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Elevated interleukin 6 activity in aqueous humor of cats with uveitis

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Abstract

The purpose of this study was to assess the role of interleukin 6 (IL-6) in feline uveitis by measuring IL-6 activity in the serum and aqueous humor of cats.

Serum and aqueous humor was collected from clinically normal, random source cats (n = 10); clinically normal, specific-pathogen free cats experimentally inoculated with *Toxoplasma gondii* strain ME49 and sampled sequentially for 20 months ($n = 4$); and client-owned cats with uveitis ($n = 27$). Interleukin 6 activity was measured in each sample. Client-owned cats with uveitis were also evaluated for evidence of present or prior exposure to *T. gondii*, feline leukemia virus, feline immunodeficiency virus, and feline coronaviruses.

Interleukin 6 activity was non-detectable or low in serum from cats of each group. Interleukin 6 activity was not detected in aqueous humor of clinically normal cats. Interleukin 6 activity was detected in 22/27 (81.5%) aqueous humor samples from cats with uveitis, with a range of 28.9 U ml^{-1}–15702.9 U ml^{-1} (mean = 1911.9 U ml^{-1}, SD = 3946.7 U ml^{-1}). Serologic evidence of exposure to *T gondii*, feline immunodeficiency virus, feline leukemia virus, or a coronavirus was present in 21/27 (77.8%) cats with uveitis. Interleukin 6 was detected in the aqueous humor of 18/21 (85.7%) and 3/6 (50%) of the cats with and without serologic evidence of exposure to one to the infectious diseases, respectively. Statistically significant increases in mean IL-6 activity in aqueous humor were found for cats with any evidence of infection with *T. gondii*, for cats with *T. gondii* antigen in aqueous humor and for cats with coronavirus antibody titers $\geq$ 1:100. Aqueous humor IL-6 activity was greater than corresponding serum IL-6 activity in 21/27 cats.

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These results show that IL-6 is produced intraocularly in some cats with uveitis and that IL-6 may be a mediator of uveitis in cats. © 1997 Elsevier Science B.V.

Keywords: *Toxoplasma gondii*; Toxoplasmosis; Feline; Uveitis; Interleukin 6

1. Introduction

Inflammation of the iris, ciliary body, and choroid (uveitis) has been associated with a number of infectious diseases of cats, including *Toxoplasma gondii*, feline leukemia virus (FeLV), feline immunodeficiency virus (FIV), feline infectious peritonitis virus (FIP), and the systemic mycoses (English et al., 1990; Chavkin et al., 1992; Dubey et al., 1993; Williams, 1994). There are no pathognomonic ophthalmic signs for these diseases. Histopathologic examination of ocular tissues documents fungal elements, *T. gondii*, and the characteristic vasculitis induced by FIP in some cases of feline uveitis; lymphocytes and plasma cells infiltrating the iris and ciliary body, without an obvious etiology, is detected most commonly (Davidson et al., 1990; Peiffer and Wilcock, 1991). Presence of lymphocytic and plasmacytic infiltrates suggests an immune-mediated component in the pathogenesis of uveitis in cats.

Interleukin 6 (IL-6) is a cytokine produced by many cells including activated T-lymphocytes and B-lymphocytes as well as monocytes, macrophages, fibroblasts, and endothelial cells triggered by the appropriate stimuli (Kishimoto, 1989). Induction of acute phase reactive proteins and mediation of the final differentiation of B-lymphocytes into antibody producing cells are two major biological functions of IL-6 (Kishimoto, 1989; Gilgor et al., 1988). Interleukin 6 is a pro-inflammatory mediator. Elevated IL-6 activity has been detected in aqueous humor from people with Fuchs' heterochromic cyclitis (Murray et al., 1990), toxoplasmosis (Murray et al., 1990), Vogt-Koyanagi-Harada disease (Norose et al., 1994), and onchocerciasis (van der Lelij et al., 1991), and from the vitreous humor from people with proliferative vitreoretinopathy (Kauffmann et al., 1994). The inflammatory mediators involved in feline uveitis have not been evaluated. The purpose of this study was to assess the role of IL-6 in feline uveitis by measuring IL-6 activity in the serum and aqueous humor of cats.

2. Methods

2.1. Sample groups

Three groups of cats were selected for study: (1) clinically normal, random source cats ($n = 10$); (2) clinically normal, specific-pathogen free (SPF) cats purchased from a commercial laboratory ($n = 6$); and (3) client-owned cats with clinical evidence of uveitis ($n = 27$). Experimental animal maintenance was in accordance with current federal guidelines, the ARVO statement for use of animals in ophthalmic and vision research, and experimental protocols approved by a campus-wide animal care and use committee.

2.2. Experimentally induced *T. gondii* infection

Toxoplasma gondii strain ME49 is maintained by serial passage in strain CF-1 mice in our laboratory. Six weeks after subcutaneous inoculation with 100 tissue cysts of *T. gondii* strain ME49, the brains were collected from infected mice following induction of anesthesia and euthanasia by cervical dislocation. Brain tissue was homogenized in sterile 0.9% NaCl by repeatedly drawing the tissue through a 22 gauge needle. Tissue cysts were quantitated and after having food withheld for 14 h, Group 2 cats were inoculated orally with 1.0×10^3 tissue cysts.

2.3. Experimental design

Each cat in the study was examined by a board-qualified or board-certified member of the American College of Veterinary Ophthalmologists using slit lamp biomicroscopy and indirect ophthalmoscopy. Group 1 and Group 2 cats were determined to be free of intraocular inflammation. Group 3 cats were determined to be free of overt external causes of uveitis including foreign bodies, corneal ulcers, or keratitis.

Blood was collected from each cat by jugular venipuncture with serum collected by centrifugation and stored at −20°C or −70°C until assayed. Each cat was sedated or anesthetized and aqueous humor was slowly aspirated from the anterior chamber through a 27 gauge needle inserted at the corneoscleral limbus. For Group 2 cats ($n = 4$), the left eye was sampled. For Group 3 cats, the eye with the most severe inflammation was sampled. Aqueous humor (0.2–0.4 ml) was placed in a sterile EDTA tube (1.5 ml draw) and frozen at −20°C or −70°C until assayed. Slit-lamp biomicroscopy, indirect ophthalmoscopy, serum collection, and aqueous paracentesis was performed on 4/6 cats in Group 2 before inoculation with *T. gondii* and on weeks 4, 8, 12, and 20 postinoculation (PI). Fecal examinations were performed using sugar solution centrifugation daily for 30 days PI and then monthly on samples from all cats. From all cats in Group 2, whole blood (10 ml) was collected into nonpreserved heparin week 26 PI.

2.4. Serologic assays

Each serum and aqueous humor sample was assayed for *T. gondii*-specific IgM, *T. gondii*-specific IgG, *T. gondii*-specific antigens, total IgM, and total IgG (Lappin et al., 1992a). The Goldmann-Witmer coefficient (C value) for determination of local production of *T. gondii*-specific antibody in aqueous humor was calculated by multiplying the ratio of *T. gondii*-specific IgM or IgG in aqueous humor to serum by the ratio of total IgM or IgG in serum to aqueous humor. A positive C value is consistent with the presence of *T. gondii*-specific antibody in aqueous humor; a C value > 1 is consistent with local production of *T. gondii*-specific antibody in aqueous humor (Lappin et al., 1992a). Serum from each cat was assayed for antibodies against feline immunodeficiency virus (Cite[R] Combo, IDEXX Corp., Portland, ME), feline leukemia virus p27 antigen (Cite[R] Combo, IDEXX Corp., Portland, ME), and antibodies against feline coronaviruses (Post et al., 1978).

2.5. Interleukin 6 bioassay

Feline IL-6 activity was measured by bioassay, using the IL-6-dependent murine cell line 7TD1 (American Type Culture Collection, Rockville, MD). This cell line has been shown previously to respond to recombinant and native IL-6 (human, rat and murine) and not to other cytokines including interleukin 1, interleukin 2, interleukin 4, and tumor necrosis factor alpha (van Snick et al., 1986). We have found that this cell line also responds by proliferation to a factor present in the supernatant of LPS-stimulated feline macrophage cultures and to a lower degree, to a factor present in supernatants from stimulated feline astrocyte and endothelial cell cultures (Elmslie et al., 1991). Due to the high degree of interspecies IL-6 homology, we believe that 7TDI cells therefore also respond to feline IL-6. A similar bioassay has been reported previously for measuring feline IL-6 activity (Goitsuka et al., 1990).

The bioassay was done as described previously for rat IL-6 (Elmslie et al., 1991). In brief, 5×10^4 7TD1 cells were added to triplicate wells in a 96 well plate containing 100 μl media in which test sera, aqueous humor, or supernatants were diluted, using serial 5-fold dilutions (starting dilutions — serum: 1:10; aqueous humor: 1:100; supernatants: 1:10) Serum and aqueous humor were treated at 56° for 30 min to inactivate complement. The 7TD1 cells were incubated for 96 h and the number of viable cells per well quantitated by MTT assay (Hansen et al., 1989). Interleukin 6 was measured at the highest dilution causing proliferation of 7TD1 cells and quantitated by comparison with a standard curve generated using human recombinant IL-6 (Boehringer-Mannhein Biochemicals, Indianapolis, IN). The assay was sensitive to 1.0 units ml^{-1} IL-6. Results were expressed as the average IL-6 activity for triplicate wells for a given dilution. Human recombinant IL-6 was added to media and three serum samples from SPF cats to give a concentration of 50 units ml^{-1} and the samples assayed for IL-6 as described. IL-6 activities were greater in each serum sample than the media control showing that feline sera does not inhibit the IL-6 assay described herein.

2.6. In vitro peripheral blood mononuclear cell stimulation

Peripheral blood mononuclear cells were separated from heparinized whole blood collected Week 26 PI from Group 2 cats (Lappin et al., 1992b). Peripheral blood mononuclear cells were separated from heparinized whole blood collected from six, specific pathogen free, *T. gondii*-seronegative adult cats to serve as controls. Concanavalin A, *T. gondii* tachyzoite antigens, and *T. gondii* secretory antigens were used to stimulate triplicate wells containing 2×10^5 cells from each *T. gondii*-infected and *T. gondii*-seronegative cat as previously described (Lappin et al., 1992b). Following a 96 h incubation period, supernatants were collected and assayed for IL-6 activity. Lymphoblast transformation in response to mitogen or antigen was assessed by MTT assay and stimulation indices (SI) were calculated (Hansen et al., 1989).

2.7. Statistical evaluation

Statistical analyses were conducted to determine whether the serum and aqueous humor IL-6 activities differed between Group 3 cats with and without serologic evidence

of present or prior infection by *T. gondii*, FIV, FeLV, or a coronavirus. Parametric and nonparametric procedures were applied. The IL-6 activities in aqueous humor were log-transformed in order to achieve a more normal distribution of the data; serum IL-6 values were evaluated both as logtransformed and nontransformed data. A two-sample *t* test was used to test the null hypothesis that no differences in the means of various classes of cats existed. The non-parametric Mann-Whitney *U* test was used as a second measure of statistical difference between groups in view of the small sample sizes and inherent assumption of the *t* test. All statistical analyses were conducted with the Statistical Analysis System for the personal computer (Statistical Analysis Systems Institute Inc., Version 6.04, Cary, NC). Significance was defined at $P < 0.05$.

3. Results

3.1. Group 1

Group mean serum IL-6 results from clinically normal, random source cats was 102.7 ± 132.5 U ml^{-1} (range, 0–462.1 U ml^{-1}). Interleukin-6 was not detected in aqueous humor from any cat.

3.2. Group 2

Each of the experimentally inoculated cats shed oocysts in feces by Day 7 PI; oocyst shedding was not detected after Day 16 PI. Polysystemic signs of disease were not observed. Localized retinitis developed in the left eye of one of the four cats used for IL-6 measurement; the lesion was quiescent by Week 8 PI. Detectable *T gondii*-specific serum IgM titers and IgG titers developed in 3/4 cats and 4/4 cats, respectively (Table 1). Interleukin-6 was not detected in serum on any sample date.

Toxoplasma gondii-specific IgM was not detected in aqueous humor. *Toxoplasma gondii*-specific IgG C values > 1 were detected in aqueous humor of each cat with peak values occurring on Week 8 or 12 PI. After inoculation with *T. gondii*, IL-6 activity greater than pre-inoculation activity was detected in the aqueous humor of 4/4 cats on at least one sample date. On Week 20 PI, IL-6 was detected in aqueous humor from 4/4 cats; *T. gondii*-specific IgG was not detectable. Of the ten aqueous humor samples with *T. gondii*-specific IgG *C* values > 1, eight had detectable IL-6 activity.

3.3. Group 3

Of the 27 cats, 25 had anterior segment inflammation characterized by aqueous flare or iritis and two had chorioretinitis without ophthalmologic evidence of anterior segment inflammation. Interleukin 6 was detected in the serum and aqueous humor of most cats with uveitis (Table 2). Interleukin 6 was detected in 22/27 aqueous humor samples (81.5%) with a range of 28.9 U ml^{-1}–15702.9 U ml^{-1} (mean = 1911.9 U ml^{-1}, SD = 3946.7 U ml^{-1}). Interleukin 6 was detected in 22/27 serum samples (81.5%) with a range of 5.4 U ml^{-1}–145.3 U ml^{-1} (mean = 24.8 U ml^{-1}, SD = 32.4 U ml^{-1}).

Table 1
Aqueous humor and serum results from cats experimentally inoculated with *Toxoplasma gondii* strain ME49

Bleed/ Cat#	Serum IL-6 (U ml^{-1})	Aqueous IL-6 (U ml^{-1})	TG IgM titer	TG IgG titer	TG IgM *C*-value	TG IgG *C*-value
Pre-inoculation						
1	ND	72	neg	neg	neg	neg
2	ND	ND	neg	neg	neg	neg
3	ND	ND	neg	neg	neg	neg
4	ND	ND	neg	neg	neg	neg
Week 4						
1	ND	78	neg	128	neg	neg
2	ND	ND	64	512	neg	neg
3	ND	240	64	512	neg	19.8
4	ND	150	neg	256	neg	10.4
Week 8						
1	ND	150	512	1024	neg	40.7
2	ND	75	neg	4096	neg	37.8
3	ND	74	neg	512	neg	165.0
4	ND	ND	neg	1024	neg	60.5
Week 12						
1	ND	72	128	1024	neg	16.3
2	ND	ND	neg	2048	neg	78.7
3	ND	155	neg	512	neg	214.0
4	ND	50	neg	1024	neg	9.2
Week 20						
1	ND	76	neg	1024	neg	neg
2	ND	69	neg	1024	neg	neg
3	ND	250	neg	4096	neg	neg
4	ND	71	neg	4096	neg	neg

TG: *Toxoplasma gondii*; IL-6: interleukin 6; ND: not detectable; neg: negative.
T. gondii IgM and IgG titers determined by ELISA and reported as reciprocal titers.
T. gondii IgM and IgG *C*-values determined by calculating the Goldman-Witmer coefficient. *C*-values > 1 are consistent with local antibody production in the aqueous humor.

Interleukin 6 activity was greater in aqueous humor than in the corresponding serum sample for 21/27 cats.

Serologic evidence of exposure to *T. gondii*, FIV, FeLV, or a coronavirus (Table 2) was present in 21/27 cats (77.8%). Interleukin 6 was detected in the aqueous humor of 18/21 (85.7%) and 3/6 (50%) of the cats with and without serologic evidence of exposure to one to the infectious diseases, respectively. The majority of the cats had *T. gondii* IgM, IgG, or antigens in serum (20/27 cats; 74.1%). Interleukin 6 was detected in the aqueous humor of the majority of cats with *T. gondii*-specific IgM, IgG, or antigens in serum (18/20 cats; 90%). The two *T. gondii*-seropositive cats negative for IL-6 in aqueous humor had *T. gondii*-specific antigens in serum without IgM or IgG. All cats with *T. gondii* antigens in aqueous humor ($n = 6$) or *T. gondii* *C* values > 1 in aqueous humor ($n = 12$) had detectable IL-6 in aqueous humor. Most cats with detectable coronavirus IgG in serum had detectable IL-6 in aqueous humor (10/11 cats; 90.9%). Of these ten cats, nine had *T gondii* antibodies or antigens in serum. The

Table 2
Mean serum and aqueous humor interleukin 6 results from cats with naturally-occurring uveitis stratified by infectious disease serologic results

Cat group	Sample size	Serum IL-6 (U ml^{-1})		Aqueous IL-6 (U ml^{-1})	
		Mean (SD)	Range	Mean (SD)	Range
All cats	27	24.77 (32.37)	ND–145.3	1911.88 (3946.7)	ND–15702.9
Seronegative cats [a]	6	15.17 (14.9)	ND–38.8	395.15 (592.6)	ND–1176.1
Any TG test positive	20	28.9 (35.9)	ND–145.3	1739.51 (3489.7)	ND–15702.9
TG antigen in aqueous	6	28.62 (25.0)	6.0–66.7	4565.32 (5587.2)	813.4–15702.9
TG *C*-value > 1	12	20.3 (22.7)	ND–61.9	582.3 (651.0)	28.9–2270.5
TG IgM *C*-value > 1	8	15.6 (19.3)	ND–51.9	654.9 (722.9)	159.5–2270.5
TG IgG *C*-value > 1	6	27.6 (25.2)	ND–61.9	442.9 (451.7)	28.9–1181.0
TG IgM and IgG *C*-values > 1	2	23.2 (17.2)	6.0–40.4	454.4 (376.9)	187.9–720.9
Coronavirus IgG ≥ 1:100 [b]	11	27.66 (44.0)	ND–145.3	2310.0 (4248.3)	ND–14459.7
FeLV positive [b]	1	7.3		3855.8	
FIV positive [b]	2	6.0 (0)		558.25 (230.0)	395.6–720.9

[a] Serologically negative for *T. gondii*, coronavirus, FeLV, and FIV.
[b] The FeLV-positive cat was positive for *T. gondii* antigens in serum and aqueous and coronavirus antibodies in serum, the FIV-positive cats were seropositive for *T gondii*, and 10/11 coronavirus-positive cats were positive for *T gondii*.
TG: *Toxoplasma gondii*; IL-6: interleukin 6; ND: not detectable. *T. gondii* IgM and IgG titers determined by ELISA and reported as reciprocal titers. *T. gondii* IgM and IgG *C*-values determined by calculating the Goldman-Witmer coefficient. *C*-values > 1 are consistent with local antibody production in the aqueous humor. *T. gondii* antigen levels determined by ELISA. FIV: feline immunodeficiency virus. Results determined by an ELISA for the detection of IgG antibodies against FIV in serum. FeLV: feline leukemia virus. Results determined by an ELISA for the detection of p27 antigen in serum. FIP: feline infectious peritonitis virus. Results determined by an IFA for the detection of IgG antibodies against coronaviruses in serum and are presented as reciprocal titers.

FIV-seropositive cats and the FeLV-seropositive cat had detectable IL-6 in aqueous humor; these cats had *T. gondii* antibodies, *T. gondii* antigens or coronavirus IgG in serum.

Serum and aqueous humor IL-6 results were stratified by infectious disease test results; the group mean and range of aqueous humor IL-6 values varied greatly (Table 2). No statistically significant differences were found in mean serum IL-6 activities when cats without serologic evidence of infection by *T. gondii*, FIP, FIV, or FeLV were compared with various classes of cats with evidence of such infections. Log transformation of the serum IL-6 activities produced similar findings. However, several statistically significant differences (t test) were found in the mean activities of IL-6 in aqueous humor when seronegative and various classes of infected cats were compared. Statistically significant increases in mean IL-6 activities were found for cats with any evidence of infection with *T. gondii* ($P = 0.04$), for cats with *T. gondii* antigen in aqueous humor ($P = 0.02$) and for cats with coronavirus antibody titers > 1:100 ($P = 0.048$). Non-parametric testing (Wilcoxon) confirmed the direction of the differences found with the t test, although only the IL-6 activity in cats with *T. gondii* antigen in aqueous humor was significantly different ($P = 0.01$) with the less powerful procedure.

3.4. In vitro peripheral blood mononuclear cell stimulation

Toxoplasma gondii-infected cats (mean SI = 8.03, SD = 2.04) and *T. gondii*-naive cats (mean SI = 10.9, SD = 3.4) had similar lymphoblast transformation in response to concanavalin A. The group mean stimulation index in response to tachyzoite antigens was greater ($P < 0.05$) for *T. gondii*-infected cats (mean SI = 3.73, SD = 2.14) than *T. gondii*-naive cats (mean SI = 2.58, SD = 0.6) suggesting an antigen-specific result. Significant differences in IL-6 activity in supernatants from *T. gondii*-infected cat peripheral blood mononuclear cells stimulated with concanavalin A (mean IL-6 = 1491.7, SD = 718.2) or *T. gondii* antigens (tachyzoite antigen mean IL-6 = 878.2, SD = 912.3; secretory antigen mean IL-6 = 1073.5, SD = 885.0) were not detected.

4. Discussion

Increased IL-6 activity was detected in aqueous humor of most cats with uveitis but not clinically normal cats. Cats experimentally inoculated with *T. gondii* developed only minimal increases in detectable IL-6 activity in aqueous humor even when the eyes appeared to be immunologically active as demonstrated by local antibody production (IgG *C* values > 1). These cats did not have evidence of anterior segment inflammation and the one cat with retinochoroiditis was minimally affected. These results suggest that IL-6 in aqueous humor of cats is associated primarily with inflammation supporting results from studies in rabbits (de Vos et al., 1992), rats (Hoekzema et al., 1991), and people (Murray et al., 1990; Norose et al., 1994; van der Lelij et al., 1991).

Detection of higher IL-6 activity in aqueous humor than serum of most cats with uveitis suggests that the IL-6 was produced locally in ocular tissues. Injection of endotoxin into rats results in IL-6 activity that is greater in aqueous humor than serum (Hoekzema et al., 1991; de Vos et al., 1994). These findings are likely due either to preferential intraocular IL-6 production or relative lack of IL-6 down regulation in the eye compared with peripheral blood cells. The source of intraocular IL-6 synthesis during uveitis is unknown but has been hypothesized to be from resident ocular cells including macrophages, endothelial cells, and epithelial cells as well as infiltrating monocytes and neutrophils that enter the eye during inflammation (de Vos et al., 1992).

Failure to detect IL-6 activity in aqueous humor from 5/27 cats with uveitis suggests that IL-6 is not the only inflammatory mediator of uveitis in cats. Tumor necrosis factor alpha, interleukin 1, interleukin 2, and gamma interferon have been detected in ocular fluids from people with uveitis (de Vos et al., 1992). Tumor necrosis factor appears to be involved with mediation of endotoxin-induced uveitis in rats (de Vos et al., 1992). Alternately, presence of other cytokines in aqueous humor (e.g. transforming growth factor beta) may have inhibited IL-6 activity in the bioassay. Timing of sample collection may also have affected our results. Aqueous humor IL-6 activity declines rapidly in rats with endotoxin-induced uveitis following clearance of endotoxin (de Vos et al., 1994).

Infection of mice with *T. gondii* results in the expression of IL-6 mRNA in central nervous system tissues (Hunter et al., 1992a; Hunter et al., 1992b), increased IL-6

activity in serum and cerebrospinal fluid (Schluter et al., 1993), and induction of IL-6 production by T-lymphocytes (Chardes et al., 1993). These results document that *T. gondii* is capable of inducing the production of IL-6 in several murine tissues. Our *T. gondii* inoculated experimental cats failed to develop increased IL-6 activity in serum and peripheral blood mononuclear cells collected on Week 26 failed to produce significant IL-6 activity when exposed to *T. gondii* tachyzoite antigens. It is possible these results are related to the strain and dose of *T. gondii* or samples collection times studied here.

Cats with ocular signs of toxoplasmosis are more likely to have *T. gondii* IgM in serum than cats with non-ocular signs of toxoplasmosis or healthy cats (Chavkin et al., 1992; Lappin et al., 1992a). *Toxoplasma gondii* IgM has never been documented in aqueous humor of healthy cats with experimentally-induced toxoplasmosis (Chavkin et al., 1994) but is the most common antibody produced in aqueous humor of cats with naturally-occurring uveitis (Lappin et al., 1992b). Interleukin 6 has been shown to be a potent cofactor with IL1 in the stimulation of IgM synthesis (Kunimoto et al., 1989). It is possible that in some cats with uveitis, IL-6 potentiates the production of *T. gondii* IgM in aqueous humor. The finding of high IL-6 activity in aqueous humor only during uveitis may partially explain the detection of *T. gondii* IgM only in aqueous humor of cats with uveitis. Interleukin 6 reverses interferon-gamma mediated activation of murine macrophages and promotes replication of *T. gondii* (Beaman et al., 1994) which may explain our finding of *T. gondii* antigens in the aqueous humor of cats with the highest IL-6 activity.

The lack of pathognomonic ophthalmic signs, the failure of serologic evidence of present or prior infection by FeLV, FIV, feline coronaviruses, or *T. gondii* to directly correlate with intraocular inflammation, and the presence of serologic evidence of mixed infections makes direct association of aqueous humor IL-6 activity with a specific infectious agent impossible in this study. Based on Group 3 results in this study, it appears likely that most, if not all causes of feline uveitis induce intraocular synthesis of IL-6.

References

Beaman, M.H., Hunter, C.A. and Remington, J.S., 1994. Enhancement of intracellular replication of *Toxoplasma gondii* by IL-6: interactions with IFN-gamma and TNF-alpha. J. Immunol., 153: 4583–4587.

Chardes, T., Veige-Roussel, F., Merelee, P., Mevelec M.N., Buzoina-Gatel, D. and Bout, D., 1993. Mucosal and systemic cellular immune responses induced by *Toxoplasma gondii* antigens in cyst orally infected mice. Immunology, 78: 421–429.

Charkin, M.J., Lappin, M.R., Poweli, C.C., Roberts, S.M., Parshall, C.J. and Reif, J.S., 1992. Seroepidemiologic and clinical observations of 93 cases of uveitis in cats. Prog. Vet. Comp. Ophthalmol., 2: 29–36.

Chavkin, M.J., Lappin, M.R., Powell, C.C., Cooper, C.M. and Munana, K.R., 1994. *Toxoplasma gondii*-specific antibodies in the aqueous humor of cats with toxoplasmosis. Am. J. Vet. Res., 55: 1244–1249.

Davidson, M.G., Nasisse, M.P., English, R.V, Wilcock, B.P. and Jamieson, V.E., 1990. Feline anterior uveitis: a study of 53 cases. J. Am. Anim. Hosp. Assoc., 27: 77–83.

de Vos, A.F., Hoekzema, R. and Kijlstra, A., 1992. Cytokines and uveitis, a review. Curr. Eye Res., 6: 581–597.

de Vos, A.F., van Haren, M.A.C., Verhagen, C., Hoekzema, R. and Kijlstra, A., 1994. Kinetics of intraocular tumor necrosis factor and interleukin-6 in endotoxin-induced uveitis in the rat. Invest. Ophthalmol. Vis. Sci., 35: 1100–1106.

Dubey, J.P. and Carpenter, J.L., 1993. Histologically confirmed clinical toxoplasmosis in cats: 100 cases (1952–1990). J. Am. Vet. Med. Assoc., 203: 1556–1566.

Elmslie, R.E., Ogilvie, G.K., Dow, S.W. et al., 1991. Evaluation of a biologic response modifier derived from *Serratia marcescens*: effects on feline macrophages and usefulness for the prevention and treatment of viremia in feline leukemia virus-infected cats. Mol. Biother., 3: 231–238.

English, R.V., Davidson, M.G., Nasisse, M.R., Jamieson, V.E. and Lappin, M.R., 1990. Intraocular disease associated with feline immunodeficiency virus infection in cats. J. Am. Vet. Med. Assoc., 196: 1116.

Gilgor, T., Andus, T., Klapproth, J., Hirano, T., Kishimoto, T. and Heinrich, P.C., 1988. Induction of rat acute-phase proteins by interleukin 6 in vivo. Eur. J. Immunol., 18: 717–721.

Goitsuka, R., Ohashi, T., Ono, K., Yasukawa, K., Doishibara, Y., Fukui, H., Ohsugi, Y. and Hasegawa, A., 1990. IL-6 activity in feline infectious peritonitis. J. Immunol., 144: 2599–2603.

Hansen, M.B., Nielson, S.E. and Berg, K., 1989. Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. J. Immunol. Methods, 119: 203–206.

Hoekzema, R., Murray, P.I., Van Haren, M.A.C., Helle, M. and Kijlstra, A., 1991. Analysis of interleukin-6 in endotoxin-induced uveitis. Invest. Ophthalmol. Vis. Sci., 32: 88–95.

Hunter, C.A., Roberts, C.W., Murray, M. and Alexander, J.A., 1992a. Detection of cytokine mRNA in the brains of mice with toxoplasmic encephalitis. Parasite Immunol., 14: 405–413.

Hunter, C.A., Roberts, C.W. and Alexander, J., 1992b. Kinetics of cytokine mRNA production in the brains of mice with progressive toxoplasmic encephalitis. Eur. J. Immunol., 22: 2317–2322.

Kauffmann, D.J.H., van Meurs, J.C., Mertens, D.A.E., Peperkamp, E., Master, C. and Gerritsen, M.E., 1994. Cytokines in vitreous humor: interleukin-6 is elevated in proliferative vitreoretinopathy. Invest. Ophthalmol. Vis. Sci., 35: 900–906.

Kishimoto, T., 1989. The biology of interleukin-6. Blood, 74: 1–10.

Kunimoto, D.Y., Nordan, R.P. and Strober, W., 1989. IL-6 is a potent cofactor of IL-1 in lgM synthesis and of IL-5 in IgA synthesis. J. Immunol., 143: 2230–2235.

Lappin, M.R., Roberts, S.M., Davidson, M.G., Powell, C.C. and Reif, J.S., 1992a. Enzyme-linked immunosorbent assays for the detection of *Toxoplasma gondii*-specific antibodies and antigens in the aqueous humor of cats. J. Am. Vet. Med. Assoc., 201: 1010–1016.

Lappin, M.R., Gasper, P.W., Rose, B.J. and Powell, C.C., 1992b. Effect of primary phase feline immunodeficiency virus infection on cats with chronic toxoplasmosis. Vet. Immunol. Immunopathol., 35: 121–131.

Murray, P.I., Hoekzema, R., van Haren, M.A.C., de Hon, F.D. and Kijlstra, A., 1990. Aqueous humor interleukin-6 levels in uveitis. Invest. Ophthalmol. Vis. Sci., 31: 917–920.

Norose, K., Yano, A., Wang, X.C. et al., 1994. Dominance of activated T cells and interleukin-6 in aqueous humor in Vogt-Koyanagi-Harada disease. Invest. Ophthalmol. Vis. Sci., 35: 33–39.

Peiffer, R.L. and Wilcock, B.P., 1991. Histopathologic study of uveitis in cats: 139 cases (1978–1988). J. Am. Vet. Med. Assoc., 198: 135–138.

Post, I.E., Wellenstein, R.C. and Clarke, R.D., 1978. Serologic tests for feline infectious peritonitis using TGE virus antigen. In: Proceedings, 21st Ann. Meet. Am. Assoc. Vet. Lab. Diagnost., pp. 427–436.

Schluter, D., Deckert-Schluter, M., Schwendemann, G., Brunner, H. and Hof, H., 1993. Expression of major histocompatibility complex class II antigens and levels of interferongamma, tumour necrosis factor, and interleukin-6 in cerebrospinal fluid and serum in *Toxoplasma gondii*-infected SCID and immunocompetent C.B-17 mice. Immunology, 78: 430–435.

van der Lelij, A., Rothova, A., De Vries, J.P., Vetter, J.C.M., Van Haren, M.A.C., Stilma, J.S. and Kijlstra, A., 1991. Analysis of aqueous humour in ocular onchocerciasis. Current Eye Res., 10: 169–175.

van Snick, J., Cayphass, Umik, A. et al., 1986. Purification of NH2-terminal amino acid sequence of a T-cell derived cytokine with growth factor activity for B-cell hybridomas. Proc. Natl. Acad. Sci. USA, 83: 9679–9683.

Williams, D., 1994. Local and systemic implications of feline ocular disease: iridal lesions. Fel. Pract., 22: 22–30.