and 1 week (C57BL/6: Figs. 2G–I; BALB/c: Figs. 3G–I).

FIGURE 1. The methodological approach for SD-OCT image analyses. (A) An en face SD-OCT image of a BALB/c mouse eye; volumetric 4 x 4 mm B-scans were taken from the inferior to superior anterior segment, as shown (white arrow). Representative scans of the anterior segment. 1 week post corneal injury and topical application of CpG, are shown to define the parameters used to quantify the anterior segment cellular response (B–D) consisting of: KP (B, white arrows); AC cells (C, arrowheads); lens cell (D, black arrow). Representative scans used to calculate corneal thickness are shown for a BALB/c eye at baseline (E–H): (E) The central image frame was denoted as C0. The frames midway between the superior and inferior-limbus and C0 were defined as the reference superior (denoted as PS0) or inferior (denoted as PI0) image frames, respectively. (F) Central corneal thickness (green double-headed arrow) was taken as the mean measure of 11 frames, being C0 and the five adjacent superior and inferior image frames (as shown in [E]). (G) Superior PCT (yellow double-headed arrow) was taken as the mean measure of three adjacent frames (as shown in [E]). All PCT measurements represent unadjusted values taken directly from the SD-OCT images as shown. (H) Inferior PCT (blue double-headed arrow) was calculated similarly. Representative images of a BALB/c cornea are shown for corneal haze quantification (I–L). (I) En face SD-OCT corneal image of a volumetric 1.4 x 1.4 mm B-scan. (J) A representative z-stack of the central cornea. (K) For each z-stack, the pixel intensity profile was measured across a vertical cross-section (from epithelium to endothelium) at the corneal apex and at 10 lateral locations, positioned approximately 10-μm apart (vertical black lines). (L) A representative two-dimensional plot of the mean pixel intensity of the corneal depth (defined as a "haze profile"), being the pixel intensity values across each cross-section averaged over the repeated measurements and normalized to mean background pixel intensity.
In CpG-treated eyes, the cellular response persisted at the 1-week timepoint (Figs. 2I, 4).

In a similar pattern to the C57BL/6 strain, saline-treated BALB/c eyes did not show an anterior segment inflammatory response at any time point (Figs. 3A, 3G). Lipopolysaccharide-treated BALB/c eyes did not demonstrate any significant increase in the number of KPs (arrows), AC cells (arrowheads) or total anterior segment cells compared with the saline-treated group (Figs. 3B, 3D–F, 3J–L). A significant cellular response was evident in CpG-compared with LPS-treated eyes at 24 hours (Figs. 3C–F), with more cells evident inferiorly than superiorly. CpG-treated eyes demonstrated a persistent inflammatory cellular response in the AC at 1 week (Fig. 4).

To confirm that KPs were adherent to the corneal endothelium, rather than an artifact due to supine positioning during imaging, a random cohort of CpG-treated eyes (n = 3) were reimaged 5 minutes after 180° rotation. Anterior chamber cells, KPs, and total anterior segment cells were counted in the artificial superior and inferior hemifields and compared with the counts from the original, prone positions. There was no significant difference in any of these quantifications (data not shown).

**Corneal Thickness**

Central anterior segment SD-OCT scans (defined in Fig. 1F) for C57BL/6 mice (Fig. 5) and BALB/c mice (Fig. 6), are shown for 0 hours, 24 hours, and 1 week after topical inoculation with saline, LPS, or CpG. Mean CCT differed between strains at baseline, with C57BL/6 mice having slightly thinner corneas than BALB/c mice (C57BL/6; 121 ± 2 μm, n = 18 versus BALB/c; 127 ± 1.5 μm, n = 22; P < 0.05).

In C57BL/6 mice, saline- (Fig. 5A) and LPS-treated (Fig. 5B) eyes showed no significant change to CCT (Fig. 5G) or PCT (Figs. 5H–I) at 24 hours compared with baseline. Generalized
Corneal thickening was evident in CpG-treated eyes (Fig. 5C; CCT: white arrowheads, PCT: black arrowheads); there were significant increases in CCT (Fig. 5G), superior PCT (Fig. 5H) and inferior PCT (Fig. 5I) compared with both baseline and the other treatment groups at 24 hours. At 1 week, CCT and PCT in saline (Fig. 5D), LPS (Fig. 5E), and CpG (Fig. 5F; CCT: white arrows, PCT: black arrows) eyes were similar, indicative of a generalized normalization of corneal thickness in CpG eyes between 24 hours and 1 week.

The time-course and pattern of corneal thickness changes differed in BALB/c mice. In BALB/c corneas, there was no significant difference in thickness at 24 hours post treatment across any treatment group (Figs. 6A–C) or compared with baseline (Figs. 6G–I). At 1 week, CCT and PCT in saline (Fig. 5D), LPS (Fig. 5E), and CpG (Fig. 5F; CCT: white arrows, PCT: black arrows) eyes were similar, indicative of a generalized normalization of corneal thickness in CpG eyes between 24 hours and 1 week.

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Corneal Haze

Central corneal stromal haze was assessed using SD-OCT z-stacks (defined in Fig. 1I) for C57BL/6 (Fig. 7) and BALB/c mice (Fig. 8). Representative z-stacks and haze profiles are shown at 0 hours, 24 hours, and 1 week after topical inoculation. Mean stromal haze at baseline differed between strains, with BALB/c mice exhibiting approximately 20% higher stromal haze values (C57BL/6: 60 ± 4 mean pixel intensity, n = 18; versus BALB/c; 71 ± 3 mean pixel intensity, n = 20; P < 0.05).

In saline-treated C57BL/6 eyes, central corneal stromal haze did not vary over the time course of the experiment (Figs. 7A, 7D, 7G, 7J; 9A). In LPS- (Figs. 7E, 7H, 7K) and CpG-treated (Figs. 7F, 7I, 7L) eyes at 24 hours, enhanced corneal reflectivity (i.e., higher mean pixel intensity), was evident, particularly in
the stroma, compared with baseline (Figs. 7B, 7C). By 1 week, this haze response in LPS-treated and CpG-treated eyes had returned to within baseline levels (Fig. 9A).

The central corneal stromal haze response differed considerably in BALB/c eyes. At 24 hours and 1 week, there was no significant change to corneal haze in saline-treated eyes (Figs. 8D, 8G, 8J, 9B) compared with baseline (Fig. 9A). Lipopolysaccharide- (Figs. 8B, 8E, 8H, 8K) and CpG-treated (Figs. 8C, 8F, 8I, 8L) eyes demonstrated an overall enhanced haze response at 1 week compared with 0 hours (Fig. 9B). CpG-treated eyes demonstrated a more exaggerated haze response at 1 week compared with 24 hours (Fig. 8I), which was accompanied by enhanced corneal thickness (Figs. 6F, 6G).

DISCUSSION

In this study, we used SD-OCT to investigate in vivo differences in strain and TLR ligand-dependent inflammatory responses in the cornea and anterior segment. While the time-course of inflammatory cell infiltration and corneal stromal haze were similar between C57BL/6 and BALB/c mice across treatment groups, there were distinct strain differences in the time course of corneal thickness changes and responsiveness to specific TLR ligands.

The infiltration of neutrophils and macrophages into the mouse cornea and anterior segment in response to injury and application of TLR4 and TLR9 ligands has been demonstrated previously using immunohistochemistry by our laboratory and others.6,7 Adopting SD-OCT to monitor and quantify inflammatory cells in the AC of patients with uveitis and keratitis is
With an axial resolution of 2.3 μm, SD-OCT has a superior resolving capacity for inflammatory cell detection in the AC compared with traditional techniques such as slit-lamp biomicroscopy. Spectral-domain OCT also has the advantage of enabling visualization of the anterior chamber when limiting factors, such as corneal edema, may be present.

While SD-OCT has been used to monitor the posterior segment of the rodent eye in disease models, including retinal degeneration and autoimmune uveoretinitis, few studies have examined experimentally-induced anterior segment pathology. In this study, analyses of the cellular infiltrate on SD-OCT images of whole anterior segments demonstrated similar responses in C57BL/6 and BALB/c mice, with the inflammatory response in both strains being most prominent with the TLR9 ligand (CpG) and less pronounced with the TLR4 ligand (LPS). These in vivo data quantitatively confirm previous ex vivo observations of TLR9 ligand–induced anterior segment inflammation, and extend this information by demonstrating that acute cellular responses are conserved across the two strains and that anterior segment inflammatory cells persist for at least 1 week. The different severities of the CpG and LPS responses likely relates to the activation of distinct TLR pathways. Lipopolysaccharide is used to mimic the inflammatory response that occurs during acute, gram-negative bacterial infections, whereas CpG elicits responses similar to those observed during chronic viral infections such as HSV-1 keratitis. The differences in the cellular responses in the mouse cornea elicited by these two commonly used TLR agonists have been recently described.

The topographic distribution of inflammatory cells was consistent in both mouse strains, with a predilection for KPs and AC cells within the inferior hemifield of the anterior segment. To our knowledge this feature has not been previously reported in animal models of anterior eye inflammation, possibly owing to the inherent limitations of cell quantification techniques that are not undertaken in vivo. Our findings are consistent with recent descriptions of the distribution of AC cells in human uveitis determined with OCT. These data support the utility of SD-OCT for accurately characterizing anterior ocular inflammation in experimental investigations and highlight striking parallels between clinical disease manifestations and experimental TLR-activated sterile ocular inflammation.

Previously, SD-OCT has been used to quantify age-related and TLR ligand-mediated corneal thickness changes in mice and to assess corneal reflectivity during a sterile wound healing response. At baseline, the adult BALB/c cornea is approximately 10% thicker than the C57BL/6 cornea. Our data show strain-dependent differences in baseline corneal thickness that support this study. Corneal thickness changes using OCT imaging have been reported in an
infectious model of P. aeruginosa keratitis in C57BL/6 mice. In this study, we report strain-dependent differences in corneal thickness changes in BALB/c and C57BL/6 mice following TLR9 ligand–induced inflammation. While corneal thickness in C57BL/6 mice peaked at 24 hours post treatment and had returned to baseline levels within 1 week, BALB/c corneas showed a delayed corneal thickness response, with a dramatic (approximately 30%) increase in thickness and evidence of a hyperreflective, subepithelial band at 1 week that may reflect an anterior stromal scarring response.

The differences in the time course of corneal inflammatory signs support the reported genetic differences in immunological and wound healing responses in these two mouse strains. Genetic differences in TLR9 gene expression in response to P. aeruginosa corneal infection have been reported in C57BL/6 and BALB/c mice. In general, C57BL/6 mice, which are considered to be susceptible to experimental P. aeruginosa corneal infection, also have increased expression of TLR9 mRNA at 24 hours post infection. In the present study, topical application of TLR9 ligand elicited a different pathological corneal response in BALB/c compared with C57BL/6 mice. These data suggest that similar to different susceptibilities of mouse strains to corneal infections such as HSV-1 and Pseudomonas keratitis, TLR9-mediated corneal pathology may similarly manifest differently in these mouse strains. The increased susceptibility of the BALB/c cornea to edema and subepithelial opacification may be an important consideration when choosing mouse strains to investigate models of corneal inflammation in which TLR9 signaling has been implicated.

Corneal stromal haze and opacification are clinical hallmarks of corneal pathology that can develop following postsurgical inflammation. Our data demonstrate a slightly higher baseline corneal haze value in BALB/c than C57BL/6 mice. While this difference was subtle, it does highlight the importance of calculating baseline haze values across treatment groups and over time. This consideration is important for researchers comparing corneal stromal haze responses in different mouse strains.

Our findings also imply strain-dependent differences in corneal inflammatory and wound healing responses that warrant further investigation. In a mouse model of central epithelial abrasion, similar to that used in this study, corneal wounds healed faster in C57BL/6 compared with BALB/c mice. While our analyses did not directly measure corneal re-epithelialization, the degree of corneal thickening, development of stromal haze and presence of sup epithelial opacities were examined, with all being exacerbated in the BALB/c strain compared with the C57BL/6 strain. Future

**FIGURE 7.** Central corneal haze following corneal TLR4 and TLR9 activation in C57BL/6 mice. Representative SD-OCT z-stacks at baseline (0 hours: [A–C]), 24 hours (D–F), and 1 week (G–I) post corneal injury and the topical application of saline (A, D, G), LPS (B, E, H), and CpG (C, F, I). Representative “haze profiles” are shown for saline (J), LPS (K), and CpG-treated eyes (L). At each time-point; increased corneal stromal haze was evident in LPS- and CpG-treated eyes at 24 hours; however, this had returned to within baseline levels by 1 week. Legend: endo, corneal endothelium; epi, corneal epithelium; stroma, corneal stroma.
investigations will seek to assess whether strain-dependent differences in corneal re-epithelialization exist.

In this study, we report time- and strain-dependent differences in susceptibility to corneal and anterior segment inflammatory changes that are most pronounced with TLR9 activation. Our findings demonstrate the strength of SD-OCT in providing high-resolution, noninvasive, in vivo imaging of the anterior segment to longitudinally quantify and monitor anterior ocular inflammation in an experimental animal model. These data provide an essential foundation for future longitudinal studies of anterior segment pathology in mice and demonstrate the value of using SD-OCT to effectively monitor anterior segment inflammation, as is frequently reported in the posterior segment of the eye.

**Figure 8.** Central corneal haze following corneal TLR4 and TLR9 activation in BALB/c mice. Representative SD-OCT z-stacks at baseline (0 hours: [A–C]), 24 hours ([D–F]), and 1 week ([G–I]) post corneal injury and the topical application of saline ([A, D, G]), LPS ([B, E, H]), and CpG ([C, F, I]). Representative “haze profiles” are shown for saline- ([J]), LPS- ([K]), and CpG-treated ([L]) eyes at each time point. Increased corneal stromal haze was evident in LPS-treated eyes at 1 week. In CpG-treated eyes, there was an initial significant increase in corneal haze at 24 hours, which was exaggerated at 1 week.

**Figure 9.** Comparison of central corneal stromal haze in C57BL/6 and BALB/c mouse strains following LPS- and CpG-treatment. Strain-dependent differences were evident in the time-course of change for the corneal stromal haze response. (A) In C57BL/6 mice, both LPS- and CpG-treatment resulted in a significant increase in stromal haze at 24 hours compared with 0 hours; by 1 week this response had normalized to within baseline levels. (B) In BALB/c mice, there was no change to stromal haze at 24 hours with LPS; however, significantly increased haze was observed with CpG treatment. At 1 week, LPS-treated eyes demonstrated a significant increase in stromal haze compared with baseline and CpG-treated eyes demonstrated a more pronounced haze response. Data presented are means ± SEM of 6 to 9 mice per group. *P < 0.05, **P < 0.001, ***P < 0.0001.
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