Diverse Mediators Modulate the Chloride Ion Fluxes That Drive Lacrimal Fluid Production

Shivaram Selvam, Austin K. Mircheff, and Samuel C. Yiu*

Department of Ophthalmology, Doheny Eye Institute, and Department of Physiology and Biophysics, Keck School of Medicine of the University of Southern California, Los Angeles, California

Correspondence: Samuel C. Yiu, The Johns Hopkins Hospital, The Wilmer Eye Institute, 600 N. Wolfe Street, Wilmer 110, Baltimore, MD 21287; syiu2@jhmi.edu.

Current affiliation: *Wilmer Eye Institute, Johns Hopkins University, Baltimore, Maryland.

Submitted: May 15, 2012
Accepted: March 5, 2013

Citation: Selvam S, Mircheff AK, Yiu SC. Diverse mediators modulate the chloride ion fluxes that drive lacrimal fluid production. *Invest Ophthalmol Vis Sci*. 2013;54:2927–2933. DOI:10.1167/iovs.12-10202

PURPOSE. To learn whether locally expressed and systemic mediators might modulate the cholinergically induced transepithelial Cl^- fluxes that underlie lacrimal fluid production.

METHODS. Reconstituted epithelial monolayers were exposed to a submaximal dose of the muscarinic agonist, carbachol (CCh), or to one of several paracrine mediators for 18 hours, then acutely stimulated with an optimal dose of CCh. Secretory Cl^- fluxes were assessed as negative short-circuit currents (I_{SC}).

RESULTS. Exposure to IL-6 at concentrations of 1 and 10 ng/mL and IL-1 β at 10 ng/mL significantly decreased CCh-induced Cl^- secretion. Prolactin decreased CCh-induced Cl^- secretion, but the extent of the decrease diminished as the prolactin concentration increased from 20 to 200 ng/mL. CCh, 10 μM , prevented CCh, 100 μM , from eliciting Cl^- secretion. Exposure to histamine, 10 mM, prevented formation of confluent monolayers. Exposure to histamine, 1 mM, decreased CCh-induced Cl^- secretion, whereas exposure to 5-HT, 1 mM, potentiated CCh-induced Cl^- secretion.

CONCLUSIONS. Chronic exposure to inflammatory cytokines may significantly impair cholinergically induced lacrimal fluid production. Concentrations of prolactin within the high range of normal values also may impair fluid production, but this effect is reversed at levels associated with pregnancy. Autonomic neurotransmitters and paracrine mediators that signal through different G protein-coupled receptors appear to exert varying influences, which range from complete suppression to potentiation of cholinergically induced fluid production. Thus, some hormones and paracrine mediators may impair secretion in apparently homeostatic glands as well as diseased glands, whereas mediators produced by certain immune cell infiltrates may actually enhance fluid formation.

Keywords: Schirmer's test, lacrimal gland fluid production, paracrine mediators

Dry eye disease is one of the most common forms of morbidity encountered in ophthalmic and optometric practices. It appears that lacrimal fluid production can be decreased by anticholinergic actions of commonly prescribed systemic medications¹; moderately elevated levels of serum prolactin²; age-related, anatomic atrophy of the lacrimal acinar epithelium; stenosis of elements of the intraglandular duct system^{3,4}; obstruction within or at the orifices of the main excretory ducts; and physiologic dysfunction associated with lymphocytic infiltration.

Lymphocytic infiltration is common. One study found infiltrates in 20% of glands from females of reproductive-age, young males and elderly males, and in 65% of glands from elderly females.⁵ Another report indicates that infiltrates were present in 83% of orbital glands from both males and females over 40 years of age.⁶ These estimates are 3-fold or more higher than estimates of the prevalence of dry eye disease.^{7,8} The actual disparity between histopathology and physiologic dysfunction might be even more pronounced, given that recent work^{9–11} suggests that dry eye disease most often begins when age- and hormone-related changes in meibomian gland function¹² alter the thickness or composition of the lipid layer of the tear film¹³ and that lacrimal fluid production subsequently decreases.

The examples of Sjögren's disease and von Mikulicz's disease suggest a general explanation for the evident disparity. The

focal, B-cell-, and CD4⁺ T-cell-rich infiltrates of Sjögren's disease can be associated with physiologic dysfunction even before gross changes in acinar- and ductal architecture become evident, whereas pervasive infiltration by the IgG₄-expressing plasmacytes of von Mikulicz's disease can have little impact on lacrimal fluid production.¹⁴ Thus, some of the more common immunopathologic processes may impair physiologic function, and some may not.

Studies of biopsied labial salivary glands from patients with Sjögren's disease and of salivary and lacrimal glands of rodent models thought to mimic Sjögren's pathophysiology have provided evidence that both autoantibodies and also paracrine mediators released by the infiltrating immune cells can cause intact-appearing parenchymal tissues to be functionally quiescent.^{15–17} Anti-M₃ acetylcholine receptor (M₃AChR) autoantibodies, present in sera of patients with Sjögren's syndrome, acutely suppress cholinergically induced elevations of cytosolic Ca²⁺ in human labial salivary glands.¹⁸ Nitric oxide, typically present in inflamed tissues, acutely potentiates, but then persistently attenuates, cholinergically induced elevations of cytosolic Ca²⁺ in labial salivary gland preparations.¹⁹ The inflammatory cytokines, IL-1 α , IL-1 β , and TNF- α , inhibit cholinergically induced glycoprotein secretion in ex vivo murine lacrimal gland models.²⁰

Exposure to elevated levels of prolactin, which functions as both a paracrine mediator and a hormone, causes acinar cells to redirect cholinergically induced merocrine protein secretion from the exocrine direction to the paracrine direction, without altering the total amount secreted.^{21,22} Exposure to mediators that are not cytokines but, rather, G protein-coupled receptor agonists, can also interfere with cholinergically induced Ca^{2+} elevation and exocrine β -hexosaminidase secretion. These include the muscarinic cholinergic agonist, carbachol (CCh), at a submaximal dose,²³ and the biogenic amines, histamine, and serotonin (5-HT).²⁴

The mechanisms of fluid secretion are largely distinct from the mechanism of merocrine protein secretion. They depend on H_2O transport proteins (aquaporins [AQPs])^{25–27} and ion transport proteins^{28–32} arrayed in the cells' apical- and basal-lateral plasma membranes. The ion transport proteins in the acini include Na^+/H^+ exchangers (NHE),²⁹ $\text{Cl}^-/\text{HCO}_3^-$ exchangers (AE),³⁰ and $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporters (NKCC1)³¹—which function in the basal-lateral plasma membranes, Cl^- selective channels (CIC3 and CFTR)^{32,33}—which function in the apical membranes,³⁴ and Na^+/K^+ exchange pumps (Na,K-ATPase), which some evidence indicates function in both the apical- and the basal-lateral membranes.²⁸ The mechanism in the acini secretes Cl^- ions into the lumens, thereby generating a negative transepithelial voltage difference; the voltage difference presumably drives Na^+ ions into the lumens via the zonulae occludens and zonulae adherens that link adjacent epithelial cells,³⁵ and the osmotic imbalance that results from the net secretion of Cl^- and Na^+ draws H_2O through the epithelium.

The ion transport proteins are largely quiescent in resting cells, and evidence indicates that each cycles between a relatively small plasma membrane pool and a much larger intracellular pool distributed through the endosomes and trans-Golgi network.^{36–38} Therefore, mediators that cause lacrimal physiologic dysfunction may do so by interfering with neurotransmitter receptor signaling or intracellular signal transduction; with the biosynthetic turnover of one or more key transport proteins; with the proper plasma membrane insertion of one or more transport proteins; or with the activation of one or more transport proteins.

The microscopic scale of the lacrimal acinus-duct unit has made it difficult to determine whether the mediators that suppress agonist-induced Ca^{2+} elevation or merocrine protein secretion exert the predicted influences on either fluid production or the ion fluxes that drive fluid production. The introduction of a new ex vivo model, an epithelial monolayer reconstituted from isolated rabbit lacrimal gland acinar cells,³⁹ has now made it possible to address this question. The magnitude of the secretory Cl^- flux the monolayer generates can be measured as the amount of current—referred to as the short-circuit current (I_{SC})—that must be delivered through an external circuit to eliminate the voltage difference across the monolayer. The results described in the following text indicate that some mediators can suppress I_{SC} ; some exert no influence; some potentiate it; and one, prolactin, exerts biphasic, concentration-dependent influences.

METHODS

Animals

Female New Zealand white rabbits weighing approximately 4 kg were obtained locally (Irish Farms, Norco, CA). They were maintained for up to 5 days in the Doheny Eye Institute vivaria, a facility fully accredited by the American Association for Laboratory Animal Science, and euthanized as described

previously.³⁹ All protocols conformed to the ARVO Resolution on the Use of Animals in Ophthalmic and Vision Research and were approved by the Institutional Animal Care and Use Committee.

Materials

Standard six-well microporous polyester membrane cell culture inserts were obtained commercially (Snapwell; Costar, Corning, NY). A serum-free fully defined hepatocyte culture medium kit was obtained commercially (Hepato-STIM; BD Biosciences, Medford, MA).

Reconstituted Epithelial Monolayers

Procedures for isolation and culture of lacrimal gland acinar cells in polyester culture inserts (Snapwell; Costar) were as described previously.³⁹ After 2 days, the mediators to be tested were added to both apical and basal chambers, and cells were maintained for 18 to 20 hours more.

Short-circuit currents (I_{SC}) were measured as described previously (Ussing Chamber Systems; Warner Instruments, Holliston, MA).³⁹ Briefly, Ag-AgCl voltage-sensing electrodes and Ag wire current-passing electrodes were connected by agar bridges containing 3 M KCl and interfaced via headstage amplifiers (DM-MC6; Physiologic Instruments, La Mesa, CA) to a microcomputer-controlled voltage/current clamp (VCC-MC6; Physiologic Instruments). Voltage-sensing electrodes were matched to within 1 mV asymmetry and corrected by an offset-removal circuit. The resistances of the medium and blank filter inserts were electrically compensated using a series compensation circuit.

Inserts with confluent monolayers were equilibrated in bicarbonated Ringer's solution (BRS) for approximately 1 hour, then gently mounted in water-jacketed apparatus (Ussing Chamber Systems; Warner Instruments) maintained at 37°C. The chamber on each side contained 4 mL BRS continuously bubbled with 95% O_2 –5% CO_2 gas lifts. The samples were allowed to stabilize for 15 to 30 minutes. Once the bioelectric properties reached steady state, agonist (CCh, histamine, or 5-HT) was added to the basal chamber, and I_{SC} was recorded every second for at least 3 minutes. Measurements of I_{SC} during the first 3 minutes after agonist additions were summed, and differences between the summed I_{SC} for control and treated wells were tested for statistical analysis. Acinar cell preparations that failed to form confluent monolayers, determined by examination under an inverted light microscope (Nikon DIAPHOT; Nikon Inc., Garden City, NY), were discarded.

Statistical Analysis

Data presented are means $\pm$ SE or ranges of replicate samples for each concentration of each mediator tested. ANOVA and multiple comparison analyses were performed (SigmaPlot 12; SyStat, San Jose, CA).

RESULTS

Negative I_{SC} can represent secretion of negative ions or absorption of positive ions. Reconstituted lacrimal epithelial monolayers generate a negative I_{SC} in response to stimulation with 100 μM CCh; studies with selective inhibitors indicate that it is attributable to a secretory flux of Cl^- ions, rather than an absorptive flux of Na^+ ions.³⁹ Unstimulated monolayers consistently generate a small positive I_{SC} ; the ionic basis of this current has not been determined, but current models of

lacrimal fluid formation suggest that it represents a low basal rate of K^+ secretion.³²

As shown in Figure 1, 18-hour exposure to IL-6 at concentrations of 1 and 10 ng/mL and exposure to IL-1 β at a concentration of 10 ng/mL decreased CCh-induced I_{SC} by 40%.

As shown in Figure 2, 18-hour exposure to prolactin (doses [ng/mL] 20, 100, and 200) decreased CCh-induced I_{SC} by 42%, 32%, and 15%, respectively.

As shown in Figure 3, exposing monolayers to 10 μ M CCh for 18 hours prevented them from generating a negative I_{SC} when subsequently stimulated with 100 μ M CCh. The treated monolayers initially decreased the positive I_{SC} from its baseline value, presumably by opening apical Cl^- -selective channels. This was followed by a small, transient overshoot of the positive I_{SC} , presumably due to the opening of apical K^+ channels. I_{SC} then returned to its baseline value. Thus, chronic exposure to 10 μ M CCh, which almost completely prevents an optimal dose of CCh from elevating Ca^{2+} or eliciting protein secretion,²³ also appears to prevent the optimal dose from eliciting Cl^- secretion.

As shown in Figure 4A, acute stimulation with 10 mM histamine elicited a small, transient positive I_{SC} , and neither histamine nor 5-HT elicited a negative I_{SC} . Combining acute histamine or 5-HT stimulation with acute CCh stimulation appeared to exert variable effects on the time courses of CCh-induced I_{SC} changes but not to alter the net I_{SC} .

As shown in Figure 4B, 18-hour exposure to 1 μ M histamine had no effect on CCh-induced I_{SC} , but exposure to 1 mM histamine reduced CCh-induced I_{SC} by 40%. Exposure to 10 mM histamine disrupted the epithelial barrier, eliminating the capacity to generate I_{SC} . Exposure to 1 μ M 5-HT had no significant effect on CCh-induced I_{SC} . Strikingly, however, exposure to 1 mM 5-HT potentiated CCh-induced I_{SC} by 78%.

DISCUSSION

Levels of IL-1 α ,⁴⁰ IL-1 β ,⁴¹ and IL-6⁴⁰⁻⁴² are increased in labial salivary glands from patients with Sjögren's disease. The finding that chronic exposure to IL-1 β or IL-6 impairs cholinergic activation of the transepithelial Cl^- currents that drive lacrimal fluid production accords with findings that IL-1 β is one of several inflammatory cytokines that inhibit protein secretion by murine lacrimal gland fragments.²⁰ Interestingly, when labial salivary glands are removed from patients with Sjögren's disease and placed in primary culture, they exhibit normal Ca^{2+} elevations in response to cholinergic stimulation. Autoimmune IgG against the M_3AChR suppress cholinergically induced elevations of Ca^{2+} , but the inhibition can be reversed by removal of the IgG.¹⁸ Although the present study did not address Ca^{2+} signaling, it is noteworthy that chronic exposure to IL-1 β or IL-6 reduced cholinergically activated I_{SC} reflecting Cl^- secretory fluxes durably (i.e., after the monolayers had been transferred to fresh media and allowed to equilibrate).⁴³

A rabbit model of induced autoimmune dacryoadenitis was recently used to assess the impact of the inflammatory process on abundances of mRNAs for transport proteins and on the abundances and plasma membrane expression levels of the several proteins that could be detected with available antibodies. The abundances of mRNAs for the Na,K-ATPase subunit isoforms, $NKA\alpha_1$, $NKA\beta_1$, $NKA\beta_2$, $NKA\beta_3$,⁴⁴ $NKCC1$,³⁴ $CFTR$,³⁴ the $ClC2$ γ -subunit,³⁴ $AQP4$, and $AQP5$ ⁴⁵ were all significantly decreased. The abundances of $NKCC1$, $CFTR$, and $AQP5$ were also decreased. In contrast, the abundances of $NKA\alpha_1$, $NKA\beta_1$, $NKA\beta_2$, $NKA\beta_3$, and $AQP4$ were, paradoxically, increased. The transporter plasma membrane pools evident in immunofluorescence microscopy appeared largely unaltered. These findings may offer clues to the nature of the durable changes in transport function described earlier. They seem to suggest that chronic exposure to an inflammatory

FIGURE 1. Influences of 18-hour exposure to IL-6 or IL-1 β on I_{SC} induced by acute stimulation (arrows) with 100 μ M CCh. The control monolayer was not exposed to added cytokines before measurement of I_{SC} . n = number of trials with replicate monolayers. All monolayers were from the same acinar cell preparation and were processed in parallel. P values refer to the significance of differences from the control determined by the Holm-Sidak method for pairwise multiple comparisons.

FIGURE 2. Influences of overnight exposure to prolactin on CCh-induced negative I_{sc} . The control monolayer was not exposed to added prolactin during the 18 hours before measurement of I_{sc} . n = number of replicate monolayers. All monolayers were from the same acinar cell preparation and were processed in parallel. P values refer to differences from control determined by the Holm-Sidak method for pairwise multiple comparisons. * I_{sc} after exposure to 200 ng/mL prolactin was significantly different from I_{sc} after exposure to 20 ng/mL prolactin and 100 ng/mL prolactin ($P \leq 0.008$), but not different from control I_{sc} . Arrows indicate the time when the agonist was added.

milieu can cause secretory dysfunction by decreasing biosynthetic turnover rates and intracellular pools of critical transport proteins rather than by grossly decreasing the transport proteins' plasma membrane-expressed pools.

The finding that prolactin has a biphasic, dose-related influence on CCh-induced Cl^- secretion may have several implications for the interpretation of previously reported clinical and laboratory findings. The added prolactin concentration of 20 ng/mL approximates the limit of the high range of normal serum prolactin values. Suppression of CCh-induced Cl^- secretion at this dose suggests a plausible mechanistic explanation for the report that increasing levels of serum prolactin within the range of normal values are strongly and highly significantly associated with decreased lacrimal function in females, both during the reproductive years and after menopause and independently of their use of hormone replacement therapy.² Serum prolactin levels are moderately increased in females with Sjögren's syndrome,^{46–48} and levels of a high molecular weight form of prolactin are increased in the salivary glands of patients with Sjögren's disease.⁴⁹ Therefore, either hormonal prolactin or paracrine prolactin might be predicted to be an additional factor contributing to lacrimal physiologic dysfunction in Sjögren's disease.

The inhibitory influence of prolactin decreased as the prolactin dose increased to the level characteristic of pregnancy.⁵⁰ This finding is of interest in view of reports that

Schirmer's scores decreased⁵¹; rose Bengal staining scores increased⁵¹; and basal rates of lacrimal gland fluid production determined by cannulation of main excretory ducts decreased in term-pregnant rabbits, whereas rates of cholinergically induced fluid production increased,²¹ and the concentration of protein in the cholinergically induced fluid decreased.²¹ The decrease of lacrimal gland fluid protein concentration can be attributed to the prolactin-induced redirection of merocrine secretion from the exocrine direction to the paracrine direction.^{22,52} However, the decrease of basal fluid production rates in a setting of increased rose Bengal staining and an evidently competent fluid production mechanism suggests that the sensorimotor loop that acutely regulates fluid production is altered in pregnancy.

Prolonged exposure to 10 μ M CCh does not disrupt the integrity of the epithelial monolayers, but it causes cytopathologic changes^{23,24}; it alters membrane vesicle-mediated intracellular traffic in a way that might alter the proteolytic processing of potential autoantigens⁵³; and it significantly decreases cytosolic Ca^{2+} elevation, protein secretion,^{23,24} and Cl^- secretion (Fig. 3) in response to an optimal dose of CCh. The authors previously proposed that chronic exposure to CCh might model chronic exposure to agonistic M_3AChR autoantibodies.²³ This hypothesis appears untenable in view of the finding, noted earlier, that the inhibitory effects of anti- M_3AChR IgG on Ca^{2+} elevations in human labial salivary glands are acutely reversible.^{17,18} However, the diverse and profound effects that the low dose of CCh exerts in the ex vivo models suggest that it might be productive to consider increased signaling in the sensorimotor loop a potential factor in the development of lacrimal physiologic dysfunction subsequent to meibomian gland dysfunction.

Prolonged exposure to 1 mM histamine appeared to have slight inhibitory effects on cholinergically induced protein secretion,²⁴ but it decreased Cl^- secretion by 40% (Fig. 4). This finding suggests that some types of immune cell infiltrates may produce settings in which fluid production rates are decreased but fluid protein concentrations are increased. At 10 mM, histamine significantly decreased protein secretion,²⁴ and it prevented monolayer formation, effectively preventing cells from generating vectorial ion fluxes. Exposure to 1 mM 5-HT decreased protein secretion by 50%,²⁴ and it suppressed Ca^{2+} elevations more dramatically,²⁴ but it potentiated Cl^- secretion by 60%. Thus, there may be other settings in addition to pregnancy in which fluid production rates are increased but fluid protein concentrations are decreased.

Histamine and 5-HT are mast cell mediators as well as neurotransmitters. Mast cells are present in normal rodent

FIGURE 3. Influence of 18-hour exposure to CCh, 10 μ M, on I_{sc} induced by acute stimulation with the optimal CCh dose, 100 μ M. Control monolayers were not exposed to CCh during the 18 hours before measurement of I_{sc} . n = number of replicate trials from the acinar cell preparation for which data are presented. Monolayers from each of seven separate acinar cell preparations studied were treated with 10 μ M CCh, most in parallel with the other mediators. Similar results were observed in every preparation. Arrows indicate the time when the agonist was added.

A. Acute **B. 18 hr Exposure**

FIGURE 4. (A) Influences of acute stimulation with histamine or 5-HT on basal- and CCh-induced I_{sc} . None of the monolayers was exposed to histamine, 5-HT, or CCh prior to measurement of I_{sc} . Neither histamine nor 5-HT acutely stimulated Cl secretion in the replicate trials indicated by the traces. (B) Influences of 18-hour exposure to histamine or 5-HT on I_{sc} induced by acute stimulation with CCh. Neither mediator altered Cl secretion at doses of 1 μM . At doses of 1 mM, histamine impaired CCh-induced Cl secretion, whereas 5-HT potentiated it. Results for each condition were pooled from monolayers from two separate acinar cell preparations. n = total number of replicate monolayers. P values in *inset* indicate significance of differences from control determined by the Holm-Sidak method for pairwise multiple comparisons. Arrows indicate the time when the agonist was added. Double arrows indicate that when there are two agonists, they are both added at the same time.

lacrimal glands.^{54,55} They can also be detected in rabbit and human lacrimal glands, but they are much less frequent than in rodent lacrimal glands. Serotonergic neurons are present in lacrimal glands of several species.⁵⁴ Moreover, G protein-coupled receptor agonists, histamine, 5-HT, and CCh, may be emblematic of the actions of broad categories of noncytokine mediators produced by different types of infiltrating immune cells, as well, perhaps, of commonly prescribed systemic medications.

Acknowledgments

The authors thank Alan Yu for advice and generous access to equipment and facilities in his laboratories at the Keck School of Medicine of the University of Southern California during the course of this work, and Laurie Dustin, also of the Keck School of Medicine, for advice on statistical procedures.

Supported by National Eye Institute/National Institutes of Health Grants EY003040, EY005801, and EY015457 and by grants from Allergan, LLC, and Research to Prevent Blindness, Inc.

Disclosure: **S. Selvam**, None; **A.K. Mircheff**, Allergan (F, C); **S.C. Yiu**, None

References

- Wong J, Lan W, Ong LM, Tong L. Non-hormonal systemic medications and dry eye. *Ocul Surf*. 2011;9:197-226.
- Mathers WD, Stovall D, Lane JA, Zimmerman MB, Johnson S. Menopause and tear function: the influence of prolactin and sex hormones on human tear production. *Cornea*. 1998;17:353-358.
- Obata H, Yamamoto S, Horiuchi H, Machinami R. Histopathologic study of human lacrimal gland. Statistical analysis with special reference to aging. *Ophthalmology*. 1995;102:678-686.
- Damato BE, Allan D, Murray SB, Lee WR. Senile atrophy of the human lacrimal gland: the contribution of chronic inflammatory disease. *Br J Ophthalmol*. 1984;68:674-680.
- Waterhouse JP. Focal adenitis in salivary and lacrimal glands. *Proc R Soc Med*. 1963;56:911-917.
- Nasu M, Matsubara O, Yamamoto H. Post-mortem prevalence of lymphocytic infiltration of the lacrimal gland: a comparative study in autoimmune and non-autoimmune diseases. *Pathology*. 1984;143:11-15.
- Schaumberg DA, Sullivan DA, Buring JE, Dana MR. Prevalence of dry eye syndrome among US women. *Am J Ophthalmol*. 2003;136:318-326.
- Moss SE, Klein R, Klein BE. Prevalence of and risk factors for dry eye syndrome. *Arch Ophthalmol*. 2000;118:1264-1268.
- Lemp MA, Crews LA, Bron AJ, Foulks GN, Sullivan BD. Distribution of aqueous-deficient and evaporative dry eye in a clinic-based patient cohort: a retrospective study. *Cornea*. 2012;31:472-478.
- Sullivan BD, Whitmer D, Nichols KK, et al. An objective approach to dry eye disease severity. *Invest Ophthalmol Vis Sci*. 2010;51:6125-6130.
- Lemp MA, Bron AJ, Baudouin C, et al. Tear osmolarity in the diagnosis and management of dry eye disease. *Am J Ophthalmol*. 2011;151:792-798.
- Knop E, Knop N, Millar T, Obata H, Sullivan DA. The international workshop on meibomian gland dysfunction: report of the subcommittee on anatomy, physiology, and pathophysiology of the meibomian gland. *Invest Ophthalmol Vis Sci*. 2011;52:1938-1978.
- Mathers WD, Daley TE. Tear flow and evaporation in patients with and without dry eye. *Ophthalmology*. 1996;103:664-669.
- Tsubota K, Fujita H, Tsubota K, Takeuchi T. Mikulicz's disease and Sjögren's syndrome. *Invest Ophthalmol Vis Sci*. 2000;41:1666-1673.
- Fox RI, Maruyama T. Pathogenesis and treatment of Sjögren's syndrome. *Curr Opin Rheumatol*. 1997;9:393-399.
- Humphreys-Beher MG, Brayer J, Yamachika S, Peck AB, Jonsson R. An alternative perspective to the immune response in autoimmune exocrinopathy: induction of functional quiescence rather than destructive autoaggression. *Scand J Immunol*. 1999;49:7-10.
- Dawson LJ, Fox PC, Smith PM. Sjögren's syndrome—the non-apoptotic model of glandular hypofunction. *Rheumatology (Oxford)*. 2006;45:792-798.
- Dawson LJ, Stanbury J, Venn N, Hasdimitrakis B, Rogers SN, Smith PM. Antimuscarinic antibodies in primary Sjögren's syndrome reversibly inhibit the mechanism of fluid secretion by human submandibular salivary acinar cells. *Arthritis Rheum*. 2006;54:1165-1173.
- Caulfield VL, Balmer C, Dawson LJ, Smith PM. A role for nitric oxide-mediated glandular hypofunction in a non-apoptotic model for Sjögren's syndrome. *Rheumatology (Oxford)*. 2009;48:727-733.
- Zoukhri D, Hodges RR, Byon D, Kublin KL. Role of proinflammatory cytokines in the impaired lacrimation associated with autoimmune xerophthalmia. *Invest Ophthalmol Vis Sci*. 2002;43:1429-1436.
- Ding C, Chang N, Fong YC, et al. Interacting influences of pregnancy and corneal injury on rabbit lacrimal gland immunoarchitecture and function. *Invest Ophthalmol Vis Sci*. 2006;47:1368-1375.
- Wang Y, Chiu CT, Nakamura T, et al. Elevated prolactin redirects secretory vesicle traffic in rabbit lacrimal acinar cells. *Am J Physiol Endocrinol Metab*. 2007;292:E1122-E1134.
- Qian L, Wang Y, Xie J, et al. Biochemical changes contributing to functional quiescence in lacrimal gland acinar cells after chronic ex vivo exposure to a muscarinic agonist. *Scand J Immunol*. 2003;58:550-565.
- McDonald ML, Wang Y, Selvam S, et al. Cytopathology and exocrine dysfunction induced in ex vivo rabbit lacrimal gland acinar cell models by chronic exposure to histamine or serotonin. *Invest Ophthalmol Vis Sci*. 2009;50:3164-3175.
- Raina S, Preston GM, Guggino WB, Agre P. Molecular cloning and characterization of an aquaporin cDNA from salivary, lacrimal, and respiratory tissues. *J Biol Chem*. 1995;270:1908-1912.
- Matsuzaki T, Suzuki T, Koyama H, Tanaka S, Takata K. Aquaporin-5 (AQP5), a water channel protein, in the rat salivary and lacrimal glands: immunolocalization and effect of secretory stimulation. *Cell Tissue Res*. 1999;295:513-521.
- Ishida N, Hirai SI, Mita S. Immunolocalization of aquaporin homologs in mouse lacrimal glands. *Biochem Biophys Res Commun*. 1997;238:891-895.
- Dartt DA, Møller M, Poulsen JH. Lacrimal gland electrolyte and water secretion in the rabbit: localization and role of (Na⁺ + K⁺)-activated ATPase. *J Physiol*. 1981;321:557-569.
- Mircheff AK, Ingham CE, Lambert RW, Hales KL, Hensley CB, Yiu SC. Na⁺/H⁺ antiporter in lacrimal acinar cell basal-lateral membranes. *Invest Ophthalmol Vis Sci*. 1987;28:1726-1729.
- Lambert RW, Bradley ME, Mircheff AK. pH-sensitive anion exchanger in rat lacrimal acinar cells. *Am J Physiol Gastrointest Liver Physiol*. 1991;260:G517-G523.
- Walcott B, Birzgalis A, Moore LC, Brink PR. Fluid secretion and the Na⁺, K⁺, 2Cl⁻ cotransporter (NKCC 1) in mouse exorbital lacrimal gland. *Am J Physiol Cell Physiol*. 2005;289:C860-C867.
- Ubels JL, Hoffman HM, Srikanth S, Resau JH, Webb CP. Gene expression in rat lacrimal gland duct cells collected using laser

- capture microdissection: evidence for K⁺ secretion by duct cells. *Invest Ophthalmol Vis Sci*. 2006;47:1876–1885.
33. Ding C, Parsa L, Nandoskar P, Zhao P, Wu K, Wang Y. Duct system of the rabbit lacrimal gland: structural characteristics and role in lacrimal secretion. *Invest Ophthalmol Vis Sci*. 2010;51:2960–2967.
 34. Nandoskar P, Wang Y, Wei R, et al. Changes of chloride channels in the lacrimal glands of a rabbit model of Sjögren syndrome. *Cornea*. 2012;31:273–279.
 35. Mircheff AK. Lacrimal fluid and electrolyte secretion: a review. *Curr Eye Res*. 1989;8:607–617.
 36. Yiu SC, Lambert RW, Bradley ME, et al. Stimulation-associated redistribution of Na, K-ATPase in rat lacrimal gland. *J Membr Biol*. 1988;102:185–194.
 37. Gierow JP, Yang T, Bekmezian A, et al. Na-K-ATPase in lacrimal gland acinar cell endosomal system: correcting a case of mistaken identity. *Am J Physiol Cell Physiol*. 1996;271:C1685–C1698.
 38. Bradley ME, Azuma KK, McDonough AA, Mircheff AK, Wood RL. Surface and intracellular pools of Na, K-ATPase catalytic and immuno-activities in rat exorbital lacrimal gland. *Exp Eye Res*. 1993;57:403–413.
 39. Selvam S, Thomas PB, Gukasyan HJ, et al. Transepithelial bioelectrical properties of rabbit acinar cell monolayers on polyester membrane scaffolds. *Am J Physiol Cell Physiol*. 2007;293:C1412–C1419.
 40. Fox RI, Kang HI, Ando D, Abrams J, Pisa E. Cytokine mRNA expression in salivary gland biopsies of Sjögren's syndrome. *J Immunol*. 1994;152:5532–5539.
 41. Boumba D, Skopouli FN, Moutsopoulos HM. Cytokine mRNA expression in the labial salivary gland tissues from patients with primary Sjögren's syndrome. *Br J Rheumatol*. 1995;34:326–333.
 42. Ogawa N, Dang H, Lazaridis K, McGuff HS, Aufdemorte TB, Talal N. Analysis of transforming growth factor beta and other cytokines in autoimmune exocrinopathy (Sjögren's syndrome). *J Interferon Cytokine Res*. 1995;15:759–767.
 43. Zhu Z, Stevenson D, Schechter JE, Mircheff AK, Atkinson R, Trousdale MD. Lacrimal histopathology and ocular surface disease in a rabbit model of autoimmune dacryoadenitis. *Cornea*. 2003;22:25–32.
 44. Ding C, Lu M, Huang J. Na(+)/K(+)-ATPase in the lacrimal glands of rabbits and its changes during induced autoimmune dacryoadenitis. *Mol Vis*. 2011;17:2368–2379.
 45. Ding C, Nandoskar P, Lu M, Thomas P, Trousdale MD, Wang Y. Changes of aquaporins in the lacrimal glands of a rabbit model of Sjögren's syndrome. *Curr Eye Res*. 2011;36:571–578.
 46. El Miedany YM, Ahmed I, Moustafa H, El Baddini M. Hyperprolactinemia in Sjögren's syndrome: a patient subset or a disease manifestation? *Joint Bone Spine*. 2004;71:203–208.
 47. Taiym S, Haghighat N, Al-Hashimi I. A comparison of the hormone levels in patients with Sjögren's syndrome and healthy controls. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2004;97:579–583.
 48. Haga HJ, Rygh T. The prevalence of hyperprolactinemia in patients with primary Sjögren's syndrome. *J Rheumatol*. 1999;26:1291–1295.
 49. Steinfeld S, Rommes S, François C, et al. Big prolactin 60 kDa is overexpressed in salivary glandular epithelial cells from patients with Sjögren's syndrome. *Lab Invest*. 2000;80:239–247.
 50. McNeilly AS, Friesen HG. Prolactin during pregnancy and lactation in the rabbit. *Endocrinology*. 1978;102:1548–1554.
 51. Ding C, Lu M, Huang J. Changes of the ocular surface and aquaporins in the lacrimal glands of rabbits during pregnancy. *Mol Vis*. 2011;17:2847–2855.
 52. Schechter J, Carey J, Wallace M, Wood R. Distribution of growth factors and immune cells are altered in the lacrimal gland during pregnancy and lactation. *Exp Eye Res*. 2000;71:129–142.
 53. Rose CM, Qian L, Hakim L, et al. Accumulation of catalytically active proteases in lacrimal gland acinar cell endosomes during chronic ex vivo muscarinic receptor stimulation. *Scand J Immunol*. 2005;61:36–50.
 54. Williams RM, Singh J, Sharkey KA. Innervation and mast cells of the rat exorbital lacrimal gland: the effects of age. *J Auton Nerv Syst*. 1994;47:95–108.
 55. Rios JD, Horikawa Y, Chen L, et al. Age-dependent alterations in mouse exorbital lacrimal gland structure, innervation and secretory response. *Exp Eye Res*. 2005;80:477–491.