Lacrimal gland inflammatory cytokine gene expression in the botulinum toxin B-induced murine dry eye model

Choul Yong Park,1,2 Wenjuan Zhuang,1 Kaevalin Lekhanont,1 Cheng Zhang,1 Marisol Cano,1 Woo-Seok Lee,1 Peter L. Gehlbach,1 Roy S. Chuck1

(The first two authors contributed equally to this publication)

1Department of Ophthalmology, Johns Hopkins University, School of Medicine, Baltimore, MD; 2Department of Ophthalmology, Dongguk University, School of Medicine, Ilsan, South Korea

Purpose: To determine the effect of keratoconjunctivitis sicca, induced by botulinum toxin-B (BTX-B), on the inflammatory cytokine gene expression in the lacrimal gland (LG). And to determine the effect of various topical anti-inflammatory agents on the resulting cytokine levels.

Methods: Forty-two mice (eight-week-old, female, CBA/J) were divided into six groups. Four groups were injected with BTX-B into both lacrimal glands, one group was injected with saline into both LG (Sal, n=7), and one group served as an un.injected control (Con, n=7). The four groups of BTX-B injected mice were then assigned to a treatment group: 1. no additional treatment (BTX), 2. artificial tear treatment (AT), 3. Cyclosporine A (CSA) treatment, and 4. fluorometholone (FM) treatment (n=7 in each group). Corneal fluorescein staining was evaluated one, two, and four weeks after injection. LGs were harvested after two weeks (groups Con, Sal, and BTX) and four weeks (groups AT, CSA, and FM) after injection. Gene microarray analysis for inflammatory cytokines and their receptors, real time reverse-transcriptase polymerase chain reaction (RT-PCR), and immunofluorescent staining with anti-mouse CD3e monoclonal antibody were then performed on LG tissue.

Results: BTX-B injection into the LG effectively induced dry eye in mice two and four weeks following injection. Microarray data identified the proinflammatory cytokines interleukin (IL)-1, tumor necrosis factor (TNF)-α, IL-12, and macrophage migration inhibitory factor (MIF) and the anti-inflammatory cytokines IL-10 and toll-interacting protein (Tollip) as candidates for validation by real time RT-PCR. MIF and IL-12 expression were elevated in BTX-B injected mice at weeks 2 and 4 regardless of treatment. Tollip and IL-1 expressions were increased in some groups after BTX-B injection regardless of the treatment type. Other cytokines showed no significant changes. LG structures were well maintained without significant T lymphocyte infiltration in all groups.

Conclusions: Ocular surface change induced by BTX-B injection resulted in an altered expression of various inflammatory cytokines in our murine dry eye model. Alteration of the pathology-induced cytokine profile by topical therapy is reported.

Dry eye syndrome is a complex inflammatory disease characterized by unstable tear film, ocular surface epithelial disease and inflammation, lacrimal gland inflammation, and secretory dysfunction [1,2]. The cornea and lacrimal gland together with other tissues of the surface of the eye and the associated sensory, sympathetic, and parasympathetic nerves form a functional unit to maintain the health of the ocular surface [3,4]. Dysfunctional tear production results in diminished tear volume or altered tear composition both of which can promote ocular surface inflammation. The resulting inflammation is mediated by both release of proinflammatory cytokines from ocular surface cells and decreased production of anti-inflammatory factors [5-8].

Hyperosmolarity in tears resulting from lacrimal gland dysfunction can lead to the release of IL (Interleukin) -1, IL-6, IL-8, tumor necrosis factor (TNF)-α, and MMP (matrix metalloproteinase) -9 from ocular surface epithelium [9-12]. These inflammatory cytokines have been reported to be present on the ocular surface even in the absence of ocular surface inflammation [13]. Ocular surface trauma (micro or macro) activates the delicate neural reflex arc that coordinates corneal requirements and lacrimal gland secretion [14]. Corneal injury involving the corneal nerves is known to alter gene expression in the lacrimal gland and to result in decreased tear production and secretion [15,16]. An excellent example is dry eye syndrome following corneal refractive surgery [17,18]. Many inflammatory cytokines have been implicated in dry eye syndrome either on the ocular surface or in the lacrimal gland [9,10,12,19,20]. Inflammatory cytokines alone have been reported to cause damage to the lacrimal gland [21].

We have recently reported a murine dry eye model that results from an injection of the neurotoxin botulinum type B (BTX-B) into the lacrimal gland [22]. It is known that interruption of the parasympathetic innervation to the lacrimal gland either from the cutting of preganglionic nerves or secondary
to trigeminal ganglion ablation diminishes tear production and is associated with ocular surface and lacrimal gland structure changes [23]. We have shown that decreased tear production persists for one month following BTX-B injection and is associated with persistent corneal fluorescein staining [22].

In this report, we hypothesize that ocular surface change induced by BTX-B injection into the lacrimal gland gives rise to an altered expression of various pro- and anti-inflammatory cytokines within the lacrimal gland and that the induced imbalance may contribute to the pathogenesis of the non-Sjögren type, dry eye syndrome. In exploring this hypothesis, we have quantified selected inflammatory cytokine levels (selection based on microarray data) in the lacrimal gland and have quantitatively assessed expression change following BTX-B injection. In an effort to extend the findings to clinical application, we have determined the effects of topical artificial tear, cyclosporine A, and fluorometholone on the ocular surface of our dry eye model and have tested the hypothesis that the observed corneal surface changes are associated with changes in the measured inflammatory cytokine levels in the lacrimal gland.

**METHODS**

**Mouse dry eye model:** Forty-two, eight-week-old, female CBA/J mice (Jackson Labs, Bar Harbor, ME) were used in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Johns Hopkins University (JHU).

Seven mice were used as controls without any injection into the lacrimal gland, another seven mice were injected with saline into the lacrimal gland, and the remaining 28 mice were injected with botulinum toxin type B (BTX-B, Myobloc™, Elan Pharmaceuticals Inc., South San Francisco, CA). BTX-B or saline injection into lacrimal glands was performed as previously described [22]. Briefly following sedation with ketamine/xylazine (45 mg/kg and 4.5 mg/kg, respectively), transconjunctival injection of saline (0.05 ml) or BTX-B (0.05 ml, 20 mU) into the intraorbital lacrimal gland was performed in both eyes under an operating microscope with custom-made 33 gauge needles (Hamilton, Reno, NV).

To confirm dry eye, corneal fluorescein staining (1 μl of 1% sodium fluorescein, Sigma, St. Louis, MO) was evaluated with digital photography (Nikon digital camera fitted with a macro lens) taken one week, two weeks, and four weeks after injection. Corneal fluorescein staining was measured in three randomly selected mice from each group and classified using a grading system, which is based upon the area of corneal staining. When the total area of punctuate staining was designated as grade 0, there was no punctuate staining; when the area was designated as grade 1, there was equal to or less than one-eighth of the corneal area stained; when it was designated as grade 2, there was equal to or less than one-fourth of the corneal area stained; when it was grade 3, there was equal to or less than one-half of the corneal area stained, and when it was grade 4, there was greater than one-half of the entire area of the cornea stained [24]. The animals were euthanized at two time points: two weeks and four weeks after BTX-B injection, and the lacrimal glands were surgically harvested.

**Botulinum type B effect on inflammatory cytokine gene expression:** Seven mice without injection (group Con), seven mice with saline injection (group Sal), and seven mice with BTX-B injection (group BTX) were euthanized at the two week point without receiving any topical medication to evaluate the effect of BTX-B on inflammatory cytokine genes expression in the lacrimal gland.

<table>
<thead>
<tr>
<th>Gene Name (Gene Bank Accession No.)</th>
<th>Sequence</th>
<th>PCR product (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-actin (NM_007393)</td>
<td>SuperArray BioScience Corp. Catalog no. PPM02945A</td>
<td>154</td>
</tr>
<tr>
<td>MIF (NM_010798)</td>
<td>SuperArray BioScience Corp. Catalog no. PPM02985A</td>
<td>118</td>
</tr>
<tr>
<td>Tollip (NM_023764)</td>
<td>SuperArray BioScience Corp. Catalog no. PPM06269A</td>
<td>177</td>
</tr>
<tr>
<td>IL-10 (NM_010548)</td>
<td>SuperArray BioScience Corp. Catalog no. PPM03017A</td>
<td>178</td>
</tr>
<tr>
<td>IL-12a (NM_008351)</td>
<td>SuperArray BioScience Corp. Catalog no. PPM03019A</td>
<td>92</td>
</tr>
<tr>
<td>TNF-α (M_11731)</td>
<td>forward/reverse</td>
<td>TCA GCC TCT TCT CAT TCC TG</td>
</tr>
<tr>
<td>IL-1b (NM_008361)</td>
<td>forward/reverse</td>
<td>TGA GCT GAA AGC TCT CCA CC</td>
</tr>
</tbody>
</table>

MIF: Macrophage migration inhibitory factor, IL-1b: Interleukin 1B, IL-12a: Interleukin-12A, TNF-α: Tumor necrosis factor alpha, Tollip: Toll interacting protein, bp: base pair
Topical dry eye medications: Twenty-one mice, all of which were injected with BTX-B, were randomized into three groups to receive twice daily topical treatment with either 0.1% fluorometholone eyedrops (FML®, Allergan, Irvine, CA; group FM), 0.05% cyclosporine A eyedrops (Restasis®, Allergan, Irvine, CA; group CSA), or artificial tears (Refresh tears®, Allergan, Irvine, CA; group AT) one week after the injection for three weeks. The animals were euthanized four weeks after injection, and the lacrimal glands were harvested.

RNA extraction and microarray analysis: Total RNA was extracted from the harvested lacrimal glands using the RNeasy Protect Mini Kit (Qiagen, Valencia, CA) according to the manufacturer’s instructions. The homogenate from three lacrimal glands randomly selected from seven animals in each group were pooled. RNA integrity was determined by ultraviolet (UV) spectrophotometry and agarose gel electrophoresis. Purified cRNA was synthesized using the TrueLabeling-AMP™ 2.0 kit (SuperArray Bioscience Corp, Frederick, MD) according to the manufacturer’s instructions. Briefly, cDNA was synthesized from 1 µg of extracted RNA, and cRNA synthesis and amplification were performed using this cDNA with overnight incubation. Biotinylated UTP (Roche Molecular Biochemical, Indianapolis, IN) was used to label the newly synthesized cRNA. After the purification of synthesized cRNA following the manufacturer’s instructions, a total 3 µg of cRNA were used for hybridization of the microarray membrane (Oligo GEArray® Mouse Inflammatory Cytokines and Receptors Microarray, catalog number OMM-011, SuperArray Bioscience Corp, Frederick, MD). After overnight hybridization, the membrane was washed and treated with alkaline phosphatase-streptavidin buffer solution. Chemiluminescent detection was performed using a CCD camera (Kodak Image Station 4000MM, Eastman Kodak Company, Rochester, NY) for 60 min. Microarray membranes were analyzed using GEArray Expression Analysis Suite Software (SuperArray Bioscience Corp., Frederick, MD).

First strand cDNA synthesis and real time polymerase chain reaction: The microarray expression level of individual genes was validated using real time RT-PCR. First-strand complementary cDNA was synthesized from total RNA with a commercially available kit (ReactionReady™ First Strand cDNA Synthesis Kit, SuperArray Bioscience Corp., Frederick, MD) as described by the manufacturer. The reaction was performed with annealing at 70 °C for 3 min followed by reverse
transcription reaction at 37 °C for 60 min. Heating at 95 °C for 5 min was then applied to hydrolyze the RNA and to inactivate the reverse transcriptase. Seven lacrimal glands from seven different mice in each group were used separately for the real time polymerase chain reaction (PCR) experiment. PCR was performed using a commercial kit (RT² Real-Time™ SYBR Green, SuperArray Bioscience Corp., Frederick, MD) for β-actin, macrophage migration inhibitory factor (MIF), toll-interacting protein (Tollip), TNF-α, IL-1b, IL-10, and IL-12a. Each reaction tube contained template DNA (120 ng) and 1 µl (100 nmol/l final concentration) of forward and reverse primers (Table 1). A negative control was assembled using the same concentrations of reagents except template DNA. Samples were amplified in a thermocycler (LightCycler® 2.0 Instrument, Roche Applied Science, Indianapolis, IN) for 40 cycles of 15 s at 95 °C, 30 s at 55 °C, and 30 s at 72 °C. The expression level of the individual gene was assessed by using comparative Ct (ΔΔCt) method.

Histology and immunofluorescence staining: Seven lacrimal glands from seven different mice in each group were immersed in formalin-PBS (1:10 dilution, Protocol®, Fisher Diagnostics, Middletown, VA), fixed overnight at 4 °C, em-
bedded in O.C.T. media (Tissue-Tek®, Sakura, Torrence, CA), and instantly frozen with liquid nitrogen. Blocks were cryosectioned with a thickness of 7 µm. To determine if any inflammatory cell infiltration into the lacrimal gland was present, the tissue sections were stained with H&E (hematoxylin and eosin) and hamster anti-mouse CD3e monoclonal antibody (eBioscience, San Diego, CA). For immunofluorescent staining, they were successively incubated with blocking serum and primary antibody (1:20 dilution) overnight at 4 °C after the sections were dried at room temperature for 10 min. Goat anti-hamster secondary antibody conjugated with Cy-3 (1:200; Jackson ImmunoResearch, West Grove, PA) was then applied for 1 h at room temperature. Nuclear counter staining was performed with Hoechst 33258 dye (1:2000; Molecular Probes, Eugene, OR) for 60 s and mounted (Fluorescence mounting media, Dako, Carpinteria, CA). Lacrimal glands from six-week-old MRL/Mpj mice were used as positive controls for CD3e immunostaining. Specimens were viewed with a standard fluorescence microscope.

**Statistical analysis:** SPSS version 11.0 for Windows was used for statistical analysis. The Mann-Whitney U Test was used for analysis of differences between two groups, and the Kruskal-Wallis test was used to compare mean values among more than two different groups. p-values less than 0.05 were considered statistically significant.

**RESULTS**

**Corneal staining:** As reported previously, BTX-B induced a significant increase in corneal staining two weeks after injection (mean±SD; 2.17±1.17) compared to the non-injection (Con) and saline injection (Sal) groups (mean±SD; 0.17±0.41 and 0.50±0.55, respectively; p=0.004, Kruskal-Wallis test; Figure 1 and Figure 2). One week of treatment with topical eyedrops resulted in no significant improvement in corneal...
staining from any of the drops tested. However, after three weeks of treatment, the fluorometholone-treated group (mean±SD; 0.67±0.82) showed significant improvement in corneal staining as compared to artificial tear treatment (mean±SD; 2.50±1.38; p=0.026, Mann-Whitney Test). Cyclosporine A (mean±SD; 1.17±0.75) showed a trend toward decreased corneal staining that did not reach statistical significance with the number of subjects tested in this study, (p=0.094, Mann-Whitney Test; Figure 1 and Figure 2).

Gene microarray: To investigate the change in inflammatory cytokine expression levels that are associated with the modeled ocular surface condition, we planned to strategically select relevant candidate genes for further study. To identify these relevant genes, gene microarray methods were used to analyze murine lacrimal gland tissue for inflammatory cytokines and their cognate receptors. These studies identified several cytokines related to inflammation as well as their cognate receptors that were moderately to highly expressed in murine lacrimal glands in all six groups (Figure 3). Among the highly expressed genes were the proinflammatory cytokines MIF, complement component 3 (C3), and IL-12a. The anti-inflammatory genes IL-10, IL-10rb, and Tollip were also detected. Other proinflammatory cytokines and receptors such as IL-6ra, IL-6st, IL-1r1, Ccr2, and IL-20 were also detected at lower levels. MIF, IL-10, IL-12, and Tollip were selected for further real time reverse-transcriptase polymerase chain reaction (RT-PCR) analysis. In addition, IL-1 and TNF-α were selected for validation by real time RT-PCR.

Real time reverse-transcriptase polymerase chain reaction: The mRNA expression levels of MIF, Tollip, IL-10, IL-12a, IL-1b, and TNF-α as well as the housekeeping gene, β-actin, were evaluated by real time RT-PCR methods in all six groups (n=7 lacrimal glands per group). The MIF expression level significantly increased in the BTX-B injected groups (BTX, AT, CSA, and FM) compared to BTX-B noninjected groups (Con and Sal). Though the corneal staining of group FM was similar to groups Con and Sal, the treatment did not normalize the MIF expression level (Figure 4). The expression level of IL-12 and Tollip showed increased expression levels in the four-week samples (groups AT, CSA, and FM) compared to the control level (group Con). (Figure 5). The expression level of IL-1 increased in BTX-B treated groups (groups BTX, AT, CSA, and FM) compared to the level in the control group, but they failed to reach statistical significance when compared to the saline injection group (Figure 6). Though there was some fluctuation of expression levels, real time RT-PCR did not reveal any significant change in the gene expression of IL-10 and TNF-α (Figure 7).
Immune fluorescence staining and histology: There have been reports that T lymphocyte-mediated inflammation in the lacrimal gland is important in Sjögren type dry eye syndrome and other proposed animal models of dry eye. Our current experiment indicates that T lymphocyte attractant chemokine, MIF, is constitutively expressed in the lacrimal glands. Therefore, we analyzed the lacrimal glands of our dry eye model with both H&E and immunofluorescent staining using anti CD3e monoclonal antibody to determine if any significant inflammatory cell infiltration into the lacrimal glands, especially T lymphocytes, was present. Lacrimal glands from all six groups contained well preserved acinar structure and showed no significant T lymphocyte infiltration (Figure 8).

**DISCUSSION**

The main lacrimal gland is an important participant in a neurally connected functional unit that also consists of the cor-

---

**Figure 6**. Real time reverse-transcriptase polymerase chain reaction for Toll interacting protein and interleukin 1. Real time RT-PCR revealed that Tollip (A) and IL-1 (B) expression is elevated in some of the BTX-B-injected lacrimal glands compared to either the non-injection group or saline injection group. This elevation was not significantly affected by topical treatment (AT, CsA, and FM). The relative expression levels were calculated by the comparative Ct method with the setting group Con as 1.00; n=7 in each group. The p-value was calculated by Kruskal-Wallis test using LSD after ranks. In A, the asterisk indicates a p=0.36 control versus “CSA” and p=0.31 control versus “FM”. In B, the asterisk indicates a p=0.34 Sal versus “BTX”, p=0.37 Sal versus “CSA”, and p=0.38 Sal versus “FM”.

**Figure 7**. Real time reverse-transcriptase polymerase chain reaction for interleukin 10 and tumor necrosis factor alpha. Real time RT-PCR for IL-10 (A) and TNF-α (B) revealed no significant differences in gene expression in all groups. The relative expression levels were calculated by the comparative Ct method setting group Con as 1.00; n=7 in each group. The Kruskal-Wallis test using LSD after ranks was employed for statistical analysis.
Figure 8. Histologic analysis. Hematoxylin and eosin (H&E) staining of lacrimal glands showed preservation of normal acinar structure and a lack of inflammatory cell infiltration in three specimens from two weeks (A-C) and another three specimens from four weeks (G-I). Immunofluorescent staining with CD3e monoclonal antibody showed no significant T lymphocyte infiltration in all specimens (D-F and J-L). H&E and immunofluorescent staining of lacrimal glands from MRL/Mpj mice as a positive control showed diffuse T lymphocytes infiltration (red) with nuclear counter staining (blue; M and N). A and D: control, B and E: saline injection, C and F: BTX-B injection, G and J: artificial tear treatment, H and K: cyclosporin A treatment, I and L: flurometholone treatment.
nea, conjunctiva, accessory lacrimal glands, and meibomian glands. Compromise of the ocular surface affects lacrimal support of the ocular surface [25] while compromise of the lacrimal gland affects the ocular surface by disruption of normal tear production [26,27]. Here we report that BTX-B injection into the murine lacrimal gland induces corneal surface changes that mimic keratoconjunctivitis sicca in humans. In this study, we have determined that normal lacrimal gland constitutively expresses multiple pro- and anti-inflammatory cytokines in the presence of a normal ocular surface and that cytokine expression levels can be altered by changes in the condition of the ocular surface with the treatment of standard topical dry eye medications.

Specifically, we found several pro- and anti-inflammatory cytokines are increased in their expression after BTX-B treatment. The expression levels of MIF, IL-12, IL-1, and Tollip were included amongst them. Although the levels of TNF-α and IL-10 at the ocular surface of other experimental dry eye models has been reported significantly changed in correlation with the severity of the disease [7,9,11,12,14,28-30], we could not find any significant change of its gene expression level in the lacrimal gland. The increase of the pro-inflammatory cytokine, MIF, was very interesting. Its expression level was higher in BTX-B injected lacrimal glands two weeks after injection than in the non-injected and the saline-injected control groups. This elevation was not normalized, even with topical steroid medication, which decreased the surface staining significantly at four weeks. Another proinflammatory cytokine, IL-12, was also detected to be increased in BTX-B injected lacrimal glands after two weeks and was not normalized despite various topical treatments either. Unfortunately, the increased IL-12 expression levels failed to reach any statistical significance when compared to saline injection groups (saline control). We could also detect the change in expression levels of the anti-inflammatory cytokine, Tollip, and the proinflammatory cytokine, IL-1, in several groups after BTX-B injection, but it was not as strong as the change detected in MIF. Finally, in spite of the elevated expression levels of various inflammatory cytokines within the lacrimal gland including MIF, a potent T lymphocyte attractant, the normal lacrimal gland structure remained well maintained and lacked any significant inflammatory cell infiltration. This may be because the immuno-regulatory mechanisms normally prevent autoimmune activation in the lacrimal glands and override the influences of the cytokine changes [31,32]. Alternatively, this finding may imply the directed secretion of various inflammatory cytokines into the tear fluid rather than diffusely into the lacrimal gland stroma.

As is the nature of biologic phenomenon, the changes in the studied cytokines did not correlate exactly with the grades of corneal staining in this experiment. However, it is interesting that the most favorable surface condition at four weeks, the fluorometholone treatment eyes (group FM), displayed the least change of cytokine expression levels compared to the eyes treated with the other topical agents (groups AT and CSA).

Proinflammatory cytokines in the lacrimal gland have been reported to decrease tear production via neuronal and hormonal effects [14,28,33]. They have been reported to shut down efferent nerve endings and also to play a role in the conversion of androgen into estrogen [33]. In addition, reports of ocular surface improvement with anti-inflammatory gene transfer into the lacrimal gland in the auto-immune dacryoadenitis model together with the reports of elevated IL-1 and MIF levels in tear films of ocular surface inflammatory disease suggest that lacrimal gland cytokines are secreted into the tear film [7,19,20,34].

Recently our group found a high expression of macrophage migration inhibitory factor (MIF) in murine acinar cells of healthy mice [unpublished data]. MIF has been reported to be a potent activator of T lymphocytes in many inflammatory diseases such as glomerulonephritis, atherosclerosis, uveitis, atopic dermatitis, adult respiratory distress syndrome, and endotoxin shock [35-40]. Furthermore, MIF’s action as an endogenous, counter-regulatory mediator for glucocorticoid action has also been suggested [41]. Thus, MIF within the tear film may be a potent signal to recruit T lymphocytes to the ocular surface leading to the damage of the cornea and conjunctiva. Despite marked clinical advances in topical drugs for treating dry eye syndrome, many non-Sjögrens dry eye patients still suffer with poor response to these agents. Considering our current results, a new therapeutic approach targeting MIF may be a promising avenue for these refractory dry eye patients. For example, functional blockade of MIF through the use of neutralizing anti-MIF antibody suppressed septic shock or delayed-type hypersensitivity in experimental animals and reduced renal injury in immunologically induced kidney disease [42-44].

It is interesting that lacrimal gland expression of toll interacting protein (Tollip) was increased with four-week topical artificial tear treatment. Tollip was first reported as a component of the IL-1 receptor signaling pathway and further investigation revealed its role in the toll-like receptor (TLR) signaling pathway [45,46]. Although the mechanism is not fully understood, Tollip is suggested to serve to limit the production of proinflammatory mediators during inflammation and infection via the inhibition of NF-κB signaling of IL-1 and maintain immune cells in their quiescent states [47,48].

There are certainly limitations in this investigation. The sample sizes are relatively small. In addition, because the protein level of these various cytokines in pure lacrimal fluid was not measured, the effects of message translation into protein were not assessed. Despite these limitations, this is the first study to our knowledge to investigate various inflammatory cytokine expression levels within lacrimal glands in an animal model of keratoconjunctivitis sicca using a gene microarray for candidate gene selection.

In summary, we have found that ocular surface change is associated with change in expression levels of various proinflammatory and anti-inflammatory cytokines in murine lacrimal glands. These findings may serve as evidence in support of a functional interconnection between the ocular surface and the main lacrimal gland. Based on these findings, further investigation directed at cytokine modulation as a therapeutic approach to dry eye syndrome is a logical approach.
ACKNOWLEDGEMENTS
This work was supported by NIH Grant EY000412-04, Stark-Mosher Center for Cataract and Corneal Diseases, a Research to Prevent Blindness Inc. unrestricted grant, and a RPB Career Development Grant (P.L.G.).

REFERENCES
35. Brown FG, Nikolic-Paterson DJ, Hill PA, Isbel NM, Dowling J,


