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Age-related Defects in Ocular and Nasal Mucosal Immune System and the Immunopathology of Dry Eye Disease

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Abstract

Dry eye disease (DED) is a prevalent public health concern that affects up to 30% of adults and is particularly chronic and severe in the elderly. Two interconnected mechanisms cause DED: (1) an age-related dysfunction of lacrimal and meibomian glands, which leads to decreased tear production and/or an increase in tear evaporation; and (2) an age-related uncontrolled inflammation of the surface of the eye triggered by yet-to-be-determined internal immunopathological mechanisms, independent of tear deficiency and evaporation. In this review we summarize current knowledge on animal models that mimic both the severity and chronicity of inflammatory DED and that have been reliably used to provide insights into the immunopathological mechanisms of DED, and we provide an overview of the opportunities and limitations of the rabbit model in investigating the role of both ocular and nasal mucosal immune systems in the immunopathology of inflammatory DED and in testing novel immunotherapies aimed at delaying or reversing the uncontrolled age-related inflammatory DED.

Dry eye disease (DED) (Figure 1) is a prevalent public health concern that affects an estimated 25 million people in the United States.[1] Among these, over five million individuals (50 years and older) experience chronic and severe DED symptoms.[2–5] DED is one of the most common reasons that people visit their ophthalmologist and optometrist.[4,5] With the increase in life expectancy, the overall burden of DED for the United States

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DECLARATION OF INTEREST

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healthcare system is expected to double in the next decades from its current cost of around $3 billion annually.[6–8]

Lacrimal and meibomian glands, corneal and conjunctival epithelia, and tear film coordinate to control the response to internal and external insults on the ocular surface and preserve its integrity and function.[9–16] When the integrity of one of these ocular surface compartments is disrupted, this may lead to DED, thus compromising the normal function of the visual system. Two interconnected external and internal mechanisms cause DED[17–22]: (1) an age-related decrease in tear production and/or an increase in tear evaporation that triggers a sustained ocular surface inflammation, which in the elderly can lead to blindness from corneal epithelial barrier disruption, ulceration, and scarring[23–26] and (2) an age-related inflammation of the surface of the eye, triggered by yet-to-be-determined immunopathological mechanisms, independent of tear deficiency. This inflammation compromises the function of lacrimal and meibomian glands, which reduces tear film stability and osmolarity, leading to inflammatory DED.[27–31]

The word “inflammation” comes from the Latin *inflammo*, which means “I set alight” or “I ignite.” Currently, there is no specific immunotherapy for inflammatory DED, and patients must rely on sustained nonspecific artificial tears, anti-inflammatory steroids[32,33] or cyclosporine-based[34–37] drugs (e.g. Restasis) to reduce the uncontrolled ocular surface inflammation. However, many of these treatments must be taken consistently for years and often many times a day. There is an urgent need to develop a specific and long-lasting immunotherapy to reduce uncontrolled inflammatory DED. Evidence suggests that inflammatory DED is associated with an ocular surface inflammatory imbalance (Figures 1 and 2), characterized by a decrease in ocular surface-resident anti-inflammatory regulatory T cells (T_{reg}) and an increase of proinflammatory $CD4^+$ Th1/Th17 cells (Figures 2 and 6). However, key knowledge gaps still remain, including the mechanisms of age-related deterioration and dysregulation of the ocular mucosal immune system that leads to severe inflammatory DED in the elderly; and the role of nasal associated lymphoid tissue (NALT) in inflammatory DED.

The present review focuses on the opportunities and limitations of a rabbit DED model in studying the role of aging ocular and nasal mucosal immune systems in the immunopathology of inflammatory DED. For more details on other aspects of DED, such as disruption of homeostatic mechanisms in DED, anti-inflammatory therapy in DED, mucosal autoimmune disease and DED, role of Toll-like receptors in DED, the prevalence and risk factors of DED in the elderly, and relevance of animal models for the research into the immunophysiological processes regulating the functions of the lacrimal gland, we direct the readers to the following excellent reviews by Dana et al.,[16,38] Pflugfelder et al.,[13] De Paiva et al.,[13] McDermott et al.,[17,21] Perez et al.,[1,39–41] and Mercheff et al.[42–44]

POTENTIAL IMMUNOPATHOLOGICAL MECHANISMS THAT LEAD TO SEVERE AND CHRONIC INFLAMMATORY DED IN THE ELDERLY

Dry eye disease, as defined by the Report of the International Dry Eye Workshop,[45] occurs in two major types: tear-deficient forms (including Sjögren syndrome and non-Sjögren tear-

deficient) and evaporative forms. Sjögren syndrome, which is the classic form of exocrine deficiency associated with keratoconjunctivitis sicca (KCS), is characterized by a chronic inflammatory infiltration of the lacrimal glands, predominantly by $CD4^+$ T cells.[46–49] The exact factors that incite the infiltration of lacrimal glands by inflammatory $CD4^+$ T cells remain unknown. In addition, the pathogenic mechanisms in DED are not limited to lacrimal inflammation. Age-related interruption of neuronal stimulation of tear secretion, defects in transmembrane and secretory mucin expression, and meibomian gland dysfunction (MGD) contribute to various forms of KCS.[9–12]

Three independent human studies have recently shown that tears from DED patients contain significantly increased concentrations of IL-1β, IL-6, IL-8, IL-17, and TNF-α proinflammatory cytokines that correlated with severity of the disease.[50–52] All three studies directly associated DED severity with increased ocular inflammation, independent of the evaporative effect. The ocular surface epithelium expresses receptors to all of these cytokines. Changes in the balance of Th2 cytokine IL-13 have a homeostatic role in promoting goblet cell differentiation. In contrast, Th1 cytokine IFN-γ antagonizes IL-13 function and promotes apoptosis and squamous metaplasia of the ocular surface epithelia. IL-17 promotes corneal epithelial barrier disruption. Therapies that maintain normal IL-13 signaling or suppress IFN-γ and IL-17 proinflammatory cytokines might reduce the severity and chronicity of inflammatory DED.

However, more studies are needed to further elucidate the underlying cellular and molecular immune mechanism(s) that lead to the increased inflammation and pathogenesis of severe inflammatory DED seen in the elderly.[17–22] An expansion of $CD4^+CD25^{(low)}GITR^{(+)}$ T_{reg} cells has been detected in inflamed tissues of the salivary glands of Sjögren syndrome patients.[53,54] This expansion may represent a regulatory attempt to reduce excessive inflammation.[53–55] We hypothesize that severe inflammatory DED seen in the elderly is associated with a lack of sufficient number and/or function of ocular surface-resident T_{reg} cells, an increase in the number and/or function of ocular surface-resident proinflammatory $CD4^+$ Th1/Th17 cells, and a high basal level of proinflammatory mediators, such as metaloproteinases (MMPs) produced by ocular surface dendritic cells (DCs).[56] These factors increase the levels of damaging proinflammatory cytokines, chemokines, and MMPs in tears, which lead to severe inflammatory DED seen in the elderly.

Dendritic cells (DCs) act as a bridge between innate and adaptive immune responses.[57] Dendritic cells lining the ocular surface epithelium are critical in initiating effective immune responses to harmful pathogens while maintaining tolerance against harmless antigens. We have recently discovered that systemic DCs from the elderly fail to prevent immune response to harmless antigens.[56] Ocular surface epithelial cells (ECs) and DCs are present in close proximity and there is continuous interaction between the two types of cells. Strikingly, there is a scarcity of information regarding the interaction between ECs and DCs, particularly in aged lung. This is an area of high significance since lung ECs and DCs have a profound effect on the functions of each other. For example, proinflammatory cytokines, such as type I and type I interferons, are secreted by DCs in response to infections act on epithelial cells of the mucosa and upregulate the expression of antiviral molecules such as MHC-I.[58] Cytokine secretion by DCs also increases the permeability of the epithelial cell

barrier to allow infiltration of other immune cells, by reducing the expression of tight junction proteins.[59] Most of the information regarding functions of ocular DCs has been derived from mouse models. The studies on human ocular DCs, particularly in the aged population, are hampered by a number of ethical and practical factors, such as the scarcity of obtaining ocular tissues from healthy aged individuals. Biopsies of the cornea, conjunctiva, and meibomian and lacrimal glands are not a routine procedure. Furthermore, the number of ocular DCs is too small to perform mechanistic and functional studies. Exploring the interface between aging and the ocular surface epithelial cells and dendritic cells in health and DED and evaluating how the failure of immune homeostatic processes in elderly leads to chronic and severe DED remain to be elucidated.

AGE-RELATED DETERIORATION AND DYSREGULATION OF THE OCULAR MUCOSAL IMMUNE SYSTEM AND ITS ASSOCIATION WITH CHRONIC AND SEVERE INFLAMMATORY DED

After age 50, the severity of inflammatory DED increases exponentially with every decade.[60–62] DED has a considerable negative impact on visual function and quality of life, particularly in the elderly.[63–65] As illustrated in Figures 1 and 6, the pathophysiology of DED involves both external and internal factors: (1) external desiccating stress, caused by hot, dry, windy environments or high altitudes that adversely affect tear film stability and tear osmolarity by damaging the ocular surface and sensory corneal nerves, and initiating a cascade of ocular inflammation that leads to DED[66–68] and (2) yet-to-be-determined, age-related internal immunopathological mechanisms, independent of the evaporative effect, that compromise the function of lacrimal and meibomian glands and the lacrimal drainage system, reducing the tear film quality and quantity, which in turn would lead to ocular surface inflammation and inflammatory DED.[19,69–73] This view is supported by our recent finding that older individuals have elevated basal levels of proinflammatory mediators produced by immune cells,[74–76] have less anti-inflammatory T_{reg} cells, have more $CD45^+$ immune leukocyte infiltration at the ocular surface, compared to young individuals,[77–81] and have more proinflammatory $CD4^+$ T cells (see Figure 3 and reference 82). Human aging is characterized by a chronic, low-grade inflammation, a phenomenon known as “inflammaging”.[83–85] Inflammaging is a highly significant risk factor for both morbidity and mortality in elderly people, as most if not all age-related diseases share an inflammatory pathogenesis. We are currently investigating age-related specific mechanisms that lead to ocular and nasal mucosal immune deterioration (i.e. immunosenescence) and dysregulation (i.e. “inflammaging”) associated with DED.

We hypothesize that age-associated increases in the number and/or function of resident proinflammatory T helper type 1 (Th1) and, preferentially, Th17 effector cells are associated with a decrease in the number and/or function of anti-inflammatory $Foxp3^+CD4^+CD25^+$ T_{reg} cells in ocular and nasal mucosal immune system, leading to chronic and severe inflammatory DED in the elderly. Recent human studies suggest that Th17 cells are important players in inflammatory DED.[86–90] In contrast to the older inflamed eye, the normal young eye is populated by an abundance of conjunctiva-resident T_{reg} cells, which might suppress Th1/Th17-mediated inflammation, thus contributing to ocular surface

homeostasis[91] (as illustrated in Figures 1 and 6). Reduced numbers of T_{reg} cells or defects in their functionality have been documented in several human inflammatory diseases.[92–94] We recently found that normal palpebral conjunctivas from healthy young rabbits contain a higher frequency of functional $Foxp3^+CD4^+CD25^+$ T_{reg} cells, compared to spleen and PBMC[93,95] (Figure 4). We also found that conjunctiva-derived T_{reg} cells efficiently suppressed inflammatory $CD4^+$ Th1 cells.[93] We are currently investigating the potential age-associated defects in the number and function of ocular-surface-resident $Foxp3^+CD4^+CD25^+$ T_{reg} cells in both humans and rabbits, and whether a decrease in the number and/or function of "aged" T_{reg} cells is associated with an increase in Th1/Th17 proinflammatory cells and severe inflammatory DED.

A reliable animal model that simulates human DED would be useful, not only for investigation of the immunopathologic mechanisms of DED, but also in developing a novel, safe, and powerful immunotherapy with the potential to produce a sustained clinical response to inflammatory DED. The paragraphs below describe several animal models of dry eye with a focus on the opportunities and limitations of the rabbit model.

ANIMAL MODELS OF DRY EYE: OPPORTUNITIES AND LIMITATIONS

Numerous animal models have been developed to reflect the multiplicity of immunopathophysiologic mechanisms involved in DED (see Report of the International Dry Eye Workshop [RIDEW, 2007]).[96,97] Several animal models mimicking different immunopathological mechanisms that cause inflammatory DED have been proposed: surgical extirpation of the lacrimal gland, mechanical inhibition of blinking with a blepharostat in rabbits, induced progressive lacrimal gland inflammation resembling the development of Sjögren syndrome in certain strains of mice, pharmacologic blockade of cholinergic muscarinic receptors in the lacrimal glands, housing mice in desiccating environments, surgical denervation, botulinum toxin injection, topical benzalkonium chloride treatment, topical cholinergic blockade, and ex vivo to *in vivo* transfer of activated immune cells. Understanding the characteristics, benefits, and limitations of these animal models will help address specific mechanisms and develop new treatment strategies for DED.

Mouse Model

The mouse model has contributed enormously to the study of inflammatory DED. It is the model most commonly used to study the mechanisms of DED, because of the diversity of knockout and transgenic strains and wide availability of immunological reagents. Thus, it is not surprising that the mouse model has contributed enormously to the study of inflammatory DED. Many transgenic and gene-targeted mouse strains have been used to characterize the immune mechanisms leading to inflammatory DED. Dana and collaborators recently reported that desiccating environmental stress and systemic muscarinic acetylcholine receptor (mAChR) blockade induced clinical signs of DED in the mouse model.[98–101] However, desiccating environmental stress appears to impart higher conjunctival $CD3^+$ T-cell infiltration, greater Th17 cell activity and T_{reg} cell dysfunction than mAChR blockade.[98] mAChR blockade also decreased tear secretion more severely than

desiccating environmental stress.[98] Systemic mAChR blockade attenuated Th17 activity and enhanced Th2 and T_{reg} cells responses without affecting Th1 activity.[98] Thus, *in vivo* inhibition of mAChRs variably affects $CD4^+$ T-cell subsets and desiccating environmental stress and systemic mAChR blockade appeared to induce DED through different immunopathogenic mechanisms.[98] Transdermal scopolamine application and exposure to a continuous airflow are used to induce dry eye in female mice in a particular mouse model of KCS.[102–106] Scopolamine induces a pharmacologic blockade of cholinergic (muscarinic) receptors in the lacrimal gland and therefore is used to decrease aqueous production, whereas the airflow mimics environmental stressing conditions (with resultant increased evaporation).

Below are listed opportunities and limitations of some murine lacrimal inflammatory models that mimic Sjögren syndrome. The nonobese diabetic (NOD) mouse model shows an infiltration of predominantly $CD4^+$ Th1 cells in the lacrimal gland as well as submandibular and thyroid glands. The appearance of autoimmune diabetes before autoimmune exocrinopathy in the NOD mouse suggests that it can be used as a model of secondary, but not primary Sjögren syndrome.[107,108] However, the complex immunopathological mechanisms involved in the NOD mouse remain to be fully elucidated. Evidence also indicates that the immunopathology of lacrimal and salivary glands is secondary to metabolic changes, unrelated to primary diseases (i.e., rheumatoid arthritis, autoimmune thyroiditis, and system lupus erythematosus), that typically give rise to secondary Sjögren syndrome.[109–112] The MLR/lpr mouse models of Sjögren syndrome exhibit lacrimal gland infiltrates characterized by a predominance of $CD4^+$ T cells. In contrast to the NOD model, the extent of the lacrimal gland inflammation is significantly greater in female than in male mice, resembling the difference observed in human Sjögren syndrome.[109,113–115] The immunopathology of MLR/lpr mouse models is unique, in that the predominance of IL-4 and B7-2 (also known as CD86) within the lacrimal gland lesions of MRL mice suggests a largely Th2-type response, distinct from that in the NOD model. However, cholangiocytes from Fas-deficient MRL/lpr mice did not show inergic agonist-induced apoptosis, and epithelial cells in biopsied labial salivary glands from patients with Sjögren syndrome are not hyperresponsive to chol MLR/lpr inergic agonists. The MLR/lpr mouse model has been used to test the hypothesis that a reduction of the quantity of parasympathetic fibers, or an alteration in the neurotransmitters in the healthy tissue of the lacrimal gland, is responsible for tear decrease in patients with Sjögren syndrome.[3,9]

Overall, these data suggest the existence of potentially divergent and complex immunopathological mechanisms of lacrimal-associated inflammatory DED in the mouse models. Several other mouse models are also associated with a predominant $CD4^+$ T-cell infiltration of the lacrimal gland. The TGF-β1 knockout mouse, for example, has shown significant inflammatory cell infiltrates in the lacrimal gland between the ages of 2 and 4 weeks, whereas the globe itself exhibits a normal structure and phenotype on histologic examination. Several factors complicate the use of these mice for study of ocular surface disease: (1) no definitive data have been produced relating the lacrimal gland infiltration to altered tear secretion in these mice; (2) the absence of specific lymph nodes, together with a variety of other serious immune defects, including depressed baseline immunoglobulin production and isotype switching, defective T-cell function, and faulty homing responses,

confounds the study of the effect of lacrimal insufficiency on the eye, as each of these factors alone or in combination may affect the cornea and ocular surface.

Endocrine control of lacrimal secretion has been proposed in humans but to date there have been no animal models that show spontaneous development of DED due to a specific endocrine imbalance. Genetically modified mice are expected to aid in the study of the influence of a specific hormone(s) on the tear film homeostasis. In an early study, Sullivan and Dana reported that no inflammation exists in rat lacrimal glands 15–31 days after orchiectomy and pituitary removal.[116] The authors also report that no aqueous tear deficiency was apparent in patients receiving anti-androgen therapy. They conclude that androgen deficiency may promote the progression of Sjögren syndrome and its associated lacrimal gland inflammation, meibomian gland dysfunction, and severe dry eye. However, androgen insufficiency alone is not sufficient to cause lacrimal gland inflammation or aqueous tear deficiency in nonautoimmune animals and humans.[116]

The tear film is constantly exposed to multiple external factors, including variable temperatures, airflow, and humidity, which stimulate or retard its evaporation. Lipids are produced by the meibomian glands and spread onto the aqueous phase that covers the ocular surface, thus protecting the tear film from excessive evaporation. Meibomian gland dysfunction (MGD) is among the leading factors that cause DED.[117–120] A better understanding of the progression of MGD may facilitate the development of effective therapeutic strategies against DED. In particular, comparative analysis of immunological features of MGDs in health and disease may reveal important insights into the immunopathology of MGD. In this regard a reliable animal model is crucial. Abnormal keratinization of the meibomian gland has been shown in various animal models of MGD, including the rabbit epinephrine-induced MGD, the primate polychlorinated biphenyl-induced MGD, and the rhino mouse genetic MGD models.[121] Recently a mouse model for characterizing glandular changes in obstructive MGD has been described by Nichols and coworkers (Abstract ARVO, 2014). In this model, obstruction of the meibomian gland orifices produced stasis of the meibum, which ultimately induced alterations in the morphology of the glands and glandular dropout characteristic of clinical MGD. This is a convenient and economical animal model that can be used for investigating therapeutic agents for treatment of evaporative DED. These models provide new insights into the pathophysiologic mechanisms of hyperevaporative DED and help to identify potential targets for novel gene therapy.

Other mouse models of lacrimal insufficiency and evaporative DED have been proposed.[102–106] Although there are significant benefits to studying sicca-related ocular surface disease in mice that lack concomitant systemic immune dysfunction, this model remains to be optimized, as it does not adequately control important environmental factors such as temperature and humidity factors that can have profound effects on the exposed ocular surface. Moreover, the airflow generated by an air fan placed 6 inches in front of the mice’s cage for 10 h a day for 12 consecutive days may be a source of stress for mice that may affect other immune responses and hence affect the data on the tear film and ocular surface homeostasis.

Rat, Cat, and Dog Models

Experimental immune dacryoadenitis has also been produced in Lewis rats by sensitization with a single intradermal administration of an extract of lacrimal gland in complete Freund's adjuvant (CFA) and simultaneous intravenous injection of killed *Bordetella pertussis*. No data have been published about the effect of this procedure on the ocular surface. Surgical removal of the main lacrimal gland in dogs and cats leads to a decrease in basal tear production as measured by the Schirmer test, but it does not cause significant changes in ocular surface signs, even after a long follow-up. Monkeys have one main lacrimal gland with an anatomic structure similar to that in humans. The removal of the lacrimal gland has been demonstrated to decrease tear secretion, but without causing any reproducible ocular surface damage. It is likely that compensatory tear production by the accessory lacrimal glands alone may be sufficient to maintain a stable tear layer.

WHAT CAN A RABBIT MODEL TEACH US ABOUT THE IMMUNOPATHOLOGY OF CHRONIC INFLAMMATORY DED?

The rabbit has recently emerged as a viable and reliable model to study DED (Figure 3E). For experimental study of DED characteristics, a rabbit model is more suitable, because it presents decreased tear secretion and ocular surface changes, has a longer lifespan, and offers greater accessibility to the ocular surface tissues.[122–136] For studying specific causes of DED, such as defects of neuronal reflex loops, environmental changes, or evaporative DED, the rabbit model appears to recapitulate many underlying pathophysiologic mechanisms observed in humans. Because of the large exposed ocular surface in rabbits compared with mice, standard DED clinical tests, such as tear breakup time and fluorescein or rose Bengal staining of the ocular surface, can be much more easily performed in rabbits. Below are opportunities and limitations of rabbit DED models.

Meibomian Gland Rabbit Model

One of the major causes of DED is meibomian gland dysfunction (MGD), which shows increased prevalence with aging.[137] Besides apparent autoimmune/inflammatory reactions, with yet-to-be-determined mechanism and target antigen(s), the MGD is also caused by hyperkeratinization of the ductal epithelium of the meibomian gland and reduced quantity and/or quality of meibum.[137] The meibum is the holocrine product that stabilizes and prevents the evaporation of the tear film.[137] Gilbard and collaborators first proposed a rabbit model of DED in which the orifices of meibomian glands were individually closed by cauterization.[138] In this rabbit model, they found not only a significant decrease in conjunctival goblet cell density and corneal epithelial glycogen levels by 12 weeks, but also the presence of inflammatory immune cells within the bulbar conjunctiva after 20 weeks. Although this rabbit model is helpful in studying the effect of meibomian gland dysfunction on the ocular surface, in order to reflect physiological conditions, the model could be improved in an environment in which temperature, humidity, and airflow are constantly monitored and controlled, such as the Controlled Adverse Environment chamber.[98,139–143] This would more closely reflect the clinical setting, where it has been demonstrated that the rate of tear film evaporation from the ocular surface is temperature-, humidity-, and airflow-

dependent. Orchiectomy and ovariectomy in rabbits have been used to study the influence of hormones on the structure and function of the lacrimal and meibomian glands, as discussed in reference[144]. In rabbits, closing the lacrimal gland excretory duct and surgically removing the nictitating membrane and harderian gland can cause an increase in tear osmolarity at postoperative day 1, accompanied with significant decrease in conjunctival goblet cell density by 8 weeks.[127,145,146]

Lacrimal Gland Rabbit Model

Early studies by Wood and collaborators established that transient infection of the cornea is followed by increased lymphocytic infiltration of the lacrimal gland.[147–150] More recently, Mircheff and collaborators have proposed a rabbit model in which infection of the lacrimal gland with a replication-deficient adenovirus vector initiates a relatively mild acute inflammatory response, which progresses through at least two quite distinct phases and then evolves into a chronic, low-grade inflammation.[151] Exposure to hot, dry weather causes lymphocyte aggregates to accumulate; notably, those aggregates are associated with a cytokine mRNA expression profile similar to the profile in the second phase of the acute response to adenovirus infection.[152] When transduced prolactin is expressed in lacrimal glands that already contain substantial numbers of resident immune cells, it induces the immune cells to upregulate IFN-γ expression and proliferate.[153–155] These findings lead to the hypothesis that the resident immune cell population contains physiologically relevant numbers of natural killer (NK) and natural killer T (NKT) cells. The lacrimal glands of normal adults contain resident immune cells that appear to accumulate through the responses to normal environmental stress and ocular adenovirus infections in addition to dIgA-secreting plasmacytes.

Neural Reflex Rabbit Model

The ocular surface (cornea, conjunctiva, and accessory lacrimal glands), meibomian glands, and main lacrimal gland are interconnected by neural reflex loops that produce an integrated “functional unit.” Neural control of lacrimal secretion has also been well established for many decades in both animal models and humans (reviewed in references[3,156,157]). A New Zealand albino rabbit DED model, which putatively mimics a blockade of the neural reflex loops involved in maintaining the normal tear physiology, has been created by administrating the anticholinergic agent atropine. Similar to scopolamine, atropine is a competitive muscarinic acetylcholine receptor antagonist.[158–160] The antimuscarinic effect of topical 1% atropine sulfate has been shown in rabbits to significantly reduce lacrimal secretion within 2 days and to induce corneal epithelial defects by 3 days. A short-term rabbit model for hyperevaporative DED has been created by preventing rabbits from blinking through the placement of lid specula or sutures.[122–136] After 2 h of desiccation induced by lid specula, dry spots appear on the rabbit corneal epithelial surface and stain with methylene blue. Because of the acuteness of the induced DED and the use of anesthetics (which themselves can decrease tear secretion), this rabbit model is not optimal for studying KCS pathogenesis, which is a chronic event. However, in these rabbits, corneal epitheliopathy develops within a few hours, and hence such rabbits can be used to evaluate the effect of artificial tears or other therapies aimed at delaying the evaporative loss of the preocular tear film.

Inflammatory DED Rabbit Model

We recently introduced a novel rabbit model to study defects of the aging ocular and nasal immune systems that led to severe inflammatory DED, as seen in the elderly (Figure 3E). We selected rabbits because numerous similarities exist between the rabbit and human ocular and nasal immune system in both homeostasis and inflammatory diseases[34,36,91,93,125,161–170]: (1) several immune-mediated ocular surface diseases, including inflammatory DED are similar in rabbits and humans[91,93,170,171]; (2) rabbit conjunctiva-associated lymphoid tissues (CALT) closely resemble the human CALT[172–175]; (3) similar to humans, rabbit palpebral conjunctiva contains an abundance of conjunctival lymphoid follicles (CLF)[173,176]; (4) from a practical standpoint rabbits possess relatively large conjunctival surfaces and lacrimal glands offering abundant ocular mucosal tissue for immune cell studies; (5) because of the large exposed ocular surface in rabbits compared to mice, standard DED clinical tests, such as tear breakup time and fluorescein and rose bengal staining, can be much more easily performed in rabbits; (6) for practical reasons, surgical closure of the nasolacrimal ducts (NLDs) to determine the role of NALT in inflammatory DED is much easier in rabbits than in mice[122–136]; and (7) during the past several years we have been studying the rabbit ocular mucosal immune system (OMIS).[93,95] We recently found that conjunctiva from young rabbits contains an abundance of functional T_{reg} cells that produce anti-inflammatory cytokines, such as IL-10 and TGF-β, and suppress excessive ocular surface-resident Th1 $CD4^+$ T-cell responses[93,177] (Figures 4 and 5). This apparent natural expansion of T_{reg} cells in young conjunctiva may represent a regulatory mechanism to suppress potential excess ocular surface inflammation. Based on the above published data, together with findings from others, we are currently testing the hypothesis that advanced age could contribute to a reduction in ocular surface-resident anti-inflammatory T_{reg} cells and an expansion of proinflammatory Th1/Th17 cells in aging conjunctiva. Such a mechanism may lead to the apparent increase of ocular surface inflammation and severity of DED seen in aging eyes.

Although the state of the art in rabbit immunological tools still lag behind that of the mouse and human, the rabbit is still widely used in studies for mucosal immunity.[178] Several immunological reagents (e.g. polyclonal and monoclonal antibodies specific to rabbit effector T cells [CD3, CD4, and CD8], regulatory T cells [CD25, GITR, CTLA4, Foxp3], macrophages [RAM-11], and dendritic cells [CD11c, CD11b]) are now commercially available and we have confirmed the usefulness of these immunological reagents to perform many immunological studies[91,93] (Figures 3–5). We and others are developing an experimental rabbit model of DED that uses a desiccation-controlled room that allows low humidity and high airflow, which increases tear evaporation and leads to the clinical signs of DED.[122–136] Using this novel rabbit model and armed with the necessary rabbit immunological expertise and reagents, we are now poised to investigate the immune mechanisms of age-related deterioration and dysregulation of the OMIS that lead to inflammatory DED, and the role of NALT in inflammatory DED.

A recent study demonstrates autoimmune/inflammatory disease in rabbits resembling Sjögren syndrome following an injection of autologous peripheral blood lymphocytes that were stimulated *in vitro* with epithelial cells obtained from the contralateral excised gland

into the lacrimal gland. The histopathologic pictures of the treated lacrimal glands were similar to those in patients with Sjögren syndrome, with predominantly $CD4^+$ T-cell infiltrates. A continuous decrease in tear production and stability and an increase in rose bengal staining of the ocular surface were recorded in eyes injected with activated PBLs and in the excised contralateral lacrimal gland that indicated generalized autoimmune/inflammatory responses. Nevertheless, it remains to be verified whether the acinar cells (the putative APCs of this model) in this rabbit model lack professional bone marrow–derived APCs that can stimulate the $CD4^+$ T cells. Further studies are required to elucidate the exact mechanism(s) that lead to increased ocular inflammation in general and, in particular, the chronicity and severity of inflammatory DED in the elderly.[122–136] An expansion of $CD4^+CD25^{(low)}GITR^{(+)}$ T_{reg} cells has been detected in inflamed tissues of salivary gland of Sjögren syndrome patients.[53,54] This expansion may represent a counter-regulatory attempt to reduce excessive inflammation.[53–55] As illustrated in Figure 6, together with related reports by others,[17,19,41,50,125,151,161,179–181] we hypothesize that severe inflammatory DED seen in the elderly is associated with lack of sufficient number and/or function of ocular surface-resident T_{reg} cells, and an increase in the number and/or function of ocular surface-resident proinflammatory $CD4^+$ Th1/Th17 cells. This will lead to an increase in the levels of damaging proinflammatory cytokines, chemokines, and MMPs in tears, which leads to severe inflammatory DED seen in the elderly.

Curcumin, extracted from *Curcuma longa* (turmeric), is a recently discovered natural dietary anti-inflammatory compound.[182–185] Curcumin reverses production of proinflammatory cytokines[186] and downregulates MMP-9.[187,188] Curcumin treatment of LPS-matured DC arrests their maturation into a tolerogenic phenotype that preferentially promotes the suppressive function of T_{reg} cells.[189] Oral treatment with curcumin alleviates intestinal inflammation by inhibiting proinflammatory Th1 cytokines.[183] However, curcumin has never been investigated in ocular inflammation. Anti-CD3 mAbs have been successfully used clinically to alleviate the severity of many inflammatory diseases by decreasing activated Th1/Th17 and increasing tissue-resident T_{reg} cells.[190–199] We are currently investigating whether curcumin will synergize with the anti-CD3 mAbs to alleviate the severity of ocular inflammation in the rabbit DED model, by interfering with Th1 cytokines, arresting maturation of DC, and promoting T_{reg} cells (as illustrated in Figure 6).

Advantage of a Rabbit Model in Studying the Potential Role of Nasal Mucosal Immune System in Chronic DED

The rabbit could represent a reliable and convenient animal model to study the role of both ocular and nasal mucosal immune systems in inflammatory DED.[82] Because of the above obvious ethical and practical considerations in obtaining human ocular and nasal mucosal tissues (see section on Inflammatory DED Rabbit Model above), we selected rabbits because numerous similarities exist between rabbit and human ocular and nasal immune systems in homeostasis and in inflammatory diseases.[34,36,91,93,125,161–170] Although severe nasal inflammatory response has been seen in the elderly,[200,201] its contribution to ocular inflammation remains to be determined. We hypothesize that deterioration of immune responses of aging nasal-associated lymphoid tissue (NALT) contributes to severe ocular inflammation. We are currently using a desiccating stress-induced rabbit model of DED

(Figure 3E) to address the following pivotal questions: Is there an effect of aging on ocular mucosal inflammation that leads to DED? What are the age-related mechanisms that lead to severe inflammatory DED? and Does aging NALT contribute to ocular inflammation and severity of inflammatory DED? As mentioned above performing surgical closure of the nasolacrimal ducts (NLDs) to determine the role of NALT in inflammatory DED is much easier in rabbits than in mice.[122–136]

The ocular mucosal immune system (OMIS)[91] is also known as eye-associated lymphoid tissue (EALT)[202,203] and drainage-associated lymphoid tissue (DALT).[204,205] The NALT and OMIS are physically interconnected through a channel called the tear duct (also known as the nasolacrimal duct or NLD).[206] Tears and topically applied solutions often drain down small channels (canaliculi) on the inner side of the eye into the tear sac. From this sac they flow down the NLD into the nose.[24,207,208] Inversely, NLDs drain intranasally administrated solutions to the mucosal surface of the eye and thus the OMIS. In the elderly, natural nasolacrimal occlusion (NLO) decreases tear draining and minimizes DED (reviewed in reference [209]). This unique anatomical connection between the OMIS and NALT systems prompted us to test whether OMIS and NALT are also immunologically interdependent and whether this interdependence affects the inflammatory DED. These studies are made possible by our novel rabbit model of DED, the first preclinical animal model in which NLDs can be surgically closed similar to that seen in humans and that allows for statistical analysis of potential clinical interventions. Using this model, we recently found that surgical closure of NLDs in rabbits significantly decreased the inflammatory responses of conjunctiva-derived IFN-γ-producing $CD4^+$ T-cell responses elucidated following topical or intranasal immunization (see Figure 3 and reference 82), suggesting that NALT and OMIS are immunologically interconnected. However, it remains to be determined whether and how NALT controls the ocular surface inflammation in general and in inflammatory DED, and whether such interdependence is eroded by age and hence leads to severe DED in the elderly. We are currently using a rabbit model of DED (young and old animals) to determine whether NLD closure will reduce ocular inflammation and alleviate the severity of inflammatory DED.

STRUCTURE, COMPONENTS, AND FUNCTION OF THE OCULAR MUCOSAL IMMUNE SYSTEM

The chief functional units of the ocular mucosal immune system are the conjunctiva, cornea, and DLNs.[202] The major T-cell inductive site of the OMIS is the conjunctiva,[172,202,210–212] which forms a continuous lymphoid tissue that covers most of the external eye. The cornea, in contrast to the conjunctiva, is considered an “immune-privileged” compartment of the eye with a minimum of lymphoid cells.[213–217] Under physiologic conditions, the cornea relies on neighboring lymphoid tissues and local lymphoid cell follicles for protection to maintain its transparency, allowing the passage of light to the retina.[69,172] Under inflammatory conditions, such as during DED, the cornea must also rely on the support of neighboring lymphoid tissues and local lymphoid cell follicles for immune protection and immune modulation.[69,172] When the topographical distribution of conjunctiva lymphoid tissue is projected onto the ocular surface, it overlies the surface of the cornea during eye closure, and

is hence in a suitable position to assist in corneal immunity and immunoregulation.[172] On one hand, corneal inflammation often induces the development of organized conjunctival leukocytic aggregates.[218,219] On the other hand, conjunctival immune cells have been shown to influence corneal immune response and the course of inflammation.[220] Likewise, it is clinically known that long-term eye closure can alleviate corneal inflammation.[221] Conjunctiva resident T_{reg} cells can suppress "aggressor" Th1 and Th17 inflammatory cells in the cornea and resolve DED, as recently suggested by Siemasko et al.[94,222,223] Clearly, the OMIS is intimately involved in corneal immune responses since it is a major source for immune cells in the cornea. Besides the organized lymphoid follicles of the conjunctiva, the draining preauricular lymph nodes are the major T-cell inductive sites. To better understand corneal immunity, immunopathology, inflammation, and ultimately design therapeutic strategies against inflammatory ocular surface diseases such as DED, we need to investigate the OMIS immune cell composition and function, including how conjunctiva T_{reg} cells interact and regulate inflammatory Th1 and Th17 cells.

The ocular mucosal immune system (secretory glands and lymphoid tissues at ocular and nasal mucosal surfaces) is largely separate from the peripheral systemic immune system (bone marrow, spleen, and lymph nodes). This system maintains the homeostasis of ocular surface while avoiding inflammation that would impair eye function. The ocular mucosal surfaces contain the major classical antigen-presenting cells (APCs), including dendritic cells (DC), macrophages (MΦ), and B cells, as well as nonclassical APCs (corneal and conjunctival epithelial cells). In general, the ocular mucosal immune system can be divided into mucosal inductive sites and mucosal effector sites. Antigens (pathogens and allergens) are encountered, taken up by APCs, processed, and presented to T cells at mucosal inductive sites, which are usually local lymph nodes (cervical, preauricular, and submandibular lymph nodes). Antigen-specific Tcells are induced in these lymph nodes and then travel back to ocular mucosal effector sites where they function.[47,87,224–228]

Another source of ocular mucosal surface protection is locally secreted antibodies (sIgA). Roughly 95% of secreted mucosal antibodies are IgA (sIgA or dIgA), while IgG subclasses account for only about 5%.[16,17,229,230] Within the common mucosal immune system, certain sites may facilitate a more far-reaching distal mucosal immune response than others in a sort of mucosal immune hierarchy.[231–234] For example, intranasal immunization induces IgA not only within the nose and salivary glands, but also on the eye surface.[229,231] Several reports suggest that intranasal immunization may be more effective than ocular administration in eliciting tear IgA antibody responses, indicating that NALT can serve as an inductive site for ocular mucosal IgA responses.[235–238] Greater than 99% of the tear IgA is synthesized locally in the lachrymal gland and most of the IgA in rat tears appear to recognize respiratory and gut microbiome.[229,239,240] The vascular structure of ocular mucosa (both blood and lymphatic vasculatures) and the pathways for lacrimal gland drainage and tear flow also provide unique anatomical conduits and intercommunication between the nasal associated lymphoid tissue (NALT) and the ocular mucosal immune system (OMIS) that are thought to be immunologically connected and interdependent.[82] The integrated nature of OMIS and NALT systems is important for the development of ocular immunotherapeutic strategy against inflammatory DED, and it is hoped that intranasally delivered immunotherapies will provide, or at least contribute to, protection against inflammatory

DED. In rats, topical ocular delivery of a particulate antigen (Ag) results in Ag uptake that is greatest at the ocular sites, particularly the conjunctiva, but there is also Ag uptake in NALT.[3,33–36] In some cases, the inductive site for Ag-specific IgA stimulation was traced to NALT rather than to the ocular surface.[37] Therefore, it was suggested that NALT functions as a primary inductive site for ocular immune responses, at least in rodent models.[241–246] However, this remains unresolved for humans, in which the complex interaction between OMIS and NALT, in both the normal and inflammatory situations, is not yet fully elucidated.

OMIS includes the protective barrier of the conjunctival and corneal epithelium, resident immune cells of the epithelia (DC and LC) and corneal stroma (various bone marrow-derived cells), the conjunctiva-associated lymphoid tissue (CALT), and the lacrimal glands.[241–246] These structures interact in ocular immunity, tolerance, and inflammation. OMIS involves a complex set of interactions between local and systemic immunocompetent and parenchymal cells that communicate through specialized cell surface receptors and soluble mediators to protect the surface of the eye.[91] Only a small number of researchers are involved in studying OMIS, also known as eye-associated lymphoid tissue (EALT).[15,202,247–250] The role of the role NALT in generating protection against inflammatory DED remains to be fully elucidated.

The Conjunctiva and Conjunctival Lymphoid Follicles in Inflammatory DED

The conjunctiva and the lacrimal gland are key elements in the OMIS. The conjunctiva forms a continuous mucosal surface that extends from the eyelid margin to the cornea and makes contact with airborne Ags, pathogens, and periocular tear film.[15,91,202,247–250] The conjunctiva and the lacrimal gland are postulated to play an active role in both inductive and effector functions and contain IgA^+ plasma cells, secretory sIgA, and T cells, which produce the inflammatory cytokines and chemokines that play a major role in inflammatory DED.[166,251] Conjunctival immunocompetent cells include those from the lymphoid system (lymphocytes) and those from the myeloid system (macrophages, polymononuclear leukocytes, eosinophils, mast cells, basophils), fibroblasts, epithelial cells, vascular endothelial cells, and professional APCs (macrophage, dendritic cells, Langerhans cells, and B cells). Human and rabbit conjunctiva contain an abundance of lymphoid derived cells.[15,91,202,247–250]

Because of the crucial role of $CD4^+CD25^+$ regulatory T cells in immune regulation in other mucosal surfaces,[252–254] it is important to determine the presence and percentage of these naturally occurring T_{reg} cells in conjunctiva compared to other lymphoid systems, such as the spleen. A better understanding of the function of T_{reg} cells in controlling OMIS inflammation should help in protecting from DED. We have recently demonstrated that conjunctiva from young rabbits contain an abundance of $CD4^+CD25^+$ T_{reg} cells, suggesting an anti-inflammatory role of these cells in protecting the surface of the eye.[93,177]

Role of OMIS in Protecting the Corneal Surface

Because the cornea, a nonlymphoid organ, normally contains few immune-competent cells, it relies on surrounding OMIS tissues, such as conjunctiva, the draining preauricular lymph nodes, and the tear film for immune protection.[93] The conjunctiva and its secretions

comprise one of the main immune tissues that protect the cornea. Along with the lacrimal gland, they provide soluble mediators, including lysozyme, sIgA, cytokines, chemokines, and complement, via the tear film.[91] The palpebral conjunctiva can detect corneal Abs and pathogens, and prime respective OMIS-derived B and T effector cells or distribute protective factors, such as secretory IgA.[241–246]

CONCLUDING REMARKS

- Inflammatory dry eye disease (DED), an immune disorder of largely unknown underlying immunopathological mechanisms, is characterized by sustained ocular surface inflammation, and disruption of the corneal epithelial barrier (see Report of the International Dry Eye Workshop (RIDEW, 2007).[255–257] In the elderly, DED can lead to severe corneal ulceration and scarring that can lead to vision loss.
- A reliable animal model is needed to investigate the internal immunopathological mechanism(s) leading to inflammatory DED, and to develop a specific and long-lasting immunotherapy to reduce the chronic and severe inflammatory DED in the elderly. Numerous animal models have been developed, each with unique characteristics and limitations.
- The mouse model has contributed enormously to the study of inflammatory DED. It is the model most commonly used to study the mechanisms of DED, because of the diversity of knockout and transgenic strains and wide availability of immunological reagents and many transgenic and gene-targeted mouse strains.
- Rabbit has recently emerged as a leading translational animal model that mimics the severity and chronicity of inflammatory DED. The rabbit model presents many opportunities to investigate the role of nasal mucosal immune systems in the immunopathology of inflammatory DED and to test novel nasal immunotherapies aimed at delaying/reversing the uncontrolled chronic and severe of age-related inflammatory DED. Ongoing efforts in our laboratory are trying to develop a safe and long-lasting specific immunotherapy. We are currently investigating whether curcumin will synergize with the anti-CD3 mAbs to alleviate the severity of ocular inflammation in the rabbit DED model by interfering with Th1 cytokines, arresting maturation of DC, and promoting T_{reg} cells (as illustrated in Figure 6).
- We hypothesize that severe inflammatory DED seen in the elderly is associated with lack of sufficient number and/or function of ocular surface-resident T_{reg} cells; an increase in the number and/or function of ocular surface-resident proinflammatory $CD4^+$ Th1/Th17 cells; and high basal level of proinflammatory mediators, such as metaloproteinases (MMPs), produced by ocular surface DCs.[56] These increase the levels of damaging proinflammatory mediators, leading to severe inflammatory DED seen in the elderly.

- The anatomical connection between OMIS and NALT suggests immunological connection and interdependency.[91,258,259] The integrated nature of OMIS and NALT systems may be important for ocular surface inflammation and inflammatory DED.[91,93,245,259–262] Topically administrated solutions to the ocular surface are conducted by NLDs into the inferior meatus of the nose, where it reaches the NALT system.[91,259,263] Inversely, intranasally administrated immunotherapy solutions are also drained by the NLDs to the mucosal surface of conjunctiva where it reaches the OMIS system.[91,263–266] The anatomical details of rabbit nasolacrimal ducts (NLDs), the connecting overpass between OMIS and NALT, are illustrated in Figure 3 and described in references 91, 82, and 263. Our recent findings indicate that NALT and OMIS are immunologically interdependent,[82] suggesting that NALT is an important factor that affects the severity of inflammatory DED. We are currently testing this hypothesis in the rabbit model of inflammatory DED.
- It is important to determine the role of aging ocular and nasal mucosal immune systems in the severity of inflammatory DED seen in the elderly. This should help the development of a novel, safe, and powerful immunotherapy with the potential to produce a sustained clinical response to lessen or ameliorate inflammatory DED.

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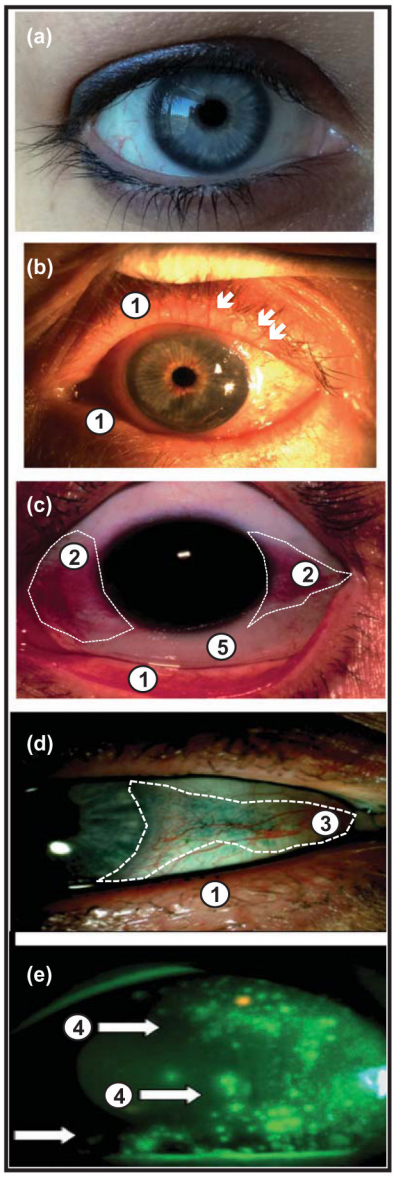

FIGURE 1.

Dry eye is a part of the natural aging process. (a) Normal young eye. (b–e) Up to 30% of people over age 50 experience some symptoms of dry eyes. (b) Dry eye in an elderly patient with conjunctival hyperemia, irregular tear film, and inflamed meibomian glands (arrows). (b–d) Conjunctival and corneal epithelial degeneration (1). In (c) rose bengal (2) and (d) lissamine green (3) staining shows uptake in degenerating ocular surface cells in the zone of exposure. (e) Severe dry eye in an elderly patient showing uptake of fluorescein stain by the basement membrane of the corneal epithelium and zones of epithelial cell (4) loss (arrows). Palpebral conjunctiva = (1). Bulbar conjunctiva = (5).

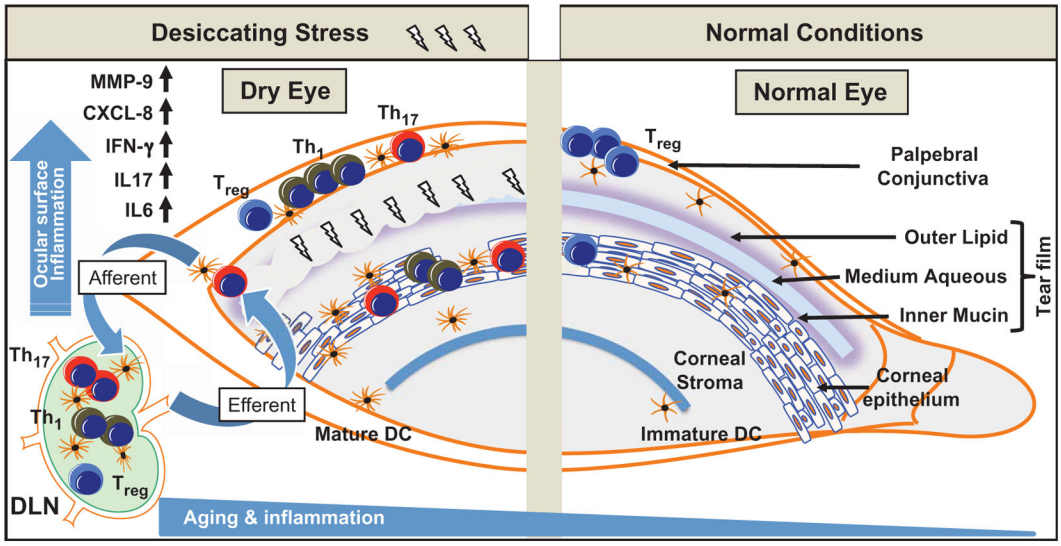

FIGURE 2.

Major immunopathological cellular and molecular players in inflammatory dry eye disease. Dry eye (left) is induced by desiccating stress, which leads to disruption in tear film and to maturation of ocular surface-resident dendritic cells (DC), which migrate to palpebral conjunctiva and to draining lymph nodes (DLN) through the afferent vessels. This triggers activation/expansion of proinflammatory $CD4^+$ Th1/Th17 cells in palpebral conjunctiva and DLN. These proinflammatory Th1 and Th17 cells will then migrate through the efferent vessels into the palpebral conjunctiva, cornea, and lacrimal and meibomian glands, leading to a disruption of epithelial barriers associated with an increase in ocular surface inflammation and DED. Low number and/or dysfunction of palpebral conjunctiva-resident T_{reg} cells seen in the elderly cannot help regulate inflammatory Th1/Th17 cells. Thus, aging promotes inflammatory Th1 and Th17 cells to produce damaging IFN-γ and IL-17 and mature DC to produce inflammatory mediators, which exacerbate ocular inflammation and increase severity of DED in the elderly. Normal eye (right) (i.e. homeostatic) is characterized by T_{reg} cells normally suppressing proinflammatory Th1/Th17 cells, thus controlling excessive ocular inflammation.

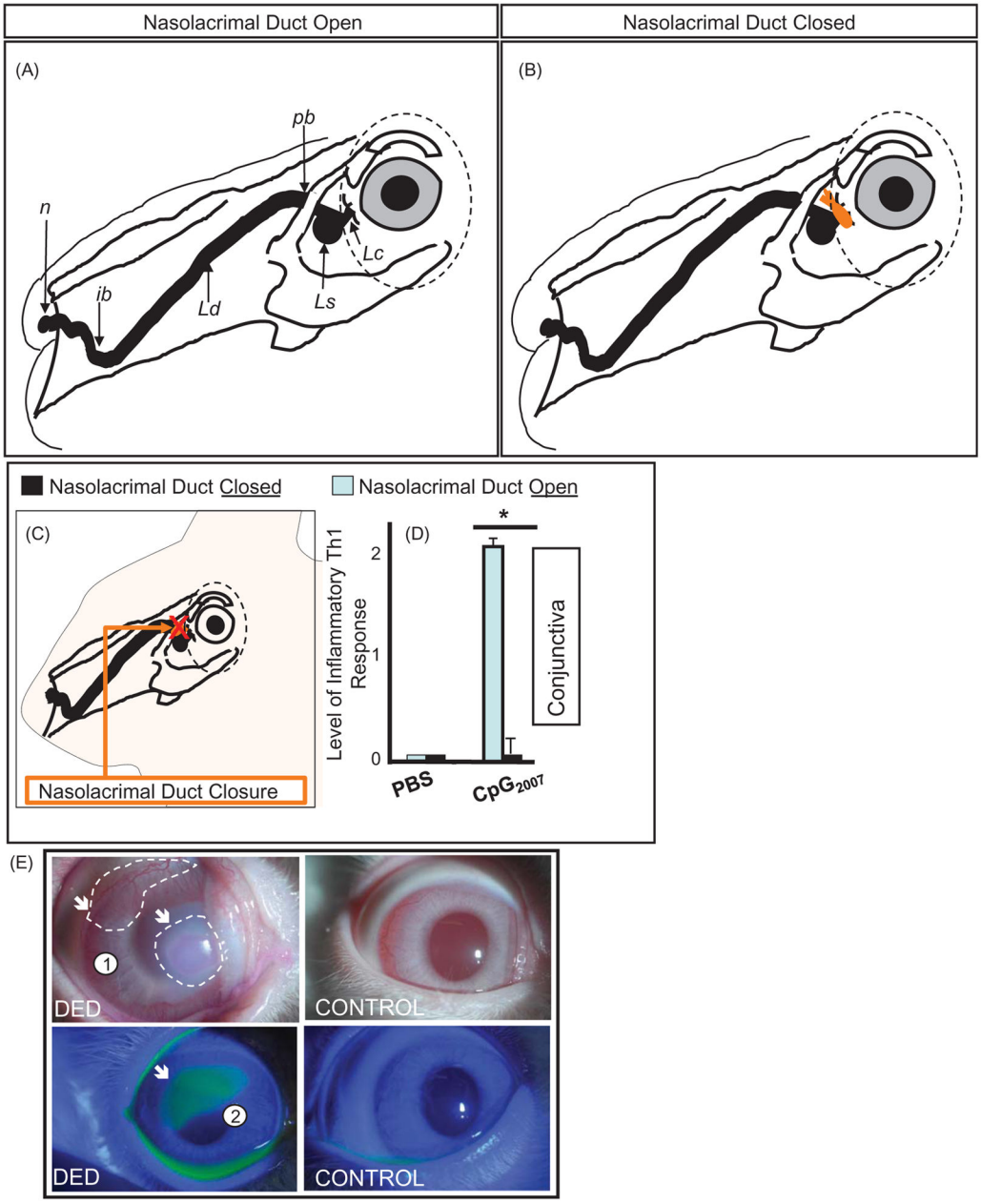

FIGURE 3.

Schematic representation of rabbit nasolacrimal system. (A) Nasolacrimal canal open and (B) nasolacrimal canal closed. The two sharp bends, the proximal maxillary bend (pb) and the bend at incisor tooth (ib), are indicated. The nasolacrimal canal system is composed of lacrimal canaliculi (Lc), lacrimal sac (Ls), nasolacrimal duct (Ld), and the nasal meatus (n). (C) Surgical closure of the nasolacrimal duct in rabbits (D) substantially reduces the level of IFN-γ produced by conjunctiva-derived inflammatory T cells following topical application of TLR9 agonist (CpG2007). (E) Representative images of old rabbit DED. Unstained (top) and fluorescein stained (bottom) rabbit corneas showing ocular surface epithelial disease (1), and altered corneal epithelial barrier (2) in old dry eye (left) compared with healthy control

young eyes (Control). Sustained corneal inflammation and disruption of the ocular surface epithelial barrier in old DED rabbits lead to corneal ulceration and scarring (2). See reference 82 for details.

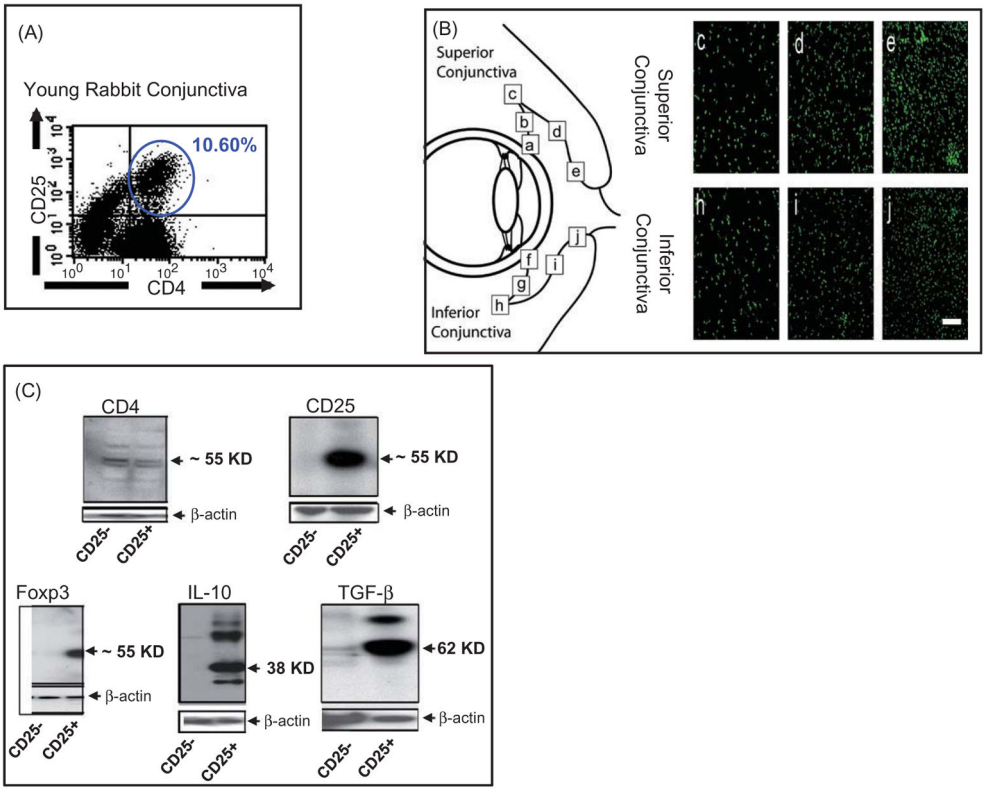

FIGURE 4.

(A) High numbers of $CD4^+CD25^+$ T_{reg} cells detected in conjunctiva of young rabbits. (B) $CD4^+CD25^+$ regulatory T cells are abundant in both superior and inferior healthy conjunctiva of young rabbits. (C) $CD4^+CD25^+$ regulatory T cells from young rabbit conjunctiva are Foxp3 positive and produce high levels of IL-10 and TGF-β. See references 93 and 95 for details.

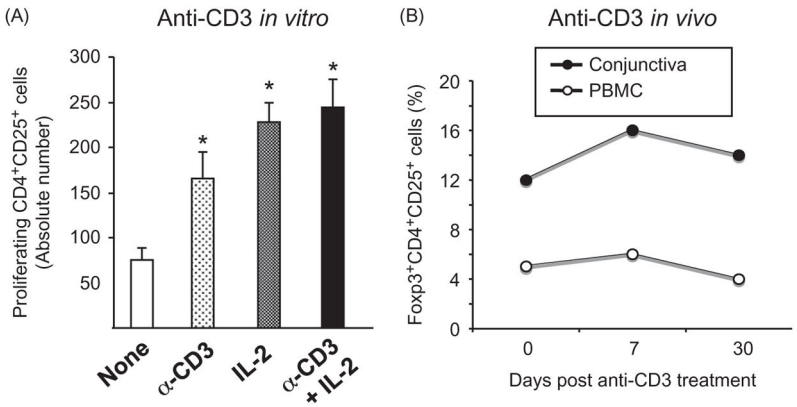

FIGURE 5.

Anti-CD3 mAb immunotherapy significantly induces the expansion of rabbit conjunctival $CD4^+CD25^+$ T_{reg} cells: (A) CFSE-labeled $CD4^+CD25^+$ T_{reg} cells ($5 \times b10^4$ cells per well) were left unstimulated (*None*) or stimulated *in vitro* with soluble anti-CD3 (1 μg/mL), IL-2 (5 ng/mL), or both in culture media for 6 days. Cells were harvested and stained with anti rabbit CD4-PE and CD25-FITC and analyzed by FACS. Values in each bar indicate the average number of proliferating $CD4^+CD25^+$ T_{reg} cells of two sets ± SD. *$p<.05$ when stimulated and unstimulated cells are compared. (B) Percentage of $CD4^+CD25^+$ T cells in inflamed conjunctiva and PBMC of rabbits receiving anti-CD3 treatment (3 doses, 20 μg each at 2-week intervals). The percentage of $CD4^+$ cells expressing CD25 is shown before (day 0), during (day 7), and after (day 30) treatment with anti-CD3. There was a significant increase in the percentage of conjunctival $CD4^+CD25^+$ ($p = .0016$) and peripheral $CD4^+CD25^+$ ($p<.005$) T cells at day 7 post-treatment.

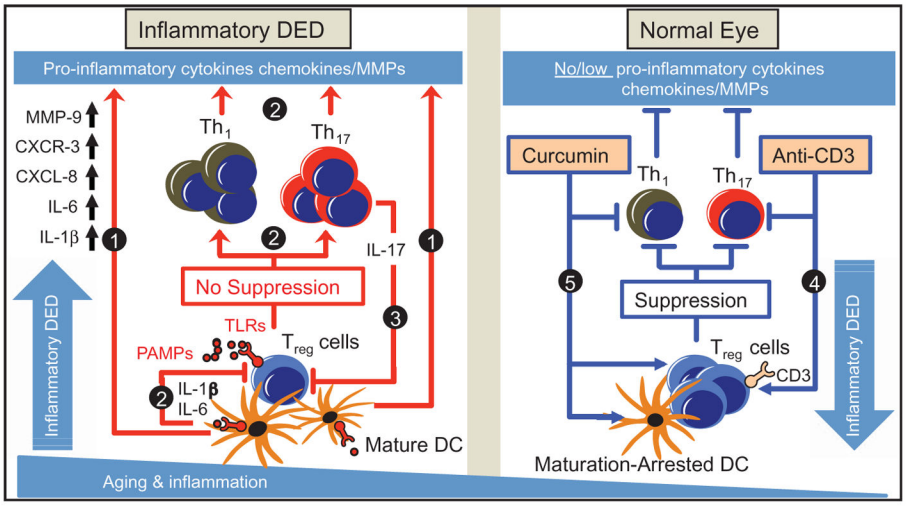

FIGURE 6.

Potential mechanisms of inflammatory DED in elderly (*left)* and development of novel targeted immunotherapies to alleviate severity of inflammatory DED (right). Desiccating stress induces (→) DC maturation through TLRs that produce relatively high basal levels of damaging proinflammatory mediators (IL-1β, IL-6, CXCL-8, CXCR-3, and MMP-9) (1). Proinflammatory cytokines (e.g. IL-1β and IL-6) reduce the suppressive function of $CD4^+CD25^+$ T_{reg} cells (|—), which fail to suppress the ongoing production of damaging proinflammatory cytokines/chemokines produced by $CD4^+$ Th1 and Th17 cells (2). Th17 cytokines/chemokines antagonize T_{reg} cell suppressive function (3) and a further expansion of proinflammatory Th1 and Th17 cells in ocular surface that leads to inflammatory DED. On the right, we hypothesize that (a) the mechanisms (1), (2), and (3) are exacerbated by age; and (b) subconjunctival injection of anti-CD3 mAbs (4), which eliminate activated Th1 and Th17 (|—) and enrich T_{reg} cells (→), and/or treatment with curcumin (5), which induces maturation-arrested DC, expands T_{reg} cells (→), and blocks Th1 (|—), would alleviate the severity of inflammatory DED in the elderly.