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The Pathology of Dry Eye: The Interaction Between the Ocular Surface and Lacrimal Glands

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Background. Most dry-eye symptoms result from an abnormal, nonlubricative ocular surface that increases shear forces under the eyelids and diminishes the ability of the ocular surface to respond to environmental challenges. This ocular-surface dysfunction may result from immunocompromise due to systemic autoimmune disease or may occur locally from a decrease in systemic androgen support to the lacrimal gland as seen in aging, most frequently in the menopausal female. **Hypothesis.** Components of the ocular surface (cornea, conjunctiva, accessory lacrimal glands, and meibomian glands), the main lacrimal gland, and interconnecting innervation act as a functional unit. When one portion is compromised, normal lacrimal support of the ocular surface is impaired. Resulting immune-based inflammation can lead to lacrimal gland and neural dysfunction. This progression yields the OS symptoms associated with dry eye. **Therapy.** Restoration of lacrimal function involves resolution of lymphocytic activation and inflammation. This has been demonstrated in the MRL/lpr mouse using systemic androgens or cyclosporine and in the dry-eye dog using topical cyclosporine. The efficacy of cyclosporine may be due to its immunomodulatory and antiinflammatory (phosphatase inhibitory capability) functions on the ocular surface, resulting in a normalization of nerve traffic. **Conclusion.** Although the etiologies of dry eye are varied, common to all ocular-surface disease is an underlying cytokine/receptor-mediated inflammatory process. By treating this process, it may be possible to normalize the ocular surface/lacrimal neural reflex and facilitate ocular surface healing.

Key Words: Dry eye—Keratoconjunctivitis sicca—Sjögren's syndrome—Ocular surface—Lacrimal gland—Tear film—Animal model—Lymphocytic infiltration—Inflammation—Autoimmunity—Neural traffic—Apoptosis—Cytokine—Neural transmitter—Neural peptide—Cyclosporine—Androgen.

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Dry-eye symptoms arise from a series of etiologies and are manifest with varying severity in different patients, making accurate diagnosis and disease-specific treatment difficult. The use of artificial tears is palliative at best, resulting in a reduction of ocular surface eyelid shear forces and some transient symptomatic relief. As recently as 1995, the National Eye Institute/Industry Workshop on Clinical Trials in Dry Eyes, under the chairmanship of Dr. Michael A. Lemp, defined specific subtypes of dry eye to standardize clinical tests used in the diagnosis and design of clinical studies (1). As research in this area proceeds, the key question remains: What causative factor(s) initiates the sequence of events resulting in the clinical symptoms experienced by the patient?

This review organizes observations that the ocular surface (cornea, conjunctiva, accessory lacrimal glands, and meibomian glands), the main lacrimal gland, and the interconnecting neural reflex loops comprise a functional unit (Fig. 1) whose parts act together and not in isolation. In the normal individual, when afferent nerves of the ocular surface are stimulated, a reflex results in immediate blinking, withdrawal of the head, and secretion of copious amounts of reflex tears from the main lacrimal gland. This aqueous secretion contains proteins as well as water. Similarly, ocular-surface irritation due to environmental factors (contact lens, low humidity, wind, etc.) results in chronic stimulation of the nerves of the ocular surface and increased secretory activity in the main and accessory lacrimal glands. In individuals suffering from dysfunction of any part of this functional unit, the tear production is no longer adequate to provide the volume and composition of normal tears necessary for homeostasis and repair.

The remainder of this article discusses this functional unit as part of homeostatic maintenance of the normal ocular-surface physiology and how it is altered in dry eye. Overall, this system has probably developed through evolution to maintain the optical properties of the cornea.

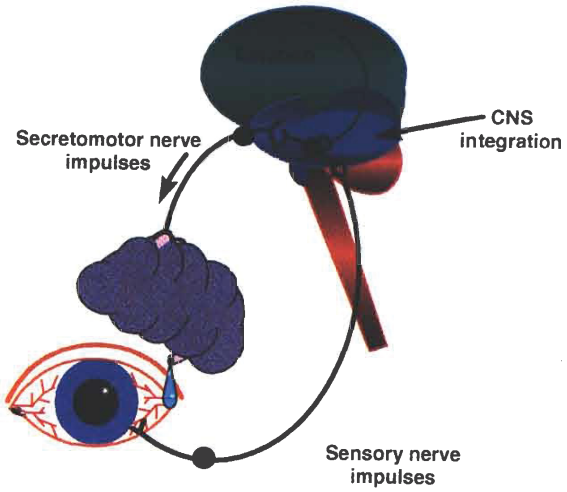

FIG. 1. Schematic illustration of lacrimal gland/ocular surface servomechanism. The ocular surface (cornea, conjunctiva, accessory lacrimal glands, and meibomian glands), the main lacrimal gland, and the interconnecting neural reflex loops comprise a functional unit whose parts act together and not in isolation.

PHYSIOLOGY OF THE NORMAL FUNCTIONAL UNIT

The ocular surface is continuously challenged by the shear forces of blinking across its surface (2), as well as several environmental factors including air currents, low humidity–induced desiccation, and foreign bodies (including contact lenses). Shear forces over the ocular surface applied during blinking (12–15 per minute) can cause significant trauma to a nonlubricated ocular surface (2). Additionally, the ocular surface is constantly confronted with organisms including bacteria and viruses. In normal individuals, the ocular surface remains intact and is able to repair the damage from these insults. Rapid repair of a normal ocular surface has been demonstrated with rose bengal staining. Staining that results from the superficial trauma induced by contact with an impression cytology membrane will no longer be observed after 24 h, indicating that there is a reparative process that actively restores the normal surface barrier (3). This healing is possibly due to the presence of a trophic surface environment driven by constituents of a normal noninflammatory tear film. The presence of tear proteins including IgA, lactoferrin, lysozyme, and growth factors such as epidermal growth factor and transforming growth factor β (TGF-β) at the appropriate concentrations are important for normal ocular-surface maintenance. Mucins produced by corneal epithelial cells and goblet cells in the conjunctival epithelium represent a main defense mechanism against various microtrauma. The wing and basal cells of the corneal epithelium have been shown to express mucins after wounding (4).

Stimulation of the ocular surface initiates neural signals resulting in aqueous tear secretion and possibly stimulation of mucin and lipid secretion. The ocular surface is exquisitely innervated, with the cornea having a density of free nerve endings ~20–40 times that of tooth pulp (5). Corneal sensation is very acute, centrally processed, and interpreted solely as pain (5,6). We believe that so-called basal tearing (7) results from continuous

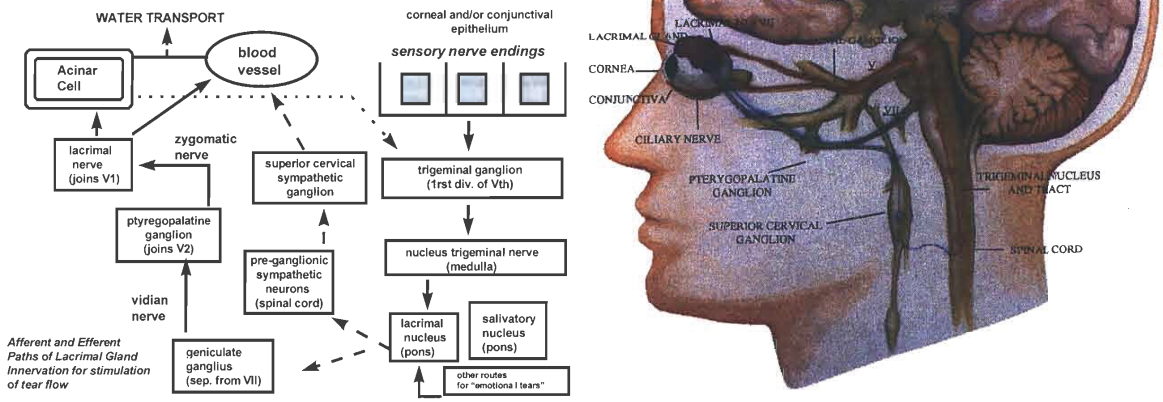

FIG. 2. Afferent (solid red lines) and efferent (solid blue lines, parasympathetic; dotted blue lines, sympathetic) pathways of lacrimal gland innovation for stimulation of tear flow.

stimulation of the corneal surface by environmental factors, even though these signals occur below the level of perception in normal individuals. The conjunctiva does not transmit sensations as acutely as the cornea but is known to convey sensations of itch as well as some temperature fluctuations. The neural pathway for corneal stimulatory reflex has been partially elucidated (Fig. 2). Sensory (afferent) traffic from the cornea and conjunctiva travels along the ophthalmic branch (1) of the trigeminal nerve (V) through the trigeminal ganglion into the spinal trigeminal nucleus located in the brainstem. The initial synapse occurs and neurons then course up to the midbrain (pons) where central processing (integration) occurs. Other neural traffic travels down to the preganglionic sympathetic neurons in the spinal cord and then to the superior cervical ganglion, located in the paravertebral sympathetic chain. Efferent fibers from the pons extend, via the facial nerve (VII), to the pterygopalatine ganglion located adjacent to the orbit where they again synapse and these neurons send fibers to the lacrimal gland where they release secretagogues that modulate water and protein transport. Sympathetic fibers from the superior cervical ganglion also enter the lacrimal gland. It is important to note that the neural control of accessory lacrimal glandular secretion as well as conjunctival goblet cell secretion is only now being investigated. Work by Seiffert and Spitznas (8) demonstrated that the accessory glands are innervated, and Dartt et al. (9) also showed that the conjunctival goblet cells are innervated and respond to the presence of vasoactive intestinal peptide (VIP), a parasympathetic transmitter/neuropeptide.

The lacrimal glands are at the distal end of the neural reflex. The main lacrimal gland resides just superior and temporal to the ocular globe within the orbit. The accessory glands of Wolfring and Krauss reside within the superior bulbar conjunctiva and the upper lid, respectively.

It is now known that lacrimal gland function is significantly influenced by sex hormones (10,11). Among the actions elucidated during the past decade, androgens were shown to exert essential and specific effects on maintaining normal glandular functions and to suppress inflammation in normal and autoimmune animal models (12–16).

ALTERATIONS IN THE FUNCTIONAL UNIT

The etiology of dry eye is believed to be multifactorial and can be related to deficiencies in any one of the three components of the tear film. Disruption of the functional unit will result in alterations of the quantity and composition of tear output leading to symptomatology. The major cause for dry eye in Sjögrens syndrome was reported to be a deficiency in aqueous tear production from the main and accessory lacrimal glands (1,17). Progressive lymphocytic infiltration has been found in the lacrimal glands of Sjögren's patients, and immunohistochemical studies demonstrated that these infiltrates primarily consist of CD4+ T and B cells (18,19). In the conjunctiva of dogs with spontaneous chronic idiopathic keratoconjunctivitis sicca (KCS), massive CD3+ T cells were also detected (Fig. 3) (20). Prior to this lymphocytic infiltration, it appears that a chronic alteration in nerve stimulation of the lacrimal gland may initiate glandular dysfunction. A recent study conducted in the NZB/NZW F1 (NZB) mouse demonstrated the disappearance of the varicose synaptophysin-like fibers among the lacrimal acini by age of 6–8 months, before the infiltrating lymphocytes are observed (21). This initial inflammation may result from the activation of vigilant T cells that are normally migrating through the lacrimal gland. Proinflammatory cytokines secreted by these activated lymphocytes may be a causative factor in the disruption of nerve cell function associated with operation of the functional unit. The presence of activated T cells and proinflammatory cytokines was also demonstrated in non-Sjögren's KCS patients, indicating that follicular infiltrates, associated with systemic autoimmunity, are not necessary to evoke glandular functional disruption. Lacrimal dysfunction therefore appears to be a mechanistic result of T-cell activation. Interruption of the neural signal at this juncture may be part of the same mechanism that initiates the immune responsive migration and proliferation of lymphocytes in the lacrimal gland and conjunctiva. However, if the sensory innervation to the gland is affected, the resulting release of substance P could also be stimulatory to lymphocytes. Schafer et al. (22) indicated that parasympathetic neural transmission in peripheral nerves can be inhibited by cytokines. Therefore, the proinflammatory cytokines such as interleukin (IL) 1β, IL-2, interferon (INF-γ), and tumor necrosis factor α, found in lacrimal and salivary gland biopsies of patients with autoimmune dry-eye syndrome, may inhibit neural stimulation of these target tissues and, if left unchecked, would result in glandular destruction by activated lymphocytes (23–25). In nonautoimmune dry eye, loss of neural tone results in gland atrophy and further immune activation. The result of this immune-based inflammation is an abnormal ocular surface epithelium (26).

Increased levels of inflammatory cytokines (mRNA and protein), such as IL-1β, IL-6, and IL-8 have been found in Sjögren's syndrome patients (27–29). These patients also demonstrated expression of immune activation markers HLA-DR and ICAM-1 in the conjunctival epithelium (27). Accumulated evidence indicates that the epithelial cells in the lacrimal and salivary tissues have the potential to be antigen-presenting cells. In vitro, lacrimal acinar cells have shown the ability to express major

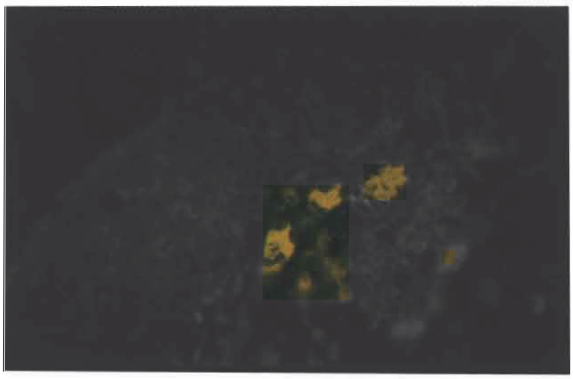

FIG. 3. The immunoreactivity of CD3 T cells was detected in the conjunctival biopsy of KCS dogs. Specific membrane stainings were found in both the epithelium and stroma.

histocompatibility complex (MHC) II after carbachol induction (30,31). In vivo, acinar cells in both the salivary gland of patients and the lacrimal gland of the MRL/lpr lymphoproliferative mouse model strongly express class II antigens (27,32,33). Additionally, a recent study using polymerase chain reaction (PCR), single-strand conformation polymorphism (SSCP) showed that some infiltrating T cells in both lacrimal and salivary glands recognize the shared epitopes on autoantigens, suggesting the importance of restricted epitopes of common autoantigens in the initiation of Sjögrens' syndrome (34). Therefore, it is reasonable to propose that the epithelial cells in inflamed lacrimal or salivary tissues are able to present autoantigens to the cell surface receptors such as T-cell antigen receptors. This mode of T-cell activation can occur locally in the absence of systemic autoimmunity.

The question then becomes, what conditions result in the inability of the ocular surface and the lacrimal glands to respond normally to chronic environmental challenges? Although still under investigation, several studies indicate that the loss in systemic androgens known to occur in pre- and postmenopausal females results in a loss of support for lacrimal secretory function and facilitation of an inflammatory environment (15,35,36).

The unique effects of androgens are presumably initiated through specific binding to receptors in the acinar nuclei of the lacrimal gland, which in turn lead to an altered expression of various cytokines and proto-oncogenes (17,34). The activity of androgens in lacrimal gland is proposed to be attributed to its ability to induce the accumulation of antiinflammatory cytokines such as TGF-β (17). Given the trophic role that androgens play in the lacrimal gland, it has been hypothesized that a

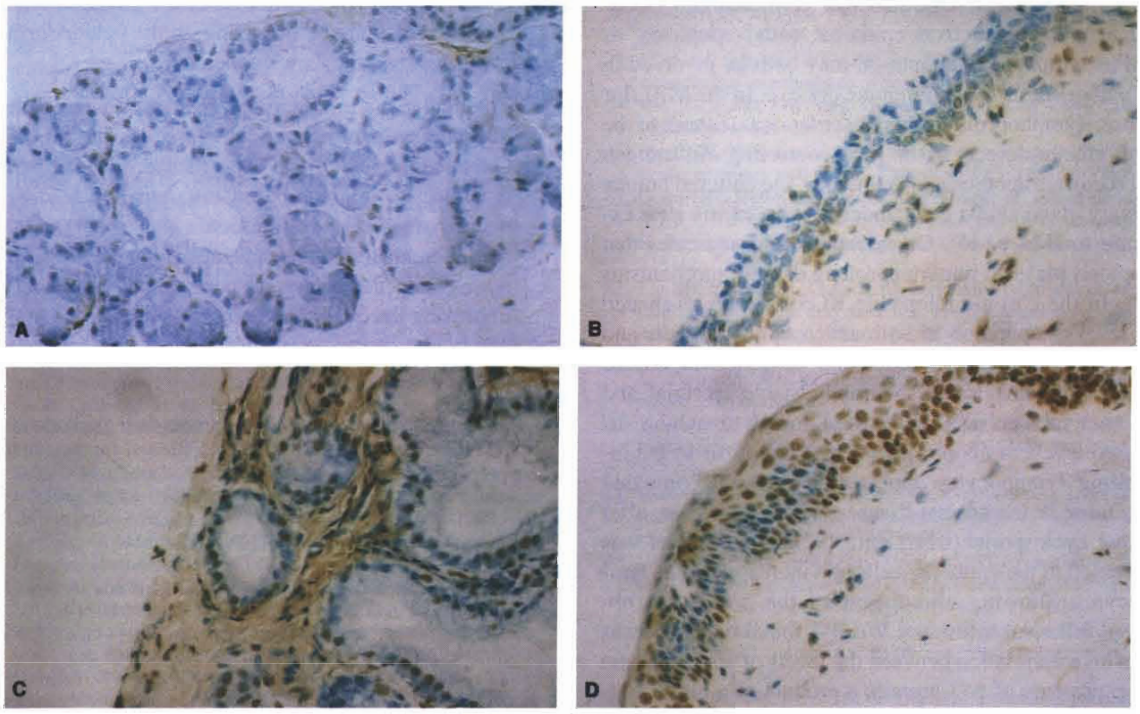

FIG. 4. The normal lacrimal **(A)** and conjunctival **(B)** epithelial cells exhibited a low level of apoptosis. In KCS dogs, apoptosis was increased in the lacrimal **(C)** and conjunctival **(D)** epithelial cells but decreased in the infiltrating lymphocytes. Note a large number of apoptotic cells were stained brown by TUNEL assay.

decrease in androgen level below a certain threshold may result in lacrimal atrophy (35). Apoptosis of the interstitial cells of the lacrimal gland was detected 4 h after withdrawal of androgen in ovariectomized rabbits with atrophic and necrotic changes in the acinar cells occurring over the ensuing several days (16). The resulting apoptotic fragments represent a source of potential autoantigens that could be subsequently presented either by interstitial antigen-presenting cells or acinar cells to CD4 cell antigen receptors and trigger the autoimmune response.

THERAPIES FOR DRY-EYE DISEASE

The immunomodulatory drug cyclosporine (37), as well as steroids (38), has been found to reduce ocular-surface rose bengal staining. Cyclosporine is most well known as an immunomodulating drug used to prevent T cell–driven rejection after organ transplantation (39) and to treat a variety of autoimmune diseases (40–42). The proposed mechanism of its action is its binding to a specific cytosolic protein, cyclophilin, which is required for the initiation of the inhibition of T-cell activities, thus preventing the production of inflammatory cytokines such as IL-2 and IFN-γ (43). Studies in the chronic idiopathic dry-eye dog demonstrated that topical ophthalmic cyclosporin A eliminates both the conjunctival and lacrimal gland lymphocytic infiltrates (20,44).

Previous studies suggested that epithelial cell (lacrimal acinar, conjunctival epithelial cells) apoptosis, as well as lymphocytic apoptosis, may both be involved in the pathogenesis of autoimmune dry eye. In the MRL/lpr mouse, lymphoproliferative disorder was found to be partly due to defects in the gene controling *Fas* antigen that mediates apoptosis (45). In vitro, the cultured human salivary gland acinar cells underwent apoptosis after exposure to IFN-γ (46). Our recent finding indicates that apoptosis plays an important role in dry-eye mechanisms (47). In the chronic idiopathic KCS dog, we evaluated the level of apoptosis in both acinar epithelial cells and lymphocytes in the lacrimal and conjunctival tissues (Gao et al., this issue). Normally stable lacrimal and conjunctival epithelial cells were found to exhibit increased levels of apoptosis, whereas apoptosis in the infiltrating lymphocytes appeared suppressed compared with those in the normal tissues (Fig. 4). However, after topical cyclosporine treatment, the apoptotic level was decreased in the epithelial cells and increased in the lymphocytes, allowing elimination of the previously observed follicular infiltrates. We also found that there was a positive correlation between the levels of apoptosis and the expression of p53 protein, a product of a tumor suppressor gene that was shown to be critical in both differentiation and apoptosis in certain cell types (48). The inhibitory activity of cyclosporine on both apoptosis and p53 expression in the epithelial cells, as demonstrated in canine KCS, implies that the apoptotic process in the lacrimal gland may be mediated by a p53-dependent mechanism.

Androgens may also serve as important therapeutics for dry eye. Systemic androgens have been shown to reduce the extent of lymphocytic infiltration in the lacrimal gland of the MRL/lpr mouse and appear to reverse the inflammation-induced disruption of acinar and ductal epithelium (49). Additionally, androgens applied to the ocular surface of dry-eye dogs also demonstrated an inhibitory effect on apoptotic level in the lacrimal gland and resolved lymphocytic infiltrates in the lacrimal acinar lobules (Stern, unpublished data).

SUMMARY

We propose that the pathology of dry eye occurs when systemic androgen levels fall below the threshold necessary to support secretory function and mainten an anti-inflammatory environment. Under these conditions, inflammation of the lacrimal glands and ocular surface can occur. Secretion of proinflammatory cytokines subsequently interfere with the normal neural connections that drive the tearing reflex. The lacrimal gland is thus effectively isolated, perhaps exacerbating atrophic alterations of the glandular tissue. These changes promote antigen presentation at the surface of the lacrimal acinar cells and increase lymphocytic infiltration of the gland. A similar series of events may be occurring on the ocular surface.

From this hypothesis we conclude:

1. The ocular surface, lacrimal gland, and interconnecting innervation act as an integrated functional unit.
2. Once the lacrimal gland loses its androgen support, it is subject to immune-based inflammation leading to neurally mediated dysfunction.
3. Antiinflammatory therapeutic agents may be capable of normalizing the ocular surface/lacrimal neural reflex, improving the quality and quantity of the tear film.
4. The ocular surface is an appropriate target for dry-eye therapeutics.

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