Review Article

Clinical impact of inflammation in dry eye disease: proceedings of the ODISSEY group meeting

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ABSTRACT.

Dry eye disease (DED) is a common, multifactorial ocular condition with major impact on vision and quality of life. It is now well recognized that the pathophysiology of chronic DED can include a cycle of inflammation involving both innate and adaptive immune responses. Recently, *in vitro/in vivo* models have been used to obtain a better understanding of DED-related inflammatory processes at molecular/cellular levels although they do not truly reproduce the complex and chronic hallmarks of human DED. In clinical DED research, advanced techniques such as impression cytology, conjunctival biopsy, *in vivo* confocal microscopy and multiplex tear analyses have allowed an improved assessment of inflammation in DED patients. This was supported by the identification of reliable inflammatory markers including matrix metalloproteinase-9, human leucocyte antigen-DR or intercellular adhesion molecule-1 in tears and impression cytology samples. One of the current therapeutic strategies focuses on breaking the inflammatory cycle perpetuating the ocular surface disease, and preclinical/clinical research has led to the development of promising anti-inflammatory compounds. For instance, cyclosporine, already approved in the United States, has recently been authorized in Europe to treat DED associated with severe keratitis. In addition, other agents such as corticosteroids, doxycycline and essential fatty acids, through their anti-inflammatory properties, show encouraging results. We now have a clearer understanding of the inflammatory processes involved in DED, and there is hope that the still emerging preclinical/clinical findings will be translated into new and highly effective therapies for patients in the near future.

Key words: cytokines – dry eye disease – HLA-DR – hyperosmolarity – inflammation

Introduction

Dry eye disease (DED) is a distressing multifactorial condition of major impact on patients' vision and quality of life, with disease symptoms that can seriously hinder daily activities. This condition affects between 5% and 35% of adults worldwide (Dry Eye Work-Shop 2007b). Triggering factors include intrinsic and extrinsic elements such as age, gender, hormones, autoimmune disorders, local environment, use of video display, contact lens wear and exposure to medications/preservatives (e.g. benzalkonium chloride [BAK]), all potentially leading to secretory and/or evaporative DED (Dry Eye WorkShop 2007a). In particular, low humidity and/or windy environmental conditions greatly contribute to ocular surface desiccation (Dry Eye WorkShop 2007a). Furthermore, dry eye sensations and symptoms were recently confirmed to be enhanced by seasonal conditions (van Setten et al. 2016).

Because of the multifactorial nature of the disease and frequent discordance between patients' symptomatology and

ocular surface clinical signs, diagnosis of DED and assessment of its severity are often challenging. Recently, the ODISSEY European Consensus Group has recommended a practical algorithm to be used in clinical settings, facilitating diagnosis of severe DED (Baudouin et al. 2014).

In 2007, the Definition and Classification Committee for the International Dry Eye Workshop highlighted the crucial roles of hyperosmolarity and inflammation in DED (Dry Eye Workshop 2007a) and how the interdependence between these factors may lead to cell apoptosis, ocular surface damage, visual impairment and other associated symptoms. This review aims not to look primarily at intrinsic and extrinsic causes of DED but rather to highlight the contribution of inflammation, where clearly present, in the course of the disease.

Diagnosis of DED relied previously on blunt tools including vital dye staining (e.g. corneal fluorescein staining—CFS), estimation of tear break-up time and Schirmer's testing. However, in recent years, there have been significant technological developments to better identify DED-related inflammation. This review will discuss the different experimental models currently available to understand this process at the molecular and cellular levels. Additionally, the latest techniques allowing the detection of inflammation on the ocular surface and in tears together with the advances made in developing anti-inflammatory therapies will be presented.

Vicious Circle of DED

Over the years, based on numerous experimental models mimicking DED and on new technologies to measure inflammation and explore biomarkers, growing evidence showed that both hyperosmolarity and inflammation could affect the ocular surface in an independent as well as in a synergistic manner. These findings led to the redefinition of DED to include the pivotal roles of these two factors in this disease (Dry Eye Workshop 2007a). Despite the multifactorial nature of DED, this disease can be chronically self-maintained through a cycle of local and systemic responses, which include inflammation (Dry Eye WorkShop 2007a). Dry eye disease

(DED) related inflammation involves both innate and adaptive immune responses. The innate immunity provides an immediate, non-specific defence response, while the adaptive (or acquired) immune system confers long-lasting immunity after an initial encounter with a specific antigen.

Triggering factors of inflammation

Ocular surface immune homoeostasis is regulated by resident lymphocytes (e.g. $CD8^+$, $\gamma\delta$ and natural killer T-cells; Bonaccorsi et al. 2015) and $CD4^+$ regulatory T-cells. These interact with anti-inflammatory factors, such as interleukin (IL)-1 receptor antagonist, transforming growth factor (TGF)-β_2 and matrix protease inhibitors like tissue inhibitor of metalloproteinase (TIMP)-1 (Gupta et al. 1996; Sobrin et al. 2000; Solomon et al. 2001; Barabino & Dana 2007; Stern et al. 2013). Stress factors including environment challenges, infections, endogenic stress, autoimmunity and genetic factors may all disturb the finely tuned homoeostatic balance existing on the ocular surface and activate an acute inflammatory response (Fig. 1; Baudouin et al. 2013; McDermott et al. 2005; Stern et al. 2013).

Increase in tear film osmolarity, possibly triggered by dysfunctional tear secretion (aqueous tear-deficient dry eye) and/or excessive water evaporation with normal lacrimal secretory function (evaporative dry eye) may lead to hyperosmotic, desiccating and mechanical/shear stresses (due to loss of hydration/lubrication), also initiating innate inflammatory events (Dry Eye WorkShop 2007a).

Furthermore, the hyperosmolar-mediated epithelial damage causes exposure and chronic stimulation of corneal nerve endings (Dastjerdi & Dana 2009; Stevenson et al. 2012). The reduction in corneal sensitivity promotes neurogenic stress, contributing to impairment of ocular surface homoeostasis (Bourcier et al. 2005; M'Garrech et al. 2013).

Increased blinking and higher reflex tear secretion can result in release of neurotrophic factors such as nerve growth factor (NGF) as well as several neuropeptides (e.g. substance P, calcitonin and neuropeptide Y), affecting immune cell degranulation, blood flow and extravasation which may lead to

neurogenic inflammation on the ocular surface and within the lacrimal gland. An inflamed lacrimal gland may produce 'toxic tears' containing pro-inflammatory cytokines, disrupting ocular surface homoeostasis and exacerbating an innate inflammatory response (Rolando et al. 2005; Dry Eye WorkShop 2007a; Mantelli et al. 2010; Lambiase et al. 2012).

Local immune responses

In the early stages of DED, exposure of corneal and conjunctival epithelia to injury induces the activation of stress-associated signalling cascades including the mitogen-activated protein kinase (MAPK) and nuclear factor κB (NFκB) pathways (Li et al. 2004, 2006; Luo et al. 2004, 2005; Stevenson et al. 2012; Stern et al. 2013), resulting in expression of pleiotropic pro-inflammatory cytokines/chemokines (e.g. tumour necrosis factor-α [TNF-α], IL-1β, IL-6, IL-8 and NGF) and matrix degrading proteases (e.g. matrix metalloproteinase [MMP]-9 and MMP-3) by corneal and conjunctival epithelial cells (Luo et al. 2004; Li et al. 2006; Na et al. 2012; Stern et al. 2013). The development of such a pro-inflammatory environment is further supported with (1) a decrease in the release of anti-inflammatory TGF-β_2 by conjunctival goblet cells in the initial stages of the disease (Pflugfelder et al. 2008a), (2) an inhibition of immune-protective cells such as regulatory T-cells (Siemasko et al. 2008; Stevenson et al. 2012), (3) an increase in MAPK, TNF and Fas-Fas ligand pathway-mediated apoptosis of epithelial and goblet cells (Yeh et al. 2003; Luo et al. 2007; Stevenson et al. 2012), and (4) a decrease in apoptosis of ocular surface inflammatory cells (Perez et al. 2009; Gao et al. 2013).

The pro-inflammatory milieu upregulates expression of receptors to inflammatory effectors (e.g. human leucocyte antigen [HLA]-DR, CD40, CD40 ligand, toll-like receptor 4 and 5 and C–C chemokine receptor 5) and adhesion receptors (e.g. intercellular adhesion molecule [ICAM]-1), facilitating inflammatory mediators' recruitment and migration from the ocular surface (Calonge et al. 2010; Redfern et al. 2015). There is also an increase in expression and activation of enzymes involved in the innate immunity (e.g.
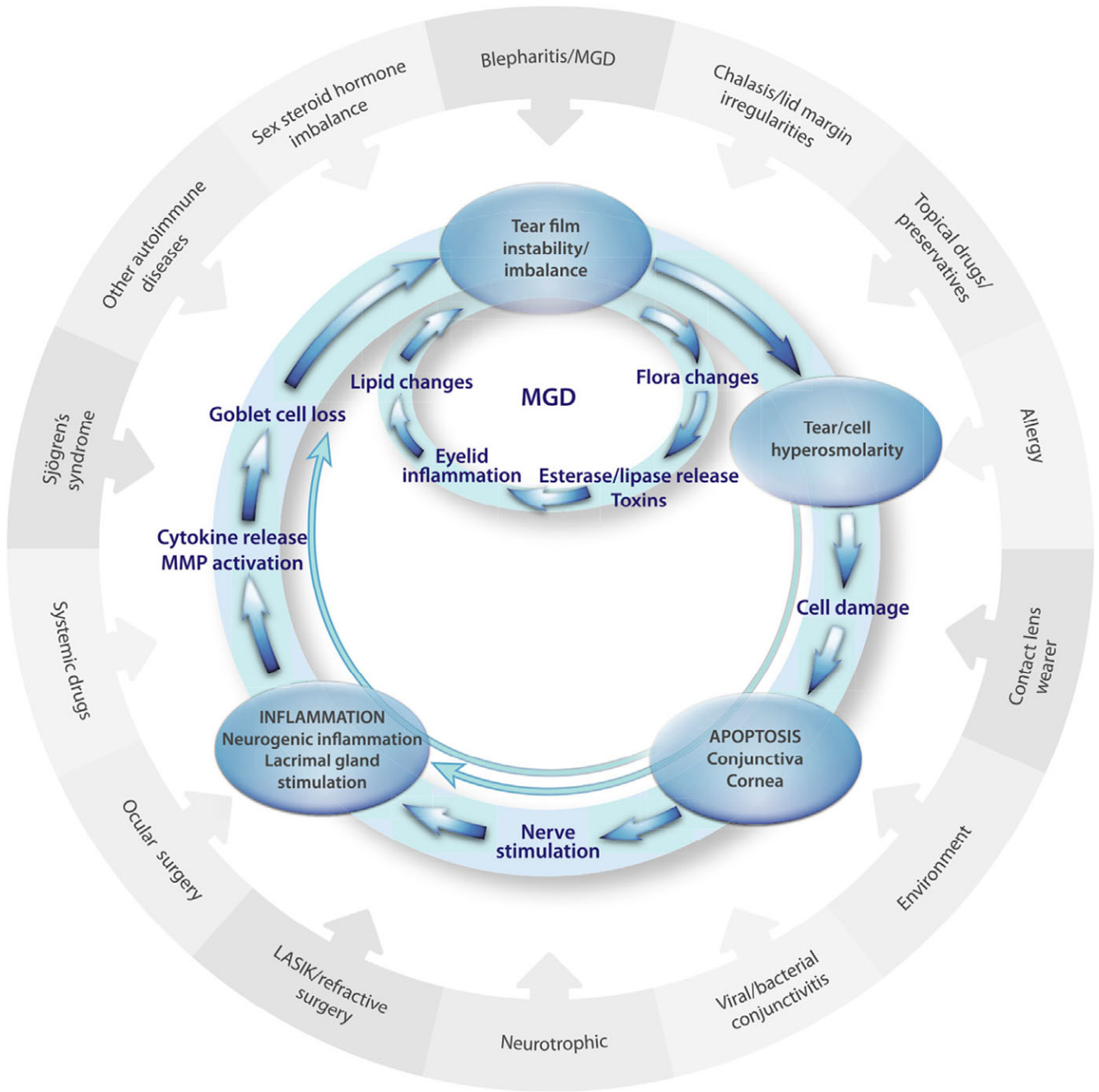
Fig. 1. Vicious circle of dry eye disease. MGD = Meibomian gland dysfunction; LASIK = Laser-assisted *in situ* keratomileusis; MMP = Matrix metalloproteinase. This diagram has been published in the reference Baudouin et al. 2013 under the Creative Commons Attribution-NonCommercial-ShareAlike License.

acidic mammalian chitinase; Musumeci et al. 2009, 2008) and in apoptosis (e.g. transglutaminase-2; Aragona et al. 2015). Together, these cascades of events contribute to amplify and perpetuate the non-self-healing innate inflammation responses, consequently resulting in cellular/tissue damage.

Epithelial-derived pro-inflammatory cytokines activate immature resident antigen-presenting cells (APCs), which are mainly dendritic cells, on the ocular surface. Mature APCs migrate to the regional lymph nodes and initiate an adaptive immune response by priming naïve $CD4^+$ T-cells including T helper (Th)1 and Th17 cells (Niederkorn et al. 2006; De Paiva et al. 2007, 2009; El Annan et al. 2009; Stevenson et al. 2012; Stern et al. 2013). Through activated angiogenesis and lymphangiogenesis, these inflammatory mediators traffic back to the ocular surface, where Th1-secreted interferon (IFN)-γ and Th17-secreted IL-17 increase cytokine production, induce epithelial and goblet cell apoptosis and alter conjunctival homoeostasis, perpetuating a chronic inflammatory process (Pflugfelder et al. 2008a).

Experimental Data Supporting DED Inflammation

Over the past two decades, experimental *in vitro* cell-based assays and *in vivo* animal models have greatly contributed

to an improved understanding of the effects of inflammation in DED (Dry Eye WorkShop 2007d; Calonge et al. 2010; Wei & Asbell 2014).

In vitro cell-based models

These assays primarily use human corneal, conjunctival or limbal epithelial cells exposed to hyperosmotic/desiccating stress, induced either by increasing osmotic conditions to 350–500 mOsm or by exposing cells to air after culture medium removal. These experimental models have allowed to easily simulate stress factors known to trigger DED and therefore to learn more about the resulting inflammatory events.

For example, exposure of human limbal/corneal epithelial cells to hyperosmotic/desiccating stress induces activation of MAPK signalling pathway and expression of cytokines (e.g. IL-1β, TNF-α, IL-8 and IL-6; Higuchi et al. 2011; Li et al. 2006) and MMPs (e.g. MMP-9, MMP-1, MMP-13 and MMP-3; Li et al. 2004). Furthermore, IFN-γ-stimulated inflammation results in increased HLA-DR and ICAM-1 expression on primary epithelial cell surface (Zhan et al. 2003).

In the past 10 years, *in vitro* three-dimensional models of human corneal epithelium have been developed and used as dry eye models after exposure to controlled environmental conditions (i.e. <40% humidity and 40°C temperature; Meloni et al. 2011; Barabino et al. 2016). Similarly, *in vitro* models of conjunctival epithelium and bioengineered lacrimal glands are also being investigated and developed in laboratories to better simulate micro-environmental conditions of both physio- and pathological ocular surfaces (Chung et al. 2007; Hirayama et al. 2013; Lu et al. 2015).

Although *in vitro* assays are relatively simple and useful models to understand ocular inflammation at molecular/cellular levels, they still fail to truly represent its complexity.

In vivo animal models

Several *in vivo* models, mainly in rodents, have been designed to study ocular surface inflammatory mechanisms (Barabino & Dana 2004; Dry Eye WorkShop 2007d; Calonge et al. 2010).

Among them, a mouse model of DED consists of pharmacological blockage of lacrimal tear production by transdermal application of scopolamine and exposure to environmental desiccating stress (Dursun et al. 2002). The observed reduction in tear production and clearance, decrease in conjunctival goblet cells and morphological changes in conjunctival epithelial cells all resemble those in human DED. The utility of this model is not that it induces DED similarly to the human disease, but that the resulting inflammation and damage share strong similarities.

Additionally, a different model exposing mice to specific low-humidity environment showed development of typical clinical signs similar to DED patients (Barabino et al. 2005). Animal models have shown systematic presence of inflammation with DED-like signs and have allowed identification of key inflammatory effectors (Calonge et al. 2010). For example, Niederkorn et al. (2006) proved that CD4$^+$ T-cells are key inflammatory players in mice exposed to environmental desiccating stress and adoptive transferred CD4$^+$ T-cells could produce keratoconjunctivitis sicca (KCS) in wild-type mice not exposed to injury. The importance of regulatory T-cells in ocular surface homoeostasis has also been studied in a desiccating stress-induced mouse model of DED (Siemasko et al. 2008). In a similar experimental model, Schaumburg et al. (2011) showed that ocular surface APCs are essential in DED initiation and development, supporting the paradigm that dry eye can result from autoimmune causes, which often involve inflammation.

Other *in vivo* models have been developed in mice to reproduce pathophysiological mechanisms observed in dry eye. For example, topical instillation of 0.2% BAK led to inflammatory changes resembling those seen in human and this BAK-induced dry eye model may potentially be useful to test anti-inflammatory therapies in DED (Lin et al. 2011). Moreover, a mouse model of aqueous tear-deficient DED was characterized after extra-orbital lacrimal gland excision, which induced decreased aqueous tear secretion, increased corneal epitheliopathy and ocular surface inflammation and immunity (Stevenson et al. 2014).

Likewise, some dog species spontaneously develop DED due to lacrimal gland problems that may be immune-mediated or might have other causes. This canine DED model caused by lacrimal gland dysfunction (both primary and/or nictitating) has been used to identify biochemical abnormalities in ocular mucins (Calonge et al. 2010) and to demonstrate positive anti-inflammatory effects of cyclosporine (CsA; Kaswan et al. 1989).

Animal models are very powerful experimental systems to simulate DED inflammation and to investigate potential treatments although none manages to reproduce all aspects of the chronic hallmark of human DED.

Clinical data supporting inflammation in DED

Over the years, techniques have been developed (1) to diagnose DED inflammation, (2) to identify and validate ocular surface inflammatory biomarkers, (3) to further understand the DED inflammatory mechanisms, and (4) to assess clinical efficacy of anti-inflammatory DED treatments.

Exploratory techniques and biomarkers

Conjunctival hyperaemia is a hallmark of ocular inflammation that can be objectively evaluated by anterior segment photography and/or with the use of grading scales (e.g. McMonnies scale; McMonnies & Ho 1991). Tear film hyperosmolarity can also be an indirect sign of inflammation. Indeed, although hyperosmolarity is regarded as the key triggering factor of ocular surface inflammation, inflammation itself may in turn lead to dysfunction of tear secretion and therefore increased osmolarity (Niederkorn et al. 2006; Dry Eye WorkShop 2007c). Currently available systems designed to measure tear osmolarity (e.g. TearLab Osmolarity System) make systematic clinical evaluation of tear film osmolarity feasible (Sullivan et al. 2004).

Matrix metalloproteinase-9 has been shown to contribute to the DED inflammatory process (Luo et al. 2004; Pflugfelder 2011). Its expression by epithelial cells and infiltrating leucocytes as well as its MMP-3 and TIMP-1-mediated enzymatic activity increases on the ocular surface of patients with

dysfunctional tear syndrome (Sobrin et al. 2000; Chotikavanich et al. 2009; Iovieno et al. 2009). Recent commercialization of a MMP-9 detection test (InflammaDry® Detector, RPS) makes it a potentially good biomarker of inflammation in DED. This device proved to be qualitatively sensitive and specific for DED diagnosis and to well-correlate with other clinical tests in two studies, although not all patients with dry eye expressed this indicator of cell damage (Sambursky et al. 2013; Messmer et al. 2014). However, poor correlation between this test and tear osmolarity was recently found in patients with mild DED, suggesting that it may be more suitable for diagnosis of moderate to severe DED (Schargus et al. 2015).

Recently, Jackson et al. (2016) found significant correlations between tear IFN-γ concentrations, tear osmolarity, total ocular surface staining and Schirmer's test score, all key clinical diagnostic parameters for DED, suggesting IFN-γ as a potential biomarker of tear hyperosmolarity associated with evaporative DED.

Hyperosmolarity induces HLA-DR overexpression in human conjunctival epithelial cells (Brignole et al. 2000; Barabino et al. 2010; Versura et al. 2011), and this upregulation may be driven by IFN-γ as shown in Sjögren's patients (Tsubota et al. 1999). In 1992, Baudouin et al. showed that impression cytology/immunohistochemistry could specifically detect HLA-DR expression and therefore local conjunctival inflammation (Fig. 2A; Baudouin et al. 1992). These findings were later confirmed in the first report showing quantification of HLA-DR expression in impression cytology specimens by flow cytometry (Baudouin et al. 1997). Furthermore, using similar techniques in dry eye samples, the number of goblet cells was shown to negatively correlate with HLA-DR expression (Pisella et al. 2000). More recently, Yafawi et al. (2013) demonstrated that impression cytology detecting HLA-DR as a biomarker of ocular surface inflammation was a sensitive, reliable, simple and noninvasive technique for investigating DED inflammation. Also, quantitative HLA-DR detection by impression cytology has been used in several DED clinical trials, and Epstein et al. (2013) have published a standard operating procedure for use of this inflammatory biomarker in multicentre clinical trials. Using impression cytology, one study showed that one drop of low-concentration clobetasone butyrate twice daily significantly decreased HLA-DR expression in Sjögren's patients (Aragona et al. 2013). Furthermore, topical CsA significantly reduced HLA-DR expression in dry eye patients (Brignole et al. 2001; Leonardi et al. 2016) and in a large randomized study, and Brignole-Baudouin et al. (2011) demonstrated that oral supplementation of omega-3 and omega-6 fatty acids decreased HLA-DR expression on conjunctival cells in DED patients. Similar to HLA-DR, ICAM-1 is upregulated on the conjunctival epithelium in ocular surface inflammation and could represent a potential biomarker (Tsubota et al. 1999). Stern et al. (2002) have identified lymphocytic infiltration and immunoreactivity for HLA-DR and ICAM-1 using conjunctival biopsy, confirming conjunctival inflammation in DED patients. Moreover, with the same technique, Kunert et al. showed that topical CsA significantly reduced the number of activated lymphocytes and increased the number of goblet cells in DED patients (Kunert et al. 2000, 2002).

High-throughput screening

Recent technological development has allowed multiplex detection of pro-inflammatory cytokines/chemokines in ocular tissues, cells and tears and identification of expression patterns
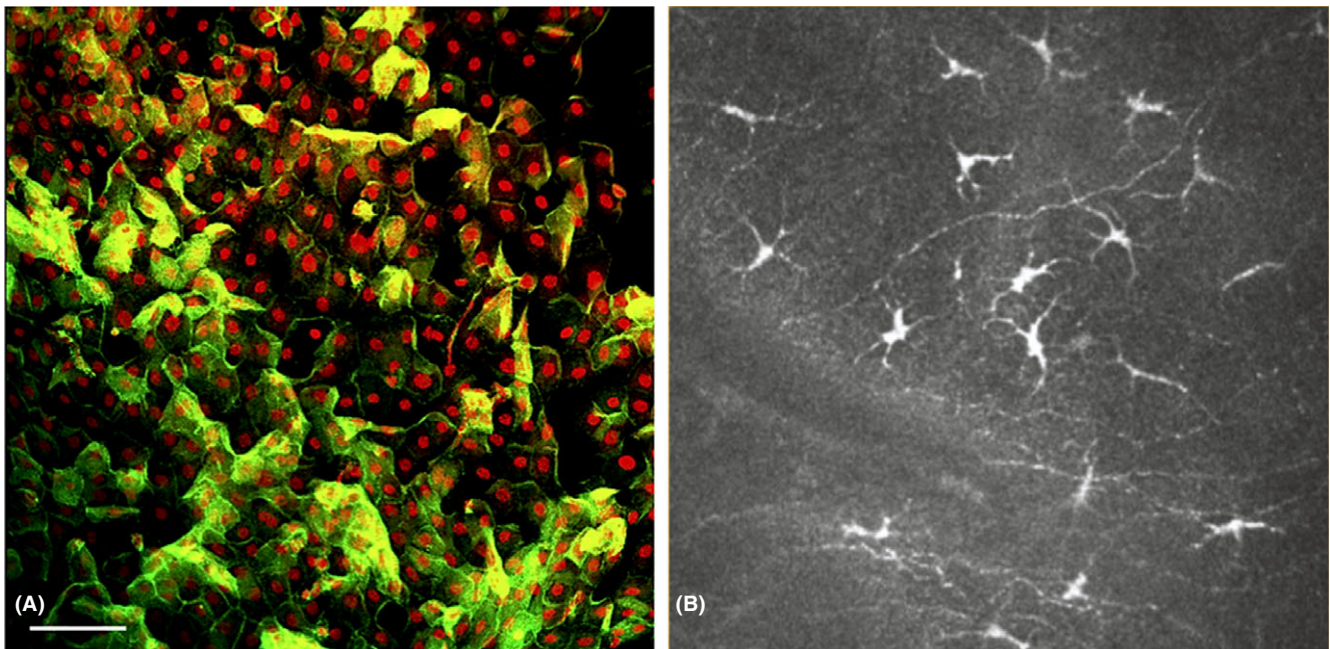
Fig. 2. Exemplary images of conjunctival/corneal samples from dry eye patients following impression cytology (A) and *in vivo* confocal microscopy (B). (A) Confocal microscopy following immunofluorescence staining for HLA-DR of conjunctival impression cytology from a dry eye patient. HLA-DR expression on the conjunctival epithelium is shown in green with nuclear staining in red (bar = 30 μm; HRT/RCM, Heidelberg, Germany). (B) *In vivo* confocal microscopy image presenting the dendritiform inflammatory cells in the conjunctiva/cornea of a dry eye patient (HRT/RCM, Heidelberg, Germany).

specific to different immune-based ocular disorders (e.g. allergy and active Sjögren's syndrome; Enriquez-de-Salamanca & Calonge 2008). For example, multiplex immunobead assays have allowed identification of specific pattern of cytokines released by corneal and conjunctival epithelia in two different mouse strains subjected to desiccating stress: C57BL/6 mice had increased tear levels of Th-1 cytokines, while BALB/c mice of Th-2 cytokines (Corrales et al. 2007; Yoon et al. 2007). These findings may suggest that blocking the production of certain cytokines or their receptors may modulate the ocular surface immune-inflammatory response in DED. Although the great amount of data generated by the multiplex-based technology may sometimes be difficult to interpret, it provides valuable information on the main ocular source for specific cytokines/chemokines.

Technological advances in mass spectrometry with proteomics, metabolomics, lipidomics and glycomics have allowed the development of analytical methods of tears and conjunctival impression. These may provide better understanding of the role of specific molecules in DED inflammation and help with diagnosis, management and treatment (Zhou et al. 2012; Soria et al. 2013).

Imaging techniques

In vivo confocal microscopy (IVCM) is a non-invasive and powerful imaging technique that allows *in vivo* visualization of the ocular surface at the cellular level (Fig. 2B). In DED, IVCM is used to observe squamous abnormalities in corneal and conjunctival epithelia, changes in subepithelial corneal nerve plexus, dysfunctional meibomian glands, as well as the presence of inflammatory cell infiltration, goblet cell density and apoptosis, all good indicators of DED inflammation (Villani et al. 2013, 2014). It has also been used to make qualitative and quantitative assessments of DED progression and severity (Qazi et al. 2014).

Another important imaging tool used in clinical practice is the anterior segment optical coherence tomography (AS-OCT). This technique is a non-contact optical system that captures cross-sectional images of the cornea and anterior chamber. It allows

quantitative analyses such as tear meniscus measurements and therefore may be useful and applicable for DED diagnosis and evaluation (Ibrahim et al. 2010; Lim 2015).

Treatments Targeting DED Inflammation

Numerous anti-inflammatory agents are being developed as treatments for DED in hopes that the vicious circle of DED may be broken by reducing the amount of inflammation on the ocular surface and in the lacrimal unit.

Corticosteroids

Dry eye disease (DED) is consensually listed by the United States Federal Regulations as steroid-responsive inflammatory conditions (Dry Eye WorkShop 2007c). Although corticosteroids are not explicitly indicated for treating DED, they are the most commonly prescribed short-term treatment for managing DED-associated inflammation.

Corticosteroids act on various inflammatory responses including ICAM-1-mediated cell adhesion, cytokines/chemokines/MMPs expression and induction of lymphocyte apoptosis (Pflugfelder 2004; De Paiva et al. 2006; Yagci & Gurdal 2014). They have been shown to clinically improve DED symptoms in several clinical trials (Dry Eye WorkShop 2007c; Aragona et al. 2013, 2015). However, their long-term use in ocular conditions is not recommended because of steroid-related side-effects such as increased intraocular pressure and cataract formation (Marsh & Pflugfelder 1999).

Cyclosporine

The immunosuppressive properties of CsA were first demonstrated in the canine spontaneous DED model (Kaswan et al. 1989). In a large multicentre study, 0.05–0.1% CsA treatment significantly reduced HLA-DR expression and to a lesser extent expression of other inflammatory and apoptotic markers in patients with moderate to severe DED (Brignole et al. 2001; Galatoire et al. 2003). A 6-month treatment with 0.05–0.1% CsA resulted in a decrease in activated lymphocytes and an increase in goblet cells in DED

patients (Kunert et al. 2000, 2002). Topical CsA increased goblet cell density and conjunctival production of immunomodulatory TGF-β_2 in DED patients (Pflugfelder et al. 2008b). In addition, 6-month treatment with 0.05% CsA decreased conjunctival IL-6 expression in patients with moderate to severe DED (Turner et al. 2000). According to available clinical data, topical CsA treatment may take 6–8 weeks to see any significant improvement in DED inflammation with no major safety concerns (Gumus & Cavanagh 2009; Aragona 2014; Yagci & Gurdal 2014).

In 2003, topical 0.05% CsA emulsion (Restasis®, Allergan) received approval from the Food and Drug Administration to increase tear production in patients whose tear production is presumed to be suppressed due to ocular inflammation associated with KCS. Restasis was never approved by the European Medicines Agency (EMA). Topical 1 mg/mL CsA cationic emulsion (Ikervis®, Santen) was approved in 2015 by the EMA and is the only CsA-containing treatment licensed in Europe. It is indicated for severe keratitis in adult patients with dry eye which has not improved despite treatment with tear substitutes.

Essential fatty acids

Several studies showed that oral administration of omega-3 and omega-6 essential fatty acids improves both DED symptoms and inflammation (Roncone et al. 2010; Deinema et al. 2016; Epitropoulos et al. 2016). Omega-6 administration has been shown to improve ocular surface signs and ocular discomfort symptoms in Sjögren's patients (Aragona et al. 2005). In addition, omega-3/omega-6 therapy improved DED signs (i.e. lissamine green staining but not tear break-up time or Schirmer's test) and reduced ocular surface expression of HLA-DR (Barabino et al. 2003). These results were confirmed in a multicentre, randomized study; supplementation with omega-3 and omega-6 fatty acids reduced HLA-DR expression in patients with DED although there was no significant difference versus placebo in ocular symptoms (Brignole-Baudouin et al. 2011). Randomized multicentre studies are currently ongoing to further investigate omega-3 fatty acids'

efficacy in DED. In addition, topical eye drops containing omega-3 fatty acids have recently become available and are currently under investigation for treating DED (Messmer 2015).

Other anti-inflammatory therapies

Other potential anti-inflammatory agents that have shown very encouraging results are currently being investigated in experimental models and/or clinical trials. For example, tetracycline and its derivatives (e.g. doxycycline) possess anti-inflammatory and anti-angiogenic properties, which make them potential candidates for treating DED inflammation. Although tetracyclines showed promising results in experimental DED, ocular rosacea and chronic meibomian gland dysfunction (Stone & Chodosh 2004; Yoo et al. 2005; De Paiva et al. 2006; De Paiva & Pflugfelder 2008), randomized, placebo-controlled clinical trials are yet to be conducted.

Another investigational molecule called SAR1118 is a lymphocyte function-associated antigen-1 antagonist. This compound inhibits T-cells' activation, adhesion, migration, proliferation and cytokine release by blocking T-cells' interaction with epithelial and endothelial cells and APCs (Murphy et al. 2011). SAR1118 showed promising results in a prospective, double-masked study in dry eye patients, improving tear production and ocular symptoms, but these positive results need to be confirmed in additional clinical trials (Semba et al. 2012).

Other anti-inflammatory compounds currently under investigation for treating DED, such as anti-inflammatory CD44, tacrolimus, voclosporin, anti-TNF-α agents, androgens and resolvins, all aim to prevent chronic inflammation by targeting specific inflammatory pathways/effectors. To date, only CsA has been approved by the American and European Regulatory Authorities for DED treatment.

There are likely multiple reasons why anti-inflammatory treatments currently only work on a subset of DED patients. Disease variability, lack of correlation of signs and symptoms, inappropriate dosage of experimental anti-inflammatory treatments and other factors contributing to disease severity like hyperosmolarity may be involved. However, the constant progress and improvement in agents, treatment paradigms and dosing are already helping some DED patients and are likely to be applicable to more as this complex disease becomes more well understood.

Conclusions

It is now well recognized that hyperosmolarity and inflammation work interdependently as key factors in some forms of DED. Not only they can act concomitantly on the ocular surface, but one may also lead to the other. Therefore, regardless of the initiating cause of this multifaceted disease, a self-sustained inflammatory response can develop on the ocular surface that can lead to chronic DED.

Over the years, several *in vitro* and *in vivo* models have been developed to get a deeper understanding of the DED inflammation process, and they continue to be used to evaluate efficacy and safety of new anti-inflammatory therapies. Furthermore, progress in advanced and powerful techniques in the identification/detection of reliable inflammatory biomarkers has allowed a better assessment of inflammation in DED patients. Identification and validation of new inflammatory markers may not only contribute to early DED diagnosis, but also help with assessment of disease severity, progression and response to treatment. These biomarkers may also allow identification of patients at risk of disease progression to a more severe stage, which may require different treatments and disease management.

One current strategy in the development of new treatments includes targeting specific inflammatory effectors/pathways to break the vicious circle of DED and therefore prevent disease chronicity and progression. The challenge to achieve a significant improvement in the management of DED explains the plethora of different components being investigated as potential anti-inflammatory treatments. To date, this approach has produced promising results from both experimental and clinical trials that led to market approval of CsA in both the United States and in Europe.

It is certainly hoped that the prolific ongoing research in the field of DED inflammation will produce highly effective diagnostic and more specific therapeutic advances that will benefit DED patients in the coming years.

References

Aragona P (2014): Topical cyclosporine: are all indications justified? Br J Ophthalmol **98**: 1001–1002.

Aragona P, Bucolo C, Spinella R, Giuffrida S & Ferreri G (2005): Systemic omega-6 essential fatty acid treatment and pge1 tear content in Sjogren's syndrome patients. Invest Ophthalmol Vis Sci **46**: 4474–4479.

Aragona P, Spinella R, Rania L, Postorino E, Sommario MS, Roszkowska AM & Puzzolo D (2013): Safety and efficacy of 0.1% clobetasone butyrate eyedrops in the treatment of dry eye in Sjogren syndrome. Eur J Ophthalmol **23**: 368–376.

Aragona P, Aguennouz M, Rania L et al. (2015): Matrix metalloproteinase 9 and transglutaminase 2 expression at the ocular surface in patients with different forms of dry eye disease. Ophthalmology **122**: 62–71.

Barabino S & Dana MR (2004): Animal models of dry eye: a critical assessment of opportunities and limitations. Invest Ophthalmol Vis Sci **45**: 1641–1646.

Barabino S & Dana MR (2007): Dry eye syndromes. Chem Immunol Allergy **92**: 176–184.

Barabino S, Rolando M, Camicione P, Ravera G, Zanardi S, Giuffrida S & Calabria G (2003): Systemic linoleic and gamma-linolenic acid therapy in dry eye syndrome with an inflammatory component. Cornea **22**: 97–101.

Barabino S, Shen L, Chen L, Rashid S, Rolando M & Dana MR (2005): The controlled-environment chamber: a new mouse model of dry eye. Invest Ophthalmol Vis Sci **46**: 2766–2771.

Barabino S, Montaldo E, Solignani F, Valente C, Mingari MC & Rolando M (2010): Immune response in the conjunctival epithelium of patients with dry eye. Exp Eye Res **91**: 524–529.

Barabino S, De Servi B, Aragona S, Manenti D & Meloni M (2016): Efficacy of a new ocular surface modulator in restoring epithelial changes in an in vitro model of dry eye syndrome. Curr Eye Res [Epub ahead of print].

Baudouin C, Haouat N, Brignole F, Bayle J & Gastaud P (1992): Immunopathological findings in conjunctival cells using immunofluorescence staining of impression cytology specimens. Br J Ophthalmol **76**: 545–549.

Baudouin C, Brignole F, Becquet F, Pisella PJ & Goguel A (1997): Flow cytometry in impression cytology specimens. A new method for evaluation of conjunctival inflammation. Invest Ophthalmol Vis Sci **38**: 1458–1464.

Baudouin C, Aragona P, Messmer EM et al. (2013): Role of hyperosmolarity in the pathogenesis and management of dry eye disease: proceedings of the OCEAN group meeting. Ocul Surf **11**: 246–258.

Baudouin C, Aragona P, Van Setten G et al. (2014): Diagnosing the severity of dry eye: a clear and practical algorithm. Br J Ophthalmol **98**: 1168–1176.

Bonaccorsi I, De Pasquale C, Campana S, Barberi C, Cavaliere R, Benedetto F & Ferlazzo G (2015): Natural killer cells in the innate immunity network of atherosclerosis. Immunol Lett **168**: 51–57.

Bourcier T, Acosta MC, Borderie V, Borras F, Gallar J, Bury T, Laroche L & Belmonte C (2005): Decreased corneal sensitivity in patients with dry eye. Invest Ophthalmol Vis Sci **46**: 2341–2345.

Brignole F, Pisella PJ, Goldschild M, De Saint Jean M, Goguel A & Baudouin C (2000): Flow cytometric analysis of inflammatory markers in conjunctival epithelial cells of patients with dry eyes. Invest Ophthalmol Vis Sci **41**: 1356–1363.

Brignole F, Pisella PJ, De Saint Jean M, Goldschild M, Goguel A & Baudouin C (2001): Flow cytometric analysis of inflammatory markers in KCS: 6-month treatment with topical cyclosporin A. Invest Ophthalmol Vis Sci **42**: 90–95.

Brignole-Baudouin F, Baudouin C, Aragona P et al. (2011): A multicentre, double-masked, randomized, controlled trial assessing the effect of oral supplementation of omega-3 and omega-6 fatty acids on a conjunctival inflammatory marker in dry eye patients. Acta Ophthalmol 89: e591–e597.

Calonge M, Enriquez-de-Salamanca A, Diebold Y, Gonzalez-Garcia MJ, Reinoso R, Herreras JM & Corell A (2010): Dry eye disease as an inflammatory disorder. Ocul Immunol Inflamm 18: 244–253.

Chotikavanich S, de Paiva CS, Li DQ, Chen JJ, Bian F, Farley WJ & Pflugfelder SC (2009): Production and activity of matrix metalloproteinase-9 on the ocular surface increase in dysfunctional tear syndrome. Invest Ophthalmol Vis Sci 50: 3203–3209.

Chung SH, Lee JH, Yoon JH, Lee HK & Seo KY (2007): Multi-layered culture of primary human conjunctival epithelial cells producing MUC5AC. Exp Eye Res 85: 226–233.

Corrales RM, Villarreal A, Farley W, Stern ME, Li DQ & Pflugfelder SC (2007): Strain-related cytokine profiles on the murine ocular surface in response to desiccating stress. Cornea 26: 579–584.

Dastjerdi MH & Dana R (2009): Corneal nerve alterations in dry eye-associated ocular surface disease. Int Ophthalmol Clin 49: 11–20.

De Paiva CS & Pflugfelder SC (2008): Rationale for anti-inflammatory therapy in dry eye syndrome. Arq Bras Oftalmol 71(6 Suppl): 89–95.

De Paiva CS, Corrales RM, Villarreal AL, Farley WJ, Li DQ, Stern ME & Pflugfelder SC (2006): Corticosteroid and doxycycline suppress MMP-9 and inflammatory cytokine expression, MAPK activation in the corneal epithelium in experimental dry eye. Exp Eye Res 83: 526–535.

De Paiva CS, Villarreal AL, Corrales RM et al. (2007): Dry eye-induced conjunctival epithelial squamous metaplasia is modulated by interferon-gamma. Invest Ophthalmol Vis Sci 48: 2553–2560.

De Paiva CS, Chotikavanich S, Pangelinan SB et al. (2009): IL-17 disrupts corneal barrier following desiccating stress. Mucosal Immunol 2: 243–253.

Deinema LA, Vingrys AJ, Wong CY, Jackson DC, Chinnery HR & Downie LE (2016): A randomized, double-masked, placebo-controlled clinical trial of two forms of omega-3 supplements for treating dry eye disease. Ophthalmology [Epub ahead of print].

Dry Eye WorkShop (2007a): The definition and classification of dry eye disease: report of the Definition and Classification Subcommittee of the International Dry Eye WorkShop. Ocul Surf 5: 75–92.

Dry Eye WorkShop (2007b): The epidemiology of dry eye disease: report of the Epidemiology Subcommittee of the International Dry Eye WorkShop. Ocul Surf 5: 93–107.

Dry Eye WorkShop (2007c): Management and therapy of dry eye disease: report of the Management and Therapy Subcommittee of the International Dry Eye WorkShop. Ocul Surf 5: 163–178.

Dry Eye WorkShop (2007d): Research in dry eye: report of the Research Subcommittee of the International Dry Eye WorkShop. Ocul Surf 5: 179–193.

Dursun D, Wang M, Monroy D, Li DQ, Lokeshwar BL, Stern ME & Pflugfelder SC (2002): A mouse model of keratoconjunctivitis sicca. Invest Ophthalmol Vis Sci 43: 632–638.

El Annan J, Chauhan SK, Ecoiffier T, Zhang Q, Saban DR & Dana R (2009): Characterization of effector T cells in dry eye disease. Invest Ophthalmol Vis Sci 50: 3802–3807.

Enriquez-de-Salamanca A & Calonge M (2008): Cytokines and chemokines in immune-based ocular surface inflammation. Expert Rev Clin Immunol 4: 457–467.

Epitropoulos AT, Donnenfeld ED, Shah ZA et al. (2016): Effect of oral re-esterified omega-3 nutritional supplementation on dry eyes. Cornea 35: 1185–1191.

Epstein SP, Gadaria-Rathod N, Wei Y, Maguire MG & Asbell PA (2013): HLA-DR expression as a biomarker of inflammation for multicenter clinical trials of ocular surface disease. Exp Eye Res 111: 95–104.

Galatoire O, Baudouin C, Pisella PJ & Brignole F (2003): [Flow cytometry in impression cytology during keratoconjunctivitis sicca: effects of topical cyclosporin A on HLA DR expression]. J Fr Ophtalmol 26: 337–343.

Gao J, Sana R, Calder V, Calonge M, Lee W, Wheeler LA & Stern ME (2013): Mitochondrial permeability transition pore in inflammatory apoptosis of human conjunctival epithelial cells and T cells: effect of cyclosporin A. Invest Ophthalmol Vis Sci 54: 4717–4733.

Gumus K & Cavanagh DH (2009): The role of inflammation and antiinflammation therapies in keratoconjunctivitis sicca. Clin Ophthalmol 3: 57–67.

Gupta A, Monroy D, Ji Z, Yoshino K, Huang A & Pflugfelder SC (1996): Transforming growth factor beta-1 and beta-2 in human tear fluid. Curr Eye Res 15: 605–614.

Higuchi A, Kawakita T & Tsubota K (2011): IL-6 induction in desiccated corneal epithelium in vitro and in vivo. Mol Vis 17: 2400–2406.

Hirayama M, Ogawa M, Oshima M et al. (2013): Functional lacrimal gland regeneration by transplantation of a bioengineered organ germ. Nat Commun 4: 2497.

Ibrahim OM, Dogru M, Takano Y, Satake Y, Wakamatsu TH, Fukagawa K, Tsubota K & Fujishima H (2010): Application of visante optical coherence tomography tear meniscus height measurements in the diagnosis of dry eye disease. Ophthalmology 117: 1923–1929.

Iovieno A, Lambiase A, Micera A, Stampachiacchiere B, Sgrulletta R & Bonini S (2009): In vivo characterization of doxycycline effects on tear metalloproteinases in patients with chronic blepharitis. Eur J Ophthalmol 19: 708–716.

Jackson DC, Zeng W, Wong CY, Mifsud EJ, Williamson NA, Ang CS, Vingrys AJ & Downie LE (2016): Tear interferon-gamma as a biomarker for evaporative dry eye disease. Invest Ophthalmol Vis Sci 57: 4824–4830.

Kaswan RL, Salisbury MA & Ward DA (1989): Spontaneous canine keratoconjunctivitis sicca. A useful model for human keratoconjunctivitis sicca: treatment with cyclosporine eye drops. Arch Ophthalmol 107: 1210–1216.

Kunert KS, Tisdale AS, Stern ME, Smith JA & Gipson IK (2000): Analysis of topical cyclosporine treatment of patients with dry eye syndrome: effect on conjunctival lymphocytes. Arch Ophthalmol 118: 1489–1496.

Kunert KS, Tisdale AS & Gipson IK (2002): Goblet cell numbers and epithelial proliferation in the conjunctiva of patients with dry eye syndrome treated with cyclosporine. Arch Ophthalmol 120: 330–337.

Lambiase A, Sacchetti M & Bonini S (2012): Nerve growth factor therapy for corneal disease. Curr Opin Ophthalmol 23: 296–302.

Leonardi A, Van Setten G & Amrane M (2016): Efficacy and safety of 0.1% cyclosporine A cationic emulsion in the treatment of severe dry eye disease: a multicenter randomized trial. Eur J Ophthalmol 26: 287–296.

Li DQ, Chen Z, Song XJ, Luo L & Pflugfelder SC (2004): Stimulation of matrix metalloproteinases by hyperosmolarity via a JNK pathway in human corneal epithelial cells. Invest Ophthalmol Vis Sci 45: 4302–4311.

Li DQ, Luo L, Chen Z, Kim HS, Song XJ & Pflugfelder SC (2006): JNK and ERK MAP kinases mediate induction of IL-1beta, TNF-alpha and IL-8 following hyperosmolar stress in human limbal epithelial cells. Exp Eye Res 82: 588–596.

Lim SH (2015): Clinical applications of anterior segment optical coherence tomography. J Ophthalmol 605729.

Lin Z, Liu X, Zhou T, Wang Y, Bai L, He H & Liu Z (2011): A mouse dry eye model induced by topical administration of benzalkonium chloride. Mol Vis 17: 257–264.

Lu Q, Al-Sheikh O, Elisseeff JH & Grant MP (2015): Biomaterials and tissue engineering strategies for conjunctival reconstruction and dry eye treatment. Middle East Afr J Ophthalmol 22: 428–434.

Luo L, Li DQ, Doshi A, Farley W, Corrales RM & Pflugfelder SC (2004): Experimental dry eye stimulates production of inflammatory cytokines and MMP-9 and activates MAPK signaling pathways on the ocular surface. Invest Ophthalmol Vis Sci 45: 4293–4301.

Luo L, Li DQ, Corrales RM & Pflugfelder SC (2005): Hyperosmolar saline is a proinflammatory stress on the mouse ocular surface. Eye Contact Lens 31: 186–193.

Luo L, Li DQ & Pflugfelder SC (2007): Hyperosmolarity-induced apoptosis in human corneal epithelial cells is mediated by cytochrome c and MAPK pathways. Cornea 26: 452–460.

Mantelli F, Micera A, Sacchetti M & Bonini S (2010): Neurogenic inflammation of the ocular surface. Curr Opin Allergy Clin Immunol 10: 498–504.

Marsh P & Pflugfelder SC (1999): Topical nonpreserved methylprednisolone therapy for keratoconjunctivitis sicca in Sjogren syndrome. Ophthalmology 106: 811–816.

McDermott AM, Perez V, Huang AJ et al. (2005): Pathways of corneal and ocular surface inflammation: a perspective from the cullen symposium. Ocul Surf 3(4 Suppl): S131–S138.

McMonnies CW & Ho A (1991): Conjunctival hyperaemia in non-contact lens wearers. Acta Ophthalmol (Copenh) 69: 799–801.

Meloni M, De Servi B, Marasco D & Del Prete S (2011): Molecular mechanism of ocular surface damage: application to an in vitro dry eye model on human corneal epithelium. Mol Vis 12: 113–126.

Messmer EM (2015): The pathophysiology, diagnosis, and treatment of dry eye disease. Dtsch Arztebl Int 112: 71–81; quiz 82.

Messmer EM, Lindenfels V, Garbe A & Kampik A (2014): Matrix-metalloproteinase-9 (MMP-9) – testing in dry eye syndrome. Invest Ophthalmol Vis Sci 55: 2001.

M'Garrech M, Rousseau A, Kaswin G, Sauer A, Barreau E, Bourcier T & Labetoulle M (2013): Impairment of lacrimal secretion in the unaffected fellow eye of patients with recurrent unilateral herpetic keratitis. Ophthalmology 120: 1959–1967.

Murphy CJ, Bentley E, Miller PE et al. (2011): The pharmacologic assessment of a novel lymphocyte function-associated antigen-1 antagonist (SAR 1118) for the treatment of keratoconjunctivitis sicca in dogs. Invest Ophthalmol Vis Sci 52: 3174–3180.

Musumeci M, Bellin M, Maltese A, Aragona P, Bucolo C & Musumeci S (2008): Chitinase levels in the tears of subjects with ocular allergies. Cornea 27: 168–173.

Musumeci M, Aragona P, Bellin M, Maugeri F, Rania L, Bucolo C & Musumeci S (2009): Acidic mammalian chitinase in dry eye conditions. Cornea 28: 667–672.

Na KS, Mok JW, Kim JY, Rho CR & Joo CK (2012): Correlations between tear cytokines, chemokines, and soluble receptors and clinical severity of dry eye disease. Invest Ophthalmol Vis Sci 53: 5443–5450.

Niederkorn JY, Stern ME, Pflugfelder SC, De Paiva CS, Corrales RM, Gao J & Siemasko K (2006): Desiccating stress induces T cell-mediated Sjogren's Syndrome-like lacrimal keratoconjunctivitis. J Immunol 176: 3950–3957.

Perez P, Anaya JM, Aguilera S et al. (2009): Gene expression and chromosomal location for

susceptibility to Sjogren's syndrome. J Autoimmun 33: 99–108.

Pflugfelder SC (2004): Antiinflammatory therapy for dry eye. Am J Ophthalmol 137: 337–342.

Pflugfelder SC (2011): Tear dysfunction and the cornea: LXVIII Edward Jackson Memorial Lecture. Am J Ophthalmol 152: 900–909 e901.

Pflugfelder SC, de Paiva CS, Li DQ & Stern ME (2008a): Epithelial-immune cell interaction in dry eye. Cornea 27(Suppl 1): S9–S11.

Pflugfelder SC, De Paiva CS, Villarreal AL & Stern ME (2008b): Effects of sequential artificial tear and cyclosporine emulsion therapy on conjunctival goblet cell density and transforming growth factor-beta2 production. Cornea, 27: 64–69.

Pisella PJ, Brignole F, Debbasch C et al. (2000): Flow cytometric analysis of conjunctival epithelium in ocular rosacea and keratoconjunctivitis sicca. Ophthalmology 107: 1841–1849.

Qazi Y, Aggarwal S & Hamrah P (2014): Image-guided evaluation and monitoring of treatment response in patients with dry eye disease. Graefes Arch Clin Exp Ophthalmol 252: 857–872.

Redfern RL, Barabino S, Baxter J, Lema C & McDermott AM (2015): Dry eye modulates the expression of toll-like receptors on the ocular surface. Exp Eye Res 134: 80–89.

Rolando M, Barabino S, Mingari C, Moretti S, Giuffrida S & Calabria G (2005): Distribution of conjunctival HLA-DR expression and the pathogenesis of damage in early dry eyes. Cornea 24: 951–954.

Roncone M, Bartlett H & Eperjesi F (2010): Essential fatty acids for dry eye: a review. Cont Lens Anterior Eye 33: 49–54; quiz 100.

Sambursky R, Davitt WF 3rd, Latkany R, Tauber S, Starr C, Friedberg M, Dirks MS & McDonald M (2013): Sensitivity and specificity of a point-of-care matrix metalloproteinase 9 immunoassay for diagnosing inflammation related to dry eye. JAMA Ophthalmol 131: 24–28.

Schargus M, Ivanova S, Kakkassery V, Dick HB & Joachim S (2015): Correlation of tear film osmolarity and 2 different MMP-9 tests with common dry eye tests in a cohort of non-dry eye patients. Cornea 34: 739–744.

Schaumburg CS, Siemasko KF, De Paiva CS, Wheeler LA, Niederkorn JY, Pflugfelder SC & Stern ME (2011): Ocular surface APCs are necessary for autoreactive T cell-mediated experimental autoimmune lacrimal keratoconjunctivitis. J Immunol 187: 3653–3662.

Semba CP, Torkildsen GL, Lonsdale JD, McLaurin EB, Geffin JA, Mundorf TK, Kennedy KS & Ousler GW (2012): A phase 2 randomized, double-masked, placebo-controlled study of a novel integrin antagonist (SAR 1118) for the treatment of dry eye. Am J Ophthalmol 153: 1050–1060 e1051.

van Setten G, Labetoulle M, Baudouin C & Rolando M (2016): Evidence of seasonality and effects of psychrometry in dry eye disease. Acta Ophthalmol 94: 499–506.

Siemasko KF, Gao J, Calder VL, Hanna R, Calonge M, Pflugfelder SC, Niederkorn JY & Stern ME (2008): In vitro expanded CD4$^+$CD25$^+$Foxp3$^+$ regulatory T cells maintain a normal phenotype and suppress immune-mediated ocular surface inflammation. Invest Ophthalmol Vis Sci 49: 5434–5440.

Sobrin L, Liu Z, Monroy DC, Solomon A, Selzer MG, Lokeshwar BL & Pflugfelder SC (2000): Regulation of MMP-9 activity in human tear fluid and corneal epithelial culture supernatant. Invest Ophthalmol Vis Sci 41: 1703–1709.

Solomon A, Dursun D, Liu Z, Xie Y, Macri A & Pflugfelder SC (2001): Pro- and anti-inflammatory forms of interleukin-1 in the tear fluid and conjunctiva of patients with dry-eye disease. Invest Ophthalmol Vis Sci 42: 2283–2292.

Soria J, Duran JA, Etxebarria J, et al. (2013): Tear proteome and protein network analyses reveal a novel pentamarker panel for tear film characterization in dry eye and meibomian gland dysfunction. J Proteomics 78: 94–112.

Stern ME, Gao J, Schwalb TA et al. (2002): Conjunctival T-cell subpopulations in Sjogren's and non-Sjogren's patients with dry eye. Invest Ophthalmol Vis Sci 43: 2609–2614.

Stern ME, Schaumburg CS & Pflugfelder SC (2013): Dry eye as a mucosal autoimmune disease. Int Rev Immunol 32: 19–41.

Stevenson W, Chauhan SK & Dana R (2012): Dry eye disease: an immune-mediated ocular surface disorder. Arch Ophthalmol 130: 90–100.

Stevenson W, Chen Y, Lee SM, Lee HS, Hua J, Dohlman T, Shiang T & Dana R (2014): Extraorbital lacrimal gland excision: a reproducible model of severe aqueous tear-deficient dry eye disease. Cornea 33: 1336–1341.

Stone DU & Chodosh J (2004): Oral tetracyclines for ocular rosacea: an evidence-based review of the literature. Cornea 23: 106–109.

Sullivan B, Whitmer D, Nichols KK et al. (2010): An objective approach to dry eye disease severity. Invest Ophthalmol Vis Sci. 51: 6125–6130.

Tsubota K, Fujihara T, Saito K & Takeuchi T (1999): Conjunctival epithelium expression of HLA-DR in dry eye patients. Ophthalmologica 213: 16–19.

Turner K, Pflugfelder SC, Ji Z, Feuer WJ, Stern M & Reis BL (2000): Interleukin-6 levels in the conjunctival epithelium of patients with dry eye disease treated with cyclosporine ophthalmic emulsion. Cornea 19: 492–496.

Versura P, Profazio V, Schiavi C & Campos EC (2011): Hyperosmolar stress upregulates HLA-DR expression in human conjunctival epithelium in dry eye patients and in vitro models. Invest Ophthalmol Vis Sci 52: 5488–5496.

Villani E, Mantelli F & Nucci P (2013): In-vivo confocal microscopy of the ocular surface: ocular allergy and dry eye. Curr Opin Allergy Clin Immunol 13: 569–576.

Villani E, Baudouin C, Efron N et al. (2014): In vivo confocal microscopy of the ocular surface: from bench to bedside. Curr Eye Res 39: 213–231.

Wei Y & Asbell PA (2014): The core mechanism of dry eye disease is inflammation. Eye Contact Lens 40: 248–256.

Yafawi R, Ko M, Sace FP & John-Baptiste A (2013): Limitations of an ocular surface inflammatory biomarker in impression cytology specimens. Cutan Ocul Toxicol 32: 46–53.

Yagci A & Gurdal C (2014): The role and treatment of inflammation in dry eye disease. Int Ophthalmol 34: 1291–1301.

Yeh S, Song XJ, Farley W, Li DQ, Stern ME & Pflugfelder SC (2003): Apoptosis of ocular surface cells in experimentally induced dry eye. Invest Ophthalmol Vis Sci 44: 124–129.

Yoo SE, Lee DC & Chang MH (2005): The effect of low-dose doxycycline therapy in chronic meibomian gland dysfunction. Korean J Ophthalmol 19: 258–263.

Yoon KC, De Paiva CS, Qi H, Chen Z, Farley WJ, Li DQ & Pflugfelder SC (2007): Expression of Th-1 chemokines and chemokine receptors on the ocular surface of C57BL/6 mice: effects of desiccating stress. Invest Ophthalmol Vis Sci 48: 2561–2569.

Zhan H, Towler HM & Calder VL (2003): The immunomodulatory role of human conjunctival epithelial cells. Invest Ophthalmol Vis Sci 44: 3906–3910.

Zhou L, Zhao SZ, Koh SK, Chen L, Vaz C, Tanavde V, Li XR & Beuerman RW (2012): In-depth analysis of the human tear proteome. J Proteomics 75: 3877–3885.

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