Effects of feline herpesvirus type 1 on tear film break-up time, Schirmer tear test results, and conjunctival goblet cell density in experimentally infected cats

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Objective—To determine the effect of feline herpesvirus type 1 (FHV-1) on tear film break-up time (TFBUT) and Schirmer tear test (STT) values in cats with primary experimental infection and to determine the relationship between TFBUT and STT values and conjunctival goblet cell density (GCD).

Sample Population—9 specific-pathogen–free cats of approximately 6 months of age.

Procedures—6 cats were inoculated with FHV-1; 3 control cats were sham inoculated. Clinical and histologic evidence of conjunctivitis and TFBUT, GCD, and STT values were assessed at multiple times until postinoculation day (PID) 29.

Results—In infected cats, mean clinical and histologic conjunctivitis scores peaked at PID 7 and remained above baseline at PID 29. In control cats, these 2 variables did not change from baseline throughout the study. Mean TFBUT declined rapidly in infected cats up to PID 15 and at PID 29 remained less than baseline, less than for control cats, and below reference range values. Mean STT value for infected cats at PID 29 was increased from baseline but was within the reference range and not different from the value for control cats. Mean GCD in infected cats declined precipitously by PID 7 and remained below reference range values at PID 29. Mean GCD in control cats remained unchanged for the duration of the study period.

Conclusions and Clinical Relevance—FHV-1 induced qualitative tear film abnormalities in experimentally infected cats, as measured by TFBUT and GCD. Assessment of TFBUT provided a reasonable clinical estimate of GCD. (Am J Vet Res 2009;70:394–403)

The preocular tear film in cats is a trilaminar structure composed of an outer lipid layer produced by the meibomian glands, a middle aqueous layer produced by the orbital lacrimal gland and gland of the third eyelid, and an inner mucin layer produced mainly by the conjunctival goblet cells. All 3 layers are critical for maintaining ocular health. A deficiency in the aqueous layer manifests clinically as keratoconjunctivitis sicca, is diagnosed on the basis of STT values, and is termed a quantitative tear film abnormality. Cats are uncommonly given a diagnosis of keratoconjunctivitis sicca. Deficiency of the lipid or mucin layer is referred to as a qualitative tear film abnormality and causes instability of the preocular tear film, with premature breakup of the tear film and resultant corneal desiccation. Qualitative tear film disorders are more challenging to diagnose. Histologic examination of meibomian gland biopsy specimens can be used indirectly to infer lipid dysfunction if findings are abnormal but are of less diagnostic value if normal. Conjunctival biopsy specimens permit enumeration of conjunctival goblet cells, which is considered an indirect assessment of preocular...
mucin. The TFBUT is a more practical measure of tear film stability and, in the absence of blepharitis, can be considered an indirect measure of preocular mucin.  

Feline herpesvirus type 1 is a common cause of upper respiratory tract and ocular diseases in kittens and cats. Disease is typically self-limiting following primary infection, but can become chronic or recurrent in some cats. Clinical, histologic, and immunologic changes that occur during natural and experimental FHV-1 infections are well characterized.\(^b\)\(^-\)\(^q\) Additionally, tear film dysfunction and accelerated TFBUT have been reported for cats with naturally occurring conjunctivitis, some of which were infected with FHV-1.\(^b\)\(^-\)\(^f\) However, little is known about the effects of FHV-1 infection on the tear film. Given the potential relationships between FHV-1, keratoconjunctivitis, and tear film dysfunction, we hypothesized that FHV-1 infection would be associated with alterations in STT values, GCD, and tear film stability, which may delay recovery from infection. Therefore, the purpose of the study reported here was to determine the effects of FHV-1 infection over time on preocular mucins (as measured by TFBUT and GCD) and STT values. Furthermore, the clinical value of TFBUT assessment was evaluated by examining correlations between TFBUTs and other indices such as clinical disease severity, STT values, and GCD during experimental primary FHV-1 infection.

### Materials and Methods

#### Study population and examination techniques

Four male and 5 female specific-pathogen–free domestic shorthair cats of approximately 6 months of age and without known history of ocular or systemic illness were included in this study. Six cats (4 male and 2 female) were infected with FHV-1; the remaining 3 cats (all female) served as uninfected control cats. Because the 6 infected cats were also involved in an unrelated study, and to avoid the potential for inadvertent FHV-1 infection of the control cats, data were collected from the control cats after the infected cats in a separate experiment. Therefore, the person performing clinical scores was not blinded as to treatment group. However, care was taken to ensure that the procedures were performed in an identical manner for both groups and the pathologist was blinded as to treatment group when reviewing conjunctival biopsy specimens.

Prior to inclusion in the study, it was determined that all cats were free of serum antibodies to FeLV and FHV-1 and serum antigens of FIV. Following a 24-hour period for acclimatization, all cats underwent complete physical and ophthalmic examinations. General physical examination included assessment of rectal body temperature, pulse rate, and respiratory rate, along with thoracic auscultation and clinical assessment of hydration, mucous membrane color, and behavior. Ophthalmic examination included assessment of pupillary light reflexes, palpebral reflex, menace response, slit-lamp biomicroscopy, STT values, TFBUT, and fluorescein staining of the cornea, in that order. All cats were also assessed on the basis of findings on CBC, serum biochemistry analysis, and urinalysis. Following collection of baseline data, 6 cats were inoculated with a total of 3.2 × 10⁷ plaque-forming units of strain 727 (passage 8) FHV-1 divided approximately equally among both nares and both conjunctival fornices. This virus is a plaque-purified field isolate that has been verified as FHV-1 by use of results of immunofluorescence with FHV-1–specific antisera\(^5\) and verified by use of PCR assay to be uncontaminated by Mycoplasma spp,\(^6\) Chlamydophila felis,\(^7\) and feline calicivirus,\(^8\) any of which might have confounded the clinical disease produced. This dose has been used in previous studies\(^7\)\(^-\)\(^9\) and reliably produces clinically overt but self-limiting disease in young adult specific-pathogen–free cats. The 3 control cats underwent sham inoculation with an equal volume of uninfected viral culture media applied as for infected cats. No cats received any topical (ophthalmic) or systemic treatment during the study. All cats were maintained and handled in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research, and all experimental procedures were approved by the University of California, Davis, Institutional Animal Care and Use Committee.

Once daily for the first 21 days after inoculation and again at 29 days after inoculation, cats underwent general physical examinations as described for the baseline examination. One day before FHV-1 or sham inoculation and at 3, 6, 9, 12, 15, 19, and 29 days after inoculation, each cat received a complete ophthalmic examination as described for the baseline examination. Prior to and once daily following inoculation, clinical signs of ocular or respiratory tract disease were scored according to a published scoring system.\(^3\) Severity of conjunctivitis was assigned a score of 0 (none) through 3 (moderate to severe). Severity of blepharospasm was assigned a score of 0 (none) through 4 (eye completely closed). Severity of ocular discharge was assigned a score of 0 (none) through 3 (marked mucopurulent discharge). Sneezing was assigned a score of 0 (absent) or 1 (present). Severity of nasal discharge was assigned a score of 0 (none) through 3 (marked mucopurulent discharge). Total clinical disease score was calculated by adding all scores for the patient and ranged from 0 (normal) to 24. Serologic testing for FHV-1 was repeated in all cats at 21 days after inoculation to verify FHV-1 infection in infected cats and to rule out accidental FHV-1 infection in control cats.

Following baseline measurement, quantitative and qualitative tear film variables were assessed every 3 days for 21 days, and again at 29 days after inoculation. Aqueous tear production, measured as millimeters of wetting in 1 minute, was measured for each eye by placing a standardized STT strip\(^4\) into the ventrolateral conjunctival sac for 1 minute. For each group, the STT values for all cats were obtained with STT strips from the same lot number; however, the STT strips used for the infected cats were from a different lot number than those used for the infected cats. The TFBUT was assessed by instilling fluorescein stain onto the dorsolateral bulbar conjunctiva and holding the eyelids closed for a few seconds. Eyelids were then opened, and the dorsolateral corneal surface was observed by use of a slit-lamp biomicroscope\(^a\) at 16× magnification and with light emitted through a cobalt blue filter. The TFBUT was defined as the time from eyelid opening to the first
signs that the tear film was breaking up, evident as the appearance of a dark spot within the fluorescent green tear film. A single reading was performed for each cat at each time point. A stopwatch was used to ensure accurate timing for STT values and TFBUT; the latter was recorded to a hundredth of a second. Determination of STT values and TFBUTs was performed exclusively by 1 author (CCL).

As part of a separate study, conjunctival samples were obtained for DNA analysis from each cat by rotating a cytology brush approximately 5 times in each ventromedial conjunctival fornix following induction of local anesthesia by topical application of 1 drop of 0.5% proparacaine hydrochloride ophthalmic solution. Conjunctival cytology brush samples were collected once daily for 9 days, then once every other day for the remainder of the study period.

**Conjunctival biopsy specimen collection and evaluation**—Immediately prior to FHV-1 or sham inoculation and at 7, 14, 21, and 29 days after inoculation, conjunctival biopsy specimens were obtained from the ventromedial conjunctival fornix of alternating eyes of each cat following induction of topical anesthesia with 1 drop of 0.5% proparacaine hydrochloride ophthalmic solution augmented by application of 2% lidocaine hydrochloride gel. Each tissue specimen was approximately 3 × 3 × 3 mm. Conjunctival specimens were placed into tissue cassettes with the epithelial surfaces oriented upward, then placed in neutral-buffered 10% formalin for histologic evaluation. When multiple specimens were collected or tests were performed on the same day, the order was always ophthalmic examination, STT value determination, TFBUT determination, conjunctival cytology brush sample collection, and finally conjunctival biopsy specimen collection.

Formalin-fixed conjunctival biopsy specimens were paraffin embedded and sectioned to a thickness of 4 µm. Serial sections were stained with H&E and PAS and evaluated by use of light microscopy. Sections stained with PAS were examined by 1 author (CCL) who quantified goblet cells. For each PAS-stained section, 50 consecutive epithelial cells were counted and the ratio of goblet to epithelial cells (ie, GCD) was determined. All H&E-stained sections were examined by 1 author (CMR) and evaluated for the severity, type, and location of conjunctival inflammation. Inflammation was scored as 0 when inflammatory cells were absent or if there were sparsely scattered, individual inflammatory cells. In the absence of inflammation, presence of 1 to 2 subepithelial lymphoid follicles was considered normal. Inflammation was scored as 1 if there were scattered aggregates or diffusely distributed low numbers of inflammatory cells. Inflammation was scored as 2 if there were focally large or diffusely moderate numbers of inflammatory cells with or without mild distortion of tissue architecture. If there was diffuse infiltration or effacement of the mucosa by large numbers of inflammatory cells with distortion of tissue architecture, inflammation was scored as 3. Inflammation was further categorized on the basis of the predominant cell types present (neutrophilic, lymphoplasmacytic, or mixed) and predominant location (epithelial, submucosal, or mixed).

**Data analysis**—Total clinical disease scores were defined as the sum of scores for all scored categories for each cat, with a maximum possible total disease score being 24. The clinical score for conjunctivitis for each eye was defined as the sum of scores for chemosis and conjunctival hyperemia for that eye, with a maximum possible score being 3/eye. Tear film break-up times, STT values, and clinical score for conjunctivitis for each eye of each cat were treated as independent variables for all statistical analyses. Univariate and multivariate analyses of the marginal and joint effects of group and either time or biopsy status of eye on dependent variables were performed by use of a repeated-measures ANOVA. For all variables, baseline data collected prior to inoculation were compared with terminal data collected at 29 days after inoculation by use of the paired t test. Terminal data were compared between the infected and control groups by use of the Student 2-tailed t test. Unless otherwise stated, data are presented as mean ± SD. Significance was set at a value of P < 0.05 for all analyses.

## Results

Results of baseline CBC, serum biochemistry analysis, and urinalysis were within reference limits for all cats. None of the control cats seroconverted with respect to FHV-1 or developed corneal ulcers or evidence of respiratory tract disease. However, all control cats developed intermittent clinical signs consistent with low-grade conjunctivitis (blepharospasm, ocular discharge, and conjunctival hyperemia). Two of the 3 control cats had these signs for the first few days following biopsy specimen collection but then had no clinical evidence

![Figure 1](image.png)
of disease (total disease score = 0) from 18 to 29 days after inoculation. The third control cat had more persistent signs of conjunctivitis following development of excessive granulation tissue at the site of conjunctival biopsy specimen collection on 1 occasion only.

By contrast, all cats inoculated with FHV-1 sero-converted, developed dendritic corneal ulcers, and had other ocular and upper respiratory tract signs typical of FHV-1 infection, including conjunctival hyperemia, ocular discharge, blepharospasm, and sneezing. Corneal ulcers first became apparent between 9 and 15 days after inoculation. Ulcers were present in 1 cat at 9 days after inoculation; in 5 cats at 12, 15, and 19 days after inoculation; and in 1 cat at 29 days after inoculation. No infected cats developed overt granulation tissue following biopsy.

Mean clinical score for conjunctivitis at baseline was 0 for all cats in both groups. Over the course of the study, there was a significant increase in mean clinical score for conjunctivitis in the infected (P < 0.001) but not the control (P = 0.39) cats (Figure 1). In addition to this variation over time, important differences were observed between the infected and control groups. Over the course of the study, mean clinical score for conjunctivitis of infected cats was significantly (P = 0.002) higher than that of control cats. An effect of biopsy on the magnitude of these differences was observed in infected cats, resulting in significantly (P = 0.004) higher clinical score for conjunctivitis for eyes that underwent biopsy within the past 7 days, compared with eyes that did not undergo biopsy within the previous week. This biopsy effect was not observed in control cats (P = 0.078). At the end of the study (29 days after inoculation), mean clinical score for conjunctivitis for infected cats was 1.2 ± 0.8, remained significantly (P = 0.017) increased, compared with baseline (0 ± 0); however, 3 eyes of 2 cats had no clinical evidence of conjunctivitis at that time. By contrast, mean clinical score for conjunctivitis for control cats at 29 days after inoculation (0.2 ± 0.3) was not significantly (P = 0.42) altered from baseline (0 ± 0) and only 1 eye from 1 cat had clinical evidence of mild conjunctivitis (disease score = 1) at 29 days after inoculation. At 29 days after inoculation, mean clinical score for conjunctivitis did not differ significantly (P = 0.086) between control (0.2 ± 0.3) and infected cats (1.2 ± 0.8).

Total disease score at baseline for each infected and control cat was 0. Because disease in control cats was typically limited to the eyes that recently underwent biopsy, mean total disease score was approximately equivalent to mean clinical score for conjunctivitis in the control group for the entire study period. However, for infected cats, total disease score was higher than clinical score for conjunctivitis for most of the study period. Mean total disease score of control cats never exceeded 3 and did not differ significantly (P = 0.43) from baseline or from 0 at any time during the study (Figure 2). By contrast, mean total disease score of infected cats changed significantly (P < 0.001) over time, increasing rapidly from approximately 1 day after inoculation, peaking at 7 days after inoculation, and then declining gradually as all cats recovered. However, at the end of the study (29 days after inoculation), mean total disease score of infected cats (4.5 ± 3.0) remained significantly (P = 0.013) greater than at baseline (0 ± 0) and only 1 cat was considered clinically normal. By contrast, 2 of 3 control cats were considered clinically normal at
29 days after inoculation, and the mean total disease score for all control cats at study end (0.3 ± 0.6) was not significantly altered (P = 0.42) from baseline (0 ± 0). Over the study period, total disease score for infected cats was significantly (P < 0.001) higher than for control cats; however, by 29 days after inoculation, mean total disease score for control (0.3 ± 0.6) and infected (3.8 ± 3.5) cats did not differ significantly (P = 0.14).

Mean TFBUT at baseline of infected cats (22.49 ± 3.89 seconds) was slightly greater than reference range values, whereas mean TFBUT for control cats (9.62 ± 2.06 seconds) was slightly less than reference range values. Following inoculation, mean TFBUT of infected cats declined rapidly up to 15 days after inoculation and remained low at the final measurement at 29 days after inoculation (Figure 3). Following logarithmic transformation of these data, a significant (P = 0.001) decrease in TFBUT was identified for infected cats during the first 19 days after inoculation. These changes in TFBUTs were uniform among all infected cats, with TFBUT (averaged times for right and left eye) for all 6 cats less than reference range values from 15 to 29 days after inoculation. Unlike infected cats, mean TFBUT of control cats did not change significantly (P = 0.68) over the course of the study. There was no overall evidence of a biopsy effect on TFBUT in either the control (P = 0.55) or infected cats (P = 0.12). At study end (29 days after inoculation), mean TFBUT for control cats (12.23 ± 1.66 seconds) was not significantly (P = 0.001) altered from baseline (9.62 ± 2.06 seconds) and was within reference range values.

Following inoculation, mean STT value at baseline (8 ± 4 mm/min) for infected cats was less than reference range values, whereas for control cats (14 ± 6 mm/min), it was within the reference range (Figure 4). Throughout the study, mean STT values for infected and control cats varied widely. There was no overall evidence of a biopsy effect on STT values in either control (P = 0.21) or infected cats (P = 0.51). At the end of the study (29 days after inoculation), the mean STT value for control cats (14 ± 7 mm/min) was not significantly (P = 0.64) altered from baseline (14 ± 6 mm/min) and remained within the reference range. At this same time point, the mean STT value for infected cats (16 ± 3 mm/min) was significantly (P = 0.003) increased from baseline (8 ± 4 mm/min) but was within the reference range. A significant (P = 0.69) difference was not detected between the mean STT values for control cats (14 ± 7 mm/min) and infected cats (16 ± 3 mm/min) at the end of the study.

All cats from both groups developed histologic evidence of conjunctivitis. For infected cats, the mean histologic score for conjunctivitis changed significantly (P < 0.001) over time (Figure 5). The mean histologic score for conjunctivitis peaked at 7 days after inoculation (3.0 ± 0), and remained significantly (P = 0.038) above baseline (1.6 ± 0.3) at 29 days after inoculation (1.5 ± 0.6). Conjunctival inflammation in all infected cats was severe at 7 days after inoculation and was defined by
conjunctiva. There was predominantly lymphoplasmacytic and subepithelial infiltration, and the submucosa and epithelium were involved in all cases. Unlike infected cats, mean histologic score for conjunctivitis in control cats did not change significantly ($P = 0.62$) from baseline over the course of the study and had decreased to $0.7 \pm 0.6$ at both 21 and 29 days after inoculation. At all time points except for baseline, histologic score for conjunctivitis in infected cats was significantly ($P < 0.001$) higher than in control cats.

Mean GCD (number of goblet cells/50 epithelial cells) at baseline for infected cats ($34 \pm 7$) and control cats ($29 \pm 25$) was similar to the published reference value for cats. Following logarithmic transformation of the data, a significant ($P < 0.001$) decline was detected in mean GCD of infected cats over the course of the study; GCD had decreased precipitously by the first biopsy (7 days after inoculation; 0 ± 0) and remained less than the published reference values for the duration of the study (Figure 6). Goblet cell density for infected cats was significantly lower ($P < 0.001$) than for control cats at all but the first time point. At the end of the study (29 days after inoculation), mean GCD for infected cats ($4 \pm 3$) remained significantly ($P = 0.001$) less than for control cats ($29 \pm 25$). Median (25th to 75th percentile) GCD of infected cats at study end ($4 \pm 3$) was significantly ($P = 0.048$) less than for control cats ($34 \pm 12$ to 42$).

**Discussion**

Data from this study indicate that primary FHV-1 infections in naïve cats are associated with a rapid and persistent decrease in TFBUT, increase in STT values, and dramatic loss or sometimes complete absence of conjunctival goblet cells. These effects persisted beyond the time at which cats appeared to have otherwise recovered, suggesting that FHV-1 infection causes persistent and clinically important decreases in tear film stability and mucin content that might easily be overlooked unless specifically tested for. This was manifested in the present study as a precipitous and persistent decrease in mean TFBUT in infected cats from slightly greater than reported reference range values prior to viral inoculation to less than baseline values within 9 days after inoculation. This decrease persisted for at least the duration of this study so that, at 29 days after inoculation; conjunctival biopsy specimens from 1 infected cat remained devoid of goblet cells through the end of the study (Figure 7). By contrast, mean GCD in control cats remained unchanged for the duration of the study period ($P = 0.73$). Goblet cell density for infected cats was significantly lower ($P < 0.001$) than for control cats at all but the first time point. At the end of the study (29 days after inoculation), mean GCD for infected cats ($4 \pm 3$) remained significantly ($P = 0.001$) less than for control cats ($29 \pm 25$). Median (25th to 75th percentile) GCD of infected cats at study end ($4 \pm 3$) was significantly ($P = 0.048$) less than for control cats ($34 \pm 12$ to 42$).

**Figure 6**—Mean ± SD GCD for 6 cats experimentally inoculated with FHV-1 (black circles) and 3 cats sham inoculated with media only (white circles). Dotted line indicates published reference value.

**Figure 7**—Photomicrographs of sections of conjunctival biopsy specimens from a cat infected with FHV-1. A—Normal conjunctiva prior to viral inoculation (baseline; day 0). Goblet cells are numerous within the conjunctival epithelium. There is no evidence of inflammation. B—Marked neutrophilic exudate and diffuse ulcers of the conjunctival epithelium at 7 days after inoculation. C—Squamous metaplasia of the conjunctival epithelium at 29 days after inoculation. The architecture of the epithelium is disorganized, compared with the architecture at baseline. Although there is evidence of goblet cell regeneration, GCD (number of goblet cells/50 epithelial cells) is markedly decreased, compared with the GCD at baseline. PAS stained; bar = 50 µm.
inoculation, mean TFBUT for infected cats remained less than reported reference range values and significantly less than baseline TFBUT and mean TFBUT for control cats.

In humans, TFBUT is considered an indirect measure of precorneal mucins, which are produced mainly by the conjunctival goblet cells with lesser contributions from corneal epithelial cells. However, to our knowledge, this correlation has not been thoroughly assessed in cats. Therefore, in the present study, conjunctival biopsies were performed to elucidate relationships among GCD, TFBUT, and histologic signs of disease in cats. Prior to inoculation, the mean TFBUT was less for control cats than for infected cats, and both values were slightly outside the published reference range. The reason for the difference between groups is unknown but may represent high interindividual variation in TFBUT in cats and the relative lack of data on reference range values. For these reasons, assessment of the changes in mean TFBUT in both groups is likely to be more clinically relevant than interpretation of absolute values. Following viral inoculation of cats in the present study, GCD and TFBUT both declined at first and then simultaneously had some signs of recovery near the end of the study. By contrast, neither the TFBUT nor the GCD in control cats varied significantly from baseline values over the same period. The temporal and direct association between TFBUT and GCD observed in infected cats supports the hypothesis that TFBUT can be used as a clinical tool to approximate conjunctival GCD in cats. Clinically, the TFBUT is easier to measure and offers results sooner, compared with conjunctival biopsy specimen collection and histologic evaluation. Additionally, conjunctival biopsy causes or exacerbates conjunctivitis at least for a short period, whereas TFBUT determination is noninvasive.

Although TFBUT and GCD in infected cats declined synchronously early in the study, TFBUT continued to decline after GCD decreased to 0. This suggests that factors other than GCD may have contributed to the decline in TFBUT seen here. The TFBUT also can be affected by preservatives within ophthalmic irrigating solutions, the volume of fluorescein solution instilled, and irregularities of the corneal surface. Because the same eyewash solution was used for all cats throughout the study, this variable likely would not have influenced TFBUT comparisons. In research settings, TFBUT is sometimes expressed as an average of multiple readings. We elected to perform single but highly standardized assessments for each eye at each time point to better reflect the way the test is performed in clinical settings. Although a standard technique was always used for TFBUT measurements in this study, we did not measure the amount of fluorescein instilled within the conjunctival sac. However, the effects of fluorescein volume on TFBUT plateau when the instilled volume is moderate, as was used in the present study. Therefore, this variable would have been expected to affect readings minimally and approximately equally throughout the study. Corneal irregularities, in particular corneal ulcers, also may have exerted an effect on TFBUT in this study. All infected cats developed superficial corneal ulcers in the latter half of this study. Although it would be expected that corneal ulcers would result in more rapid TFBUTs by increasing corneal surface irregularity, no consistent association between presence of ulcers and changes in TFBUTs was detected in individual cats in this study. This may be in part attributed to small sample size; however, location of corneal ulcers may also play a role in TFBUT alterations. Intuitively, corneal ulcers located at or near the area from which the TFBUT is being recorded (in this study the dorsolateral aspect of the cornea) would be expected to exert more influence on TFBUT than ulcers located farther away from the area of the cornea being studied.

Baseline GCDs for cats in the present study were similar to published values for clinically normal cats. Following viral inoculation, GCD declined rapidly, and goblet cells were undetectable by 7 days after inoculation, the time at which peak total disease scores and maximum histologic and clinical evidence of conjunctivitis were seen. This decline in GCD was persistent, with goblet cells also being undetectable at 21 days after inoculation. These results are in accordance with previous findings associating conjunctival inflammation with loss of conjunctival goblet cells. Although these earlier reports linked keratoconjunctivitis with decreased GCD, the direct role of FHV-1 was not fully explored and a causal relationship was not established. The hypothesis that decreased GCD is a result, rather than a cause, of keratoconjunctivitis in these cats is supported by the rapid disappearance of goblet cells in infected cats at the first sample point following viral inoculation and the observation that GCD in control cats did not change significantly during this study. Data collected in both the control and infected cats suggest that conjunctival goblet cells are able to regenerate in the presence of low-grade inflammation; however, it appears that goblet cell repair lags behind improvements in histologic and clinical evidence of conjunctivitis and even further behind improvements in total disease score. The persistence of conjunctivitis and decreased goblet cell numbers despite improving total disease score suggests that careful assessment of the conjunctiva will provide a better indicator of tear film quality during FHV-1 infection than observation of other signs used here, such as blepharospasm or ocular discharge. However, clinical evidence of conjunctival inflammation is not always an accurate predictor of GCD because improvements in clinical conjunctivitis occur before GCD returns to normal.

The lipid portion of the tear film secreted by the meibomian glands also contributes to tear film stability. Abnormalities of the lipid component could therefore also have influenced TFBUTs in this study. Although not included in the disease scoring scale used in the present study, all infected cats did develop some degree of blepharitis that may have resulted in meibomian gland dysfunction. Meibometry might have permitted better assessment of the contributions of altered tear lipid and tear mucin on TFBUT; however, this was not available at the time, and to our knowledge, reference range values for cats have not been established. Although the nature and extent of lipid alteration in this study cannot be determined, it could be hypothesized that tear lipid abnormalities would have been
most severe at 7 days after inoculation, when total disease score peaked, then improved over the remainder of the study period. On the basis of this assumption, if lipids had exerted an important effect on tear film stability in this study, TFBUTs would have been expected to improve at 7 days after inoculation. Rather, TFBUTs continued to decline through, and stay low beyond, 15 days after inoculation. This suggests that any influence of tear film lipids on TFBUTs in this study was less important than effects caused by altered tear mucins.

Baseline STT values in infected cats were less than the reported reference range values for cats, whereas control STT value readings were within this range. The difference in baseline measurements may have been influenced by the use of STT strips from 2 lot numbers for control and infected cats. However, because STT strips of the same lot number were used for all measurements within each group, differences in absorptive capacity of STT strips would not have accounted for the changes over time in aqueous tear production seen in this study. Although diurnal variation may influence STT values, this effect would have been minimal in the present study because all STT values were recorded at approximately the same time each day in both groups of cats. Cats had been transported to the housing area 24 hours prior to commencement of the study. To our knowledge, the effect of increased sympathetic tone on tear production in cats has not been studied; therefore, increased sympathetic tone secondary to stress associated with relocation may have contributed to low baseline tear production. However, in other studies in cats, STT values have been determined without acclimatization, and no observable influences of sympathetic tone were seen. Also, this effect was not observed in the control cats of our study, despite similar handling. Results of 1 study revealed a wide variation in STT values for clinically normal cats. Because the findings from baseline ophthalmic and physical examinations were within reference limits, it seems likely that normal variation may account for the low baseline STT values seen in some cats in this study. This may also explain the lack of discernible changes in STT values of control cats during the course of this study.

Primary infection with FHV-1 was reported to cause temporary but significant decreases in STT values in 5 of 30 experimentally infected cats. This was hypothesized to be secondary to lacrimal adenitis or ducal occlusion. By contrast, we demonstrated a significant increase in the mean STT value of infected cats above baseline at the final time point of the study (29 days after inoculation). However, there are important differences in methodology between these 2 studies. All 5 cats that experienced decreased STT values in the previous study also received subconjunctivally administered corticosteroids, which are associated with more severe clinical signs of herpetic infection and protracted viral shedding, compared with untreated cats. Additionally, the time points at which STT values were measured in the present study may, in part, explain why aqueous deficiency was not seen. Decreased STT values were first observed 23 days after infection in the previous study, whereas the present study examined STT values in the more acute period following primary infection and only once after 21 days. Ocular discomfort from ulcerative herpetic keratoconjunctivitis would be expected to cause increased lacrimation and increased STT values as seen in this study. However, if this were the only cause of increased aqueous production, STT values should have decreased over time as clinical scores for total disease and conjunctivitis declined. Rather, STT values were significantly greater than baseline at 29 days after inoculation. Considering the concurrent evidence of tear film instability (decreased TFBUT and GCD) in cats in the present study, it is possible that increased production of the aqueous portion of the tear film occurred during primary FHV-1 infection to compensate for tear film instability resulting from mucin deficiency. In further support of this hypothesis, neither the STT values nor the TFBUTs in control cats changed significantly during this study. Regardless of cause, given that STT values typically remained within the reference range during this study, it is reasonable to conclude that an STT value within reference range should not be used to infer tear film stability or ocular health.

Although the conjunctivae of 2 infected cats (3 eyes) were clinically normal by the end of the present study, TFBUTs in both of these cats remained abnormally low despite this apparent return to clinical normality. Additionally, in infected cats, mean TFBUT at 29 days after inoculation remained significantly less than at baseline. These data suggest that recovery from tear film instability induced by experimental primary herpetic keratoconjunctivitis lags behind apparent clinical recovery. The decreased TFBUT seen in the present study is consistent with that reported for cats and dogs with naturally occurring keratoconjunctivitis of herpetic and nonherpetic origin. Two of these studies followed patients through to resolution of clinically evident disease. While the TFBUTs were not documented for the cats following resolution of ocular surface disease, in contrast to cats in the present study, the TFBUT of dogs in the other study returned to normal after clinical disease resolved. Therefore, our data indicate that clinical monitoring, including measurement of TFBUT, and treatment for herpetic keratoconjunctivitis should be continued beyond resolution of other clinical ocular abnormalities. Observation of cats for longer than 29 days after inoculation would likely be required to see return of TFBUT to normal.

It is possible that some of the interventions performed in the present study such as biopsy or cytology brush sample collection of the conjunctiva may have caused or worsened some of the changes observed by removing goblet cells and exacerbating clinical or histologic evidence of conjunctivitis. For this reason, a sham-inoculated control group underwent the same procedures and all data were assessed for the effect of biopsy. Outcome of this assessment revealed a significant effect of biopsy on clinically scored conjunctival disease in infected cats only. However, the magnitude of this effect was unlikely to be clinically important because the difference in conjunctival clinical score between cats that recently underwent biopsy and cats that did not undergo biopsy was always < 1. Further, mean clinical score for conjunctivitis was higher for infected cats than for control cats at all time points, and mean
TFBUT of control cats did not decline during the study as it did in infected cats. No relationship was identified between recent (within 7 days) biopsy and TFBUT, further demonstrating that FHV-1 infection, not the biopsy procedure, was responsible for the TFBUT changes seen in this study.

To our knowledge, the effects of medications typically used to treat cats undergoing FHV-1 infection (such as systemically or topically administered antiviral and antibacterial agents) on tear film stability have not been assessed. Given the association between conjunctivitis and goblet cell deficiency, it is plausible that anti-inflammatory medications may be useful for improving tear film stability in cats with keratoconjunctivitis. However, the use of such agents in cats with herpetic keratoconjunctivitis is likely to be contraindicated because these agents dampen host immune responses and may therefore worsen disease related to FHV-1. Cyclosporine is an immunomodulating drug that is associated with increased conjunctival mucin stores and decreased conjunctivitis when applied topically. This makes it potentially attractive as a treatment for keratoconjunctivitis secondary to FHV-1. To our knowledge, there are no studies examining the relationship between cyclosporine and FHV-1; however, some effects of cyclosporine in humans affected by the closely related human herpes simplex virus have been described. Unfortunately, results of such studies are ambiguous. There is evidence that topically applied cyclosporine potentiates herpes simplex virus–induced keratitis in humans, presumably via inhibition of host immune responses. However, there are also experimental and clinical reports that indicate an improvement of herpes simplex virus–induced keratitis when cyclosporine is used in conjunction with an antiviral drug.

Artificial tear (specifically mucin replacement) formulations represent the most important method of managing tear film instability without the risk of adverse effects seen with anti-inflammatory and immunomodulatory treatment. Mucinomimetics have been associated with resolution of goblet cell–deficient keratoconjunctivitis. By inhibiting premature tear film evaporation, mucinomimetics offer improved ocular comfort and prevent exacerbation of conjunctival inflammation. For example, sodium hyaluronate provides prolonged corneal retention time and may therefore worsen disease related to FHV-1. Cystosoft cytology brush, Medical Packaging Corp, Camarillo, Calif.

References


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