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EFFECT OF INFLAMMATION ON LACRIMAL GLAND FUNCTION

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Abstract

The lacrimal gland is the main contributor to the aqueous layer of the tear film. It secretes proteins, electrolytes and water, which helps to nourish and protect the ocular surface. Lacrimal gland secretion is primarily under neural control, which is achieved through a neural reflex arc. Stimuli to the ocular surface activate afferent sensory nerves in the cornea and conjunctiva. This in turn activates efferent parasympathetic and sympathetic nerves in the lacrimal gland to stimulate secretion. Sex steroid hormones are also important regulators of lacrimal gland functions. A decrease or lack of lacrimal gland secretion is the leading cause of aqueous tear deficient dry eye syndrome (DES). It has been suggested that DES is an inflammatory disorder that affects the ocular surface and the lacrimal gland. In several pathological instances, the lacrimal gland can become a target of the immune system and show signs of inflammation. This can result from autoimmune diseases (Sjögren's syndrome), organ transplantation (graft versus host disease), or simply as a result of aging. The hallmarks of lacrimal gland inflammation are the presence of focal lymphocytic infiltrates and increased production of proinflammatory cytokines. The mechanisms leading to lacrimal gland dysfunction are still poorly understood. Apoptosis, production of autoantibodies, hormonal imbalance, alterations in signaling molecules, neural dysfunction, and increased levels of proinflammatory cytokines have been proposed as possible mediators of lacrimal gland insufficiency in disease states.

Keywords

lacrimal gland; tears; Sjögren's syndrome; cytokines; dry eye syndrome

I. Introduction

1. Anatomy of the lacrimal gland

The anatomy of the main lacrimal gland has been extensively reviewed elsewhere (Dartt, 1989, Dartt, 1994). It is a multilobular tissue composed of acinar, ductal, and myoepithelial cells. The acinar cells account for 80% of the cells present in the lacrimal gland and are the site for synthesis, storage, and secretion of proteins. Several of these proteins have antibacterial (lysozyme, lactoferrin) or growth factor (epidermal growth factor, transforming growth factor α , keratocyte growth factor) properties and are crucial to the health of the ocular surface (Dartt, 1989, Dartt, 1994). The primary function of the ductal cells is to modify the primary fluid secreted by the acinar cells and to secrete water and electrolytes (Mircheff, 1994). The myoepithelial cells contain multiple processes, which surround the basal area of the acinar and ductal cells. The myoepithelial cells, which contain α -smooth muscle actin, are thought to contract and force fluid out of the ducts and onto the ocular surface. This has been shown to occur in the salivary and mammary glands, but there is no evidence for this hypothesis in the lacrimal gland.

Other cell types present in the lacrimal gland include plasma cells, B and T cells, dendritic cells, macrophages, bone marrow-derived monocytes, and mast cells (Dua et al., 1994, Wiecek et al., 1988, Zierhut et al., 2002). Immunoglobulin A (IgA)-positive plasma cells

account for the majority of the mononuclear cells in the lacrimal gland (Dua et al., 1994, Wieczorek et al., 1988). These cells synthesize and secrete IgA, which then is transported into acinar and ductal cells and secreted by these epithelial cells as secretory IgA (Sullivan and Sato, 1994, Zierhut et al., 2002).

2. Regulation of lacrimal gland secretion

a. Neural control—The lacrimal gland is innervated by the parasympathetic and sympathetic nervous system (Botelho, Hisada and Fuenmayo, 1966, Sibony et al., 1988). Although scarce, sensory nerves are also present in the lacrimal gland (Botelho et al., 1966). Nerves are located in close proximity with acinar, ductal, and myoepithelial cells as well as blood vessels, and hence can control a wide variety of lacrimal gland functions (Botelho et al., 1966, Sibony et al., 1988). It is well documented that stimulation of lacrimal gland secretion occurs through a neural reflex arc originating from the ocular surface (Botelho, 1964). Indeed, stimuli to the ocular surface activate afferent sensory nerves in the cornea and conjunctiva that in turn activate efferent parasympathetic and sympathetic nerves in the lacrimal gland to stimulate secretion (Botelho, 1964, Dartt, 1994) (Figure 1). Neurotransmitters and neuropeptides released by the lacrimal gland nerves include acetylcholine, vasoactive intestinal peptide (VIP), norepinephrine, neuropeptide Y (NPY), substance P (SP), and calcitonin gene related peptide (CGRP). Each of these neuromediators interacts with specific receptors present on the surface of lacrimal gland cells to elicit a specific response (Hodges and Dartt, 2003). Acetylcholine and norepinephrine are the most potent stimuli of lacrimal gland protein, water and electrolytes secretion (Dartt, 2004, Hodges and Dartt, 2003). Acetylcholine binds to cholinergic M₃ muscarinic receptors and norepinephrine binds to α - and β -adrenergic receptors (Hodges and Dartt, 2003, Mauduit, Jammes and Rossignol, 1993). The intracellular signaling pathways activated by these receptors have been well defined and recently reviewed (Dartt, 2004, Hodges and Dartt, 2003).

b. Hormonal control—Hormones from the hypothalamic-pituitary-gonadal axis exert a profound impact on lacrimal gland structure and function and have been reviewed elsewhere (Sullivan, 2004, Sullivan, Block and Pena, 1996). Hypophysectomy or anterior pituitary ablation results in glandular atrophy (Azzarolo et al., 1995, Sullivan et al., 1996). Adrenocorticotrophic hormone (ACTH), α -melanocyte stimulating hormone (α -MSH), prolactin, androgens, estrogens, and progestins have been shown to influence lacrimal gland functions (Leiba et al., 1990, Mircheff et al., 1992, Sullivan et al., 1996). In addition, glucocorticoids, retinoic acid, insulin, and glucagon are known to affect various aspects of the lacrimal gland (Petersen, 1976, Rocha et al., 2000, Ubels, Dennis and Lantz, 1994). The receptors for α -MSH, prolactin, androgens, estrogens, progestins, glucocorticoids, retinoic acid, and insulin are either transcribed and/or translated in lacrimal tissue (Hann, Taturo and Sullivan, 1989, Leiba et al., 1990, Mircheff et al., 1992, Rocha et al., 2000, Ubels, Sulahian and Viel, 1997). Arginine vasopressin (AVP), a peptide produced in the posterior pituitary whose primary physiologic role is fluid homeostasis, is also present in the lacrimal gland acinar and ductal cells (Djeridane, 1994).

Androgens are potent hormones that stimulate the secretion of secretory IgA, an important component of the mucosal immune system of the eye (Sullivan et al., 1996). Androgens have been shown to account for many of the gender-related differences seen in the lacrimal gland (Azzarolo et al., 1997, Sullivan et al., 1999).

3. Inflammatory disorders of the lacrimal gland

In several pathological instances, the lacrimal gland can become a target of the immune system and show signs of inflammation. This can occur as a result of autoimmune diseases (Sjögren's syndrome), following bone marrow transplants (graft versus host disease), or simply as a result

of aging. These will be discussed in the next sections. Lacrimal gland inflammation results in insufficient secretion leading to dry eye syndrome. There are two major types of dry eye syndrome: aqueous-deficient dry eye (which will be discussed in this review) due to lacrimal gland diseases and evaporative dry eye which is mainly due to meibomian gland diseases (Lemp, 1995).

a. Sjögren's syndrome—Sjögren's syndrome (SS) is the most commonly under-diagnosed autoimmune disease. It is not uncommon for there to be a delay of 5 to 10 years after symptom onset before a diagnosis is made (Asmussen, 2001, Oxholm and Asmussen, 1996). Moreover, the prevalence of this syndrome varies widely depending on the criteria for classification, but it is estimated that over 1 million North Americans, mostly women, suffer from this disease (Fox, 2000). The etiology of SS, although still ill-defined, is thought to be multifactorial, involving viral, neural, genetic, and environmental factors (Fox, Törnwall and Michelson, 1999, Mariette, 2002, Mariette, 2003). The fact that SS affects almost exclusively females (> 90%) highlights the role of the endocrine system (sex steroid hormones) in this disease (Sullivan et al., 1999).

SS is a systemic inflammatory disease affecting primarily the lacrimal and salivary glands. It may exist as a primary disorder (primary SS) or can be associated with other autoimmune diseases (secondary SS) such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), or systemic sclerosis (Fox, 1992). SS is a T cell-driven autoimmune disease and is characterized by focal lymphocytic infiltration of the lacrimal and salivary glands (Fox, 1992). The majority of these T cells are autoreactive CD4⁺ that seem to surround B cells (Fox, 1992).

Another hallmark of SS is the presence of circulating autoantibodies presumably produced by B cells (Fox and Stern, 2002). Patients with SS frequently present autoantibodies to both organ and non-organ-specific autoantigens. The most commonly detected autoantibodies are those directed against the ribonucleoproteins Ro/SSA and La/SSB and are in fact included as a criterion for diagnosis (Vitali et al., 2002). Other autoantibodies include those against α -fodrin and M₃ muscarinic receptors (Gordon et al., 2001, Haneji et al., 1997). Antibodies against α -fodrin have been proposed to play a role in the initiation/perpetuation of the immune attack against the lacrimal gland. However, the mechanism(s) by which this could be achieved is still not clear. α -Fodrin autoantibodies were also proposed as a reliable diagnostic tool for SS (Witte et al., 2000) but recent studies have cast doubt about their usefulness (Ruffatti et al., 2004, Witte et al., 2003, Zandbelt et al., 2004). The potential role of M₃ muscarinic autoantibodies in interfering with lacrimal gland functions will be discussed later.

In addition to autoantibodies, increased expression of several proinflammatory cytokines is also common in SS patients (Fox et al., 1994, Mariette, 2002). These inflammatory mediators might play an important role, not only by perpetuating the immune attack against the lacrimal gland, but also by impairing its functions.

The treatment of the ocular aspects of SS is mainly symptomatic (Fox, 2000). Ocular treatments include the use of artificial tears, topical autologous serum eye drops, or punctal plug occlusion (Fox, 2003). Recently, oral pilocarpine and cevimeline, two cholinergic muscarinic agonists, were shown to stimulate salivation in SS patients and received FDA approval (Fox, 2002). The effect of both agonists on tear production in SS is still unclear. Topical cyclosporine A was shown to suppress ocular inflammation and restore tear production in severe cases of KCS (Stevenson, Tauber and Reis, 2000) and recently received FDA approval. The effect of neutralizing inflammatory cytokines is discussed later.

b. sarcoidosis—Sarcoidosis is a chronic systemic disorder of unknown origin with an estimated prevalence ranging from 1 to 40 cases per 100,000 population (Bresnitz and Strom, 1983). It is characterized by the presence of noncaseating granulomas in multiple organs with the lungs being involved most frequently (Baughman, Lower and du Bois, 2003). Other organs include the spleen, liver, lymph nodes, skin, and salivary and lacrimal glands (Baughman et al., 2003, Jones, 2002).

The vast majority of sarcoidosis patients suffer from dry eye and dry mouth (Drosos et al., 1989, Drosos et al., 1999, Ramos-Casals et al., 2004). The involvement of the salivary and lacrimal glands in sarcoidosis and the fact that this disease shares several of the extraglandular features of SS makes it difficult to differentiate between the two diseases. In fact, Ramos-Casals et al. showed in a study of 59 patients that sarcoidosis and SS coexisted in 28 cases while in the remaining patients sarcoidosis mimicked SS (Ramos-Casals et al., 2004).

Sarcoidosis is also a T cell-driven disease. Scattered lymphocytic infiltrates are very common in sarcoidosis but, in contrast to SS, do not form foci (Drosos et al., 1989, Drosos et al., 1999, Ramos-Casals et al., 2004). Another finding in sarcoidosis is the increased production of circulating proinflammatory cytokines, especially TNF α (Agostini, Meneghin and Semenzato, 2002, Barnard and Newman, 2001, Baughman and Iannuzzi, 2003, Baughman et al., 2003).

Corticosteroids are indicated for the treatment of cardiac, nervous system, severe ocular, and symptomatic or progressive pulmonary involvement in sarcoidosis (Baughman et al., 2003). Specific TNF α antagonizing biological agents such as Infliximab and Etanercept are being tested in patients with sarcoidosis with mixed success. Infliximab has been shown to produce clinical improvement and reduce the requirement for corticosteroids in a small number of patients with sarcoidosis (Baughman and Iannuzzi, 2003). However, the effects of these treatments on sarcoidosis-associated dry eye have not been documented.

c. Chronic graft-versus-host disease—Graft-versus-host disease (GVHD) develops after hematopoietic stem cell transplantation performed on patients suffering from hematologic malignancies (Kansu, 2004). This disease can be acute or chronic showing different onsets and clinical features. Chronic GVHD usually develops 100 days after transplantation and is characterized by signs and symptoms similar to autoimmune diseases (Kansu, 2004). Dry eye is the most frequent ocular complication associated with chronic GVHD occurring in 50% to 70% of cases (Anderson and Regillo, 2004, Calissendorff, el Azazi and Lonnqvist, 1989, Mencucci et al., 1997, Ogawa and Kuwana, 2003, Ogawa et al., 1999).

The histopathologic features of the lacrimal gland in chronic GVHD include prominent fibrosis and an increase in stromal fibroblasts (Jack et al., 1983, Ogawa and Kuwana, 2003). As in SS and sarcoidosis, chronic GVHD is a T cell-driven disease with CD4⁺ and CD8⁺ T cells detected in periductal areas of the lacrimal gland (Ogawa et al., 2003, Ogawa et al., 2001). There is also a dysregulation of cytokine production with increased production of proinflammatory cytokines (Antin and Ferrara, 1992).

As with SS, current therapies for chronic GVHD-associated dry eye are still mainly symptomatic. They include the use of artificial tears, topical steroids, immunosuppressants, autologous serum eye drops, or punctual plug occlusion (Ogawa and Kuwana, 2003). Anti-cytokine therapy is also being tested on GVHD patients but the effects on dry eye have not been documented (Antin et al., 1994, Jacobsohn and Vogelsang, 2004).

d. Aging—The prevalence of aqueous-deficient type of dry eye is known to be high among the elderly (Dalzell, 2003, Moss, Klein and Klein, 2000, Schaumberg et al., 2003, Schaumberg,

Sullivan and Dana, 2002). Studies in animals have shown that lacrimal gland secretion in response to several neural agonists decreases with increasing age (Bromberg, Cripps and Welch, 1986, Bromberg and Welch, 1985, Draper et al., 1998, Ríos et al., 2005). Other studies have shown that, with age, the lacrimal gland undergoes dramatic structural changes highlighted by inflammation (Adeghate, Draper and Singh, 2002, Benson, 1964, Damato et al., 1984, Draper et al., 1998, Obata et al., 1995, Ríos et al., 2005, Singh, Draper and Adeghate, 2002, Walker, 1958, Williams, Singh and Sharkey, 1994). Increased focal infiltration by T and B cells, increased numbers of mast cells, and increased accumulation of lipofuscin in the lacrimal gland occur with aging (Adeghate et al., 2002, Benson, 1964, Damato et al., 1984, Draper et al., 1998, Obata et al., 1995, Ríos et al., 2005, Singh et al., 2002, Walker, 1958, Williams et al., 1994).

Aging is also associated with increased production of proinflammatory cytokines (Krabbe, Pedersen and Bruunsgaard, 2004). Increased amounts of IL-1 β and TNF α were found in lacrimal glands of old, but not young mice (J. Ríos, personal communication). Additionally, as in SS, sarcoidosis and chronic GVHD, the lacrimal gland from aged individuals shows signs of atrophy and increased fibrosis (Damato et al., 1984, Obata et al., 1995, Ríos et al., 2005).

e. Other—Lacrimal gland deficiency is associated with several other systemic and autoimmune diseases. Although the lacrimal gland is not the primary target in these diseases, inflammation of this gland is often seen. These diseases include, but are not limited to, hepatitis C (De Vita et al., 2002, Ramos-Casals et al., 2002, Ramos-Casals et al., 2001), acquired immunodeficiency syndrome (AIDS) due to infection by human immunodeficiency virus (HIV) (Chronister, 1996, DeCarlo et al., 1995), thyroid disease (Eckstein et al., 2004, Gilbard and Farris, 1983, Patel and Lundy, 2002, Punzi and Betterle, 2004), and diabetes (Goebbels, 2000, Grus et al., 2002, Nepp et al., 2000, Palmowski and Ruprecht, 1995).

The fact that the lacrimal (and salivary) gland is involved in both hepatitis C and HIV infections reinforces the possible involvement of viruses in the pathogenesis of autoimmune diseases of the lacrimal gland (Flescher and Talal, 1991, Ramos-Casals et al., 2002, Ramos-Casals et al., 1999). Indeed, it has been suggested that an initial infection and/or reactivation of Epstein-Barr virus, cytomegalovirus, or herpes virus-6, all of which have been detected in biopsies from SS patients, might be a cause of SS (Fox, 1992, Mariette, 1995, Mariette, 1998, Pflugfelder et al., 1993, Sullivan et al., 1997).

f. Summary—Despite the fact that the diseases discussed above have different etiopathologies, they all result in dry eye due to lacrimal gland inflammation. Although the type and nature of lymphocytic infiltrates in these diseases differed and in some cases the infiltrates were absent, the resulting symptoms were the same. One common denominator that these diseases have is the dysregulation of cytokine production often reflected by increased production of proinflammatory cytokines and/or decreased production of anti-inflammatory cytokines.

4. Mechanisms of lacrimal gland dysfunction

Apoptosis, hormonal imbalance, production of autoantibodies, alterations in signaling molecules, neural dysfunction, and increased levels of proinflammatory cytokines have been proposed as possible mediators of lacrimal gland insufficiency and will be discussed in the following sections.

a. Apoptosis—Apoptosis of the acinar and ductal epithelial cells of the salivary and lacrimal glands has been proposed as a possible mechanism responsible for the impairment of secretory function (Manganelli and Fietta, 2003, Mariette, 2003). Apoptotic cell death of the epithelial

cells is probably due to activation of several apoptotic pathways involving Fas (Apo-1/CD95), FasL (FasL/CD95L), Bax, caspases, perforin, and granzyme B. It might also be due to an imbalance in the expression of pro-apoptotic (Fas, Bax) versus anti-apoptotic factors (Bcl-2, Bcl-X) (Tapinos et al., 1998).

Inflamed lacrimal gland epithelial cells express several cytokines (IL-1, TNF α), protooncogenes (c-myc), autoantigens (Ro, La, α -fodrin), and costimulatory molecules (B71, B72) that can play a role in apoptotic cell death (Tapinos et al., 1998). Cytotoxic T cells through the release of proteases, such as perforin and granzyme B, or the interaction of Fas ligand expressed by T cells, with Fas on epithelial cells, can lead to apoptosis (Manganelli and Fietta, 2003, Mariette, 2003). Intriguingly, the infiltrating lymphocytes are apoptosis-resistant despite the fact that they express the apoptosis-inducer factor, Fas. This might be due in part to the high expression of Bcl-2 (anti-apoptotic factor) in these cells (Tapinos et al., 1998).

Some studies suggested that apoptosis of the lacrimal and salivary gland epithelial cells in SS is responsible for the decreased secretory function of these glands (Humphreys-Beher et al., 1999, Tsubota et al., 2003). However, other studies showed limited roles for apoptosis in SS (Ohlsson et al., 2001, Van Blokland et al., 2003). The different experimental approaches used in these studies might explain some of the discrepancies.

Although apoptosis does occur in inflamed lacrimal and salivary glands, its impact on the function of these tissues is still controversial. It is very likely that the resistance of the infiltrating lymphocytes to apoptosis, rather than apoptosis of the epithelial cells *per se*, could have important consequences on lacrimal and salivary gland physiology.

b. Hormonal imbalance—As discussed earlier, hormones have a profound impact on lacrimal gland structure and function and may be involved in susceptibility of this tissue to disease (Sullivan et al., 1997, Sullivan et al., 1999). An imbalance or dysfunction of the neuroendocrine system may play an important role in dry eye associated with lacrimal gland diseases (Johnson and Moutsopoulos, 2000, Sullivan et al., 1997). Several studies have documented the central role of androgens in the support of lacrimal gland functions (Rocha et al., 1998, Sullivan et al., 1996, Toda et al., 1998). Interestingly, androgen insufficiency alone does not cause lacrimal gland inflammation or decreased aqueous tear production, rather it is suggested that androgen deficiency may promote the progression of lacrimal gland inflammation (Sullivan et al., 1999). Reduced levels of circulating androgens were found in SS patients (Sullivan, 2004, Sullivan et al., 2003). Androgens have potent anti-inflammatory properties and were shown to increase tear production in animals as well as in humans (Azzarolo et al., 1999, Rocha et al., 1998, Sullivan and Edwards, 1997, Sullivan, Rocha and Sato, 1995). The effects of androgens on the lacrimal gland are mediated by androgen receptors within the epithelial cells to alter the expression of pro- and anti-apoptotic factors as well as cytokines (inhibits proinflammatory cytokine production and enhances that of anti-inflammatory ones) (Sullivan, 1997).

On the other hand, estrogens and prolactin are known to possess pro-inflammatory properties (Sullivan et al., 1996). Several studies have shown increased relative levels of estrogens and/or prolactin in autoimmune diseases (Allen et al., 1996, Johnson and Moutsopoulos, 2000, Mathers et al., 1998, Nagler and Pollack, 2000, Taiym, Haghighat and AlHashimi, 2004). Furthermore, another study showed that women using hormone replacement therapy (particularly estrogen alone) were at greater risk of developing dry eye syndrome (Schaumberg et al., 2001, Schaumberg et al., 2003).

In summary, most of the studies highlighted above suggest that a decline in androgen levels might play an important role in the susceptibility of the lacrimal gland to inflammation. Also,

given the abundant literature, it is very likely that androgen-based therapies could be a viable option to decrease inflammation and restore tear production in SS-associated dry eye.

c. Autoantibodies—Production of autoantibodies is a hallmark of autoimmune diseases. In the case of autoimmune diseases of the lacrimal gland, several autoantibodies have been described. The most commonly detected autoantibodies in SS patients are those directed against the ribonucleoproteins Ro/SSA and La/SSB (Fox, 1992). Other autoantibodies include those against α -fodrin and M₃ muscarinic receptors (Gordon et al., 2001, Haneji et al., 1997). Anti-Ro/SSA, La/SSB and α -fodrin antibodies have no direct impact on lacrimal gland secretion and will not be discussed.

Hu et al. and Yamamoto et al., using non-obese diabetic (NOD) mice as a model for SS, were the first to document the presence of autoantibodies against cell surface receptors (Hu et al., 1994, Yamamoto et al., 1996). These included autoantibodies against the β -adrenergic (Hu et al., 1994) and the muscarinic receptors (Yamamoto et al., 1996). Later, several investigators reported the presence of autoantibodies against M₃ muscarinic receptors in SS patients (Bacman et al., 1998, Bacman et al., 1998, Cavill, Waterman and Gordon, 2004, Li et al., 2004, Perez Leiros et al., 1999, Reina et al., 2004, Waterman, Gordon and Rischmueller, 2000).

The role of these autoantibodies in the impaired function of the lacrimal gland is still controversial. Some studies showed that these autoantibodies have agonistic (i.e., stimulating) activity (Bacman et al., 1998, Bacman et al., 1998, Reina et al., 2004) whereas others showed that they have antagonistic (i.e., inhibitory) activity (Cavill et al., 2004, Li et al., 2004, Nguyen et al., 2000, Waterman et al., 2000). This discrepancy might be due in part to the way the effects of these autoantibodies were tested as well as the source of autoantibodies used. The studies showing agonistic effects of M₃ autoantibodies used sera from primary SS patients as a source and were tested on lacrimal gland acinar cell preparations (Bacman et al., 2001, Bacman et al., 1998, Bacman et al., 1998). The studies that showed antagonistic activities either used artificially raised antibodies (Cavill et al., 2004, Nguyen et al., 2000) or tissues other than the lacrimal or salivary glands (bladder, colon smooth muscle) (Cavill et al., 2004, Waterman et al., 2000). Furthermore, a recent study showed that anti-muscarinic antibodies could not be detected in SS patients using conventional immunological methods (western blotting and ELISA) although the authors found a reversible reduction in agonist-stimulated calcium elevation in salivary acinar cells (Dawson et al., 2004).

In summary, although the presence of anti-muscarinic antibodies in SS patients is not questionable, their role in impairing lacrimal (and salivary) gland secretion is still controversial. Furthermore, the clinical efficacy of muscarinic agonists (pilocarpine and cevimeline) on SS patients' salivary glands would indirectly argue against an inhibitory effect of anti-muscarinic antibodies (Fox, 2002).

d. Alterations in signaling molecules—A plausible explanation for the loss of lacrimal gland secretion in disease states would be the inability of the acinar and ductal cells to respond to neural and hormonal stimuli. Studies in NOD mice showed a decreased responsiveness of salivary glands to adrenergic agonists, a down-regulation of β -adrenergic receptors along with their intracellular signaling components (Hu and Humphreys-Beher, 1995, Hu et al., 1994). The same group showed a decreased responsiveness of salivary glands to cholinergic stimulation (Yamamoto et al., 1996). Again, it was suggested that this was due to a down-regulation of muscarinic receptors (Yamamoto et al., 1996). However, recent studies performed on SS human salivary gland acinar cells showed that the response to cholinergic stimulation was at best comparable, if not higher, than that obtained from healthy controls (Dawson et al., 2001, Pedersen et al., 2000).

Our own studies showed that acinar cells isolated from inflamed lacrimal and salivary glands, of a murine model of SS, not only responded to both cholinergic and adrenergic stimulations, but the responses were exaggerated when compared to control cells (Zoukhri et al., 1998). The up-regulated response followed the pattern of disease progression in this animal model. In support of supersensitivity of diseased glands to exogenous neurotransmitter stimulation, a recent study showed an up-regulation of muscarinic receptors in salivary glands of SS patients (Beroukas et al., 2002). We hypothesized that the supersensitivity of diseased lacrimal and salivary glands to exogenous stimulation might be due to the inability of their nerves to release their neurotransmitters, which make both glands behave as if denervated (Zoukhri et al., 1998). We later showed that diseased lacrimal and salivary gland nerves are not able to release their neurotransmitters (Zoukhri and Kublin, 2001).

Stimulation of lacrimal gland cholinergic muscarinic receptors leads to activation of protein kinase C (PKC) (Hodges and Dartt, 2003). PKC is a family of 11 closely related isoforms of which five (PKC α , - δ , - ϵ , - λ , and - μ) are expressed in the lacrimal gland and play a major role in cholinergic-induced protein secretion (Zoukhri et al., 1997, Zoukhri et al., 1997). One immunohistochemical study reported a weak or absent staining for three isoforms of PKC in SS salivary glands (Törnwall et al., 1997). This finding could not be duplicated in lacrimal glands from NOD mice (Tensing et al., 2004). Whether this is true for human lacrimal glands awaits further investigation.

Another molecule whose distribution was reported to be defective in SS lacrimal glands is aquaporin-5 (Tsubota et al., 2001). Aquaporins are a family of water channels comprised of at least ten members in mammals, four of which (aquaporin-1, -3, -4, and -5) are expressed in the lacrimal gland (Moore et al., 2000, Verkman, 2003). These proteins are believed to play a role in water movement in the lacrimal gland (Verkman, 2003). A recent study showed defective cellular trafficking of lacrimal gland aquaporin-5 in SS, linking it to decreased lacrimal gland secretion in these patients (Tsubota et al., 2001). However, other studies failed to report such a defect in SS glands (Beroukas et al., 2001). Furthermore, genetic studies using knock-out mice lacking aquaporin-1, -3, -4, or -5, showed that deletion of these proteins did not affect basal or agonist-stimulated tear fluid production or chloride concentration, providing direct evidence against a role for aquaporins in lacrimal gland fluid secretion (Moore et al., 2000).

In summary, lacrimal (and salivary) gland acinar cells from SS retain their ability to respond to exogenous agonist stimulation. Whether or not some of the intracellular signaling components are defective in SS is still open for debate. Nevertheless, normal responsiveness of exocrine tissues can explain the effectiveness of muscarinic agonists in treating sicca symptoms in SS patients (Fox, 2002).

e. Neural dysfunction—As discussed above, lacrimal gland secretion is under tight neural control (Figure 1). Therefore, a loss of innervation in the inflamed lacrimal gland could be another attractive hypothesis to explain the associated loss of lacrimal gland secretion (Hakala and Niemela, 2000). Several studies have shown that this is not the case (Konttinen et al., 1992, Konttinen et al., 1992, Tornwall et al., 1994). We have conducted an extensive study using a murine model of SS prior to onset of disease, early, middle, and at later stages of lacrimal gland disease (Zoukhri, Hodges and Dartt, 1998). We showed that although parasympathetic, sympathetic, and sensory nerves were absent in fibrotic areas of the lacrimal gland, they were present in non-fibrotic areas (Zoukhri et al., 1998) (Figure 2). Furthermore, since we used antibodies against neurotransmitters, our results also suggested that the remaining nerves contained neurotransmitters (Zoukhri et al., 1998) (Figure 2). Hence, the lack of lacrimal gland secretion in SS cannot be explained by a loss of neural support. In fact we found that the remaining nerves were not able to release their neurotransmitters and that this correlated with the lack of lacrimal gland protein secretion (Zoukhri and Kublin, 2001).

In another study, we investigated the effect of aging on lacrimal gland innervation (Ríos et al., 2005). We used BALB/c mice at 3, 8, 12, 24, and 32 months of age. We found that up to 12 months, the density and distribution of parasympathetic and sympathetic nerves was indistinguishable from that seen in young animals (Ríos et al., 2005). However, starting at 24 months, the innervation decreased, which coincided with a decline in acetylcholine release from the lacrimal gland (Ríos et al., 2005). In contrast, we found that neural, cholinergic, and adrenergic agonist stimulated lacrimal gland secretion decreased at eight months of age and continued at this depressed rate for 12 to 32 months (Ríos et al., 2005). Hence, it was concluded that reduced lacrimal gland secretion with aging could not be explained solely by a decrease in innervation. It is believed that, as is the case in SS, proinflammatory mediators might play a role in inhibiting lacrimal gland secretion during aging.

In summary, even in the worst cases of lacrimal gland inflammation, the autonomic innervation is not completely lost. However, accumulating evidence suggest that these remaining nerves are not able to release their neurotransmitters, which leads to impaired lacrimal gland secretion.

f. Proinflammatory cytokines—A common denominator in all inflammatory diseases of the lacrimal gland is the increased production of proinflammatory cytokines. These cytokines not only perpetuate the immune attack on the lacrimal gland by stimulating lymphocyte recruitment and proliferation but also, as discussed below, they interfere with the normal functions of the gland. In this section, the role of two potent proinflammatory cytokines, IL-1 β and TNF α , in the impaired function of the diseased lacrimal gland will be described.

Several reports have shown that the amounts of IL-1 β and/or TNF α are increased in lacrimal glands from SS, GVHD, sarcoidosis, and aging individuals (Agostini et al., 2002, Antin and Ferrara, 1992, Baughman and Iannuzzi, 2003, Willeke et al., 2003). Studies in animal models of these diseases have also shown increased levels of IL-1 β and TNF α in the lacrimal gland (Zoukhri et al., 2002). It is of interest that some studies have shown that these cytokines were not only being produced by the invading immune cells, but also by the lacrimal gland epithelial cells themselves (Robinson et al., 1998, Zoukhri et al., 2002) (Figure 3). This could have important consequences because it implies that, after the onset of lacrimal gland inflammation, the epithelial cells become active participants in perpetuating the inflammatory state and can no longer be viewed as bystanders. This is further supported by the fact that lacrimal gland acinar cells can act as professional antigen presenting cells and stimulate the proliferation of T cells (Guo et al., 2000, Guo et al., 2000, Hakala and Niemela, 2000). Therefore, strategies aimed only at halting the immune infiltration might not be successful in restoring lacrimal gland function if they are not combined with strategies geared towards neutralizing the local production of inflammatory cytokines.

In several inflammatory diseases it was shown that IL-1 β and TNF α can directly inhibit neural activity (Collins et al., 1992, Cunningham et al., 1996, Jacobson, McHugh and S.M., 1997, Swain, Blennerhassett and Collins, 1991). Our studies have shown that exogenous addition of IL-1 β or TNF α inhibited neurotransmitter release which lead to impaired lacrimal gland secretion (Zoukhri et al., 2002). The effects of IL-1 β were specific as they could be blocked by an IL-1 neutralizing antibody (Zoukhri et al., 2002). Furthermore, we showed that the amounts of IL-1 β and its receptor (IL-1R type 1, the signaling receptor) were up-regulated in the lacrimal gland of a murine model of SS (Zoukhri et al., 2002) (Figure 4). We also showed that the lacrimal gland epithelial cells were a source of IL-1 β (Figure 3) and expressed the IL-1R type 1 (Figure 5) (Zoukhri et al., 2002). These findings coupled with the fact that inflamed lacrimal gland nerves are not able to release their neurotransmitters (Zoukhri and Kublin, 2001) suggest that endogenously produced proinflammatory cytokines might be responsible for this blockade.

Increased amounts of proinflammatory cytokines are found in the tears of SS patients (Pflugfelder et al., 1999, Solomon et al., 2001, Tishler et al., 1998). Activation of the sensory nerves in the cornea and conjunctiva is a crucial component of the neural reflex arc that regulate lacrimal gland secretion (Botelho, 1964, Dartt, 1994). Although not proven, it is tempting to speculate that proinflammatory cytokines could inhibit ocular surface sensory nerve activity, as they do to the parasympathetic and sympathetic nerves of the lacrimal gland. This hypothesis is supported by the lower corneal sensitivity found in SS patients (Adatia et al., 2004). In this scenario, increased production of proinflammatory cytokines would inhibit both the afferent as well as the efferent systems thus shutting down communication between the ocular surface and the lacrimal gland (Figure 6).

Another potential mechanism by which proinflammatory cytokines might affect lacrimal gland function is by altering sex steroid metabolism. As discussed earlier, sex steroids play an important role in the immunology and physiology of the lacrimal gland (Azzarolo et al., 1997, Sullivan, 2004). It has been shown that proinflammatory cytokines can increase the conversion of androgens into estrogens (Sullivan, 1997). This would result in decreased androgen and increased estrogen levels, a situation associated with SS as well as other inflammatory diseases (Johnson and Moutsopoulos, 2000, Sullivan, 2004, Sullivan et al., 2003, Taiym et al., 2004). As discussed earlier, decreased levels of androgens and/or increased levels of estrogens have been linked to lacrimal gland inflammation and tear deficiency in SS (Sullivan, 2004, Sullivan et al., 2002). Additionally, proinflammatory cytokines can also alter the metabolism of other hormones and peptides that regulate lacrimal gland homeostasis including ACTH, arginine vasopressin, and prolactin (Chikanza, Petrou and Chrousos, 2000, Sternberg, 2001, Yasin et al., 1994).

Studies in animals showed that delivery of a gene encoding an inhibitor of TNF α suppressed experimentally-induced lacrimal gland inflammation (Zhu et al., 2003). Similarly, delivery of a gene encoding IL-10 (an anti-inflammatory cytokine) also suppressed experimentally induced lacrimal gland inflammation (Zhu et al., 2004). Infliximab, a chimeric mouse/human monoclonal antibody, and Etanercept, a fully human soluble TNF receptor fusion protein, are two drugs known to neutralize TNF α and are FDA-approved for the treatment of RA (Maini and Taylor, 2000). In an open label pilot study of 16 SS patients, Infliximab (3 mg/kg) improved several symptoms including dry eye and dry mouth (Steinfeld, Demols and Appelboom, 2002, Steinfeld et al., 2001). However, a randomized, double-blind, placebo-controlled trial showed no beneficial effects of Infliximab (5 mg/kg) on SS patients (Mariette et al., 2004). Studies using Etanercept also gave somewhat conflicting results: minimal effects (Zandbelt et al., 2004) versus no effect at all (Sankar et al., 2004). Although it is not clear why the results differed between these clinical trials, tissue penetration might be one possibility. Infliximab and Etanercept are rather large molecules and may not reach the lacrimal and salivary glands.

In summary, proinflammatory cytokines can alter both the neural as well as the hormonal support of the lacrimal gland. They can also potentially inhibit the afferent branch of the neural reflex arc connecting the lacrimal gland to the ocular surface (Figure 6). Although preliminary trials aimed at neutralizing TNF α did not yield positive results, studies on the effects of neutralizing other proinflammatory cytokines or delivery of anti-inflammatory cytokines to the lacrimal gland and/or the ocular surface are warranted.

5. Conclusions

The prevalence of dry eye syndrome is very high (Moss et al., 2000, Schaumberg et al., 2003, Schaumberg et al., 2002). Dry eye is now regarded by many investigators as an inflammatory disorder affecting the ocular surface and the tear producing glands (Pflugfelder, 2004). Inflammation of the lacrimal gland resulting in dry eye is found in several systemic

diseases of autoimmune origin or not. Our knowledge of the pathogenesis of lacrimal gland inflammation is still not complete. Furthermore, treatment for dry eye remains mainly symptomatic. A further understanding of the impact of proinflammatory cytokines on the lacrimal gland and the ocular surface might lead to novel therapies targeted towards the inflammatory process. Such advancement has been achieved in other inflammatory disorders such as RA (Maini and Taylor, 2000).

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6. References

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Figure 1.

Schematic of the neural reflex arc that connects the ocular surface to the lacrimal gland. Activation of the afferent sensory nerves in the cornea and conjunctiva leads to activation of efferent parasympathetic and sympathetic nerves that signal the lacrimal gland to secrete proteins, electrolytes and water. Reprinted from *The Ocular Surface* 2004, 2: 76-91.

Figure 2. Immunolocalization of parasympathetic nerves in normal and inflamed lacrimal glands. Fluorescence micrograph showing the localization of the parasympathetic neurotransmitter, vasoactive intestinal peptide (VIP), in the lacrimal glands of 18-week old female MRL/lpr (diseased) and MRL/+ (control) mice. Upper micrographs show Hematoxylineosin (H&E) staining of the lacrimal gland sections (See also, Zoukhri et al., Clin. Immunol. Immunopathol. 1998; 89(2): 126-133).

Figure 3.

Immunolocalization of IL-1 β in the lacrimal gland. Lacrimal glands were removed from 13-week old female MRL/lpr (diseased mice, A, B, and D) and 18-week old female MRL/+ (control mice, C and E) mice. A, B, and C, micrographs depict immunofluorescence staining with an antibody against murine IL-1 β . D and E, micrographs showing negative controls in which the primary antibody was omitted. An asterisk denotes areas of lymphocytic infiltration. Reprinted from Zoukhri et al., *Invest. Ophthalmol. Vis. Sci.* 2002; 43: 1429-1436.

Figure 4.

Immunodetection of IL-1RI in lacrimal gland homogenates. Lacrimal glands were removed from 16-week old female MRL/lpr (diseased) and MRL/+ (control) mice. Acinar cells were then prepared and lymphocytes removed. Acini were homogenized and proteins separated by SDS-PAGE followed by western blotting. The molecular weight (in KDa) of protein standards is shown in the left. Asterisks denote the migration of IL-1RI. The upper band is a heavily glycosylated and slowly migrating form of the IL-1RI. Each lane represents samples from a different animal (See also, Zoukhri et al., Invest. Ophthalmol. Vis. Sci. 2002; 43: 1429-1436).

Figure 5.

Immunolocalization of IL-1RI in the lacrimal gland. Lacrimal glands were removed from 13-week old female MRL/lpr (diseased) and 18-week old female MRL/+ (control) mice. A, B, and C, micrographs depict immunofluorescence staining with an antibody against murine IL-1RI. D and E, micrographs showing negative controls in which the primary antibody was omitted. An asterisk denotes areas of lymphocytic infiltration. Reprinted from Zoukhri et al., *Invest. Ophthalmol. Vis. Sci.* 2002; 43: 1429-1436.

Figure 6.

Schematic showing the effect of proinflammatory cytokines on the neural reflex arc that connects the ocular surface to the lacrimal gland. Inhibition of neurotransmission either of the afferent sensory nerves or the efferent parasympathetic and sympathetic nerves would result in inadequate lacrimal gland secretion. Reprinted from *The Ocular Surface* 2004, 2: 76-91.