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

Kathleen S. Kunert; Ann S. Tisdale; Ilene K. Gipson

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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K ERATOCONJUNCTIVITIS sicca (KCS), or dry eye syndrome, is a frequently encountered problem in ophthalmologic practice. Keratoconjunctivitis sicca is characterized by chronic dryness of the corneal and conjunctival surfaces.[1] Patients with dry eye syndrome typically have symptoms of ocular discomfort ranging from irritation to severe pain. Redness, burning, itching, foreign body sensation, contact lens intolerance, photophobia, and blurred vision can occur.[2] The diagnosis of dry eye is difficult since it has no single characteristic sign or symptom and no single diagnostic measure. The recent committee of the National Eye Institute/Industry Workshop on Clinical Trials in Dry Eyes reported a new classification system for the various types of dry eye syndrome.[3] The 2 major categories are aqueous tear production–deficient and evaporative dry eye. The aqueous tear production–deficient category includes Sjögren-associated KCS (SS-KCS) and non-Sjögren- associated KCS (NSS-KCS).

In NSS-KCS and SS-KCS, T-cell infiltration of the conjunctiva has been observed.[4,5] Increased levels of the cytokines tumor necrosis factor (TNF) α, interleukin (IL) 1α, IL-6, IL-8, and IL-10, as well as expression of immune-activation markers such as HLA-DR, intracellular adhesion molecule 1 (ICAM-1), and CD11a, have been described in these patients.[5-8] This chronic inflammatory environment on the ocular surface is in part responsible for the characteristic pathologic alterations of the conjunctival epithelium known as squamous metaplasia,[6,9] which is accompanied by an increase in epithelial stratification, enlargement of the superficial epithelial cells, and loss of goblet cells in patients with both NSS-KCS and SS-KCS.[4,9-12] Another feature of SS-KCS is an increase in the epithelial mitotic rate.[13] It is not known, however, whether patients with NSS-KCS have an increased epithelial proliferative rate.

From the Schepens Eye Research Institute and Department of Ophthalmology, Harvard Medical School, Boston, Mass.

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.[25] 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.[21] 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.[21] 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 +5 in the same eye, where corneal staining was greater than or equal to +2; (3) a baseline Ocular Surface Disease Index[26] score of 0.1 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.[21] 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[27] 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

Currently, the only treatments available are palliative, consisting primarily of lubricating eye drops to supplement a patient's natural tears or punctal occlusion to improve the residence time of the tears that the patient can produce. Less frequently, topical corticosteroids and oral doxycycline are used, particularly for dry eye that results from meibomitis. Attempts to develop therapeutic treatments for KCS have been difficult due to the limited understanding of the underlying pathophysiologic mechanisms. Although the pathology is still not completely understood, there is now sufficient evidence to suggest that dry eye syndrome is in part the result of an underlying immune-mediated inflammation affecting the lacrimal gland and the ocular surface.[14-19] This hypothesis is further supported by results obtained in human studies using topical treatment with the immunomodulatory agent cyclosporine A (CsA) ophthalmic emulsion.[5,8,20-22] This drug prevents synthesis and/or secretion of several proinflammatory cytokines, such as TNF-α[23] and IL-6,[24] and has beneficial effects on the underlying inflammatory pathology of dry eye syndrome.[5] Clinical trials using CsA have shown improvement in objective measures of dry eye syndrome, such as Schirmer test values and corneal staining.[21,25]

Our study was designed to determine whether goblet cell numbers and the epithelial mitotic rate are altered when comparing patients with NSS-KCS and SS-KCS and to evaluate whether therapy with topical CsA influences goblet cell numbers and epithelial turnover in these 2 types of dry eye syndrome.

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 Kessing[28] 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.[5] 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 mm^2 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.[30] 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 mm^2 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.

junctival biopsy specimens of both NSS-KCS and SS-KCS groups revealed an increase in PAS-stained goblet cells compared with baseline. There were no differences observed between the 0.05% and 0.01% treatment groups, and thus, data for the 2 groups were combined. Vehicle treatment, on the other hand, resulted in a further decrease in the numbers of goblet cells.

No difference in goblet cell patterns was detected between patients with NSS-KCS and those with SS-KCS (**Figure 1** and **Figure 2**). A decrease in goblet cell numbers from normal at baseline and an increase from baseline with CsA treatment was found for both KCS subtypes (Figures 1 and 2). Figure 1 shows micrographs of PAS-stained conjunctival sections in which the goblet cells are clearly delineated from the surrounding stratified epithelial cells. Biopsy specimens are shown from 2 patients with NSS-KCS (Figure 1A-D) and 2 patients with SS-KCS (Figure 1E-H) taken at baseline and after 6 months

of treatment with either CsA or vehicle. These sections show only a few goblet cells at baseline and an increase in goblet cells with CsA treatment in both NSS-KCS (Figure 1A and B) and SS-KCS (Figure 1E and F). In contrast, sections from a patient with NSS-KCS (Figure 1C and D) and one with SS-KCS (Figure 1G and H) treated with the vehicle alone show fewer goblet cells when compared with baseline. Figure 1I shows the distribution of conjunctival goblet cells in a normal patient. There was a high density of goblet cells observed in comparison with the baseline biopsy specimens of patients with NSS-KCS and SS-KCS.

At baseline, goblet cell numbers appear to be significantly decreased in both KCS populations compared with those of normal control subjects (*P*<.001) (Figure 2A). When numbers of goblet cells at baseline were compared with numbers after treatment with either CsA or vehicle, an increase from baseline was found in the
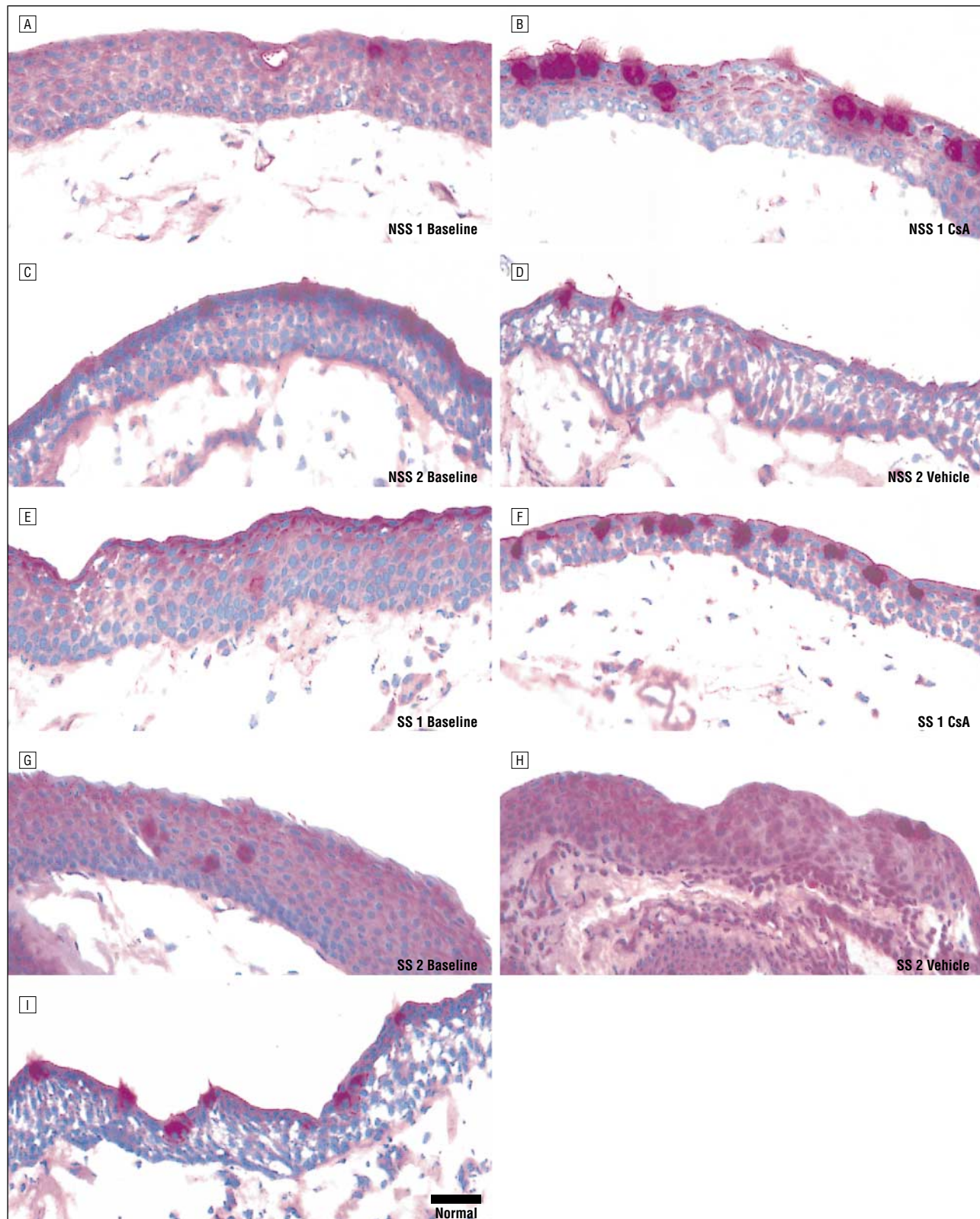
Figure 1. Goblet cells in human conjunctival biopsy specimens from 2 patients with non–Sjögren syndrome–associated keratoconjunctivitis sicca (NSS-KCS) (A-D) and 2 patients with SS-KCS (E-H). A, Patient 1 with NSS-KCS at baseline. B, Same patient after 6 months of treatment with topical cyclosporine 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.
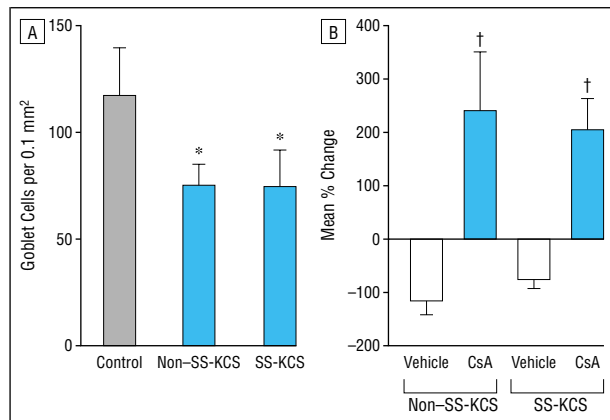
Figure 2. Goblet cell numbers per 0.1 mm² of conjunctival epithelium at baseline (A) and percent change from baseline after 6 months of treatment with either topical cyclosporine A (CsA) ophthalmic emulsion or the vehicle (B). A, At baseline, goblet cell numbers (± SEM) are shown for patients with non–Sjögren 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 numbers of goblet cells (± SEM) is shown for patients with NSS-KCS and SS-KCS. Asterisk indicates *P*<.001; dagger, *P*<.05.

CsA-treated groups. In contrast, there was a reduction from baseline in the vehicle-treated groups (Figure 2B). The numbers of goblet cells for the patients with NSS-KCS increased by 234% from baseline with CsA treatment and decreased by 114% with vehicle treatment. For the patients with SS-KCS, the numbers of goblet cells increased by 198% from baseline with CsA treatment and decreased by 75% with vehicle treatment. Pairwise comparisons favored CsA over the vehicle (*P*<.001). The increase from baseline in the numbers of goblet cells within the CsA group was statistically significant (*P*<.05) for both KCS populations.

QUANTITATION OF Ki-67–LABELED CELLS

Results of immunohistochemical analysis documented numerous Ki-67–positive cells in NSS-KCS conjunctiva at baseline compared with low numbers of Ki-67–positive cells in normal conjunctiva. In SS-KCS conjunctiva, however, numbers appeared to be similar to those in normal conjunctiva. After 6 months of treatment with either concentration of CsA, conjunctival biopsy specimens of patients with NSS-KCS revealed a decrease in Ki-67–labeled cells compared with baseline whereas vehicle treatment resulted in an increase in Ki-67–labeled cells. In contrast, no substantial change was observed for either treatment in the SS-KCS subset.

There was a difference in the Ki-67 binding pattern between patients with NSS-KCS and those with SS-KCS (**Figure 3** and **Figure 4**). Figure 3 shows representative sets of immunofluorescence micrographs for Ki-67–positive cells in conjunctival specimens of 2 patients with NSS-KCS (Figure 3A-D) and 2 patients with SS-KCS (Figure 3E-H) taken at baseline and after 6 months of treatment with either CsA or vehicle. Specific binding was mainly seen in the basal layers of the epithelium. At the baseline visit, both NSS-KCS patient biopsy specimens (Figure 3A and C) show numerous Ki-67–positive cells

whereas both SS-KCS patient biopsy specimens show only a few Ki-67–labeled cells. The specimen of the patient with NSS-KCS treated with CsA reveals a reduction in positive cells at month 6 (Figure 3B), whereas the biopsy specimen of the patient with NSS-KCS treated with the vehicle alone (Figure 3D) reveals an even higher number of Ki-67–positive cells compared with baseline (Figure 3A and C, respectively). Specimens of the 2 patients with SS-KCS, 1 treated with CsA (Figure 3F) and 1 treated with the vehicle alone (Figure 3H), indicate little difference between Ki-67–positive cells after 6 months of treatment and at baseline (Figure 3E and G, respectively). Very few Ki-67–labeled cells are seen in normal conjunctival epithelium (Figure 3I).

At baseline, the number of Ki-67–labeled cells per 100 basal epithelial cells appeared to be significantly greater in conjunctival biopsy specimens of patients with NSS-KCS when compared with normal controls (*P*<.05). In contrast, no significant difference from normal was observed for the SS-KCS population (Figure 4A). The number of Ki-67–labeled cells in patients with NSS-KCS decreased by 73% from baseline with CsA treatment and increased by 178% with vehicle treatment (Figure 4B). In the patients with SS-KCS, the number of Ki-67–labeled cells decreased by only 29% from baseline with CsA treatment and increased by 25% with vehicle treatment. Pairwise comparisons show a significant difference between CsA and vehicle treatment for the NSS-KCS (*P*<.05) but not for the SS-KCS population. Changes from baseline were significant for CsA (*P*<.001) and vehicle (*P*<.05) of the NSS-KCS but not for the SS-KCS population.

COMMENT

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] 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.[11]

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
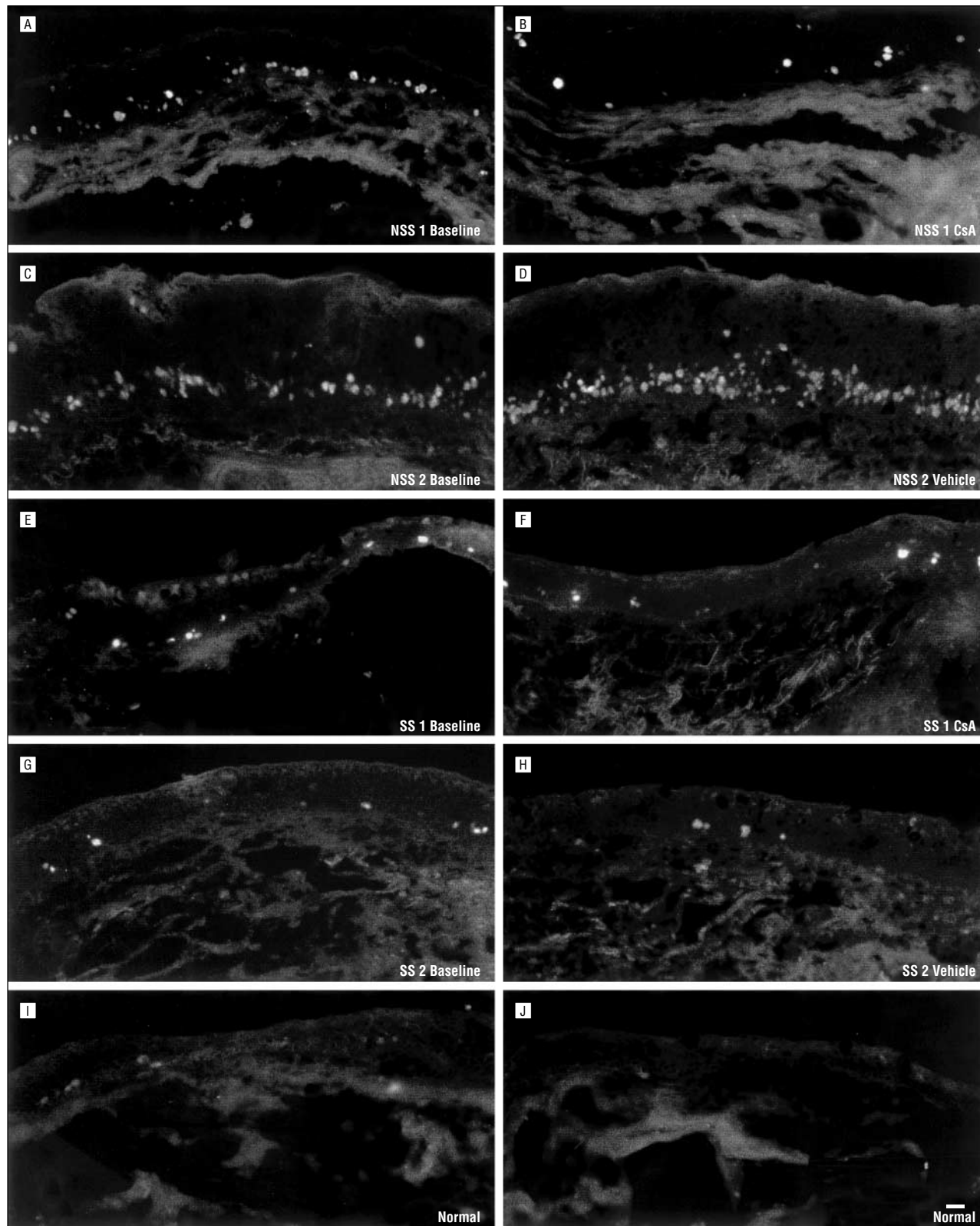
Figure 3. Immunohistochemical localization of Ki-67 antibody binding on cryosections of human conjunctiva. The antibody recognizes a nuclear antigen found only in proliferating cells. Representative biopsy specimens are shown from 2 patients with non–Sjögren syndrome–associated keratoconjunctivitis sicca (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.
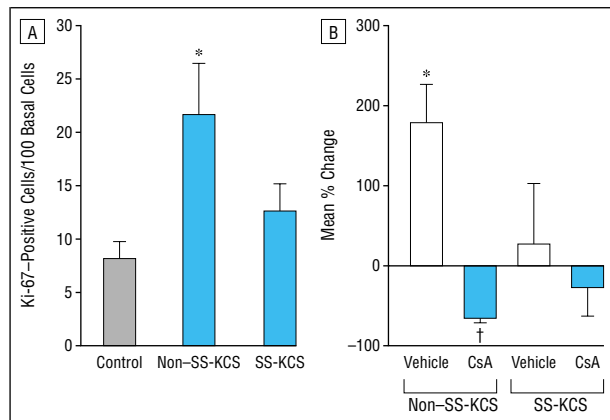
Figure 4. Ki-67–labeled cells of conjunctival epithelium at baseline (A) and percent change from baseline after 6 months of treatment with either topical cyclosporine A (CsA) ophthalmic emulsion or the vehicle (B). Data are expressed as the number of Ki-67–labeled cells per 100 basal propidium iodide–labeled 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 $P<.05$; dagger, $P<.001$.

in goblet cell numbers in patients with KCS following therapy.[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 topical 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, suggesting that the inflammatory process is still ongoing. This implies that CsA, in reducing ocular surface inflammation, 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 support this interpretation. Treatment with keratinocyte growth factor or TNF-α antibodies increases mucus production and restores goblet cells in cases of intestinal inflammation.[35,36] Alternatively, CsA may have a direct effect on goblet cell differentiation. In a human colon adenocarcinoma cell line, CsA induced a 94% increase in the volume of mucin within goblet cells.[37] Our data furthermore 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 treatment.

A second finding of this study is that there were differences 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 conjunctiva, 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 compared 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[3] found an increase in mitotic rate as shown by bromodeoxyuridine-labeling in SS-KCS. Even though we also found an increase in the number of proliferating cells in SS-KCS, this increase was not significant when compared with normal controls. Possible explanations for this observed difference might be that our study included a larger sample size and that our control subjects were mostly elderly patients undergoing cataract surgery as opposed to young human control subjects as enrolled by Jones and colleagues.

Altered lubrication and drying of the ocular surface in KCS result in ocular surface damage or epithelial wounding. Chung and colleagues[38] have shown that the conjunctival epithelium responds to corneal wounding by an increase in bromdeoxyuridine-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 stromal T-cell infiltration (predominantly CD3+ and CD4+ T lymphocytes) have been shown.[4,6] Furthermore, several studies that focused on conjunctival cytokine expression in patients with SS-KCS[4,7,18] demonstrated increased levels of inflammatory cytokines, such as IL-1α, TNF-α, IL-6, and IL-8, in the conjunctival epithelium of patients with SS-KCS compared with that of controls.[4,7,18] There are data suggesting the involvement of such inflammatory cytokines in epithelial hyperproliferation. Interleukin 6 and IL-8, for example, have been reported to influence growth and differentiation of epithelial cells and promote hyperproliferation of the epidermis in psoriasis.[39,40] Furthermore, the concentration of EGF, a cytokine that is capable of inducing proliferation or differentiation of epithelium, has been reported to be lower in the tear fluid of patients with KCS.[41] Pflugfelder and colleagues[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 inflammation increases the receptor density, making the conjunctival epithelial cells hypersensitive to mitogens. The exact 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. Perhaps there are different degrees of conjunctival inflammation or a different cytokine profile in the 2 types of KCS, which might in part explain the observed differences in the epithelial mitotic rate in the NSS-KCS and SS-KCS groups.

In reducing ocular surface abrasion as well as inflammation 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 populations 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 epithelial mitotic rate remains unclear, but different cytokine or growth factor profiles may be responsible for these differences.

Corresponding author and reprints: Ilene K. Gipson, Schepens Eye Research Institute, Boston, MA 02114 (e-mail: gipson@vision.eri.harvard.edu).

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sion from the sinus to the orbit.[1] 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.

Don O. Kikkawa, MD
La Jolla, Calif

Grant W. Heinz, MD
Tucson, Ariz

Ronald T. Martin, MD
William N. Nunery, MD
Indianapolis, Ind

Andrew S. Eiseman, MD
Washington, DC

Corresponding author and reprints: Don O. Kikkawa, MD, University of California, San Diego, Department of Ophthalmology, 9415 Campus Point Dr, La Jolla, CA 92093-0946 (e-mail: dkikkawa@ucsd.edu).

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Correction

Error in Reference. In the article titled "Goblet Cell Numbers and Epithelial Proliferation in the Conjunctiva of Patients With Dry Eye Syndrome Treated With Cyclosporine," published in the March issue of the ARCHIVES (2002;120:330-337), on page 337, in the first reference, the editors of *Principles and Practice of Ophthalmology: Clinical Practice* are Daniel M. Albert and Frederick A. Jakobiec.