Sjögren’s Syndrome: Cytokine and Epstein–Barr Viral Gene Expression Within the Conjunctival Epithelium

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Purpose. In primary Sjögren’s syndrome (SS), ocular surface changes within the conjunctival epithelium include lymphocytic infiltration, squamous cell metaplasia, and a reduction in goblet cell number. These changes may be the simple result of increased mechanical abrasion secondary to dryness. Alternatively, they may represent a local response to ocular and/or systemic inflammatory processes, perhaps in response to Epstein–Barr viral (EBV) infection, an agent recently implicated in the etiology of SS. To determine whether inflammatory processes or local infection by EBV contribute to the ocular surface pathology of SS, we examined the expression of inflammatory cell surface markers, cytokines, and EBV gene products within the ocular conjunctiva of patients with SS.

Methods. Ocular conjunctival tissue was isolated from patients with primary SS and nondry eye control patients by impression cytology or direct biopsy. These specimens were examined by immunofluorescence microscopy and reverse-transcriptase polymerase chain reaction (RT-PCR) for the expression of various markers.

Results. The authors found the frequency of expression of HLA-DR (P < 0.0001), ICAM-1 (P < 0.035), and IL-6 (P < 0.0001) to be significantly elevated in patients with primary SS versus nondry eye control patients. The IL-2 receptor and cytokines IL-1/β and IL-8 were each found to be expressed with relatively high frequency in both patient populations, whereas mRNAs encoding cytokines IL-2, IFN-γ, GM-CSF, and TGF-β were not reproducibly detectable in either population. Messenger RNA encoding a marker for passive-latent EBV infection (EBNA-1) was detected with high frequency in both SS and normal populations. The EBV IL-10 analog BCRF-1 was expressed with low frequency in the SS population; however, these levels were not significantly different from the control population. The expression of two other markers of EBV infection, latent membrane protein (LMP, a lytic and latent marker), and BZLF-1 (putative latent–lytic switch gene) was undetectable in either study population.

Conclusion. Based on the increased expression of the cell surface molecules HLA-DR and ICAM-1, and the inflammatory cytokine IL-6, the authors propose that local inflammatory processes contribute to the ocular surface changes and ocular surface dryness associated with primary SS. Invest Ophthalmol Vis Sci. 1994;35:3493–3504.
struction of their normal secretory function with resultant dryness of the mouth and eyes.\(^5\),\(^4\)

Although the immunopathology of the lacrimal gland in SS has received considerable attention, little emphasis has been placed on those pathologic changes occurring on the ocular surface. Irritation and dryness of the ocular surface has traditionally been attributed to progressive diminution of lacrimal gland function with decreased aqueous tear production and concomitant desiccation and mechanical abrasion of the corneal and conjunctival surfaces.\(^5\) Several characteristic changes have been identified in the conjunctival epithelia of patients with Sjögren’s syndrome, including: a reduction in goblet cell number,\(^5\) squamous metaplasia\(^5\) (epithelial cells display an increased cytoplasmic–nuclear ratio), and lymphocytic infiltration (predominantly T cell).\(^5\),\(^6\) Although the etiology of these ocular surface changes is unclear, a mechanical etiology is supported by a reduction in the aqueous and mucinous components of the precorneal tear film secondary to lacrimal gland destruction and diminution of the number of mucin-producing goblet cells. In addition, the autoimmune nature of the disease, combined with the infiltration of immune cells into both the conjunctiva and the lacrimal gland, support the notion that inflammatory processes play an important contributory role. Recently, Epstein–Barr virus (EBV) has been implicated as an etiologic agent of the epithelial pathology and B cell lymphoproliferation found in SS lacrimal glands. This is based upon the identification of lytic and latent phase EBV antigens and EBV genomic DNA within the lacrimal glands of patients with SS.\(^3\),\(^7\)-\(^10\) Epstein–Barr virus genomic DNA has also been detected in the tears of patients with SS\(^10\) implying that inflammatory and/or infectious processes occurring in the lacrimal gland may impact directly upon the ocular surface.

The normal precorneal tear film comprises a complex mixture of lipids, polysaccharides, and proteins that continuously bathe the ocular surface.\(^11\) The lipid component slows aqueous evaporation preventing desiccation of the underlying epithelium. The heterogeneous proteinaceous component contains mucinous proteoglycans, antibacterial agents (i.e., lactoferrin, lysozyme), growth factors (i.e., epidermal growth factor), and additional factors that have been poorly defined.\(^11\) Some of the lacrimal gland-derived factors present in the precorneal tear film may play a role in regulating normal conjunctival epithelial differentiation or in maintaining the fully differentiated epithelial surface. If this is case, inflammation of the lacrimal gland and/or ocular surface may lead to the anomalous production of secretory growth factors or cytokines that modulate gene expression within the conjunctival epithelium, perhaps leading to an altered cellular phenotype. In SS, the detection of such factors in the tear fluid is extremely difficult owing to the markedly reduced tear production of this disease. Nevertheless, indirect detection of such agents within the tear film may be accomplished by evaluating their effects upon downstream gene expression in the conjunctival epithelium. Inflammatory mediators such as interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) are known to induce the expression of cell surface markers such as class II HLA (HLA-DR) and intercellular adhesion molecule-1 (ICAM-1) in a variety of cell types, including epithelial cells and keratinocytes.\(^12\)-\(^14\) Moreover, cytokines produced early in the immune response frequently induce the expression of other cytokines that are downstream in the inflammatory cascade. To investigate the possibility that inflammatory processes contribute to the ocular surface changes found in SS, we compared the expression of specific cytokine and cell surface markers (class II HLA [HLA-DR], ICAM-1, interleukin-1β [IL-1β], IL-2, IL-2 receptor [IL-2R], IL-6, IL-8, IFN-γ, granulocyte–monocyte colony stimulating factor [GM-CSF] and transforming growth factor [TGF-β]) in the ocular conjunctivae of patients with SS and normal (non-dry eye) patients.

In addition, to investigate further the putative role of EBV as an etiologic agent of SS, we evaluated the expression of EBV gene products representing different stages of EBV infection (Epstein–Barr nuclear antigen-1 [EBNA-1], late membrane protein [LMP], BCRF-1, and BZLF-1) in similar populations of SS and normal patients.

**MATERIALS AND METHODS**

**Materials**

Impression cytology was performed with nitrocellulose acetate (HAWP 304 FB, Millipore, Bedford, MA) or Biopore membranes (Millipore) Reverse transcriptase–PCR kits were purchased from Perkin–Elmer Cetus (Norwalk, CT). All oligonucleotide PCR primers and oligonucleotide hybridization probes were based on human sequences and were obtained from Clontech (human cytokine MAPping Amplimer Sets, Palo Alto, CA) for IL-1β,\(^15\) IL-2,\(^16\) IL-2R,\(^17\) IL-6,\(^18\),\(^19\) IL-8,\(^20\) IFN-γ,\(^21\) TGF-β,\(^22\) and GM-CSF\(^23\) (original references cited) or were synthesized de novo (Applied Biosystems automated DNA–RNA synthesizer model 394) for G\(_\omega\),\(^24\) IL-6,\(^18\),\(^19\) ICAM-1,\(^25\) EBV-LMP,\(^25\) EBV-EBNA-1,\(^27\) EBV-BCRF-1,\(^27\) and EBV-BZLF-1.\(^27\) The oligonucleotide sequences of PCR primers used in this study are indicated in Table 1. Southern hybridizations were performed with HyBond nylon membranes (Amer sham, Arlington Heights, IL) and the lumiphos 550 nonradioactive detection system (Boehringer–Mannheim, Indianapolis, IN). The polyclonal rabbit anti-human IL-6 antiserum was from Genzyme (Cam-
were applied to the inferior bulbar conjunctiva several times. AM-1 antisera were obtained from Becton-Dickinson (Mountainview, CA) and Dr. Timothy Springer (Harvard Medical School, Boston, MA), respectively. Reagents were of reagent grade quality.

The DNA sequences of the primers utilized for RT-PCR amplification are depicted. Orientations (5'-primer and 3'-primer) refer to the mRNA sequence. The predicted genomic and cDNA PCR amplified DNA product sizes are also indicated.

* The genomic sequence contains introns but has not been fully characterized and no product is obtained by PCR amplification of genomic DNA.

bridge, MA), and the FITC-conjugated goat antimouse and goat anti-rabbit antisera were from Sigma (St. Louis, MO). Mouse anti-human HLA-DR and ICAM-1 antisera were obtained from Becton-Dickinson (Mountainview, CA) and Dr. Timothy Springer (Harvard Medical School, Boston, MA), respectively. Recombinant IL-6 was purchased from BRL (Gaithersburg, MD). All other supplies were obtained commercially and were of reagent grade quality.

Conjunctival Impression Cytology and Biopsy

This research was conducted by medically qualified personnel in strict accordance with the University of Miami School of Medicine Institutional Review Board in accordance with the tenets of the Declaration of Helsinki. Informed consent was obtained after the nature and possible consequences of the study were explained. Conjunctival biopsy tissue for immunofluorescence analysis was obtained from the medial bulbar conjunctiva directly for PCR amplification under the following conditions: initial denaturation —2 min at 94°C, 5 minute Schirmer test without anesthesia ^ 5 mm of wetting, resulting strip wetting, interpalpebral conjunctival plus corneal Rose-Bengal staining, serum autoantibody titers (rheumatoid factor ^ 1:160 and/or ANA ^ 1:160), and squamous metaplasia of the bulbar conjunctival epithelium noted by impression cytology. None of the patients in this study received systemic immunosuppressive therapy.

Isolation of RNA, cDNA Synthesis, and PCR Amplification

Impression cytology samples from the inferior bulbar conjunctiva were placed immediately in denaturing guanidinium solution, vortexed, and either frozen at −20°C for up to 1 month or used immediately for RNA isolation as described by Chomczynski and Sacchi. First-strand cDNA was synthesized from one-third of the total RNA isolated from each impression cytology sample in a 20-µl reaction mixture as directed by the manufacturer (Perkin—Elmer Cetus). After cDNA synthesis, each entire first-strand cDNA reaction mixture was diluted fivefold (100 µl final) and used directly for PCR amplification under the following conditions: 5 µl diluted first-strand cDNA reaction mixture, 1 µM 5'-oligonucleotide primer, 1 µM 3'-oligonucleotide primer, 100 µM each dATP, dGTP, dCTP, dTTP, 1.25 units Taq polymerase, 2 mM MgCl₂, 10 mM Tris-HCl pH 8.3, and 50 mM KCl in a 50-µl reaction. PCR amplification was performed using a Perkin—Elmer Cetus DNA Thermal Cycler under the following conditions: initial denaturation —2 min at
95°C, one cycle; amplification —1 minute at 60°C, 1 minute at 72°C, 45 seconds at 95°C, 50 cycles (35 cycles for G<sub>sa</sub>); strand extension —1 minute at 60°C, 10 minutes at 72°C. Positive control template DNA was obtained from Clontech (IL-1β, IL-2, IL-2R, IL-6, IL-8, IFN-γ, TGF-β, and GM-CSF), amplified from human peripheral blood mononuclear cells (DTJ; ICAM-1, G<sub>sa</sub>) or amplified from EBV infected Raji or B-95<sup>1</sup> cells (EBNA-1, LMP, BCRF-1, and BZLF-1; cell line catalog #CCL86 and #1612CRL, respectively, ATCC, Rockville, MD). Distilled water was substituted for template DNA in the negative control samples. After amplification, reaction mixtures were extracted twice with ether, concentrated by vacuum centrifugation to 15 to 25 μl, and the entire reaction was loaded into 15% agarose gels and resolved by electrophoresis. The cDNA and predicted genomic sizes are indicated in Figure 1 (IL-1β, IL-2, IL-2R, IL-6, IL-8, IFN-γ, TGF-β, and GM-CSF provided by Clontech; ICAM-1, G<sub>sa</sub>) and Genbank, G<sub>sa</sub> (25) and LMP, EBNA-1, BZLF-1 and BCRF-1 (20,27). Amplified material was visualized by ethidium bromide staining and recorded with Polaroid photography. The results were interpreted and quantified by visual inspection by two independent observers (DTJ and SCP). Each PCR amplified specimen (representing each eye separately) was scored independently and was considered positive if a PCR product was visually detectable with the unaided eye by ethidium bromide staining. Scores were averaged in rare cases of minor discrepancy, and data from the two patient populations were analyzed for statistical significance with Fisher's exact test.

Pilot studies using serial dilutions of previously cloned G<sub>sa</sub> template DNA at 35 and 50 cycles of amplification indicated a dynamic working range of six logs (10 fg to 10 ng) over which qualitative differences in the amount of starting template DNA were detectable by this assay system (data not shown). Minor variations were observed in the levels of G<sub>sa</sub> DNA amplified from patient samples at both 35 cycles (data not shown) and 50 cycles (Fig. 2) of amplification. Although the results presented in this article are not rigorously quantitative, these studies indicate that levels of initial template cDNA obtained from the impression cytology patient samples were within the dynamic operating range of the assay system. Large differences in the amount of starting template, indicative of variability in the amount of tissue isolated by impression cytology or in the yield of the RNA/cDNA preparation, were not apparent in these assays with G<sub>sa</sub>.

**Southern Analyses**

RT-PCR products were resolved on agarose gels, transferred to nylon membranes under alkaline conditions, and probed with specific oligonucleotides or cDNA sequences by chemiluminescent, nonradioactive detection methods as described by the manufacturer (Boehringer–Mannheim). Hybridization conditions were as follows: prehybridization in buffer A (5 × SSC [1 × SSC = 150 mM sodium chloride, 15 mM sodium citrate], 1% blocking reagent, 0.1% N-lauroylsarcos-
FIGURE 2. RT-PCR agarose gel electrophoresis. Representative panels of RT-PCR products resolved on agarose gels and stained with ethidium bromide. The mRNA used for amplification was isolated from impression cytology samples of normal patients and patients with SS. (a) IL-2, (b) IL-2 receptor, (c) IL-6, (d) Gm. Predicted RT-PCR product sizes are indicated on the right of each gel panel. OD = Right eye; OS = left eye. For Gm, the predicted PCR amplified product sizes are as follows: mRNA (cDNA) = 435 bp, genomic DNA = 915 bp.

Note: For Gm, the results for patients 1 and 2 with are not presented in Figure 2 but were similar to the others shown, and no detectable material was amplified from patient 5 with SS for Gm. This sample was not included in any of the statistical calculations of this study.
cells of the patient with SS. Altogether, comparison of 8 normal patients and 11 patients with SS revealed that both HLA-DR and ICAM-1 were expressed at significantly elevated levels by conjunctival epithelial tissue of the SS population compared with the normal population (HLA-DR, \( P < 0.001 \); ICAM-1, \( P < 0.035 \)). These studies indicate that conjunctival epithelial cells of patients with SS exhibit increased expression of these two different cell surface markers of the inflammatory response.

**PCR Evaluation of Cytokine and ICAM-1 Expression**

We evaluated the expression of a panel of cytokines in the ocular conjunctiva of 10 patients with SS versus 11 normal patients by RT-PCR. To ensure that mRNA was indeed isolated from each impression cytology sample and that cDNA and not genomic DNA was amplified by RT-PCR, each impression cytology sample was first analyzed for mRNA encoding the \( \alpha \) subunit of the stimulatory GTP-binding protein, \( G_s \), using oligonucleotide primers designed to span several introns. \( G_s \) was chosen because it is expressed ubiquitously \( ^{29,30} \) and at a moderate messenger copy number per cell (DTJ, unpublished observation, 1988). Amplification of bona fide \( G_s \) cDNA versus genomic DNA was verified by PCR product size (Fig. 2d) and Southern hybridization analysis with a \( G_s \) specific cDNA probe (data not shown). These studies demonstrate the vast majority of RT-PCR amplified material originated from \( G_s \) cDNA and not genomic DNA. Moreover, detectable levels of \( G_s \) mRNA were present in all but one of the samples (this sample was not included in any statistical analyses but is presented for completeness), and the variability of mRNA recovery between impression cytology samples was small because roughly comparable amounts of \( G_s \) cDNA were amplified from each impression cytology sample (based on ethidium bromide staining).

We next compared the expression of several different inflammatory cytokines between the two patient populations. For each cytokine, we anticipated one of three possible scenarios: The marker would be expressed with similar frequency in both patient populations; the marker would not be expressed in either population; or the marker would exhibit differential expression with elevated levels in one population and reduced or undetectable levels in the other. The first two scenarios reveal nothing about the role of the given cytokine in SS. In contrast, results consistent with the third scenario suggest that a given cytokine is positively or negatively associated with the SS disease process. Representative examples of each situation are presented in Figure 2. Interleukin-2 was expressed at undetectable levels in both populations (by ethidium bromide staining [Fig. 2a] and Southern hybridization...
More important, these studies indicate the frequency in the SS population was confirmed by PCR product size and Southern hybridization. The authenticity of the ICAM-1 RT-PCR product for each product with specific cDNA or oligonucleotide probes (data not shown). The greater frequency of ICAM-1 expression found in normal patients by RT-PCR as compared with immunofluorescence is not surprising considering the greater sensitivity of PCR compared with immunofluorescence data presented in Figure 2.

The authenticity of the amplified EBNA-1 product was confirmed by PCR product size and Southern hybridization analyses with an EBNA-1 specific oligonucleotide probe (data not shown). Altogether, these studies do not support latent or lytic infection of the ocular surface as an etiologic factor in the ocular surface pathology of primary SS. However, they do indicate the majority of patients in this study harbor latent EBV infection of the conjunctival epithelium.

**DISCUSSION**

In primary SS, the conjunctival epithelium undergoes several pathologic changes including squamous meta-
Impression Cytology

A  Non-Immune Serum  IL-6 (10x)  IL-6 (40x)

Conjunctival Tissue Sections

Phase Immunofluorescence

Normal  Sjögren's

FIGURE 3. IL-6 Immunofluorescence. (a) Representative immunofluorescence analysis of an impression cytology sample from a patient with SS stained with nonimmune or IL-6 specific antiserum. Powers of magnification were as indicated (nonimmune serum was x10). (b) Comparison of phase-contrast and immunofluorescence for representative conjunctival biopsy samples from a normal patient and a patient with SS stained with IL-6 specific antiserum. Preadsorption of the IL-6 specific antiserum with recombinant IL-6 effectively eliminated the immunofluorescent signal observed in the SS samples (data not shown).

TABLE 3. Summary of RT-PCR Amplification for EBV Gene Products

<table>
<thead>
<tr>
<th>PCR Product</th>
<th>Normal (n = 22) (%)</th>
<th>SS (n = 20) (%)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMP</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>EBNA-1</td>
<td>95</td>
<td>75</td>
<td>NS (P = 0.087)</td>
</tr>
<tr>
<td>BCRF-1</td>
<td>0</td>
<td>5</td>
<td>NS (P = 0.48)</td>
</tr>
<tr>
<td>BZLF-1</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
</tbody>
</table>

Impression cytology samples from 11 normal (22 eyes) and ten SS (20 eyes) patients were analyzed for cytokine mRNA expression by RT-PCR. NS = not statistically significant. Results are summarized from data similar to that depicted in Figure 2. For statistical purposes each eye was scored independently.

* P-values were derived by comparing the two patient populations using Fisher’s exact test.

Although the etiology of these changes has not been established, several mechanisms have been proposed, including local vitamin A deficiency, mechanical trauma due to the markedly reduced preocular aqueous and mucinous tear layer, elevated tear film osmolarity, local infection with Epstein–Barr virus, and inflammation. This study was undertaken to address the latter two hypotheses. Our findings of aberrant expression of class II HLA antigens (HLA-DR), ICAM-1, and the inflammatory cytokine IL-6 in the conjunctival epithelia of patients with primary SS imply that, in addition to mechanical trauma, immunologic processes may contribute to the pathologic changes occurring on the ocular surface in this disease.
Cell surface molecules, including cell adhesion molecules and antigen presenting molecules (HLA), mediate specific cell–cell interaction and recognition that is used by the immune system for precise immunoregulatory control. In this way, the destructive capacity of the inflammatory response is limited and directed to areas of foreign invasion. We report here that two cell surface markers of the inflammatory response, HLA-DR and ICAM-1, are expressed with greater frequency in the conjunctival epithelium of patients with primary SS compared with normal controls. These two markers of the inflammatory response are not normally expressed by cells of epithelial origin, but their expression can be induced by a variety of inflammatory mediators including IL-1, IL-3, IL-6, GM-CSF, IFN-γ, and TNF-α.12–14 The elevated expression of these two markers in primary SS supports the notion that the ocular surface epithelium is exposed to locally or distantly produced mediators of the inflammatory cascade. Although IFN-γ is the principal mediator known to induce the expression of both these markers, we were unable to detect local expression of IFN-γ mRNA in conjunctival epithelium from either normal patients or patients with SS. Recently, it has been shown that inflamed lacrimal glands of patients with SS produce IFN-γ at levels significantly greater than normal patients.38 This implies that increased levels of HLA-DR and ICAM-1 found in the conjunctiva of primary patients with SS may represent a response to lacrimaly derived IFN-γ. This hypothesis is difficult to test, however, because the markedly reduced tear output of patients with SS makes it virtually impossible to collect a sufficient quantity of tears to perform assays to detect inflammatory cytokines.

Tissue sampling by impression cytology is potentially limited because this technique removes only the superficial layers of the conjunctiva.38 In addition to demonstrating alterations in gene expression, these immunofluorescence studies validate the use of impression cytology as a less invasive means of studying the conjunctiva of patients with SS because similar results were obtained with both impression cytology and full-thickness biopsy specimens.

Primary and secondary SS are known to be associated with particular HLA serotypes (HLA-DR3 and HLA-DR4, respectively).39 Although the patients in this study all conformed to a diagnosis of primary SS, the HLA-DR antiserum used in this study did not permit discrimination between these two subtypes. As a consequence, it was not possible to determine the subtype of HLA-DR upregulated in the patients with primary SS presented in this study.

We have demonstrated the inflammatory cytokine IL-6 is expressed with greater frequency at both the mRNA and protein level in the conjunctival epithelium of patients with primary SS. Various agents are known to induce IL-6 expression including IL-1, IL-2, IL-4, IL-6, TNF-α, IFN-γ, platelet-derived growth factor, bacterial endotoxin, and several viruses (including herpes viruses).40 Moreover, in response to the appropriate signal, a number of different cell types have been shown to produce IL-6, including endothelial cells, fibroblasts, B lymphocytes, and keratinocytes.40,41 Functionally, IL-6 is a pleitropic immunologic mediator with functions that overlap to some extent with cytokines IL-1 and TNF-α.32,42 The properties of IL-6 include pyrogenic and antiviral activity, stimulation of B cell antibody production, induction of cytotoxic T cell differentiation, and stimulation of keratinocyte proliferation. In addition, depending on the specific cell type, IL-6 may enhance, have no effect, or inhibit the differentiation of epithelial cells.44 Interleukin-6 also functions as an autoimmune growth factor for EBV-immortalized B cells.45,46 Interleukin-6 is known to effect changes in gene expression via a membrane-bound receptor second messenger cascade coupled to a specific nuclear transcription factor (nuclear factor IL6).47,48 It has recently been reported that IL-6 increases ICAM-1 expression in dermal dendritic cells and endothelial cells of human foreskin cultures.49 Although we have found increased expression of both IL-6 and ICAM-1 by conjunctival epithelial cells in primary SS, the precise role of IL-6 in the ocular immunopathology of SS is unclear. Perhaps IL-6 is produced in response to lacrimally derived IFN-γ present in the tears of patients with SS. This combination of cytokines may lead to the induction of cell surface molecules (i.e., ICAM-1) and the associated lymphocytic infiltration of the conjunctival epithelium observed in SS. Alternatively, IL-6 may inhibit maturation of conjunctival epithelial cells leading to the hyperplastic state found in SS (see below).

The immunopathologic changes in the conjunctival epithelium of primary SS bear similarities to those previously described in the epidermal plaques of psoriasis. Elevated expression of IL-6 mRNA and protein has been detected in the suprabasal layers of psoriatic epidermal plaques where it has been postulated to serve as an autocrine growth factor for keratinocyte growth.30 It is possible that IL-6 plays a similar role as an autocrine growth factor in the conjunctival epithelium of SS. In this study, we demonstrate that IL-6 expression is greatest in the superficial layers of the conjunctiva where it may play a role in modulating normal cellular maturation. Recently, we have noted a marked increase in the number of epithelial cell layers in bulbar conjunctival biopsies of primary patients with SS and found expression of the hyperproliferation associated cytokeratin K16 in a significantly greater number of conjunctival epithelial specimens from primary patients with SS versus normal control.
patients (manuscript in preparation). Clinical and cyto-
tologic data suggest there is a lack of normal terminal
differentiation of the conjunctival epithelium in SS.
This is based upon intense rose bengal staining of
the ocular surface consistent with an absence of the
normal mucin coating as well as the marked reduction
in goblet cell number. It has recently been suggested
that the mucin layer may be produced, in part, by
terminally differentiated epithelial cells of the con-
junctiva. The role of IL-6 in these processes, if any,
is not known at present. The combination of elevated
IL-6 levels and the capacity of IL-6 to perhaps induce
epithelial and keratinocyte proliferation while inhib-
iting maturation suggest that IL-6 may be contribut-
ing to the hyperproliferative state of the conjunctiva
in primary SS. Future research is required to determine
whether IL-6 may be contributing to these pathologic
changes.

We also evaluated the hypothesis that EBV infec-
tion of the conjunctival epithelium contributes to the
ocular surface pathology of primary SS. Epstein–Barr
virus is known to be associated with an epithelial hy-
gerplastic condition of the lateral aspect of the tongue
termed oral hairy leukoplakia where elevated expres-
sion of lytic-cycle EBV antigens is found in the super-
facial layers of tongue epithelium. Interestingly, we
found evidence of EBNA-1 mRNA expression in im-
pression cytology specimens from both normal and
SS conjunctiva. These results imply that passive–latent
infection of the conjunctiva occurs in most individu-
als. Alternatively, the EBNA-1 template mRNA may
be derived from EBV infected B cells present in the
superficial layers of the conjunctival epithelium. His-
to logical studies indicate that B lymphocytes reside pri-
marily in the basal layers of the conjunctiva, whereas
expression cytology removes the superficial layers
and is less likely to contain B lymphocytes. In contrast
to EBNA-1, we were unable to detect significant differ-
ces in the expression of any active-latent or lytic
cycle markers in our patient populations suggesting
that EBV does not play a direct role in the epithelial
pathology of primary SS.

The ocular surface changes in patients with severe
aqueous tear deficiency have traditionally been attrib-
uted to mechanical irritation from reduced tear pro-
duction. The results of this study suggest that, in addi-
tion to mechanical irritation secondary to dryness, lo-
cal immunopathologic changes of the conjunctival
epithelium also contribute to the ocular surface dis-
ease of primary SS. Although we have evaluated the
local expression of several cytokines within the ocular
conjunctiva of patients with SS, this survey has not
been exhaustive and other cytokines and growth fac-
tors may provide important contributions to the ocu-
lar pathology of this disorder. The tears represent an-
other potential source of immunomodulatory factors
and normal tears are known to contain growth factors
and biologic response modifiers such as epidermal
growth factor and retinol. It is possible that these fac-
tors play an essential role in the normal differentia-
tion of the conjunctival epithelium into a normal lubricat-
ing mucosal surface that contains both goblet cells
and epithelial-associated mucin. Perhaps the absence
of appropriate differentiation factors and/or the ele-
vated expression of inflammatory cytokines within the
tears promotes the morphologic and immunopatho-
logic changes observed in the conjunctival epithelium
of primary SS. Future therapy of primary SS may be
directed to replacing deficient factors or inhibiting
such anomalously overexpressed factors as IL-6.

Key Words
Sjögren’s syndrome, cytokine, interleukin-6, Epstein-Barr vi-
rus, dry eye disorder

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References
1. Pflugfelder SC, dniels T, Whitcher J. Sjögren’s Syn-
2. Pflugfelder SC, Tseng SCG, Pepose JS, et al. Epstein-
Barr virus infection and immunologic dysfunction in
patients with aqueous tear deficiency. *Ophthalmology.*
1990;97:313–323.
3. Pepose JS, Akata RF, Pflugfelder SC, et al. Mononu-
clear cell phenotypes and immunoglobulin gene re-
arrangements in lacrimal gland biopsies from patients
with Sjörgen’s Syndrome. *Ophthalmology.* 1990;97:
1599–1605.
4. Adamson TC, Fox RI, Frisman DM, et al. Immunohis-
tochemical analysis of lymphoid infiltrates in primary
Sjörgen’s Syndrome using monoclonal antibodies. J
5. Pflugfelder SC, Huang AJW, Feuer W, et al. Conjunc-
tival cytologic features of primary Sjögren’s Syndrome.
biopsy in Sjögren’s Syndrome: Correlations between
histological and immunohistochemical features. *Histo-
7. Pflugfelder SC, Crouse CA, Monroy D, et al. Epstein-
Barr virus and the lacrimal gland pathology of Sjög-
of Epstein-Barr virus DNA in lacrimal and salivary
glands of patients with Sjögren’s Syndrome. ARVO


