Dry Eye: Inflammation of the Lacrimal Functional Unit

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Core Messages

- Dry eye (LKC, lacrimal keratoconjunctivitis) can best be defined as a disease resulting in altered tear film composition
- Regardless of the aetiology, systemic autoimmune disease such as Sjögren's syndrome or local autoimmune diseases such as keratoconjunctivitis sicca, patients will have an immune based inflammation of the ocular surface and lacrimal glands
- Tears are secreted by the "lacrimal functional unit" composed of the ocular surface, the main and accessory lacrimal glands and the interconnecting innervation
- Current concepts of the tear film define it as a mucin/aqueous gel covered by a layer of lipid
- A decrease in systemic androgens associated with aging or various pathologies has been found to be one of the predisposing factors behind the initiation of LKC
- Effects on the ocular surface in patients with LKC include an increase in apoptosis (programmed cell death)

2.1 Introduction

2.1.1 Basics

Dry eye disease has traditionally been defined as a tear deficiency. Research over the past decade, however, has led to an appreciation of the central role that ocular surface inflammation plays in dry eye disease. Dry eye may now be viewed as a syndrome in which an unstable tear film of altered composition fails to support ocular epithelial health and instead promotes ocular surface inflammation.

Recent research has also led to recognition that the ocular surface and tear secreting glands act in concert as a functional unit [33, 41]. The lacrimal functional unit maintains ocular surface health by a homeostatic mechanism: sensory neural input from the ocular surface is integrated and directed to the secretory apparatus in order to manipulate the volume and composition of the tear film (Fig. 2.1).

Dry eye disease can arise in a number of different ways. For example, Sjögren's syndrome, a systemic autoimmune disease, probably initiates dry eye by autoimmune-mediated inflammation of the lacrimal glands, causing altered tear volume and composition (including proinflammatory cytokines), in turn leading to inflammation of ocular surface tissues. In another example, disease of the meibomian glands, which secrete the lipid layer protecting the tear film, may initially impact tear composition by allowing excess evaporation, later causing ocular surface inflammation, which can impact neural control of the lacrimal glands. Interconnectedness within the lacrimal functional unit means that the varied causes of dry eye disease all eventually manifest an unstable, proinflammatory tear film, which leads to inflammation of the ocular surface (Fig. 2.2), including apoptosis of epithelial cells within the main and accessory lacrimal glands and the conjunctiva, and chronic firing of ocular surface sensory nerves, experienced as ocular pain. In the past, the ocular surface disease associated with dry eye has been called keratoconjunctivitis sicca;

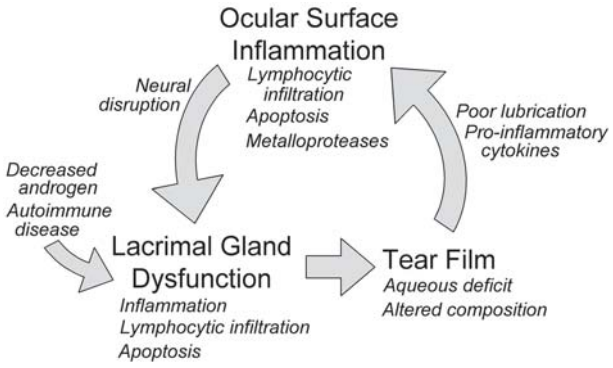

Fig. 2.1. The lacrimal functional unit maintains the health of ocular surface tissues by a homeostatic mechanism. A properly refreshed tear film provides protection, lubrication and a trophic environment for the ocular surface epithelia. Tear film components are secreted by the lacrimal and meibomian glands, and from conjunctival goblet cells, under neural control derived from afferent innervation of the ocular surface. Glandular function is also influenced by hormonal regulation

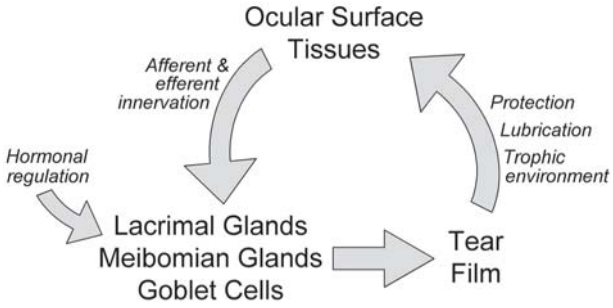

Fig. 2.2. Lacrimal keratoconjunctivitis results from dysfunction of the lacrimal functional unit. Ocular surface inflammation is promoted by a proinflammatory tear film, and by increased osmolarity due to a deficit of aqueous production. Lacrimal gland inflammation, accompanied by lymphocytic infiltration and apoptosis, is responsible for the unrefreshed tear film and its altered composition. Inflammatory effects on neural control of gland function, hormonal imbalance, and autoimmune disease can all contribute to lacrimal gland dysfunction

however, the term lacrimal keratoconjunctivitis (LKC) seems a more accurate description, in that dryness is not initially evident, and will be used in this article.

Estimates of the prevalence of dry eye vary widely, probably because of different criteria used in epidemiological studies. Prevalence estimates range from 0.7% of subjects 65 years and older that had irritation symptoms together with two clinical signs of LKC, to 15% who self-reported ocular dryness symptoms [26, 38]. Prevalence of dry eye disease increases with age, is greater in women, and may increase over time due to the advent of refractive surgeries such as LASIK (laser-assisted intrastromal keratomileusis). The disease pathophysiology can be initiated by the interruption of corneal sensory nerves that occurs during LASIK, causing dysfunction of the lacrimal functional unit [7]. Costs to society, such as time lost from work and the expense of medical care, must be substantial as well. As a suggestion of how substantial, worldwide sales of artificial tears exceeded $500 million in 2002.

2.1.2 Lacrimal Functional Unit

Tear flow is reflexively regulated by the lacrimal functional unit (Fig. 2.1). The lacrimal functional unit comprises the ocular surface, including the cornea, conjunctiva, and meibomian glands, the main and accessory lacrimal glands, and the neural network that connects them [33, 41]. Its overall purpose is to maintain corneal clarity and the quality of the image projected onto the retina. Corneal clarity depends, in turn, on the integrity of the tear film and the health of the ocular surface.

The lacrimal functional unit operates by a homeostatic mechanism. The status of the ocu-

lar surface is monitored by sensory nerves carrying information to the lacrimal centre in the brain stem. Autonomic secretomotor nerves direct secretory tissues and glands, including the main and accessory lacrimal glands, the meibomian glands, and the conjunctival goblet cells. The major variables which can be adjusted to influence the system's status (and thereby maintain or return to stasis) are the volume and composition of the tear film [33, 41].

The tear film serves four important functions: it provides a smooth optical surface for normal vision, it maintains ocular surface comfort, it protects ocular surface tissues from environmental and infectious insults, and it contain factors important for maintenance of epithelial cell health. The tear film and the anterior surface of the cornea combine to provide approximately 80 % of the refractive power for the eye's focusing mechanism. Small changes in tear film stability and volume can result in tear film break-up, causing optical aberrations that can significantly degrade the quality of vision, primarily by decrease in contrast sensitivity. Tear film break-up likely contributes to the visual fatigue and photophobia experienced by many LKC patients [4]. Ocular surface comfort depends on the tear film's lubricating properties, which decrease the shear forces exerted by the superior lid margin during a normal blink cycle [6]. The mucin layer of the tear film is critical for this lubrication. The tear film protects the ocular surface, the most environmentally exposed mucosal surface of the body, from extremes of temperature and humidity, allergens, irritants and infectious agents. The surface lipid layer, secreted by the meibomian glands, prevents evaporation of the aqueous component and consequent increases in osmolarity of the tear film in adverse environments. Some of the proteins present in the tear film, such as immunoglobulin A, lactoferrin, lysozyme, and peroxidase, help resist bacterial or viral infections. Because the corneal epithelium lacks vasculature, it is dependent on tear film electrolytes and oxygen for tissue health, and on tear film growth factors to stimulate the constant regeneration of the corneal epithelium and for wound healing. Antioxidants in the tear film help maintain a reducing environment and scavenge free radicals.

Traditionally the tear film was envisioned as three distinct components: a mucin layer coating the surface epithelium, an aqueous layer making up the majority of the tear film, and a thin lipid layer sitting on top to slow evaporation. That view has evolved to the currently proposed structure of a mucin/aqueous gel containing electrolytes, proteins, and regulatory factors that decreases in density toward the lipid layer (Table 2.1) [6, 33]. The mucin component functions as a surfactant for the ocular surface, allowing the tear film to spread evenly over the hydrophobic epithelium. It includes the glycocalyx, composed of transmembrane mucins anchored to the epithelial cell surface [11], and soluble mucins, shed by epithelial cells and secreted by conjunctival goblet cells and the lacrimal glands [16]. Soluble mucins interact with the glycocalyx and the aqueous component to form a water-trapping gel. The mucin component may also help prevent adherence of inflammatory cells, bacteria, and debris to the ocular surface [11]. The aqueous component solubilizes oxygen, electrolytes, and numerous proteins and regulatory factors. Normal tear osmolarity, about 300 mOsm/l, is important to maintain normal epithelial cell volume, for maintenance of correct nerve membrane potential, and for cellular homeostasis and secretory function. The main and accessory lacrimal glands secrete the aqueous component of tears, although their relative contributions to tear volume are unresolved. The main lacrimal gland is responsible for reflex tearing, which can flush infective or irritating particles from the ocular surface. The composition of the lipid layer is complex, with polar lipids found mostly at the lipid-aqueous interface, and non-polar lipids found at the lipid-air interface. The very diverse array of lipids found in the tear film are secreted by the meibomian glands, whose ducts exit just anterior to the mucocutaneous junction of the lids. Blinking helps to spread the lipid layer uniformly over the tear film surface, a process assisted by the low surface tension of the lipid-air interface.

The lacrimal functional unit is provided information about the status of the ocular surface by afferent innervation via the first (ophthalmic) division of the trigeminal ganglion (or the second division for the lower lid). The

Table 2.1. Components of the tear film

Component	Secreted by:	Functions
Lipid	Meibomian glands	Minimize evaporation
Aqueous	Main and accessory lacrimal glands	Solubilize mucins, electrolytes, proteins Flush irritants (reflex tears)
Mucin	Goblet cells, epithelia, lacrimal glands	Lubrication; surfactant between hydrophobic epithelium and aqueous component

cornea, the most densely innervated epithelial surface in the body, and the rest of the ocular surface epithelia are populated by sensory neural receptors of a morphologically unspecified type, called free nerve endings. Sensations evoked are painful in nature, but aside from relatively infrequent traumatic events such as debris on the corneal surface, they are usually subconscious, and an individual is unaware of sensory input from the ocular surface. Reflex tearing and eyelid closure are the obvious responses to stimulation of corneal nerves.

Nerves from the parasympathetic sphenopalatine (pterygopalatine) ganglion are associated with the secretory glands of the lacrimal functional unit. Parasympathetic cholinergic nerves are primarily responsible for signalling reflex tear secretion, and acetylcholine (M_3) receptors are present on secretory epithelia of lacrimal glands and on mucin-producing goblet cells in the conjunctiva [5]. Other parasympathetic neurotransmitters have also been detected near the lacrimal epithelium and meibomian glands [13]. Evidence for nerves of sympathetic origin has been found for the main and accessory lacrimal glands, the meibomian glands, and conjunctival goblet cells. Maintenance of the lacrimal gland secretory environment is also regulated by serum-derived factors, including androgen, oestrogen, progesterone, cortisol, insulin, thyroxin, and growth factors [43].

Lacrimal glands are composed of numerous lobules with secretory acini and ducts that converge into excretory ducts. The acini appear as rosettes of polarized columnar secretory epithelial cells in cross section whose apical surfaces terminate in the central lumen. The mid and apical regions of acinar cells contain numerous secretory granules of protein products to be released, whereas the basal regions contain the nucleus surrounded by a prominent endoplasmic reticulum and Golgi apparatus. When a neurotransmitter molecule binds a cognate receptor on the exterior of the acinar cell's basolateral membrane, it activates heterotrimeric G proteins on the cytoplasmic side [13, 25]. Their G_α subunits dissociate, exchange GDP for GTP, and initiate a cascade of intracellular regulatory events leading to Ca^{2+} influx and elevated cAMP. These mediators cause preformed transport vesicles (derived from the Golgi apparatus), containing proteins destined for secretion, to fuse with the apical cell membrane of the acinar cell, releasing the vesicle contents [44]. Secretion of water by lacrimal epithelial cells depends mostly on osmotic pressure generated by secretion of electrolytes, although secretion of proteins and mucins may contribute. The same receptor binding event that triggers protein secretion activates at least seven ion transporters that function together to secrete Na^+, K^+, and Cl^- ions, resulting in secretion of water into the lacrimal ducts [45].

2.2 Specific Pathologies of the Lacrimal Functional Unit

2.2.1 Dysfunction of the Afferent System

Decreased afferent sensory input from the ocular surface results in decreased lacrimal function and epithelial mucin production, which in turn can lead to LKC. For example, patients with familial dysautonomia (Riley-Day syndrome), a

hereditary sensory and autonomic neuropathy that causes corneal anaesthesia, produce a reduced amount of tears when crying and suffer from severe LKC. Their reflex lacrimation response to irritants such as onion odour is absent; however, affected children produce abundant tears after parenteral administration of a cholinergic agonist, indicating that lacrimal gland function is retained.

Surgical damage or amputation of trigeminal afferent nerves is a common cause of reduced corneal sensation. It has long been recognized that surgical sectioning of the trigeminal ganglion (to relieve trigeminal neuralgia) leads to LKC. Experimental trigeminal ablation in animal studies decreased conjunctival goblet cell density and corneal epithelial glycogen, and resulted in morphological alterations of ocular surface epithelia similar to those characteristic of LKC. Ocular surgical procedures that decrease corneal sensation include penetrating keratoplasty, photorefractive keratectomy, and LASIK.

Several ocular and systemic diseases cause trigeminal dysfunction and decreased tear production. Herpes zoster ophthalmicus can reduce corneal sensation in the distribution of the first division of the trigeminal nerve, and herpes simplex keratitis can result in sectoral or diffuse reduction of corneal sensation; both conditions can decrease tear production [12]. Diabetes mellitus can cause a polyneuropathy that reduces corneal sensation and causes secondary tear deficit and LKC. Reduced corneal sensation and aqueous tear deficiency are considered risk factors for diabetic keratoepitheliopathy.

2.2.2 Dysfunction of the Efferent System

Dysfunction of the efferent component of the lacrimal functional unit can affect autonomic nerves that stimulate tear secretion (secretory fibres), or those that regulate eyelid blinking and the tear drainage pump, or both. A common cause of efferent dysfunction is use of systemic anticholinergic medications, such as antihistamines, antispasmotics, antiemetics, and antidepressants [26]. Interestingly, a mechanism of secretory dysfunction found in Sjögren's syndrome may mimic the effects of anticholinergics: circulating autoreactive antibodies interact with M_3 acetylcholine receptors on lacrimal gland secretory cells [2, 15]. Age-related generalized dystrophy of the parasympathetic nervous system can also decrease secretory drive.

2.2.3 Glandular Dysfunction

Dysfunction of lacrimal and other secretory glands of the lacrimal functional units can result from a number of conditions (Table 2.2).

Sjögren's syndrome is a major cause of dry eye. It is a systemic autoimmune disease, primarily of females, characterized by progressive lymphocytic infiltration of lacrimal and salivary glands, which results in aqueous tear deficiency, LKC, and dry mouth. It can develop secondarily to connective tissue disease, most commonly rheumatoid arthritis. Specific autoantibodies are usually detectable. Lacrimal dysfunction and LKC are typically more severe in Sjögren's syndrome patients than in non-Sjögren's patients [31]. Several mechanisms contribute to lacrimal dysfunction in Sjögren's syndrome. The lymphocytic infiltrate, predominately B and T helper ($CD4^+$) cells, along with a lesser proportion of cytotoxic T cells ($CD8^+$), is accompanied by disruption of normal lacrimal gland architecture, with loss or dysfunction of secretory acini and proliferation of ductal epithelia [30]. Inflammatory cytokines released by infiltrating lymphocytes and by diseased epithelial cells can inhibit stimulation of lacrimal secretion [interleukin (IL)-1], or may promote apoptosis of lacrimal secretory epithelia [tumour necrosis factor (TNF)-α, interferon [INF]-γ, IL-12 and IL-18] [19, 49]. Among the autoantibodies developed by Sjögren's patients are antibodies which bind the M_3 acetylcholine receptor on secretory epithelial cells in lacrimal and salivary glands, and are proposed to inhibit their neural stimulation [2, 15]. Aqueous tear deficiency and altered tear composition, including hyperosmolarity and elevated concentrations of proinflammatory cytokines in tears,

promote inflammation and additional pathological changes of other ocular surface tissues, contributing to LKC [41].

Decreased androgen levels and increased oestrogen levels are associated with Sjögren's syndrome (and with other autoimmune diseases as well), consistent with the fact that 90–95% of Sjögren's patients are female [43]. The exact mechanisms are not yet elucidated, but may involve the pervasive influences of these hormones on innate and adaptive immunity.

Dry eye is a complication of acute and chronic graft-versus-host disease. In this condition, lacrimal production may be obstructed by accumulation of normal-appearing granules, together with amorphous material and cellular debris, in the acinar and ductule lumens. Tear deficiency in patients suffering graft-versus host disease may be accompanied by severe LKC, conjunctival scarring, and corneal epithelial defects [29].

Age-related degeneration, including pathological changes such as atrophy, and lobular, diffuse, and periductal fibrosis, may be the most common cause of decreased lacrimal function [8]. Decreased androgen levels [43] and aging effects on corneal sensory nerves may contribute to age-related lacrimal gland dysfunction. Immunological mechanisms may be involved as well, although circulating autoantibodies are not evident in non-Sjögren's LKC.

2.3 Lacrimal Keratoconjunctivitis Inflammation

Dysfunction of the lacrimal functional unit leads to ocular surface epithelial disease, which has been traditionally called keratoconjunctivitis sicca. We feel that the newly introduced term, lacrimal keratoconjunctivitis (LKC), more completely describes the array of pathological features associated with this syndrome (Table 2.3). Although our view of ocular surface pathology resulting from secretory dysfunction is incomplete, changes in tear fluid composition are clearly central in its development. As the lacrimal functional unit fails, tear concentrations of growth factors [for example, epidermal growth factor EGF)] and anti-inflammatory factors decrease [28], and concentrations of proinflammatory cytokines, that can originate from diseased lacrimal glands, meibomian glands, or ocular surface epithelia, increase [32, 40]. Tear concentrations of the soluble mucin, MUC5AC, are decreased in Sjögren's patients. Increased levels of proteases, including plasmin and matrix metalloproteinases (MMPs), profoundly impact tear composition and corneal epithelial integrity [39, 40]. Inflammation and neural sensitization result, together with ocular surface epithelial disease, the most clinically recognizable manifestation of LKC.

Table 2.3. Pathophysiology of the ocular surface in lacrimal keratoconjunctivitis

Tear film
Tear film break-up, poor lubrication
Increased osmolarity and proinflammatory cytokines
Cornea
Loss of epithelial barrier function
Conjunctiva
Squamous metaplasia
Apoptosis of epithelial cells (especially goblet cells)
T-lymphocyte infiltration

Table 2.2. Conditions associated with lacrimal dysfunction

Efferent dysfunction
Anticholinergics, anti-M_3 autoantibody
Lacrimal inflammation
Lymphocytic infiltration
Proinflammatory cytokines
Apoptosis
Hormonal imbalance
Decreased androgen/increased estrogen
Cicatricial obstruction of lacrimal ducts
Due to autoimmune and inflammatory diseases

2.3.1 Corneal Epithelial Disease

The decreased tear production and altered tear composition associated with dysfunction of the lacrimal functional unit contribute to poor lubrication of the ocular surface and a destabilized tear film. This is evident clinically as rapid tear break-up (see Sect. 1.4), with visible discontinuities in the tear film [24, 34]. The unstable tear film is associated with corneal epithelial surface irregularities that are detectable by computerized videokeratoscopy [4]. The blurred, fluctuating vision, photosensitivity, and reduced contrast sensitivity reported by LKC patients likely result from effects of corneal epithelial disease.

The normal corneal epithelium is much less permeable than the conjunctival epithelium because of its barrier function, which is critical for corneal smoothness and clarity. Disruption of the barrier function, assessed clinically by increased permeability to fluorescein dye, is a well-recognized feature of LKC: corneal epithelial permeability in untreated dry eye patients is 2.7–3 times greater than in patients with normal tear function. Studies of the rabbit cornea showed that it is impermeable to molecules larger than 3 kDa in molecular weight, and that the barrier to permeability lies entirely in the epithelium. The permeability of reepithelialized corneal tissue (after wounding) correlated with the degree of surface vascularization: conjunctival epithelium transdifferentiated into a corneal phenotype with minimal vascularization was relatively impermeable, whereas vascularized epithelium retaining a conjunctival phenotype displayed increased permeability characteristic of conjunctival epithelium [14]. Disruption of the mucin layer associated with cell membranes of apical corneal epithelial cells may compromise their barrier function [9]. Death, loss, or dysfunction of well differentiated apical corneal epithelial cells, as can occur in LKC, exposes the relatively permeable subapical layer of less well differentiated cells, obviating the corneal epithelial barrier function.

The corneal epithelial barrier function also depends on the integrity of tight junctional complexes between adjacent cells of the apical epithelium. Exposure of cultured corneal epithelial cells to low concentrations of surfactants or to a proinflammatory stimulus disrupted tight junctions [47]. Altered expression of tight junction complex proteins, such as ZO-1, ZO-2, and occludin, or their proteolytic degradation, appeared to be responsible for the disruption. Hyperosmolar stress and proinflammatory cytokines, conditions found on the ocular surface in LKC, activate transcription factors NF-KB and AP-1, which regulate tight junction development, and such conditions also increase expression of matrix metalloproteinase-9 (MMP-9) by the corneal epithelium [1, 39]. MMP-9 is increased in the tear film of LKC patients, and it is known to cleave tight junction proteins such as occludin, suggesting it plays a role in disruption of the barrier function. Although much remains to be learned, the findings so far suggest an explanation of how the proinflammatory ocular surface environment of LKC disrupts corneal epithelial barrier function.

Neurogenic inflammation of the ocular surface can occur by activation of or damage to the numerous unmyelinated axons that innervate the cornea. Released substance P and calcitonin gene-related peptide (CGRP) can act on anterior segment vascular elements, leading to inflammation and migration of immune cells from the vascular space to the ocular surface. Neurogenic inflammation may contribute to the ocular irritation symptoms of LKC.

2.3.2 Conjunctival Epithelial Disease

Squamous metaplasia, a condition of hyperproliferation and abnormal differentiation of the conjunctival epithelium, occurs in a variety of ocular surface inflammatory diseases, including LKC [31].

An important feature of squamous metaplasia is significantly decreased numbers of mucin-producing goblet cells in both Sjögren's and non-Sjögren's LKC patients. Consistent with this, tear concentrations of the goblet cell-specific soluble mucin, MUC5A, were reduced in

these patient populations [48]. Immunological analysis showed an altered distribution or glycosylation of membrane-bound mucins on apical conjunctival epithelial cells of patients with dry eye symptoms compared with normal patients, which correlated with rose bengal staining, another sign of dry eye disease [3]. Reduced membrane-bound and soluble mucin levels probably impair the spreading capability of the tear film, contributing to tear film break-up.

Hyperproliferation implies an elevated mitotic rate, which was indicated in the bulbar conjunctival epithelium of Sjögren's syndrome patients by increased epithelial stratification, DNA synthesis, and cell proliferation [17]. An elevated mitotic rate was also documented by increased immunostaining for the cell cycle associated protein KI-67 in bulbar conjunctival epithelial biopsies of non-Sjögren's LKC patients compared with those of normal patients [20]. Together with accelerated mitotic rates, increased expression of genes for transglutaminase 1, involucrin, filagrin, and the cytokeratin pair 1/10 in conjunctival tissue from Stevens-Johnson syndrome and ocular cicatricial pemphigoid patients with severe squamous metaplasia [27] may suggest alterations in the differentiation program of conjunctival epithelial cells.

LKC patients exhibit accelerated apoptosis of conjunctival epithelial cells, which is most severe in the goblet cell-rich areas of the bulbar conjunctiva. This phenomenon has been investigated experimentally in dry eye dogs that spontaneously develop LKC and in a mouse model where dry eye is induced by systemically administered anticholinergic agents coupled with a desiccating environment. Apoptosis is readily detected in both systems by indirect staining for chromosomal DNA released as a consequence of cell death [10, 46]. Interestingly, high level expression of the apoptotic indicator proteins fas, fasL, and p53 in conjunctival tissue of dry eye dogs was reversed by treatment with the immunomodulatory drug, cyclosporin A [10]. Similarly, experimentally induced apoptosis was observed in murine conjunctival epithelium (as compared with uninduced control animals), and the induced apoptosis could be reversed by administration of cyclosporin A [46].

Retinoid deficiency can inhibit proper differentiation of ocular surface epithelial cells, resulting in squamous metaplasia and a reduced concentration of mucin-secreting conjunctival goblet cells [36]. Alkaline or acidic chemical insult, in addition to possible damage to the epithelium, can destroy conjunctival goblet cells, and damage lacrimal and meibomian gland ducts. Reduced soluble mucin concentration may destabilize the tear film, and can lead to LKC.

2.3.3 Inflammation

Numerous studies, together with the therapeutic response of LKC to anti-inflammatory drugs, underscore the importance of inflammation in its pathogenesis. Cellular and soluble mediators act in a number of ways in a series of complex interactions to promote and modulate ocular surface inflammation. Some mediators act as chemokines, stimulating chemotaxis of migrating inflammatory cells to sites of inflammation on the ocular surface. Other mediators stimulate expression of adhesion molecules such as ICAM-1 on conjunctival vasculature and epithelial cells. These act by binding proteins called integrins on the surfaces of chemoattracted inflammatory cells to help retain them at sites of inflammation. Certain cytokines and other mediators activate inflammatory cells once they arrive to begin a proinflammatory program of gene expression, secretion of more mediators, and other functions. Other activities of inflammatory mediators include alteration of epithelial proliferation and differentiation, stimulation of protease production and activation (see Sect. 1.3.2), promotion of apoptosis (see Sect. 1.3.2), and sensitization of ocular surface pain receptors.

T-lymphocyte infiltration clearly indicates a well-developed inflammatory process. T-cell infiltration of the conjunctival epithelium and substantia propria is found in both Sjögren's and non-Sjögren's LKC [31, 42]. Not only are T-cell numbers elevated, but the population is shifted from predominately cytotoxic T_{Killer} cells (CD8 marked) to T_H cells (T-helper cells;

CD4 marked), which also display increased levels of CD11a (an α-integrin subunit) and CD23 (an IgE receptor) on their surfaces, indicators of an activated state [21]. Treatment of LKC patients with the immunomodulatory drug cyclosporin A decreases the numbers of T cells in the conjunctiva.

Increased levels of a number of proinflammatory cytokines, including IL-1α and β, IL-6, TGFβ1, and TNF-α, have been detected in the conjunctival epithelium of LKC patients, as well as in tear fluid (IL-1α and β, IL-6) [18, 32, 40]. Evidence suggests that at least some of these cytokines are synthesized by activated conjunctival epithelial cells. Correlating with this proinflammatory cytokine spectrum, increased IL-1β and a decreased ratio of IL-1α to IL-1 receptor antagonist (IL-1Ra) were observed in conjunctival epithelium [40]. Elevated levels of the cell surface immune activation markers ICAM-1, HLA-DR, and CD40/CD40 ligand (CD40L) are also present in the conjunctival epithelium of both Sjögren's and non-Sjögren's LKC patients [18, 46].

Exactly how ocular surface inflammation in LKC arises is not completely understood. Clearly, systemic autoimmune disease, such as Sjögren's syndrome, or androgen deficiency predispose some classes of patients, and desiccating environmental stress appears to be an important trigger. Consistent with this, human corneal epithelial cells respond to a hyperosmolar environment (as would result from desiccation of the tear film) by activating a cascade of stress associated protein kinases, which in turn activate transcriptional regulators of inflammatory cytokine and MMP production. Inflammatory mediators produced by ocular surface epithelial cells could initiate or contribute to an inflammatory cascade leading to dysfunction of other components of the lacrimal functional unit, for example, tear-secreting glands. Cytokines from ocular surface epithelial cells could also affect proliferation, differentiation, or apoptosis of other epithelial cells. Finally, the proinflammatory cytokines IL-1α, TNF-α, and TGF-β1 are potent stimulators of MMP production (including gelatinases, collegenases, and stromelysins) by cultured human corneal epithelial cells [22, 23]. In addition to their degradation of tight junction proteins (see Sect. 1.3.1), MMPs can proteolytically activate latent proinflammatory cytokines such as IL-1β, TNF-α, and TGF-β, and neural peptides such as substance P. Although only parts of the overall picture are visible at present, evidence is accumulating that epithelial cells, in addition to T lymphocytes, are important and direct participants in the ocular surface inflammation characteristic of LKC.

2.4 Diagnosis

Diagnosis of LKC or dry eye disease is complicated by symptoms that are common to other ocular surface disorders as well. However, two sorts of complaints are suggestive of dry eye: exacerbation of ocular irritation by environmental stress, such as the low humidity of airplanes, smoky environments, or drafts from air conditioners, and exacerbation by activities that require prolonged visual attention, such as reading or viewing a video display terminal. A history consistent with Sjögren's syndrome, or with other autoimmune disorders, should also raise the possibility of dry eye. To diagnose LKC or dry eye with confidence, objective tests are necessary, and many clinicians prefer to use multiple tests.

An unstable tear film, resulting from inflammatory compositional alterations of the aqueous, mucin and lipid components, is the hallmark of dry eye [41]; therefore measurement of the tear film break-up time (TBUT) is probably the most important clinically used objective test for LKC or dry eye because it assesses tear film stability in a nearly direct manner. The test is conducted by introducing fluorescein into the lower conjunctival sac by micropipette, insertion of a fluorescein strip wetted with saline, or, perhaps better, a commercial pre-wetted fluorescein strip that minimizes the volume change of the tear film. The patient is asked to blink, and the time interval between a complete blink and the appearance of the first dry spot or discontinuity in the precorneal tear film (viewed through a yellow filter) is recorded. Topical anaesthesia and lid holding are discouraged be-

cause they reduce tear break-up time. Three repetitions are recommended, and although there is no consensus, an average value of less than 10 s is considered abnormal by many. Dry spots or discontinuities are thought to occur where the tear film thins to the extent that the lipid layer "sinks" into the mucin layer because of their proximity and similar hydrophobicity, and the aqueous layer retracts locally from the hydrophobic spot; however, this explanation is not universally accepted. Anomalous results may be caused by discontinuities of the corneal surface that cause persistent tear break-up in a single location. Tears deficient in either aqueous, mucin, or lipid components may exhibit tear film instability; therefore the test does not distinguish lacrimal dysfunction from meibomian gland dysfunction.

Non-invasive alternative methods of measuring tear film break-up substitute a regular pattern reflected from the tear film for instillation of fluorescein. These methods avoid artefacts that are possible with the fluorescein method, but require specialized instrumentation, for example, computerized videokeratoscopy.

The Schirmer test measures tear volume and/or production. The test is performed by insertion of a standardized strip of filter paper over the lid margin at the junction of the medial and lateral third of the lower lid. Tear production is measured by the number of millimetres of the paper strip wetted in 5 min. A Schirmer I (without anaesthesia) value of 5 mm/5 min or less indicates dry eye; however, because of variability in the test, this cut-off value may not be especially sensitive for diagnosis. Several variations of the Schirmer test have attempted to improve its reproducibility. Instillation of topical anaesthesia prior to sampling tear production (sometimes called the Schirmer II test) minimizes reflex tear production. Use of a phenol red-impregnated cotton thread instead of a strip of filter paper is less irritating; wetting is readily visualized by colour change due to the slightly alkaline pH of tears.

Meibomian gland disease usually does not show a decrease in aqueous tear production. It can be evaluated by biomicroscopic examination of the glands, looking for ductal orifice metaplasia, reduced expressibility of secretions, increased turbidity and viscosity of expressed secretions, and dropout of glandular acini.

Diagnostic dye staining is a simple and practical method to evaluate the severity of ocular surface damage in LKC. Fluorescein dye stains the epithelium and underlying tissue in areas where barrier function has been disrupted due to death or desquamation of the apical epithelium. It also stains the stroma at sites of other epithelial defects. The barrier function of healthy corneal epithelium prevents such penetration. Fluorescein is introduced from a strip as for the tear break-up test, and viewed through a yellow filter. A cornea with LKC shows a number of stained dots and perhaps some confluent staining areas or some more linear staining patterns characteristic of filamentary keratitis. Several grading schemes have been proposed to quantify the severity of corneal staining, which correlates well with other measures of LKC [4].

Rose bengal, applied from a strip or as a solution, stains the conjunctiva more effectively than the cornea. It stains devitalized epithelial cells and also healthy epithelial cells that are not protected by a normal mucin layer; therefore, it evaluates the protective status of the preocular tear film [35]. The classic rose bengal staining pattern for dry eye is two triangles (nasal and temporal) in the interpalpebral conjunctiva – the region that is usually exposed when the eyes are open. Rose bengal staining also correlates well with other objective measures of LKC, such as the Schirmer test.

Impression cytology uses cellulose acetate filter strips applied with gentle pressure to different areas of the conjunctiva to obtain superficial cells for analysis. Topical anaesthesia is recommended for patient comfort. Goblet cell density and epithelial morphology can be readily assessed from the filters. For example, the extent and severity of squamous metaplasia can be graded based on loss of goblet cells, enlargement and increased ratio of cytoplasm to nucleus of superficial epithelial cells, and increased keratinization relative to normal samples. Squamous metaplasia of the bulbar conjunctiva is especially prevalent in Sjögren's syndrome LKC, although it can occur in a variety of other dry eye conditions [31].

Impression cytology is a highly sensitive means to detect pathologic changes on the conjunctival surface, and thereby confirm a clinical diagnosis. Impression cytology samples can also be immunostained to detect cell surface mucins as well as specific markers for particular types of infiltrating inflammatory cells. Routine use of impression cytology in clinical settings is probably limited by lack of facilities to stain and microscopically examine the filters.

Summary for the Clinician

- **Irritation from desiccating environments or from prolonged visual concentration suggests dry eye**
- **Autoimmune disease is an important predisposing factor**
- **Diagnosis is complicated by varying symptoms – multiple objective tests for dry eye are recommended**
- **Tear break-up time and the Schirmer test evaluate tear film stability and tear volume/production, respectively**
- **Fluorescein staining of the cornea and rose bengal staining of the conjunctiva reveal the severity of damage to ocular surface epithelia**

2.5 Therapies

Traditional therapies for dry eye disease or LKC are mostly aimed at augmenting the depleted tear film. Recently, an anti-inflammatory therapy that treats the underlying disease process has become commercially available.

The first goal of therapy is to minimize factors that exacerbate dry eye or LKC. Use of systemic anticholinergic medications (antihistamine and antidepressants), which may decrease tear production, should be minimized or eliminated. Exposure to desiccating environments should be avoided, where possible. Lowering computer displays to decrease the interpalpebral aperture, and periodic breaks from reading or computer work to close one's eyes may help.

Artificial tear solutions contain polymers, electrolytes, and buffering agents to mimic normal tear viscosity, osmolarity and pH. Aqueous preparations may be used as required for temporary improvement of symptoms; preservative-free preparations (lacking benzalkonium chloride) are recommended for patients who use tears more than 4 times per day. Newer emulsion tears, which contain a lipid component, are also effective, but the number of doses may be limited to about four per day because excessive use may cause blurred vision. Artificial tears may temporarily relieve symptoms and discomfort, but they do not reverse conjunctival squamous metaplasia, indicating that they do not treat the underlying pathology of dry eye.

Punctal occlusion is a useful and practical therapy for conserving tears. Occlusion by semipermanent plugs of silicone or thermolabile polymers inserted into the punctal orifice has the advantage of being readily reversible. Punctal plugs can improve symptoms and ocular surface dye staining. Permanent punctal occlusion by thermocautery or radio frequency needle can be performed using a topical anesthetic. If punctal occlusion is performed, it is still important to resolve inflammation within the lacrimal functional unit in order to avoid retaining proinflammatory tears on the ocular surface.

Anti-inflammatory therapy should be considered for moderate or severe dry eye (e.g. any patient with corneal epithelial disease), or for patients with symptoms that persist despite artificial tear use and improvements in their environment. A recently FDA-approved therapy for ocular inflammation associated with LKC contains cyclosporin A 0.05% in an emulsion vehicle (Restasis, Allergan, Inc.). Cyclosporin A is an anti-inflammatory compound that acts by binding an intracellular protein (cyclophilin) involved in a regulatory cascade that ultimately controls transcription factors required for T-cell activation and cytokine production. Cyclophilin binding of cyclosporin A also inhibits an early event in mitochondrially mediated apoptosis. Clinical trials of cyclosporin A 0.05% instilled BID showed significantly greater improvement in two objective measures of dry eye disease or LKC, corneal fluorescein staining and anaesthetized Schirmer test values, than was observed for the vehicle [37]. Clinical improve-

ment was accompanied by decreased expression of immune activation markers and apoptosis markers, and decreased numbers of T lymphocytes in the conjunctiva of cyclosporin A-treated patients, indicating a reversal of the inflammatory disease underlying LKC [21]. It should be noted that the emulsion vehicle itself showed provided significant short-term improvements in symptoms, but failed to improve the indicators of inflammation in conjunctival tissue. The excellent safety profile of cyclosporin A permits prolonged BID dosing.

Topically administered corticosteroids have been reported to improve dry eye signs and symptoms in several studies. However, because of increased risks of elevated intraocular pressure, cataract formation, and infection, they should be used in short pulses or minimal doses.

Whether or not punctal occlusion or anti-inflammatory therapy is employed, the therapeutic regimen should attempt to eliminate or minimize environmental factors that may contribute to dry eye or LKC and should include artificial tears as a palliative treatment.

Summary for the Clinician

- **Minimize environmental factors and medications that can exacerbate dry eye**
- **Artificial tears are useful for temporary relief of symptoms**
- **Punctal occlusion can help conserve tears, but inflammation must also be addressed, to avoid retention of inflammatory tears on the ocular surface**
- **Anti-inflammatory therapy that treats the underlying pathology should be considered for moderate to severe dry eye**

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