Characterization of uveitis induced by use of a single intravitreal injection of bacterial lipopolysaccharide in cats

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Objective—To investigate the use of a single intravitreal injection of bacterial lipopolysaccharide (LPS) to experimentally induce uveitis in cats.

Animals—7 young male European shorthair cats that were considered physically and ophthalmologically healthy.

Procedures—In each cat, LPS was injected intravitreally into 1 eye; the contralateral eye was injected with the preparation vehicle. During a period of 45 days, both eyes were evaluated by means of clinical evaluation; assessment of the integrity of the blood-aqueous humor barrier (determined via measurement of protein concentration and cell content in samples of aqueous humor); functional analysis (via electroretinography); and following euthanasia, histologic examination of the retinas.

Results—In LPS-treated eyes, several clinical signs were observed until day 45 after injection. Compared with vehicle-treated eyes, intraocular pressure was significantly lower and protein concentration and the number of infiltrating cells were significantly higher in LPS-treated eyes. Mean amplitudes of scotopic electroretinographic a- and b-waves were significantly reduced in eyes injected with LPS, compared with findings in eyes injected with vehicle. At 45 days after injection, LPS-induced alterations in photoreceptors and the middle portion of the retina were detected histologically.

Conclusion and Clinical Relevance—Results indicated that a single intravitreal injection of LPS in eyes of cats induced clinical, biochemical, functional, and histologic changes that were consistent with the main features of naturally occurring uveitis. This technique may be a useful tool in the investigation of new treatment strategies for uveitis in cats. (Am J Vet Res 2008;69:1487–1495)

Abbreviations

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<tr>
<th>AH</th>
<th>Aqueous humor</th>
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<tr>
<td>ERG</td>
<td>Electroretinography</td>
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<td>IOP</td>
<td>Intraocular pressure</td>
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<td>LPS</td>
<td>Lipopolysaccharide</td>
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Acute or chronic uveitis is a common ophthalmic disorder that constitutes one of the main causes of irreversible blindness in domestic cats. Although uveitis in cats may develop as a result of corneal ulceration, penetrating wound, or blunt trauma, infectious systemic diseases are the most frequent causes. Feline immunodeficiency virus, feline infectious peritonitis virus, FeLV, Toxoplasma gondii, Cryptococcus neoformans, Histoplasma capsulatum, Coccioides immitis, Candida albicans, and Blastomyces dermatis are the most common infectious agents implicated in the disease. However, an underlying cause is not identified in a substantial number of cats with uveitis. Uveitis affects primarily the uveal tract, but other ocular critical structures can also be affected. Regardless of etiology, conjunctival hyperemia, corneal edema, aqueous flare, hypHEMA, hypopyon, iritis, miosis, and a decrease of IOP are the main clinical signs in affected cats. In addition, inflammation of the posterior segment (choroid and retina) of the eye may provoke retinal detachment and degeneration, with consequent blindness.

At present, treatment of cats with uveitis generally involves administration of corticosteroids, which is directed toward reduction of pain and lesions in ocular tissues. However, the immunosuppressive effect of corticosteroids may contribute to the development of the systemic disease, and their chronic use could result in corticosteroid-induced glaucoma. Nonsteroidal anti-
inflammatory drugs are also indicated for uveitis treatment, but they may delay the reparative process and coagulation and may induce acute renal insufficiency and gastrointestinal hemorrhage or ulceration.\textsuperscript{2,6} Moreover, it has been recently determined that only 33\% of cats with systemic disease–associated uveitis respond to this treatment.\textsuperscript{7}

A novel method for experimental induction of uveitis in cats would greatly facilitate the understanding of the cellular events involved in ocular inflammation as well as find application in the development of new treatment strategies. In cats, techniques for experimental induction of uveitis have included inoculations of \textit{T} gonii via the carotid artery;\textsuperscript{4} topical ocular application of pilocarpine;\textsuperscript{6} or paracentesis of the anterior chamber of the eye.\textsuperscript{6,12} Bhatia et al\textsuperscript{11} used endotoxin-induced uveitis in cats to assess the efficacy of anti-inflammatory compounds; however, few details of the ocular consequences of LPS administration were provided in that report.

In rats and rabbits, acute ocular inflammation can be induced via intravitreal administration of LPS, a component of gram-negative bacterial outer membranes.\textsuperscript{12-14} Lipopolysaccharide enhances the expression of various inflammatory mediators, such as interleukin-6,\textsuperscript{13} tumor necrosis factor-\alpha,\textsuperscript{18} prostaglandin E\textsubscript{2},\textsuperscript{17} and monocyte chemotactic protein-1,\textsuperscript{18} as well as the production of nitric oxide;\textsuperscript{15} all of these contribute to the inflammatory process in the eye, which results in the breakdown of the blood-ocular barrier and leukocyte infiltration. In addition, T lymphocytes and dendritic cells have been detected in uveal tissues from eyes with endotoxin-induced uveitis.\textsuperscript{20,21}

Although endotoxin-induced uveitis in rats\textsuperscript{12} was originally used as an experimental model of anterior uveitis in humans, there is increasing evidence that it also involves inflammation of the posterior segment of the eye with recruitment of polymorphonuclear leukocytes and macrophages that adhere to the retinal vasculature and infiltrate the vitreous cavity.\textsuperscript{3,23} Because uveitis in cats frequently leads to severe vision loss or blindness, we considered it worthwhile to investigate and characterize a novel method of experimental induction of uveitis (ie, a single intravitreal injection of bacterial LPS) in this species.

**Materials and Methods**

**Animals**—All animal procedures were conducted in strict accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. Cats were obtained from the research colony of the Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Provincia de Buenos Aires. Seven young European shorthair male cats (mean weight, 2.5 ± 0.5 kg) that were seronegative for \textit{T} gonii, FIV, and FeLV were housed individually in a temperature- and light-controlled environment (fluorescent lights were turned on and off automatically in 12-hour cycles). The cats were fed a balanced diet; water was available ad libitum. Cats were adapted to human contact for 4 to 6 weeks. Prior to inclusion in the study, each cat’s physical and ophthalmologic health conditions were determined on the basis of results of general and ocular examinations. Ocular examination included Schirmer tear testing,\textsuperscript{4} fluorescein staining,\textsuperscript{1} applanation tonometry,\textsuperscript{4} biomicroscopy,\textsuperscript{4} indirect\textsuperscript{4} and direct\textsuperscript{4} ophthalmoscopy in dilated eyes, and ERG.\textsuperscript{3}

**Intravitreal injections**—Cats were anesthetized via IM administration of ketamine hydrochloride (15 mg/kg) and xylazine hydrochloride (1.5 mg/kg). By use of a disposable 1-mL syringe with a 25-gauge needle, 20 μg of LPS from \textit{Salmonella} serovar Typhimurium\textsuperscript{4} in 100 μL of sterile saline (0.9% NaCl) solution was injected intravitreally into 1 eye and the contralateral eye was injected intravitreally with 100 μL of sterile saline solution (vehicle). A drop of topical anesthetic (0.5% sterile propocaaine hydrochloride ophthalmic solution\textsuperscript{3}) was instilled before injections. Intravitreal injections were performed at a location 5 mm posterior to the limbus and were directed toward the optic nerve to avoid trauma to the lens.

**Clinical study**—During a period of 45 days after injection of vehicle or LPS, cats were evaluated periodically for signs of ocular pain, conjunctival hyperemia, chemosis, keratic precipitates, aqueous flare, hypopyon, hyphema, miosis, iridal swelling, iris heterochromia, synechiae, and cataracts, as well as for vitreal opacity, retinal detachment, chorioretinitis, and optic neuritis by use of a slit lamp and via direct and indirect ophthalmoscopy. Clinical severity of the inflammatory signs was assessed at days 1, 3, 5, 8, 10, 12, 15, 17, 19, 22, 24, 26, 29, 33, 35, 40, and 45 following injection of vehicle or LPS and was graded on a scale from 0 to 3 (Appendix). Miosis was quantified in reference to the pupil size. Severity of other clinical signs was graded by assignment of scores of 0 (absent) or 1 (present), scores of 0 to 2 (0 = absent; 1 = moderate; and 2 = severe), or scores of 0 to 3 (0 = absent; 1 = mild; 2 = moderate; and 3 = severe). For synechiae, the criterion was based on the number of synechiae (0 = absent; 1 = 1 synechia; 2 = 2 synechiae; and 3 ≥ 3 synechiae) and Tyrndall effect was quantified in accordance with the scale described by Hogan.\textsuperscript{24} Despite changes in the anterior portions of the eyes, it was possible to observe alterations induced by LPS in the posterior portions because in all examined eyes, any cataracts were mainly immature (not complete) and miosis was severe but not complete even at 24 hours after the injection. Each eye was examined at each time point by 3 observers (MJDs, PHS, and DCF), who were unaware of the treatment applied; clinical signs were graded, and a cumulative score was assigned to each eye by each observer. The mean of the cumulative clinical scores assigned by the 3 observers was recorded as the clinical score for a given eye at a given time point. At each time point, the mean values from vehicle- or LPS-injected eyes were averaged to compute the group mean ± SE clinical score.

**IOP assessment**—Tonometric measurements were performed in awake cats by a single investigator (MJDs) by use of an applanation tonometer.\textsuperscript{4} Cats were manually restrained, and a drop of topical anesthetic agent was applied to the cornea immediately before tonometry. Five independent IOP readings were obtained from each eye, and IOP was determined as the mean of these readings. Among all cats, no significant differences were observed.
between the right and the left eyes before injections. Assessments of IOP were performed in each eye at 1, 3, 5, 8, 10, 12, 15, 17, 19, 22, and 24 days after intravitreal injection of vehicle or LPS. Intraocular pressure measurements were performed at the same time each day (between 11 AM and noon) to correct for diurnal variations.22

Inflammatory cells and protein concentration in AH—On days 2, 7, 14, 21, and 28 after injection of vehicle or LPS, a sample (400 µL) of AH was obtained from both eyes via paracentesis performed by use of a 23-gauge needle. The number and type of infiltrating cells and protein concentration were determined in each sample. For cell counting and typing, AH samples were centrifuged at 800 × g for 3 minutes at 4°C. Then, 350 µL of supernatant was extracted for protein concentration measurement, and the remainder was suspended in 20 µL of sodium and potassium EDTA salts (0.342 mol/L; pH, 7.2) for cell counting and typing. Red cells were lysed via 1:10 dilution in 5% acetic acid. Inflammatory cells were counted by use of a Neubauer camera and light microscopy. The number of cells per field (equivalent of 0.1 µL) was counted, and the number of cells per microliter of AH was obtained by averaging the results of 4 fields and referring to the sample volume before centrifugation. For cell typing, a drop of the same sample was deposited onto a slide, dried by air, and stained with Giemsa stain. Protein content was measured by the method of Lowry et al.,26 with bovine serum albumin as a standard.

ERG—On day 30 after injection of vehicle or LPS, ERG activity was assessed in the cats. After 120 minutes of dark adaptation, each cat was anesthetized via IM administration with ketamine and xylazine. Phenylephrine hydrochloride (2.5%) and 1% tropicamide1 were applied to both eyes to dilate the pupils; the corneas were intermittently irrigated with balanced salt solution1 to prevent keratopathy. Each cat was placed facing the stimulus at a distance of 20 cm. A reference electrode was placed through the ear, a grounding electrode was attached to the occipital crest, and a contact lens electrode (ERG jet electrode™) was placed in the central cornea. Anesthesia did not rotate the globes, and eyelids were fixed by use of a blepharostat. A 16-W red light was used to enable accurate electrode placement. This maneuver did not significantly affect dark adaptation and was switched off during the recordings. Electroretinograms were recorded from both eyes simultaneously, and 10 responses to a flash of white light (5 milliseconds; 0.2 Hz) from a photic stimulator (light-emitting diodes) set at maximum brightness (350 candela••• without a filter) were amplified, filtered (1.5-Hz low-pass filter; 1,000-Hz high-pass filter; notch activated) and averaged.1 The a-wave amplitude was measured as the difference in amplitude between the recording at onset and the trough of the negative deflection, and the b-wave amplitude was measured as the difference in amplitude between the trough of the a-wave to the peak of the b-wave. Electroretinographic responses were averaged for each run (10 tests). Runs were repeated 3 times with 5-minute intervals to confirm consistency, and the mean of these runs was used for subsequent analysis.

Histologic examination—Forty-five days after the injection of vehicle or LPS, both eyes were enucleated by use of a transconjunctival enucleation technique in 5 cats that were anesthetized and immediately euthanized via IV administration of pentobarbital sodium (80 mg/kg) and diphenylhydantoin sodium (10 mg/kg).22 Enucleated globes were immediately placed in fixative (4% formaldehyde in 0.1M phosphate buffer; pH, 7.3) for 10 minutes. Then, globes were incised with a razor blade to allow a better access of fixative to the interior structures. After fixation for 48 hours, eyes were transsected on the medial side (1 mm from the limbus). Eyes were sectioned along the horizontal meridian through the optic nerve head, and photomicrographs were obtained 1.5 mm from the optic nerve head center. Retinal sections (5-µm thick) were stained with H&E stain.

Statistical analysis—All data are presented as mean ± SE. Statistical analyses of results (ie, clinical score, ERG a- and b-wave amplitude, and number of inflammatory cells and protein concentration in AH) for each group at each time point were made by use of a Student t test or a 2-way ANOVA followed by a Tukey test. For all analyses, a value of P < 0.05 was considered significant.
Results

Clinical scores—During the study period, clinical severity scores were assessed in vehicle- and LPS-injected eyes of 7 cats on 16 occasions (at days 1, 3, 5, 8, 10, 12, 15, 17, 19, 22, 24, 26, 29, 35, 40, and 45 after injection). Mean clinical scores for each group of treated eyes were calculated (Figure 1). In all eyes injected with LPS (without exception), signs of ocular inflammation developed, whereas no signs of uveitis were evident in vehicle-injected eyes. The clinical score for LPS-injected eyes reached maximal values (mean scores, 27 to 21) from days 1 to 12 after injection; LPS-associated effects were most commonly signs of pain, conjunctival hyperemia, chemosis, hypopyon, hyphema, miosis, Tyndall effect, iridal swelling, tumefaction and rubecosis, vitritis, chorioretinitis, and partial secondary blindness (Figure 2). The earliest sign was miosis, which was apparent 1 hour after LPS injection. From days 15 to 29, although the clinical score still remained high (mean score, 18 to 13), clinical signs in the posterior segment of LPS-injected eyes (eg, cataract, vitritis, and active chorioretinitis) predominated during this period. From days 35 to 45 after injection, the mean clinical score decreased slowly (to approx 9); this phase was characterized by the presence of sequelae (eg, cataract, vitreal opacity, and focal retinal degeneration).

WBC count—At 1 day after injection with vehicle in one eye and LPS in the other eye, mean WBC count in 7 cats (9,854 ± 420 cells/µL) did not differ significantly from reference values for healthy cats. Similar results were obtained at 7 and 14 days after injections (eg, 9,324 ± 720 cells/µL and 9,968 ± 840 cells/µL, respectively).

IOP assessments—Intraocular pressures in both vehicle- and LPS-injected eyes were assessed 1, 3, 5, 8, 10, 12, 15, 17, 19, 22, and 24 days after injections (Figure 3). At days 1 through 12, IOP values were significantly lower in LPS-injected eyes, compared with vehicle-injected eyes. After this period, no significant differences in IOP between vehicle- and LPS-injected eyes were detected.

Number and types of inflammatory cells and protein concentration in AH—in samples of AH from eyes treated with LPS or vehicle, numbers of inflammatory cells and protein concentrations were assessed at 2, 7, 14, 21, and 28 days after injection. The mean ± SE protein concentration in AH obtained from vehicle-injected eyes was approximately 0.49 ± 0.04 mg/mL at each time point, which was within reference range for this species. In LPS-injected eyes, the AH protein concentration was 41.97 ± 4.57 mg/mL, 20.59 ± 6.27 mg/mL, 11.04 ± 2.95 mg/mL, 1.46 ± 0.20 mg/mL, and 1.15 ± 0.24 mg/mL at days 2, 7, 14, 21, and 28, respectively. At each time point, the value was significantly (P < 0.01; Tukey test) higher in LPS- than in vehicle-injected eyes.

Figure 2—Representative photographs of typical gross changes that developed with time in the eyes of 4 cats that were used in a study of LPS-induced uveitis. One eye of each cat received an intravitreal injection of 20 µg of LPS in 100 µL of sterile saline solution, and the other eye received an intravitreal injection of a similar volume of saline solution (vehicle). The number beneath each image indicates the day after injection that the image was obtained. A—In cat 2, the left eye was treated with vehicle and the right eye was treated with LPS; in the LPS-injected eye, miosis and iris heterochromia (day 1), hypopyon (day 1 and 5), and synechia (days 1, 5, 15, and 29) developed. B—In cat 5, the left eye was treated with vehicle and the right eye was treated with LPS; in the LPS-injected eye, miosis (days 1 and 5) and iridal swelling (days 1, 5, and 19) developed. C—In cat 4, miosis and hyphema (day 5) developed in the right LPS-injected eye. D—In cat 1, miosis and an immature cataract (day 15) developed in the right LPS-injected eye.

Figure 3—Mean ± SE values of IOP (determined via applanation tonometry) in 7 healthy cats at days 1, 3, 5, 8, 10, 12, 15, 17, 19, 22, and 24 following intravitreal injection of 20 µg of LPS in 100 µL of sterile saline solution (black circles) in one eye and intravitreal injection of a similar volume of saline solution (vehicle; white circles) in the other eye (7 eyes/group). *Value in LPS-injected eyes was significantly (Student t test; P < 0.01) lower than the value in vehicle-treated eyes at this time point. †Value in LPS-injected eyes was significantly (Student t test; P < 0.05) lower than the value in vehicle-treated eyes at this time point.
The maximal effect of LPS on AH protein concentration was observed at 2 days after injection.

No inflammatory cells were observed in AH from vehicle-injected eyes at any time point. In LPS-injected eyes, the number of cells was 1,870 ± 341, 537 ± 216, 44 ± 36, 27 ± 26, and 4 ± 4 at days 2, 7, 14, 21, and 28, respectively. Two days after the injection, the number of infiltrating cells was significantly higher (Tukey test; P < 0.01) in LPS- than in vehicle-injected eyes. Seven days after the injection, the number of infiltrating cells decreased but was still significantly higher (Tukey test; P < 0.01) in LPS- than in vehicle-injected eyes. The maximal effect of LPS on inflammatory cell number in AH was observed at 2 days after injection.

Regarding cell type, polymorphonuclear cells predominated at day 2 after LPS injection (1,790 ± 180 polymorphonuclear cells/µL of AH and 271 ± 37 lymphocytes/µL of AH), whereas lymphocytes were most abundant at day 7 (112 ± 10 polymorphonuclear cells/µL of AH and 412 ± 50 lymphocytes/µL of AH).

**ERG findings**—To analyze the functional state of retinas in vehicle- or LPS-injected eyes, scotopic ERGs in both eyes of each cat were recorded before and at 30 days after treatment (Figure 4). The mean amplitudes of scotopic ERG a- and b-waves in all eyes before treatment and in the eyes injected with vehicle or LPS were calculated (Figure 5). The injection of LPS decreased scotopic ERG a- and b-wave amplitudes, although their respective latencies remained unchanged (data not shown). Mean a- and b-wave amplitudes in LPS-injected eyes were significantly (Tukey test; P < 0.01) less than preinjection values or values in vehicle-injected eyes.

**Histologic findings**—At 45 days after treatment, retinas from both eyes of 3 cats were collected and sections were examined via light microscopy (Figure 6). No signs of inflammation were evident in the anterior segment of eyes injected with LPS (data not shown), but signs of retinal inflammation were observed. The injection of LPS caused formation of folds and loss of photoreceptors. In addition, a high number of inflammatory cells that were located mainly in the inner retinal regions and disorganization of the axons from the nerve fiber layer were observed. In vehicle-injected eyes, no signs of inflammation were detected in any ocular structure.

**Discussion**

The results of the present study indicated that a single intravitreal injection of LPS in cats induced major clinical, biochemical, functional, and histologic alterations in the treated eyes. No changes of any clinical importance developed in contralateral eyes injected with the saline solution vehicle. In several species, methods of experimental induction of uveitis that involve IV, intraperitoneal, or footpad administration of LPS have been developed.27-28 In the study of this report, we used an intravitreal injection of endotoxin because this maneuver avoids development of inflammation systemically and allows the use of the contralateral eye as a control, thereby decreasing the risk of treatment-related death and minimizing the number of animals used. The study cats were injected with vehicle in 1 eye and with LPS in the contralateral eye, and yet the WBC counts did not differ significantly from reference values in healthy cats. Moreover, no signs of febrile response to the experimental treatment were observed in these cats. On the basis of AH cell number and protein concentration, clinical scores, and IOP values of vehicle-injected eyes, effects of LPS were statistically significant at 2 days after injection. On the basis of AH cell number and protein concentration, clinical scores, and IOP values of vehicle-injected eyes, effects of LPS were statistically significant at 2 days after injection.
ject ed eyes, it seems unlikely that injection of LPS in 1 eye affected the contralateral eye. Taken together, these results support the fact that intravitreal injection of LPS in 1 eye of cats induced unilateral ocular inflammation, which affected ocular structures in the ipsilateral side without major contralateral ocular or systemic effects.

The use of a scoring system offers a means with which a quantitative measure of clinical signs and characterization of their temporal course can be achieved. In the present study, the clinical consequences of LPS injection during the 45-day period were assessed by use of a scoring system and could be apportioned to approximately 3 phases: from days 1 through 12, from days 13 through 29, and from day 33 until the end of the study (day 45), respectively. In the first phase, mostly signs of changes in the anterior segment of the LPS-injected eyes were observed, whereas in the last phase, sequelae in the posterior segment were most noticeable.

Allbaugh and Davidson reported that paracentesis of the anterior chamber of the eye provokes ocular inflammation in adult Beagles, and Rankin et al determined that paracentesis of the anterior chamber induces breakdown of the blood-aqueous barrier in cats. However, in our experimental conditions, repetitive paracentesis did not induce ocular inflammation in cats as evidenced by the findings (protein concentration, cell number, clinical scoring, and IOP) obtained from vehicle-injected eyes. Differences between the results of those previous investigations and those of our study may have resulted from differences in immunologic responses between cats and dogs, as well as differences in volumes of AH extracted in these studies (0.8 to 1.1 mL vs 400 µL in our study).

Similar to findings in cats with naturally occurring uveitis, IOP was significantly lower in LPS-injected eyes (compared with vehicle-injected eyes) up to 12 days after injection in the cats of the present study, which was approximately the same period during which clinical scores were highest. However, the ocular hypertension that typically develops in eyes with chronic uveitis (secondary glaucoma or hypertensive uveitis) was not evident in LPS-injected eyes, suggesting that the trabecular meshwork was not chronically affected in those eyes. Moreover, the fact that IOP did not differ between vehicle- and LPS-injected eyes during the last phase of the inflammatory process suggests a spontaneous recovery of anterior segment structures, which was also supported by the histologic analysis of retinal tissue sections.

Regardless of the administration method used, injection of LPS is known to provoke an increase in protein concentration and cell number in AH in rabbits, rats, and mice. As expected, the intravitreal injection of LPS in cats in the present study provoked a significant increase in both variables, supporting a disruption of the blood-ocular barrier integrity. However, few studies have previously addressed the temporal course of changes in these variables after injection of the endotoxin, and largely only short-term (24 hours to 7 days) consequences were reported. In our investigation, significant differences in AH protein concentration between LPS- and vehicle-injected eyes were evident during the 45-day study period; at 2 days after injection, LPS induced an approximately 60-fold increase in AH protein concentration, compared with the value in vehicle-injected eyes. Afterwards, the concentration in LPS-injected eyes decreased but still remained slightly (and significantly) higher than the concentration in vehicle-injected eyes on day 28. In vehicle-injected eyes, AH protein concentration was within reference range for this species. Injection of LPS induced an increase in cell number in AH samples; the maximal value was detected at 2 days after LPS injection. The number of cells in LPS-injected eyes progressively decreased and was similar to the number of cells in vehicle-injected eyes at day 14 after experimental treatment. These results suggest that a single intravitreal injection of LPS in cats induces considerable disruption of the blood-ocular barrier and that the duration of that effect is longer than previously estimated. In addition, assessment of the types of cells in the AH revealed a predominance of polymorphonuclear cells (associated with innate immunity) at 2 days after injection of LPS, whereas at day 7, the most abundant cells were lymphocytes (associated with adaptive immunity). Therefore, it seems likely that the intravitreal injection of LPS triggered both innate and adaptive immune responses in the cats of the present study.

Figure 6—Representative photomicrographs of transverse sections of retinas from a cat in which one eye received an intravitreal injection of 100 µL of sterile saline solution (vehicle; A) and the other eye received an intravitreal injection of 20 µg of LPS in 100 µL of sterile saline solution (B and C) 45 days earlier. In the eye injected with vehicle, the retinal architecture appears normal. In the eye injected with LPS administration, alterations in photoreceptors and middle portion of the retina are apparent. Notice the inward foldings of the external segments of photoreceptors (asterisks; B) and inflammatory cell infiltration in the inner portion of the retina (arrow; C). H&E stain. Bar in panels A and B = 100 µm; bar in panel C = 50 µm.
The fact that the concentration of protein in AH samples remained increased for a longer period than did the number of cells in those samples is compatible with partial repair of the disrupted blood-ocular barrier during last phases of the inflammatory process. Although macrophages are the predominant infiltrating cell detected during the last phases of uveitis in mice,^3^ cells in AH samples collected at 7 days after injection of LPS from cats in the present study were mostly lymphocytes. There is no ready explanation for this discrepancy, but it seems possible that species-specific factors could account for it. In fact, a lymphoplasmacytic infiltrate within the iris and ciliary body is typical of many cats with uveitis.^3^

Originally, endotoxin-induced uveitis was considered exclusively an experimental model of anterior uveitis. However, it is now known that endotoxin-induced uveitis is also associated with effects in the vascular layer and the posterior segment of affected eyes, indicating that this is an experimental model of panuveitis. Studies^22,23^ in mice and rabbits have revealed that LPS induces inflammation-mediated retinal injury. The results of the present study extend this presumption to cats because retinas from eyes injected with LPS were chronically affected at functional and histologic levels.

Electroretinography has been successfully used to noninvasively and objectively measure retinal function following a wide range of retinal insults (eg, toxic agents, infection, vasculopathy, and photic lesions) in several species.^40–43^ To assess the effect of LPS on retinal function in the cats of the present study, scotopic flash ERGs were performed. Compared with values in vehicle-injected eyes, significant reductions in ERG a- and b-wave amplitudes were detected in LPS-treated eyes, suggesting that sequelae of ocular inflammation induced by LPS could be located in both outer and middle regions of the retina. In agreement with this suggestion, attenuation of the ERG a- and b-wave amplitudes in various animals, including humans, with clinical or experimental uveitis has also been observed.^44–46^ However, as stated previously, there are no reports of the long-lasting functional sequelae associated with endotoxin-induced uveitis to our knowledge. It should be noted that before the injections of LPS or vehicle in eyes of the cats of the present study, we had ascertained that flash ERGs in all 2 eyes of each cat were similar. Thus, the differences in ERG data from LPS- and vehicle-injected eyes are valid indicators of the effects of LPS. No attempt was made in the present study to assess further changes of the ERG activity.

Several lines of evidence indicate that there is a close relationship between the degree of ocular inflammation and the depression of the ERG. The a-wave of the flash ERG is classically thought to represent photoreceptor activity, whereas the b-wave reflects bipolar and Muller cell functions. Although the assessments of retinal function and histologic characteristics were not performed at the same interval after injection (ie, 30 and 45 days after the injection of vehicle or LPS, respectively) in the present study, the results also support a correlation between retinal morphology and the ERG activity in LPS-injected eyes. Retinal lesions were formed by inward foldings of photoreceptor external segments, and lymphocytes were present in the inner region of the retina; these findings were indicative of damage to the outer, middle, and inner regions of the retina. Notably, these retinal alterations were evident at the postinjection time point at which no inflammatory signs were observed in the anterior segment. Although this result does not eliminate the possibility of functional impairment in anterior segment structures, the fact that IOP was restored in the last phase of the inflammatory process could suggest recovery of the ocular anterior segment from LPS-induced damage.

In the present study, the long-term consequences of intravitreal injection of LPS were investigated, and results indicated that this experimental treatment reproduces key features of naturally occurring uveitis in cats, including histologic and functional sequelae in the retina. In a previous report,^27^ ocular toxoplamosis in cats was experimentally induced via inoculation of a low number of organisms from a mildly virulent strain of *T gondii* into the common carotid artery. That procedure and the technique investigated in our study differ in several aspects. The pathogenicity of *T gondii* depends on the virulence of the strain, which could make it difficult to reproduce the experimental model in different laboratories, whereas LPS is a highly accessible standard reactive. Mild inflammation in the anterior segment was observed following inoculation with *T gondii*, whereas intravitreal injection of LPS triggered more generalized ocular inflammation. Experimentally induced ocular toxoplasmosis was associated with the breakdown of the retinal epithelium cell barrier, whereas LPS-induced uveitis was associated with disruption of both anterior and posterior blood-ocular barriers. Finally, ocular toxoplasmosis is a specific form of ocular disease, whereas uveitis induced via intravitreal injection of LPS has the general features of diseases caused by a greater variety of etiopathogenic agents.

Among species, uveitis has several common characteristics. However, many antiuveitic medications that are successfully used in humans and dogs are not tolerated well by cats; therefore, the selection of recommended drugs is limited in the latter species. Results of the present study indicated that uveitis induced via intravitreal injection of LPS in cats is associated with clinical, biochemical, histologic, and functional changes consistent with the main features of naturally occurring uveitis. The technique may have application in pharmacologic studies to develop new treatment strategies for cat with uveitis.

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b. Schirmer tear test strips, Schering-Plough Animal Health Corp, Union, NJ.

c. Love Sudamericana Laboratory, Buenos Aires, Argentina.
d. Tono-Pen XL, Mentor, Norwell, Mass.
e. Slit lamp HLS 150, Heine Optotechnik, Herrsching, Germany.
f. 20-D lens, Ocular Instruments, Bellevue, Wash.
g. Heine Omega 130, Heine Optotechnik, Herrsching, Germany.
h. Heine Beta 200, Heine Optotechnik, Herrsching, Germany.
i. Akomic BIO-PC, Akomic, Buenos Aires, Argentina.
j. Sigma, St Louis, Mo.
k. Anestalcom, Alcon Laboratories, Buenos Aires, Argentina.
l. Alcon Laboratories, Buenos Aires, Argentina.
m. LKC Technologies, Gaithersburg, Md.
n. Euthanyle, Brouwer, Buenos Aires, Argentina.
Appendix

Score system used to grade the clinical severity of LPS-induced uveitis in cats.

<table>
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<th>Clinical sign</th>
<th>Score</th>
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<td>Pain</td>
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<td>Photophobia</td>
<td>0</td>
</tr>
<tr>
<td>Blepharospasm</td>
<td>0</td>
</tr>
<tr>
<td>Epiphora</td>
<td>0</td>
</tr>
<tr>
<td>Conjunctival hyperemia</td>
<td>0</td>
</tr>
<tr>
<td>Chemosis</td>
<td>0</td>
</tr>
<tr>
<td>Keratic precipitates</td>
<td>0</td>
</tr>
<tr>
<td>Corneal edema</td>
<td>0</td>
</tr>
<tr>
<td>Corneal vascularization</td>
<td>0</td>
</tr>
<tr>
<td>Tyndall effect</td>
<td>0</td>
</tr>
<tr>
<td>Hyphema</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>≤ 33% of the anterior chamber</td>
<td>1</td>
</tr>
<tr>
<td>&gt; 33% of the anterior chamber</td>
<td>2</td>
</tr>
<tr>
<td>Hypopyon</td>
<td>None</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>≤ 33% of the anterior chamber</td>
<td>1</td>
</tr>
<tr>
<td>&gt; 33% of the anterior chamber</td>
<td>2</td>
</tr>
<tr>
<td>Miosis</td>
<td>None</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>Mild</td>
<td>1</td>
</tr>
<tr>
<td>Moderate</td>
<td>2</td>
</tr>
<tr>
<td>Severe</td>
<td>3</td>
</tr>
<tr>
<td>Maximal</td>
<td>4</td>
</tr>
<tr>
<td>Iridial swelling</td>
<td>0</td>
</tr>
<tr>
<td>Iridial hyperemia or ruberosis</td>
<td>0</td>
</tr>
<tr>
<td>Synchiae</td>
<td>0</td>
</tr>
<tr>
<td>Iris heterochromia</td>
<td>0</td>
</tr>
<tr>
<td>Cataract</td>
<td>0</td>
</tr>
<tr>
<td>Lens subluxation or luxation</td>
<td>0</td>
</tr>
<tr>
<td>Vitritis</td>
<td>0</td>
</tr>
<tr>
<td>Chlororretinitis (active or inactive)</td>
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</tr>
<tr>
<td>Optic neuritis</td>
<td>0</td>
</tr>
<tr>
<td>Negative menace response</td>
<td>0</td>
</tr>
<tr>
<td>Pupillary light reflex</td>
<td>3</td>
</tr>
<tr>
<td>Fast</td>
<td>0</td>
</tr>
<tr>
<td>Slow</td>
<td>1</td>
</tr>
<tr>
<td>Absent</td>
<td>3</td>
</tr>
</tbody>
</table>

Maximum cumulative clinical score 50

Severity of clinical signs was graded by assignment of scores of 0 (absent) or 1 (present), scores of 0 to 2 (0 = absent; 1 = mild; 2 = moderate; and 3 = severe). Miosis was quantified in reference to the pupil size. For synechiae, the criterion was based on the number of synechiae (0 = absent, and 3 = ≥ 3).

References

32. Bosch-Morell F, Roni J, Marin N, et al. Role of oxygen and


