*Cornea 19(4): 492–496, 2000.*


# Interleukin-6 Levels in the Conjunctival Epithelium of Patients with Dry Eye Disease Treated with Cyclosporine Ophthalmic Emulsion

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**Purpose.** To evaluate interleukin-6 (IL-6) levels in the conjunctival epithelium of patients with moderate to severe dry eye disease before and after treatment with cyclosporin A ophthalmic emulsion (CsA) or its vehicle. **Methods.** Conjunctival cytology specimens were obtained from a subset of patients enrolled in a 6-month randomized, double-masked clinical trial of the efficacy and safety of topical CsA at baseline and after 3 and 6 months of B.I.D. treatment with 0.05% cyclosporine emulsion ($n = 13$), 0.1% cyclosporine emulsion ($n = 8$), or vehicle ($n = 10$). RNA was extracted and a competitive reverse transcriptase polymerase chain reaction (RT-PCR) was used to evaluate the levels of mRNA encoding the inflammatory cytokine IL-6 and a housekeeping gene, G3PDH. Levels of IL-6 and G3PDH were measured and compared. **Results.** There was no change from baseline in the level of G3PDH after 3 or 6 months in any group. IL-6 normalized for G3PDH (IL-6/G3PDH ratio) was not different from baseline at 3 months but showed a significant decrease from baseline in the group treated with 0.05% CsA ($p = 0.048$) at 6 months. No significant between-group differences were noted and no correlation was observed between the change in IL-6/G3PDH and corneal fluorescein staining. **Conclusions.** This preliminary, small-cohort study showed a decrease in IL-6 in the conjunctival epithelium of moderate to severe dry eye patients treated with 0.05% CsA for 6 months. The observed decrease suggests that dry eye disease involves immune-mediated inflammatory processes that may be decreased by treatment with topical ophthalmic cyclosporine.
**Key Words:** Conjunctiva—Cyclosporin A—Dry eye disease—Interleukin-6—Keratoconjunctivitis sicca.

Specific evidence of chronic immune activation of the conjunctival epithelium in patients with dry eye suggests that cell-mediated inflammatory processes may play an important role in the pathogenesis of dry eye disease.[1–4] These immunopathologic findings include increased expression of immunologic adhesion molecules (i.e., HLA class II and ICAM-1 antigens) and inflammatory cytokines (IL-1, IL-6, IL-8, and TNF-α) in these patients.[5–7] Among the inflammatory cytokines evaluated, levels of interleukin-6 (IL-6) showed the greatest elevation in eyes with dry eye disease when compared to normal eyes.[6]

Further evidence that immune-mediated inflammatory processes are involved in the pathogenesis of dry eye disease comes from reports that topical use of the immunomodulatory agent cyclosporine can improve the signs and symptoms of dry eye.[8–10] However, it has not been determined whether the use of topical cyclosporine can cause measurable decreases in any of the inflammatory markers that are associated with dry eye disease.

The purpose of this study was to evaluate the level of the inflammatory cytokine IL-6 in the conjunctiva of patients with keratoconjunctivitis sicca enrolled in a randomized trial of two different concentrations of cyclosporin A ophthalmic emulsion (CsA) and a castor oil emulsion vehicle. IL-6 was chosen because previous studies have shown that the level of IL-6 mRNA is elevated in the conjunctival epithelium of patients with Sjögren's syndrome keratoconjunctivitis sicca.[5,6] Furthermore, the level of IL-6 mRNA was found to decrease in bronchoalveolar lavage cells obtained from patients with acute lung transplant rejection who were treated with aerosolized cyclosporine.[11]

## PATIENTS AND METHODS

### Patients

Evaluation of the level of IL-6 mRNA in the conjunctiva was performed on conjunctival cytology specimens from a subset of patients enrolled in a prospective, double-masked, vehicle-controlled, multi-center clinical trial of topical cyclosporine emulsion (CsA) for the treatment of dry eye disease.[9] Adult patients of either sex were eligible for participation if they presented with a diagnosis of moderate to severe dry eye disease as defined by the following criteria:

1) Schirmer test without anesthesia of ≤5 mm/5 minutes in at least one eye (If Schirmer test without anesthesia = 0 mm/5 minutes, then Schirmer with nasal stimulation had to be > 3 mm/5 minutes in the same eye.);
2) sum of corneal and interpalpebral conjunctival staining of ≥ +5 in the same eye where corneal staining was ≥ +2;
3) a baseline Ocular Surface Disease Index© (OSDI©) score of 0.1 with no more than three responses of "not applicable;" and
4) a score of ≥ 3 on the Subjective Facial Expression Scale.

The OSDI© is a questionnaire consisting of 12 questions (each

Submitted September 13, 1999. Revision received January 8, 2000. Accepted January 15, 2000.
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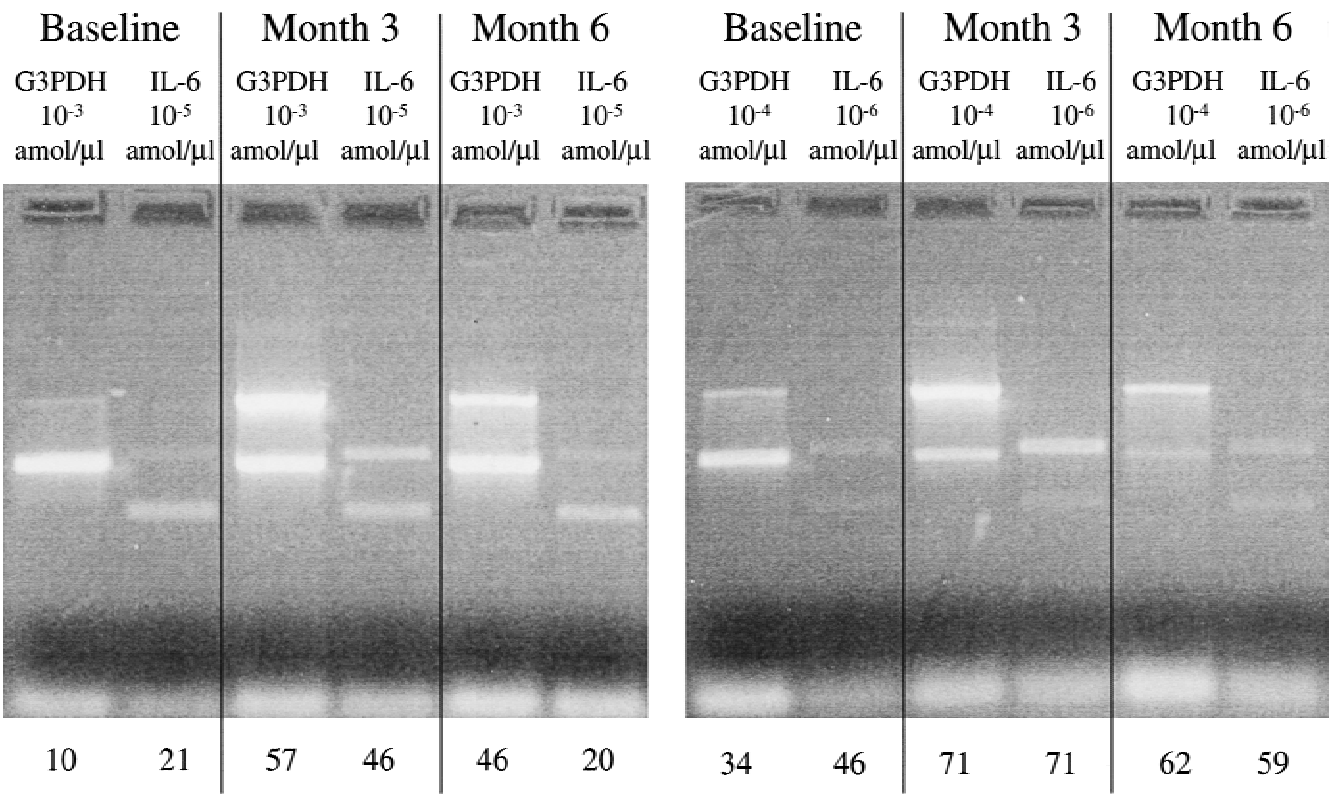


**FIG. 1.** Ethidium bromide-stained agarose gel. Initial PCR was performed using high and low concentrations of mimics for G3PDH ($10^{-3}$ and $10^{-4}$ attomole/µL), and IL-6 ($10^{-5}$ and $10^{-6}$ attomole/µL). The percentage of product (G3PDH or IL-6) per total amplified product (gene + mimic) in the lane is given at the bottom. amol = attomole.

rated from 0 = none of the time to 4 = all of the time) for the evaluation of the impact of a patient's dry eye disease on his or her vision-related functioning. The overall score was calculated by dividing the sum of the responses for all questions answered by the total possible score and then multiplying by 100. Thus, overall scores ranged from 0 = no disability to 100 = complete disability.

To be eligible for enrollment, signs and symptoms of dry eye disease must have presented despite conventional management, which may have included artificial tear drops, gels and ointments, sympathomimetic agents, parasympathomimetic agents, and punctal occlusion. Eligible patients were enrolled if they were deemed capable of following the study protocol and were considered likely to complete the treatment period and to return for all scheduled visits, if they had normal lid position and closure, and if they had a best-corrected early treatment of diabetic retinopathy study visual acuity score of +0.7 logmar or better in each eye.

Patients were excluded from the study if they had used systemic or topical ophthalmic cyclosporine within 90 days before the study. Other exclusion criteria included the presence or history of any systemic or ocular disorder or condition (including ocular surgery, trauma, and disease) that could possibly interfere with the interpretation of the study results; current or recent use of topical ophthalmic or systemic medications that could affect a dry eye condition; known hypersensitivity to any component of the study or procedural medications; required contact lens wear during the study; recent (within 1 month) or anticipated use of temporary punctal plugs during the study; permanent occlusion of lacrimal puncta within 3 months of the study; or if they were pregnant, lactating, or planning a pregnancy. Patients were also excluded if they appeared to have end stage lacrimal gland disease (Schirmer reading with nasal stimulation of < 3 mm/5 minutes) or if their dry eye disease was secondary to the destruction of conjunctival goblet cells or scarring. Any patient who no longer met the criteria for moderate to severe dry eye (as defined above) after completing the 2-week run-in phase was excluded from enrollment in the treatment phase of the study.

Conjunctival epithelial samples were obtained at baseline, at 3 months, and at 6 months from four participating sites in the United States. Informed consent was obtained from all patients before the study. The study complied with the Tenets of the Declaration of Helsinki. Conjunctival epithelial samples were obtained by impression debridement with a nitrocellulose membrane from the temporal bulbar conjunctiva of the "worse" eye. The "worse" eye was defined as the eye with the worse Schirmer tear test value (without anesthesia) and the worse sum of corneal and interpalpebral conjunctival staining. If both eyes were comparable, then the right eye was used. The membranes were placed in Trizol RNA lysis buffer (Life Technologies, Rockville, MD, U.S.A.) and immediately frozen at −80°C. Samples were shipped in dry ice by a commercial overnight delivery service to the central laboratory at the University of Miami School of Medicine. Upon arrival, samples were placed in a −80°C freezer until the RNA was extracted.

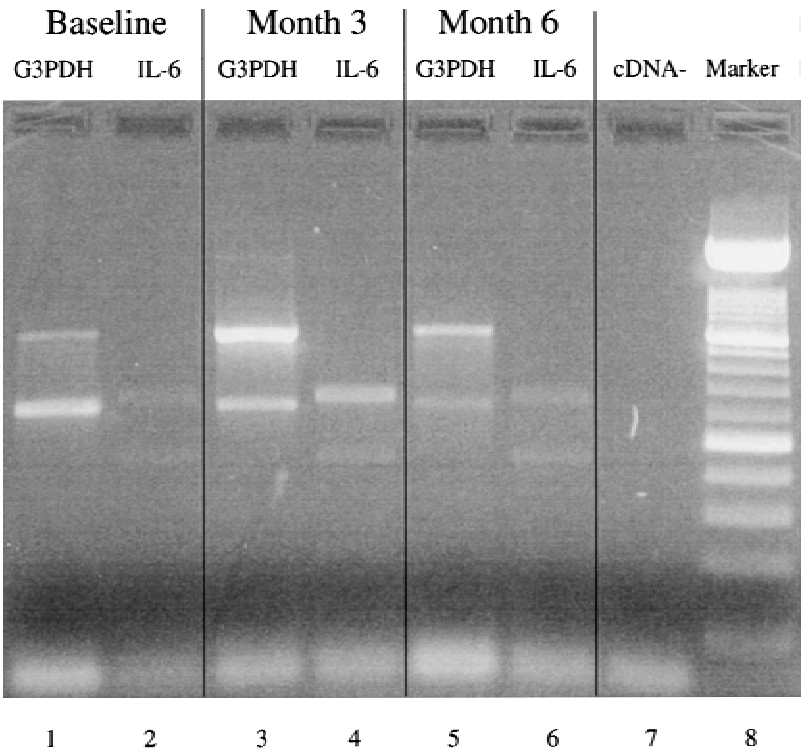


**FIG. 2.** Example of final gels for each patient RNA sample. G3PDH primers were reacted with $10^{-4}$ attomole/μL mimic, and IL-6 with $10^{-6}$ attomole/μL mimic. The upper band in each lane is the amplified product and the lower band is the amplified mimic. Lanes 1, 3, and 5 are G3PDH and the G3PDH mimic; lanes 2, 4, and 6 are IL-6 and the IL-6 mimic. Lanes 1 and 2 are baseline, lanes 3 and 4 are month 3, lanes 5 and 6 are month 6, lane 7 is cDNA-(PCR reaction mixture without cDNA), and lane 8 is the DNA marker IX (Boehringer, Indianapolis, IN, U.S.A.).

## RNA Extraction and cDNA Synthesis

RNA was extracted from the conjunctival epithelial samples using a previously reported protocol.[5] Complementary DNA (cDNA) was synthesized with a first strand cDNA synthesis kit (Boehringer, Indianapolis, IN, U.S.A.) using 1 μg of total RNA. Reverse transcriptase negative controls were performed for each RNA sample. The efficiency of cDNA synthesis was assessed by evaluating the level of cyclophilin mRNA in each sample by polymerase chain reaction (PCR) amplification using 35 cycles. We added 2 μL of cDNA to the PCR reaction mixture containing 5 μL of 10× reaction buffer, 1 μL deoxy-nucleotide triphosphates (10 mmol/L deoxyadenosine triphosphate, 10 mmol/L deoxycytidine triphosphate, 10 mmol/L deoxyguanosine triphosphate, and 10 mmol/L deoxythymidine triphosphate), 39.7 μL sterile water, 0.3 μL Taq DNA polymerase (5 units/μL), and 2 μL of primers specific for cyclophilin.[12] The upstream cyclophilin primer sequence is 5′ ATG GTT AAC CCC ACC GTG TTC GAC 3′, and the downstream primer sequence is 5′ CTG GAT TGC AGA GTT AAG TTT 3′. Cyclophilin is a constitutively expressed gene in all human cells that serves as the cyclosporine-binding protein.[13] The PCR products were separated by 1.6% agarose gel electrophoresis and the intensity of the band was visually graded from 0 (no band) to 4+ (strong). cDNA samples that yielded 2+, 3+, or 4+ bands were used for competitive reverse transcriptase polymerase chain reaction (RT-PCR) analysis of IL-6 and G3PDH, a housekeeping gene, levels. cDNA samples that yielded 0 or 1+ bands were resynthesized.

## Semiquantitative Analysis of mRNA Levels by Competitive RT-PCR

The relative levels of RNA transcripts encoding the inflammatory cytokine IL-6 or the housekeeping protein G3PDH were evaluated by a competitive PCR technique. This technique was performed by adding 2 μL of sample cDNA to the PCR mixture described above, containing 2 μL of primers specific for IL-6 or

**TABLE 1.** *Average percent of product*

| | | % G3PDH in lane | | | | | % IL-6 in lane | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mimic $10^{-3}$ attomole | | Mimic $10^{-4}$ attomole | | | Mimic $10^{-5}$ attomole | | Mimic $10^{-6}$ attomole | | |
| Cyclosporine dose | Mo | Mean | Std. dev. | Mean | Std. dev. | *p* value | Mean | Std. dev. | Mean | Std. dev. | *p* value |
| Vehicle | 0 | 47 | 20 | 74 | 19 | 0.002 | 21 | 13 | 41 | 27 | 0.008 |
| | 3 | 31 | 33 | 48 | 43 | 0.051 | 17 | 18 | 29 | 24 | 0.005 |
| | 6 | 32 | 22 | 70 | 28 | 0.001 | 4 | 6 | 9 | 10 | 0.091 |
| 0.05% | 0 | 46 | 22 | 70 | 32 | 0.005 | 34 | 26 | 61 | 30 | 0.005 |
| | 3 | 27 | 22 | 46 | 43 | 0.026 | 26 | 22 | 41 | 34 | 0.081 |
| | 6 | 53 | 18 | 77 | 16 | 0.003 | 34 | 27 | 53 | 28 | 0.004 |
| 0.10% | 0 | 37 | 20 | 57 | 31 | 0.012 | 12 | 15 | 37 | 17 | 0.002 |
| | 3 | 29 | 25 | 35 | 38 | 0.388 | 16 | 19 | 31 | 25 | 0.003 |
| | 6 | 24 | 17 | 40 | 22 | 0.025 | 12 | 16 | 32 | 26 | 0.009 |

**TABLE 2.** *Change in percent G3PDH from baseline*

| Group | 3-mo mean | SD | *p* value | Range | 6-mo mean | SD | *p* value | Range |
|---|---|---|---|---|---|---|---|---|
| Vehicle | −12 | 31 | 0.4 | −61 to 17 | 13 | 25 | 0.14 | −15–21 |
| 0.05% CsA | −15 | 33 | 0.2 | −72 to 28 | 3.7 | 27 | 0.6 | −38–64 |
| 0.10% CsA | −4.4 | 27 | 0.6 | −45 to 47 | 12 | 33 | 0.3 | −55–35 |

G3PDH.[6] Both the IL-6 and G3PDH PCR reactions were run for 35 cycles. The primer sequences for IL-6 and G3PDH were chosen to span an intron to confirm that the PCR product was due to amplification of cDNA, not genomic DNA. The size of the amplified IL-6 cDNA product was 627 base pairs, and the size of the amplified G3PDH cDNA product was 983 base pairs. Each reaction tube used to amplify the IL-6 cDNA was spiked with a specifically designed "mimic" template (Clontech, Palo Alto, CA, U.S.A.) that contained DNA sequences that were complimentary to the IL-6 PCR primers. These primer-specific sequences were ligated to the 5′ and 3′ ends of a nonspecific DNA fragment (in this case, a portion of the v-erbB gene). The mimic templates compete with the IL-6-specific cDNA sequences in the conjunctival epithelial specimens for the reaction primers and they have a different length (in base pairs) after PCR amplification than the cytokine amplification product. The added mimics also served as internal controls in each reaction tube. Two concentrations of mimic ($10^{-5}$ and $10^{-6}$ attomole/μL) were used for each sample. PCR reactions for G3PDH were also spiked with two concentrations ($10^{-3}$ and $10^{-4}$ attomole/μL) of a mimic with G3PDH primer-specific sequences. PCR reactions for IL-6 and G3PDH were performed on baseline (month 0), month 3, and month 6 samples, simultaneously for each subject. The levels of PCR products were evaluated by 1.6% agarose gel electrophoresis. Gels were stained with ethidium bromide (10 mg/mL) and were photographed with a Polaroid camera (Cambridge, MA, U.S.A.). Baseline, month 3, and month 6 PCR products for IL-6 and G3PDH (and their mimics) from each subject were run together on one agarose gel (Fig. 1). Two final gels (one for the lower and the other for the higher mimic concentration) were run for each subject (Fig. 2). The mimic concentration yielding measurable bands for IL-6 and G3PDH that allowed comparisons between at least two time points (baseline to month 3, or baseline to month 6, or month 3 to month 6) was used for the final analysis.

Photographs of each gel were scanned with a Hewlett Packard ScanJet (Palo Alto, CA, U.S.A.). The integrated optical density of the IL-6, G3PDH, and their mimic bands in the scanned images was measured. Levels of IL-6 and G3PDH in each lane were expressed as a fraction of total amplified product in each lane (e.g., the percentage of IL-6 = IL-6/[IL-6 + IL-6 mimic]). Levels of G3PDH in each treatment group were compared over time to determine whether the amount of G3PDH changed significantly with CsA treatment. Levels of IL-6 were evaluated at each time point (baseline, month 3, and month 6) in relation to the level of G3PDH in each sample by dividing the percentage of IL-6 by the G3PHD (normalized IL-6).

**TABLE 3.** *Change in %IL-6/%G3PDH ratio from baseline*

| | CsA 0.05% | CsA 0.1% | Vehicle | *P* value[a] |
|---|---|---|---|---|
| Day 0[b] | 1.144 ± 1.005 (n = 14) | 1.401 ± 1.198 (n = 12) | 0.846 ± 0.663 (n = 10) | 0.438 |
| Change from baseline | | | | |
| Month 3 | 0.036 ± 0.643 n = 10 | −0.095 ± 1.430 n = 10 | 2.269 ± 5.524 n = 7 | 0.221 |
| Within[c] | p = 0.862 | p = 0.839 | p = 0.319 | |
| Month 6 | −0.626 ± 1.025 n = 13 | −0.384 ± 1.248 n = 8 | −0.460 ± 0.749 n = 10 | 0.853 |
| Within | *p* = 0.048[c] | *p* = 0.413 | *p* = 0.084[d] | |

[a] Among-group *p* values from one-way analysis of variance.
[b] Values are mimic corrected ratios of IL-6 to G3PDH.
[c] Within-group *p* value from paired-t-test.
Note. CsA = cyclosporine ophthalmic emulsion. Values shown are mean ± standard deviation. A negative value indicates a decrease from baseline.

## Statistical Analysis

A two-tailed paired Student *t* test was used to confirm that IL-6 and G3PDH, as a percentage of the product plus mimic total, decreased with sample dilution for each group at each follow-up period. Within group comparisons of G3PDH and IL-6, changes were performed with the two-tailed paired Student *t* test. Changes in the normalized IL-6 percentage from baseline to 3- and 6-month follow-up were calculated for each subject and between-group differences in the extent of change were examined with one-way analysis of variance.

# RESULTS

## Mimic as a Competitive Internal Control

Mimics were added to the PCR reactions to act both as a positive internal control for the PCR reaction and as a competitive template used for calculating the percentage of amplified cytokine or housekeeping protein. To confirm the competitive nature of the mimic, the percentage of IL-6 and G3PDH in each lane was compared using high and low mimic concentrations. For each treatment group and time point, the percentage of IL-6 and G3PDH increased as the concentration of added mimic decreased (Table 1).

## Changes from Baseline

There were no significant differences between G3PDH levels at months 0, 3, or 6 in any treatment group (Table 2). This demonstrates that the levels of the housekeeping protein (G3PDH) are not changed by CsA treatment.

The levels of IL-6 were then normalized to the G3PDH levels to identify treatment-related changes in IL-6 mRNA levels. At 6 months posttreatment, a significant decrease in the ratio of the percentage of IL-6 to G3PDH was observed in the group treated with 0.05% CsA, but not in the vehicle or 0.10% CsA treatment groups (Table 3). The ratio of the percentage of IL-6 to G3PDH was not significantly different from baseline at month 3 in any treatment group. There were no significant differences in the between-group ratios of the percentage of IL-6 to the percentage of G3PDH.

## DISCUSSION

The most important finding of the present study was that normalized IL-6 levels significantly decreased from baseline after 6 months of treatment with 0.05% CsA, whereas the decreases seen after treatment with either 0.1% CsA or vehicle were not statistically significant. The decrease in this inflammatory factor by cyclosporine is consistent with the results of another study conducted on conjunctival biopsies taken from a separate cohort of patients that participated in this multi-centered clinical trial. Kunert et al.[14] demonstrated that there was a significant decrease in the immune activation markers HLA-DR and CD11a after 6 months of treatment with 0.05% CsA, whereas these same markers increased in the vehicle group. Other studies have demonstrated that increased levels of IL-6, and other inflammatory markers, are associated with dry eye disease.[5–7,15] For example, a study by Pflugfelder et al.[6] used the same assay as the present study to demonstrate that the levels of IL-1α, IL-6, IL-8, TNF-α, and TNF-β1 RNA found in the conjunctival epithelium of Sjögren's syndrome patients were significantly greater than those found in control patients. However, the present study and the other study performed on this patient population[14] are the first to demonstrate that a topical ophthalmic treatment for dry eye disease (cyclosporin A) can significantly decrease several markers of inflammation.

In the present study, no statistically significant between-group differences were seen in IL-6 levels. Thus, it is not possible to rule out regression to the mean as the source of the IL-6 decrease.[16] However, a more likely explanation may be a large therapeutic effect observed in the vehicle group. Disruption of epidermal barrier function results in increased levels of several inflammatory cytokines, including TNF-α, IL-1, and IL-6, in the epidermis.[17] A therapeutic effect of the oil-in-water vehicle of this cyclosporine emulsion might be expected, as topical application of certain lipid mixtures can accelerate epidermal barrier recovery after defined barrier insults in mice.[18]

A very small amount of RNA was obtained from the samples provided for this study. Consequently, a highly sensitive competitive RT-PCR assay was used to evaluate the levels of IL-6 and a constituently expressed gene, G3PDH. The assay was designed to determine the relative amount of each of these mRNAs in the sample when compared to a competitive template (mimic) that was added to each of the PCR reaction tubes. The validity of this approach is supported by the fact that the levels of G3PDH were not affected by either the study medication or the vehicle.

In conclusion, the findings of the present study demonstrate that topical CsA decreases the levels of IL-6, an inflammatory cytokine that is upregulated in the conjunctiva of patients suffering from dry eye disease.

## REFERENCES


1. Stern ME, Beuerman RW, Fox RI, Gao J, Mircheff AK, Pflugfelder SC. The pathophysiology of dry eye: the interaction between the ocular surface and lacrimal glands. *Cornea* 1998;17:584–9.
2. Pflugfelder SC, Wilhelmus KR, Osato AS, Matoba AY, Font RL. The autoimmune nature of aqueous tear deficiency. *J Ophthalmol* 1986; 93:1513–7.
3. Damato BE, Allan D, Murray SB, Lee WR. Senile atrophy of the human lacrimal gland: the contribution of chronic inflammatory disease. *Br J Ophthalmol* 1984;68:674–80.
4. Williamson J, Gibson AAM, Wilson T, Forrester JV, Whaley K, Dick WC. Histology of the lacrimal gland in keratoconjunctivitis sicca. *Br J Ophthalmol* 1973;57:852–8.
5. Jones DT, Monroy D, Ji Z, Atherton SS, Pflugfelder SC. Sjögren's syndrome: cytokine and Epstein-Barr viral gene expression within the conjunctival epithelium. *Invest Ophthalmol Vis Sci* 1994;35:3493–504.
6. Pflugfelder SC, Jones D, Ji Z, Afonso A, Monroy D. Altered cytokine balance in the tear fluid and conjunctiva of patients with Sjögren's syndrome keratoconjunctivitis sicca. *Curr Eye Res* 1999;19:201–11.
7. Raphael M, Bellefgih S, Piette JCH. Conjunctival biopsy in Sjögren's syndrome: correlations between histological and immunohistochemical features. *Histopathology* 1988;13:191–202.
8. Gunduz K, Ozdemir O. Topical cyclosporin treatment of keratoconjunctivitis sicca in secondary Sjögren's syndrome. *Acta Ophthalmol* 1994;72:435–442.
9. Sall K, Stevenson OD, Mundorf TK, Reis BL, CsA Phase 3 Study Group. Two multicenter, randomized studies of the efficacy and safety of cyclosporine ophthalmic emulsion in moderate to severe dry eye disease. *Ophthalmology* 2000;107:631–9.
10. Laibovitz RA, Solch S, Andriano K, O'Connell M, Silverman MH. Pilot trial of cyclosporine 1% ophthalmic ointment in the treatment of keratoconjunctivitis sicca. *Cornea* 1993;12:315–23.
11. Iacono AT, Smaldone GC, Keenan RJ, et al. Dose-related reversal of acute lung rejection by aerosolized cyclosporine. *Am J Respir Crit Care Med* 1997;155:1690–8.
12. Haendler B, Hofer-Warbinek R, Hofer E. Complementary DNA for human T-cell cyclophilin. *EMBO J* 1987;6:947–50.
13. Ryffel B, Woerly G, Greiner B, Haendler B, Mihatsch MJ, Foxwell BM. Distribution of the cyclosporin binding protein cyclophilin in human tissues. *Immunology* 1992;72:399–404.
14. Kunert KS, Tisdale AS, Stern ME, Smith JA, Gipson IK. Effect of cyclosporine ophthalmic emulsion on the number of inflammatory cells and goblet cells in conjunctival biopsies of patients with dry eye [ARVO abstract]. *Invest Ophthalmol Vis Sci* 1999;40(4):S771.
15. Tsubota K, Fujihara T, Saito K, Takeuchi T. Conjunctival epithelium expression of HLA-DR in dry eye patients. *Ophthalmologica* 1999; 213:16–19.
16. Fleiss JL. *The design and analysis of clinical experiments.* New York: Wiley, 1986.
17. Wood LC, Stadler K, Liou A, et al. Barrier disruption increases gene expression of cytokines and the 55kD TNF receptor in murine skin. *Exp Dermatol* 1997;6:98–104.
18. Man MQ M, Feingold KR, Thorfeldt CR, Elias PM. Optimization of physiological lipid mixtures for barrier repair. *J Invest Dermatol* 1996; 106:1096–101.