Goblet Cell Numbers and Epithelial Proliferation in the Conjunctiva of Patients With Dry Eye Syndrome Treated With Cyclosporine

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Objectives: To compare conjunctival goblet cell numbers as well as epithelial turnover in patients with non–Sjögren syndrome–associated keratoconjunctivitis sicca (NSS-KCS) and those with SS-KCS before and after 6 months of treatment with topical cyclosporine A (CsA) ophthalmic emulsion.

Methods: Conjunctival biopsy specimens from 16 patients with NSS-KCS and 12 with SS-KCS were obtained at baseline and after 6 months' therapy with CsA or vehicle alone. Conjunctival biopsy specimens were also obtained from 11 normal subjects. Periodic acid–Schiff staining determined the number of goblet cells present. Immunofluorescence microscopy for Ki-67 localization was used to evaluate the number of actively cycling cells.

Results: Periodic acid–Schiff staining showed fewer goblet cells at baseline in both dry eye populations when compared with normal subjects \( P < .001 \). After 6 months of CsA treatment, conjunctival biopsy specimens of both NSS-KCS and SS-KCS groups revealed an increase in goblet cells compared with baseline \( P < .05 \). More Ki-67–positive cells were observed in NSS-KCS conjunctiva at baseline than in normal conjunctiva \( P < .05 \) whereas numbers of these cells in SS-KCS conjunctiva were similar to normal at baseline. After 6 months of CsA treatment, conjunctival biopsy specimens of NSS-KCS revealed a decrease in Ki-67–labeled cells compared with baseline \( P < .001 \). In contrast, no substantial change was observed for CsA treatment in patients with SS-KCS.

Conclusions: Treatment of dry eye syndrome for 6 months with topical CsA resulted in an increase in goblet cell numbers in patients with NSS-KCS and SS-KCS and a decrease in epithelial turnover in those with NSS-KCS. Reducing ocular surface inflammation might have an effect on the proliferative activity of the epithelium.

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SUBJECTS AND METHODS

SUBJECTS

Conjunctival biopsy specimens from 16 patients with NSS-KCS and 12 with SS-KCS were obtained at the baseline visit and after 6 months of twice-daily therapy with 0.05% or 0.1% CsA or the vehicle alone. This subject group was randomly chosen from a double-masked, vehicle-controlled clinical study designed by Allergan Inc (Irvine, Calif) to investigate the efficacy and safety of topical CsA in the treatment of moderate to severe dry eye syndrome. The study was conducted in compliance with Good Clinical Practices, investigational site institutional review board regulations, sponsor and investigator obligations, informed consent regulations, and the Declaration of Helsinki. Potential patients signed a prescreening informed consent and a second written informed consent prior to actual enrollment. The protocol for this study is described briefly below. Adult patients of either sex were eligible for participation if they were diagnosed as having moderate to severe KCS as defined by the following criteria: (1) Schirmer test reading without anesthesia of less than or equal to 5 mm/5 min in at least 1 eye (if reading was 0 mm/5 min, then Schirmer reading with nasal stimulation had to be greater than 3 mm/5 min in the same eye); (2) sum of corneal and interpalpebral conjunctival staining of greater than or equal to +2; (3) a baseline Ocular Surface Disease Index score of 0 with no more than 3 responses of “not applicable”; and (4) a score of greater than or equal to 3 on the Subjective Facial Expression Scale. Signs and symptoms must have been present despite conventional management. Individually packaged preservative-free artificial tears (REFRESH Lubricant Eye Drops; Allergan Inc) were provided as an adjunctive treatment to be used as frequently as needed. Patients were excluded from the study if they had participated in an earlier clinical trial with CsA ophthalmic emulsion or had used systemic or topical ophthalmic CsA within 90 days prior to the study. Other exclusion criteria were the presence or history of any systemic or ocular disorder or condition (including ocular surgery, trauma, and disease); current or recent use of topical ophthalmic or systemic medications that could affect a dry eye condition; known hypersensitivity to any component of the drug or procedural medications such as stains or anesthetics; 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 the patients 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 min) or if their dry eye syndrome was secondary to the destruction of conjunctival goblet cells or scarring.

A retrospective diagnosis of SS was used with modified criteria reported by Vitali et al to ensure that a consistent definition of SS was assigned to the patients enrolled. Diagnosis included presence of at least one of the following autoantibodies in sera: antinuclear antibody, rheumatoid factor, and SS autoantibodies class SS-A (Ro) and class SS-B (La). In addition, oral and ocular symptoms were used to classify patients with SS.

Full-thickness conjunctival biopsy specimens of a standard size (2 × 3 mm) were removed from the “worse” eye by surgeons following standard procedure. The “worse” eye was defined as the eye with the lowest Schirmer tear test reading (without anesthesia) and the lowest sum of corneal and interpalpebral conjunctival staining. If both eyes were comparable, then the right eye was used. At the baseline visit, the conjunctival biopsy specimen was taken from the inferonasal quadrant of the bulbar conjunctiva, close to the midline. At the 6-month visit, the specimen was removed from the same eye but from the inferotemporal quadrant of the bulbar conjunctiva, also close to the midline. Conjunctival biopsy specimens from 11 control subjects (generously supplied by C. Steven Foster, MD, Massachusetts Eye and Ear Infirmary, Boston, Mass) were also obtained. Patients with 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 the patients were pregnant, lactating, or planning a pregnancy were also excluded.

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RESULTS

QUANTITATION OF GOBLET CELLS

In general, PAS staining documented fewer goblet cells at baseline in both KCS populations than in normal control subjects. After 6 months of treatment with CsA, con-
examined. Controls were patients aged 47 to 89 years who were undergoing ocular surgery for conditions unrelated to ocular surface disease. Exclusion criteria for controls included evidence of ocular surface disease or of trauma during the past 6 months, age younger than 18 years, the presence of dry eye syndrome, or the intake of medications known to affect the ocular surface. Full-thickness conjunctival biopsy specimens were taken from the eye at the time of surgery from the superotemporal bulbar region. This site was chosen based on the article by Kessing28 demonstrating that this region has goblet cell numbers comparable with superotemporal bulbar and inferotemporal/nasal bulbar quadrants of the normal human conjunctiva. Prior to biopsy, standard measures were followed to ensure sterility of the operated eye, including preparation with a drop of 5% povidone-iodine solution in the eye followed by the use of 10% povidone-iodine on the skin.

TISSUE PROCESSING FOR PERIODIC ACID–SCHIFF STAINING AND IMMUNOHISTOCHEMISTRY

After removal, biopsy specimens from patients with NSS-KCS and SS-KCS taken at day 0 and after 6 months of treatment with either CsA or vehicle, and from controls, were immediately frozen in OCT embedding compound (Tissue-Tek; Miles Laboratories; Elkhart, Ind) and stored at −80°C until patient-matched 6-month biopsy specimens were obtained and similarly frozen. Six-micrometer sections were taken from each block, mounted on gelatin-coated slides, and processed for periodic acid–Schiff (PAS) and immunohistochemistry. To minimize differences due to experimental conditions, sectioning of tissue blocks and histological and immunohistochemical experiments were done as pairs of biopsies, pretreatment and posttreatment.

PAS STAINING TO DETERMINE NUMBER OF GOBLET CELLS

Periodic acid–Schiff staining of conjunctival biopsy specimens of patients with NSS-KCS, SS-KCS, and control patients was performed by conventional techniques to determine the numbers of goblet cells present. Image analysis was performed as previously described.3 Counting was done in a masked fashion by 2 independent observers. Counts were recorded for 3 images of conjunctival epithelium from each biopsy specimen (original magnification ×20), and numbers of goblet cells/0.1 mm² of epithelium in patients with NSS-KCS, SS-KCS, and normal patients were compared.

IMMUNOHISTOCHEMISTRY FOR Ki-67 NUCLEAR ANTIGEN

Immunohistochemical localization of Ki-67 nuclear antigen—a marker of actively cycling cells—on conjunctival sections of biopsy specimens of patients with NSS-KCS, SS-KCS, and control patients was done as previously described.29 Secondary antibody controls that omitted the primary antibody for all biopsy specimens were run. As described previously, labeled cells were counted in all layers overlying 100 basal epithelial cells.29 Counting was done in a masked fashion by 2 independent observers. Counts were recorded for 3 images of conjunctival epithelium from each biopsy specimen (original magnification ×20), and numbers of goblet cells per 0.1 mm² of epithelium in patients with NSS-KCS, SS-KCS, and normal patients were compared.

STATISTICAL METHODS

The same statistical methods were applied for goblet cell numbers and Ki-67–positive cells, comparing NSS-KCS and SS-KCS subpopulations. Baseline characteristics were tabulated and summarized by patient populations. Overall differences among patient populations were tested using a 2-way analysis of variance for continuous variables and the Fisher exact test for categorical variables. Percent changes in the numbers of goblet cells and Ki-67–positive cells were summarized using descriptive statistics (ie, sample size, mean, SEM, minimum, maximum, and median). A 1-way analysis of variance with main effect for treatment was used to test for differences in percent change from baseline. If the test for among-group differences in main effect was significant, then all pairwise comparisons were made. Within-group changes from baseline were analyzed by the paired t test method.
Figure 1. Goblet cells in human conjunctival biopsy specimens from 2 patients with non–Sjögren syndrome–associated keratoconjunctivitis sicca (NSS-KCS) (A–D) and 2 patients with SS-KCS (E–H). A, Patient 1 with NSS-KCS at baseline. B, Same patient after 6 months of treatment with topical cyclosporine A (CsA) ophthalmic emulsion. C, Patient 2 with NSS-KCS at baseline. D, Same patient after 6 months of treatment with the vehicle alone. E, Patient 1 with SS-KCS at baseline. F, Same patient after 6 months of treatment with CsA. G, Patient 2 with SS-KCS at baseline. H, Same patient after 6 months of treatment with the vehicle alone. I, Normal goblet cells in normal human conjunctiva. Periodic acid–Schiff; bar = 50 µm. All micrographs are the same magnification.
There have also been some attempts to look at changes in goblet cell numbers in dry eye syndrome following several treatment modalities. These include therapy with retinol palmitate ophthalmic solution and hypotonic electrolyte solutions, both demonstrating an increase in goblet cell numbers after 6 months of treatment with CsA in both KCS subsets. In concordance with our data, several groups have found a decrease in the numbers of goblet cells in both forms of aqueous-deficient dry eye.4,9,12

Goblet cell densities are thought to be very sensitive indicators of ocular surface disease.31 In eyes with KCS, the first evidence of ocular surface injury is a decrease in conjunctival goblet cells. As the disease progresses in severity, goblet cell numbers decrease further, resulting in squamous metaplasia, enlargement of the epithelial area, and occasional keratinization of the ocular surface.31

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In this study, immunohistochemical analysis was used to evaluate goblet cell numbers and epithelial mitotic rates in patients with NSS-KCS and those with SS-KCS at baseline and following treatment with topical CsA or the vehicle. We demonstrated that treatment of patients with KCS using topical CsA for 6 months resulted in an increase in goblet cell numbers for both patients with NSS-KCS and SS-KCS, and a decrease in epithelial turnover in patients with NSS-KCS.

We observed significantly fewer goblet cells at baseline compared with normal specimens and an increase in goblet cell numbers after 6 months of treatment with CsA in both KCS subsets. In concordance with our data, several groups have found a decrease in the numbers of goblet cells in both forms of aqueous-deficient dry eye.4,9,12

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Figure 3. Immunohistochemical localization of Ki-67 antibody binding on cryosections of human conjunctiva. The antibody recognizes a nuclear antigen found only in proliferating cells. Representative biopsy specimens are shown from 2 patients with non-Sjögren syndrome–associated keratoconjunctivitis sicca (NSS-KCS) (A–D) and 2 patients with SS-KCS (E–H). A, Patient 1 with NSS-KCS at baseline. B, Same patient after 6 months of treatment with topical cyclosporine A (CsA) ophthalmic emulsion. C, Patient 2 with NSS-KCS at baseline. D, Same patient after 6 months of treatment with the vehicle alone. E, Patient 1 with SS-KCS at baseline. F, Same patient after 6 months of treatment with CsA. G, Patient 2 with SS-KCS at baseline. H, Same patient after 6 months of treatment with the vehicle alone. I, Normal Ki-67–labeled cells in normal human conjunctiva. J, Normal, negative biopsy result in which the primary antibody was omitted. Periodic acid–Schiff; bar=50 µm. All micrographs are the same magnification.
in goblet cell numbers in patients with KCS following
therapy.\textsuperscript{32-34} However, comprehensive studies of goblet
cell density in patients with dry eye syndrome treated with
topical CsA are lacking. In this study, we demonstrate a
significant increase in goblet cell numbers following topi-
cal treatment with CsA. Treatment with the vehicle alone,
on the other hand, leads to an even further decrease in
goblet cells during the 6-month treatment course, sug-
gestig that the inflammatory process is still ongoing. This
implies that CsA, in reducing ocular surface inflamma-
tion, might help to restore conjunctival goblet cells, which
secrete mucins that prevent the formation of dry spots
associated with KCS.

Data from studies of the gastrointestinal tract sup-
port this interpretation. Treatment with keratinocyte
growth factor or TNF-\(\alpha\) antibodies increases mucus pro-
duction and restores goblet cells in cases of intestinal in-
flammation.\textsuperscript{35,36} Alternatively, CsA may have a direct effect
on goblet cell differentiation. In a human colon adeno-
carcinoma cell line, CsA induced a 94\% increase in the
volume of mucin within goblet cells.\textsuperscript{37} Our data furth-
ermore suggest that there is no difference in goblet cell
numbers between NSS-KCS and SS-KCS, either at baseline or
after CsA treatment, indicating that both forms of aqueous-
deficient dry eye syndrome may benefit from CsA treat-
ment.

A second finding of this study is that there were dif-
ferences in the epithelial mitotic rate between patients
with NSS-KCS and SS-KCS at baseline and after 6 months
of treatment with CsA. More Ki-67–positive cells were
observed in NSS-KCS conjunctiva at baseline compared
with normal conjunctiva whereas in SS-KCS conjuncti-
va, numbers were similar to normal. After 6 months of
CsA treatment, conjunctival biopsy specimens of NSS-
KCS revealed a decrease in Ki-67–labeled cells when com-
pared with baseline. In contrast, no substantial change
was observed for CsA treatment in SS-KCS. It should be
noted, however, that sample sizes for each group were
relatively small.

Jones and colleagues\textsuperscript{3} found an increase in mitotic
rate as shown by bromodeoxyuridine-labeling in SS-
KCS. Even though we also found an increase in the num-
ber of proliferating cells in SS-KCS, this increase was not
significant when compared with normal controls. Pos-
sible explanations for this observed difference might be
that our study included a larger sample size and that our
control subjects were mostly elderly patients undergo-
ing cataract surgery as opposed to young human control
subjects as enrolled by Jones and colleagues.

Altered lubrication and drying of the ocular sur-
face in KCS result in ocular surface damage or epithelial
wounding. Chung and colleagues\textsuperscript{38} have shown that
the conjunctival epithelium responds to corneal wounding
by an increase in bromodeoxyuridine-labeled cells in the
bulbar conjunctival epithelium of rats. In addition to the
mechanical surface abrasion secondary to aqueous tear
deficiency, local inflammatory processes contribute to the
ocular surface disease associated with KCS. In NSS-KCS
as well as in SS-KCS, conjunctival epithelial and stro-
mal T-cell infiltration (predominantly CD3+ and CD4+T
lymphocytes) have been shown.\textsuperscript{3,6} Furthermore, sev-
eral studies that focused on conjunctival cytokine ex-
pression in patients with SS-KCS\textsuperscript{7,18} demonstrated in-
creased levels of inflammatory cytokines, such as IL-1\(\alpha\),
TNF-\(\alpha\), IL-6, and IL-8, in the conjunctival epithelium of
patients with SS-KCS compared with that of controls.\textsuperscript{4,7,18} There are data suggesting the involvement of
such inflammatory cytokines in epithelial hyperprolif-
eration. Interleukin 6 and IL-8, for example, have been
reported to influence growth and differentiation of ep-
ithelial cells and promote hyperproliferation of the epi-
dermis in psoriasis.\textsuperscript{39,40} Furthermore, the concentra-
tion of EGF, a cytokine that is capable of inducing prolifera-
tion or differentiation of epithelium, has been reported to
be lower in the tear fluid of patients with KCS.\textsuperscript{31} Pflug-
felder and colleagues\textsuperscript{18,22} also found a decrease in tear EGF
and an increase in the expression of EGF receptors in the
conjunctiva of patients with SS-KCS. Perhaps inflamma-
tion increases the receptor density, making the conjunc-
tival epithelial cells hypersensitive to mitogens. The ex-
act role of cytokines in KCS, however, and the possible
difference in cytokine profiles between the 2 types of KCS,
NSS-KCS and SS-KCS, remains to be elucidated. Per-
haps there are different degrees of conjunctival inflam-
amation or a different cytokine profile in the 2 types of
KCS, which might in part explain the observed differ-
ences in the epithelial mitotic rate in the NSS-KCS and
SS-KCS groups.

In reducing ocular surface abrasion as well as in-
flammation by decreasing the production and release of
inflammatory cytokines, CsA may help to reconstitute
homeostasis of the conjunctival epithelium, resulting in
an increase in goblet cells as seen in both KCS popula-
tions and a decrease in epithelial turnover as seen in our
patients with NSS-KCS. The differential response to
CsA treatment of the 2 types of KCS in terms of epithe-
lial mitotic rate remains unclear, but different cytokine
or growth factor profiles may be responsible for these
differences.

Figure 4. Ki-67–labeled cells of conjunctival epithelium at baseline (A) and
percent change from baseline after 6 months of treatment with either topical
cyclosporine A (CsA) ophthalmic emulsion or the vehicle (B). Data are
expressed as the number of Ki-67–labeled cells per 100 basal propidium
iodide–labeled cells. A. At baseline, Ki-67–labeled cells (± SEM) are shown
for patients with non–Sjögren syndrome–associated keratoconjunctivitis
sicca (NSS-KCS) (n=16) and SS-KCS (n=12) in comparison with a normal
patient population (n=11). B. Percent change from baseline at month 6 after
treatment with either CsA or the vehicle for the number of Ki-67–labeled cells
(± SEM) is shown for patients with NSS-KCS and SS-KCS. Asterisk indicates
\(P\textless.05\); dagger, \(P\textless.001\).
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sion from the sinus to the orbit. None of our cases had any evidence of sinus disease, and it is more likely that any sinus opacification seen is secondary to adjacent inflammation from the dacryocystitis or unrelated sinus disease. Other possible causes include hematogenous spread from other systemic sources and, in some cases, a primary orbital cellulitis that can extend into the lacrimal sac without the dacryocystitis necessarily being causal.

In summary, our series of orbital cellulitis and abscess secondary to dacryocystitis has been presented. Orbital cellulitis and abscess can rapidly progress to an intraconal abscess and can cause severe visual sequelae if untreated. Prompt recognition and appropriate surgical management of this condition are necessary to prevent vision loss. Prior dacryocystitis is a risk factor for developing orbital extension, and patients with prior episodes of dacryocystitis who elect not to have a lacrimal bypass operation should be warned of these potential consequences.

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