them for outpatients, a license by an appropriate national body may be required in certain countries. The protocol used for the production of serum eye drops determines their composition and efficacy. An optimized protocol for the production was recently published.[113] Concentrations between 20% and 100% of serum have been used. The efficacy seems to be dose-dependent.

Because of significant variations in patient populations, production and storage regimens, and treatment protocols, the efficacy of serum eye drops in dry eyes has varied substantially between studies.[113] Three published prospective randomized studies with similar patient populations (predominantly immune disease associated dry eye (ie, Sjogren syndrome) are available. When comparing 20% serum with 0.9% saline applied 6 times per day, Tananuvat et al found only a trend toward improvement of symptoms and signs of dry eyes,[114] whereas Kojima et al reported significant improvement of symptom scores, fluorescein-breakup time (**FBUT**), and fluorescein and rose bengal staining.[115]

A prospective clinical cross-over trial compared 50% serum eyedrops against the commercial lubricant previously used by each patient. Symptoms improved in 10 out 16 patients, and impression cytological findings improved in 12 out of 25 eyes.[116] Noda-Tsuruya and colleagues found that 20% autologous serum significantly improved TFBUT and decreased conjunctival rose bengal and cornea fluorescein staining 1-3 months postoperatively, compared to treatment with artificial tears, which did not change these parameters.[117] Additional reports of successful treatment of persistent epithelial defects—where success is more clearly defined as "healing of the defect"—with autologous serum substantiate the impression that this is a valuable therapeutic option for ocular surface disease.[118]

2. Salivary Gland Autotransplantation

Salivary submandibular gland transplantation is capable of replacing deficient mucin and the aqueous tear film phase. This procedure requires collaboration between an ophthalmologist and a maxillofacial surgeon. With appropriate microvascular anastomosis, 80% of grafts survive. In patients with absolute aqueous tear deficiency, viable submandibular gland grafts, in the long-term, provide significant improvement of Schirmer test FBUT, and rose bengal staining, as well as reduction of discomfort and the need for pharmaceutical tear substitutes. Due to the hypoosmolarity of saliva, compared to tears, excessive salivary tearing can induce a microcystic corneal edema, which is temporary, but can lead to epithelial defects.[110] Hence, this operation is indicated only in end-stage dry eye with an absolute aqueous tear deficiency (Schirmer-test wetting of 1 mm or less), a conjunctivalized surface epithelium, and persistent severe pain despite punctal occlusion and at least hourly application of unpreserved tear substitutes. For this group of patients, such surgery is capable of substantially reducing discomfort, but often has no effect on vision.[119,120]

E. Anti-Inflammatory Therapy

Disease or dysfunction of the tear secretory glands leads to changes in tear composition, such as hyperosmolarity, that stimulate the production of inflammatory mediators on the ocular surface.[31,121] Inflammation may, in turn, cause dysfunction or disappearance of cells responsible for tear secretion or retention.[122] Inflammation can also be initiated by chronic irritative stress (eg, contact lenses) and systemic inflammatory/autoimmune disease (eg, rheumatoid arthritis). Regardless of the initiating cause, a vicious circle of inflammation can develop on the ocular surface in dry eye that leads to ocular surface disease. Based on the concept that inflammation is a key component of the pathogenesis of dry eye, the efficacy of a number of anti-inflammatory agents for treatment of dry eye has been evaluated in clinical trials and animal models.

1. Cyclosporine

The potential of cyclosporine-A (**CsA**) for treating dry eye disease was initially recognized in dogs that develop spontaneous KCS.[123] The therapeutic efficacy of CsA for human KCS was then documented in several small, single-center, randomized, double-masked clinical trials.[124,125] CsA emulsion for treatment of KCS was subsequently evaluated in several large multicenter, randomized, double-masked clinical trials.

In a Phase 2 clinical trial, four concentrations of CsA (0.05%, 0.1%, 0.2%, or 0.4%) administered twice daily to both eyes of 129 patients for 12 weeks was compared to vehicle treatment of 33 patients.[126] CsA was found to significantly decrease conjunctival rose bengal staining, superficial punctate keratitis, and ocular irritation symptoms (sandy or gritty feeling, dryness, and itching) in a subset of 90 patients with moderate-to-severe KCS. There was no clear dose response; CsA 0.1% produced the most consistent improvement in objective endpoints, whereas CsA 0.05% gave the most consistent improvement in patient symptoms (Level 1).

Two independent Phase 3 clinical trials compared twice-daily treatment with 0.05% or 0.1% CsA or vehicle in 877 patients with moderate-to-severe dry eye disease.[127] When the results of the two Phase 3 trials were combined for statistical analysis, patients treated with CsA, 0.05% or 0.1%, showed significantly ($P < 0.05$) greater improvement in two objective signs of dry eye disease (corneal fluorescein staining and anesthetized Schirmer test values) compared to those treated with vehicle. An increased Schirmer test score was observed in 59% of patients treated with CsA, with 15% of patients having an increase of 10 mm or more. In contrast, only 4% of vehicle-treated patients had this magnitude of change in their Schirmer test scores ($P < 0.0001$).

CsA 0.05% treatment also produced significantly greater improvements ($P < 0.05$) in three subjective measures of dry eye disease (blurred vision symptoms, need for concomitant artificial tears, and the global response to treatment). No dose-response effect was noted. Both doses of CSA exhibited an excellent safety profile with no significant systemic or ocular adverse events, except for transient burning

symptoms after instillation in 17% of patients. Burning was reported in 7% of patients receiving the vehicle. No CsA was detected in the blood of patients treated with topical CsA for 12 months. Clinical improvement from CsA that was observed in these trials was accompanied by improvement in other disease parameters. Treated eyes had an approximately 200% increase in conjunctival goblet cell density.[128] Furthermore, there was decreased expression of immune activation markers (ie, HLA-DR), apoptosis markers (ie, Fas), and the inflammatory cytokine IL-6 by the conjunctival epithelial cells.[129,130]The numbers of CD3-, CD4-, and CD8-positive T lymphocytes in the conjunctiva decreased in cyclosporine-treated eyes, whereas vehicle-treated eyes showed an increased number of cells expressing these markers.[131] After treatment with 0.05% cyclosporine, there was a significant decrease in the number of cells expressing the lymphocyte activation markers CD11a and HLA-DR, indicating less activation of lymphocytes compared with vehicle-treated eyes.

Two additional immunophilins, pimecrolimus and tacrolimus, have been evaluated in clinical trials of KCS.

2. Corticosteroids

a. Clinical Studies

Corticosteroids are an effective anti-inflammatory therapy in dry eye disease. Level I evidence is published for a number of corticosteroid formulations. In a 4-week, double-masked, randomized study in 64 patients with KCS and delayed tear clearance, loteprednol etabonate 0.5% ophthalmic suspension (Lotemax [Bausch and Lomb, Rochester, NY]), q.i.d., was found to be more effective than its vehicle in improving some signs and symptoms.[132]

In a 4-week, open-label, randomized study in 32 patients with KCS, patients receiving fluorometholone plus artificial tear substitutes (**ATS**) experienced lower symptom severity scores and lower fluorescein and rose bengal staining than patients receiving either ATS alone or ATS plus flurbiprofen.[133]

A prospective, randomized clinical trial compared the severity of ocular irritation symptoms and corneal fluorescein staining in two groups of patients, one treated with topical nonpreserved methylprednisolone for 2 weeks, followed by punctal occlusion (Group 1), with a group that received punctal occlusion alone (Group 2).[134] After 2 months, 80% of patients in Group 1 and 33% of patients in Group 2 had complete relief of ocular irritation symptoms. Corneal fluorescein staining was negative in 80% of eyes in Group 1 and 60% of eyes in Group 2 after 2 months. No steroid-related complications were observed in this study.

Level III evidence is also available to support the efficacy of corticosteroids. In an open-label, non-comparative trial, extemporaneously formulated nonpreserved methylprednisolone 1% ophthalmic suspension was found to be clinically effective in 21 patients with Sjogren syndrome KCS.[135] In a review, it was stated that "...clinical improvement of KCS has been observed after therapy with anti-inflammatory agents, including corticosteroids."[136]

In the US Federal Regulations, ocular corticosteroids receiving "class labeling" are indicated for the treatment "...of steroid responsive inflammatory conditions of the palpebral and bulbar conjunctiva, cornea and anterior segment of the globe such as allergic conjunctivitis, acne rosacea, superficial punctate keratitis, herpes zoster keratitis, iritis, cyclitis, selected infective conjunctivitides, when the inherent hazard of steroid use is accepted to obtain an advisable diminution in edema and inflammation." We interpret that KCS is included in this list of steroid-responsive inflammatory conditions.[137-140]

b. Basic Research

Corticosteroids are the standard anti-inflammatory agent for numerous basic research studies of inflammation, including the types that are involved in KCS. The corticosteroid methylprednisolone was noted to preserve corneal epithelial smoothness and barrier function in an experimental murine model of dry eye.[141] This was attributed to its ability to maintain the integrity of corneal epithelial tight junctions and decrease desquamation of apical corneal epithelial cells.[142] A concurrent study showed that methylprednislone prevented an increase in MMP-9 protein in the corneal epithelium, as well as gelatinase activity in the corneal epithelium and tears in response to experimental dry eye.[141]

Preparations of topically applied androgen and estrogen steroid hormones are currently being evaluated in randomized clinical trials. A trial of topically applied 0.03% testosterone was reported to increase the percentage of patients that had meibomian gland secretions with normal viscosity and to relieve discomfort symptoms after 6 months of treatment compared to vehicle.[143] TFBUT and lipid layer thickness were observed to increase in a patient with KCS who was treated with topical androgen for 3 months.[144] Tear production and ocular irritation symptoms were reported to increase following treatment with topical 17 beta-oestradiol solution for 4 months.[145]

3. Tetracyclines

a. Properties of Tetracyclines and Their Derivatives

1) Antibacterial Properties

The antimicrobial effect of oral tetracycline treatment analogues (eg, minocycline, doxycline) has previously been discussed by Shine et al,[146] Dougherty et al,[147] and Ta et al.[148] It is hypothesized that a decrease in bacterial flora producing lipolytic exoenzymes[146,148] and inhibition of lipase production[147] with resultant decrease in meibomian lipid breakdown products[146] may contribute to improvement in clinical parameters in dry eye-associated diseases.

2) Anti-Inflammatory Properties

The tetracyclines have anti-inflammatory as well as antibacterial properties that may make them useful for the management of chronic inflammatory diseases. These agents decrease the activity of collagenase, phospholipase A2, and several matrix metalloproteinases, and they de-

crease the production of interleukin (**IL**)-1 and tumor necrosis factor (**TNF**)-alpha in a wide range of tissues, including the corneal epithelium.[149-151] At high concentrations, tetracyclines inhibit staphylococcal exotoxin-induced cytokines and chemokines.[152,153]

3) Anti-angiogenic Properties

Angiogenesis, the formation of new blood vessels, occurs in many diseases. These include benign conditions (eg, rosacea) and malignant processes (eg, cancer). Minocycline and doxycycline inhibit angiogenesis induced by implanted tumors in rabbit cornea.[154] The anti-angiogenic effect of tetracycline may have therapeutic implications in inflammatory processes accompanied by new blood vessel formation. Well-controlled studies must be performed, at both the laboratory and clinical levels, to investigate this potential.[155]

b. Clinical Applications of Tetracycline

1) Acne Rosacea

Rosacea, including its ocular manifestations, is an inflammatory disorder, occurring mainly in adults, with peak severity in the third and fourth decades. Current recommendations are to treat rosacea with long-term doxycycline, minocycline, tetracycline, or erythromycin.[156] These recommendations may be tempered by certain recent reports that in women, the risk of developing breast cancer and of breast cancer morbidity increases cumulatively with duration of antibiotic use, including tetracyclines.[157,158] Another large study did not substantiate these findings.[159]

Tetracyclines and their analogues are effective in the treatment of ocular rosacea,[160,161] for which a single daily dose of doxycycline may be effective.[162] In addition to the anti-inflammatory effects of tetracyclines, their ability to inhibit angiogenesis may contribute to their effectiveness in rosacea-related disorders. Factors that promote angiogenesis include protease-triggered release of angiogenic factors stored in the extracellular matrix, inactivation of endothelial growth factor inhibitors, and release of angiogenic factors from activated macrophages.[155,163]

Tetracyclines are also known to inhibit matrix metalloproteinase expression, suggesting a rationale for their use in ocular rosacea.[164] Although tetracyclines have been used for management of this disease, no randomized, placebo-controlled, clinical trials have been performed to assess their efficacy.[153]

2) Chronic Posterior Blepharitis: Meibomianitis, Meibomian Gland Dysfunction

Chronic blepharitis is typically characterized by inflammation of the eyelids. There are multiple forms of chronic blepharitis, including staphylococcal, seborrheic (alone, mixed seborrheic/staphylococcal, seborrheic with meibomian seborrhea, seborrheic with secondary meibomitis), primary meibomitis, and others, like atopic, psoriatic, and fungal infections.[165] Meibomian gland dysfunction (**MGD**) has been associated with apparent aqueous-deficient dry eye. Use of tetracycline in patients with meibomianitis has been shown to decrease lipase production by tetracycline-sensitive as well as resistant strains of staphylococci. This decrease in lipase production was associated with clinical improvement.[147] Similarly, minocycline has been shown to decrease the production of diglycerides and free fatty acids in meibomian secretions. This may be due to lipase inhibition by the antibiotic or a direct effect on the ocular flora.[146] One randomized, controlled clinical trial of tetracycline in ocular rosacea compared symptom improvement in 24 patients treated with either tetracycline or doxycycline.[166] All but one patient reported an improvement in symptoms after 6 weeks of therapy. No placebo group was included in this trial.

A prospective, randomized, double-blind, placebo-controlled, partial crossover trial compared the effect of oxytetracycline to provide symptomatic relief of blepharitis with or without rosacea. Only 25% of the patients with blepharitis without rosacea responded to the antibiotic, whereas 50% responded when both diseases were present.[167] In another trial of 10 patients with both acne rosacea and concomitant meibomianitis, acne rosacea without concomitant ocular involvement, or seborrheic blepharitis, minocycline 50 mg daily for 2 weeks followed by 100 mg daily for a total of 3 months significantly decreased bacterial flora ($P = 0.0013$). Clinical improvement was seen in all patients with meibomianitis.[148]

Because of the improvement observed in small clinical trials of patients with meibomianitis, the American Academy of Ophthalmology recommends the chronic use of either doxycycline or tetracycline for the management of meibomianitis.[165] Larger randomized placebo-controlled trials assessing symptom improvement rather than surrogate markers are needed to clarify the role of this antibiotic in blepharitis treatment.[153] Tetracycline derivatives (eg, minocycline, doxycycline) have been recommended as treatment options for chronic blepharitis because of their high concentration in tissues, low renal clearance, long half-life, high level of binding to serum proteins, and decreased risk of photosensitization.[168]

Several studies have described the beneficial effects of minocycline and other tetracycline derivatives (eg, doxycycline) in the treatment of chronic blepharitis.[146,147,168,169] Studies have shown significant changes in the aqueous tear parameters, such as tear volume and tear flow, following treatment with tetracycline derivatives (eg, minocycline). One study also demonstrated a decrease in aqueous tear production that occurred along with clinical improvement.[170]

A recently published randomized, prospective study by Yoo Se et al compared different doxycycline doses in 150 patients (300 eyes) who had chronic meibomian gland dysfunction and who did not respond to lid hygiene and topical therapy for more than 2 months.[171] All topical therapy was stopped for at least 2 weeks prior to beginning the study. After determining the TFBUT and Schirmer test scores, patients were divided into three groups: a high dose group (doxycycline, 200 mg, twice a day), a low dose group (doxycycline, 20 mg, twice a day) and a control group (placebo). After one month, TFBUT, Schirmer scores, and

Table 2. Dry eye severity grading scheme

Dry Eye Severity Level	1	2	3	4*
Discomfort, severity & frequency	Mild and/or episodic occurs under environ stress	Moderate episodic or chronic, stress or no stress	Severe frequent or constant without stress	Severe and/or disabling and constant
Visual symptoms	None or episodic mild fatigue	Annoying and/or activity limiting episodic	Annoying, chronic and/or constant limiting activity	Constant and/or possibly disabling
Conjunctival injection	None to mild	None to mild	+/–	+/++
Conjunctival staining	None to mild	Variable	Moderate to marked	Marked
Corneal staining (severity/location)	None to mild	Variable	Marked central	
Corneal/tear signs	None to mild	Mild debris↓ meniscus	Filamentary keratitis, mucus clumping↑ tear debris	Filamentary keratitis, mucus clumping↑ tear debris
Lid/meibomian glands	MGD variably present	MGD variably present	Frequent	Trichiasis, keratinization, symblepharon
TFBUT (sec)	Variable	≤ 10	≤ 5	Immediate
Schirmer score (mm/5 min)	Variable	≤ 10	≤ 5	≤ 2

*Must have signs AND symptoms. TBUT: fluorescein tear break-up time. MGD: meibomian gland disease

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symptoms improved. Both the high- and low-dose groups had statistically significant improvement in TFBUT after treatment. This implies that low-dose doxycycline (20 mg twice a day) therapy may be effective in patients with chronic meibomian gland dysfunction.

3) Dosage and Safety

Systemic administration of tetracyclines is widely recognized for the ability to suppress inflammation and improve symptoms of meibomianitis.[172,173] The optimal dosing schedule has not been established; however, a variety of dose regimens have been proposed including 50 or 100 mg doxycycline once a day,[174] or an initial dose of 50 mg a day for the first 2 weeks followed by 100 mg a day for a period of 2.5 months, in an intermittent fashion.[146-148,170] Others have proposed use of a low dose of doxycycline (20 mg) for treatment of chronic blepharitis on a long-term basis.[171] The safety issues associated with long-term oral tetracycline therapy, including minocycline, are well known. Many management approaches have been suggested for the use of tetracycline and its derivatives; however, a safe but adequate option in management needs to be considered because of the new information regarding the potentially hazardous effects of prolonged use of oral antibiotics. A recent study suggested that a 3-month course of 100 mg of minocycline might be sufficient to bring significant meibomianitis under control, as continued control was maintained for at least 3 months after cessation of therapy.[170]

In an experimental murine model of dry eye, topically applied doxycycline was found to preserve corneal epithelial smoothness and barrier function.[141] It also preserved the integrity of corneal epithelial tight junctions in dry eyes, leading to a marked decrease in apical corneal epithelial cell desquamation.[142] This corresponded to a decrease in MMP-9 protein in the corneal epithelium and reduced gelatinase activity in the corneal epithelium and tears.[141]

F. Essential Fatty Acids

Essential fatty acids are necessary for complete health. They cannot be synthesized by vertebrates and must be obtained from dietary sources. Among the essential fatty acids are 18 carbon omega-6 and omega-3 fatty acids. In the typical western diet, 20-25 times more omega-6 than omega-3 fatty acids are consumed. Omega-6 fatty acids are precursors for arachidonic acid and certain proinflammatory lipid mediators (PGE2 and LTB4). In contrast, certain omega-3 fatty acids (eg, EPA found in fish oil) inhibit the synthesis of these lipid mediators and block production of IL-1 and TNF-alpha.[175,176]

A beneficial clinical effect of fish oil omega-3 fatty acids on rheumatoid arthritis has been observed in several double-masked, placebo-controlled clinical trials.[177,178] In a prospective, placebo-controlled clinical trial of the essential fatty acids, linoleic acid and gamma-linoleic acid administered orally twice daily produced significant improvement in ocular irritation symptoms and ocular surface lissamine green staining.[179] Decreased conjunctival HLA-DR staining also was observed.

Table 3. Dry eye menu of treatments

Artificial tears substitutes
Gels/Ointments
Moisture chamber spectacles
Anti-inflammatory agents (topical CsA and corticosteroids, omega-3 fatty acids)
Tetracyclines
Plugs
Secretogogues
Serum
Contact lenses
Systemic immunosuppressives
Surgery (AMT, lid surgery. tarsorrhaphy, MM & SG transplant)

G. Environmental Strategies

Factors that may decrease tear production or increase tear evaporation, such as the use of systemic anticholinergic medications (eg, antihistamines and antidepressants) and desiccating environmental stresses (eg, low humidity and air conditioning drafts) should be minimized or eliminated.[180-182] Video display terminals should be lowered below eye level to decrease the interpalpebral aperture, and patients should be encouraged to take periodic breaks with eye closure when reading or working on a computer.[183] A humidified environment is recommended to reduce tear evaporation. This is particularly beneficial in dry climates and high altitudes. Nocturnal lagophthalmos can be treated by wearing swim goggles, taping the eyelid closed, or tarsorrhapy.

Table 4. Treatment recommendations by severity level

Level 1:
Education and environmental/dietary modifications
Elimination of offending systemic medications
Artificial tear substitutes, gels/ointments
Eye lid therapy

Level 2:
If Level 1 treatments are inadequate, add:
Anti-inflammatories
Tetracyclines (for meibomianitis, rosacea)
Punctal plugs
Secretogogues
Moisture chamber spectacles

Level 3:
If Level 2 treatments are inadequate, add:
Serum
Contact lenses
Permanent punctal occlusion

Level 4:
If Level 3 treatments are inadequate, add:
Systemic anti-inflammatory agents
Surgery (lid surgery, tarsorrhaphy; mucus membrane, salivary gland, amniotic membrane transplantation)

Modified from: International Task Force Guidelines for Dry Eye[185]

IV. TREATMENT RECOMMENDATIONS

In addition to material presented above, the subcommittee members reviewed the Dry Eye Preferred Practice Patterns of the American Academy of Ophthalmology and the International Task Force (**ITF**) Delphi Panel on dry eye treatment prior to formulating their treatment guidelines.[184,185] The group favored the approach taken by the ITF, which based treatment recommendations on disease severity. A modification of the ITF severity grading scheme that contains 4 levels of disease severity based on signs and symptoms was formulated (Table 2). The subcommittee members chose treatments for each severity level from a menu of therapies for which evidence of therapeutic effect has been presented (Table 3). The treatment recommendations by severity level are presented in Table 4. It should be noted that these recommendations may be modified by practitioners based on individual patient profiles and clinical experience. The therapeutic recommendations for level 4 severity disease include surgical modalities to treat or prevent sight-threatening corneal complications. Discussion of these therapies is beyond the scope of this report.

V. UNANSWERED QUESTIONS AND FUTURE DIRECTIONS

There have been tremendous advances in the treatment of dry eye and ocular surface disease in the last two decades, including FDA approval of cyclosporin emulsion as the first therapeutic agent for treatment of KCS in the United States. There has been a commensurate increase in knowledge regarding the pathophysiology of dry eye. This has led to a paradigm shift in dry eye management from simply lubricating and hydrating the ocular surface with artificial tears to strategies that stimulate natural production of tear constituents, maintain ocular surface epithelial health and barrier function, and inhibit the inflammatory factors that adversely impact the ability of ocular surface and glandular epithelia to produce tears. Preliminary experience using this new therapeutic approach suggests that quality of life can be improved for many patients with dry eye and that initiating these strategies early in the course of the disease may prevent potentially blinding complications of dry eye. It is likely that future therapies will focus on replacing specific tear factors that have an essential role in maintaining ocular surface homeostasis or inhibiting key inflammatory mediators that cause death or dysfunction of tear secreting cells. This will require additional research to identify these key factors and better diagnostic tests to accurately measure their concentrations in minute tear fluid samples. Furthermore, certain disease parameters may be identified that will identify whether a patient has a high probability of responding to a particular therapy. Based on the progress that has been made and the number of therapies in the pipeline, the future of dry eye therapy seems bright.

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(Parenthetical codes following references indicate level of evidence, as described in Table 1. CS = Clinical Study; BS = Basic Science.)

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Research in Dry Eye:
Report of the Research Subcommittee of the International Dry Eye WorkShop (2007)

ABSTRACT Members of the dry eye subcommittee reviewed research into the basic mechanisms underlying dry eye disease. Evidence was evaluated concerning the tear film, lacrimal gland and accessory lacrimal glands, ocular surface epithelia (including cornea and conjunctiva), meibomian glands, lacrimal duct system and the immune system. Consideration was given to both animal and human research data. Results are presented as a series of information matrices, identifying what is known and providing supporting references. An attempt is made to identify areas for further investigation.

KEY WORDS DEWS, dry eye, Dry Eye WorkShop, mechanisms of dry eye, pathology of dry eye

I. INTRODUCTION

Members of the Research Subcommittee were grouped according to their particular areas of expertise and asked to review the evidence for the basic mechanisms of dry eye pathology within that area. To facilitate this, a standardized template was developed (the DEWS Research Committee Report Form—Appendix 1 [access at: www.tfos **EDITOR; INSERT COMPLETE INFO**]), which members used to present their findings. Based on the information derived from the returned reports, information matrices were developed.

Evidence related to the tear film, lacrimal gland and accessory lacrimal glands, ocular surface epithelia (including cornea and conjunctiva), meibomian glands, lacrimal duct system, and the immune system was evaluated. Consideration was given to both animal and human research data. Results are presented in a matrix of information that identifies what is known, with supporting references, and identifies areas for further investigation.

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Proprietary interests of Subcommittee members are disclosed on pages (EDITOR; INSERT PAGE NUMBERS)

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II. GOALS OF THE RESEARCH SUBCOMMITTEE

Goals of the Research Subcommittee were as follows:

A. To consider whether there is sufficient evidence to define the basic mechanisms underlying dry eye disease.
   1. To summarize the state of knowledge about primary alterations and/or secondary responses of the following ocular and systemic components that contribute to tear film dysfunction.
      a. Tear film
      b. Lacrimal gland and accessory lacrimal glands
      c. Ocular surface epithelia, cornea, conjunctiva
      d. Meibomian gland
      e. Lacrimal duct system
      f. Immune system
   2. To construct an information matrix to identify areas where knowledge is insufficient and to determine if there are common pathologies across the syndrome.
   3. To identify areas where clinical information is available or lacking.

B. Based on data derived from Part A, to answer Question 2: Is the state of basic knowledge on mechanisms of dry eye sufficient to determine how these give rise to disease symptoms?

C. Develop, if possible, definitions of the mechanism of dry eye pathology or develop major hypotheses on the mechanism that can be tested.

III. THE TEARS AND TEAR FILM

A. Human Disease

The evidence presented at the last dry eye workshop report (National Eye Institute [**NEI**]/Industry Workshop of 1995, hereafter referred to as the "1995 Workshop") indi-

OUTLINE

cated that tear film osmolarity is increased in all forms of dry eye (**DE**) and that tear volume and certain lacrimal tear proteins, such as lysozyme and lactoferrin, are decreased in aqueous-deficient dry eye.[1] An evaporative form of dry eye was also recognized, caused, for example, by a decreased integrity of the tear film lipid layer.

New evidence since the 1995 Workshop indicates that meibomian lipid composition and distribution is altered in DE and a number of bioactive tear proteins, including plasmin, matrix metalloproteinases (**MMPs**), defensive molecules, and phospholipase A2 IIa in DE are increased. There is also an increase in inflammatory cytokines in non-Sjogren syndrome (**NSS**) dry eye, as well as in Sjogren syndrome (**SS**) dry eye, and a decrease in goblet cell mucin MUC5AC in keratoconjunctivitis sicca (**KCS**) and SS (Table 1).

Given the sparsity of information available about the changes in the composition of the tear film listed above, it is unclear how the changes in human tear composition relate to tear dysfunction. To better understand the mechanism of dry eye disease, there is need for proteomic, lipidomic, and glycomic analyses of the tears from large, well-defined, staged, and age-matched patients or subject populations, to develop biomarkers specific to dry eye disease. Progress has been made in developing proteomic baseline studies of tear proteins, but studies comparing normal and dry eye tears are lacking.[41-44] Mass spectrometry is a powerful analytical tool for identification[45] of molecules and compounds, and it is being used to develop a standard lipid profile of normal tears and to identify specific component differences in the tears from DE models.

The application of mass spectrometry to the characterization and identification of the lipids of the meibomian gland secretions is demonstrating that the previously reported compositions are in need of revision. Complicating these efforts is the observation that the lipids are very diverse in class and functionality. Different analytical approaches for isolation and detection are needed to differentiate lipid classes.

High throughput mass spectroscopic and glycan array methodologies are now available for glycomic analysis, and these could be used to analyze tear glycans in normal and DE patients. Similarly, determination of ratios and amounts of membrane-associated and secreted mucins in tear film is necessary. It will also be important to determine the relationship between various measures of tear stability (eg, tear film breakup time [**TFBUT**]) and the mucin and lipid quantity and character of the tears.

Abbreviations used in text and tables

↑ = Increase in/increased
↓ = Decrease in/decreased
Δ = Change in/changes to
−/− = Homozygous null mouse
− = totally depleted
ACAT-1 = Acyl-CoA:cholesterol acyltransferase-1
Auto-AG = Autoantigen
BUT = Breakup time
CALT = Conjunctiva-associated lymphoid tissue
Chr Bleph = Chronic blepharitis
CIC = Cicatrizing disease
Conj = Conjunctiva/conjunctival
Cont lens = Contact lens
DE = Dry eye
DES = Dry eye syndrome
EDA = Ectodermal dysplasia
ENV STR = Environmental stress
epi = Epithelia/epithelial
Epi. Diff/sq metaplasia = Epithelial differentiation/squamous metaplasia
GVHD = Graft vs host disease
KCS = Keratoconjunctivitis sicca
Lac = Lacrimal
Meibom = Meibomian
↓MG = Loss of meibomian glands
MGD = Meibomian gland dysfunction
NSS = Non-Sjogren syndrome
NSS/ACQ = Aqueous-deficient non-Sjogren syndrome
Nasolac = Nasolacrimal
NLD = Nasolacrimal duct
RA-MGD = Retinoic acid induced MGD
SCOP = Scopolamine
siRNA = Small interfering RNA
Spont DE = Spontaneous dry eye
SS = Sjogren syndrome
TALT = Tear duct-associated lymphoid tissue
TBUT = Tear breakup time
Undif KCS = undifferentiated keratoconjunctivitis sicca
↓Vit A = Vitamin A deficient
–Vit A = Vitamin A totally depleted

Table 1. Information matrix: human tear film

	KCS*	NSS	SS	MGD	Androgen Deficiency	Contact Lens/DE	Refs Refs
Tear Volume/Osmolarity:							
↑ Osmolarity, ↓ Volume	✓	✓	✓	✓	✓	✓	2-6
↑ Evaporation	✓			✓			1, 7-9
↓ Meniscus	✓	✓	✓	✓	✓	✓	5, 10-13
Correlation: Evaporation to osmolarity & lipid layer	✓						14, 15
↓ BUT, ↑ Surface tension	✓	✓	✓	✓	✓	✓	5, 12, 16-20
Mucins:							
↓ Glycoproteins, MUC5AC	✓		✓	✓			21-23
Lipids:							
Δ Lipid patterns, Distribution			✓	✓			24, 25
↓ Polar lipids	✓						26
↓ Lipid layer, ↑ Evaporation	✓						14
Proteins:							
Δ Proteins	✓						27, 28
↑ Plasmin levels	✓						29
↑ MMPs				✓			30, 31
↑ Inflammation markers, PRPs	✓			✓			32
↓ Lactoferrin							33
↑ Nine defensive molecules				✓			34
↓ Lysozyme, Lactoferrin							35
↑ Phospholipase A2 IIa	✓					✓	36, 37
Inflammatory Mediators:							
Proinflammatory cytokines: IL-1, IL-6, IL-8, TNF-α				✓			38-40

*Type not defined

B. Animal Models of Dry Eye

Animal models discussed at the 1995 Workshop included a rabbit model in which the meibomian and lacrimal glands and the nictitans were ablated, which caused tear hyperosmolarity and ocular surface damage, mimicking the features of human DE.

New models and findings since the 1995 Workshop include: 1) mouse models of DE that employ scopolamine and environmental, dessicating stress that show increases in inflammatory cytokines and osmolarity in their tears; 2) neurturin-deficient mice that develop DE and have increased inflammatory mediators in their tear film; 3) a rabbit lacrimal gland ablation model that shows that treatment with dexamethasone reverses the decreased TFBUT and ocular surface damage; and 4) rabbit lacrimal gland denervation models that produce altered tear protein and lipid profiles (Table 2).

One critical area of investigation with respect to the existing evidence presented regards the need to correlate tear osmolarity, tear breakup, and the inflammatory stress

Table 2. Information matrix: animal tear film

	Rabbit	Mouse	Refs
Tear Vol/Osmolarity			
↑ Osmolarity + ↓ Tear volume	–Meibomian glands	Scop & Env Str	48-49
↑ Osmolarity, ↑ surface injury	–Lacrimal gland		50
↓ BUT, ↓ surface injury with dexamethasone	–Lacrimal gland		51
Lipids			
↑ Acylglycerols	–Lacrimal gland/nictitans		45
Lipids in rabbit/human match	–Lacrimal gland/nictitans		45
Proteins			
↓ Protein	–Nerves		52
↑ IL-1β		–Neurturin	53

response. To that end, immortalized human corneal and conjunctival epithelial cell lines are now available that have differentiation characteristics of native epithelia.[46,47] They will be useful to study effects of tear osmolarity, inflammatory mediators, and DE tears on surface epithelia.

Mass spectrometry, lipidomics, and proteomics in animal models of dry eye should be done to provide insight into the DE condition. Comparison of animal tear proteomes, lipidomes, and glycomes will help ascertain the most appropriate human-relevant models (eg, total chloroform extractables of rabbit tears match closely those of human tears).[45]

IV. OCULAR SURFACE

A. Human Disease

Aspects of dry eye surface pathology discussed at the 1995 Workshop included the lack of epithelial barrier function as demonstrated by increased dye uptake (with no data available on mechanism), an increased tear film osmolarity causing ocular surface damage, a loss of conjunctival goblet cells, and an increased squamous metaplasia of the surface epithelial cells (morphological observations).

New evidence since that report indicates that there are alterations in cell-surface and secreted mucins and in keratinization-related proteins expressed by epithelial cells. There also are alterations in corneal innervation density and sensitivity. Studies document increased conjunctival epithelial cell turnover. Evidence indicates that conjunctival epithelial cells are active in the immune response and are a source of inflammatory mediators[85] (Table 3).

Despite what is known, information about the tear film and ocular surface in dry eye disease is still deficient. It would be of value to determine the conjunctival epithelial proteome and glycome in a well-defined, staged, dry eye population compared to age- and sex-matched controls to identify common changes in apical surface components with disease. It is desirable to determine if age and sex, or a combination thereof, influence the effects of environmental stress on ocular surface epithelia. It is important to determine any genetic predictors of susceptibility to DE. Finally, a comparison of early intermittent stages of the disease to chronic disease may distinguish primary pathways causing DE from secondary responses associated with the disease.

B. In Vitro and Animal Models

Information gathered from in vitro and animal models as of the 1995 Workshop identified lack of barrier function as demonstrated by dye uptake in several animal models of dry eye, loss of goblet cells in several animal models of dry eye, and keratinization of ocular surface epithelium in vitamin A deficiency.

Since the 1995 Workshop, investigations have identified the role of membrane-associated mucins as a protective barrier (human epithelial cells in vitro), increased cell turnover (mouse experimental dry eye), and increased expression

Table 3. Information matrix: human ocular surface

	Undif KCS	NSS/ACQ	SS	CIC	↓ Vit A	Cont Lens	LASIK	Refs
Corneal and conj. epi. cell damage as indicated by dye penetrance — Fluorescein, lissamine green, rose bengal	✓	✓	✓	✓	✓	✓	✓	Well established
Mucins:								
↓ Goblet cells	✓	✓	✓	✓	✓	↑	✓	54-61
↓ MUC5AC	✓		✓					22, 23
Mucin glycosylation altered	✓					✓		62-65
Δ Glycosyltransferases				✓				66
Δ Membrane-associated mucins		✓	✓					22, 57, 65, 67
Δ Conj. Cell-Epithelial:								
↓ Microplicae			✓					68
Filamentary keratitis	✓							69
↑ Stratification	✓			✓				66, 70
Epi proliferation			✓					71
Δ Nuclear/chromatin structure	✓		✓					72-74
↑ Apoptosis	✓	✓	✓					75
Δ Innervation		✓	✓				✓	76-80
↑ Infection	✓							35, 81
↑ Keratinization related proteins			✓		✓			82-84
Inflammatory markers on conj. epi. cells	✓	✓	✓					75, 85

Table 4. Information matrix: animal ocular surface epithelium

	In vitro/human oc surf epi	Rabbit	Mouse	Rat	Dog	Refs
Goblet cells; mucins/glycoproteins:						
Rose bengal penetrance	–MUC16					86
↓ Goblet cells, MUC5AC		–Vit A –Meibomian gland –Neurotrophic keratitis	Scop & env str –/– Neurturin –/– I κβ–ζ	–Vit A		42, 53, 87-91
Δ Mucin glycosylation					Spont. DE	92
↓ Membrane associated mucins	–Vit A –Serum		–/– Neurturin	–Vit A		53, 89, 93, 94
↓ Glycogen		–Meibomian gland –Lacrimal gland –Neurotrophic keratitis				48, 50, 88
Epi. Diff/sq. Metaplasia:						
↑ Keratinization		–Vit A			Spont. DE	95-97
↑ Conj epi proliferation			Scop & env str			90
↑ Apoptosis			Scop & env str			53, 98
↑ Inflammatory cytokines/MMPs:						
	+Hyperosmolar str		–/– Neurturin Scop & env str + Hyperosmolar str			49, 53, 99-101
Reversal of ocular surface defects/inflammation without meibomian gland:						
			EDA knockin			102

of inflammatory cytokines (mouse experimental dry eye). New mouse models have been developed as useful tools to study molecular mechanisms of ocular surface damage. Mouse models in which the lacrimal and/or meibomian glands are dysfunctional have allowed better characterization of ocular surface pathology (staining, goblet cell density, etc. [Table 4]).

Given what is now known, additional research is needed to determine the role of ocular surface disease in the mechanism of tear dysfunction. A comparison of human and mouse tear and apical epithelial surface proteomes/glycomes would identify common components for validation of the animal models and facilitate interpretation of dry eye model data. Inducible models of specific dry eye diseases and models of chronic disease should be further developed. Importantly, mechanisms of goblet cell differentiation from epithelial stem cells and mechanisms of goblet cell loss need to be characterized, as goblet cell loss characterizes all forms of DE. It would be helpful to develop functional tests in vitro using siRNA techniques to elucidate the contribution of different cell surface molecules to the maintenance of corneal epithelial barrier function. Advanced genetic manipulation techniques using knockout, knockin, and knockdown animals to perform functional tests in standardized animal models of dry eye should be explored. Determination of the basis of fluorescein, lissamine green, and rose bengal staining is needed. It would be worthwhile to determine if epithelial-stromal interactions influence development of DE.

V. IMMUNE SYSTEM

A. Human Disease

Evidence from the 1995 Workshop indicated that SSDE is the result of an autoimmune disease in which response to autoantigens causes inflammatory destruction of the lacrimal tissue. The new evidence since the 1995 report indicates that proinflammatory cytokines and T-cell populations are increased in conjunctival tissue and lacrimal tissue in NSSDE as well as in SSDE. Chemokines and their receptors are increased in dry eye. Dry eye in graft vs host disease (**GVHD**) is associated with inflammation and immune cell infiltration of the lacrimal gland and ocular surface epithelia. The disease is also characterized by fibrosis associated with fibroblast and bone marrow-derived cell infiltration. It is clear that ocular surface epithelial cells can modulate inflammatory responses (Table 5).

Information is still lacking about the role played by the immune system in human tear dysfunction in DE. There is little or no information about the changes in cornea (vs tear film or conjunctiva) or the early changes in and role of immune factors causing disease. It is not known which changes are primary and which are secondary, information that is required in order to determine "cause and effect."

There is a need to determine more precisely the role of immunomodulatory proteins and peptides present in cornea and tear film (TGF-β, α-MSH, IL-1Ra, etc.) and to delineate the role of innate immunity in dry eye disease (including lactoferrin, lysozyme, toll-like receptors, complement, kinin-kininogen, arachidonic acd metabolites, neuropeptides).

Table 5. Information matrix: human immune system/dry eye

	Undifferentiated KCS	NSS	Rosacea DE	SS	GVHD	Refs
Conjunctiva:						
↑ CD3, CD8 cells				✓	✓	103
↑ CD4 and T cells		✓		✓	✓	104-108
↑ Chemokine CCR5 receptor	✓	✓		✓	✓	109, 110
↑ Fas		✓				69
↑ ICAM-1					✓	111
Conjunctiva and Tears:						
↑ IL-1, TNF-α and IL-8, IL-6			✓	✓		38-40
Conjunctiva and Lacrimal Gland:						
↑ MHC class II, HLA-DR	✓	✓		✓	✓	75, 105, 107, 110-113
↑ CD40, CD40 ligand, CD80, CD86	✓	✓		✓	✓	75, 107
Fibrosis					✓	107, 108, 114
Lacrimal Gland:						
Lacrimal gland: ↑ CD4, T & B cells	✓			✓	✓	108, 115-117
↑ ICAM-1	✓				✓	107, 118
Inflammatory infiltrate		✓		✓		119, 120
Shared autoantigens, lacrimal & salivary gland		✓				115
↑ Fas-Fas ligand, IL-1β, IL-6, IFN-γ, vascular cell adhesion molecule-1 & intercellular adhesion molecule-1 infiltrating lymphocytes, apoptosis		✓				121-123

B. In Vitro/Animal Models of Dry Eye—Immune System

The models and findings of the 1995 Workshop confirmed that cyclosporine A is effective in the treatment of a spontaneous canine dry eye model. New evidence available since the 1995 report indicates that IFN-γ can upregulate HLA-DR and ICAM-1 in human conjunctival cells, indicating that ocular surface cells can respond to and modulate inflammation. Mouse models of dry eye that employ either scopolamine and environmental stress or environmental stress alone show that ocular surface stress can induce the inflammatory/T-cell alterations seen in human dry eye. Evidence suggests that inflammation induced by desiccating stress is mediated by T-cells[126] (Table 6).

What questions can be answered or what promising types of basic research need to be done in model systems to determine the role of the immune system in the mechanism of tear dysfunction in DE? There is a dearth of information regarding understanding the role of T cells in the early immunopathogenesis of the ocular surface (vs lacrimal gland) disease in DE. The extent to which the ocular surface disease is T-cell-mediated needs to be clarified. It is also necessary to determine the role of autoimmunity in this disorder and the nature of the autoantigens. Studies are needed to characterize the effect of inflammatory cytokines on mucin genes and proteins. Delineation of the role of the innate immune system in dry eye syndrome is also needed (including

Table 6. Information matrix: animal immune system

	In vitro Animal	Rabbit	Mouse	Dog	Refs
IFN-γ ↑ HLA-DR, ICAM-1	Conj Primary Culture				124
Inflammation ↑ Conj, lacrimal gland apoptosis			Scop & Env Str	Spont. DE	96, 98
IFN-γ in TH1-type inflammations and DE			Scop & Env Str, Env Str		118, 125
T cells mediate local inflammation to eye drying			Scop & Env Str		126
Lac Inflammation & DE					
↑ T cells, CD4 especially			Autoimmune dacryoadenitis		127
↑ CD3 T cells; CD8, CD4			GVHD Model		128
↑ ICAM-1			MRL/lpr mice		118
↑ MHC class II		DE			129

lactoferrin, lysozyme, complement, kinin/kininogen, arachidonic acid metabolites, neuropeptides, toll-like receptors, and surfactant protein-D).

VI. HYPOTHESIS OF THE MECHANISM OF ACUTE AND CHRONIC INFLAMMATION IN DRY EYE DISEASE

The Cullen Symposium on Corneal & Ocular Surface Inflammation (Baylor College of Medicine, Houston. TX, January, 2005, *The Ocular Surface*, Vol. 3, Supplement) attempted to provide a unified mechanistic view of acute and chronic ocular surface inflammation (Figure 1), including that seen in DE.[130]

1) *Acute:* Irritation of the ocular surface (viral, bacterial, environmental) leads to rapid vascular endothelial selectin expression and diapedesis of non-primed (non-targeted) T-cells into the conjunctiva.

2) *Chronic:* Challenge to the ocular surface (over time) leads to activation and drainage of antigen-presenting (including dendritic) cells to lymphoid organs permitting T-cells to be primed and capable of targeting the ocular surface.

3) Symptoms correlate primarily with corneal epithelial damage, thought to be due to cumulative damage mediated by cytotoxic effects of inflammatory and pro-apoptotic stimuli, and hyperosmolarity. Concomitant with epithelial loss/devitalization is the stimulation of corneal nociceptive nerve endings

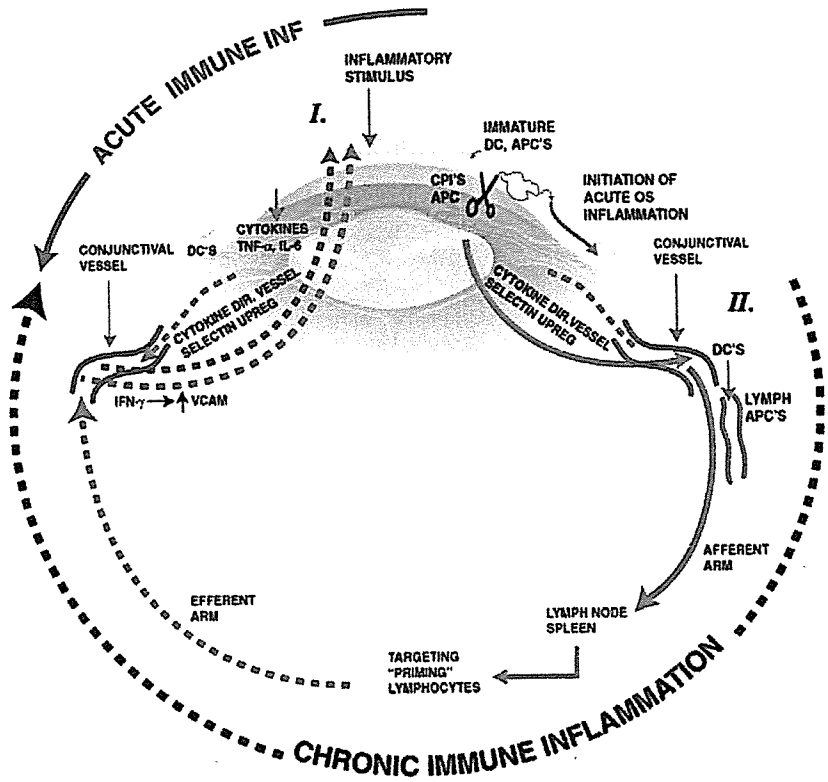

Figure 1. Hypothesis of the mechanism of acute and chronic immune inflammation.

I. Inflammatory stimuli (microbial antigens, trauma, UV light, hyperosmolar stress) initiate acute immune inflammation by stimulating production and release of inflammatory cytokines (eg, IL-1, TNF-α, and IL-6) by the ocular surface epithelial cells, which activate immature antigen presenting cells (APCs) and increased expression of adhesion molecules (eg, ICAM-1) and selectins by the conjunctival vascular endothelium, which facilitates recruitment of inflammatory cells to the ocular surface.

II. Chronic immune inflammation, which involves procurement and processing of antigens by ocular APCs that migrate to the regional lymph nodes and spleen via conjunctival lymphatics and veins, respectively, and prime naive T-cells. Primed CD4 T-cells travel to the conjunctiva, where they adhere to activated vascular endothelium and enter the tissue through diapedesis. Cytokines produced by activated T-cells, such as IFN-γ, amplify the immune response by increasing adhesion molecules (eg, VCAM) expression by conjunctival blood vessels.

APCs = antigen presenting cells; CPIs = corneal proteases; DC = dendritic cell; TNF-α = tumor necrosis factor alpha; IL-6 = interleukin 6; IFN-γ = interferon gamma. (Reprinted from McDermott AM et al. Pathways of corneal and ocular surface inflammation: a perspective from the Cullen Symposium. Ocul Surf 2005;3(4):S131-S138.)

VII. LACRIMAL/ACCESSORY LACRIMAL GLANDS/ NASOLACRIMAL DUCT

A. Human Disease

Evidence from the 1995 Workshop indicated that the lacrimal glands of SSDE patients are infiltrated by lymphocytes and that tear secretion is decreased in volume. Some evidence suggested a potential Epstein-Barr virus infection link to dry eye, although this area was controversial. It was known that occluding the nasolacrimal duct improves ocular surface staining in DE.

Evidence accumulated since the 1995 Workshop has identified the lymphocyte types, Fas-Fas ligand expression, and apoptotic markers in lacrimal glands of SS patients. There is some evidence to suggest a link between hepatitis C and HIV infection with NSDE and SSDE. An autoantibody to the M3 muscarinic acetylcholine receptor has been identified, and increased serum levels correlate with decreased nasally stimulated Schirmer value and increased rose bengal staining score. There is an increase in lacrimal mucin in DE (Tables 7 and 8).

Questions remain to be answered about the role of the lacrimal gland, the accessory lacrimal glands, and the nasolacrimal duct in dry eye. Based on the current level of information, it would be useful to compare the lacrimal proteome in a population of well-characterized age/sex-matched normals to that of DE patients, as well as to compare the lacrimal proteomes of different KCS in order to identify potential biomarkers of the disease types.

Information is particularly lacking about the accessory lacrimal glands and the nasolacrimal duct in humans with dry eye disease. All histologic and immunohistochemical

data on accessory lacrimal glands are from normal tissue; no information is available regarding the glands in dry eye of any type. We do not know the extent to which they are affected in DE; because they are embedded in subconjunctival tissue at the ocular surface, they are an important therapeutic target for topical, lacrimal secretagogues. Gene expression in accessory glands, compared to the main lacrimal glands, is not defined. The relative contributions of accessory and main lacrimal glands to basal tear secretion or impairment of tear secretion are not known, and there is need for comparison of accessory and lacrimal gland gene expression.

Table 7. Information matrix: human lacrimal gland/nasolacrimal duct

	KCS	SS	GVHD	Aging	Refs
Lacrimal Gland					
Inflammatory infiltrate		✓	✓		107, 108, 119, 120
Shared autoantigens, lacrimal and salivary gland		✓			115
↑ FAS-FAS ligand, IL-1β, IL-6, IFN-γ, VCAM-1, ICAM-1, Infiltrating lymphocytes, apoptosis		✓			121-123
Viral etiology of hepatitis C, HIV, Epstein Barr	✓	✓			131-135
Autoantibodies to M3 muscarinic acetylcholine receptors		✓			136
Correlation: Serum autoantibody levels to Schirmer with nasal stimulation and rose bengal/ fluorescein staining		✓			137
↑ MUCs 4, 5AC & 5B in human lacrimal gland (4 cadavers with dry eye)				✓	138
↓ Innervation in lacrimal glands	✓	✓			139
↑ Fibrosis				✓	140
Nasolacrimal Ducts (NLD)					
Occluding nasolac. syst. (puntum plugs, etc.) improves oc. surf. DE	✓	✓			>100 refs.
DE & nasolac diseases occur frequently in middle to advanced-age women	✓	✓			141

Likewise, information is lacking on the nasolacrimal duct function in dry eye disease. Long-term studies of the benefit of punctal occlusion are lacking. Yen et al[150] found that ocular surface sensation and tear production decreased after temporary punctal occlusion in normal subjects. However, in normal subjects, there appears to be an autoregulatory mechanism that returns tear production and tear clearance to preocclusion levels 14 to 17 days after punctal occlusion, a mechanism that seems to be lacking in DE patients.[150] Thus, it could be suggested that the absorption of tear fluid components into the blood vessels of the surrounding cavernous body[151,152] could provide a signal for tear fluid production that ceases when tears are lacking. Studies are needed to characterize feedback systems in the nasolacrimal duct epithelia and blood vessels and their connections to the ocular surface system.

B. In Vitro/Animal Models

In the 1995 Workshop report, mouse models of SS had been identified, in which lacrimal inflammation was shown to be reduced by androgens.

Since the 1995 report, studies have been done with microarray analysis, showing dramatic changes in lacrimal gland gene expression after acute corneal injury in the mouse. Cytokines and chemokines have been identified in a mouse model of SS, as well as altered cholinergic function and neurotransmitter release. Alpha-fodrin has been identified as an autoantigen in the NFS mouse model of SS, and ICA69 is the autoantigen identified in the NOD mouse model of SS. Muscarinic receptors are autoantigens for SS in a rat model. It has also been demonstrated that nasolacrimal ducts can absorb labeled cortisol, an indication that absorption of tear components can occur within the duct cholesterol (Table 9).

To validate animal models of dry eye, it may be important to characterize and compare the lacrimal gland transcriptome anad proteome in both human and mouse. Comparing the proteomes of lacrimal glands from normal and DE mice could also be informative. It is also important to determine which signaling pathways are altered to cause the decrease in lacrimal gland secretion that occurs in aging

Table 8. Information matrix: human accessory lacrimal gland (not DE relevant)

	Refs.
Acinar structure similar in accessory and main glands	142, 143
Secretory immune system of accessory and main gland similar	142, 144, 145
Innervation of accessory and main gland similar	146, 147
Protein secretion and signaling pathways similar in accessory and main glands	145, 148, 149

Table 9. Information matrix: animal lacrimal gland/nasolacrimal duct

	In Vitro	Rabbit	Mouse	Rat	Dog	Refs
Lacrimal Gland:						
Coculture of lacrimal acinar cells/ lymphocytes activates lymphocytes and cause inflammation in host lacrimal gland	Lacrimal gland					153-157
↑ Lymphocytic infiltration, CD4, CD8; ↑ Fas, Fas-Ligand & cytokine			MRL/lpr mouse NOD mouse model of SS			158-166
Androgens ↓ inflammation, are immunosuppressive & decrease androgen receptors			MRL/Mp-lpr/ lpr mice; NZB/NZW F1 Mouse	Exp. autoimmune dacryoadenitis	Dog DE	161, 167-176
Lacrimal gland autoantigen or extract causes lymphocytic infiltration in lacrimal gland			Mouse in vivo	Rat in vivo		172, 173, 177, 178
Cholinergic function altered Sjögren's syndrome ICA69 is autoantigen			NOD mouse model of SS			179, 180
Lymphocytic infiltration blocks lacrimal gland secretion by preventing nerve release of neurotransmitters in Sjögren's syndrome			MRL/lpr mouse model of SS			181
α-fodrin is an autoantigen for the lacrimal gland and causes Sjögren's syndrome			NFS Mouse model of SS			182
↑ vulnerability to herpes infection				Cells of female lacrimal gland		174
Δ Lacrimal gland gene express. in corneal injury			Normal mouse			183
Nasolacrimal duct (NLD):						
^{3}H-cortisol incorporated from NLDs into rabbit blood		Absorpt. of lipophillic substances fr. tear fluid by epi. of NLDs		No absorption of lipophillic substance from tears by epi. of NLDs		184, 185
Anatomy useful for investigating NLDs		Comparative studies			Comparative studies	184-186
↓ Secretion ↓ Innervation ↑ Lipofusci			Aging model			187

mouse or rat models. Yet to be determined in animal models is the role of myoepithelial cells in lacrimal gland dysfunction. It may be useful to determine, using the autologous lymphocyte rabbit model, if exposure of cryptic antigens through errors in recycling initiates SS. Determination of the cellular mechanisms used to induce autoimmune disease in the lacrimal gland could also employ the autologous lymphocyte rabbit model. This model could also be used to determine if the exocytotic process for protein secretion is a target for lacrimal gland dysfunction and to determine the role of lacrimal gland duct cells in lacrimal gland dysfunction through laser capture microdissection.

With regard to the nasolacrimal ducts, information is lacking regarding cells of the ducts, and cell lines of nasolacrimal duct epithelium are not currently available. Questions to be answered in animal models include whether the absorption of tear fluid components into the blood vessels of the cavernous body surrounding the nasolacrimal ducts changes or ceases in dry eye models, and what happens to drained tear fluid in the nasolacrimal passage.

VIII. MEIBOMIAN GLAND

A. Human Disease

The 1995 Workshop report documented decreased and/or altered meibomian lipids in DE, as well as morphologic abnormalities of the gland acini and tubules.

New evidence since the 1995 report identifies keratinization of ductal epithelium, orifice metaplasia, and reduced quality of meibomian gland secretions in people during aging, in patients taking antiandrogen therapy, and/or in women with Complete Androgen Insensitivity Syndrome (Androgen Deficiency). Correlations have been made be-

tween nutrient intake (eg, omega 3 fatty acids, vitamin B6, vitamin D) and the polar lipid profiles of meibomian gland secretions in women with SS. It has been determined that meibomian gland disease may be a contributing factor in over 60% of all dry eye patients (Table 10).

Information is still lacking about the role of the human meibomian gland in the tear dysfunction of dry eye. Factors influencing meibomian duct keratinization should be explored further, with the hypothesis (not new) that duct hyperkeratinization is a common factor and key event leading to meibomian gland disease (**MGD**) in both primary and secondary MGD.

Some clues may derive from the literature concerning epinephrine toxicity in the rabbit and, perhaps more relevantly, retinoid toxicity in humans. Clues may also be derived from an insubstantial but interesting literature suggesting that conjunctivitis (eg, allergic, chronic) or SS dry eye are associated with MGD, with the implication that mediators (proinflammatory or otherwise) might be transferred across the conjunctiva to the meibomian glands and ducts.

Investigative approaches could include:

1) A review of the literature of keratinization processes in multiple epithelia;
2) A review of the mechanism of retinoid action and genetically regulated processes involved with keratinization, in mucosae, transitional epithelia (like the meibomian ductal epithelium) and in skin;
3) A comparative review of potential points of interaction of signaling pathways under retinoid control and pathways under adrenergic, particularly alpha adrenergic, control, with respect to the keratinization process;
4) Attention to the histochemistry and electronhistochemistry of keratinization at the cellular levels. Markers of keratinization;
5) A search for retinoids or other compounds capable of blocking or reversing the action of anti-acne retinoid compounds;
6) Clinical studies of the comparative frequency of MGD in eyes treated with adrenergic agonists for glaucoma, particularly where agonists are used unilaterally.

We need to know the minimum number of glands required to provide an adequate lipid layer for tear film function and the molecular mechanisms leading to loss or to morphologic abnormalities of the meibomian gland. Determining how the lipid layer is attached to the aqueous layer and whether this changes in DE is important, as is defining the role of lipocalin and other lipid carriers in tear film stability. We need a comprehensive qualitative and quantitative evaluation of the meibomian gland secretions of normal subjects and DE patients, obtained with modern analytical techniques, in particular, using liquid chromatography/mass spectrometry to determine if the molar ratio of the critical lipid species that are present in the meibomian gland secretions changes with the development of DE. It would be helpful to create an artificial model of the tear film lipid layer that mimics the lipid composition of the meibomian gland secretions collected from normal subjects and has similar biophysical properties. Questions exist as to the etiology of meibomian gland obstruction, eg, why doesn't a chalazion form with every obstruction?

Additionally, we need to know more about age-related changes in meibomian gland function and the relationship between meibomian gland obstruction and nutrition. The role of lipids in lubricity of the lid and ocular surfaces should be clarified. Is there a role of the lid wiper and lid wiper epitheliopathy within MGD?

B. In Vitro/Animal Models

Relatively little was known about animal models for MGD at the time of the 1995 Workshop other than that keratinization of the duct epithelium existed in the epinephrine rabbit models. Since then, new models and findings have provided the knowledge that androgen deficiency, which in humans is associated with meibomian gland dysfunction, alters the lipid profiles of meibomian gland secretions, and causes tear film instability and evaporative dry eye. Androgen deficiency in mice and rabbits is as-

Table 10. Information matrix: human meibomian gland

	KCS	Chr Bleph	MGD	NSS	SS	Androgen Deficiency	Aging	Cont Lens	Refs
Meibomian gland loss/ obstruction/distortion Decreased secretions		✓	✓	✓18.5%	✓60%		✓	✓	6, 188-195
Δ Lipid profiles						✓	✓	✓	36, 196-198
Keratinization, orifice metaplasia						✓	✓		5, 10
Melting pt. of lipid 3° higher than normal			✓						199
Bacterial strains associated with Chr Bleph		✓							200
↑ Fluorescein, rose bengal			✓						195
Δ Lipid layer; ↑ Thickness	✓					✓	✓	✓	36, 197, 198, 201, 202

Table 11. Information matrix: animal meibomian gland

	Rabbit	Mouse	Hamster	Refs
↓ MG, Conj. erythema	RA-MGD model	−/− EDA	RA-MGD model	102, 203, 204
↑ Ductal keratinization	MGD/epinephrine model			205
↑ Sterols and ceramides	MGD/epinephrine model			206
Atrophic MG with ocular surface damage		−/− ACAT-1		207
↓ Androgens Δs Lipids, gene expression in meibomian gland	castrated male model	castrated male model		208-210

sociated with altered lipid profiles and gene expression in meibomian glands (Table 11).

A number of questions remain to be answered, and basic research using model systems is needed to determine the role of the meibomian gland in various forms of DE and in the mechanism of tear dysfunction. Most importantly, we need to determine the structure and composition of the lipid layer and its change in experimental MGD. It is necessary to determine which components of the meibomian secretion actually spread on the tear film and what change in composition is required to effect a significant change in the melting point and expressibility of oil. Finally, we need to understand the structure of the lipid layer and how it changes in MGD.

IX. MECHANISMS UNDERLYING DRY EYE PATHOLOGY

Based on data derived from the information accumulated in the preceding reports, it was the opinion of the group that insufficient information was available to define the basic mechanism underlying dry eye, but that a hypothesis as to the mechanisms might be advanced. The evidence suggests that dry eye is multifactorial: factors such as age, hormonal status, genetics, sex, immune status, innervation status, nutrition, pathogens, and environmental stress alter the cellular and molecular structure/function of components of the ocular surface system. The term and concept of the *Ocular Surface System* was adopted by consensus agreement at the DEWS Meeting, Miami, Florida, May 2006.

The "ocular surface system" is defined as *the wet-surfaced and glandular epithelia of the cornea, conjunctiva, lacrimal gland, accessory lacrimal glands, nasolacrimal duct and meibomian gland, and their apical and basal matrices, linked as a functional system by both continuity of epithelia, by innervation, and the endocrine and immune systems* (For further explanation see Gipson, 2007[211]). Also included in the ocular surface system are portions of the eye lids. The rationale for the description of the unit as the *Ocular Surface System* is several-fold. First, the primary functions of the system are to provide a smooth refractive surface to the cornea (the ocular surface) and to protect and maintain that surface. Thus, the name *Ocular Surface System* is linked to its primary function at the ocular surface. Second, all the epithelia of the ocular surface are in continuity and derived embryologically from surface ectoderm. The corneal and conjunctival epithelium are in continuity through the ductal epithelium, with the lacrimal gland, glandular epithelium, as is the case with the accessory lacrimal glands, the meibomian gland, and the nasolacrimal system. The glandular systems are essentially invaginations from and specializations of the ocular surface epithelium. Thirdly, all regions of the epithelia produce components of the tear film. The functions of the various regions of the continuous epithelia are integrated by the nervous system, endocrine system, immune system, and vascular system, and are supported by the connective tissue with its resident cells. Finally, dry eye disease affects and is detected on the ocular surface.

*The term *Ocular Surface System* represents an elaboration of the *Lacrimal Functional Unit*, which has been previously described by Stern, Pflugfelder, and Beuerman[212-215] and is discussed in detail elsewhere in this supplement (Chapter 1: Definition and Classification).[216] Alterations in one or several components of the ocular surface system or its secretions results in changes in the tear film or corneal epithelial surface composition (eg, tear osmolarity, volume), leading to susceptibility to desiccation and epithelial damage (as evidenced by dye penetrance). Epithelial damage leads to release of inflammatory mediators. Attendant inflammation amplifies and sustains further damage by chronic deregulation of the ocular surface system.

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*Note added by writing committee