Expression Patterns of Sialylated Epitope Recognized by KL-6 Monoclonal Antibody in Ocular Surface Epithelium of Normals and Dry Eye Patients

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PURPOSE. To determine the association of the KL-6 epitope (sugar moiety) with MUC1 and its distribution on the ocular surface of human non–dry and dry eyes.

METHODS. The human ocular surface was examined immunohistochemically and immunoelectron microscopically using monoclonal antibody (mAb) KL-6, which recognize a carbohydrate epitope of MUC1. The expression patterns of KL-6 epitope in corneal and conjunctival cells were examined by impression cytology from 24 non–dry eye volunteers and 43 dry-eye patients.

RESULTS. In the cornea, bulbar conjunctiva, and limbus epithelium, mAb KL-6 reacted to the apical cell membrane of superficial cells and the intercellular space of superficial and wing cells. No immune reactivity of mAb KL-6 was observed in the basal plasma membrane of basal epithelial cells. Results of impression cytology indicated that the corneal epithelium of 13 of 24 non–dry eyes was weakly stained by mAb KL-6, whereas 42 of 43 dry eyes showed a mosaic pattern. In non–dry eyes, 19 of 24 bulbar conjunctival epithelia expressed the KL-6 epitope in a honeycomb pattern. In mild (17/19) and moderate (17/17) dry eye conjunctiva, the KL-6 epitope showed a mosaic pattern. However, the expression of KL-6 epitope decreased in severe dry eyes, showing a mosaic pattern in three of seven patients and labeling a few cells weakly in four of seven patients.

CONCLUSIONS. These findings suggest that there is an upregulation of the sialylated KL-6 epitope of MUC1 by apical corneal and conjunctival cells in mild and moderate dry eyes. This upregulation may in part alleviate the consequences caused by goblet cell mucin dysfunction in dry eyes. It is noteworthy that the KL-6 epitope is downregulated in the conjunctiva of severe dry eyes, a phenomenon that may be explained in part by the malfunction of conjunctival epithelial cells. (*Invest Ophthalmol Vis Sci.* 2004;45:2212–2217) DOI:10.1167/iovs.03-0988

Human MUC1, a membrane-associated mucin similar to the Muc1s of animal species, is a large glycoprotein that contains a variable number of tandem repeats in the extracellular domain, which extends 200 to 500 nm from the cell surface.[1,2] MUC1 mucin exists in mucosal epithelium of the respiratory and digestive systems as well as the keratoconjunctival epithelium of the ocular surface.[3–5] Because of the abundant hydrophilic polysaccharide side chains of its extracellular domain, MUC1 can serve as a lubricant in situ. The role of its intracellular carboxyl domain remains elusive; however, increasing evidence indicates that this carboxyl terminal peptide may participate in signaling.[1,6]

In the eye, MUC1 and -4 are expressed by the ocular surface epithelia (i.e., cornea and conjunctiva), whereas MUC5AC expression is limited to conjunctiva.[6] It has been suggested that the cooperation between transmembrane mucin and secretory mucin is necessary for the stability of the tear film.[7] MUC1 is thought to be as important as MUC4[8] in conjunction with MUC5AC[9–12] for the formation of tear-film. Of interest, vitamin A–deficient rats exhibited a downregulation of rMuc4 and rMuc5AC, but rMuc1 expression maintained the same.[13] It has been reported that the secretion of mucin by goblet cells decreases in dry eye syndrome.[14–16] However, it remains unknown whether there is an alteration of MUC1 expression in patients with dry eye.

It has been determined by immunohistochemistry that a mAb KL-6, prepared from a hybridoma cell line using a lung adenocarcinoma cell line, reacts to a sugar moiety of MUC1. However, there is a lack of biochemical data to establish unequivocally the correlation of the KL-6 epitope with the level of MUC1 expression.[17,18] In this report, we identified that the KL-6 epitope was a sialylated sugar moiety of MUC1 and examined its location in the ocular surface epithelium (i.e., cornea and conjunctiva of normal eye bank eyes and patients with dry eye, by immune electron microscopy and impression cytology with mAb KL-6). Our results indicate that KL-6 epitope expression is upregulated in all dry eye cornea and in the mild and moderate dry eye conjunctiva, but downregulated in severe dry eye conjunctiva.

MATERIALS AND METHODS

This study was conducted in compliance with the tenets of the Declaration of Helsinki. Human ocular tissues were obtained from the Northwest Lion Eye Bank (Seattle, WA) and the Cincinnati Eye Bank. The Monoclonal KL-6 antibody, which reacts to the sugar chain antigen of MUC1, was obtained from a mouse hybridoma cell line prepared with a lung adenocarcinoma cell line, as previously described.[17,19]

Western and Immunoprecipitation-Western Immunoblot Analyses

Epithelia from human cornea and conjunctiva were lysed in a buffer solution containing 50 mM Tris-HCl (pH 7.4), 250 mM NaCl, 25 mM NaF, 5 mM EDTA, 0.1% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride (PMSF), and a cocktail of protease inhibitors (Sigma-Aldrich, St. Louis, MO). For immunoprecipitation, lysates were incubated with protein G Sepharose (Amersham Pharmacia Biotech, Piscataway, NJ) for 1 hour to remove the nonspecific proteins that bound to protein G. Supernatants were incubated with 10 ng/mL mAb KL-6 for 2 hours, followed by incubation with protein G Sepharose for 1 hour to precipitate the antigen-antibody complex.

Tissue lysates and immunoprecipitation were then boiled in 0.5 mL 2× SDS gel-loading buffer (100 mM Tris-HCl [pH 6.8], 200 mM dithiothreitol, 4% SDS, 0.2% bromophenol blue, and 20% glycerol). Aliquots (equivalent to 50 μg of original tissue) were subjected to Western blot on 10% SDS-polyacrylamide gels (Bio-Rad, Hercules, CA) and transferred onto polyvinylidene difluoride (PVDF) membrane (Millipore Corp., Bedford, MA) by electroblot. Membranes were treated with or without 0.5 U/mL neuraminidase (Sigma-Aldrich) in 50 mM sodium acetate and 10 mM calcium acetate (pH 5.5) for 1 hour at 37°C. After preincubation with 5% dry milk in TBS (0.15 M NaCl and 50 mM Tris-HCl buffer [pH 7.4]), membranes were incubated with mAb KL-6 (1 ng/mL) or antibodies against tandem repeat antigens: anti-MUC1 (100 ng/mL; Neomarkers, Fremont, CA), anti-MUC4 (200 ng/mL; Santa Cruz Biotechnology, Santa Cruz, CA) or anti-MUC5AC (100 ng/mL; Neomarkers). The membranes were washed three times with TBS and then incubated with the secondary antibody conjugated with horseradish peroxidase (1:10,000 dilution; Vector, Burlingame, CA). Immunoreactivity was visualized with chemiluminescence (ECL plus; Amersham Pharmacia Biotech), according to the manufacturer's protocol.

Immunohistochemical and Immunoelectron Microscopic Study of MUC1 Glycoprotein on the Human Ocular Surface

Five human ocular tissues were fixed overnight in 4% paraformaldehyde, 0.1% glutaraldehyde, and 0.2% picric acid in 0.1 M phosphate buffer solution (pH 7.4), and 50-μm sections were cut on a microslicer (Dosaka EM, Tokyo, Japan). Immunohistochemical analysis was performed on free-floating sections (Vectastain ABC Elite kit; Vector). MUC1 glycoprotein was determined by mAb KL-6. After preincubation in normal horse serum, sections were reacted with the primary antibody (20 ng/mL) for 24 hours at 4°C. After incubation with biotinylated horse anti-mouse IgG (Vector) for 2 hours, sections were incubated with 3% H_2O_2 for 30 minutes, then with streptavidin and biotinylated horseradish peroxidase (Vector) for 90 minutes. The final chromogenic reaction was achieved by incubation in 0.05% 3,3-diaminobenzidine tetrahydrochloride (Sigma-Aldrich) and 0.01% hydrogen peroxide. Ocular sections were fixed with 1% osmium tetroxide for 1 hour, contrasted en bloc with 2% uranyl acetate in 70% alcohol, dehydrated and flat-embedded in Durkupan (Fluka AG, Buchs, Switzerland) on siliconized glass. The ocular sections were analyzed with a light microscope (model AX70; Olympus, Tokyo, Japan) and photographed using 32 ASA black-and-white film (Fuji, Tokyo, Japan).

Corneal, conjunctival, and limbal epithelia were dissected and embedded for preparing ultrathin sections. The sections were further contrasted with lead citrate for electron microscopic examination.

Microslicer sections incubated with normal mouse serum were used as control.

Impression Cytology of Patients with Dry Eye

Impression cytology was performed in 43 eyes from 43 patients with dry eye (average age 61.5 ± 2.0 years, 3 men and 40 women), including 17 patients with Sjögren's syndrome, and in 24 volunteers (average age, 64.0 ± 2.7 years, 2 men and 22 women) with no ocular surface disease. The patients with dry eye were divided into three groups on the basis of dry eye severity (i.e., mild, moderate, and severe, using rose bengal staining).[20,21]

After informed consent, impression cytology was performed as previously reported.[22] Filter membrane (Durapore pore size 0.45 μm; Millipore) was cut into 3 × 8-mm segments and autoclaved before use. After administration of topical anesthesia (0.5% proparacaine hydrochloride; Santen Pharmaceutical, Osaka, Japan), the membrane was placed on the temporal cornea and conjunctiva. Some membranes derived from patients who were first subjected to rose bengal staining were photographed using 100 ASA reversal color film (Fuji Film) before analysis, as described later. The specimens were fixed overnight in 4% paraformaldehyde in 0.1 M phosphate buffer solution (pH 7.4) and subjected to immune reactions as described earlier. The filter membranes were incubated with streptavidin and biotinylated horseradish peroxidase (Vector) for 90 minutes. The final chromogenic reaction was achieved by incubation in diaminobenzidine solution (Histofine; Nichirei, Tokyo, Japan) for 5 minutes at 4°C. The membranes were air dried, mounted in filter membrane mounting medium (Taiho, Tokyo, Japan), and analyzed with a light microscope (model AX70; Olympus). Photographs were taken in the peripheral corneal epithelium 2 mm from the limbus and in the bulbar conjunctival epithelium 3 mm from the limbus. All photographs were taken at the same exposure time using a charge-coupled device camera (model DP 50; Olympus). Immunoprecipitate density of each cell was analyzed with NIH image software (version 1.62, developed by Wayne Rasband the National Institutes of Health and available at http://rsb.info.nih.gov/nih-image/download.html). The filter membranes were also subjected to periodic acid Schiff (PAS) staining as follows: after incubation in 1% periodic acid for 10 minutes, the membranes were reacted in Schiff's reagent for 10 minutes and subsequently incubated three times for 5 minutes each in 2% sodium sulfite. Filters were re-embedded in paraffin and cross-sections were prepared.

RESULTS

Western Blot Analysis

To characterize mAb KL-6, Western immunoblot was performed with tissue extracts of human corneal and bulbar conjunctival epithelium. Figure 1A shows a diffuse pattern of immunoreactivity in a range of molecular weights greater than 200 kDa, as described before.[3,19] Neuraminidase treatment abolished KL-6 immunoreactivity, whereas immunoblots of anti-MUC1, anti-MUC4, and anti-MUC5AC tandem repeat antibodies remained prominent (Fig. 1B). Glycoproteins precipitated by mAb KL-6 reacted with anti-MUC1 antibody, but not anti-MUC4 and anti-MUC5AC tandem repeat antibodies (Fig. 1C). The result suggests that the KL-6 epitope is associated a sialylated epitope on MUC1.

Immunohistochemical and Immunoelectron Microscopic Study of MUC1 Glycoprotein in the Human Ocular Surface

Light Microscopic Examination. In corneal epithelium, mAb KL-6 immunopositive deposits were predominantly found in the plasma membrane of superficial cells (Fig. 2A, arrows), and weak immunoreactivity was also observed in the plasma membrane of wing cells. The mAb KL-6 immune reactivity
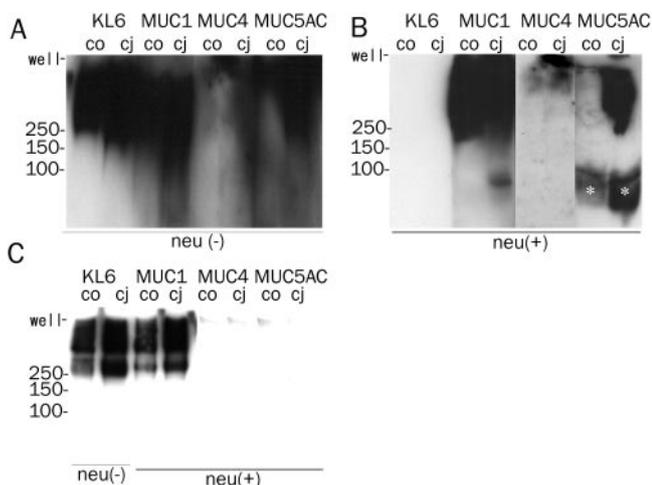
FIGURE 1. Western blot analysis of epithelium of cornea (co) and bulbar conjunctiva (cj) using anti-KL-6, anti-MUC1, MUC4, and MUC5AC antibodies. Tissue extracts were subjected to Western blot in 10% PAGE-SDS, the membranes were treated (A) without and (B) with neuraminidase (neu) before incubation with antibodies. Without neuraminidase treatment, KL-6 antibody and antibodies specifically to the tandem repeats of MUC1, -4, and -5AC reacted to antigens showed broad bands at more than 200 kDa. After neuraminidase treatment, reactivity of mAb KL-6 was abolished, whereas anti-MUCs antibodies still reacted to the antigens. The protein bands (✱) at molecular weights smaller than 100 kDa are probably from nonspecific cross-reactivity of anti-MUC5AC antibody. (C) Immunoprecipitate by mAb KL-6 was reacted by anti-MUC1 tandem repeat antigen antibody, but not by anti-MUC4 or -MUC5AC. well-, bottom level of gel wells.

showed a decreased gradient from superficial cells to wing/suprabasal basal cell layers, but was negative in the basal plasma membrane of basal cells (Fig. 2A, arrowheads). In the bulbar conjunctiva and limbal epithelium, strong mAb KL-6 immunoreactivities were detected in the plasma membrane of superficial and wing cells, whereas the basal plasma membrane of basal cells remained negative (Figs. 2C, 2E).

Electron Microscopic Examination. In the cornea, bulbar conjunctiva and limbus epithelium, KL-6 immunoreactivities were detected in apical plasma membranes of superficial cells (Fig 2B), and in the intercellular space of superficial and wing cells (Figs. 2B, 2D). Weak immune reactivity was also detected in the lateral cell–cell interface (Fig. 2F). No immunopositive staining was observed in the basal plasma membrane of basal cells.

MUC1 Sugar Chain Antigen Expression in Patients with Dry Eye

To examine the possibility that the expression pattern of sialylated antigen may change on the ocular surface of patients with dry eye, impression cytology was performed with mAb KL-6. The patients were divided into three groups: mild dry eye ($n = 19$), moderate dry eye ($n = 17$), and severe dry eye ($n = 7$) based on the score of rose bengal staining.

To examine the feasibility of impression cytology to detect MUC1, filter membranes prepared from normal patients were stained by mAb KL-6 and PAS before preparation of paraffin-embedded sections examined with a microscope. As shown in Figures 3A and 3B, the filter membrane absorbed mucin secreted by goblet cells (Fig. 3A, asterisks), was stained by PAS (arrowheads), and trapped superficial cells (Fig. 3B, arrows) and subsuperficial cells of the conjunctiva (Fig. 3A, arrows). mAb KL-6 immunoreactivity was found in the apical and/or basal plasma membranes (Fig. 3B, arrows) of superficial cells of

the conjunctival epithelium. Figures 3C, 3D, and 3E respectively, show representative patterns of rose bengal, KL-6, and KL-6/PAS staining in conjunctiva use in impression cytology from a patient with moderate dry eye. The rose bengal and PAS staining showed an identical pattern, whereas some rose bengal/PAS–positive cells were not labeled or were weakly labeled by mAb KL-6 (arrowheads).

The corneal epithelium of 13 of 24 non–dry eye volunteers was weakly stained (Fig. 3F) and the other 11 samples showed weak mosaic patterns of the KL-6 epitope stained by the mAb. A mosaic KL-6 pattern was observed in 42 of 43 patients with dry eye examined. Figures 3H, 3J, and 3L showed the representative mosaic patterns in which cells expressing different levels of KL-6 epitope intermingled. The incidence of mosaic KL-6 pattern in corneal epithelium significantly increased in dry eye compared with that in non–dry eye (Fisher's exact $P < 0.0001$).

In the bulbar conjunctival epithelium, many PAS-positive mucin granules secreted by goblet cells were observed in all non–dry eye subjects (Fig. 3G, arrowheads), 7 of 19 patients with mild dry eye had mucin granules (Fig. 3I), and 16 of 17 with moderate dry eye (Fig. 3K) and all with severe dry eye (Fig. 3M) showed a much decreased PAS stain. In comparison, 19 of 24 non–dry eye subjects showed a honeycomb KL-6 staining pattern[16] at the interface of superficial cells, 2 of 24 showed a mosaic pattern, and 3 of 24 had weak staining, whereas the mosaic pattern showed increased incidence in mild (17/19) and moderate (17/17) dry eye (Fisher's exact test, $P < 0.0001$). Figure 3I and 3K showed representative mosaic staining of conjunctiva from mild and moderate dry eye. In contrast, in severe dry eye the number of specimens (3/7) showing mosaic pattern decreased (compare with mild and moderate dry eye: Fisher's exact test, $P = 0.037$), another four specimens had very little expression of the KL-6 epitope and only a few weakly positive cells were visible (Fig. 3M). The observation suggested a reverse correlation of mucin secretion and expression of KL-6 epitope by conjunctival epithelium in mild and moderate dry eye.

To further examine the aforementioned possibility, the immune reactivity of mAb KL-6 was determined in the photographs of impression cytology using the NIH Image analysis program. Mean densities of KL-6–immunopositive deposit on the filter membranes was significantly increased in the cornea in dry eye (Fig. 4A). Conversely, mean densities of mAb KL-6 immunopositive deposit increased in mild and moderate dry eye conjunctiva, but decreased in severe dry eye conjunctiva in comparison with that of mild and moderate dry eye (Fig. 4B).

DISCUSSION

The results of the present study showed that the KL-6 epitope of MUC1 glycoprotein is a silylated sugar moiety. The expression of the KL-6 epitope is upregulated in the corneal epithelium of all dry eye patients, whereas in the bulbar conjunctival epithelium it is upregulated in mild and moderate dry eye, but diminishes in severe dry eye. Inatomi et al.[3] showed MUC1 core protein expression by human corneal and conjunctival epithelial cells using in situ hybridization and immunofluorescence. Our data of Western analysis and immunoelectron microscopy data are consistent with the notion that both corneal and conjunctival epithelial cells synthesize MUC1 (Figs. 1, 2). Western blot analysis, as shown in Figure 1, unequivocally established that KL-6 epitope indeed associates with MUC1. Use of mAb KL-6 has an advantage in identifying the presence of MUC1 in situ because of its ability to react the exposed sialylated antigen.

Immunoelectron microscopy detected the KL-6 epitope in the cell membrane of superficial cells and intercellular spaces.
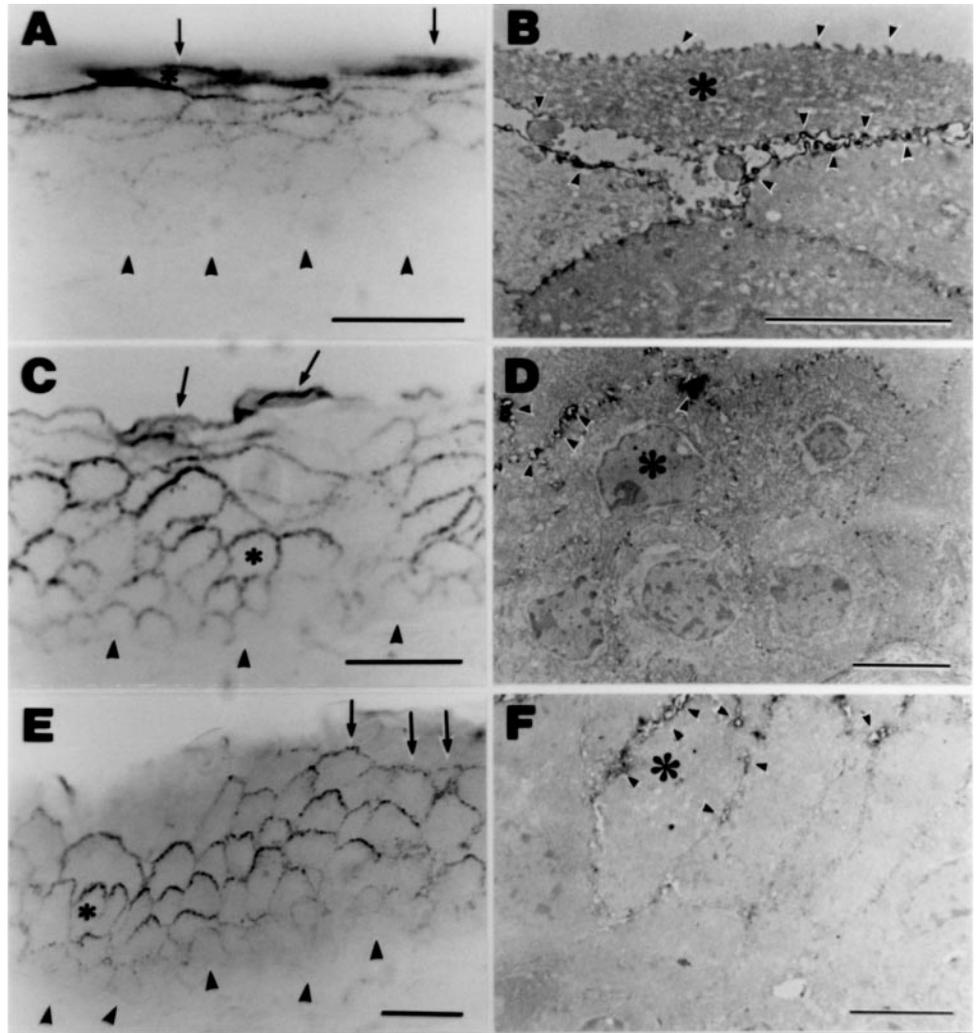
FIGURE 2. Immunohistochemical study using mAb KL-6 in human cornea (**A**, **B**), limbus (**C**, **D**), and bulbar conjunctiva (**E**, **F**). Light micrographs of 50-μm sections (**A**, **C**, **E**) and electron micrographs (**B**, **D**, **F**) are shown. (✽) Same cells of individual specimens examined by both light and electron microscopy. In the cornea, dense KL-6–immunopositive deposits were observed in apical and basal cell membranes of superficial cells (**A**, *arrows*). Weak positive deposits were observed in cell membrane of wing cells and basal cells, except for the basal membrane of basal cells (**A**, *arrowheads*). Corneal electron micrograph shows superficial and subsuperficial cell membrane stained by mAb KL-6 (**B**, *arrowheads*). In limbus and bulbar conjunctiva, superficial (**C**, **E**, *arrows*) and wing cell membranes had immunopositive deposits, expressed in the intercellular space (**D**, **F**, *arrowheads*). Apical membranes of basal cells were weakly stained, and no immunopositive deposits were found in basal membrane (**C**, **E**, *arrowheads*). Scale bars: (**A**, **C**, **E**) 20 μm; (**B**, **D**, **F**) 5 μm.

It has been suggested that in addition to its involvement in tear film formation on the ocular surface, MUC1 may have other functions attributable to its unique hydrophilic properties similar to that of MUC4. MUC1 and -4 distributions are similar[8] and may share some common functions. For example, transfection of MUC1 and -4 suppressed cell aggregation of cultured cells.[23–25] It has also been demonstrated that MUC1 antisense oligonucleotides induce cell–cell and cell–surface adhesion in breast cancer cells.[26,27] Therefore, intercellular MUC1 may play a role in modulating cell mobility and cell migration by suppressing cell–cell adhesion.

Our results with the mAb KL-6 suggest that in severe dry eye the formation of the sialylated sugar moiety may be compromised. Garcher et al.[28] reported that the immune reactivity of mAb CA19-9 against a glycoprotein of malignant tumor cells was significantly decreased in the bulbar conjunctival epithelium in dry eye patients. It is also known that MUC5AC is primarily secreted by goblet cells, whose synthesis by conjunctival epithelium is impaired in dry eye. Thus, it is likely that the upregulation of the KL-6 epitope derives from increased MUC1 core protein expression by conjunctival epithelial cells of mild and moderate dry eye patients and by dry eye corneal epithelium. This increased expression may result from altered gene expression and/or differentiation of conjunctival epithelial cells.

In most normal bulbar conjunctiva, the mAb KL-6 staining showed a honeycomb pattern representing the hexagonal shape of terminal differentiated superficial cells. This observa-

tion is consistent with the finding that the KL-6 epitope is also present in the lateral intercellular space (Fig. 2). It is possible that the pathogenesis of mild and moderate dry eye may result in the alteration of terminal differentiation and perturbation of the hexagonal shape of superficial conjunctival cells,[29,30] which can in part explain the mosaic pattern of the KL-6 epitope (Figs. 3I, 3K). In severe dry eye, the expression of the KL-6 epitope by the conjunctiva was suppressed (Fig. 3M).

In dry eye, ocular surface epithelial tissue is under increased mechanical stress due to the loss of mucin, which serves as a lubricant, and the stress causes accelerated peeling off of epithelial cells. Therefore, cell division and cell migration may increase to accommodate the increased loss of superficial cells. The condition may stimulate the upregulation of MUC1 core protein and lead to an increase in the KL-6 epitope in mild and moderate dry eye. However, in the bulbar conjunctival surface epithelium of severe dry eye, expression of the KL-6 antigen of MUC1 glycoprotein was suppressed, implicating advanced perturbation on ocular surface epithelium differentiation. Excessive stimulation and stress to the bulbar conjunctival epithelium may perturb conjunctival epithelial differentiation. This suggestion is supported by our observation of moderate dry eye in which rose bengal–positive superficial cells[31–33] were MUC1 glycoprotein hypoexpressive and PAS-staining positive, implicating the existence of large amounts of other glycoproteins in the corneal epithelium (Figs. 3C–E). In contrast, the mosaic KL-6 expression pattern in dry eye cornea may be due to upregulated, but uneven, MUC1 expression. However, this
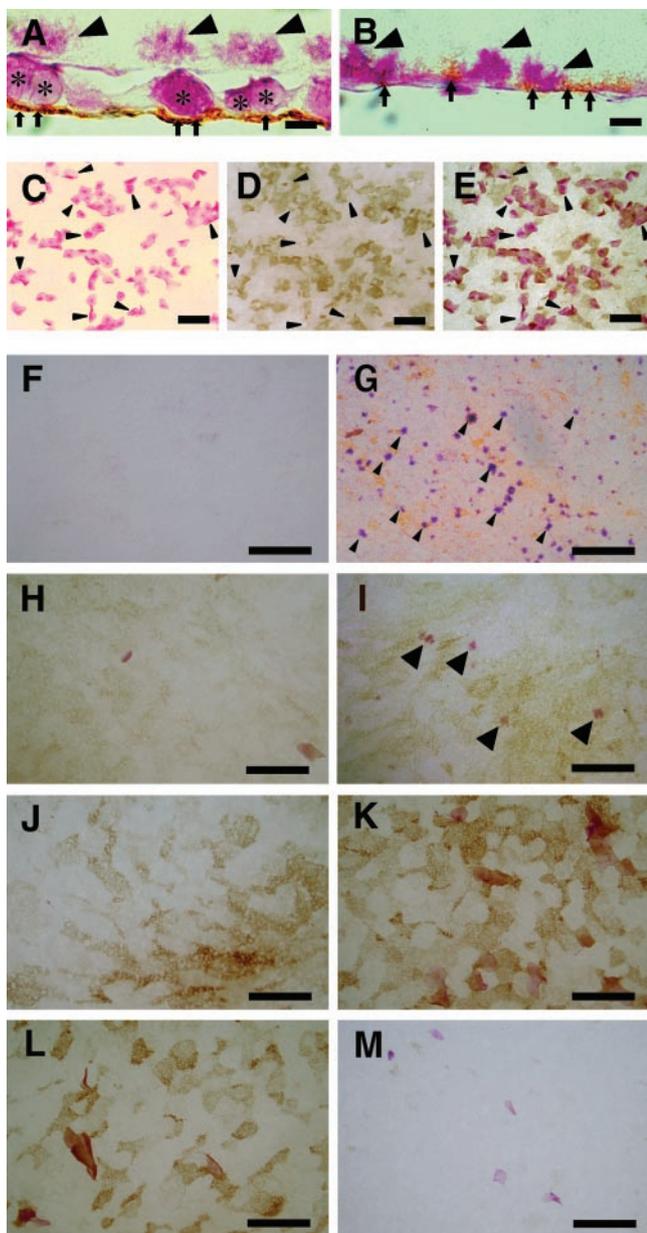
MUC1 upregulation is insufficient to return toward normal the shortened tear film break up time observed in dry eye in which the expression of other mucins is severely compromised.[29,30,34] For example, Danjo et al.[35] showed mucinlike glycoprotein alteration in dry eye. Using mAb H185 staining pattern, it was demonstrated that superficial cell mucin and

FIGURE 3. MUC1 glycoprotein expression in non–dry eye and dry eye. Filter membranes prepared by impression cytology were stained with PAS and the mAb KL-6. Sections (5 μm) were prepared from filters of non–dry eye conjunctiva (**A**, **B**). The membrane filter primarily picked up superficial cells (**B**, *arrows*) and absorbed secreted mucin (**A**, **B**, *arrowheads*), and occasionally whole goblet cells (**A**, **✱**). Note that the mAb KL-6 did not label goblet cell mucin. Rose bengal–stained bulbar conjunctival epithelia (**C**, *small arrowheads*) in moderate dry eye are weakly stained by mAb KL-6 (**D**), but were still PAS positive (**E**). Non–dry eye corneal epithelium (**F**) was weakly stained by the mAb KL-6. A prominent mosaic pattern of KL-6 was observed in the corneal epithelium of moderate (**J**) and severe (**L**) dry eye and a weak mosaic pattern of KL-6 was observed in mild dry eye (**H**). In bulbar conjunctival epithelium, a honeycomb pattern was seen in non–dry eye (**G**); a mosaic pattern without strong positive cells in mild dry eye (**I**); a mosaic pattern with strong positive cells in moderate dry eye (**K**); and only a few weak positive cells in severe dry eye (**M**). PAS-positive mucin secreted by goblet cells was observed in non–dry eye (**G**), only a few in mild dry eye (**I**), and none in moderate (**K**) and severe (**M**) dry eye. Scale bars: (**A**, **B**) 10 μm; (**C**-**M**) 100 μm.
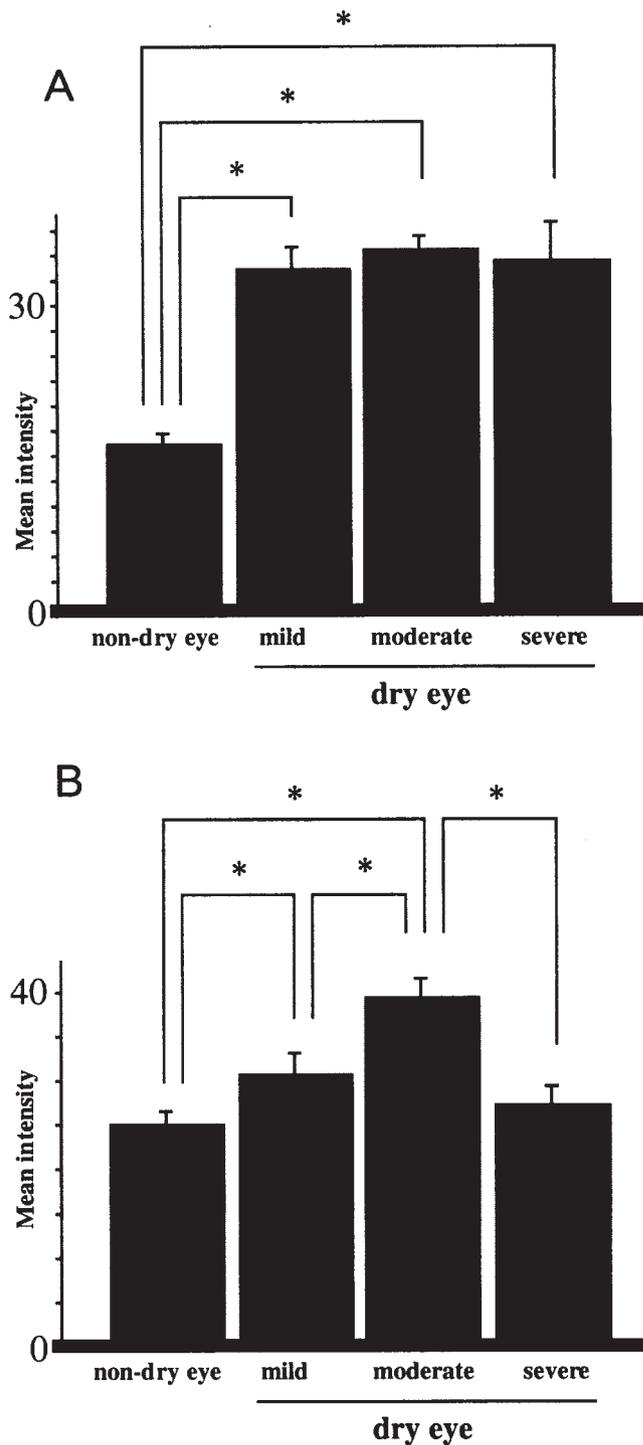
FIGURE 4. Histogram of KL-6–positive cells. Densities of KL-6 immune reactivity on filter membranes were measured by NIH Image. Densities in the temporal cornea were significantly increased in dry eye (**A**). In contrast, mean KL-6–immunopositive deposit densities increased in mild and moderate dry eye conjunctiva, but did not significantly increase in severe dry eye conjunctiva (**B**). (*$P < 0.05$, unpaired t-test).

goblet cell mucin glycosylation patterns were altered in severe dry eye. In our present studies of severe dry eye, we did not detect goblet cell mucin secretions. The reasons for this discrepancy are not known. It is possible, however, that the differences may arise from the small number of patients examined and/or the duration of the disease.

Taken together, our results demonstrate that the decrease of PAS staining of mucin secreted by goblet cells is accompanied by an increase of KL-6 epitope expression in conjunctiva of mild and moderate dry eyes. It is likely that MUC1 may be upregulated as evidenced by the increase in KL-6 immune activities, which may partially compensate for the decreased MUC5AC secretion by goblet cells and alleviate the dry eye symptoms. However, in severe dry eye, the expression of the KL-6 epitope diminished in the bulbar conjunctiva, implicating the advanced loss of the conjunctival epithelium's ability to secrete mucin.

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