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Review Article

Dry eye disease and inflammation

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  Dry eye disease is a devastating and multifactorial disorder of the tear-film layer and ocular surface. Multiple symptoms include multiple eye irritation, and visual disturbances, and signs have tear-film instability, along with the potential for injuring the ocular surface. Accumulating evidence indicates that dry eye disease is accompanied by hyperosmolarity of the tear film and inflammation of the ocular surface microenvironment. Most cases of dry eye disease are secondary to any of a vast array of inflammatory conditions and disorders, including auto- and allo-immune diseases, infection, aging, neuroinflammation, and sterile Inflammation. Sterile inflammation is induced by several different kinds of non-infectious triggers, including altered cellular and tissue states. New results have implicated damage-associated molecular patterns, microorganisms, and neurotransmitters in primary dry eye disease. Furthermore, in conjunction with inflammatory and fibrotic changes, the renin angiotensin system and epithelial mesenchymal transition may be involved in disease-associated and immune-mediated dry eye disease, such as chronic GVHD-related dry eye. Collectively, studies show that at least some and possibly many factors influence the condition of the ocular surface, leading to chronic inflammation that exacerbates dry eye symptoms in a vicious cycle. Since the pathway for each type of dry eye disease may differ, and specific pathways may be responsible for the disease in individual patients, patient-tailored therapies should be developed in the future. In this review article, we focus on emerging concepts on the role of the inflammatory process in dry eye disease.

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Introduction

  Dry eye disease is a devastating and multifactorial disorder that affects 20-30% of the population worldwide[1]. The accompanying discomfort and impact on patients' vision can limit their activities of daily living and reduce their quality of life[2]. Given its high incidence, then, dry eye disease constitutes a major public health and welfare problem that needs to be addressed.

  Clinical and basic research on dry eye disease is quite active, and this topic has drawn considerable interest, be-

cause dry eye disease affects patients in several major fields of medicine, including autoimmune disease, infection, aging, and neuroinflammation. Classically, dry eye disease has been conceptualized solely as a problem in tear fluid deficiency; however, recent research advances suggest that considerable cases of dry eye disease involves a local autoimmune-mediated dysfunction that is likely to contribute to the pathology. Although the precise cellular and molecular mechanisms that result in dry eye disease have not been fully elucidated, it is increasingly clear that immune-mediated inflammation plays a key role in the initiation and development of this enigmatic disease. Currently, certain dysfunctions of the immune system have been linked to a cluster of chronic dry eye diseases. In this review article, we focus how dry-eye disease is linked to ocular surface immunology and inflammation.

Homeostasis of the ocular surface

The ocular surface consists of a tear-film layer, lacrimal glands, accessory glands, corneal conjunctiva, nasolacrimal ducts, and meibomian glands; these components maintain the homeostasis of the ocular surface and protect the eye from invading pathogens and other environmental challenges. The mucosal membrane of the ocular surface is anatomically continuous with the lacrimal gland, conjunctiva, meibomian glands, and lacrimal drainage system, and it contains a mucosal immune tissue called the "eye-associated lymphoid tissue"(EALT)[3].

The homeostatic mucosal immune system includes dendritic cells, macrophages, mast cells, lymphocytes, fibroblasts, and soluble immune mediators that collectively perform immune surveillance. The conjunctiva also has several defense systems that protect the integrity of the surface epithelium and its mucin layer, including antimicrobial peptides and secreted IgA, which adhere to the mucin layer. The role of IgA has been elucidated in some detail. IgA-producing plasma cells reside in the subepithelial stroma of the lacrimal gland and in the ocular surface mucosal membrane. Secreted IgA dimers bind the IgA receptor on the basement membrane of basal epithelia, are taken up and transported them intracellularly and are thereafter excreted into the tear film by the ocular surface epithelia along with other secretory components[4]. When the ocular immune homeostasis is disrupted, the integrity of the ocular surface can break down, resulting in inflammatory mucosal disease and causing dry eye disease[3].

Immune-mediated inflammation and dry eye disease

Inflammatory diseases are multifactorial disorders determined, like many disorders, by genetic vulnerabilities and environmental triggers. In fact, genetic and environmental "cross-talk" probably results in the integration of triggers, leading to the development of dry eye disease. Inflammation is an adaptive response of the immune system that is influenced by many different persistant stimuli and conditions[5]. One hallmark of inflammation, T cell infiltration, occurs in animal models of dry eye disease and human patients, regardless of the presence of systemic autoimmune diseases[6, 7]. These findings led to the proposal that dry eye may result from a localized autoimmune disease that itself originates from an imbalance in the protective immunoregulatory and proinflammatory pathways of the ocular surface[8].

In the early phase of inflammatory dry eye disease, irritation of the ocular surface by stimuli such as microbial antigens, microtrauma, UV light, or hyperosmotic stress activates the immune inflammatory signaling pathway, by stimulating epithelial cells of the ocular surface to produce and release inflammatory cytokines such as IL-1α, IL-1β, IL-6, IL-8 and TNF-α. The inflammatory cytokines further amplify proinflammatory cytokine and chemokine production and recruit other inflammatory cells, which collectively activate the expression of adhesion molecules and conjunctival vascular endothelial molecules; these molecules further facilitate the recruitment of inflammatory cells to the ocular surface, thus generating an inflammatory microenvironment[9]. Chronic irritation may develop under the influence of one or several triggers, causing late acute or chronic inflammation, which can lead to dry eye disease.

Chronic immune inflammation of the eye involves the aquisition and processing of antigens by ocular antigen presenting cells (APC)s, which migrate to the draining lymph nodes via conjunctival afferent lymphatics and veins, where they prime naïve T cells and induce Th1 and Th17 polarization[10]. Primed CD4$^+$ T cells then migrate into the conjunctiva, where they adhere to the activated vascular endothelium and enter the tissue parenchyma. Cytokines such as IFN-γ and IL-17, produced by activated T cells, amplify the immune response by increasing the expression of adhesion molecules such as VCAM, VEGF-C, and VEGF-D on conjunctival blood vessels, which leads to ocular surface damage[9, 11]. IFN-γ also alter mucins on the ocular surface; these alterations are implicated in epithelial cell apoptosis, goblet

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cell loss, and squamous dysplasia[12]. IL-17 increases MMP3/MMP9 expression and corneal epithelial barrier dysfunction[11].

Although these changes do not always occur in all types of dry eye disease, immune-mediated inflammation, including the inappropriate activation of innate and acquired immunity, is a critical factor in developing and perpetuating the disease.

Genetic factors

The major histocompatibility complex (MHC) region on human chromosome 6 is the most critical autoimmune susceptibility locus identified to date, and is associated with the greatest number of autoimmune diseases. In addition, non-MHC gene alleles that have been implicated in autoimmune disease are mostly immune-associated genes, suggesting that these diseases result, at least in part, from allelic variations in immune-associated genes[13]. However, among monozygotic twins with an autoimmune disease, only 40% are both affected, indicating that genetics alone cannot be responsible for the development of these diseases[14]. Furthermore, since allelic variations in immune genes evolved as a result of environmental selection, it is reasonable to expect that environmental factors may influence the development of immune-mediated diseases[13].

In the field of dry eye research, genetic dissociation of dacryoadenitis and sialadenitis has led to the identification of common and disease-specific genetic susceptibility loci for Sjögren's syndrome mouse model[15]. Stevens Johnson syndrome (SJS), a rare disorder often caused by a reaction to medication, is often accompanied by a severe form of chronic inflammatory dry eye disease characterized by conjunctival fibrosis, trichiasis, limbal stem cell deficiency, and infection. A possible association between SJS/toxic epidermal necrolysis (TEN) and a disordered innate immunity has been revealed by genetic analysis[16]. Recently, several studies have suggested that a splicing variant of MUC1 influences the lubrication and protection of the ocular surface against inflammation, and estrogen receptor polymorphisms have likewise been implicated in some cases of dry eye disease[17, 18]. Collectively, these reports suggest that a large number of genetic variants may influence the degree of dry eye severity and the specific type of disorder.

Environmental factors

Environmental factors such as air speed, humidity, patho-gens, smoking, toxins, UV, diet, antibiotics, microbiota, and vitamin D levels related to sun exposure and diet can influence inflammation on the mucosal membrane, leading to dry eye[13, 19]. Long-term stimulation by environmental factors probably leads to dry eye disease as a result of chronic inflammation. In addition, the mucosal barrier on the ocular surface interacts with a large number of microorganisms, including commensal bacteria, and immune dysfunction and/or an abnormal response to microbiota are reported to cause ocular surface inflammation[20]. It was recently shown that the ocular-surface epithelial cells play a critical role in triggering a cross-reactive immune response to environmental stimuli. It is still unknown how epithelial cells collect the information that determines an inflammatory or non-inflammatory response to the microbiota and thus preserve conjunctival homeostasis. Nonetheless, current evidence suggests that a dysregulated immunomodulatory function of these epithelial cells may contribute to the development of inflammation in the mucosal surface barrier which results in dry eye disease.

Dry eye disease and the tear film layer, epithelium, and stroma

1)Tear film osmolarity and tear lipid layer

Hyperosmolarity is usually caused by reduced aqueous tear flow, resulting from lacrimal failure and/or increased evaporation from the tear film. Increased evaporation is induced by environmental conditions of low humidity and high air flow. In addition, an important clinical cause is dysfunction of the meibomian glands which are on the eyelids that release oils and mucins, leading to an unstable tear film lipid layer[1, 21]. The quality of oils released by the meibomian glands is modified by the action of esterases and lipases produced by normal eyelid commensal bacteria, whose numbers increase in blepharitis. Reduced aqueous tear flow may also be induced by certain systemic drugs, including anti-histamines and anti-muscarinic agents. The common cause of tear hyperosmolarity is inflammatory lacrimal gland damage, which is seen in autoimmune disorders such as Sjögren's syndrome, graft-versus-host disease (GVHD), Stevens-Johnson syndrome, ocular cicatricial pemphigoid and non-Sjögren's syndrome; in these cases, the damage may be owing to aberrant immune responses or a failure of immune regulation. Ocular inflammation causes tissue destruction and can result in a potentially irreversible neurosensory block. Inflammation is promoted by low tissue androgen levels.

2)Dry eye disease and the mucin/aqueous layer

Anti-bacterial molecules in the aqueous tear film layer serve as modulators of innate immunity. Altered lysosome, lactoferrin, lipocalin, secreted IgA, secreted phospholipase A, and antimicrobial proteins such as defensins and cathelicidin are reported to contribute to dry eye disease[4]. Mucins produced by the ocular surface include soluble mucins and membrane-spanning mucins. Gel-forming mucins, including MUC5AC, are secreted by goblet cells and are the main components of all mucous layers, including that of the ocular surface[22]. Membrane-spanning mucins, including MUC1, MUC4, and MUC16 are produced by the endoplasmic reticulum and delivered by secretory vesicles of conjunctival epithelia. They represent the major components of the ocular surface glycocalyx, a protective mucin-containing structure[22-24]. Decreased mucin formation and/or secretion or abnormal mucins contribute to the onset and persistence of dry eye disease, especially by affecting tear instability and contributing to the inflammation associated with mechanical stress.

3)Dry eye disease and the ocular surface epithelium

Studies have shown that ocular-surface epithelial cells can regulate ocular-surface inflammation[25]. Disruption of the ocular-surface barrier induces inflammation, which is likely to lead to dry eye disease. Epithelial injury caused by dry eye disease stimulates corneal nerve endings, generating discomfort for the patient. The loss of normal mucins at the ocular surface contributes to these symptoms by increasing friction between the eyelids and eye itself. In addition, the increased corneal neural input triggers neural reflex responses, and during this period of increased irritation, the upregulated stimulation of the reflex circuit may lead to neurogenic inflammation within the meibomian gland. Epithelial cells interface with an extracellular matrix, and breakdown of the basement membrane stimulates interactions between the intercellular epithelial cytoskeleton and extracellular matrix components, leading to cytoskeletal rearrangement[26]. These conditions also may induce dry eye disease-promoting changes in epithelial function.

4)Dry eye disease and the stroma

The epithelial stroma may play a major role in supporting epithelial cell and tear film layer functions. Some immune competent cells normally exist in the epithelial stroma and provide immune surveillance. The breakdown of capillaries and activation of vascular and lymphatic cells in the stroma may be an initial step in activating the immunological cascade that eventually leads to dry eye disease[27, 28]. The sustained migration of inflammatory cells from blood vessels into the epithelium plays a key role in perpetuating immune-mediated dry eye[28].

Dry eye disease and the renin angiotensin system

The renin angiotensin system (RAS) was originally reported as a blood pressure regulator, and termed "systemic RAS". Later studies recognized that RAS could also exist as a local system in tissues that functions in a proinflammatory cascade; local RAS is termed "tissue RAS"[29]. RAS activation of the innate and acquired immune systems can lead to tissue damage by the immune response to angiotensin II, for which T cells and macrophages are important effectors. In atherosclerosis, angiotensin II promotes the adhesion and infiltration of monocytes/macrophages by up-regulating adhesion molecules and chemokines. The role of RAS in inflammation has been shown in mice fed a high-fat diet, in which adipose tissue expresses macrophage-derived monocyte chemotactic protein and is infiltrated by macrophages; blocking the effects of RAS with the angiotensin II receptor antagonist Valsartan reduces these inflammatory and metabolic consequences of the high-fat diet. Tissue RAS is also found to be present in the lacrimal gland[30]. In addition, the frequency of CD45+ inflammatory cells increases in the cGVHD-affected lacrimal gland and is decreased by an AT1R antagonist[31], suggesting that RAS is linked to the inflammatory cascade underlying dry eye disease.

Dry eye disease and reactive oxygen species

Aging is often defined as the accumulation of diverse deleterious changes occurring in cells and tissues with advancing age that are responsible for increased risk of disease and death. Aging at the cellular level, termed "cellular senescence" is marked by chromosomal changes such as genomic instability and reduced telomere length. Cellular senescence is explained by various theories, which include the genetic programming and oxidative damage among others[32-34].

Oxidative stress and inflammation are reported to be involved in the development of age-related dysfunction of the lacrimal glands and ocular surface, leading to dry eye dis-
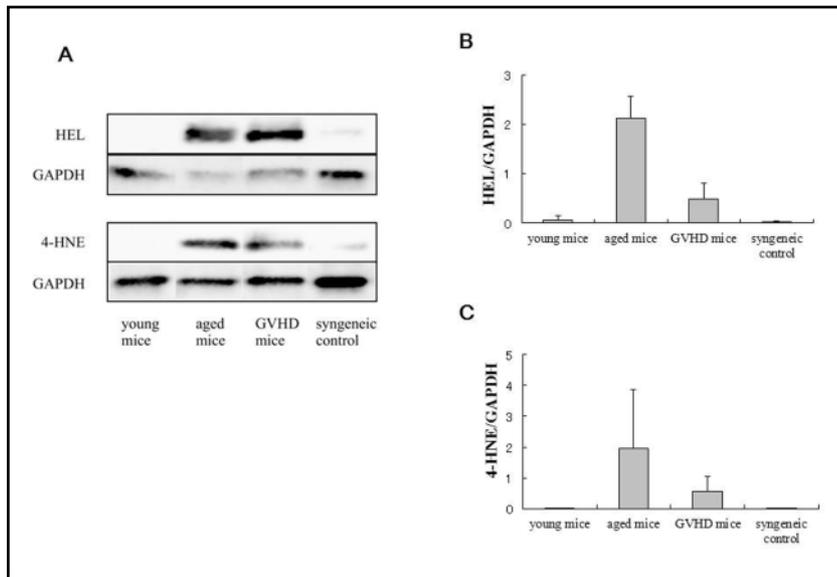
Fig.1 Protein expression of HEL and 4-HNE in the lacrimal glands in aged and cGVHD model mice

(A) The cropped blots are representative of samples from the young mice (n=3), aged mice (n=3), cGVHD mice (n=3) and syngeneic control (n=2). The expression of HEL and 4-HNE in the aged and cGVHD mice was notably higher than in the young mice. The full-length gels and blots are shown in the supplementary figure 3. (B, C) The corresponding quantitative data of HEL (B), and 4-HNE(C), normalized to the internal control, GAPDH, are shown. Bars indicate standard deviation. (Reprints from reference 32, with permission from Scientific Reports.)
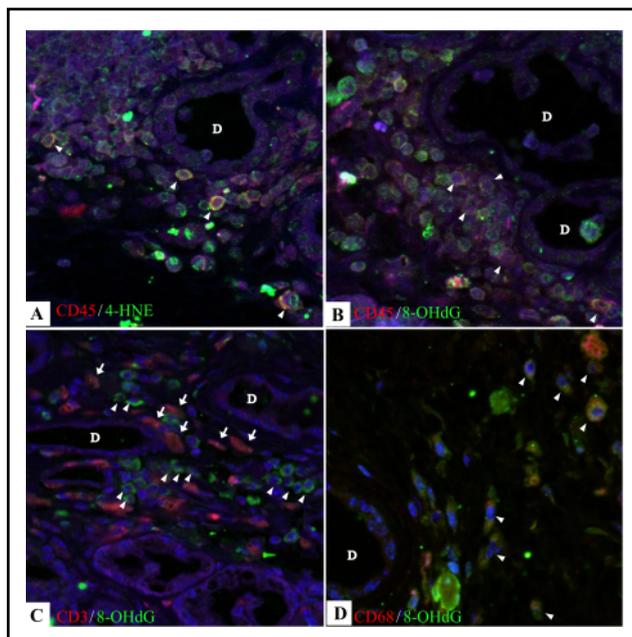
Fig.2 Macrophages are possible cells expressing the oxidative stress markers in the cGVHD model mice

Immunofluorescence double staining of CD45 and 4-HNE (A), CD45 and 8-OHdG (B), CD3 and 8-OHdG (C) and CD68 and 8-OHdG (D) on a specimen from animal model of cGVHD. Arrowheads: cells co-expressing a corresponding marker and 8-OHdG. A, Acini; D, duct. (A-D) Original magnification. X400 (Reprints from reference 32, with permission from Scientific Reports.)

ease[32, 33]. Reactive oxygen species (ROS) are produced by several endogenous pathways that trigger the inflammatory response[5]. Our group has observed that such aging-associated changes can progress rapidly in patients with severe cGVHD-associated dry eye[34]. In addition, the risk of conjunctival carcinoma increases after hematopoietic stem cell transplantation (HSCT), much as in elderly patients. In our study comparing cGVHD and aging in mouse models, some infiltrating cells in the ocular stroma of both the cGVHD and aging mice expressed markers for oxidative stress and aging, whereas acinar cells and ductal cells barely expressed these markers (Fig. 1). Given that the greatest accumulation of the products of oxidative damage was found in infiltrating immune cells and one of the candidate is macrophage (Fig. 2), rather than in parenchymal cells, we analyzed when the damage occurred relative to the onset of cGVHD. We found that the accumulation of these products correlated with the onset of cGVHD.

Oxidative damage of immune cells may be the earliest sign of immune aging. Recently, macrophages were reported to be promoters of age-related diseases such as atherosclerosis, cancer, and macular degeneration[35]. Other studies have shown that oxidative stress in mitochondria can activate ROS, leading to inflammasome activation and the production of proinflammatory cytokines IL-1 and IL-18, resulting in inflammation and signs of aging[36]. Our results suggest that macrophages contribute to the generation of oxidative stress, inflammasome activation, and cytokine production that lead to parenchymal cell destruction, a process that is similar to the etiology of certain age-related diseases.

Dry eye disease and the inflammasome

Like its role in aging, chronic inflammation, in this case of

the ocular surface, is a key element in the pathogenesis of dry eye disease, yet the factors that trigger and sustain the inflammation remain largely elusive. Inflammasomes are cytoplasmic caspase-1-activating protein complexes that promote the maturation and secretion of the proinflammatory cytokines IL-1 β and IL-18. The most-studied inflammasome complex is NLRP3. It is activated by intracellular foreign bodies, and induces inflammation in response. ROS are known to be involved in these cascades.

In previous reports on immune-mediated dry eye in a mouse model, mitochondrial alterations were implicated as central to the disease process. Mitochondria contribute to the process of inflammation, and pro-inflammatory mediators apparently alter mitochondrial function to amplify their effect. Both of these processes may increase mitochondrial oxidative stress, resulting in a vicious inflammatory cycle that exacerbates immune-mediated dry eye disease. Moreover, damage-associated molecular patterns derived from mitochondria may promote inflammasome formation and caspase-1 activation; aberrant mitochondrial autophagy may also cause inflammation. Interventions aimed at controlling excessive oxidative stress within mitochondria may prove both preventive and therapeutic in controlling inflammation associated with dry eye disease.

Dry eye disease and neurogenic inflammation

Symptoms of neurogenic inflammation correlate primarily with corneal epithelial damage, which is believed to represent the cumulative cytotoxic damage mediated by inflammatory and pro-apoptotic stimuli, as well as hyperosmolarity caused by reduced tear secretion. The epithelial damage also causes the stimulation of corneal nociceptive nerve endings[9, 37], and such damage may explain why cases of dry eye are not always correlated with signs of ocular-surface damage, changes in tear dynamics, or ocular-surface findings.

Recent studies show that dry-eye symptoms can be accompanied by non-specific corneal pain, and a dysregulated corneal pain system can be a central pathogenic feature of dry eye disease[38]. Although dry eye is partially caused by inflammation, most patients do not show conjunctival hyperemia or edema, and whether the traditional symptoms of inflammation can be used to evaluate dry eye has been increasingly called into question.

In the classic literature, inflammation is described as a principal response of the body with hallmarks of swelling, redness, pain, and fever, which lead to organ dysfunction. However, the inflammation associated with chronic dry-eye disease may be the result of sterile inflammation and different types of inflammatory inducers, including altered cellular and tissue states[5]. Furthermore, dry eye disease may be caused by neurogenic inflammation, triggered by nociceptive stimuli and resulting in plasma extravasation, hypersensitivity, and the local activation of immune cells. Nerve growth factor, substance P, calcitonin gene-related peptide, neuropeptide Y, and vasoactive intestinal peptide are the main players in this complex mechanism[37]. These neurotransmitters are found in the tear-film layer and have a possible association with dry eye disease. Immune cells related to neuroinflammation include mast cells, neutrophils, macrophages, and lymphocytes, which can interact with neuropeptides and transmitters, leading to local inflammation.

As in other mucosal surfaces, neurogenic inflammation and innate immunity may work together to protect the ocular surface[36]. Recent advances suggest that neuromediators interacting with the complex molecular and cellular structure of the ocular surface may play a critical role in initiating the immune response and regulating its chronicity. Therefore, strategies aimed at managing neuroinflammation may provide a new approach for the prevention and treatment of at least some dry eye conditions[38]. A similar situation may occur after LASIK or cataract surgery, when corneal nerve endings are injured. In these cases, surgery-related dry eye may be associated with the release of neurotransmitters from the damaged nerve endings that cause neurogenic inflammation[36]. In addition to chronic pain syndromes caused by neuroinflammation, symptoms of dry eye cause suffering among patients with several systemic autoimmune diseases, presbyopia, and postmenopausal symptoms.

A recent exciting discovery was that a cold-transducing ion channel, the transient receptor potential cation channel subfamily M member 8 (TRPM8) is responsible for the regulation of basal tearing at the ocular surface[39]. This receptor molecule is induced by hyperosmolarity of the tear film, and it lowers the temperature on the ocular surface just before a dry spot appears. Persistant hyperosmolairty in dry eye disease may be link to TRPM8 alteration, probably leading to decrease blinking and exacerbate the severity of dry eye.

Dry eye disease and extracellular DNA

Sterile inflammation, triggered by a wide variety of stimuli,

including altered cellular and tissue states, may be associated with chronic dry eye disease. Recently, an interesting study focused on extracellular DNA (eDNA), a damage-associated molecular pattern recognized in dry eye disease[40]. Besides T cell infiltration, antigen presenting cell (APC) activation, and inflammatory cytokines, additional stresses, such as eDNA, that signal danger to the tissue are associated with increased severity and persistence of dry eye disease. eDNA is released at the ocular surface by superficial dead and dying corneal and conjunctival cells that are shed into the tear film layer and release their DNA. The authors of the study found that a nuclease deficiency in tear fluid leads to the accumulation of eDNA and neutrophil extract traps (NETs) such as cathelicidin and LL-37 in the precorneal tear film, resulting in ocular surface inflammation accompanying severe dry eye disease. The authors propose that LL-37 binds eDNA, carrying it into ocular-surface cells and leading to activation of the TLR9-MyD88 pathway, a type-1 interferon response that triggers adaptive immunity, the core inflammatory process in chronic dry eye disease[41]. This article suggests a new understanding of the mechanisms that can underlie dry eye disease.

Disease-associated dry eye: Epithelial mesenchymal transition and inflammation

1)cGVHD after hematopoietic stem cell transplantation

Allogeneic hematopoietic stem cell transplantation (HSCT) is a potential curative treatment for hematological malignancies. However, the success rate is hampered by cGVHD, which can result in a severely diminished quality of life. Ocular GVHD, i.e., dry eye after HSCT, is linked to both an immune-mediated dry eye condition and to sterile inflammation.

A pattern-recognition receptor may be an important source of GVHD-related ocular inflammation. In HSCT recipients with cGVHD-related dry eye, irradiation or massive chemotherapy prior to HSCT and the inflammatory cell infiltration that follows it induce the release of large amounts of proinflammatory cytokines and apoptotic cells into the ocular microenvironment. Cross-reactions between the donor and recipient immune cells generate a "cytokine storm", which compromises the mucosal barriers on the ocular surface, and precedes the onset of GVHD-related dry eye.

Recently, several studies have reported that the epithelial-mesenchymal transition (EMT), a normal process in em-

bryonic development, contributes to various fibrotic diseases of the kidney, lung, and liver, and to cancer metastasis[42]. For example, 40% of the fibroblasts in cases of kidney fibrosis arise from epithelial cells via local EMT triggered by inflammatory stress. EMT is characterized by the loss of apical/basal cell polarity and cell-to-cell adhesions, followed by the acquisition of a mesenchymal phenotype, i.e., the cells take on a migratory phenotype, acquire invasive capabilities, and express mesenchymal markers. EMT is triggered by various stimuli, including irradiation, hypoxia, ROS, inflammatory cytokines such as transforming growth factor-β and fibroblast growth factor[42, 43], disruption of the basal lamina, and exposure of the cytoplasm to extracellular matrix[26]. These EMT triggers also participate in cGVHD pathogenesis after HSCT. In a clinical setting, the damage caused by total body irradiation before HSCT and the migrating inflammatory cells generated by the irradiation and by HSCT itself cause the release of substantial amounts of proinflammatory cytokines. This "cytokine storm" acts on grafted T cells, prompting them to attack host antigens. In addition, ROS-mediated organ injury following allogeneic bone marrow transplant has been reported.

Among the processes that could account for cGVHD-related dry eye, EMT is a good candidate, because the ocular-surface epithelium loses the ability to secrete mucins when the cells undergo the transition to mesenchyme[26, 44]. Notably, rearrangement of cytoskeletal actin filaments is necessary for EMT, but an intact cytoskeleton is required to guide secretory vesicles to the ocular surface and to generate microvilli. Since cGVHD may trigger the EMT of ocular epithelium, the conjunctival microvilli may be abnormal and unable to secrete membrane-spanning mucins[44]. In ocular cGVHD, the production of both gel-forming mucins, including MUC1, and the ocular surface glycocalyx, including MUC1, MUC4, and MUC16, is much reduced. It is likely that cytotoxic T cells cause the basement membrane of the ocular epithelium to break down, allowing direct interactions between the cytoskeleton of epithelial cells and stromal extracellular matrix components, which trigger EMT in HSCT recipients.

To better understand the immune processes involved in cGVHD-associated dry eye, the lacrimal glands of patients with this disease have been analyzed for the expression of cell-surface molecules associated with T-cell activation. In cGVHD patients, CD4+ and CD8+ T cells are mainly detected in the periductal areas of the lacrimal glands. Periductal fibroblasts expressing CD34 and HLA-DR as well as adhe-

sion molecules such as CD54, and costimulatory molecules such as CD40, CD80, and CD86, i.e., the full complement of surface molecules necessary for antigen presentation, colocalize with the CD4+ and CD8+ cells[45]. The subset of CD4+ and CD8+ T cells that reside in the periductal area express CD54 and probably represent cells that were recently activated upon their recognition of allo- or autoantigenic peptides, presented by functional APCs in or near the periductal area. In fact, the colocalization of these fibroblasts and T cells raises the possibility that a subset of stromal fibroblasts function as APCs in the periductal area, although the APCs that seem most likely to induce T-cell activation in this area are mononuclear infiltrates, such as macrophages and B cells, which constitutively express HLA-DR, CD54, and the costimulatory molecules. Furthermore, despite the stromal fibroblasts' expression of the requisite surface molecules for T-cell activation, whether they have the ability to efficiently process and present antigens is unclear. Because the stromal fibroblasts are attached not only to T cells, but also to B cells and macrophages, an alternative role for periductal fibroblasts in the pathogenic immune effects of cGVHD is as accessory cells which are nonhematopoietic cells that provide the appropriate environment for the differentiation, proliferation, and activation of hematopoietic cells[44] that support the activation of T cells and other inflammatory cells.

2)Sjögren's syndrome

Sjögren's syndrome is characterized by lymphocytic infiltration into lacrimal and salivary glands, resulting in dry eye and dry mouth. Autoreactive CD4+ T cells or B cells are believed to contribute to the pathogenesis of this disease. Recently, the role of macrophages has drawn more attention in various inflammatory phenomena, and their involvement in autoimmune disease has been reported. Macrophages interact intimately with CD4+ T cells, serving as both T cell-directed phagocytes and T cell-activating APCs; that is, macrophages present antigenic peptides complexed with MHC class II to antigen-specific CD4+ T cells, and CD4+ T cells activate macrophages.

Macrophages secrete a variety of proinflammatory cytokines, including IL-1, that play a critical role in promoting the development of squamous metaplasia[46], which is a representative finding influenced by chronic stress and irritation on ocular surface, one of the characteristic features of dry eye . Infiltration of CD68+ macrophages into the lacrimal gland

of patients with Sjögren's syndrome has been reported[47]. In the lacrimal glands, interferon-γ and IL-17 can directly activate infiltrated macrophages and cause exocrinopathy. In clinical settings, lacrimal gland swelling requires the differential diagnosis of Sjögren's syndrome, B cell lymphoma, sarcoidosis, and IgG4-related ophthalmic disease which is charachterized by lymphoplasmacytic infiltration and storiform fibrosis in target organs as well as an increased level of serum IgG4 (>135 mg/dL)[48].

New treatments for dry eye disease

Artificial tears, hyarulonic acid, vitamin A, methylcellulose, and autologous serum eye drops are currently available for the topical treatment of dry eye. Surgical treatments for dry eye disease include occlusion by the insertion of punctual plugs and surgical punctal occlusion. Moisture aids, and therapeutic contact lenses, can help to alleviate dry-eye symptoms. In addition, treatments of the eyelid, including warm compresses, lid hygiene, and the use of a lubricant ointment, can improve symptoms from meibomian gland dysfunction, which often accompany dry eye disease.

Besides currently available treatments, topical and systemic corticosteroids and immunosuppressants such as cyclosporine may be considered. The results of clinical trials on the use of immunosuppressant drugs as a treatment for dry eye have already been reported and the results of the studies suggest that these therapies alleviate dry eye. However, whether these interventions should be applied to all types of dry eye disease is unclear. At present, immunosuppressants and corticosteroids for dry eye have not been approved for the treatment of dry eye in Japan. Alternatively, two new mucin-stimulating eye drops have been developed and approved by the Japanese Welfare Ministry. One is diquafosol, a P_2Y_2 receptor agonist that can stimulate water secretion from conjunctival epithelial cells and mucin secretion from conjunctival goblet cells via the P_2Y_2 receptors, and has been shown to be effective and safe for the treatment of dry eye syndrome[49]. This eye drop has just approved also in South Korea. The other agent is rebamipide, an antiinflammatory drug that promotes mucin production[25] and helps regenerate the damaged epithelial barrier[50]. Therapies targeted at correcting tear-film abnormalities, based on a detailed understanding of these abnormalities, have also been proposed by the Japanese Dry Eye Society, led by N. Yokoi and K. Tsubota. In addition to using topical and/or systemic medications, it is very important to evaluate and

monitor the psychological state and history of dry eye patients, because they often suffer from unexpected chronic pain, complex anxiety disorders, irritability, and a number of several systemic diseases.

Conclusion

Future therapeutic goals include regeneration of the dysfunctional ocular surface and lacrimal gland. Broad-based translational research that extends from bench to bedside and makes use of clinical results from bedside to bench will augment current analyses of the mechanisms underlying dry eye disease, and will lead to the development of new anti-inflammatory or other specific interventions, based on these mechanisms.

Conflicts of interest

The authors declare that there are no conflicts of interest to be disclosed.

CV

Yoko Ogawa graduated and received the MD degree from Keio University School of Medicine in 1980 and 1990, respectively. She has been an associate Investigator in the Division of Cellular Signaling, the Institute for Advanced Medical Research, Keio University School of Medicine and is currently a Project Associate Professor in the Department of Ophthalmology, Keio University School of Medicine. Her interests include ocular-surface immunology and pathogenic fibrosis in the pathogenesis of chronic graft versus host disease after hematopoietic stem cell transplantation and other immune mediated dry eye, including ocular cicatricial pemphigoid, Stevens Johnson syndrome, Sjögren's syndrome, and IgG4-related disease.

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