Review Article

The role of oxidative stress and inflammation in the pathogenesis of dry eye

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Dry eye is a multifactorial disease of the tears and the ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface. Increased osmolarity of the tear film and inflammation in the lacrimal system and ocular surface are important operational factors in the genesis of some dry eye disorders. Mechanical abrasion secondary to tear deficiency may create an inflammatory environment where conjunctival epithelial cells and lymphocytes are stimulated to produce and secrete various cytokines into the tear film. Elevated cytokine levels within the tear film, combined with reduced concentrations of essential lacrimal-gland derived factors such as epidermal growth factor (EGF) and retinol create an environment in which terminal differentiation of the ocular surface epithelium is impaired. The histopathologic changes in the minor salivary gland and lacrimal gland biopsy are characterized by focal and/or diffuse lymphoid cell infiltrates and parenchymal destruction. The majority of cells in glandular biopsy specimens are CD4+ cells with a small proportion of CD8+T-cells.

Apoptosis of acinar cells and inflammatory cells are also key events in relation to development of dry eyes. We previously showed the presence of apoptosis of acinar cells, FasL expression in lacrimal glands and that the FasL expression highly correlated with glandular function especially in those patients without glandular enlargement. FasL expression of infiltrating lymphocytes were low in the lacrimal glands of patients with SS with enlarged exocrine glands where the lacrimal gland function was well preserved even with massive lymphocyte invasion. In other words, the infiltration of lymphocytes alone did not cause glandular dysfunction. Apoptosis of acinar cells may explain these differences. Oxidative stress is recently receiving the attention of researchers and clinicians as a possible mechanism in the development of dry eye disease. Tsubota et al have shown, for the first time in the literature, increases in oxidative stress markers, changes in antioxidant-related gene expression, and discordance in differentiation capacity in corneal epithelia in dry eye conditions, suggesting a strong relationship between the accumulation of oxidative stress and the etiology of corneal epithelial alterations in blink-suppressed dry eye. The management of oxidative stress may provide a new approach for the prevention and therapeutic treatment for dry eye syndromes. The increased awareness of oxidative stress related to disease and the need to measure the delicate balance that exists between free radicals and the systems in place to regulate them has given rise to a demand for new research tools.

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Introduction

Dry eye is a multifactorial disease of the tears and the ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface. Increased osmolarity of the tear film and inflammation in the lacrimal system and ocular surface are important operational factors. The current article will review inflammatory mechanisms involved in the pathogenesis of dry eyes.

Inflammatory Background in Dry Eyes

Many exocrine glands, the ocular surface and the lacrimal glands are affected by androgen hormones. Androgen receptor protein exists within the epithelial cell nuclei of the human exocrine glands, meibomian gland, conjunctiva and cornea. Androgens influence their structural organization and functional activity, and have a profound impact on the immunology, molecular biology and secretory capacity of the lacrimal gland[1]. Such proven androgen effects led to the thinking that androgen deficiency states such as SS, SLE, RAs, aging, and the use of anti-aging medications may be very important in the pathogenesis of dry eyes[2]. The androgen binding to the receptors in the acinar nuclei of the lacrimal gland leads to an altered expression of numerous cytokines and protooncogenes. Androgens in the exocrine glands induce accumulation of anti-inflammatory cytokines such as transforming growth factor-beta (TGF-β)[2]. The reduction of the androgen level below a certain threshold may result in the release of proinflammatory cytokines such as interleukin beta (IL-1β), IL-2, interferon-γ, and tumor necrosis factor (TNF)-α by the lymphocytes trafficking the gland[3]. Evidence has shown that progressive CD4+ T-cell and B-cell infiltration occurs in lacrimal and salivary glands of animal models of dry eye and in patients with SS, clinically presenting as conjunctival injection and corneal epithelial damage in most instances (Fig.1A,B)[4]. Alterations of the nerve fibers among the glandular acini are superimposed on the inflammatory process in these patients. It is also possible that the central nervous system plays an important role in the cascade of events that occur in SS where the amount of tear flow is initially decreased through cellular infiltration of the tear gland. Increased friction between the lids and dryness of the cornea may result in exfoliation of the ocular surface epithelium resulting in constant repeated C-fiber nerve stimulation which becomes dominant in time and acts in combination with central parasympathetic inhibition for tear flow. This could also explain the clinical and experimental discrepancies of the concept of infiltration and destruction of the lacrimal gland as a single cause in tear flow depression[5].

Under normal circumstances, lymphocytes trespassing the lacrimal or an exocrine gland are expected to undergo apoptosis. In the presence of inflammation, however, the apoptotic response is aborted, and the lymphocytes release proinflammatory cytokines and inflammatory cytokines such as IL-1β, IL-6 and IL-8[6,7]. The increased expression of HLA-DR and intercellular adhesion molecule (ICAM) in the conjunctiva, for instance, has been reported to be associated with "homing" of more T-cells and cytokine release within the lacrimal gland, increasing the level of inflammation[8]. Both human and animal studies indicate the involvement of IL-10 in the pathogenesis of primary SS and mice transgenic for IL-10 develop a Fas-ligand mediated exocrinopathy that resembles SS[9,10].

Inflammation—Ocular Surface Interactions

Pflugfelder et al reported that IL-1α and β, IL-6, IL-8, TGF-β1, and TNF-α were expressed at elevated levels in the conjunctival epitheliae of patients with SS compared with dry eye controls. Mechanical abrasion secondary to tear deficiency may create an inflammatory environment where conjunctival epithelial cells and lymphocytes are stimulated to produce and secrete various cytokines into the tear film. Elevated cytokine levels within the tear film, combined with reduced concentrations of essential lacrimal-gland derived factors such as epidermal growth factor (EGF) and retinol create an environment in which terminal differentiation of the ocular surface epithelium is impaired. As a consequence, the epithelium displays increased mitotic activity, and loses the ability to express protecting molecules including membrane bound mucin, MUC-1. Indeed, relevant conjunctival impression cytology work to support this hypothesis reveal that "snake-like chromatin cells", increased grades of squamous metaplasia, decreased goblet cell density and inflammatory cells are encountered in SS patients[11-13]. The ocular surface changes in SS dry eyes are frequently associated with corneal hypoesthesia, which creates a vicious cycle. These would all imply that anti-inflammatory medications that suppress the inflammatory component of this cascade may ameliorate the ocular surface disease and discomfort experienced by SS patients (Fig.2A,B).

The Role of Apoptosis in dry eye disease

Autoimmune diseases are mostly characterized by tissue destruction and functional decline due to autoreactive T-cells that escape self-tolerance. Although the specificity of cytotoxic T-lymphocyte function has been an important issue of organ specific autoimmune response, the mechanisms responsible for tis-

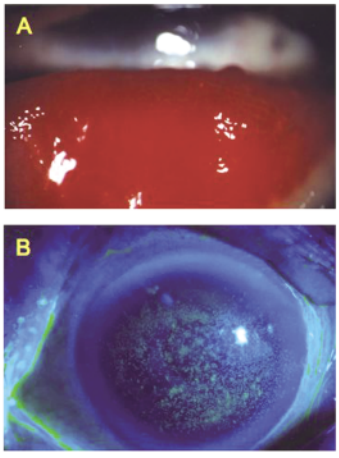

Fig.1
A: Note the conjunctival injection in a patient with severe dry eye
B: Note the corneal epithelial damage in the patient with severe dry eye (fluorescein staining)

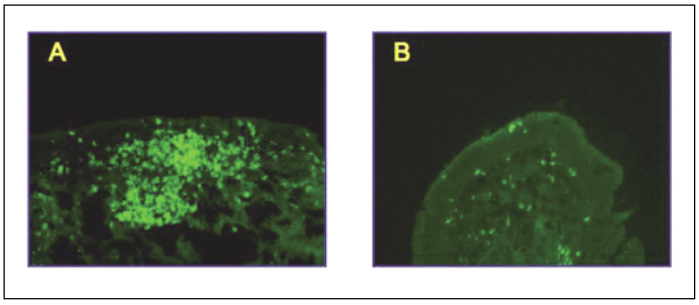

Fig.2
A: Note extensive CDS3+ lymphocyte infiltration in the lacrimal gland of a patient with secondary Sjögren's syndrome (inflammatory cell density: 2291 cells/mm^2)
B: Note the reduction in the inflammatory infiltrates (762 cells/mm^2) after 6 months' of treatment with 0.05% cyclosporine A eye drops

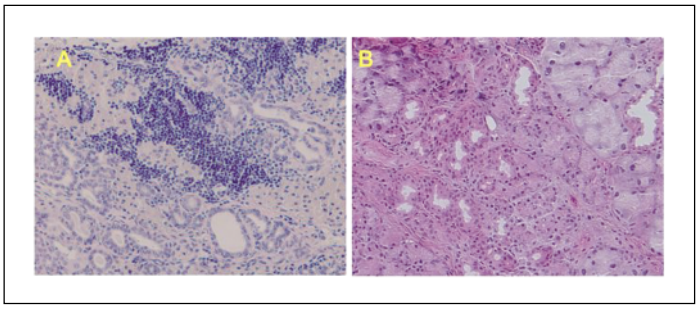

Fig.3
A: Note the extensive inflammatory cell infiltration around the acinii in the lacrimal gland of a mouse model of dry eye (SOD KO mice) (HE staining 200x magnification)
B: Note the atrophy of the acinar units in the lacrimal gland of a mouse model of dry eye (SOD KO mice)) (HE staining 200x magnification)

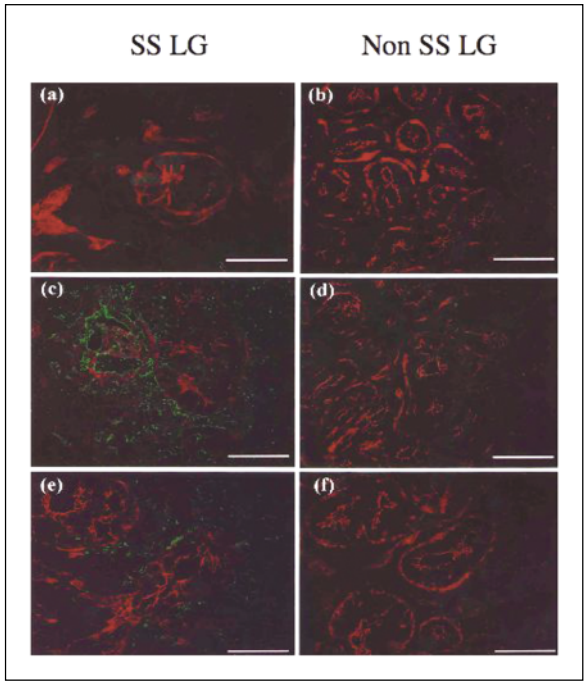

Fig.4
Quantitative evaluation and evidence of apoptotic mechanisms in SS (Reprinted from Experimental Eye Research, Vol 76, Tsubota et al, Quantitative analysis of lacrimal gland function, apoptotic figures, Fas and Fas ligand expression of lacrimal glands in dry eye patients, pp 233-240, Copyright 2007, with permission from Elsevier.)
Double staining of actin and Apo2.7, Fas or FasL in lacrimal gland. (a,c,e) Lacrimal glands from SS (b,d,f) Lacrimal glands from non-SS were stained with Apo2.7 (a,b: green), Fas (c,d: green) and FasL (e,f: green) antibody followed by rhodamine-phalloidin (red). Bars: 50 μm
(a) SS lacrimal gland: Note the (+) acinar cells
(b) Non-SS lacrimal gland: No acinar cells are stained with Apo2.7 antibody
(c) SS lacrimal gland: Note the (+) acinar cells
(d) Non-SS lacrimal gland: Some acinar cells are stained with Fas antibody
(e) SS lacrimal gland: Note (+) staining of infiltrating lymphocytes
(f) Non-SS lacrimal gland: FasL antibody did not stain any cells

sue destruction in SS remain to be elucidated. The histopathologic changes in the minor salivary gland and lacrimal gland biopsy are characterized by focal and/or diffuse lymphoid cell infiltrates (Fig.3A) and parenchymal destruction (Fig.3B). The majority of cells in glandular biopsy specimens are CD4+ cells with a small proportion of CD8+T-cells[14]. These T-cells express the $\alpha\beta$ antigen receptor and cell surface antigens associated with mature memory T-cells.

A 120 kDa organ-specific autoantigen was recently described from the salivary gland tissues of a dry eye animal model (NFS/*sld* mutant mouse thymectomized 3 days after birth (3d-TX). The sequence of the first 20 NH_2-terminal residues was found to be identical to that of cytoskeletal protein human α-fodrin[14]. Furthermore, sera from patients with SS reacted positively with purified 120 kDa antigen, and induced a proliferative response of peripheral blood lymphocytes. Purified antigen was detected from SS patients, but not from SLE or RA patients and healthy controls. These results indicate that the anti-120 kDa α-fodrin immune response plays an essential role in the development of primary SS. Recent reports have demonstrated evidences that caspase-3 is required for α-fodrin cleavage during apoptosis[14]. It has been speculated that an increase in activity of apoptotic proteases is involved in the progression of α-fodrin proteolysis during development of SS. Fodrin cleavage by caspases can potentially lead to cytoskeletal alterations and it may be of interest to remember that α-fodrin binds to ankylin, which contains a cell death domain. It has been shown that cleavage products of α-fodrin inhibit ATP-dependent glutamate and γ-aminobutyric acid accumulation into synaptic vesicles where a cleavage product can be a novel component of an unknown immunoregulatory network such as cytolinker proteins[14]. It is now also clear that the interaction of Fas with FasL regulates a large number of pathophysiological processes in apoptosis. Immunohistological studies revealed that the majority of tissue infiltrating T-cells in salivary glands bear FasL in the SS model, and epithelial duct cells express Fas antigen on their cell surface[15]. CD4+T-cells isolated from the affected glands bear a large proportion of Fas L compared to CD8+ cells in flow cytometry. Tsubota et al previously showed the presence of apoptosis of acinar cells, FasL expression in lacrimal glands (Fig.4A-F) and that the FasL expression highly correlated with glandular function especially in those patients without glandular enlargement[16]. FasL expression of infiltrating lymphocytes were low in the lacrimal glands of patients with SS with enlarged exocrine glands where the lacrimal gland function was well preserved even with massive lymphocyte invasion[16]. In other words, the infiltration of lymphocytes alone did not cause glandular dysfunction. Apoptosis of acinar cells may explain these differences.

New concepts in the pathogenesis of dry eyes: Oxidative stress and inflammation

Oxygen is the primary oxidant in metabolic reactions designed to obtain energy from the oxidation of a variety of organic molecules. Oxidative stress results from the metabolic reactions that use oxygen, and it has been defined as a disturbance in the equilibrium status of pro-oxidant/anti-oxidant systems in intact cells. This definition of oxidative stress implies that cells have intact pro-oxidant/anti-oxidant systems that continuously generate and detoxify oxidants during normal aerobic metabolism. When additional oxidative events occur, the pro-oxidant systems outbalance the anti-oxidant, potentially producing oxidative damage to lipids, proteins, carbohydrates, and nucleic acids, ultimately leading to cell death in severe oxidative stress. Researchers are now making rapid progress in understanding the role of oxidative stress in cardiovascular diseases, inflammatory diseases, dermal and ocular inflammation and arthritis, metabolic diseases such as diabetes; and diseases of the central nervous system.

Since free radicals are generally highly reactive, a few methodologies are typically required to adequately detect and quantify their existence in biological samples. To accomplish this, researchers can look at modifications to a system's regulatory mechanisms (e.g. endogenously expressed antioxidants), or look at by-products of the applied stressor (e.g. damage/modification of lipids, proteins or DNA)[17]. Researchers may also wish to look at causative factors such as the events facilitating an inflammatory cascade such as neutrophil activation and subsequent release of myeloperoxidase and/or lactoferrin. Tools for assaying the presence and quantification of some oxidative biomarkers can be summarized as: a) Lipids (MDA, HAE, LOOH, 8-Isoprostane, 8-iso- metabolite, 4-HNE) b) Proteins (Aconitase, a1-Antiproteinase, Nitrotyrosine) c) DNA (8-OHdG)[17].

Tear fluid contains various antioxidants to protect the ocular surface from radical insults such as ascorbic acid, lactoferrin, uric acid, and cysteine[18-20]. In addition to suppressed blink frequency, Tsubota et al observed decreased tear production, clearance, and tear stability with a concomitant elevation of DNA, Lipid (MDA) and 4-HNE oxidative stress markers in a jogging board dry eye mouse model as shown in Fig.5 (Nakamura S et al. IOVS 2006;47:E-Abstract 5589)[17]. It can be speculated that, because of an imbalance in the tear film status, a synergistic effect between prolonged exposure to atmospheric oxygen and insufficient supplementation of antioxidant agents may have in-

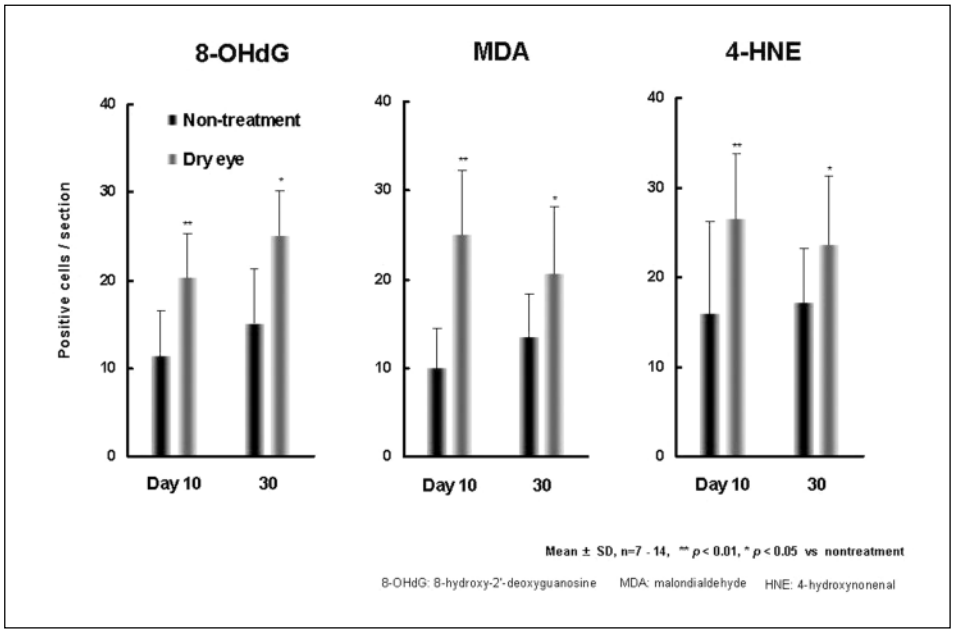

Fig.5 The number of cells positive for oxidative stress markers in the corneal epithelia of a dry eye model mouse.

Quantitative analysis of positive 8-OHdG (left), MDA (center), and 4-HNE (right) cells. Note the significant increase of all oxidative stress markers after 30 days compared to the treatment group. (Reprinted from IOVS. Nakamura S, Shibuya M, Nakashima H, Hisamura R, Masuda N, Imagawa T, Uehara M, Tsubota K. Involvement of oxidative stress on corneal epithelial alterations in a blink-suppressed dry eye. Invest Ophthalmol Vis Sci, 48(4): 1552-1558, 2007. Copyright 2007, with permission from IOVS.)

duced the overexpression of ROS production on the ocular surface.

Tsubota et al have shown, for the first time in the literature, increases in oxidative stress markers, changes in antioxidant-related gene expression, and discordance in differentiation capacity in corneal epithelia in dry eye conditions, suggesting a strong relationship between the accumulation of oxidative stress and the etiology of corneal epithelial alterations in blink-suppressed dry eye[17]. Further study is needed to elucidate the full mechanisms involved and the relative contribution of oxidative stress in patients with this type of dry eye. The management of oxidative stress may provide a new approach for the prevention and therapeutic treatment for this syndrome. The increased awareness of oxidative stress related to disease and the need to measure the delicate balance that exists between free radicals and the systems in place to regulate them has given rise to a demand for new research tools.

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