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Conjunctival Cytokine Expression in Symptomatic Moderate Dry Eye Subjects

Srihari Narayanan, William L. Miller, and Alison M. McDermott

Purpose. To compare ocular surface cytokine expression in healthy controls and subjects with moderate dry eye and to study the ability of interleukin (IL)-1β to modulate cytokine expression in cultured human conjunctival epithelial cells (CECs).

Methods. Subjective (symptom questionnaire) and objective (tear osmolality, fluorescein tear break-up time [TBUT]) measures of dry eye were determined in five healthy controls and five subjects with moderate dry eye. Tear clearance rates were measured with a fluorophotometer. Enzyme immunoassay and a cytokine bead assay were used to quantify IL-1β in tear fluid. RT-PCR was performed to detect expression of IL-1β, IL-6, IL-8, growth-related oncogene (GRO)-β, intercellular adhesion molecule (ICAM)-1, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), and ephrin A5 in conjunctival impression cytology (CIC) samples and in CECs (IOBA-NHC cell line, n = 3; primary cultured CEC, n = 3) exposed to 10 ng/mL IL-1β for 6 hours.

Results. Subjects with moderate dry eye had significantly higher symptom scores, higher tear osmolality, and shorter TBUT than healthy controls. Subjects with dry eye demonstrated slightly slower tear clearance (13.1% per minute) than healthy controls (15.4% per minute). Very low levels of IL-1β protein were detected in the tear fluid of both groups. TRAIL was constitutively expressed in CIC samples, whereas IL-1β, IL-6, and GRO-β were absent. Weak expression of IL-8 (two healthy, four dry eye), ICAM-1 (four healthy, four dry eye), and ephrin A5 (one healthy, two dry eye) was observed. IL-1β upregulated its own expression and that of IL-6, IL-8, GRO-β, and ICAM-1 in cultured CECs but not that of ephrin A5 or TRAIL.

Conclusions. The lack of major differences in ocular surface cytokine expression between the two groups of subjects implies other inflammatory pathways or etiologies are involved in moderate dry eye. Although IL-1β modulated the expression of various cytokines in cultured CECs, its absence in tear fluid and CIC samples suggests that IL-1β does not play a modulatory role in moderate dry eye. (*Invest Ophthalmol Vis Sci.* 2006;47: 2445–2450) DOI:10.1167/iovs.05-1364

Dry eye is a condition that arises because of decreased tear secretion or increased tear evaporation.[1] Tear-deficient dry eye can be broadly classified in two categories—Sjögren syndrome–associated keratoconjunctivitis sicca (SS KCS), which is associated with autoimmune disease, and non-Sjögren keratoconjunctivitis sicca (NS KCS), which is the moderate form of the disease not associated with any autoimmune disorder. All forms of dry eye result in damage to the ocular surface epithelia and are consequently associated with ocular irritation symptoms such as discomfort, dryness, grittiness, and soreness.[1,2] Although NS KCS is not a severe form of the disease, patients with NS KCS still have many symptoms[2] and demonstrate less vitality, poorer general health, and lower quality of life than healthy persons.[3] Therefore, it is essential to understand the causes of moderate dry eye to provide better therapeutic options for these patients.

The pathogenesis of the ocular surface epithelial changes in dry eye disease has been explained by theories as varied as vitamin A or other nutritional deficiency,[4–7] inflammation,[8] mechanical irritation causing inflammation,[9] hyperosmolarity,[10,11] and hormonal imbalance.[12,13] Current evidence favors a significant role for inflammation in the pathogenesis of dry eye.[8] To substantiate this hypothesis, several recent studies have investigated the presence of inflammatory cytokines and the balance between cytokines and growth factors at the ocular surface. Most studies on the inflammatory component of dry eye have largely been restricted to patients with SS KCS (aqueous-deficient dry eye) or patients with ocular rosacea (an evaporative form of dry eye).[9,14–16] However, nearly two thirds of persons with dry eye have the moderate form of the disease, NS KCS.[17]

Although the etiology of NS KCS has not been clearly established, evidence exists to support the hypothesis that inflammation is a feature of all forms of dry eye. For example, in conjunctival biopsy specimens from patients with SS KCS or NS KCS, Stern et al.[18] found lymphocytic infiltration and increased immunoreactivity for markers of inflammation and immune activation, such as intracellular adhesion molecule-1 (ICAM-1) and major histocompatibility complex (MHC) class II molecules HLA-DR and HLA-DQ. They also noted that the extent of cellular immunoreactivity did not differ significantly between SS KCS and NS KCS tissue samples. One recent study[19] found that conjunctival cells from patients with NS KCS and SS KCS demonstrate a high level of expression of inflammatory and apoptosis markers, such as HLA-DR, ICAM-1, CD40, CD40 ligand, and Apo2.7. In addition, increased levels of the inflammatory cytokine IL-6 was noted in patients with SS KCS and NS KCS.[20] The success of corticosteroids, long known to have anti-inflammatory properties, and of the immunosuppressive agent cyclosporin A in the treatment of SS KCS and NS KCS further reinforces the potential role for inflammation in dry eye disease.[20–23] These studies were among the first to suggest that in NS KCS, inflammation could contribute to the damage of the ocular surface and thus cause dry eye symptoms. Other evidence comes from our study[24] showing that subjects with NS KCS demonstrate an increased expression of the inflammation-inducible peptide human β defensin-2 (hBD-2).[25–27] The source of the inflammation in the moderate dry

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eye subjects is not clearly known. However, given that the proinflammatory cytokine IL-1 was found to upregulate hBD-2 expression in conjunctival cells,[24] the upregulation of hBD-2 in the conjunctiva of patients with moderate dry eye may involve the activity of this cytokine.

Increased levels of IL-1α and IL-1β have been shown in patients with Sjögren syndrome or ocular rosacea.[16,28] Interestingly, IL-1, which is considered to play significant roles in ocular surface immune and inflammatory responses and in wound healing,[29] exerts its proinflammatory activity in part by modulating cytokine gene expression. For example, IL-1 is known to alter the expression of a variety of cytokine genes, such as chemokines and TNF-superfamily members in corneal fibroblasts, epithelial cells, and endothelial cells.[29–33] Studying the expression of IL-1–modulated genes in human conjunctival epithelial cells will lead to a logical expansion of our knowledge on the effects of this cytokine on ocular surface epithelia. Therefore, the expression of IL-1–modulated cytokines identified in a previous study[31] (IL-1β, IL-6, IL-8, GRO-β, ICAM-1, tumor necrosis factor-related apoptosis-inducing ligand [TRAIL] and ephrin A5) was compared between healthy controls and subjects with moderate dry eye (NS KCS) and was also studied in cultured human conjunctival epithelial cells (CECs) exposed to IL-1. Some of these results have been presented in preliminary form (Narayanan S. *IOVS* 2004;45:ARVO E-Abstract 86).

Methods

Human Subjects

Five healthy controls (mean age, 33.2 ± 3.8 years; one man, four women) and five subjects with moderate dry eye (mean age, 43.8 ± 4.4 years; one man, four women) participated in the study. The study followed the tenets of the Declaration of Helsinki. Informed consent was obtained from all subjects, and the study was approved by the University of Houston Institutional Review Board. Subjective assessment of ocular irritation was conducted using a scoring system–based dry eye questionnaire.[2] A total score of 32 points or more was considered indicative of moderate dry eye. Objective assessment of the subjects included an ocular surface health assessment by biomicroscopy (including fluorescein and lissamine green staining of the cornea and conjunctiva), tear osmolality measured with a vapor pressure osmometer, tear break-up time measured with the Dry Eye Test (DET; Akorn, Chicago, IL), and tear clearance rate (TCR) measured with a fluorophotometer (Fluorotron II; Ocumetrics, Mountain View, CA). DET (Akorn) strips were used to deliver fluorescein to the ocular surface for TCR measures because these strips provide consistent delivery of approximately 1 μg fluorescein with each instillation.[34] Tear film fluorescein concentration (ng/mL) was measured 5, 10, and 15 minutes after fluorescein instillation. Fluorescein concentrations were normalized, and the exponential fluorescein decay rates were calculated (Kaleidagraph 4.0 software; Synergy Software, Reading, PA) and converted to a TCR measure. A 5-μL unstimulated tear sample was collected with a glass micropipette and stored at −80°C for IL-1β enzyme immunoassay (EIA). A 12-μL unstimulated tear sample was collected from one eye on a separate visit, stored at −80°C, and used in a tear fluid IL-1β assay with cytokine bead technology (Bio-Plex Assay; Bio-Rad, Hercules, CA). To prevent degradation by molecules such as proteases, tear fluid samples were kept on ice during tear collection from patients and then immediately transferred to −80°C for storage. Total time for collecting the tear sample ranged from 6 to 10 minutes. Reflex tearing was noted in four subjects, both subjectively (subject responded that he or she was "suddenly" tearing) and objectively (observer noted sudden increase in tear flow through the microcapillary tube). A sample was not used if it was contaminated by reflex tears. If reflex tearing was noted, subjects were given a 10- to 15-minute break, adequate time for replacement of the tear layer, before tear sample collection was resumed. All subjects were examined between 1 PM and 3 PM to minimize any variability that might have occurred with time of day. Furthermore, the results of our dry eye questionnaire[2] indicated that subjects with moderate dry eye had more symptoms toward the end of the workday and, therefore, that evaluation in the afternoon hours would provide a better reflection of their condition.

Conjunctival Impression Cytology Sample Collection

Conjunctival impression cytology (CIC) samples were collected as described previously.[24] A single drop of 0.5% proparacaine hydrochloride (Akorn, Chicago, IL) was first instilled in the eye. Then a 3 × 8-mm preautoclaved polyether sulfone membrane (Supor; Pall Gellman Sciences, East Hills, NY) was placed on the temporal bulbar conjunctiva for 5 to 10 seconds. The membrane was gently removed and was placed directly in 100-μL ice-cold reagent (TRIzol; Invitrogen, Carlsbad, CA). CIC samples were stored at −80°C until RT-PCR analysis.

Cell Culture

Normal human conjunctival epithelial cells (IOBA-NHC cell line[35]; passages 72, 75 and 79) and primary human conjunctival epithelial cells (passages 1 and 2) from three donors (55, 63, and 69 years of age) were used in these experiments.[24,35] All cell culture reagents were obtained from Invitrogen (Carlsbad, CA), unless otherwise stated. IOBA-NHC cells[35] were cultured in media, as follows: Dulbecco modified Eagle medium (DMEM)/F12 (1:1 vol/vol) containing 10% fetal bovine serum, 2 ng/mL mouse epithelial growth factor (EGF; Sigma, St. Louis, MO), 1 μg/mL bovine insulin (Sigma), 0.1 μg/mL cholera toxin (Sigma), 5 μg/mL hydrocortisone (Sigma), and 2.5 μg/mL fungizone and penicillin (5000 U/mL)/streptomycin (5000 μg/mL) mixture.[35] Human conjunctival tissue was obtained from the National Disease Research Interchange (NDRI, Philadelphia, PA), then incubated overnight at 4°C in a 1:1 vol/vol solution of EpiLife (Cascade Biologics, Portland, OR) with dispase (20 U/mL). Epithelial cells were then scraped free and seeded in media (EpiLife; Cascade Biologics) with growth supplement into 25-cm^2 flasks coated with fibronectin and collagen (FNC; AthenaES, Baltimore, MD). Cells grew to confluence by 1 week and were then passaged with the use of trypsin-EDTA.

IL-1β Treatment of Conjunctival Cells

IOBA-NHC cells were serum-starved overnight, whereas primary cultured conjunctival cells were placed in growth supplement–free media overnight before each experiment. The cells were treated for 6 hours with serum-free (IOBA-NHC cells) or supplement-free (primary cultured cells) medium alone (untreated controls) or with the addition of the proinflammatory cytokine IL-1β (10 ng/mL; R&D Systems, Minneapolis, MN). This duration was chosen based on our previous study of the effect of IL-1β on corneal epithelial cytokine expression.[31] At the completion of the treatment period, cells were trypsinized, collected in RNA lysis buffer (Qiagen, Valencia, CA) and stored at −80°C until RNA extraction.

RNA Extraction and RT-PCR

Total RNA was extracted from the CIC specimens with the use of a modified phenol-chloroform extraction procedure and used in two-step RT-PCR reactions.[24,36] Total RNA was extracted from the cell pellets with RNeasy-mini kits (Qiagen) and used in one-step RT-PCR reactions.

Based on our previous research in corneal epithelial cells,[31] the following cytokines were selected for analysis: IL-1β, IL-6, IL-8, GRO-β, ICAM-1, TRAIL, and ephrin A5. β-actin was used as the housekeeping gene. Primer sequences, expected product sizes, and RT-PCR cycle profiles were as described previously.[31] Ethidium bromide–stained 1.3% agarose gels were used to analyze the PCR products. An Alpha-imager (Alpha Innotech, San Leandro, CA) gel documentation system

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TABLE 1. Differences in Objective Dry Eye Test Results in the Two Groups of Subjects

Test	Normal	Dry Eye	*P* and Statistical Test
DET-TBUT (sec)	13.4 ± 2.2	5.4 ± 2.1	0.042*; one-way ANOVA
Osmolality (mOsm/kg)	269.4 ± 3.7	302 ± 8.8	0.008*; one-way ANOVA
Corneal fluorescein (grade)	0.40 ± 0.54	0.60 ± 0.89	0.81; Kruskal-Wallis
Conjunctival fluorescein (grade)	0.60 ± 0.54	0.54 ± 0.83	0.73; Kruskal-Wallis
Corneal lissamine green (grade)	0.20 ± 0.44	0.40 ± 0.54	0.51; Kruskal-Wallis
Conjunctival lissamine green (grade)	0.40 ± 0.54	0.60 ± 0.55	0.55; Kruskal-Wallis

$n = 5$ subjects in each group. All values are mean ± SD. DET-TBUT, Dry Eye Test tear break-up time.
* Significant difference between the two groups of subjects at $P < 0.05$.

was used to obtain a digital image of the gels and to analyze the images semiquantitatively. Controls in which either nucleic acid or reverse transcriptase was omitted were also performed, in which case no product was obtained (data not shown).

Quantitation of IL-1β in Tear Fluid

IL-1β was first quantitated in tear fluid using a commercially available IL-1β EIA (R&D Systems). Duplicate 2-μL tear samples, diluted in the assay buffer, were used to detect IL-1β in the tear fluid of healthy controls and subjects with moderate dry eye. The assay was performed according to the manufacturer's instructions.

Tear fluid IL-1β levels were also quantified by a single-plex cytokine bead assay (Bio-Plex Assay; Bio-Rad). The detection platform used was calibrated for high sensitivity (1.95–32,000 pg/mL). The sample diluent buffer alone was used as a control to account for false positives. Triplicate 4-μL tear samples, diluted in the sample diluent (supplied by the manufacturer), were used to detect IL-1β in the tear fluid of the two groups of subjects. The assay was performed according to the manufacturer's instructions.

RESULTS

Subjective and Objective Assessment

Subjective assessment with a dry eye questionnaire[2] indicated that the subjects with moderate dry eye had significant ($P = 0.011$; Student *t* test) symptoms (score [mean ± SD], 44.2 ± 5.3 points) compared with the healthy controls (score, 23.6 ± 3.4 points). Results of the objective dry eye tests are shown in Table 1. Subjects with moderate dry eye demonstrated a slightly slower rate (13.1% per minute) of tear clearance than healthy controls (15.4% per minute). Nonparametric Wilcoxon rank test for the tear clearance data did not reveal any statistically significant difference between the two groups of subjects at the 5-minute ($P = 1.00$), 10-minute ($P = 0.625$), or 15-minute ($P = 0.625$) time points. Figure 1 shows the fluorescein decay rates in the two groups as measured with a fluorophotometer (Fluorotron Master II; OcuMetrics, Mountain View, CA).

Cytokine Expression in Conjunctival Epithelial Cells

The CIC samples yielded 1 to 2 μg total RNA. The RNA extraction procedure from CIC samples was optimized before our experiments were conducted. RNA from CIC samples run on ethidium bromide–stained agarose gels demonstrated intact 28S and 18S bands without genomic DNA contamination (results not shown). Analysis of the CIC specimens (Fig. 2) showed that TRAIL mRNA was constitutively expressed, whereas IL-1β, IL-6, and GRO-β were not present in any subject (not shown). Weak expression of IL-8 (two healthy, four dry eye), ICAM-1 (four healthy, four dry eye), and ephrin A5 (one healthy, two dry eye) was also noted.

Conjunctival epithelial cells (IOBA-NHC cells and primary cultured conjunctival cells) exposed to IL-1β (10 ng/mL, 6 hours) demonstrated altered cytokine expression (Fig. 3) compared with untreated control cells exposed to the culture medium alone. IOBA-NHC cells ($n = 3$) showed an upregulation of IL-1β (1.05 ± 0.04-fold), IL-6 (1.11 ± 0.06-fold), IL-8 (1.21 ± 0.14-fold), GRO-β (1.1 ± 0.05-fold), and ICAM-1 (1.15 ± 0.05-fold) at the mRNA level, whereas primary cultured conjunctival epithelial cells showed an upregulation of

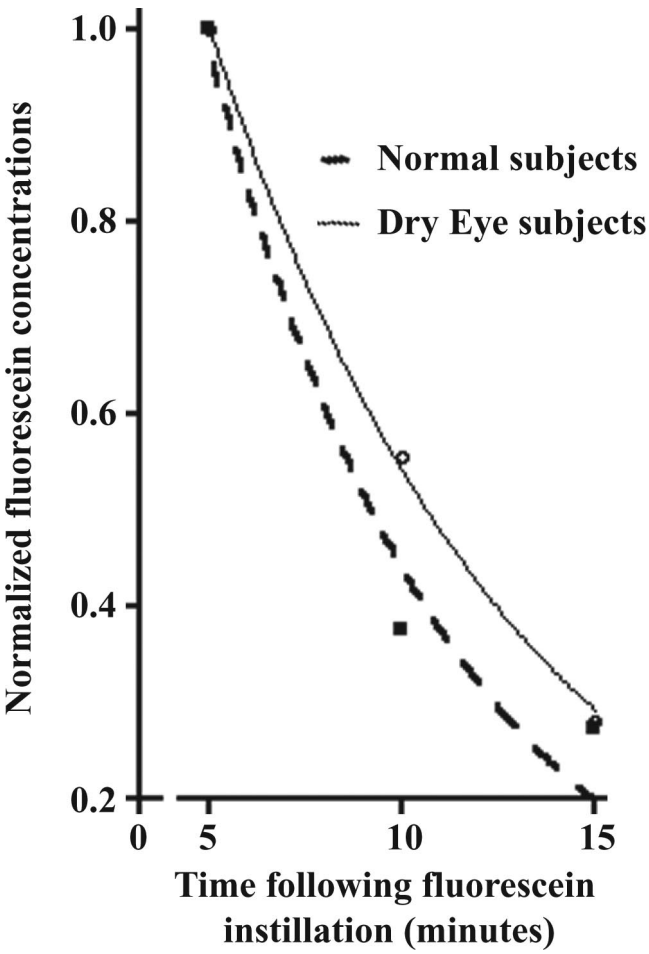

FIGURE 1. Tear clearance rates in healthy controls and subjects with moderate dry eye. Fluorescein was instilled on the ocular surface with the use of fluorescein strips, and tear film fluorescein concentration was measured with the use of a fluorophotometer 5, 10, and 15 minutes after instillation. Fluorescein concentrations were normalized, and fluorescein decay rates were calculated and converted to tear clearance rate measures. Data represent mean normalized tear film fluorescein concentrations in the two groups of subjects.

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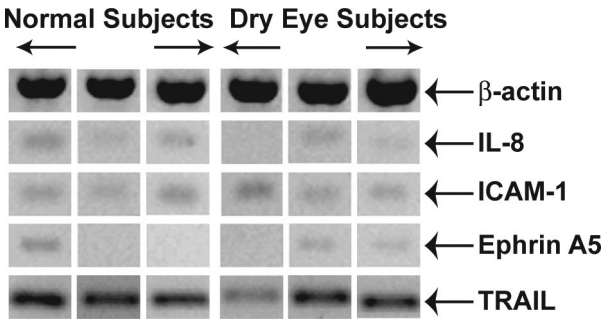

FIGURE 2. Cytokine gene expression in the conjunctival epithelium of healthy controls and subjects with moderate dry eye. Two-step RT-PCR was performed for β-actin, IL-8, ICAM-1, ephrin A5, and TRAIL on RNA extracted from conjunctival impression cytology specimens. Data are from three representative subjects in each group.

IL-1β (1.51 ± 0.29-fold), IL-6 (1.3 ± 0.11-fold), IL-8 (1.25 ± 0.04-fold), GRO-β (1.1 ± 0.05-fold), and ICAM-1 (1.1 ± 0.0-fold). The expression of ephrin A5 and TRAIL was not altered.

Tear Fluid Levels of IL-1β

IL-1β was not detected through IL-1β EIA in the tear fluid of any of the study subjects. However, the more sensitive cytokine bead assay revealed very low levels of IL-1β in the tear fluid of three healthy controls (0.62, 1.58, and 3.84 pg/mL) and three subjects with moderate dry eye (0.37, 0.47, and 7.62 pg/mL). There was no statistically significant difference ($P = 0.43$; Student t test) in tear fluid IL-1β levels between the two groups. It must be noted that two healthy controls and two subjects with dry eye had tear film IL-1β levels below the lowest standard (1.95 pg/mL) of the Bio-Plex assay.

DISCUSSION

Results of this study show that subjects with moderate dry eye did not demonstrate any major differences in ocular surface cytokine expression compared with healthy controls and that IL-1β modulated its own expression and that of IL-6, IL-8, GRO-β, and ICAM-1 in cultured human conjunctival epithelial cells.

Subjects with moderate dry eye demonstrated a significantly higher degree of ocular irritation, higher tear osmolality, shorter tear break-up time, and slightly delayed tear clearance than healthy controls, who showed no evidence of ocular surface disease. Thus, the subjects with moderate dry eye had clinical characteristics typical of patients with moderate dry eye.[1,37] Subjective and objective assessments of these subjects with dry eye matched well with those of an earlier, larger study that validated the dry eye questionnaire used here.[2] For example, the most commonly reported symptoms in subjects with moderate dry eye in the present and the previous[2] study were dryness and soreness. Although the high level of ocular irritation suggests definite ocular surface damage, there was no difference in IL-1 or IL-1–modulated ocular surface cytokine expression between the two groups. This lack of difference in inflammatory cytokine expression could imply that ocular surface damage in moderate dry eye is caused by inflammation-independent mechanisms or is mediated by cytokines other than those studied here.

In the present study, use of a commercially available EIA kit did not detect IL-1β in the tear fluid of any subject. Because the EIA kit had a restricted range of the standard curve (3.2–500 pg/mL), tear fluid IL-1β levels were measured with a more sensitive assay based on cytokine bead technology. Very low levels of IL-1β were detected in the tear film of a few subjects with the cytokine bead assay, but there was no difference between the subjects with moderate dry eye and healthy controls. Additionally, the tear film IL-1β level in two subjects in each group was below that of the lowest standard, suggesting that these subjects had extremely low or undetectable IL-1β levels in their tear film. These low levels did not agree with levels measured in human tear fluid in other published studies. For example, one study[28] showed that the IL-1β level in the tear film of subjects with dry eye was 80 to 180 pg/mL, whereas that of healthy controls was 30 pg/mL. This earlier study,[28] performed on patients with Sjögren syndrome or ocular rosacea-associated Meibomian gland disease, made use of a commercially available EIA kit similar to the one used in the present study. It should be noted that in this study,[28] IL-1β was detected in immunostained CIC specimens in 2 of 6 healthy controls and 15 of 16 subjects with Sjögren syndrome, suggesting that IL-1β protein expression in the healthy population is low and variable but that it is highly expressed in patients with Sjögren syndrome. These results demonstrate some concurrence with our data with respect to IL-1β expression in CIC samples from healthy controls, and they suggest that the definite presence of an immune-mediated disease process (such as Sjögren syndrome) may be essential to routinely detect significant conjunctival epithelial inflammatory cytokine expression. The lack of agreement between previous studies and the present investigation with respect to the presence of tear fluid IL-1β in patients with moderate dry eye cannot be definitively explained. One shortcoming of our study is the small number of participants; sampling from a greater number of healthy controls and subjects with dry eye would illuminate the variability regarding IL-1 levels in tear film. With this caveat, it can be speculated that the results of the present study could be attributed to the moderate nature of the disease in the study

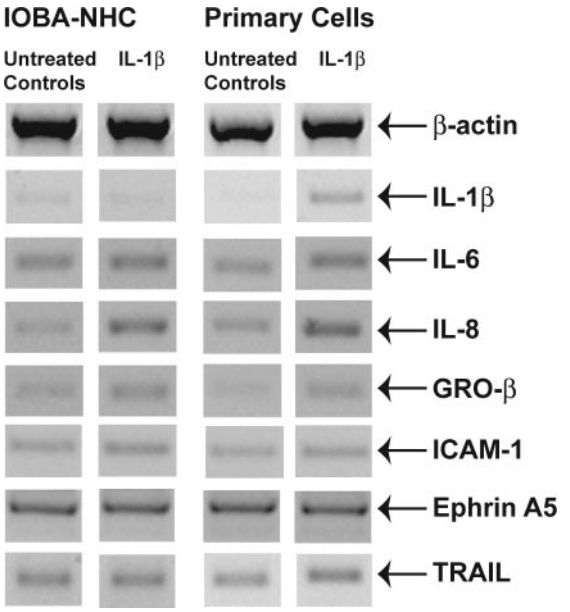

FIGURE 3. Cytokine gene expression in IOBA-NHC and primary cultured conjunctival epithelial cells after exposure to 10 ng/mL IL-1β for 6 hours. IOBA-NHC and primary cultured conjunctival epithelial cells were treated with serum-free growth media alone (untreated controls) or with the addition of 10 ng/mL IL-1β (IL-1β) for 6 hours. One-step RT-PCR was performed on samples collected from IOBA-NHC ($n = 3$) or primary cultured conjunctival epithelial cells ($n = 3$). The figure shows RT-PCR products for one sample of IOBA-NHC or primary cultured conjunctival epithelial cells for the constitutively expressed β-actin and the cytokines IL-1β, IL-6, IL-8, GRO-β, ICAM-1, ephrin A5, and TRAIL.

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subjects. The subjects with moderate dry eye in the present study demonstrated very low scores on corneal and conjunctival staining with fluorescein and lissamine green, suggesting minimal ocular surface damage. These scores, especially the low lissamine green staining scores, clearly demonstrate the moderate nature of the disease. Mean corneal and conjunctival staining scores were slightly higher in the dry eye group compared with those in healthy controls, though this difference did not reach statistical significance. It is notable from our present and previous[2] data that small differences in ocular surface health (as determined by vital dye staining) can lead to large differences in ocular irritation symptoms. Even though this small amount of ocular surface epithelial damage was adequate to cause significant dry eye symptoms in our study, it might not have been enough to upregulate IL-1β and the other inflammatory cytokines to a detectable level. The low levels of IL-1β detected in the tear fluid, coupled with the absence of IL-1β in the CIC samples, suggest that IL-1β and perhaps other inflammatory cytokines may be just below the detection level of the assays performed. Therefore, a very low grade inflammatory reaction or an inflammatory pathway mediated by proinflammatory cytokines such as TNF-α cannot be ruled out in these subjects with moderate dry eye. It can also be argued, however, that the low levels of IL-1β in the tear fluid and the absence of IL-1β in the CIC samples suggest that IL-1β may not play a role in causing the symptoms observed in patients with moderate dry eye. In keeping with this argument, for example, the upregulation of hBD-2 observed in subjects with moderate dry eye in an earlier study[28] might have been mediated by other cytokines (such as TNF-α). Clarification of this speculative interpretation awaits an investigation with a larger cohort of subjects expanded to include patients with severe dry eye disease in whom a role for IL-1 appears likely.

The present study is the first to demonstrate the expression of ephrin A5 (one healthy control, two subjects with dry eye) and TRAIL (constitutive expression) in human CIC samples and in cultured human conjunctival epithelial cells. Constitutive expression of ephrin A5 and TRAIL was observed in human conjunctival epithelial cells treated with growth media alone (untreated control cells) or with the addition of 10 ng/mL IL-1β (6 hours). However, ephrin A5 was significantly downregulated and TRAIL was upregulated in cultured human corneal epithelial cells exposed to 10 ng/mL IL-1β (6 hours).[31] Thus, the expression of these two molecules contrasts in corneal and conjunctival cells in culture. Ephrins regulate cell shape and size during development.[38] TRAIL has recently been shown to be involved in the lymphocytic destruction of the salivary gland of patients with Sjögren syndrome.[39] We observed that IL-1β modulated its own expression and that of IL-6, IL-8, GRO-β, and ICAM-1 in cultured human conjunctival epithelial cells. One previous study[40] also noted that IL-1β exposure upregulated the expression of ICAM-1 and IL-8 in primary cultured conjunctival cells, whereas another study[41] noted upregulation in ICAM-1 expression after IL-1β treatment of Chang (Wang-Kilbourne derivative) conjunctival epithelial cells. IL-1β has also been shown to induce IL-6 in primary cultured human conjunctival epithelial cells in a dose-dependent manner.[42] However, the present study appears to be the first report of the effect of IL-1β on GRO-β expression by human conjunctival epithelial cells in culture. The effects of IL-1β on conjunctival cytokine expression are comparable to those seen with corneal epithelial cells.[31] By modulating chemokines such as IL-8 and GRO-β in an analogous manner in the corneal and conjunctival epithelia, IL-1 could play an important role in corneal and conjunctival wound healing by indirectly promoting leukocyte chemotaxis. Although we did not find IL-1β expression in our human subjects, we believe that our in vitro data are applicable to other literature[28] supportive of a role for IL-1β in severe dry eye associated with Sjögren syndrome.

In summary, the results of the present study indicate that the role of IL-1β–mediated inflammation in the pathogenesis of ocular surface damage in moderate dry eye subjects is questionable. Although we found IL-1β to be a potent modulator of conjunctival cytokine expression, lack of tear fluid IL-1β and minimal changes in cytokine expression suggest that the pathogenesis of moderate dry eye likely involves pathways other than one mediated or initiated by IL-1β. However, we caution the reader that our study group was small and stress the moderate nature of the condition in our subjects. Thus, though evidence for IL-1β–mediated damage in severe dry eye is substantial, elucidation of its role in moderate disease awaits larger, preferably multicenter studies.

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