



The Balance of Th17/Treg Cells at The Ocular Surface in Dry Eye Disease

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Article info:

Received: 08.09.2022

Accepted: 19.12.2022

Keywords:

Th17,
Treg,
Dry eye,
Hyperosmolarity,
Inflammation.

Abstract

The eye is considered immune-privileged along with the brain and the testicles. To preserve their visual function, a state of tolerogenic microenvironment must be sustained by the resident immune cells to prevent aggressive inflammatory reactions. Excessive immune system stimulation or immunoregulatory mechanisms disruption at the ocular surface can result in inflammation, subsequently leading to ocular surface diseases. Dry eye is the most common type of ocular surface disorders which is characterized by unstable tear film and a variety of symptoms that adversely affects the daily activities of the patient. Despite the multifactorial nature of the dry eye, recent studies have shown that immunologic processes have a critical role in the disease's pathogenesis and progression. Herein, we focused on the involvement of Th17 and Tregs in the disease's pathophysiology and their relation to the disease progression.

1. Introduction

The prevailing consensus around dry eye disease (DED) is that it is a disorder of multifactorial nature, distinguished by impaired tear film homeostasis and a variety of symptoms produced by hyperosmolarity, inflammation of the ocular surface, and neurosensory abnormalities (Master et al., 2021; Clayton, J. A., 2018). DED affects 7% to 34% of the worldwide population and seems to be more common in older individuals hence is considered a growing health problem globally. (Barabino et al., 2021). The symptoms of dry eye are variable and can range from irritation, itching, redness, and photophobia. Cumulatively these symptoms can impair visual function and visual performance, resulting in difficulties and limitations with daily tasks including reading, writing, watching television, and driving (Baiula and Spampinato, 2021).

DED consists of two main subtypes defined based on the underlying mechanism of the disease. The first subtype, aqueous deficient dry eye (ADDE) is characterized by insufficient tear production from the lacrimal gland. This subtype can be further subclassified into non-sjögren and sjögren ADDE; Sjogren syndrome is an exocrinopathy in which the salivary, lacrimal and maybe other exocrine glands are attacked by an autoimmune process that can also affect other organs in association with other systemic diseases like rheumatoid arthritis (Akpek et al., 2019).

Other subtype is evaporative dry eye (EDE), which is the most common form of DED and is caused by conditions that affect the eyelids such as dysfunctional meibomian glands or eyelid abnormalities (Rolando and Merayo-Llodes, 2022).

DED is more common in women, with an increased prevalence after menopause (Garcia-Alfaro et al., 2021). Other risk factors include longer screen time, the prevalent use of contact lenses, exposure to air pollution, hormonal imbalance, autoimmune diseases, diabetes mellitus, infection, and ophthalmic surgery (Hasan, 2021; Gomes and Santo, 2019). Additionally, several drugs including antihistamines, beta-blockers, diuretics tricyclic antidepressant drugs, selective serotonin reuptake inhibitors, decongestants anxiolytics, antipsychotics, antiparkinsonian agents, and oral contraceptives are considered as risk factors for DED as well (Rouen and White, 2018). DED may be impacted by a variety of daily activities and social and dietary habits like smoking, which increases the incidence of DED, and the consumption of omega-3 dietary supplementation, which decreases DED prevalence (Alharbi et al., 2020).

The tear film (TF) is crucial for ocular surface health and for establishing the optimal refractive characteristics of the air/TF interface (Widjaja-Adhi, Chao, & Golczak, 2022). Three layers made up the tear film; the lipid layer, the aqueous layer, and the mucous layer. Any disruption of the tear film dynamics due to a poor composition of the lipid portion that is released by the meibomian glands or an insufficiency of the aqueous part generated by the lacrimal glands will lead to DED (Widjaja-Adhi, Chao, & Golczak, 2022).

Whether there is a decrease in the tear production or an increase in the evaporation of the tear, this results in increasing the tear osmolarity (Rao, Mohan, Gokhale, Matalia, & Mehta, 2022). Hyperosmolarity

can cause damage to the ocular surface epithelium directly and indirectly by triggering an acute inflammatory response (Bron et al., 2017). Acute response cytokines, including, IL-1 α , IL-1 β , IL-6, and TNF- α furthermore stimulate secretion of proinflammatory cytokine and chemokine, expression of adhesion molecules essential for infiltration of the innate cells and activation of the antigen presenting cells (APCs) at the ocular surface (Stern et al., 2013). The stimulation of resident, immature APC then migrates to the regional lymph nodes where it activates the T cells to effector T cells, that in turn migrate to the ocular surface to start adaptive immune response (Leonardi et al., 2021). Several clinical studies have shown elevated levels of these inflammatory cytokines in the tears of patients with DED (Pflugfelder, 2004; Nicolle et al., 2018). Consequently, all of these inflammatory responses will result in increasing both tear film hyperosmolarity and instability, and increasing damage of the ocular surface, resulting in continuance of the vicious cycle (Yamaguchi, 2018).

The diagnosis of DED depends upon several factors, including a detailed history, a questionnaire that can be filled out by the patient to assess the severity of symptoms like the ocular surface disease index (OSDI), clinical tests such as tear breakup time (TBUT), the schirmer test, tear osmolarity, and lysozyme analysis of the tears. The damage at the ocular surface can be assessed with the use of special stains such as fluorescein staining and lissamine green staining, as well as by using impression cytology, which can assess the cytological changes at the ocular surface and detect any inflammatory cells through special staining or in-vivo by the confocal microscope (Rouen et al., 2018).

2. T Helper Subsets

There are two major divisions of the immune system; the innate and the adaptive immune system. The innate immunity causes an immediate, nonspecific inflammatory response against pathogens, which is mediated by mast cells, monocytes, neutrophils, natural killer cells, and macrophages. If the injury or infection is not quickly eliminated by the cells of the innate immune system, the innate immune system recruits the adaptive immune system to promote the elimination of infection or injury. The main constituent cells of the adaptive immune system are T cells and antibody-producing B cells. T cells can be classified into two main populations: CD4⁺ T helper cells and cytotoxic CD8⁺ T cells. (Parkin & Cohen, 2001). CD4 T cells have an important role in mediating host immune responses against pathogens and in the etiology of chronic inflammation, autoimmunity, and tumors. They maintain and increase the CD8⁺ T cell response, interact with B cells to produce antibodies, and can regulate the functions of monocytes and macrophages (Luckheeram et al., 2012). In general, CD4⁺ T cells are the immune system's major response coordinators to pathogens.

Following antigenic stimulation, naive CD4⁺ T cells get activated, proliferate, and differentiate into distinct effector subgroups (Cano & Lopera, 2013).

CD4⁺ T cells can be subdivided into five distinct phenotypes; Th1 cells, Th2 cells, regulatory T cells (Treg), Th17 cells, and follicular Th cells. The type of the signals will determine the fate of the differentiation of T helper cells. Every single type of cell secretes different cytokines which are accountable for the functional roles of these cells.

3. Th17 Cells

Th17 cells were defined as a distinct subset of T helper cells by the discovery of unique differentiation and transcription factors for them (Korn et al., 2009). They can secrete several cytokines, including IL-17A/F, IL-21, IL-22, and IL-26 (Li et al., 2020). Th17 cells differentiation, expansion, and survival are dependent on a variety of cytokines and transcription factors that cooperate to promote the induction of increased Th17 numbers and inhibit the production of other T helper cell lineages (Guo & Zhang, 2021; Wu & Wan, 2020). Th cells differentiate into Th17 in the presence of IL-6 and transforming growth factor-beta (TGF- β) (Korn & Hiltensperger, 2021).

Multiple transcription factors regulate the differentiation of Th17 cells by inducing Retinoic acid receptor-related orphan nuclear receptor gamma t (ROR- γ t), interferon regulatory factor 4 (IRF4), basic leucine zipper ATF-like transcription factor (BATF), signal transducer and activator of transcription 3 (STAT3), and runt-related transcription factor 1 (RUNX1) (Aghbash et al., 2021). ROR- γ t is a key transcription factor for Th17 differentiation (Lee et al., 2015). Regarding the regulators that suppress Th17 induction, IL-2 is a critical repressor of their differentiation, and IL-2-mediated STAT5 activation preferentially inhibits IL-17 production (Li, Li, Geng, and Zhao, 2022). Th17 cells display a high degree of plasticity (Taniki et al., 2022). Th17 cells are capable of altering their transcriptional profile and trans-differentiating into regulatory type 1 cells, which are powerful immunosuppressive T cell subtypes (Tr1) (Krebs and Panzer, 2018). Th17 plays an essential role in the

eradication of fungal and extracellular bacterial infections. Additionally, Th17 can be major pathological contributor to the pathogenicity of various autoimmune disorders, such as multiple sclerosis, psoriasis, and inflammatory bowel disease (Li et al., 2020).

4. Treg Cells

Tregs are involved in preserving self-tolerance, maintaining immune homeostasis, and inhibiting autoimmune diseases (Yang et al., 2016). They make up 5% to 10% of the total CD4+ cell compartment (Matos et al., 2021). Different types of Treg cells have been identified, which can be categorized based on their developmental origin: Thymus-derived or naturally existing Treg cells are a distinct lineage that originates in the thymus and accounts for the vast majority of total Tregs (Göschl et al., 2019). The other category is peripheral Tregs, which are developed in the periphery in response to numerous antigens, whereas inducible Treg cells can be generated from naive T cells in vitro in the presence of TCR signaling with other stimuli like IL-2 and TGF- β (Scheinecker et al., 2020). Other forms of regulatory T cells exist in addition to regulatory CD4+ T cells, including T-regulatory type 1 (Tr1) cells, tissue-resident regulatory T cells, and CD8+ Treg cells. (Zhang et al., 2020).

The main Tregs transcription factor is Foxp3 (Forkhead box P3), which tightly controls Treg function and plasticity (Dong et al., 2021). These cells (Foxp3+ Tregs) are characterized by high expression of membrane marker CD25 (IL-2 receptor α -chain) and a low level of the CD127 (IL-7 receptor α -chain) (Churov et al., 2020). Lack of a functional Foxp3 protein causes severe disseminated T cell-

mediated autoimmune diseases, such as immunological dysregulation, polyendocrinopathy, enteropathy X-linked syndrome in humans (IPEX), or a similarly debilitating condition in scurfy mice (Alvarez et al., 2020).

Treg cells can perform their immunosuppressive activities by several potential mechanisms, including cell-to-cell contact-dependent and contact-independent (Miao and Zhu, 2018). Several membrane-associated regulatory factors are expressed by Treg cells to facilitate their cell-cell contact suppressive activity, such as GITR, CTLA-4, and PD-1. CTLA-4 activation can suppress the expression of CD80 and CD86 on antigen-presenting cells (Miyara and Sakaguchi, 2007). Additionally, they can highly express CD25 (IL-2R α), which binds to IL-2, and this will lead to deprivation of IL-2 from other effector T cells (Kimura, 2020; Malek and Castro, 2010). In addition to cell-contact inhibition, Tregs secrete the anti-inflammatory cytokines IL-10, IL-35, and TGF- to improve immune tolerance (Deng et al., 2019).

5. The Balance of Th17/Treg Cells

Th17 cells and Tregs perform completely different functions in the pathogenesis of inflammatory and autoimmune disorders. Whereas autoimmunity is promoted by Th17 cells, Tregs act to control it and, as a consequence of that, play a significant role in the autoimmune diseases pathogenesis by controlling the expansion/activation of autoreactive CD4⁺ T cells. Therefore, it is very important to establish Th17 and Tregs balance to prevent excessive immune activation and autoimmune responses (Zhang et al., 2021). Multiple signals, factors, epigenetic alterations, metabolic pathways, and microbiota have

been shown to regulate the plasticity of Th17 and Treg cells (He et al., 2020).

Th17 and Tregs signaling pathways are closely related and they are mediated by TGF- β (Yan et al., 2020). TGF- β in conjunction with IL-2 can promote Foxp3⁺ Treg development from naive CD4⁺ T cells in the periphery, while TGF- β in the presence of pro-inflammatory cytokines such as IL-6 stimulates the Th17 lineage (Zhu et al., 2020; Wu et al., 2020). Furthermore, retinoic acid, the key modulator of the TGF- β -driven-immune deviation, can induce Treg cell differentiation by inhibiting ROR γ t and thus suppress Th17 development (Zhu et al., 2020).

6. Significance of Th17/Treg Imbalances in Dry Eye Disease Progression

The imbalance between Th17 and Tregs can result in a variety of autoimmune diseases, chronic inflammatory disorders, and tumors (Su et al., 2013). Numerous investigations have demonstrated that DED is an autoimmune disorder resulting from an unbalanced regulatory system of the ocular surface protective immunity (Guannan et al., 2018).

Significant data suggests that the ocular surface is primarily invaded by CD4⁺ T cells, which play a crucial role in the pathogenesis of both Sjogren's and non-Sjogren's DED (Chen and Dana, 2021). Increased Th17 cells are found not only in the tissues of the ocular surface as well as in the draining lymph nodes (Fan et al., 2021). The evidence proffered by researchers is indicative of the vital role of Th17 cells, they revealed that the protein and mRNA levels of IL-6, IL-17A, and IL-23 on the ocular surfaces of DED patients increased significantly (Liu et al., 2017). Additionally, a study by Liu et al. revealed

that the ocular surface expressions of IL-6 and IL-17A were increased in the SS group compared with the other groups and had a positive correlation with the parameters of the ocular surface of DED (Liu et al., 2017). Inflammation in the conjunctiva, cornea, and lacrimal glands results in reduced production of tears and loss of the conjunctival goblet cell has been triggered via transplanting CD4⁺ T cells of mice exposed to experimental DED to T-cell-deficient nude mice were not subjected to desiccating stress (De Paiva et al., 2009). It has been shown that IL-17 increases the synthesis of both MMP-3 and MMP-9 at the corneal epithelium through binding to IL-17RA, which is a constitutively expressed receptor at the ocular surface epithelium, causing corneal epithelial barrier disruption (Chen and Dana, 2021).

Tregs play a critical role in the suppression of ocular inflammation in patients with DED (Harrell et al., 2021). Hence, Treg cell abnormalities were reported in several autoimmune disorders such as systemic lupus erythematosus, rheumatoid arthritis, and Sjögren's syndrome, all of which have been recognized to be associated with DED

(Ganesalingam et al., 2019). Also, Treg cell depletion by the use of anti-CD25 antibody or in CD25 mutant mice resulted in disease exacerbation, but normal Treg restoration in DED animals provided disease resistance, showing that Treg cells, most likely nTreg, play essential regulatory functions in the pathogenesis of DED (Chen and Dana, 2021). Disruption of the signalling of TGF- β in CD4⁺ T cells has been shown to cause a paradoxical improvement of DED in mice subjected to desiccating stress (De Paiva et al., 2011).

The severity of DED has been linked to increased expression of Th1-associated IFN- γ and Th17-associated IL-17, as well as dysfunctional Tregs (Inomata et al., 2018). Also, Chauhan et al. demonstrated that in DED animal model, Tregs are incapable of suppressing pathogenic T cell activation, and in vivo IL-17 inhibition markedly decreases both severity and progression of the disease, which is paralleled by a decrease in the Th17 cell expansion and Treg cell function reconstitution (Chauhan et al., 2009). Consequently, the deficiency of Tregs may be the key factor in the pathogenesis of DED.

The regulation of the Th17 and Tregs balance may provide a novel therapeutic target for DED. A recent study on animal models demonstrated that Rebamipide modified the balance of Th17 and Treg cells by Th17 cells inhibition and enhancing Tregs development in DED (Fu et al., 2019). Additionally, different aspects of the immune response in DED are effectively reduced by blocking IL-17A, such as reconstitution of Treg function, decrease in B cell production, and suppression of lymphangiogenesis (Fan et al., 2021).

Nowadays, significant progress has been achieved in the treatment of dry eye, from simply addressing symptoms by hydrating and moisturizing the ocular surface using artificial tears to promoting tear secretion, and anti-inflammatory and immunological regulation (Zhang et al., 2017).

Recent developments in the treatment of DED, such as Cyclosporine A (0.05%), acts to prevent apoptosis and increase the goblet cell number by acting against T lymphocytes (Adyanthaya and Abhilash, 2021). While a recent integrin antagonist, Lifitegrast, has

demonstrated a decrease in T cell recruitment, migration, and production of cytokines, suggesting its possible use in the DED treatment (Donthineni et al., 2021)

7. Conclusion

Whereas the pathogenesis of dry eye revolves around three key concepts namely tear film instability, hyperosmolarity, and inflammation, inflammation is considered as being the critical driver of dry eye pathogenesis. Hence, we infer that the balance between Th17 and Treg is very important not only in the pathogenesis of the disease but also in its prognosis and therapy. Thus enhancing the knowledge of dry eye pathogenicity could improve treatment options through the development and/or improvement of current therapeutic agents targeting immune cells and cytokines involved in the dry eye inflammatory reaction.

Conflicts of interest

The authors declare that they have no financial or other conflicts of interest with this study.

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