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The pathophysiology of dry eye disease: What we know and future directions for research

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Abstract

Clinical and laboratory studies performed over the past few decades have discovered that dry eye is a chronic inflammatory disease that can be initiated by numerous extrinsic or intrinsic factors that promote an unstable and hyperosmolar tear film. These changes in tear composition, in some cases combined with systemic factors, lead to an inflammatory cycle that causes ocular surface epithelial disease and neural stimulation. Acute desiccation activates stress signaling pathways in the ocular surface epithelium and resident immune cells. This triggers production of innate inflammatory mediators that stimulate the production of matrix metalloprotease, inflammatory cell recruitment, and dendritic cell maturation. These mediators combined with exposure of autoantigens can lead to an adaptive T-cell mediated response. Cornea barrier disruption develops by protease-mediated lysis of epithelial tight junctions leading to accelerated cell death, desquamation, an irregular poorly lubricated cornea surface and exposure and sensitization of epithelial nociceptors. Conjunctival goblet cell dysfunction and death are promoted by the T helper 1 cytokine interferon gamma (IFN-γ). These epithelial changes further destabilize the tear film, amplify inflammation and create a vicious cycle. Cyclosporine and lifitegrast, the two FDA-approved therapies inhibit T cell activation and cytokine production. While these therapies represent a major advance in dry eye therapy, they are not effective in improving discomfort and corneal epithelial disease in all patients. Preclinical studies have identified other potential therapeutic targets, biomarkers and strategies to bolster endogenous immunoregulatory pathways. These discoveries will hopefully lead to further advances in diagnostic classification and treatment.

Dry Eye – A multifactorial and self-perpetuating inflammatory disease

Knowledge regarding the pathophysiology of dry eye has advanced tremendously over the past two decades and continues to evolve. While tear disorders were traditionally classified by deficient component (e.g. aqueous or lipid), or as aqueous deficient or evaporative, the reality is most patients experiencing symptoms or signs of tear dysfunction have multiple risk factors and disease or dysfunction of more than one tear producing cells/glands that

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result in an unstable tear film.[1] Tear instability is accompanied by increased tear osmolarity (either in area of tear break-up or diffusely) which activates stress signaling pathways in the ocular surface epithelium and resident immune cells and triggers production of innate inflammatory molecules that initiate a vicious self-perpetuating cycle (Figure 1) that may lead to further decline in tear function and worse symptoms.[2, 3] The numerous extrinsic (e.g. desiccating environment, exposure) and intrinsic (e.g. aging, autoimmunity, drying medications) factors that can contribute to this inflammatory cycle demonstrate why it is often difficult to ascribe a single cause for most cases of dry eye disease and the importance of addressing all modifiable risk factors.

The ocular surface is a very unique exposed mucosa. It is covered with a specialized stratified epithelium that serves as a barrier to environmental, microbial and inflammatory insults. Next to the intestine, the conjunctival epithelium has the second highest density of mucus-producing goblet cells. It also harbors a variety of resident immune cells, such as natural killer, dendritic cells, macrophages, γδ and CD4 and $CD8^+$ T cells that function primarily in antimicrobial defense but may participate in the dry eye pathogenesis.[4–6] The cornea epithelium must withstand daily environmental challenges while maintaining clarity and comfort. The lacrimal glands and ocular surface epithelia produce an array of antimicrobial factors including, α and β defensins, IgA, lactoferrin, and lysozyme that are present in the tear film and function to maintain a paucibacterial microenvironment.[7–20] Many of the mechanisms to maintain ocular surface and glandular homeostasis are disrupted in dry eye (Figure 2). Studies performed in animal models and dry eye patients have found that desiccation is a potent stress (in the same magnitude to microbial products) to the ocular surface that initiates a secondary immune response that can lead to a vicious cycle (Figure 1).[21–27] Hyperosmolar stress has a direct pro-inflammatory effect on the ocular surface epithelium. It has been shown to activate mitogen-activated protein kinases (MAPKs), stimulate secretion of pro-inflammatory cytokines (e.g. IL-1β, TNF-α, and IL-6), chemokines and matrix metalloproteinases such as MMP-3 and MMP-9 and induce apoptosis.[22, 23, 26, 28–38] The interaction of these inflammatory mediators is complex and they have been shown to upregulate each other; thus amplifying the inflammatory cascade. For example, IL-1β stimulates the production of TNF-α and MMP-3, among other factors. [31, 32, 39, 40] In turn, TNF-α stimulates MMP-9 and MMP-3 which is a physiological activator of MMP-9.[41, 42] MMP-9 contributes to corneal barrier disruption by lysing tight junctions in the superficial epithelium.[23, 26, 43] MMP-9 knockout mice are resistant to corneal barrier disruption when exposed to desiccating stress, and MMP inhibitors, such as corticosteroids and doxycycline have shown potential in preventing desiccation induced corneal epithelial barrier disruption in animal models.[26, 43–45] A point-of-care MMP-9 detection system (InflammaDry®, RPS, Sarasota, FL) is approved for detecting elevated levels of MMP-9 in tears of dry eye patients.[46–50] Increased tear MMP-9 has also been detected in other ocular surface diseases, such as atopic and vernal keratoconjunctivitis, corneal ulceration, recurrent corneal erosions and ocular burns that also have corneal barrier disruption.[51–63] Detection of elevated tear MMP-9 provides a rationale for use of anti-inflammatory/protease therapies in these conditions.

Ocular surface epithelial cells also secrete chemokines that attract inflammatory cells. Increased levels of chemokines CCL20 (MIP3α), CXCL9 (MIG), CXCL10 (IP-10) and

CXCL11 (I-TAC) and their receptors was noted in ocular surface cells and/or tears of dry eye patients and mice with the experimentally induced dry eye.[64–68] Genetic deletion or pharmacological blockage of certain chemokines or chemokine receptors (CCL20, CCR6 or CXCR3) prevented the development of desiccation-induced ocular surface disease and decreased pathogenicity of autoreactive T cells in mouse models of dry eye.[69, 70]

Another effect of desiccation is upregulation of innate inflammatory pathways in the epithelium, including the nucleotide-binding domain, leucine-rich–containing family, pyrin domain–containing-3 (NLRP3), toll-like receptor and oxidative stress pathways.[29, 30, 71–80] Antioxidants have shown therapeutic potential for treating dry eye in preclinical culture or mouse studies and in a pilot clinical trial. [30, 34, 81–85]

Metaplasia and goblet cell loss in the conjunctival epithelium is a well-recognized feature of aqueous tear deficiency.[86–92] The most severe ocular surface diseases, such as Stevens-Johnson syndrome, mucous membrane pemphigoid (MMP), graft vs. host disease and severe alkali burns involving the conjunctiva often have complete loss of conjunctival goblet cells.[93–96] T helper cytokines have been found to modulate conjunctival goblet cell differentiation. The Th2 cytokine IL-13 stimulates proliferation and mucus production, while the Th1 cytokine IFN-γ induced goblet cell entrapment, expression of cornified envelope precursors, decreased mucus production, unresponsiveness to cholinergic stimulation, ER stress and unfolded protein response and apoptosis.[27, 97–104] In addition to producing tear-stabilizing mucins, goblet cells also produce [105, 106] immunoregulatory factors, such as TGF-β and retinoic acid.[104, 107, 108] Crosstalk between goblet cells and dendritic cells is critical to maintaining immune tolerance in mucosal tissues.[109] Goblet cell associated-passages that deliver surface antigens to the underlying dendritic cells and promote tolerance have been identified in both intestine and conjunctiva.[109, 110] Mice with deletion of the SAM pointed domain containing ETS transcription factor gene *(Spdef* knockout) are devoid of goblet cells, develop conjunctival inflammation and lose [111] immune tolerance to topically applied antigens, as has been found in other mouse dry eye models that are accompanied by goblet cell loss [109, 112–114] These studies indicate a critical role of goblet cell products in conditioning tolerogenic properties in conjunctival dendritic cells and maintaining ocular surface immune tolerance. [107, 108]

Evidence indicates that the initial innate immune response to dryness is followed by an adaptive $CD4^+$ T cell autoimmune response in mice exposed to desiccating stress and patients with Sjögren syndrome (SS) and non-SS associated aqueous tear deficiency.[115–117] While the target autoantigen(s) in this autoimmune reaction have not been identified, members of the kallikrein family have been implicated as putative antigens in some studies.[118, 119] Disrupted immune tolerance in dry eye[112–114] elicits leads to dendritic cell maturation[120] and generation of autoreactive T effector cells[70, 101, 121–124] in mouse dry eye models. Human dry eye patients have an increased number of conjunctival dendritic cells[125, 126] and a higher percentage of cells expressing the dendritic cell maturation marker HLA-DR.[127–130] Depletion of dendritic cells prevented the development of dry eye disease in mice subjected to desiccating stress.[120] Mature dendritic cells prime antigen-specific Th1 and Th17 effector T cells in the conjunctival draining lymph nodes. Several laboratories have identified interferon gamma (IFN-γ) and IL-17, produced by Th1 and Th-17 cells

respectively, as critical effector cytokines in dry eye.[27, 66, 69, 70, 99, 101, 121, 123, 124, 131–136] IFN-γ promotes conjunctival goblet cell loss and lacrimal gland acinar loss, while IL-17 cause corneal barrier disruption and lymphangiogenesis in mouse dry eye models. The disruption of immune tolerance and generation of effector T cells suggests inadequate suppression by regulatory T cells (Tregs). Indeed, dysfunctional Tregs that cannot suppress T effector activity, but produce IFN-γ and IL-17 have been observed in mouse models of dry eye induced by desiccating stress or associated with aging.[123, 137] Furthermore, adoptive transfer of either T effectors or Tregs from aged mice into naïve immunodeficient recipient mice caused goblet cell loss and lacrimal gland infiltration, while the adoptive transfer T effectors or Tregs from young mice did not, suggesting that age-related Treg dysfunction may contribute to induction of dry eye disease.[137]

Lacrimal gland (LG) inflammation and dysfunction develop with age and in the autoimmune disease Sjögren syndrome (SS).[138] The hallmarks of SS are lymphocytic infiltration of the lacrimal and salivary glands, serum autoantibodies, keratoconjunctivitis sicca and dry mouth.[139] Mouse models of SS and aging have identified a pathogenic role for $CD4^+T$ [98, 137, 140–142] and B cells.[143–146] Mouse SS models that develop dacryoadenitis tend to be Th1 skewed[147–151], whereas those that develop sialadenitis are Th17 skewed.[152–155] IFN-γ produced by the infiltrating cells increases caspase expression and causes acinar apoptosis.[150, 151, 156–158] Altered nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) signaling has been implicated in SS [159–161] and increased NFκB signaling in epithelial cells was found to promote lacrimal gland acinar apoptosis that preceded lymphocytic infiltration in a mouse SS model.[161] Infiltration with autoreactive T cells and oxidative stress have also been observed in the aged lacrimal gland, indicating that aging is associated with inflammation and is not simply a degenerative process. [98, 137, 162–166] These studies suggest that similar to the ocular surface, a vicious cycle of inflammation and apoptosis involving infiltrating cells and glandular acinar cells perpetuates LG inflammation leading to glandular dysfunction in SS and age-related dry eye.

There is an increased body of evidence demonstrating that the microbiome, the microbial community that inhabits the human body, has immunoregulatory functions. The presence of an ocular microbiome has long been suspected; however, traditional swab cultures of the conjunctiva are often negative.[17, 167] This is in sharp contrast to cultures of the lid margin and periocular skin which often grow bacteria.[168, 169] Studies using 16S genomic sequencing have demonstrated an ocular surface microbiome that may have the lowest biomass of any tissue in the body[16, 170, 171] No difference in the quantity and diversity of the ocular microbiome was noted between SS and control subjects;[16] however, significant alterations of the intestinal microbiome were noted in the same cohort with a significant decrease in commensal genera and an increase in pathogenic genera, such *as Escherichia/ Shigella and Proteobacteria.* Mice that had an antibiotic-induced depletion of the microbiome with a cocktail of five oral antibiotics prior to experimental desiccating stress developed significantly worse dry eye than control mice that did not receive antibiotics, suggesting that the intestinal microbiome can modulate ocular surface inflammation and severity of dry eye disease.[16]

Future Directions for Research

The two approved therapies for dry eye, cyclosporine and lifitegrast, target T cells which are key contributors to the pathophysiology of chronic dry eye. Cyclosporine bound to cyclophilin inhibits the activity of the serine/threonine phosphatase calcineurin which normally dephosphorylates nuclear factor of activated T cells (NFAT) after antigen binding to the T cell receptor.[172] Dephosphorylated NFAT is transported to the nucleus where it initiates transcription of T cell cytokines, notably IL-2 and IFN-γ.[172] Lifitegrast is a small molecule that inhibits binding of leukocyte-associated antigen 1 (LFA-1) on T cells to its ligand intercellular adhesion molecule 1 (ICAM1) on antigen presenting, epithelial and vascular endothelial cells and prevents the formation of the immunological synapse that is required for full T cell activation.[125] These molecules have improved dry eye signs and symptoms in clinical trials, but they are not effective in all dry eye patients and don't address acute effects of desiccation on the ocular surface, including the increased production of innate mediators and activation of the MAPK stress signaling pathways.[172–174] Therapies targeting the acute effects of desiccation would likely provide more rapid relief of eye irritation and prevent the effects of a dry, drafty environment such as an airplane cabin. Corticosteroids have shown efficacy in treating chronic dry eye and preventing irritation and cornea barrier disruption in response to a desiccating environmental challenge[175–178]; however, long-term use of corticosteroids carries risks of cataract formation and glaucoma, and therapies with steroid-like inhibitory effects on innate inflammatory pathways would represent a major advance.

Conjunctival goblet cells produce soluble mucins that stabilize the pre-corneal tear layer. They also produce factors that maintain homeostasis and immune tolerance on the ocular surface.[109, 179] The worst cornea disease develops in dry eye conditions with loss of goblet cells, such as Sjögren syndrome, Stevens-Johnson syndrome and graft-vs.-host disease.[134, 180, 181] The Th1 cytokine IFN-γ inhibits goblet cell secretion and promotes apoptosis of these cells.[133, 182] Both cyclosporine A and serum drops have been reported to increase conjunctival goblet cell density.[183, 184] Research is needed to identify therapies to maintain goblet cell number and function with aging and in dry eye conditions, particularly those associated with the most severe goblet cell loss.

Therapies to bolster endogenous natural anti-inflammatory and immunomodulatory mechanisms also appear to have promise. The Western diet is often deficient in anti-inflammatory polyunsaturated fatty acids (PUFAs).[185, 186] Oral supplementation with gamma-linolenic acid (GLA, n-6) and omega-3 (n-3) PUFAs has been found to improve ocular irritation symptoms and tear stability, inhibit conjunctival dendritic cell maturation and decreased inflammatory mediators in patients with dry eye.[187–189] Other nutritional supplements such as curcumin have potent anti-inflammatory effects and have been found to suppress IL-1β production by osmotically stressed cornea epithelial cells and dendritic cell maturation.[190, 191] Intestinal dysbiosis has been found as a risk factor for SS dry eye and mice with antibiotic-induced depletion of their microbiome developed significantly worse ocular surface disease in response to desiccating stress.[16] Supplementation with commensal microbiota have shown anti-inflammatory effects in autoimmune conditions such as inflammatory bowel disease and diabetes mellitus.[192–196] It is possible that probiotics or

metabolites of commensal bacteria could have future therapeutic potential for dry eye disease.

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Highlights

Multiple factors can promote tear instability and hyperosmolarity that trigger ocular surface and glandular inflammation. Cornea barrier disruption, conjunctival goblet cell loss, and glandular dysfunction are consequences of the dry eye inflammatory cascade.

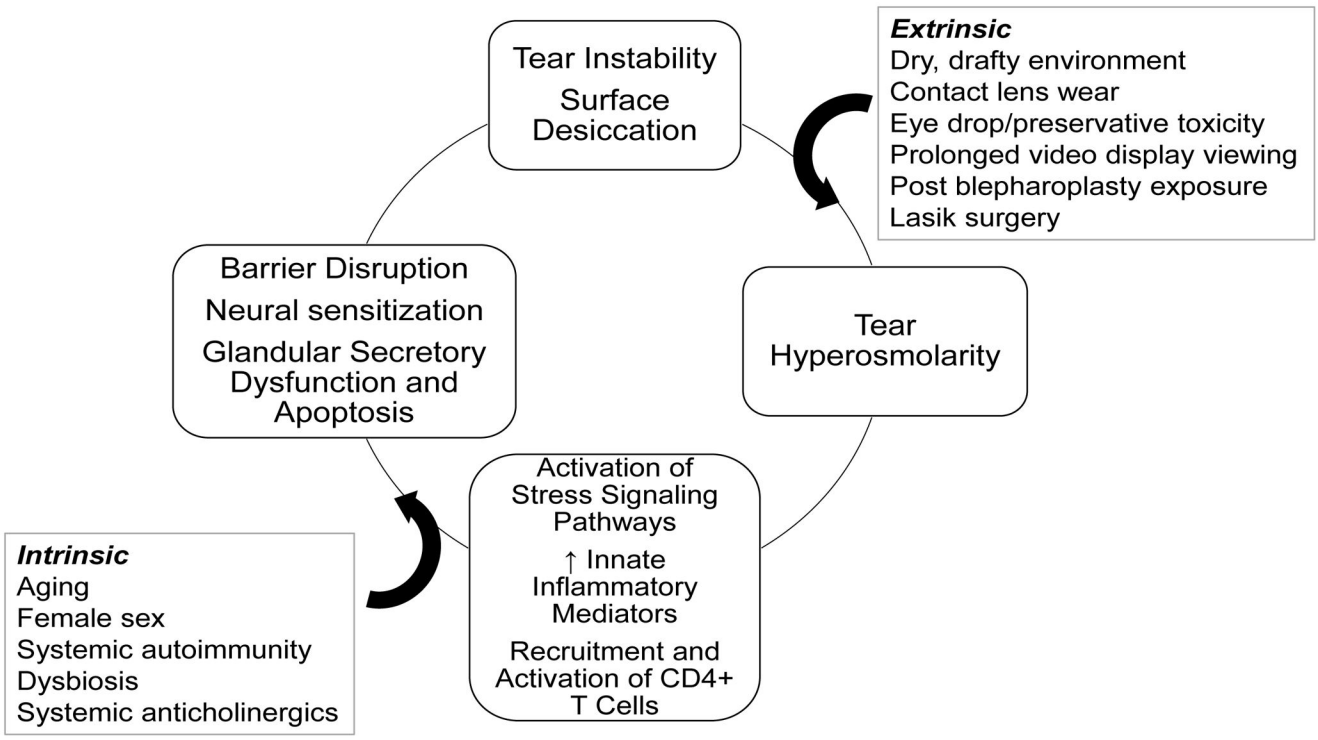

Figure 1.

Dry eye inflammatory cycle that can be initiated or amplified by extrinsic and intrinsic factors that cause tear instability and tear composition changes including hyperosmolarity that activate stress signaling pathways in the ocular surface cells which triggers production of innate inflammatory mediators which can lead to recruitment and activation of CD4+ T cells which produce cytokines that cause corneal, conjunctival and lacrimal gland epithelial disease.

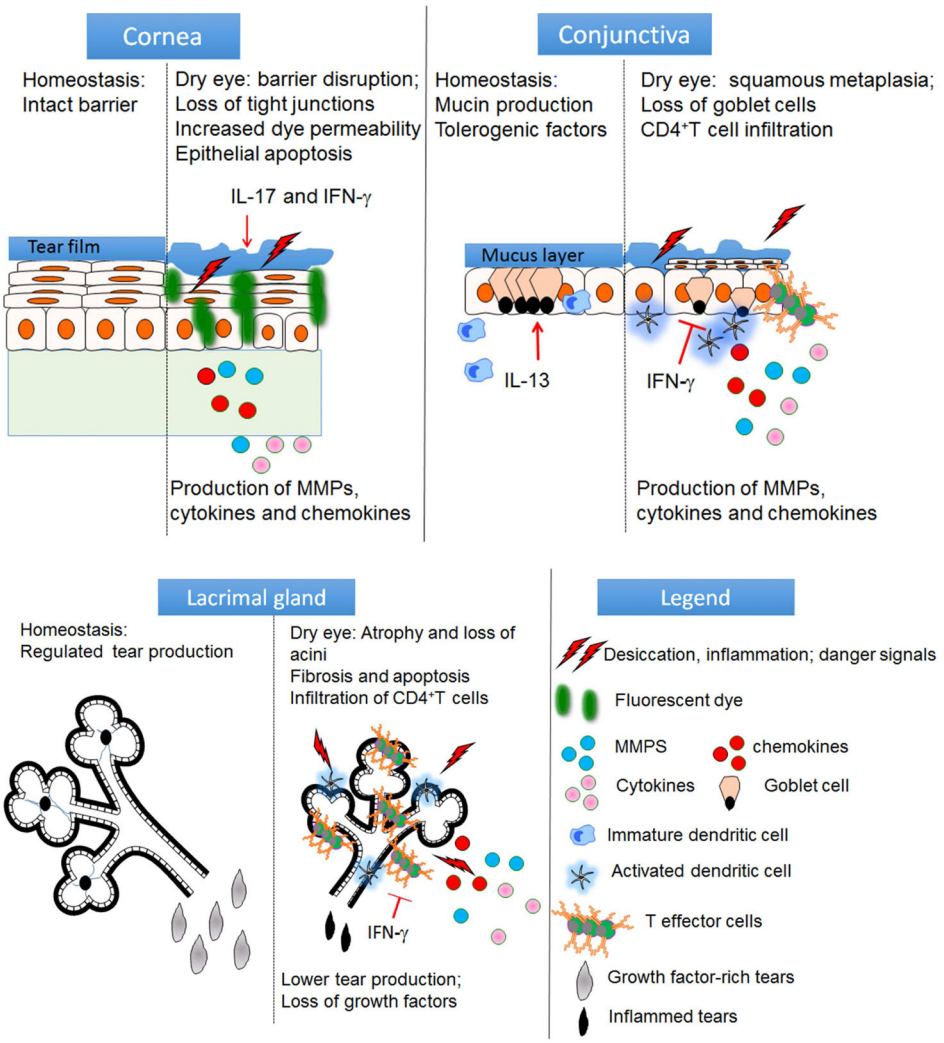

Figure 2.

Function of the cornea, conjunctival and lacrimal gland in maintaining ocular surface homeostasis (left side of each tissue) and disease relevant mediators and pathological changes in each tissue (right side of each tissue). IL-17 = interleukin 17, IL-13 = interleukin 13, IFN-γ = interferon-gamma