Chapter

Lymphocytes in Dry Eye Disease

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Abstract

The eye is a delicate organ that, along with other tissues such as the testicles and brain, is considered immune-privileged. Immune cells that reside in the eye must create a tolerogenic microenvironment to prevent unwanted aggressive inflammatory reactions that can compromise function. However, the eye is exposed to persistent environmental insult that may overwhelm immune tolerance and result in eye diseases from diverse origins (autoimmune, infectious, and inflammatory). The immune system plays a central role in the different phases of eye diseases, as alterations in immune cells in response to mechanical, chemical, or infectious stimuli initiate and amplify the immune response that lead to ocular tissue damage. Both resident and infiltrating immune cells also actively inhibit the immune response and promote tissue repair. Emerging evidence is leading to a better understanding of how and when lymphocytes, amongst other immune cells, contribute to inflammatory diseases such as dry eye disease (DED). We have compiled literature identifying the presence and participation of lymphocyte subpopulations that modulate DED from studies in both mice and humans. Notably, most mouse studies have relied on desiccant-stress-induced models (non-autoimmune DED), whereas human studies are predominantly in patients with Sjögren's syndrome (autoimmune DED).

Keywords: Dry eye disease, Lymphocytes, Inflammation, Ocular surface, Ocular therapy

1. Introduction

As a part of the central nervous system (CNS) and as an organ constantly exposed to a wide range of environmental stimuli, the eye has finely regulated immune-defense mechanisms. The eye can simultaneously respond to invading pathogens and tolerate beneficial, resident bacterial communities (ocular microbiota) while conducting its vital function, to capture luminous stimuli and transfer the signal to the brain so that they can be assimilated, and images are ultimately interpreted [1]. This finely tuned ocular function is protected by immune tolerance and defense mechanisms that are highly coordinated [2].

Physical barriers such as the blood–brain barrier (BBB) and the blood–retina barrier (BRB) prevent infiltration of systemic blood antigens into the eye protecting against a potential immune trigger [1]. Innate and adaptive immune cell populations are strategically positioned both dispersed throughout the ocular tissue and in organized follicles along the eye tissues (eye-associated lymphoid tissue (EALT)) [3]. This actively promotes a tolerogenic microenvironment, which is maintained by

specific programs displayed by these immune cells. For example, antigen-presenting cells (APCs) express low levels of MHCII and costimulatory molecules (CD80 and CD86), and resident T cells show low production of interferon gamma (IFNγ) and enhanced transforming growth factor (TGF) β and interleukin 10 (IL-10). An additional, highly efficient eye component is tear production by the lacrimal gland. Tears are complex fluids whose role is to lubricate the ocular surface by binding to the epithelial surface via the inner mucin layer and to cooperate in microbial containment (both pathogen and commensals) as tears contain antimicrobial peptides (AMPs) such as lysozyme, lipocalin, lactoferrin, and immunoglobulin A (IgA), which inhibit microbial adherence [4–6]. Therefore, diverse surveillance pathways collaborate in a coordinated fashion to maintain eye homeostasis.

Despite the effort displayed by the above-mentioned tolerance and defense mechanisms, the eye faces challenging situations where overwhelming or persistent insults may ultimately alter homeostasis resulting in ocular pathologies. Eye disease can arise from the complex interaction between host (genetics, immunity, age, and sex) and the environment (air pollution, device exposure, and unsupervised medication). Multifactorial origins ranging from infectious and inflammatory to autoimmune can result in complex, yet unrelated co-morbidities.

1.1 Dry eye disease

Ocular surface inflammatory diseases such as dry eye disease (DED), which is currently the most frequent reason for ophthalmologic visits is projected to be an increasing eye morbidity due to lifestyle changes such as prolonged device use [7, 8]. DED is a group of diseases characterized by a strong inflammatory response targeting the ocular surface (conjunctiva, cornea, and meibomian and lacrimal glands) [7]. The most updated classification subdivides DED into two broad types: tear-deficient (aquodeficient) and evaporative DED. In the aquodeficient DED subtype, malfunctioning lacrimal glands (LGs) are often diagnosed. Deficiency in LG function is strongly associated with an autoimmune response targeting the body's salivary and lacrimal glands (Sjögren's syndrome) [7]. In evaporative DED, a reduced or altered lipidic composition of tear film is thought to be responsible [8]. Meibomian gland dysfunction (MGD) can result in decreased lipidic production, which is associated with infectious (bacterial and parasite) and non-infectious (hormones and duct obstruction) processes [7].

Manifestations compatible with those observed in DED are widely reported worldwide, positioning DED as the most common eye disease. DED is the most common eye pathology because this disease can emerge as a primary phenotype; that is, a local immune response is generated and sustained in the ocular surface and draining lymph nodes [9]. DED is also found as a secondary phenotype, where both autoinflammatory (e.g., colitis) [10, 11] and autoimmune diseases (Sjögren's syndrome, rheumatoid arthritis, and lupus) present DED symptoms [12–14]. The relevant finding that DED onset precedes autoimmune and non-autoimmune diseases in several patients is puzzling, and it has attracted interest from researchers worldwide, but the pathways remain to be elucidated.

Regardless of the origin, immune cells and their secreted products are the driving force of DED [9, 15]; therefore, a comprehensive understanding of the immune response as an initiator and perpetuating factor in DED is an area of intense research. Our immune system is composed of organs, cells, and molecules performing in a highly coordinated fashion; although finetuned mechanisms of regulation exist, pathologies prove that these mechanisms are not always limiting the intensity of the immune response. From the many cellular components of the immune response participating in DED, lymphocytes constitute one important component,

which, when danger signals are detected, can be activated and become a disease-promoting player rather than homeostasis-maintaining cell type.

1.2 Lymphocyte diversity in the immune response

Lymphocytes are present in blood and lymph vessels and include innate and adaptive subtypes. All lymphocytes originate in the bone marrow (organ where all blood cells are created) from a common lymphoid precursor (CLP); however, not all lymphocytes reach mature status while in the bone marrow. For example, to achieve a mature T cell lineage, migration into the thymus is required, so they can complete their maturation through a complex process [16].

Classically, innate lymphocytes are represented by natural killer (NK) cells, whereas their adaptive counterparts include T and B cells. However, other lymphocytic subpopulations also exist in both innate and adaptive subtypes. The main difference between innate and adaptive lymphocytes is that the former express receptors encoded in the germline with limited diversity, such as toll-like receptors (TLRs) and carbohydrate-recognizing receptors (lectins), amongst others [17]. In contrast, adaptive lymphocytes express receptors generated by genetic recombination, which ultimately results in endless diversity. T and B lymphocytes perform gene rearrangements to express surface dimeric (two-chain, membrane-bound) T cell receptor (TCR) and B cell receptor (BCR), respectively. The TCR structure consists of alpha-beta chains, whereas the BCR structure is characterized by heavy and light chains forming a membrane-inserted immunoglobulin [18]. Thus, conventional innate lymphocytes are the NK cells and the conventional adaptive lymphocytes are the T and B cells.

An extended functional and phenotypical characterization of lymphocytes recently uncovered a growing diversity in lymphocytic subpopulations. A group of lymphocytes bearing low diversity TCRs and simultaneously expressing surface markers of NK was identified and named NKT cells [19]. Whereas most T cells express TCRs composed of classical alpha-beta chains, a less-abundant, mucosa-dwelling subtype of T cells express TCRs composed of gamma-delta chains, which is referred as γδ T cells [20]. Currently, both NKT and γδ T cells are considered unconventional T cells. More recently, other unconventional innate-like lymphocytes have been identified and these include the group 2 and group 3 innate lymphoid cells (ILC2s and ILC3s, respectively) [21] and mucosal-associated invariant T cells (MAITs), whose role has been widely explored in other mucosal surfaces (gut, skin, and lungs) [22].

B cells also have an innate-like counterpart; therefore, B cells are also subdivided into B1 and B2 cells. B1 cells mostly reside in the peritoneal and pleural cavity and produce low-affinity antibodies without stimulation (naturally produced antibodies), whereas B2 cells can produce high-affinity antibodies (affinity maturation process) and highly efficient memory responses [23].

Regarding their role within the immune response, all these lymphocytic cells contribute to a wide variety of both physiological and pathological processes. Lymphocytes residing and circulating during homeostatic conditions participate in immune surveillance; however, lymphocytes can be rapidly activated and collaborate in pathogen clearance. Furthermore, lymphocytes are involved in highly specialized functions such as the generation of memory responses, which allows increased intensity and efficiency in a secondary response. Lymphocytes additionally participate in the amplification of the immune response by rapidly releasing cytokines and chemokines (helper subpopulations) and preventing pathogen and tumor cells dissemination by elimination of infected or transformed cells (cytotoxic and killer types). Conversely, specific lymphocytes are also able to down-modulate

the intensity of the immune response, thus turning these cells into regulatory subtypes, which are central in the resolution phase of the inflammatory process.

As above-mentioned, lymphocytes are active players both as promoters and regulators of inflammatory diseases. In the case of DED, how lymphocytes might be involved in the immunopathology of this disease was only recently described, and the understanding of these lymphocyte-driven pathological pathways may pave the way for new therapeutic opportunities.

2. The role of lymphocytes in DED

2.1 NK cells

NK cells are an early source of cytokines, such as interferon (IFN)γ and tumor necrosis factor (TNF)α and display cytotoxic features that place them in the first-line defense against intracellular pathogens and tumor cells. NK cells are grouped into the innate arm of the immune response since they lack antigen-specific receptors such as those found on adaptive cells (i.e., TCR and BCR) [24]. To fulfill their primary cytolytic role, NK cells are equipped with killer-activating receptors (KARs) as well as killer-inhibitory receptors (KIRs), whose role is integrating external signals that modulate the release of perforin and granzyme-containing granules that eliminate target cells. NK cells additionally express pattern-recognition receptors (PRRs), cytokine and chemokine receptors, and antibody Fc fragment receptors, all of which also contribute to NK functions [24].

NK cells were reported to be present in human conjunctiva samples obtained by cytology, and DED patients showed similar numbers of NK cells, suggesting that NK cell populations are not increased in DED [25]. Moreover, mouse studies showed that NK cells are found in the healthy conjunctiva, and DED induction caused a rapid infiltration of NK cells into the ocular surface (cornea and conjunctiva) as well as NK cell expansion in the draining cervical lymph node (CLN) [26–28]. A dual role for NK cells in the immunopathology of DED has been described. On one hand, it was reported that NK cells progressively decrease upon DED induction, which was paralleled by lower levels of IL-13 and goblet cell loss [29]. Thus, NK cells were identified as an IL-13 source. In turn, IL-13 prevented goblet cell loss, assigning a protective role to NK cells during DED (**Table 1**). Additionally, cyclosporine A (CsA) administration preserved NK cells and down-regulated pathogenic IFNγ and IL-17 cytokines [29]. On the other hand, a pathogenic role for NK cells in acute mouse DED was suggested since NK depletion with either antibodies (anti NK1.1) [26, 28] or antiasialo rabbit serum [27] ameliorated DED signs as gauged by less corneal damage (**Table 1**). Mechanistically, when IFNγ-producing NK cells were depleted, a reduced expression of costimulatory molecules (CD80 and CD86) in antigen-presenting cells (APCs), such as macrophages and dendritic cells (DCs), was found [26]. In line with this, immune neutralization of NK cells reduced CXCL9, CXCL10, and CXCL11 chemokines and IFNγ [28]. Furthermore, NK cells were identified as a source of the highly pathogenic cytokine IL-17 [27]. Ablation of NK cells resulted in matrix metalloproteinase (MMP) 3 and MMP9 attenuation in the cornea. Therefore, NK cell depletion strongly impacts pathogenic cytokine and chemokine output as well as APC activation, suggesting that rapid NK cell activation and further cytokine secretion in turn activate APCs to contribute to DED.

Mouse studies suggested that NK cells reside in the ocular surface and, upon DED induction, a highly dynamic cytokine response in these cells is initiated. However, depending on the cytokine profile released by NK cells, a differential impact on the DED outcome is observed. Altogether this mouse evidence suggested

Cell type	Strain	DED type/ model	Role disease	Ref
NK cells	Female C57BL6 Female and male C57BL/6 and STAT6 KO mice	Scopolamine-induced DED	NK cells were identified as an abundant source of IL-6, IL-17, IL-23, and IFNγ cytokines. NK cell depletion ameliorated DED outcome, related to decreased MMPs expression in cornea and lower costimulatory molecules on APC surface. A beneficial role was attributed to resident NK in maintaining Goblet cells by secreting IL-13.	[26, 29, 30]
NKT cells	Female and male C57BL/6 and STAT6 KO mice	Scopolamine-induced DED	NKT cells released IL-13, which collaborated in preserving goblet cells in the ocular surface.	[29]
ILCs	N.D.	N.D.	N.D.	
γδ T cells	Female C57BL/6 and male NOD mice	Scopolamine-induced DED	Upon DED induction, γδ T cell numbers tend to decrease on conjunctiva, suggesting a regulatory role on DED.	[30, 31]
T helper cells	Female C57BL/6	Scopolamine-induced DED	Th1 cells were present in draining lymph nodes from DED-induced mice and contributed to the recruitment of Th17 cells and inflammatory macrophages polarization. Th1 cells secreted IFNγ, which in turn induced goblet cell apoptosis and antagonized IL-13 effect on squamous epithelial cells.	[32, 33]
	Female C57BL/6 Female and male C57BL6, IFN-γKO Female BALB/c	Scopolamine-induced DED	Conjunctiva and cornea epithelia created a Th17 response. Memory Th17 cells promoted chronic DED. Th17 cells induced VEGF, which resulted in ocular angiogenesis. Th17 cells were highly pathogenic in DED, since they promoted MMPs expression and corneal barrier disruption, and suppressed the Treg response.	[34–38]
T cytotoxic cells	Female Lewis rats Female C57BL/6	Autoimmune DED Scopolamine-induced DED	Rats induced with autoimmune DED showed damaged lacrimal and salivary acinar cells, accompanied by a massive T cell infiltration, where $CD8^+$ T cells dominated over $CD4^+$ T cells. A pathogenic role for $CD8^+$ T cells was postulated. Upon acute DED induction, numbers of $CD8^+$ T cells were reduced in both conjunctival epithelium and stroma, and regulatory CD8+ T cells were described in DED.	[39–41]
Treg cells	Female BALB/c mice Female C57BL/6	Scopolamine-induced DED T cell transfer mediated-DED	$CD4^+CD25^+Foxp3^+$ T regs preserved tear production and reduced mononuclear cell and neutrophil infiltration when transferred into nude mice induced with Sjögren's syndrome-like disease. Treg cells from DED mice exhibited defective suppressive ability rather than decreased numbers.	[36, 39]

Cell type	Strain	DED type/ model	Role disease	Ref
B cells	Female and male C57BL/6	Scopolamine-induced DED	Plasma cells release IgG antibodies targeting components from the lacrimal gland (Kallikrein 13). When purified, these anti-kallikrein 13 antibodies induced DED signs. B cells could be activated more efficiently by IL-17 than by IFNγ. B cells infiltrated lacrimal glands in age-related DED.	[42–44]

Table 1.
The diverse roles of lymphocytes in rodent DED.

a dual role of NK cells in DED; a switch from normal protective IL-13-mediated to a pathogenic IFNγ- and IL-17-mediated role has been proposed for the eye-resident NK population [27]. What triggers either a protective or pathogenic program in NK cells and the role of NK cells in human DED remains to be determined.

2.2 NKT cells

NKT cells are a subgroup of innate-like lymphocytes that recognize lipid antigens presented by MHC class-I-like molecule (CD1d) and are identified by the co-expression of TCR and the NK-related NK1.1 marker. NKT cells are further divided into type I NKT or invariant NKT cells (iNKT) and type II NKT cells. Type I NKT cells express a semi-invariant TCR, whereas type II NKT cells possess a more diverse TCR repertoire. Upon NKT activation by either lipid antigens or bacterial products sensed by TLRs, these cells rapidly secrete large amounts of cytokines, such as IFN-γ, TNF-α, IL-2, IL-4, IL-5, and IL-13, which modulate the function of neighbouring innate and adaptive cells. In organs such as the liver, NKT cells are abundant and the main players of the local response; however, in the eye, NKT cells are shown to be a relevant cell type [45].

Pioneer studies identified NKT cells as the resident population in the mouse ocular surface. Cells isolated through immunobeads from the mouse ocular surface under homeostatic conditions were identified using reliable NKT markers (TCR and NK1.1). Moreover, these cells were found to be an important source of IL-13; through the secretion of this molecule, NKT cells help to preserve the goblet cells, which promote ocular surface stability by producing mucins (**Table 1**) [29]. The same group of researchers subsequently confirmed their findings by identifying cells positively stained for CD3 and NK1.1 markers, a phenotype compatible with NKT cells in conjunctiva samples from healthy mice [30]. Interestingly, these authors reported that the actual number of NKT cells was higher than the number of conventional T cells (CD4 and CD8 lymphocytes), suggesting that, like in other epithelial tissues, NKT cells are abundant in the ocular surface [30].

Therefore, the evidence, although minimal but convincing, shows that NKT cells are fundamental for maintenance of the ocular surface through communication with goblet cells. Impaired crosstalk between these cells adds to the development of DED, so a protective role can therefore be inferred for NKT cells in DED.

2.3 γδ T cells

T lymphocytes bearing a TCR composed of gamma-delta chains (γδ T cells) are less abundant than αβ T cells; however, γδ T cells represent a major T cell population in the epithelial tissues such as the skin, and gastrointestinal and reproductive

tracts. As part of the intraepithelial lymphocytes (IELs), γδ T cells are central players in the protection and homeostasis of surfaces in constant contact with the external environment. Specifically in the eye, γδ T cells collaborate in maintaining ocular immune privilege [46].

Early evidence arose from studies on the non-diabetic obese (NOD) mouse strain, which, upon ageing, develop a Sjögren's-syndrome-like disease. When DED was induced in NOD mice via scopolamine delivery, symptoms such as a decreased tear volume and goblet cell density as well as increased corneal permeability were observed (**Table 1**). The authors noted a significant decline in the numbers of γδ T cells in the conjunctival epithelium during the acute phase of DED [31]. Intriguingly, using the same DED model (scopolamine administration) in a different mouse strain (C57BL/6), decreased numbers of γδ T cells present in the conjunctival epithelium were observed when DED was induced, but increased γδ T cells were visible in flow cytometry samples [30]. Moreover, a strain-dependent effect of γδ T cells on tear volume was found where C57Bl/10 J (B10) mice lacking γδ T cells presented higher tear volume compared with C57BL/6 J (B6) similarly lacking γδ T cells [47].

Regarding human DED, the role of γδ T cells has not yet been explored; however, Sjögren's syndrome patients are reported to present altered numbers of γδ T cells. One may speculate that as DED is frequently observed in Sjögren's syndrome patients, in human DED secondary to autoimmune disease, a modified γδ T cell response is expected.

Salient evidence suggests that the depletion of γδ T cells is a hallmark of experimental DED, supporting an immunoregulatory role of the γδ T cells despite them being a well-known source of pathogenic IL-17 (**Table 1**). This regulatory role is further supported by mice lacking γδ T cells not developing anterior-chamber-associated immune deviation (ACAID), and corneal grafts are more tolerated when γδ T cells are present [48]. An anti-inflammatory role for γδ T cells in DED is currently accepted.

2.4 T helper cells

T cells and B cells compose the lymphocytes of the adaptive immune system. T cells are further subdivided into T helper (Th) and T cytotoxic (Tc) groups, identified as $CD4^+$ and $CD8^+$, respectively. $CD4^+$ Th cells are abundant lymphocytes with the primary role of secreting cytokines to amplify the immune response by promoting crosstalk amongst cells. Once activated, Th cells proliferate and polarize, which means they selectively secrete specific groups of cytokines and chemokines. Currently, effector Th cells are grouped based on the cytokines released as follows: Th1 cells secrete IFNγ; Th2 cells secrete IL-4, IL-5, and IL-13; Th17 cells secrete IL-17; and Th22 cells secrete IL-22. Compared with effector Th cells, regulatory Th cells produce IL-10 and are termed type 1 T regulatory cells (Tr1). As Th cells have been shown to be highly pathogenic and contribute to inflammatory disease, specifically Th1 and Th17 cells, these subpopulations are under intense research in DED.

$CD4^+$ and $CD8^+$ T cells are less abundant on the healthy ocular surface compared to γδ T cells and it has been noted that $CD4^+$ T cells outnumber $CD8^+$ T cells. Induction of DED in rodents and in patient with DED, T cells are found to consistently infiltrate the ocular surface. In early mouse studies of DED, upon desiccant-stress-induced DED, increasing numbers of CD4+ T cells were observed in the cornea, conjunctiva, and lacrimal gland tissues [39] (**Table 1**). It was demonstrated that transferring these cells is enough to induce DED in mice lacking T and B cells, showing that $CD4^+$ T cells are largely responsible for inducing DED (**Table 1**) [39]. Additional reports also provided evidence of $CD4^+$ T cells driving autoimmune DED in autoimmune regulator-knockout (Aire KO) mice [49] and being present in

lacrimal glands in a novel autoimmune model of DED in rats [40]. This same population was found to be expanded within draining lymph nodes from DED-induced mice [32]. Similarly, human studies showed that DED patients with an autoimmune origin (Sjögren's syndrome) and with a non-autoimmune origin (non-Sjögren) presented comparable numbers of $CD4^+$ T cells assessed per immunohistochemistry using conjunctival samples [50].

Research has also focused on identifying specific subtypes of $CD4^+$ T cells involved in DED, where Th1 and Th17 cells have received the most attention since DED induction was found to cause increased transcripts of both IFNγ and IL-17 in the ocular surface [34]. Expansion of IFNγ-secreting $CD4^+$ T cells co-expressing CXCR3 and CCR5 chemokine receptors (Th1 polarized T cells) in the regional lymph nodes (submandibular and cervical) has been reported [32, 51]. Interestingly, IFNγ may be highly relevant at the onset of DED, but its role during chronic DED may be limited [49]. The relevance of Th1 in DED immunopathology can be inferred due to the detrimental effect of IFNγ on the ocular surface, since the presence of IFNγ receptor was demonstrated on the conjunctival and corneal epithelium [33]. Additionally, IFNγ is amongst the cytokines elevated in tears from DED patients and was shown to alter mucin secretion by inducing cell death in conjunctiva-residing goblet cells [52]. A diminished density of goblet cells resulting from IFNγ administration was also reported [41]. IFNγ was also found to be responsible for inducing apoptosis in lacrimal gland cells [53].

Once polarized and activated, Th2 cells secrete IL-4, IL-5, and IL-13 cytokines. Even though DED is a Th1-prone inflammatory condition, it has been reported that tear samples from DED patients contain elevated levels of IL-4, IL-5, and IL-13, suggesting activation of the Th2 pathway [54–56]. Intriguingly, Th2 cytokines were detected elevated in tears from DED-induced experimental animals [57]; however, the contribution of the cytokines to mouse DED appears to be strain-dependent, since DED induction caused different cytokine and chemokine profiles in C57BL/6 compared with BALB/c mice [58]. Although IL-13 has been shown to prevent goblet cell loss, innate lymphocytes (NK and NKT cells) were demonstrated to be the cellular source; thus, we can speculate that Th2 cells collaborate in preserving mucin-producing cells as well. However, this remains to be proven.

In terms of Th17 cells, DED induction creates a Th17-inducing microenvironment as gauged by a rapid increase in IL-6 and IL-23 expression. Th17 cells have been readily detected in draining lymph nodes from acute and chronic DED-induced mice [34, 35], and their pathogenic role is supported by experimental evidence showing that IL-17 neutralization attenuated corneal damage. Furthermore, Th17 cells emerging in experimental DED were shown to be resistant to suppression exerted by T regulatory cells and, unlike Th1 cells, Th17 cells survived longer periods of time, adding to chronic DED [36]. A wide variety of negative effects on the ocular surface have been attributed to Th17 cells and IL-17 including promotion of MMPs expression, corneal barrier damage, and induction of angiogenesis via vascular endothelial growth factor (VEGF) (**Table 1**) [37].

Another group of $CD4^+$ T cells addressed in the context of DED is the T regulatory (Treg) subtype. Treg cells are identified by the expression of the transcription factor Foxp3 and high levels of CD25 and can suppress cell proliferation. Niederkorn et al. demonstrated that the presence of $Foxp3^+$ Tregs prevented DED symptoms induced by the adoptive transfer of ocular-surface-specific T effector cells (**Table 1**) [39]. Thereafter, it was shown that a percentage of lymph node residing $Foxp3^+$ Tregs remains unaltered upon DED induction but their suppressive ability is reduced compared with their counterparts from non-DED animals, when tested *in vitro* [36]. Recently, administration of histone deacetylase inhibitors (HDACi)-containing microspheres that stabilized $Foxp3^+$ expression reduced DED signs [59].

Several $CD4^+$ T subtypes have been identified in DED, and the roles of Th1, Th17 and Tregs have been highlighted. A well-established pathogenic role of Th1 and Th17 in inducing ocular surface damage through the release of IFNγ and IL-17, respectively, has been assigned. In sharp contrast, Treg cells are responsible for restraining exacerbated inflammatory responses in the ocular surface. Therefore, the balance between these $CD4^+$ T cells subpopulations seems to be determinant for the onset and chronicity of DED.

2.5 T cytotoxic cells

Although T cytotoxic cells ($CD8^+$ T cells) also contribute by producing cytokines such as IFNγ, they are best known for their cytolytic functions. $CD8^+$ T cells contain granules with perforin and granzyme, which are delivered to the target cells with the goal of inducing cell death via membrane damage and cellular content release. A few studies have addressed the role of $CD8^+$ T cells in DED, and the evidence is controversial. It was suggested that $CD8^+$ T cells might play a regulatory role, since a significant loss of $CD8^+$ T cells in the conjunctiva was found to accompany the development of DED [30] and DED patients showed increased CD4/CD8 compared with healthy donors (**Table 2**) [25]. Furthermore, thrombospondin 1 (TSP1)-deficient mice exhibited DED symptoms accompanied by dramatic lacrimal gland cell infiltration, where $CD4^+$ T cells were significantly increased; $CD8^+$ T cells were not increased compared with those in mice expressing TSP1 [65]. Conversely, $CD8^+$ T cells were the dominant cell type in severely damaged ducts within lacrimal glands of rats induced with an autoimmune DED model [40]. In line with this,

Cell type	DED type	Role in Disease	Ref
NK cells	Non SS	A putative limited role was assumed since NK numbers were not different between healthy controls and DED patients	[25]
NKT cells	N.D.	N.D.	
ILCs	N.D.	N.D.	
γδ T cells	N.D.	N.D.	
T helper cells	SS and Non SS	Highly infiltrated in lacrimal glands from SS patients. Increase in $CD4^+$ T cells numbers correlated with dryness, hyperemia, and itching score. Th cells secreted IL-21, which in turn favors B cell transition to plasma cells. Different T cell subpopulations were associated with differential DED signs, for instance, $CD4^+CCR7^+CD45RO^-CD45RA^+$ correlated with hyperemia, whereas patients with $CD4^+CCR7^-CD45RO^+CD45RA^-CD69^-CD103^-$ cells showed reduced tear film break up time (BUT). Memory T cells ($CD4^+CD45RA^-$) correlated with corneal damage and serum Ro antibodies.	[60–64]
T cytotoxic cells	SS and Non SS	The presence of Tc cells with the phenotype $CD8^+CCR7^+CD45RO^-CD45RA^+$ positively correlated with hyperemia. Conversely, $CD8^+CCR7^-CD45RO$ and $CD45RA^+CD69^+CD103^+$ were abundant when patients largely exhibited reduced tear film	[63]
B cells	SS untreated	Produced antinuclear antibody (ANA)	[62]

SS; Sjögren's Syndrome, N.D.; not documented.

Table 2.
The role of lymphocytes in human DED.

using a mouse autoimmune model of Sjögren's syndrome, lacrimal glands presented a massive infiltration of $CD8^+$ T cells [53], and aged mice displaying ocular surface pathology (corneal irregularity and conjunctival goblet cell loss) presented increased numbers of $CD8^+$ T cells [42].

Therefore, additional studies are needed to better understand the role of $CD8^+$ T cells during DED development. In the current literature, most of the mouse studies investigate immunological changes during the acute stage of disease; however, it is well-known that DED is a chronic disease. Thus, the role of resident cells might contribute to the infiltration of additional pathogenic populations responsible for perpetuating the inflammatory process. A differential role of $CD8^+$ T cells occurring during early versus late stages of disease cannot be ruled out.

2.6 B cells

B lymphocytes complement T cells in adaptive immunity and in generating immune memory responses. B cells perform a variety of functions in homeostatic conditions and following the initiation of an adaptive immune response. These functions include cytokine release, antigen processing and presentation, and their signature role as antibody-producing cells. In mucosal surface immunosurveillance, the presence of IgA is pivotal in limiting pathogen invasion. In the EALT, B cells are present in both diffuse and organized (follicles) forms to support their function. B cells are essential in eye-associated immune responses ranging from surveillance to autoimmune-mediated diseases and allergies.

As mentioned above, human DED can arise from autoimmune diseases (Sjögren's syndrome and rheumatic) and DED was proposed to be a mucosal autoimmune disease [66]. Despite being proposed as an autoimmune disease, the role of B cells in both human and mouse DED has not been completely addressed.

In terms of studies in patients with Sjögren's syndrome, autoimmune-response-targeting exocrine glands (salivary and lacrimal) are the driving force of the disease; however, human studies mostly focused on the salivary glands rather than lacrimal glands and the ocular surface. Information learned from patients with Sjögren's syndrome linking B cell subpopulations and eye manifestations is still lacking. More thorough research on B cells and their participation during mouse DED must be conducted.

Thus far, the role of B cells in mouse models of DED may be model-dependent. When DED was induced via pharmacological inhibition of the lacrimal gland function (desiccant stress), no significant changes were observed regarding the percentage of B cells present in the tissue (**Table 1**) [30]. Likewise, the NOD autoimmune model of DED was attenuated by blocking high-mobility group box 1 (HMGB1) with neutralizing antibodies; however, no substantial changes in either the percent of B cells or in IL-10-producing B cells were found [67]. Conversely, when DED symptoms were evaluated in aged mice, without any additional chemical agent, B cell numbers were found to be increased accompanying DED development [42]. A pathogenic role of B cells in DED is supported by the findings showing that a DED-like disease can be generated by transferring antibody-containing serum (purified IgG isotype) obtained from mice previously induced with DED for three weeks [43]. The transfer of antibodies required the presence of complement proteins to cause ocular surface damage [43], suggesting that in eye tissue exposed to desiccant stress, antibodies targeting lacrimal gland components like kallikerin 13 are induced (**Table 1**). Additionally, IL-17 collaborates in B cell proliferation and plasma cells generation [44].

Recent findings show that B cells are instrumental in DED, either human or mouse; however, the only mechanism through which these B cells induce eye damage is proposed to be by secreting antibodies targeting lacrimal gland antigens.

To prove that additional pathways are also regulated by B cells resulting in Sjögren-associated and non-Sjögren-related DED, future studies are required.

3. The future players in DED

Both novel techniques (single cell sequencing and massive flow cytometry) and the discovery of novel functions have allowed the better characterization of immune cell populations, revealing a wider diversity of lymphocyte subpopulations than previously thought. As an example, mucosal-residing ILCs have emerged as central early regulators in the immune response. In the case of inflammatory diseases, including DED, lymphocyte populations have specifically received more attention than others, which does not imply that the populations that we do not yet understand are less important. Notably, MAITs and ILCs are only beginning to arise as potential drivers in eye pathologies. Few studies have, for instance, shown that extremely low numbers of ILC2s reside in the mouse cornea and are recruited upon corneal epithelial injury, where they are required for cornea tissue repair [68]. Additional studies described that cells, presumably ILCs, can be isolated from human and mouse conjunctiva [69]. In eye pathologies, MAITs were reported to be increased in acute anterior uveitis [70] and ILCs played no role in ocular infection with herpes simplex virus (HSV)2 [71]. Thus, a role for MAITs and ILCs cannot be ruled out in DED, and future evidence will further our understanding of the expanding universe of lymphocytes in DED.

4. Targeting lymphocytes as therapy for DED

The findings summarized here strongly indicate that resident lymphocytes can rapidly be activated when the microenvironment in the ocular surface is altered by the lack of tears (aquodeficient) or have altered composition (evaporative). Evidence now suggests that innate and adaptive lymphocytes regulate the onset and persistence of DED. For instance, NK are shown to switch their cytokine response, which is critical for initiating DED, whereas other innate lymphocytes such as γδ T cells and NKT cells are mostly suppressive in homeostasis, and DED may parallel the loss of these populations. Regarding the adaptive lymphocytes, T and B populations are responsible for promoting chronicity. The important role of lymphocytes during DED is also supported using diverse therapeutics aimed to attenuate lymphocyte activation; thus, controlling lymphocyte populations has long been considered an efficient therapy for DED. These strategies include diverse methods of controlling T cells response; for instance, cyclosporine A (CsA) eyedrops targeted cell proliferation [72, 73]. It has been reported that commercially available CsA formulations such as Restasis (0.05% CsA, Allergan) and Ikervis (0.1% CsA, Santen) are highly effective in treating DED, however, side effects which are thought to be vehicle-related, have been reported [74, 75]. Therefore, improving CsA delivery is the current challenge. Recently, results from phase II and phase III clinical trials have been released. Wirta *et al.* published their results from a USA-centered phase II trial (efficacy, safety and tolerability) using a water- and oil-free emulsion containing a 0.1% CsA dose termed CyclASol for 16 weeks that resulted in earlier and more effective relief in adult DED patients compared to Restasis (Allergan) administered under the same protocol [76]. In an independent study, CsA was encapsulated in nanomicelles to enhance its effectiveness. The authors hypothesized that given the hydrophobic nature of CsA that dampens aqueous solubility encapsulating CsA in nanomicelles would help to solubilize CsA and ultimately increase its efficacy.

The 0.09% CsA nanomicellar solution, termed OTX-101, was administered for 84 days in individuals (18–90 years old) and significantly increased integrity of the ocular surface [77]. Thus, CsA still remains as one of the most recommended DED therapies and these efforts to enhance its effects by using novel ways of delivery with promising results keep expectations high to achieve complete DED remission.

Additional DED therapies targeting lymphocytes include eyedrops containing anti-CD4 antibodies suppressing cell activation [78] and more recently, blocking T cell infiltration by antagonizing LFA1 (Lifitegrast) [79]. Therapies increasing regulatory lymphocytes are another method of ameliorating DED. Rebamipide, which promotes the expansion of regulatory adaptive lymphocytes, yielded promising results in autoimmune DED [80]. More sophisticated agents such as histone deacetylase inhibitor (HDACi)-containing microspheres aimed at stabilizing regulatory T cells have also shown beneficial effects on DED symptoms [59].

Thus, currently approved drugs as well as experimental evidence (NK depletion) show that regulating both innate and adaptive lymphocytes can be a complimentary therapy for strategies to restore healthy tears. The more we understand about how lymphocytes participate in DED, the greater the possibilities of mitigating DED.

5. Conclusion

There has been a tremendous breakthrough concerning DED research, from previously being considered only as a syndrome to what is now recognized as the most common eye pathology. Experimental models have been instrumental for the better understanding of DED immunopathology; unfortunately, human studies are underrepresented. Convincing evidence obtained mostly from animal studies shows that lymphocytes have important implications in DED, placing Th1, Th17, and B cells as the main pathological subtypes, which seem to be in constant competition with immune cell populations mostly displaying regulatory features such as Tregs that are ultimately responsible for lessening the intensity of disease-promoting counterparts. Contrary to the extensive work that has been done describing how adaptive cells are active players in both promoting and regulating DED, evidence is just starting to uncover surprising roles attributed to innate and innate-like lymphocytes. As we have reviewed here, strong evidence, up to now, suggests possible "program switching" in resident innate cell populations like NKs whereas other cells such as NKT and T cells rather display a regulatory role contributing to a tolerogenic microenvironment on the ocular surface. More recently, cell populations like ILCs have been described expanding upon eye tissues injury, which paves the way to uncover novel roles for these ILCs in a variety of eye pathologies, similarly to other organs. Continued research will help to clarify how these populations contribute to DED immunopathology. It is evident that additional human studies would complement and validate these findings, with the ultimate goal of identifying new therapeutic targets based on modulating lymphocyte responses. We have also shown that some of the most effective DED treatments indeed target lymphocyte populations (cyclosporine A and LFA-1 inhibitors) and current trials are aimed to develop a more efficacious way to deliver these drugs with proved benefit in DED therapy.

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References

[1] Kels, B.D., A. Grzybowski, and J.M. Grant-Kels, *Human ocular anatomy.* Clin Dermatol, 2015. **33**(2): p. 140-6.

[2] Knop, E. and N. Knop, *Anatomy and immunology of the ocular surface.* Chem Immunol Allergy, 2007. **92**: p. 36-49.

[3] Knop, E. and N. Knop, *[Eye-associated lymphoid tissue (EALT) is continuously spread throughout the ocular surface from the lacrimal gland to the lacrimal drainage system].* Ophthalmologe, 2003. **100**(11): p. 929-42.

[4] Tiffany, J.M., *The normal tear film.* Dev Ophthalmol, 2008. **41**: p. 1-20.

[5] Zhou, L., et al., *In-depth analysis of the human tear proteome.* J Proteomics, 2012. **75**(13): p. 3877-85.

[6] Knop, E., N. Knop, and P. Claus, *Local production of secretory IgA in the eye-associated lymphoid tissue (EALT) of the normal human ocular surface.* Invest Ophthalmol Vis Sci, 2008. **49**(6): p. 2322-9.

[7] Willcox, M.D.P., et al., *TFOS DEWS II Tear Film Report.* Ocul Surf, 2017. **15**(3): p. 366-403.

[8] Johnson, M.E. and P.J. Murphy, *Changes in the tear film and ocular surface from dry eye syndrome.* Prog Retin Eye Res, 2004. **23**(4): p. 449-74.

[9] Knop, N. and E. Knop, *Regulation of the inflammatory component in chronic dry eye disease by the eye-associated lymphoid tissue (EALT).* Dev Ophthalmol, 2010. **45**: p. 23-39.

[10] Cury, D.B. and A.C. Moss, *Ocular manifestations in a community-based cohort of patients with inflammatory bowel disease.* Inflamm Bowel Dis, 2010. **16**(8): p. 1393-6.

[11] Czompa, L., et al., *Corneal Manifestations of Inflammatory Bowel Disease.* Semin Ophthalmol, 2019. **34**(7-8): p. 543-550.

[12] Vehof, J., et al., *Advances, limitations and future perspectives in the diagnosis and management of dry eye in Sjogren's syndrome.* Clin Exp Rheumatol, 2020. **38 Suppl 126**(4): p. 301-309.

[13] Kemeny-Beke, A. and P. Szodoray, *Ocular manifestations of rheumatic diseases.* Int Ophthalmol, 2020. **40**(2): p. 503-510.

[14] Read, R.W., *Clinical mini-review: systemic lupus erythematosus and the eye.* Ocul Immunol Inflamm, 2004. **12**(2): p. 87-99.

[15] Clayton, J.A., *Dry Eye.* N Engl J Med, 2018. **378**(23): p. 2212-2223.

[16] Koch, U. and F. Radtke, *Mechanisms of T cell development and transformation.* Annu Rev Cell Dev Biol, 2011. **27**: p. 539-62.

[17] Janeway, C.A., Jr. and R. Medzhitov, *Innate immune recognition.* Annu Rev Immunol, 2002. **20**: p. 197-216.

[18] Nielsen, S.C.A. and S.D. Boyd, *Human adaptive immune receptor repertoire analysis-Past, present, and future.* Immunol Rev, 2018. **284**(1): p. 9-23.

[19] Makino, Y., et al., *Predominant expression of invariant V alpha 14+ TCR alpha chain in NK1.1+ T cell populations.* Int Immunol, 1995. **7**(7): p. 1157-61.

[20] Nielsen, M.M., D.A. Witherden, and W.L. Havran, *gammadelta T cells in homeostasis and host defence of epithelial barrier tissues.* Nat Rev Immunol, 2017. **17**(12): p. 733-745.

[21] Vivier, E., et al., *Innate Lymphoid Cells: 10 Years On.* Cell, 2018. **174**(5): p. 1054-1066.

[22] Pellicci, D.G., H.F. Koay, and S.P. Berzins, *Thymic development of unconventional T cells: how NKT cells, MAIT cells and gammadelta T cells emerge.* Nat Rev Immunol, 2020. **20**(12): p. 756-770.

[23] Wang, Y., et al., *B Cell Development and Maturation.* Adv Exp Med Biol, 2020. **1254**: p. 1-22.

[24] Sivori, S., et al., *Human NK cells: surface receptors, inhibitory checkpoints, and translational applications.* Cell Mol Immunol, 2019. **16**(5): p. 430-441.

[25] Barabino, S., et al., *Immune response in the conjunctival epithelium of patients with dry eye.* Exp Eye Res, 2010. **91**(4): p. 524-9.

[26] Chen, Y., et al., *Interferon-gamma-secreting NK cells promote induction of dry eye disease.* J Leukoc Biol, 2011. **89**(6): p. 965-72.

[27] Ren, G., et al., *Association of killer cell immunoglobulin-like receptor and human leukocyte antigen-C genotype with dry eye disease in a Chinese Han population.* Genet Test Mol Biomarkers, 2012. **16**(8): p. 910-4.

[28] Coursey, T.G., et al., *Desiccating stress-induced chemokine expression in the epithelium is dependent on upregulation of NKG2D/RAE-1 and release of IFN-gamma in experimental dry eye.* J Immunol, 2014. **193**(10): p. 5264-72.

[29] De Paiva, C.S., et al., *Homeostatic control of conjunctival mucosal goblet cells by NKT-derived IL-13.* Mucosal Immunol, 2011. **4**(4): p. 397-408.

[30] Zhang, X., et al., *NK cells promote Th-17 mediated corneal barrier disruption in dry eye.* PLoS One, 2012. **7**(5): p. e36822.

[31] Yoon, K.C., et al., *Desiccating environmental stress exacerbates autoimmune lacrimal keratoconjunctivitis in non-obese diabetic mice.* J Autoimmun, 2008. **30**(4): p. 212-21.

[32] El Annan, J., et al., *Characterization of effector T cells in dry eye disease.* Invest Ophthalmol Vis Sci, 2009. **50**(8): p. 3802-7.

[33] Pflugfelder, S.C., R.M. Corrales, and C.S. de Paiva, *T helper cytokines in dry eye disease.* Exp Eye Res, 2013. **117**: p. 118-25.

[34] Chen, Y., et al., *Chronic dry eye disease is principally mediated by effector memory Th17 cells.* Mucosal Immunol, 2014. **7**(1): p. 38-45.

[35] Chauhan, S.K. and *R. dana*, *Role of Th17 cells in the immunopathogenesis of dry eye disease.* Mucosal Immunol, 2009. **2**(4): p. 375-6.

[36] Chauhan, S.K., et al., *Autoimmunity in dry eye is due to resistance of Th17 to Treg suppression.* J Immunol, 2009. **182**(3): p. 1247-52.

[37] Chauhan, S.K., et al., *A novel pro-lymphangiogenic function for Th17/IL-17.* Blood, 2011. **118**(17): p. 4630-4.

[38] De Paiva, C.S., et al., *IL-17 disrupts corneal barrier following desiccating stress.* Mucosal Immunol, 2009. **2**(3): p. 243-53.

[39] Niederkorn, J.Y., et al., *Desiccating stress induces T cell-mediated Sjogren's Syndrome-like lacrimal keratoconjunctivitis.* J Immunol, 2006. **176**(7): p. 3950-7.

[40] Jiang, G., et al., *A new model of experimental autoimmune keratoconjunctivitis sicca (KCS) induced in Lewis rat by the autoantigen Klk1b22.*

Invest Ophthalmol Vis Sci, 2009. **50**(5): p. 2245-54.

[41] De Paiva, C.S., et al., *Dry eye-induced conjunctival epithelial squamous metaplasia is modulated by interferon-gamma.* Invest Ophthalmol Vis Sci, 2007. **48**(6): p. 2553-60.

[42] McClellan, A.J., et al., *Ocular surface disease and dacryoadenitis in aging C57BL/6 mice.* Am J Pathol, 2014. **184**(3): p. 631-43.

[43] Stern, M.E., et al., *Autoantibodies contribute to the immunopathogenesis of experimental dry eye disease.* Invest Ophthalmol Vis Sci, 2012. **53**(4): p. 2062-75.

[44] Subbarayal, B., et al., *IL-17 Augments B Cell Activation in Ocular Surface Autoimmunity.* J Immunol, 2016. **197**(9): p. 3464-3470.

[45] Zhu, S., H. Zhang, and L. Bai, *NKT cells in liver diseases.* Front Med, 2018. **12**(3): p. 249-261.

[46] Skelsey, M.E., J. Mellon, and J.Y. Niederkorn, *Gamma delta T cells are needed for ocular immune privilege and corneal graft survival.* J Immunol, 2001. **166**(7): p. 4327-33.

[47] O'Brien, R.L., et al., *alphabeta TCR(+) T cells, but not B cells, promote autoimmune keratitis in b10 mice lacking gammadelta T cells.* Invest Ophthalmol Vis Sci, 2012. **53**(1): p. 301-8.

[48] Xu, Y. and J.A. Kapp, *gammadelta T cells are critical for the induction of anterior chamber-associated immune deviation.* Immunology, 2001. **104**(2): p. 142-8.

[49] Chen, Y.T., et al., *Pax6 downregulation mediates abnormal lineage commitment of the ocular surface epithelium in aqueous-deficient dry eye disease.* PLoS One, 2013. **8**(10): p. e77286.

[50] Stern, M.E., et al., *Conjunctival T-cell subpopulations in Sjogren's and non-Sjogren's patients with dry eye.* Invest Ophthalmol Vis Sci, 2002. **43**(8): p. 2609-14.

[51] Yoon, K.C., et al., *Expression of CXCL9, -10, -11, and CXCR3 in the tear film and ocular surface of patients with dry eye syndrome.* Invest Ophthalmol Vis Sci, 2010. **51**(2): p. 643-50.

[52] Zhang, X., et al., *Interferon-gamma exacerbates dry eye-induced apoptosis in conjunctiva through dual apoptotic pathways.* Invest Ophthalmol Vis Sci, 2011. **52**(9): p. 6279-85.

[53] Bian, F., et al., *Altered balance of interleukin-13/interferon-gamma contributes to lacrimal gland destruction and secretory dysfunction in CD25 knockout model of Sjogren's syndrome.* Arthritis Res Ther, 2015. **17**: p. 53.

[54] Enriquez-de-Salamanca, A., et al., *Tear cytokine and chemokine analysis and clinical correlations in evaporative-type dry eye disease.* Mol Vis, 2010. **16**: p. 862-73.

[55] LaFrance, M.W., L.E. Kehinde, and R.J. Fullard, *Multiple cytokine analysis in human tears: an optimized procedure for cytometric bead-based assay.* Curr Eye Res, 2008. **33**(7): p. 525-44.

[56] Lam, H., et al., *Tear cytokine profiles in dysfunctional tear syndrome.* Am J Ophthalmol, 2009. **147**(2): p. 198-205 e1.

[57] Corrales, R.M., et al., *Strain-related cytokine profiles on the murine ocular surface in response to desiccating stress.* Cornea, 2007. **26**(5): p. 579-84.

[58] Yoon, K.C., et al., *Expression of Th-1 chemokines and chemokine receptors on the ocular surface of C57BL/6 mice: effects of desiccating stress.* Invest Ophthalmol Vis Sci, 2007. **48**(6): p. 2561-9.

[59] Ratay, M.L., et al., *Controlled release of an HDAC inhibitor for reduction of*

inflammation in dry eye disease. Acta Biomater, 2018. **71**: p. 261-270.

[60] Reinoso, R., et al., *Differential cell proliferation, apoptosis, and immune response in healthy and evaporative-type dry eye conjunctival epithelia.* Invest Ophthalmol Vis Sci, 2011. **52**(7): p. 4819-28.

[61] Choi, W., et al., *Expression of CCR5 and its ligands CCL3, −4, and −5 in the tear film and ocular surface of patients with dry eye disease.* Curr Eye Res, 2012. **37**(1): p. 12-7.

[62] Jin, L., et al., *CD4+CXCR5+ follicular helper T cells in salivary gland promote B cells maturation in patients with primary Sjogren's syndrome.* Int J Clin Exp Pathol, 2014. **7**(5): p. 1988-96.

[63] Bose, T., et al., *Tissue resident memory T cells in the human conjunctiva and immune signatures in human dry eye disease.* Sci Rep, 2017. **7**: p. 45312.

[64] Joachims, M.L., et al., *Sjogren's Syndrome Minor Salivary Gland CD4(+) Memory T Cells Associate with Glandular Disease Features and have a Germinal Center T Follicular Helper Transcriptional Profile.* J Clin Med, 2020. **9**(7).

[65] Turpie, B., et al., *Sjogren's syndrome-like ocular surface disease in thrombospondin-1 deficient mice.* Am J Pathol, 2009. **175**(3): p. 1136-47.

[66] Stern, M.E., C.S. Schaumburg, and S.C. Pflugfelder, *Dry eye as a mucosal autoimmune disease.* Int Rev Immunol, 2013. **32**(1): p. 19-41.

[67] Kim, K.H., et al., *Effects of subconjunctival administration of anti-high mobility group box 1 on dry eye in a mouse model of Sjgren's syndrome.* PLoS One, 2017. **12**(8): p. e0183678.

[68] Liu, J., et al., *Local Group 2 Innate Lymphoid Cells Promote Corneal Regeneration after Epithelial Abrasion.* Am J Pathol, 2017. **187**(6): p. 1313-1326.

[69] Yoon, C.H., et al., *Distribution of Interleukin-22-secreting Immune Cells in Conjunctival Associated Lymphoid Tissue.* Korean J Ophthalmol, 2018. **32**(2): p. 147-153.

[70] Huang, J.C., et al., *Preliminary Report on Interleukin-22, GM-CSF, and IL-17F in the Pathogenesis of Acute Anterior Uveitis.* Ocul Immunol Inflamm, 2019: p. 1-8.

[71] Hirose, S., et al., *Roles of Type 1, 2, and 3 Innate Lymphoid Cells in Herpes Simplex Virus 1 Infection In Vitro and In Vivo.* J Virol, 2019. **93**(13).

[72] de Paiva, C.S., et al., *Topical cyclosporine A therapy for dry eye syndrome.* Cochrane Database Syst Rev, 2019. **9**: p. CD010051.

[73] Kunert, K.S., et al., *Analysis of topical cyclosporine treatment of patients with dry eye syndrome: effect on conjunctival lymphocytes.* Arch Ophthalmol, 2000. **118**(11): p. 1489-96.

[74] Sall, K., et al., *Two multicenter, randomized studies of the efficacy and safety of cyclosporine ophthalmic emulsion in moderate to severe dry eye disease. CsA Phase 3 Study Group.* Ophthalmology, 2000. **107**(4): p. 631-9.

[75] Leonardi, A., et al., *Efficacy and safety of 0.1% ciclosporin A cationic emulsion in dry eye disease: a pooled analysis of two double-masked, randomised, vehicle-controlled phase III clinical studies.* Br J Ophthalmol, 2019. **103**(1): p. 125-131.

[76] Wirta, D.L., et al., *A Clinical Phase II Study to Assess Efficacy, Safety, and Tolerability of Waterfree Cyclosporine Formulation for Treatment of Dry Eye Disease.* Ophthalmology, 2019. **126**(6): p. 792-800.

[77] Goldberg, D.F., et al., *A Phase 3, Randomized, Double-Masked Study of OTX-101 Ophthalmic Solution 0.09% in the Treatment of Dry Eye Disease.* Ophthalmology, 2019. **126**(9): p. 1230-1237.

[78] Hayashi, Y., et al., *Effective treatment of a mouse model of Sjogren's syndrome with eyedrop administration of anti-CD4 monoclonal antibody.* Arthritis Rheum, 2004. **50**(9): p. 2903-10.

[79] Holland, E.J., et al., *Lifitegrast for the Treatment of Dry Eye Disease: Results of a Phase III, Randomized, Double-Masked, Placebo-Controlled Trial (OPUS-3).* Ophthalmology, 2017. **124**(1): p. 53-60.

[80] Fu, R., et al., *Rebamipide ophthalmic solution modulates the ratio of T helper cell 17/regulatory T cells in dry eye disease mice.* Mol Med Rep, 2019. **19**(5): p. 4011-4018.