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New Understandings on Pathogenesis of Dry Eye – From Animal Models to Clinical Therapy

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1. Introduction

As defined by the International Dry Eye Workshop, dry eye is a multifactorial disease of the tears and ocular surface with symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface resulting in a dysfunctional lacrimal functional unit [1]. It is one of the most common medical problems that affects 6 to 44 million people in the United States based on reported prevalence figures of 4 to 33% from large epidemiological studies [2-4].

Dry eye affects quality of vision and quality of life. It is also associated with a significant financial burden to the individual and society [5, 6]. It has been shown that dry eye significantly decreases work productivity among office workers, by preventing them from performing at their full potential [7]. The cost of work performance loss associated with dry eye was estimated to be between \$10-15 million/year in Japan [7].

The corneal surface irregularity in dry eye degrades visual function by decreasing contrast sensitivity and functional visual acuity [8]. Visual impairment is one of the 10 most common disabilities and vision impacts mobility, independence and quality of life. The presence of dry eye was found to significantly impact the ability to perform daily activities such as reading, using a computer and driving [9]. Visual impairments associated with dry eye disease have been associated with falls and hip fractures in the elderly [10], and complications from these falls is the leading cause of death from injury in men and women over the age of 65 [11]. As vision deteriorates, one's ability to perform activities of daily living becomes exceedingly more difficult. Using time trade off techniques, Schiffman and colleagues calculated that patients with severe dry eye expecting to live 10 years or more, were willing to give up 1.6 years of that

time to be disease free [12], an amount similar to patients with moderate to severe angina [13]. Even though dry eye is a chronic disease, dry eye patients often complain about daily exacerbations. One frequent complaint is worsening of irritation symptoms and vision after prolonged use of a computer or grocery shopping.

While its pathogenesis has not been fully elucidated, it has been shown that changes in tear composition including increased proinflammatory cytokines, chemokines, metalloproteinases, increased expression of immune activation and adhesion molecules by the conjunctival epithelium and increased number of T lymphocytes in the conjunctiva play a pathogenic role in both dry eye patients and in animal models [14-21]. Increased knowledge has shown that inflammation is responsible in part for the irritation symptoms, ocular surface epithelial disease including loss of goblet cell, conjunctival metaplasia, and altered corneal epithelial barrier function in dry eye. This book chapter will discuss diverse pathogenic aspects of dry eye.

2. Experimental animal models of dry eye

There are several animal models of dry eye currently used throughout the world. They have been used to evaluate the natural history of the disease, to elucidate pathogenic mechanisms (risk factors and molecular mediators, pathways) and also to evaluate efficacy of therapeutic candidates. Models of dry eye rely on decreasing tear volume and production. To achieve that, surgical excision of the lacrimal gland, injection of pro-inflammatory cytokine IL-1 or isolated lymphocytes into the lacrimal gland, pharmacological blockade of lacrimal gland secretion have been used [22-24]. Autoimmune strains have also been studied. These strains have age-related lymphocytic infiltration of the lacrimal and salivary glands and ocular surface inflammation mimicking Sjögren's syndrome to a certain extent. These include the non-obese diabetic (NOD), MRL/Lpr, NZB/W F1 mouse, and TGF- β 1, CD25 and Thrombospondin knock-out (KO) strains [25-32].

We have developed an inducible experimental murine dry eye model where wild-type mice are subjected to an environmental stress for five or ten days and lacrimal gland secretion is pharmacologically inhibited by administration of scopolamine. Mice are kept in an environmentally controlled room where relative humidity is kept below 30% at all times. Mice subjected to this experimental model develop conjunctival goblet cell (GC) loss and increased CD4+T cell infiltration in the conjunctival epithelium (Figure 1A) and increased expression of inflammatory mediators, corneal barrier disruption measured by a fluorescent dye (Figure 1B) similar to human dry eye patients (Figure 1C). Since the initial studies, this experimental model has been used extensively by us and other groups within the US but also worldwide. A significant body of evidence providing insight into the pathogenesis of dry eye has been derived from this animal model and will be discussed in more detailed in the sequential topics.

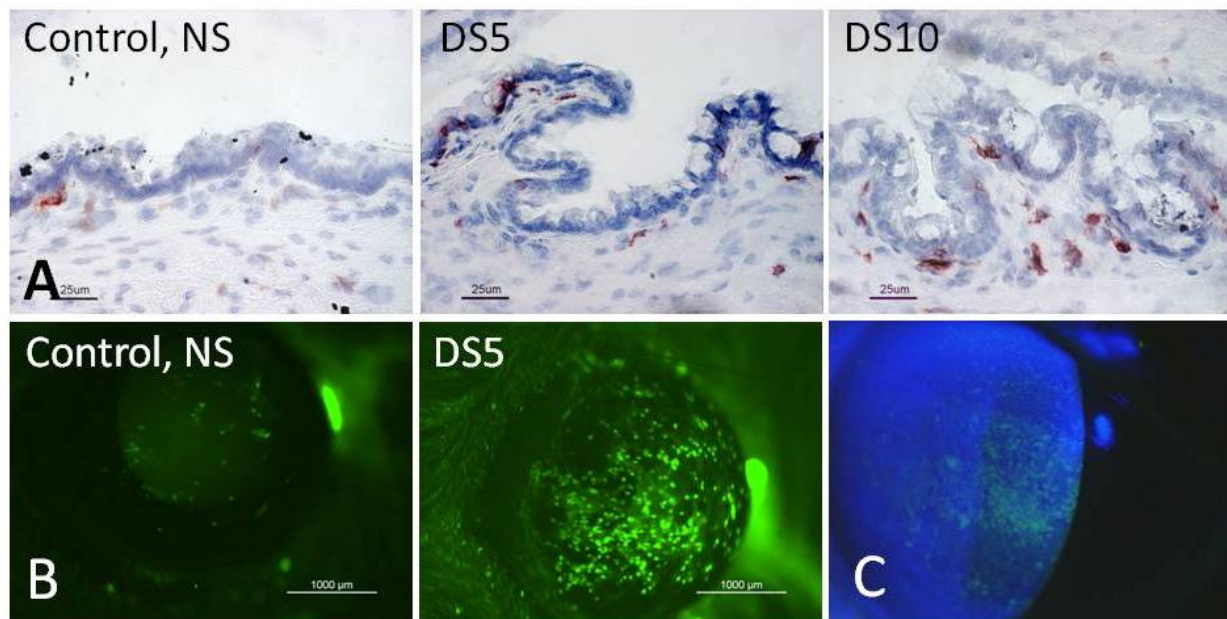


Figure 1. A. Representative images of immunohistochemical CD4⁺staining (red cells) in the goblet cell rich area of the conjunctiva of C57BL/6 mice without desiccating stress (control, non-stressed, NS) and with desiccating stress for 5 or 10 days (DS5 or DS10, respectively). **B.** Representative images of Oregon-Green-Dextran corneal staining in C57BL/6 mice without desiccating stress (control, non-stressed "NS") and with desiccating stress for 5 days (DS5). **C.** Representative image of human cornea stained with sodium fluorescein and visualized under a blue filter showing punctate dry spots in central cornea.

3. Role of tear hyperosmolarity

Hyperosmolarity has been shown to be a potent pro-inflammatory stimulus involved in the pathogenesis of the ocular surface disease of dry eye, termed keratoconjunctivitis sicca (KCS). Von Bahr was the first to suggest in 1941 that tear film osmolarity is dependent on tear secretion and evaporation, and that decreased secretion would lead to increased osmolarity [33]. Balik appears to have been the first to suggest in 1952 that the corneal and conjunctival changes in KCS could be explained on the basis of an increased "concentration of sodium chloride" in the tear film [34]. It would not be until two decades later that Mishima and colleagues were able to evaluate tear osmolarity and found an elevation of about 25 mOsm/liter in six eyes with KCS [35]. Subsequent studies reported significantly increased tear fluid osmolarity in patients with KCS, with the mean value 343 ± 32 (SD) mOsM, and the ranging up to 441 mOsM [36]. Based on its sensitivity and specificity, tear osmolarity was proposed as a gold standard diagnostic test for dry eye by Farris in 1992 [37], which was further evidenced by the fact that the use of sodium hyaluronate eye drops

with pronounced hypotonicity showed greater therapeutic efficacy on the severity of Sjögren's syndrome associated KCS than isotonic solutions [38].

In rabbit dry eye models, the elevated tear film osmolarity caused decreased corneal glycogen and reduced conjunctival goblet cell density, as well as pathological changes in the corneal epithelium, such as increased desquamation, decreased intercellular connections, blunting and loss of microvillae, cell membrane disruptions and cellular swelling [39-41]. In mice following 2 days of DS, tear volume significantly decreased from 0.066ul to 0.026ul in C57BL/6J and 0.093ul to 0.028ul in BALB/c mice while tear osmolarity concomitantly and significantly increased from 177 to 300 mOsM in C57BL/6J mice, and nearly doubled from 285 to 559 mOsM in BALB/C mice. Topical treatment of ocular surface with hypertonic saline (500 mOsM) was found to stimulate expression and production of interleukin (IL)-1 β , tumor necrosis factor (TNF) α and MMP-9 by the corneal and conjunctival epithelia, when compared with age matched controls and mice treated with iso-osmolar (305 mOsM) balanced salt solution [42, 43]. In our murine dry eye model [44], the stimulated expression of pro-inflammatory mediator, TNF- α , IL-1 β and MMP-9 was observed [45]. The mitogen-activated protein kinases (MAPKs) are well conserved signaling pathways that include extracellular signal regulated kinases (ERK), c-Jun N-terminal kinases (JNK) and p38 MAPK [46-48]. Interestingly, the levels of phosphorylated JNK1/2, ERK1/2, and p38 MAPKs in the corneal and conjunctival epithelia were markedly increased in mice treated with hypertonic saline [45].

In human corneal epithelial cultures, the expression and production of a number of pro-inflammatory mediators, IL-1 β , TNF- α , IL-8, and MMPs, including gelatinase MMP-9, collagenases MMP-1 and MMP-13, and stromelysin MMP-3 were progressively increased as the media osmolarity increased from 312 mOsM to 500 mOsM [49, 50]. Activated phosphor (p)-JNK-1/p-JNK-2 and p-ERK-1/p-ERK-2 were also detected by Western blot and peaked at 60 minutes in cells exposed to hypertonic media. The levels of p-JNK-1/p-JNK-2 and p-ERK1/p-ERK2 were positively correlated with the medium osmolarity. The inhibitors for JNK or ERK pathways, SB202190, PD98059 and doxycycline markedly suppressed the levels of phosphor-JNKs and/or ERKs, as well as these proinflammatory markers. Other investigators also showed that hyperosmolarity induced the pro-inflammatory cytokine and chemokines IL-6, IL-8 and monocyte chemoattractant protein-1 in cultured human corneal epithelial cells [51]. The efficacy of doxycycline in treating ocular surface diseases may be due to its ability to suppress JNK and ERK signaling activation and inflammatory mediator production in the corneal epithelium.

Another hallmark of dry eye is cornea and conjunctiva metaplasia, where the normal epithelium undergoes terminal differentiation and becomes keratinized [52]. The cornea is a highly transparent tissue and this abnormal keratinization process can lead to corneal irregularity and blurred vision [53, 54]. In mice, we observed that desiccating stress induces expression of cornified envelope proteins, including involucrin and small proline-rich proteins 2a and 2b in both cornea and conjunctiva [21, 55]. We also observed that stress-

associated pathway, such as JNK, ERK and p38 MAPK are activated in hours after desiccating stress starts [21, 43, 45].

We found increased levels of active phosphorylated JNK1 and JNK2 (JNK2 JNK1) in ocular surface epithelia treated with hypertonic saline *in vivo* and in cultured human corneal epithelial cells exposed to hyperosmolar media [45, 49, 56]. *In vivo*, we showed that the JNK2 protein but not JNK1, appears to have an essential role in desiccation-induced corneal epithelial disease by stimulating production of MMP-1, MMP-9, and cornified envelope precursors as JNK2KO mice were resistant to dry eye-induced changes [20].

4. Role of matrix metalloproteinases

The hallmark of dry eye disease is the increased permeability of corneal epithelium to fluorescent dyes, clinically observed as fluorescent punctate spots. Because epithelial corneal disease is responsible for the irritation and blurred vision symptoms reported by most dry eye patients, corneal epithelium health is very important. The epithelium is not bystander, but actively responds to desiccating stress by secreting inflammatory cytokines, chemokines and matrix metalloproteinases (MMPs).

Matrix degrading enzymes including MMPs have been identified as important factors in the inflammatory and wound healing response of the ocular surface, particularly in dry eye and ocular burns. Their induction during wound healing is thought to play a role in extracellular matrix remodeling, cytokine activation, and regulation of angiogenesis [57]. The MMP family includes more than 25 members that can be divided into collagenases that degrade fibrillar collagen types I, II, and III (MMP-1, -8, -13); gelatinases that degrade collagen types IV, V, and VII and X as well as decorin, fibronectin, and laminin, that are found in basement membranes (MMP-2,-9); stromelysins (MMP-3, and -10); matrilysins that degrade proteoglycans, laminin, and glycoproteins (MMP-7 and -26); and the membrane-type MMPs that are bound to epithelial cell membranes, and can activate MMPs, according to their structure and substrate specificity (MMPs 14-17, and -24) [58-61]. Collectively they are able to degrade the entire extracellular matrix and basement membranes components. Barely detected in an unwounded cornea, MMPs are strongly induced during wound healing. Among these, MMP-9 plays a prominent role being produced by stressed cornea and conjunctival epithelial cells and has both matrix degrading and pro-inflammatory activities [62-64].

Several members of MMPs have been found to increase in the corneal epithelium after experimental dry eye and MMP-9 (Figure 2) [57]. MMP-9 has been found in tear fluid of dry eye patients [65]. Of special interest, we have shown that epithelium-secreted MMP-9 after experimental dry eye activates TGF- β [66], breaks-down the apical corneal epithelial tight junctions (Figure 3), facilitating corneal barrier dysfunction [21, 57, 67] and accelerating corneal desquamation [68].

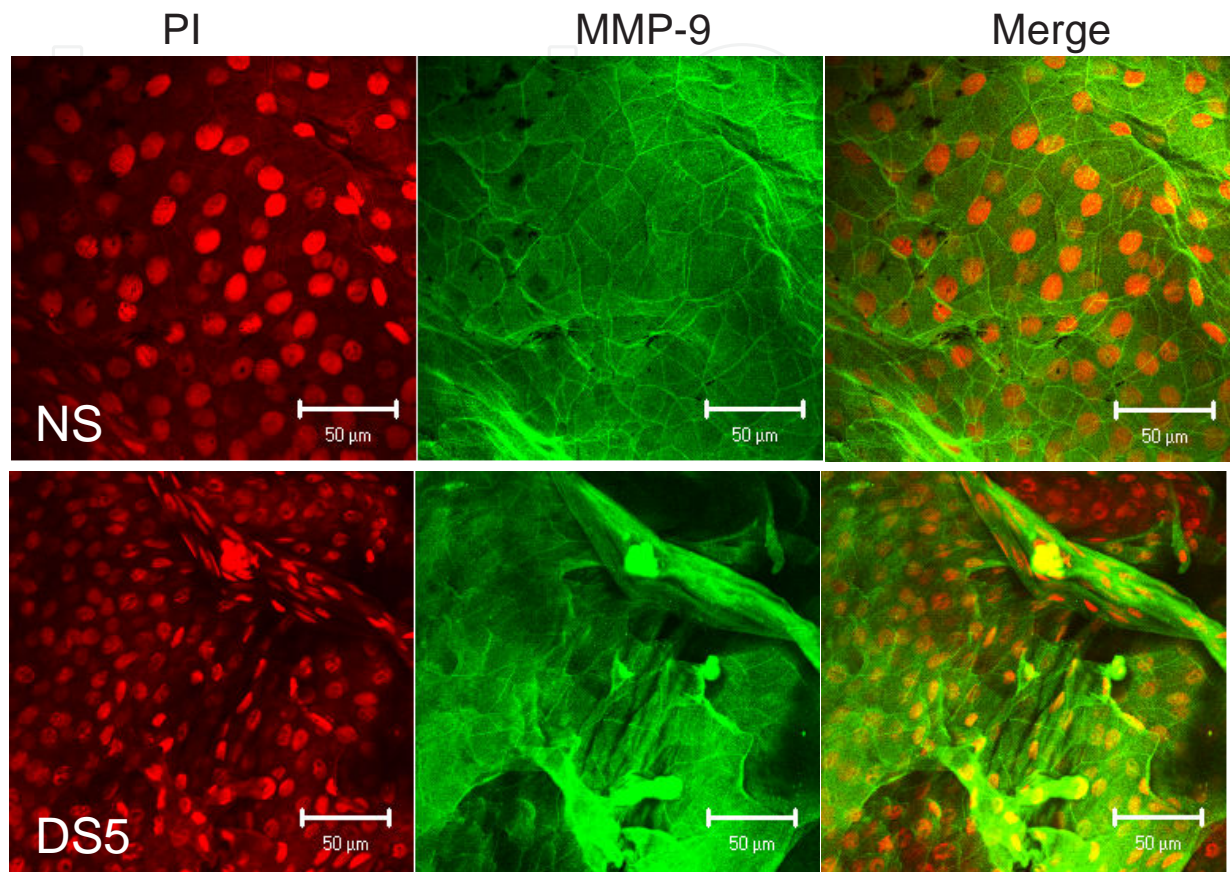


Figure 2. Laser scanning confocal microscopy of wholemount murine corneas stained for MMP-9 (in green) with propidium iodide nuclear counterstaining (PI, in red), organized into non-stressed (NS) and desiccating stress for 5 days (DS5).

MMP-9 knock-out mice are resistant to these changes, but exogenous topical administration of MMP-9 to MMP-9KO mice induced increased corneal permeability in similar range to wild-type control mice. Moreover, cultured human corneal epithelial cells treated with MMP-9 showed breakdown of tight junction proteins, notably occludin [67]. In human dry eye patients, increased MMP-9 mRNA transcripts in conjunctiva and increased MMP-9 activity in tears was noted compared to normal subjects; increased MMP-9 activity in tears positively correlated with symptom score, cornea and conjunctiva staining, low contrast visual acuity and inversely correlated with tear-break-up [54]. In a group of dry eye patients, we observed that tear MMP-9 activity levels increased as the severity of corneal disease progressed (Table 1) [54].

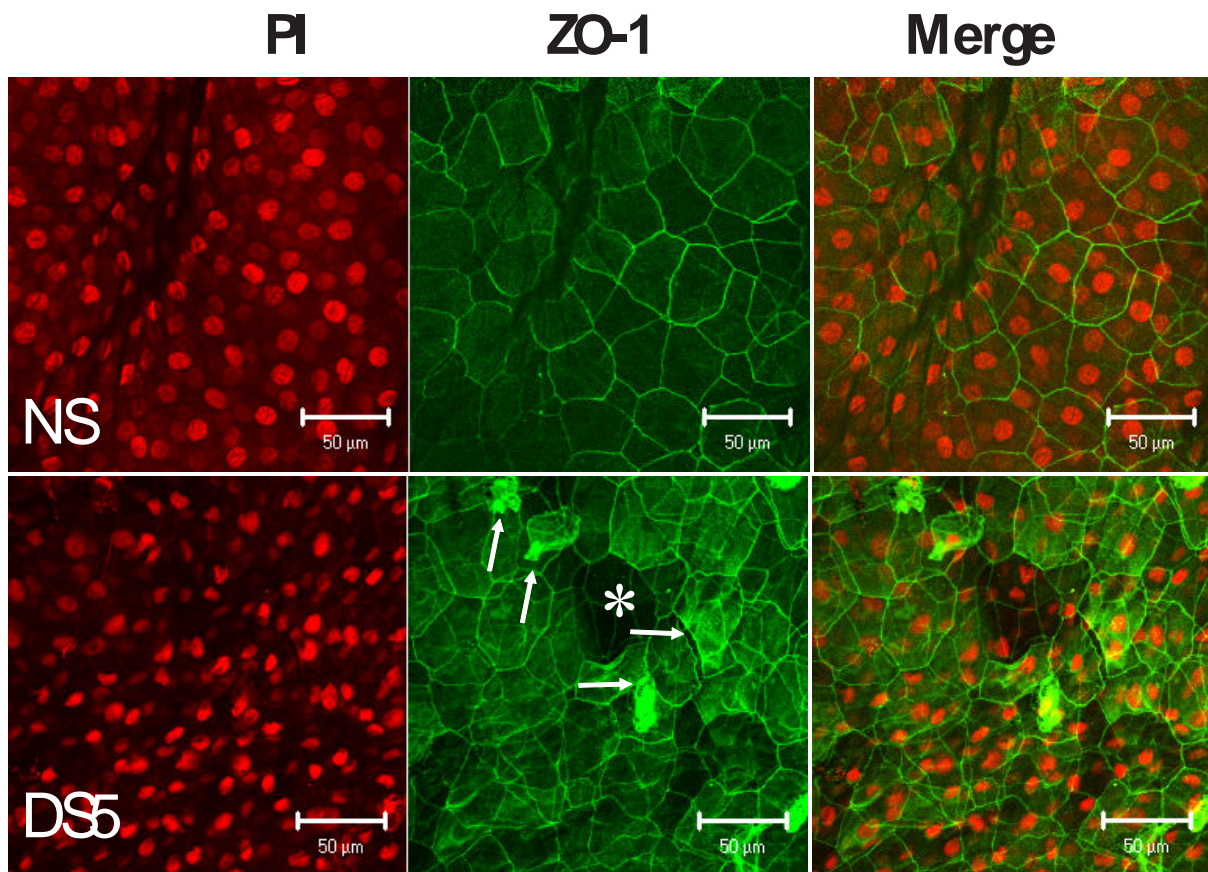


Figure 3. Laser scanning confocal microscopy of wholemount murine corneas stained for zona occludens 1 (ZO-1, in green) with propidium iodide nuclear counterstaining (PI, in red), organized into non-stressed (NS) and desiccating stress for 5 days (DS5). The NS controls show uniform cell with membrane staining while the DS5 corneas have increased apical cell loss (asterisk) and increase desquamation (arrows show either broken ZO-1 or cells that are rolling up).

Group	MMP-9 Activity (ng/mL)
Normal (n=18)	8.39 ± 4.70
Dysfunctional tear syndrome 1 (n=10)	62.40 ± 49.33**
Dysfunctional tear syndrome 2 (n=20)	58.84 ± 49.46**
Dysfunctional tear syndrome 3 (n=07)	116.99 ± 138.18**
Dysfunctional tear syndrome 4 (n=11)	360.25 ± 168.83**♦♦

Note: DTS=dysfunctional tear syndrome ** $P < 0.004$ versus normal; ♦♦ $P < 0.007$ versus normal and the other severity based DTS groups

Table 1. Tear MMP-9 activity levels among normal subjects and dry eye patients stratified by 4 levels of severity according to the Delphi Panel [69] [range from DTS1 (very mild) to DTS4 (severe dry eye)]

5. The conjunctiva acts as an exogenous lymphoid tissue

Similar to other mucosal tissues, the conjunctiva is covered with epithelium containing dendritic antigen presenting cells and a variety of intraepithelial lymphocyte (IEL) populations, lymphocytes that reside outside the lymphoid organs and in contact with epithelial cells in the gut, skin and lungs [70]. To date, several subsets of IELs have been identified in the mouse and human conjunctiva, including CD4⁺, CD8⁺, gammadelta ($\gamma\delta$)⁺ and NK⁺ cells [71-74]. The CD103 integrin has been used as a marker for IEL in different mucosal sites because it mediates homing and retention of lymphocytes to the epithelium. Its ligand, E-cadherin is highly expressed on mucosal epithelial cells [75, 76].

An important breakthrough in recent studies is the discovery of a link between the ocular surface epithelium and immune cells. Soluble factors from immune cells have been found to be either pathogenic or homeostatic to cornea and conjunctiva epithelium. Early biopsies from Sjögren's Syndrome (SS) patients have shown lymphocytic infiltration in the lacrimal gland and activated T cells have been detected in the conjunctiva dry eye patients [77-82]. Animal studies using the adoptive transfer experiment showed that dry eye can be induced in T cell deficient nude mice that had never been exposed to desiccated stress by adoptively transferring CD4⁺T cells from dry eye mouse model [83]. This landmark study showed that immunocompetent recipients will only develop disease when regulatory cells are depleted with antibody to CD25 [83]. Taken together, these experiments showed that: 1) dry eye is indeed an autoimmune disease, since goblet cell loss and CD4⁺T infiltration was seen in naïve mice receiving cells but never subjected to DS; 2) confirmed the pathogenic role of CD4⁺T cells as adoptive transfer of unfractionated or non-CD4⁺T cells had minimal effect; 3) showed that CD4⁺T cells primed *in vivo* during DS will migrate back to ocular surface tissues and LG; 4) dry eye induces a systemic immune response, as adoptive transfer of splenic CD4⁺T cells was sufficient to induce disease and 5) provided a mechanism to evaluate different components of the immune system.

T helper (Th) CD4⁺T cells have been classically divided into Th-1, Th-2 and Th-17. Th-1 responses are important for controlling viral, fungal and intracellular bacterial infections. Th-1 cells are classically identified by the production of interferon-gamma (IFN- γ). Once committed, the Th-1 cells activate macrophages and induce IgG2a production by B cells. Th-2 responses are frequently found in allergic diseases, such as asthma, and are particularly important in the host response to parasites and helminthes in the gut. Th-2 cell differentiation is promoted by interleukin (IL)-4 and it is characterized by production of IL-4, IL-13 and IL-5. The Th-2 committed cells promote IgG1 and IgE class switching and eosinophil recruitment. Th-17 cells are important in responding to extracellular bacterial and fungal pathogens, by recruiting neutrophils and macrophages to infected tissues and have been implicated in autoimmunity [84-86].

Migration of CD4⁺T cells into the conjunctival and cornea in dry eye disease may be modulated by chemokine ligands produced by the surface epithelium that increase in dryness. Pathogenic CD4 cells that infiltrate the ocular surface tissues express receptors to these ligands [18, 87, 88].

5.1. Th-1 CD4+T cells in dry eye

Th-1 committed CD4+T cells secrete IFN- γ . CXCL9, CXCL10, CXCL11, IFN- γ inducible chemokines, are highly expressed after experimental desiccating stress in both cornea and conjunctiva [87-89] which will in turn attract more Th-1 cells, serving as an amplifying mechanism.

IFN- γ has been proposed as a biomarker for dry eye disease and SS because elevated IFN- γ , either protein or RNA levels, has been detected in tears [90-97], conjunctiva [96-100], saliva [101, 102], lacrimal [26, 103-108], submandibular glands [94, 103, 109-111], and blood [112, 113].

Increased IFN- γ concentration in tears of dry eye patients detected by ELISA was reported more than a decade ago. More sensitive immunoassays, such as Luminex and antibody microarrays used in subsequent studies have confirmed these early results [90, 92-96]. In addition to dry eye, increased tear IFN- γ concentration has also been found in patients with sicca symptoms after bone marrow transplantation [90] and in tears of SS patients [91, 93]. Among the various subsets of dysfunctional tear syndrome, those with meibomian gland disease (MGD) had significantly lower IFN- γ concentration than those without MGD [91, 92] and tear IFN- γ concentration was found to correlate with corneal fluorescein staining score [91]. It is possible that inflamed lacrimal glands in patients with SS are one source for their increased tear IFN- γ .

Similarly to tears [90], increased IFN- γ has been found in saliva of SS patients and its presence correlated with sicca symptoms [101]. Interestingly, increased Th1/Th2 ratios was observed in more severe SS cases [114-116], where increased Th-2 response correlated with milder SS [101, 102, 117, 118]. Virtually every mouse autoimmune model that mimics SS or even environmentally-induced mouse dry eye models have shown increased expression of IFN- γ in LG and submandibular gland [26, 103-109, 118]. Increased expression of IFN- γ mRNA has also been observed in the conjunctiva, both in dry eye patients and mice subjected to dry eye [18, 96-100]. We have shown that IFN- γ induces conjunctiva metaplasia and apoptosis and loss of conjunctival goblet cells [97, 119, 120]. IFN- γ KO mice are resistant to dry eye induced changes but reconstitution of KO mice with exogenous IFN- γ induces goblet cell loss in similar magnitude as wild-type mice and this was accompanied by cornification and apoptosis of conjunctival epithelium [97, 120]. It has also been shown that IFN- γ significantly decreases epithelial mucin expression [121]. Adoptive transfer of CD4+T cells from donor mice exposed to desiccating stress that received anti-IFN- γ were less pathogenic to immunodeficient mice recipients, yielding less corneal apoptosis and greater number of PAS+filled goblet cells [119]. Mice that received subconjunctival injections of anti-IFN- γ antibody showed decreased corneal and conjunctival apoptosis [98, 119].

5.2. Th-17 and NK cells in dry eye

IL-17A is the signature cytokine of the new discovered Th subtype, Th-17. The differentiation of Th17 cells from naïve CD4+T cells is regulated by cytokines [122]. Transforming growth

factor- β (TGF- β) and IL-6, broadly expressed by many cell types in the body, including dendritic and epithelial cells, are dominant in the initiation of Th17 cell differentiation [122-124]. IL-23, IL-1 β and IL-21, which are products of activated dendritic cells, macrophages, activated T cell or inflamed epithelial cells, possibly expand and maintain the differentiated Th17 cells in the presence of IL-6 and TGF- β 1 [122, 125, 126]. Furthermore, signal transducer and activator of transcription 3 (STAT3) has been found to mediate the initiation of Th17 cell differentiation by these inducing cytokines [127].

TGF- β is a critical factor in Th-17 differentiation. TGF- β is a pleiotropic cytokine that can have pro- or anti-inflammatory effects depending on the context. It regulates various biologic processes such as embryonic development, cell proliferation and differentiation, extracellular matrix synthesis, immune response, inflammation, and apoptosis [128]. TGF- β 1 is produced by the human lacrimal gland (LG) and corneal and conjunctiva epithelia and has been detected in tears [129, 130]. Elevated levels of bioactive TGF- β 1 in tears and elevated TGF- β 1 mRNA transcripts in conjunctiva and minor salivary glands of human Sjögren's Syndrome (SS) patients has also been reported [18, 78, 131, 132].

We addressed the role of TGF- β in the Th-17 response in the conjunctiva by evaluating TGF- β dominant-negative TGF- β type II receptor (CD4-DNTGF β RII) mice. These mice have a truncated TGF- β receptor in CD4⁺T cells, rendering them unresponsive to TGF- β . These mice exhibit an age-related dry eye phenotype at 14 weeks of age; however, when subjected to desiccating stress, we observed that DS improved their corneal barrier function and corneal surface irregularity, increased their number of PAS+GC, and lowered CD4⁺T cell infiltration in conjunctiva. In contrast to WT, CD4-DNTGF β RII mice did not generate a Th-17 and Th-1 response, and they failed to upregulate MMP-9, IL-23, IL-17A, ROR γ T, IFN- γ and T-bet mRNA transcripts in conjunctiva. RAG1KO recipients of adoptively transferred CD4⁺T cells isolated from DS5 CD4-DNTGF β RII showed milder dry eye phenotype and less conjunctival inflammation than recipients of WT control [19].

Thrombospondin-1 (TSP-1) is an extracellular matrix protein that activates TGF- β 1. When covalently bound to TGF- β 1, the latency associated peptide (LAP) blocks its active site and renders the molecule inactive. This immature form of TGF- β 1 naturally equilibrates with its active state via detachment from LAP. Thrombospondin-1 stabilizes the covalent binding sites on the disassociated LAP, thereby preventing its interaction and subsequent inhibition of TGF- β 1 [133]. As a result, increased levels of TSP-1 in the presence of TGF- β 1 are linked to greater levels of active TGF- β 1 [133, 134]. Interestingly, similar to our findings in the CD4-DNTGF β RII mice, TSP-1KO mice had decreased corneal surface dye staining, increased number of conjunctival goblet cells and low levels of inflammatory cytokine mRNA transcripts in cornea tissue compared to WT mice. We also showed that adoptive transfer of WT bone-marrow DC into TSP-1KO reverted the TSP-1KO resistance to desiccating stress, showing that DC-derived TSP is critical for the immune dry eye phenotype [135].

The corneal epithelium responds quickly to different stressors. Cultured human corneal epithelial cells challenged by hyperosmotic media (450 mOsM), microbial components (polyI:C, flagellin, R837, and other TLR ligands) and TNF- α responded by significantly increasing expression of IL-6, TGF- β and IL-1 β and IL-23 mRNA transcripts. Interestingly,

when incubated with conditioned media of HCECs irritated by polyI:C or TNF- α , CD4⁺ T cells displayed increased mRNA levels of IL-17A, IL-17F, IL-22, CCL-20, and STAT3, increased IL-17 protein in the supernatant, and increased numbers of IL-17-producing T cells (Th17 cells). These findings demonstrate for the first time that Th17 differentiation can be promoted by cytokines produced by corneal epithelium that are exposed to hyperosmotic, microbial, and inflammatory stimuli [136].

Th17 cells can be identified by expression of CCR6 surface receptors [137-139]. CCR6 only ligand known, CCL20, is highly expressed after injuries to epithelium, including experimental desiccating stress [18]. Dry eye has been demonstrated to cause inflammation on the ocular surface, evidenced by increased levels of inflammatory cytokines (IL-1, IL-6, IL-17 and TNF- α) in the tear fluid and corneal and conjunctival epithelium, and an increased infiltration of DCs and T lymphocytes in the conjunctiva [18, 79, 83, 96, 136, 140-144]. Increased levels of IL-17, IL-23 and IL-6 were also found in saliva and salivary glands biopsies obtained from patients with the severe autoimmune dry eye condition, Sjögren's syndrome [145-147].

Evidence in mouse models of dry eye indicates that IL-17 stimulates production of MMP-3 and MMP-9 that contribute to disruption of corneal epithelial barrier function. Recent studies have shown that antibody neutralization of IL-17 ameliorated corneal barrier disruption in mice subjected to desiccating stress [18, 148] and decreased expression of MMP-3 and -9 mRNA transcripts in the corneal epithelium [18], providing a definitive link between epithelial and immune cells in this process.

5.3. Goblet Cells in dry eye

The conjunctival epithelium is part of the few tissues in the body where goblet cells are present, including the gut and the airway epithelia. Goblet cell loss is another clinical characteristic of ocular surface diseases, including dry eye, Stevens-Johnson and ocular graft-versus-host-diseases [149, 150]. Both NK and NKT cells are resident cells in conjunctiva [142]. NK cells are a subtype of lymphocytes that lack expression of the antigen receptors expressed by B and T cells; their name is derived from their ability to recognize and kill malignant cells. NKT cells are defined as NK cells that express conventional T cell receptor (TCR). Both cell types are important source of inflammatory cytokines, notably after encountering pathogens (viruses, bacteria and protozoans). NKT cells have been involved in mucosal immunity and in a variety of inflammatory/autoimmune diseases, such as experimental murine and human ulcerative colitis, asthma, multiple sclerosis and skin diseases (atopic dermatitis, psoriasis) [151-153].

Using isolation techniques, we identified that NKT-derived IL-13 is trophic factor for conjunctival goblet cells, as IL-13KO and STAT6KO strains had lower goblet cell density than their wild-type control mice [154]. In experimental murine dry eye, IL-13 significantly decreased in tears after 5 and 10 days in Th-1 prone C57BL/6 mice, while it increased in BALB/C mice that have been found to develop less severe corneal and conjunctival disease in response to desiccating stress [96].

NK cells participate in the initiation of the immune response by releasing IFN- γ [99] and IL-17A and by decreasing dendritic cell activation [142]. NK cells have been implicated in both the

regulation and immunopathogenesis of dry eye disease since they are an early source of IFN- γ during the induction phase of experimental dry eye disease [99]. Systemic depletion of NK cells prior and during DS led to a decrease in the frequency of total and activated DCs, a decrease in T helper-17(+) cells in the cervical lymph nodes and generation of less pathogenic CD4⁺T cells. B6.nude recipient mice of adoptively transferred CD4⁺T cells isolated from NK-depleted DS5 donor mice showed significantly less corneal barrier disruption, lower levels of IL-17A, CCL20 and MMP-3 in the cornea epithelia compared to recipients of control CD4⁺T cells [142].

5.4. Regulatory T Cells in dry eye

Resident CD8⁺T cells have been found in the epithelium and stroma of normal human and mouse conjunctiva [97, 155], but their function remains unknown. In non-ocular tissues, CD8⁺T cells have been found to have an immunoregulatory function. In the Lewis rat, peripheral tolerance to orally administered antigens was mediated by TGF- β secreting CD8⁺T cells [156, 157]. In the iris, CD8⁺T cells once activated in the presence of parenchymal cells, expressed and secreted enhanced amounts of TGF- β 2 [158]. In certain conjunctival inflammatory conditions, including graft-versus-host disease, Sjögren's syndrome and human and experimental murine keratoconjunctivitis, a significant decrease in CD8⁺T cells with concomitant increase in CD4/CD8 ratio in the conjunctiva has been observed [74, 97, 159].

We have identified that CD8⁺ T cells can also function as regulatory cells. CD8⁺ T cell depletion promoted generation of IL-17A producing CD4⁺T cells via activation of dendritic cells in both the ocular surface and draining cervical lymph nodes in C57BL/6 mice subjected to DS. T cell-deficient nude recipient mice receiving adoptively transferred CD4⁺T cells from CD8⁺cell-depleted donors exposed to DS displayed increased CD4⁺T cell infiltration and elevated IL-17A and CCL20 levels in the ocular surface, which was associated with greater corneal barrier disruption. Enhanced DS-specific corneal barrier disruption in CD8-depleted donor mice correlated with a Th17-mediated expression of MMP-3 and 9 in the recipient corneal epithelium. Co-transfer of CD8⁺CD103⁺Tregs did not affect the ability of DS-specific pathogenic CD4⁺T cells to infiltrate and cause ocular surface disease in the nude recipients, showing that CD8⁺cells regulate the afferent arm of DS-induced immune response. In summary, CD8⁺regulatory cells suppress generation of a pathogenic Th17 response that plays a pivotal role in DS-induced disruption of corneal barrier function [160].

6. Therapeutic strategies: from bench side to clinic

Traditionally, dry eye was treated with palliative solutions and frequent instillation of artificial tears. With the change of paradigm and recognition of the role of inflammation in the disease, several other modalities of treatment entered the pipeline. Animal models have suggested potential agents/pathways.

Anti-protease therapy is very effective in treating dry eye associated corneal epithelial disease. Previously reported studies using our experimental dry eye model demonstrated that the

MMP inhibitor doxycycline and the steroid methylprednisolone was efficacious in decreasing gelatinolytic activity and levels of MMP-9 transcripts in corneal epithelium, as well as preventing the dry eye-induced increase in inflammatory cytokines IL-1 and TNF- α [161]. Doxycycline also improved corneal surface regularity and improved corneal barrier function [21]. At the cellular level, doxycycline preserved apical epithelial cell area and the tight-junction protein occludin, resulting in a decreased number of desquamating epithelial cells from the surface of the cornea [21, 68]. The inhibitory effect of doxycycline on MMP-9 was also confirmed on osmotically stressed cultured human corneal epithelial cells [67]. While there is no FDA approved anti-inflammatory agents, both steroid and doxycycline eyedrops have also been used with success in human dry eye patients [162, 163].

It has also been shown that manipulation of afferent (migration of DC into the regional lymph nodes) or efferent (migration of differentiated cells from the nodes and into the cornea and conjunctiva) can ameliorate development of dry eye disease [137, 164-167] indicating that therapeutic strategies that interfere with various points in this immune circle may have clinical significance.

Cyclosporine A 0.01 % emulsion (CsA), the only FDA-approved drug to treat dry-eye disease has been shown to modulate several arms of the immune response by decreasing HLA-DR expression in conjunctiva of dry eye patients [16, 168, 169] and decreasing expression of IL-17A and IFN- γ in conjunctiva of animals after desiccating stress [18]. One beneficial side effect from Cyclosporine emulsion is its significant effect increasing the amount of goblet cells in human patients. We observed similar findings in our animal model as well: CsA topical treatment prevented goblet cell loss, maintained the number of NK+cells in the conjunctiva increased IL-13 mRNA in NK+cells, and decreased IFN- γ and IL-17A mRNA transcripts in NK+and NK-populations, showing that CsA can act in both epithelial and immune compartments [154].

Novel therapeutic strategies based on the interruption of migration of Th17⁺CCR6⁺cells to the ocular surface or production of IL-17 have been shown to ameliorated dry eye disease in animal models of dry eye [18, 137, 142, 148, 165]. Exogenous administration of IL-13 to wild-type mice subjected to desiccating stress for 5 days prevented DS-induced goblet cell loss [154]. Recently, it has been made clear that strategies to neutralize IFN- γ may inhibit development of corneal and conjunctival disease in experimental dry eye. Neutralization of NK cells, early producers of IFN- γ (NK cells) following desiccating stress was found to decrease corneal fluorescein staining and inflammatory cytokine expression in the cornea and conjunctiva [99, 142] and also to decrease the Th-17 response.

7. Conclusions

This book chapter described the most recent advances in understanding the pathogenesis of dry eye disease. A significant amount of work has been performed using an environmentally-induced murine dry eye model. Inflammation is now recognized as important player in dry eye. While there is just one FDA-approved drug to treat dry eye, several other drugs are in the pipeline addressing different aspects of the disease and are potential new therapeutics.

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