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Review Article

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Age-related Autoimmune Changes in Lacrimal Glands

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Abbreviations
EGF, epidermal growth factor; LG, lacrimal gland

ABSTRACT

Aging is a complex process associated with dysregulation of the immune system and low levels of inflammation, often associated with the onset of many pathologies. The lacrimal gland (LG) plays a vital role in the maintenance of ocular physiology and changes related to aging directly affect eye diseases. The dysregulation of the immune system in aging leads to quantitative and qualitative changes in antibodies and cytokines. While there is a gradual decline of the immune system, there is an increase in autoimmunity, with a reciprocal pathway between low levels of inflammation and aging mechanisms. Elderly C57BL/6J mice spontaneously show LGs infiltration that is characterized by Th1 but not Th17 cells. The aging of the LG is related to functional alterations, reduced innervation and decreased secretory activities. Lymphocytic infiltration, destruction, and atrophy of glandular parenchyma, ductal dilatation, and secretion of inflammatory mediators modify the volume and composition of tears. Oxidative stress, the capacity to metabolize and eliminate toxic substances decreased in aging, is also associated with the reduction of LG functionality and the pathogenesis of autoimmune diseases. Although further studies are required for a better understanding of autoimmunity and aging of the LG, we described anatomic and immunology aspects that have been described so far.

Keywords: Aging; Dry eye; Lacrimal gland

INTRODUCTION

With advances in medicine, vaccines and overall improvement in living conditions, the life expectancy of the population has increased considerably over the last century, and with it, the prevalence of age-related illnesses has also increased (1). Although the aging process begins from the day we are born, not all cells and organ systems lose function or age at the same rate. Although aging has been used as a synonym for loss of function that happens with chronological aging, each person may experience different declines or rate in function. Therefore, it is essential to differentiate between healthy aging from pathological aging. Pathological aging has been associated with increased low-levels of inflammation, and the term "inflamm-aging" has been coined (2). Downregulation of specific serum inflammatory markers such as IL-6 and TNF-α correlates with longevity (3,4).

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There are many theories of aging, usually divided into two general categories: 1) stochastic, related to somatic mutation, DNA repair, protein modification, mitochondrial defects, and oxidative stress, and 2) developmental genetic, which consider the mechanisms of aging as part of an elaborate process including longevity genes, accelerated aging syndromes, cellular senescence, neuroendocrine and immunologic alterations, and cell death. Many of these processes might be involved simultaneously in a complex multifactorial process involving the shortening of telomeres, inflammation, degenerative modifications, changes in DNA, and immunosenescence. It is well established that immune system function declines with aging despite a paradoxical increase in the incidence of autoimmunity. The elderly have lower responses to vaccines and increased susceptibility to viral infections (5). Many studies have demonstrated a reduction in B cells with aging in both mice and humans. Likewise, a decrease in naïve T cells and a parallel increase in memory T cell pool has been described, and it is thought to be secondary to thymus involution and increased survival of T cells in the periphery. Despite the decreased production of näive T and B cells in the elderly, the incidence of autoimmunity increases as we age (5,6).

The lacrimal gland (LG) plays a vital role in the health of the ocular surface by secreting water, growth factors and antimicrobial peptides that are critical for ocular surface (7-9). Aging remains one of the most established risk factors for developing dry eye (10,11). The ocular surface suffers many changes with aging: a decrease in goblet cell density, increased presence of inflammatory markers such as IL-6 and IL-8 in tears, a decline in Meibomian gland lipid production, Meibomian gland drop out and decreased tear volume, conjunctival and eyelid alterations (such as conjunctivochalasis, ectropion, and lid laxity) (12-14).

Because the LGs are highly susceptible to pathological aging, this review will focus on the known mechanisms of aging that affect them. We will highlight similarities and differences to Sjögren syndrome, an autoimmune disease that severely impacts LG, and causes the most severe forms of dry eye disease and is often used as a model for studying pathological dry eye.

SYSTEMIC DYSREGULATION OF THE IMMUNE SYSTEM IN AGING

Immunosenescence

The immune system declines gradually with aging, both innate and adaptive, mainly through a process called immunosenescence. It has been considered detrimental to the body due to the gradual accumulation of pro-inflammatory and inflammatory factors, which contribute to causing diseases such as cancer, autoimmune diseases and infections (15). The production of T cells and cytokines are also impaired with aging, mostly related to thymus involution (16-18). Aged human and elderly mice have compromised maturation and differentiation processes for B, T, and cells of the innate immune response (19,20). The major changes in aging are an increase in the number of memory T cells and a decrease in the number of naïve T cells, as well as diminished responsiveness of T cells to different antigenic exposures (2).

There are significant evidence corroborating changes in the adaptive immune response with aging. Over time, the composition of the T cell population shifts so that there is a decrease in the number of naïve T cells and an increase in memory T cells, which may explain the increased susceptibility to infections and diseases (5,17,21). Aging impairs this

sequence of events with changes mainly related to modulatory factors and, for this reason, can be linked to environmental modifications and lifestyle choices, such as nutrition and exercise (17,22).

Although aging leads to a decline in the normal function of $CD4^+$ T cells, Tregs have also been considered as inhibiting factor for the progression of autoimmune diseases (21,23). Increased pathogenicity and activation of aged T cells and a parallel increase in dysfunctional Tregs have been described in humans and mice (24-26). The latter seems to be a global finding in dry eye, as both inducible and naturally occurring animal models have shown increased pathogenicity of T cells and Tregs dysregulation (27-29). While it is logical that increased pathogenicity of T cells could be a consequence of dysfunctional Tregs, the literature has shown instead that activated aged effectors escape the control of Tregs, suggesting that an imbalance of effectors and dysfunctional Tregs participate in age-related diseases (24,27).

Several studies have shown alterations in signaling pathways and receptor functions with aging, such as a TCR, and TLRs. The significant changes leading to a decrease in the functional activity of T lymphocytes with aging are related to changes in transduction of TCR and CD28 receptors. There is also a decline in the expression of CD28, but not in TCR, although many studies have shown several defects in the cascade of cellular signaling even in the initial stages of TCR antigen recognition in human and mouse models (21,30). Cytokines play a significant role in the mediation of innate and adaptive immune response, controlling distinct functions related to cellular behavior, coordinating cell differentiation, proliferation, survival, apoptosis, and even gene expression, as well as many pathophysiological processes such as viral infections, autoimmune disorders, and inflamm-aging. Although many studies still need to be performed regarding cytokine receptor and signaling pathways in immunosenescence, cytokine changes during aging are well documented, such as a decrease in IL-2 production and the increase in IL-6. It has been reported that IL-2 and IL-6 receptors are altered in T cells and macrophages with aging, especially regarding the Janus Kinases and Signal Transducer and Activator Transcription intracellular signaling pathways.

Studies have shown that the qualitative and quantitative changes in the humoral immune response with age affect both the class of the antibody produced and its specificity. There is an increase in the level of mutations in Ig genes, probably related to its accumulation in the organism in aged humans (19,31). Although the diversity of Ig in B cells decreases with age, the proportion of somatic hypermutation of Ig remains the same in young and old individuals (32-34). Other studies in humans have also reported a decrease in the production of specific high-affinity antibodies against infectious agents with aging, partly due to failures in T cell signaling to B cells, CD28 expression, and reduction in somatic hypermutation and class switch recombination in the B cells of germinal centers (16,18,35). Similarly, because of the change in signal transduction with aging. There is a reduction of IgA production in aging, one of the essential antibodies that participate in mucosal immunity, followed by an increased susceptibility to infectious diseases (9). In mice, increased production of immunoglobulins has been reported and are considered to be either a normal process of increased B cell production or related to the abnormal production of autoantibodies (5,17,18). Our studies in the sera of C57BL/6J mice aged 8-wk and 15 months confirmed the existing literature showing increased Ig isoforms with aging (36), particularly IgA, IgG2b, IgG3 and IgM (**Fig. 1**). Several other immune cell types have age-related changes, and they have been well reviewed previously (17).

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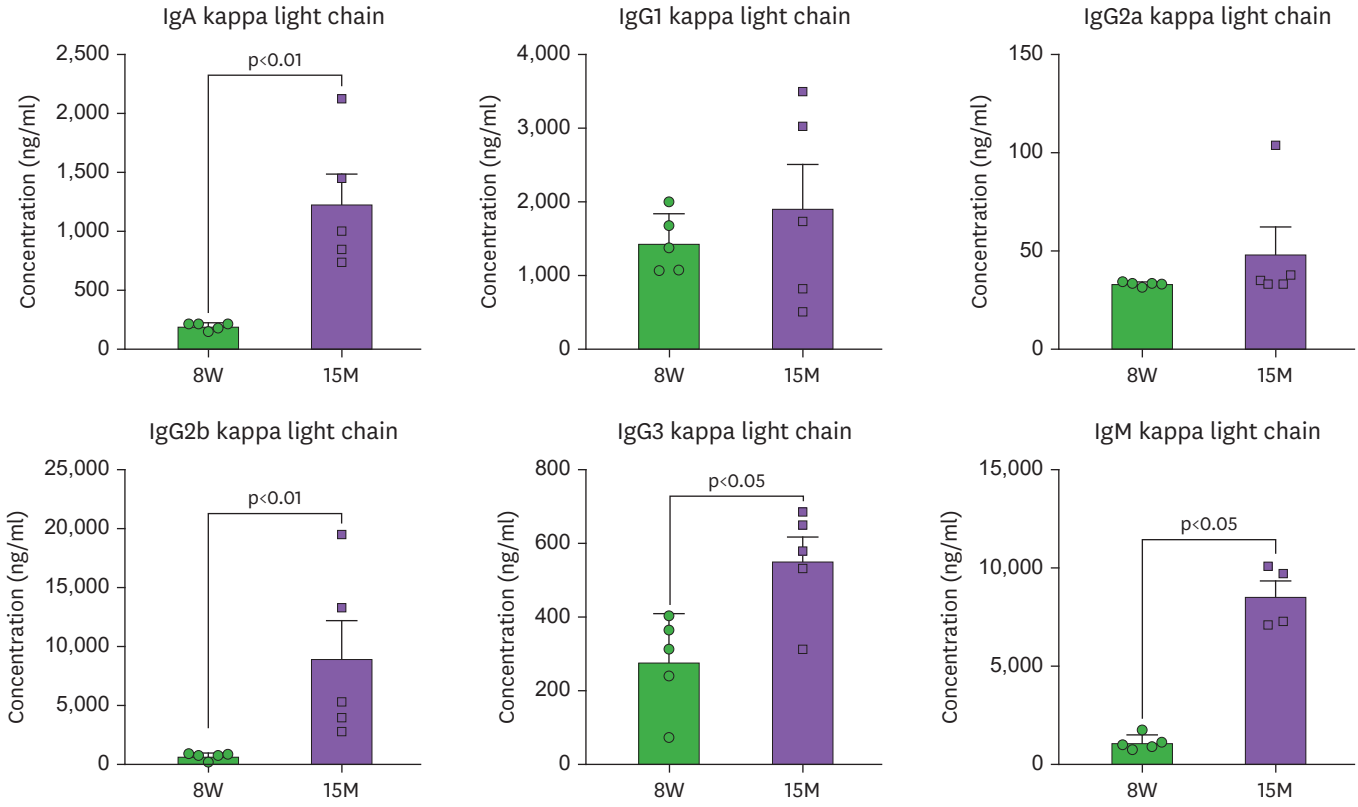

Figure 1. Aging is accompanied by a systemic increase in Igs. Sera from 8-week-old (8W) and 15-month-old (15M) female C57BL/6J mice were collected by cardiac puncture upon euthanasia and Igs were measured using Luminex assay (Mann-Whitney *U* test).

Inflamm-aging

Although inflammation with aging was initially thought of as purely a consequence of immunosenescence, more recent studies have shown a reciprocal pathway in which immunosenescence is induced by inflammation and vice versa (2). In this sense, inflamm-aging can be understood as a decrease with aging in the body's ability to deal with stressing molecules and a constant and progressive inflammatory status (2). Inflamm-aging has high mortality and morbidity as many age-related diseases have an inflammatory component. One feature of inflamm-aging is low grade and persistent chronic inflammation that may lead to tissue destruction (2). The pro-inflammatory status of aging is also caused by chronic activation of macrophages and lymphocytes. (2,21).

It has been postulated that decreased IL-2 production and T-cell proliferation in aged shifts the cytokine production from Th1 to Th2 lymphocytes by the adaptive immune system (3,25). In an attempt to compensate this imbalance, there is an increase in the production of cytokines derived from Th-1 lymphocytes, including IL-2 and IL-6, leading to this Inflamm-aging (21). Leukocytes of elderly persons produce higher amounts of IL-1, IL-6, IL-8, and TNF-α after induction with lipopolysaccharide than leukocytes from young donors (37). Increased serum levels of TGF-β in centenarians can be considered biomarker good health, while increased serum levels of IL-6 and TNF-α are predictors of disability and mortality and octogenarians and centenarians (38,39). IL-6 usually shows low or undetectable levels in most young people, progressively increasing around 50–60 years of age in both healthy individuals and pathological aging conditions, and high levels of IL-6 can be detected in centenarians (2).

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Autoimmunity and aging

One frequent finding in the aging population is the increase of autoimmunity, which is a failure in the body's self-tolerance system caused by the immune responses being abnormally triggered by antigens of the cells and tissues of the body itself. Peripheral tissues are rich in immune cells that promote immune surveillance. The LG is not different, and many immune cells have been shown to reside in normal, non-inflamed lacrimal tissue albeit these resident cells are not organized as seen in the intestine (40). These cells include T and B cells, macrophages, dendritic cells, mast cells, among others (40).

A classic example of autoimmunity is Sjögren syndrome, where lymphocytic infiltration is considered not only diagnostic criteria but also a pathognomonic sign (41). Sjögren syndrome is a chronic systemic autoimmune disease with relatively unknown etiology, more frequent in females in their fourth to fifth decades of life (9:1 female predisposition), with symptoms sometimes beginning many years before diagnosis. The primary targets are exocrine glands (salivary and LGs), resulting in dry mouth (stomatitis sicca) and dry eye (keratoconjunctivitis sicca). Changes in tear film composition, including chemokines, metalloproteinases, and inflammatory cytokines have been reported, in addition to increased T lymphocytes in the conjunctiva and LG in animal models and patients (42-46).

Interestingly, aging is an essential factor in genetic-driven models of Sjögren syndrome — from 2–12 months; see a very comprehensive list in (40); this suggests that even in predisposing genetic backgrounds, autoimmunity requires aging/time to develop. This age-effect in autoimmunity remains poorly understood, but it is generally accepted that it could be related to loss of immune tolerance with aging or could be related to an accumulation of autoreactive T cells that recognize a self-antigen, which is yet to be determined (47). Sex remains an essential variable for autoimmunity and dry eye (23,48,49). It is well known that women are predisposed to autoimmune diseases. This increased susceptibility of females compared to males can also be shown in animal models of autoimmune diseases, such as Sjögren syndrome, thyroiditis, autoimmune encephalomyelitis, systemic lupus erythematosus and diabetes (23,48). Theories for this increased prevalence range from differences in sex hormones to hormonal sex receptors, production of lipid mediators and a direct effect of XX chromosome in thymic regulatory genes such as autoimmune regulator gene (50,51). The female sex represents a higher risk of developing autoimmune diseases than other genetic or environmental risks discovered so far (52).

Another feature of autoimmunity that maybe is influenced by aging is the generation of autoantibodies. Autoantibodies are present in about 50% of patients with autoimmunity and, although the clinical application of autoantibodies is highly appreciated, further studies need to be done for a better understanding about the mechanisms related to their production and regulation in the immune system (53,54). The presence of autoantibodies in primary Sjögren syndrome patients (most common are anti-Ro/SSA and anti-La/SSB) correlates with a higher risk condition for the development of non-Hodgkin lymphoma. Other diseases are associated with different antibodies, such as anti-centromere antibodies with Raynaud's phenomenon, anti-mitochondrial antibodies with hepatic pathology (54,55).

AGING OF THE LG AND DRY EYE

C57BL/6J mice, the most widely used inbred mouse strain, spontaneously develop dry eye disease and LG infiltration with aging. These aged mice have all the hallmarks of dry eye disease, including corneal barrier dysfunction, loss of goblet cell, Meibomian gland dysfunction and increased cytokine expression (14,28,56,57). The ocular changes are visible as early as 1 year of age (28). However, only a few studies have examined the immunological changes with aging, and these are described below. Anatomic changes in the aged LG have been noted more frequently.

Anatomic changes in the aged LG

The lacrimal system is composed of the main LG, corresponding to approximately 90% of the tissue, and the accessory LGs (9,40). In humans, the main LG is composed by the orbital lobe, which lies in the lacrimal fossa on the anterior lateral part of the orbit, and palpebral lobe, in contact with the superior and lateral fornix of the conjunctiva and lies below the aponeurosis of the levator palpebrae superioris (9,40,58). Throughout life, the combination of exposure to numerous external agents and a senescent immune system may contribute to generating inflammation and structural changes in the gland, such as fibrosis, lymphocytic infiltration, ductal proliferation, cystic formation and decrease of their secretory power (9,40).

The appearance of the LG on gross specimen changes with aging. In young rats (3–5 month of age) LGs have a smooth pink color, while in elderly animals (20–24 months of age) it shows a more lobular aspect due to the infiltration of fatty and connective tissue and a very tan color (33). Macroscopic investigation of LGs from 24 month-old female C57BL/6J mice showed that numerous cysts are observed in about 20% of the cases (**Fig. 2A**, arrows). These cysts are easily ruptured when touched by forceps, and mucous secretion is then easily extruded. Histologic examinations showed that these are not real cysts, but dilated ducts (**Figure 2B**) Histological studies have reported critical age-related changes in the LG of humans, such as lymphocytic infiltration (mainly periductal), acinar atrophy, periacinar and periductal fibrosis, acinar atrophy ducts tortuosity and chronic inflammation (59). In a study done in aged rats, Draper and colleagues (60) showed that the typical acinar was predominantly composed of the serous type in young, converting initially for seromucous followed by a gradual transformation to mucous acini type in aged rats. Likewise, while young animal glands showed a high number of protein secretory granules, with aging, there is a gradual change in this pattern with more prevalent mucous secretory granules, showing a decrease in the secretory power in the LG and alteration in mitochondria shape (60) (**Fig. 3**).

According to Obata et al. (58), older women have diffuse atrophy compared to young women, which may suggest a correlation with keratoconjunctivitis sicca in post-menopause women. Also, pathological changes, such as interlobular ductal dilatation and periductal fibrosis, may be related to the LG dysfunction and decreased lacrimal film production (58). El-Fadaly et al. (61) have reported other significant changes with aging in the LGs, such as a decrease in the tear secretion and weight, and an increase in collagen area peri-acinar and periductal, ductal number and diameter, acinar area and density, and inflammation demonstrated by the elevated number of mast cells. We observed a significant decrease in the combined LG/body mass with aging in female C57BL/6J mice that can be found as early as 12 months of age (**Fig. 2C**).

Lipofuscin and lipofuscin-like pigments are considered one of the evidence of cellular senescence and autophagy failure (62,63). These autofluorescent structures can be derived

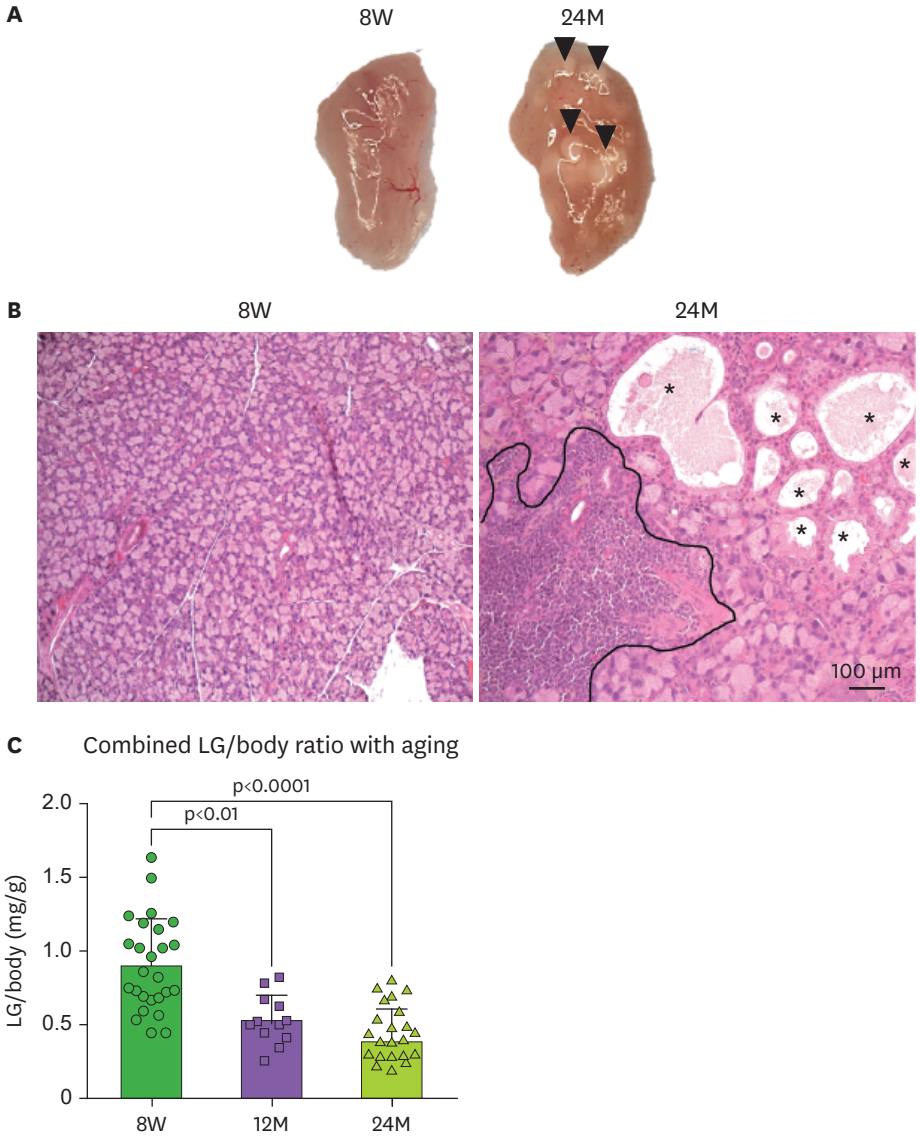

Figure 2. Pathological changes to the aged lacrimal gland (LG). (A) Macro images of 8-week (8W) and 24 months old (24M) female LG of C57BL/6 mice. Arrow heads indicate cysts. (B) Representative images of lacrimal gland sections stained with H&E. Areas of lymphocytic infiltration are demarcated in the 24M section. (C) Right and left LG wet weight/body ratio (n=19/group). One-way ANOVA followed by Sidak's multiple comparison test.
*Asterisks indicate enlarged ducts.

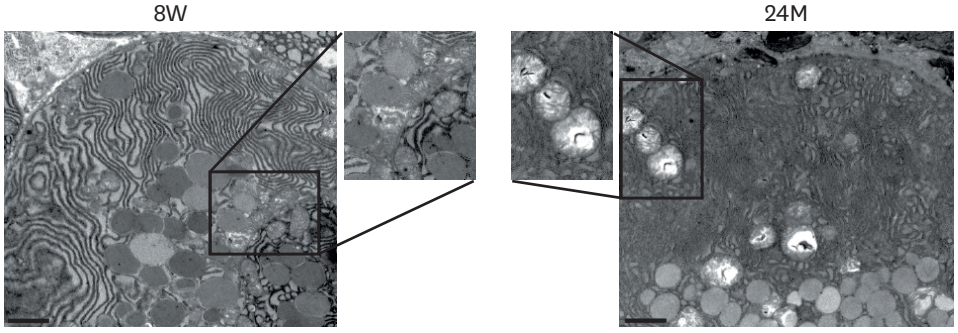

Figure 3. Transmission electron microscopic examination of lacrimal gland acinar of young (8W) and aged (24M) C57BL/6J female mice. Frequent marked structural changes in mitochondria (see insets) in aged mice were observed, including swelling and loss of cristae and disorganization. Increased number of mucous-containing granules were also observed (bar=04 μm).
8W, 8 weeks of age; 24M, 24 months of age.

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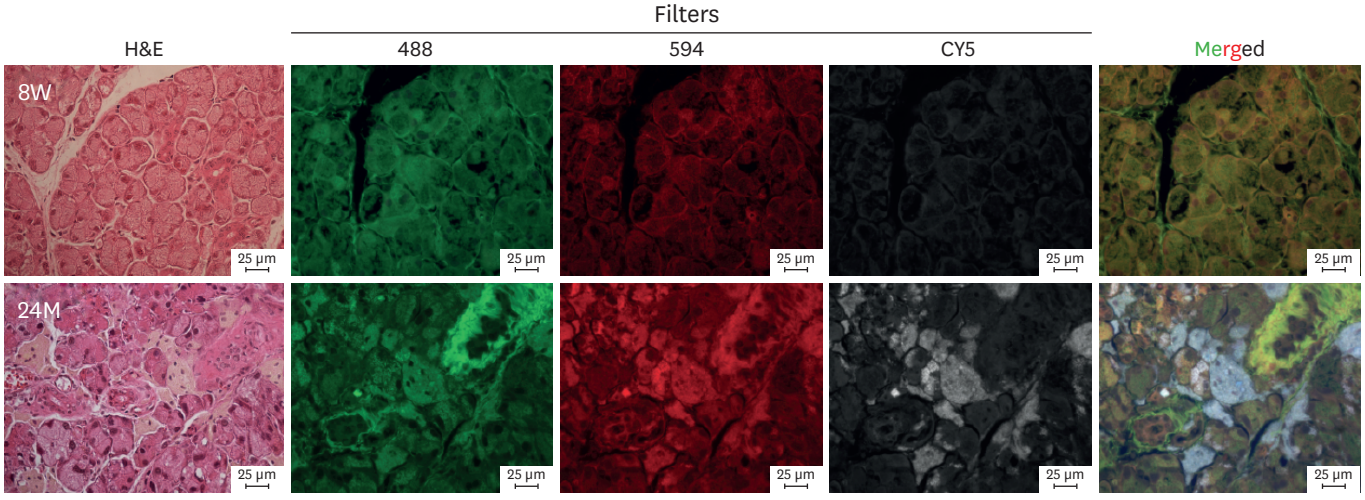

Figure 4. Lipofuscin and lipofuscin-like structures are increased in female aged C57BL/6J lacrimal gland. Paraffin-embedded lacrimal gland histologic sections were fixed in formalin and routinely processed in paraffin and stained with H&E. The same area was photographed with a color camera or with a fluorescent camera with the indicated filters. Autofluorescence in ducts is visible with the 488 and 594 filters, while distinct structures are autofluorescent with the 594 and CY5 filters. (bar=25 μm).
8W, 8 weeks of age; 24M, 24 months of age.

from the oxidative process of proteins, lipids and metal ions, and their accumulation impairs the efficacy of cellular proteolytic systems, events which are very frequent in aging (61,64). Lipofuscin and lipofuscin-like structures can make immunofluorescent studies challenging to be performed in the aged LG (64). One characteristic of these structures is auto-fluorescence across the spectrum; they can be visualized even when histologic sections are stained with hematoxylin and eosin (**Fig. 4**).

The LG is stimulated by both the sympathetic and parasympathetic nerves, and any impairment in the function of one of these systems can lead to a decreased secretion (64). The changes induced by aging initially occur by the reduction in the afferent corneal nerves function. The stimulation for the LG secretion can happen in different ways, such as light, temperature and mechanical and chemical stimuli, among others. Sensory nerves located in the cornea and conjunctiva are stimulated and send information up to the brain which then follows through efferent nerves of the sympathetic and parasympathetic systems to the LG (9,64,65). In this way, the glandular secretion is stimulated by adrenergic and cholinergic agonists released by autonomic nerves, such as acetylcholine, and noradrenaline, as well as vasoactive intestinal polypeptides. With aging, the innervation modulated by these three substances is considerably reduced (66). In a study done in mice, the secretory response of LG begins declining with increasing age at eight months, with a decrease occurring at 24 months (64). The reduction of Schirmer reflex with aging may occur due to the decline in functional structures of the LG, such as neurotransmitters or the secretory tissue itself (9). In agreement with this, it has been recently reported that substance p decreases with aging (67). Also, corneal sensitivity to chemical and mechanical stimuli also declines with age in humans and rodents (67,68).

INFLAMMATORY AND IMMUNE CHANGES IN THE AGED LG

Only a few studies have investigated the immunological alterations in the LG with aging. It has been reported that age-related LG inflammation is characterized by lymphocytic infiltration, destruction of parenchymal epithelial cells and increased secretion of

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inflammatory cytokines, ductal dilation, and glandular atrophy (27,28,64,69). An early infiltration of mast cells has been observed as early as eight months of age in rodents, followed by a late infiltration of CD4, CD8, B cells and regulatory cells (27,28,64). Flow cytometry analysis of the lymphocytic infiltration in the aged C57BL/6J LG showed a predominance of Th1 but not Th17 cells (69). This is of particular interest because IFN-γ can induce lacrimal and salivary gland acinar apoptosis (70,71), and on the ocular surface, can promote ocular surface epithelium metaplasia, blockade of goblet cell secretion and loss of goblet cells (56,72,73). 15-month-old IFN-γ knock-out mice are partially resistant to age-related goblet cell loss, indicating the importance of the Th1 pathway with aging (56). $CD4^+CXCR3^+IFN\text{-}\gamma^+$ cells accumulated in conjunctiva, draining nodes and LGs of 24-month old mice (69). Furthermore, adoptive transfer of aged $CD4^+CXCR3^+$ cells infiltrated LGs and induced goblet cell loss of immunodeficient recipients (69).

Similarly to Sjögren syndrome, it is unclear if the lymphocytic infiltration in the aged LG precedes or follows inflammation. A single injection of IL-1β into the LG was sufficient to induce an intense and reversible inflammatory response that leads to the destruction of LG acinar epithelial cells in young mice (65). A systemic cholinergic blockade is sufficient to cause upregulation of inflammatory cytokines and an increase in the presence of inflammatory cells in the LG; this is reversible after cessation of the cholinergic blockade (74,75). Interestingly, passive transfer of sera from patients with Sjögren syndrome has been shown to cause loss of LG secretion and lymphocytic infiltration in histologic sections (76). This is believed to be due to high levels of anti-muscarinic three receptor antibodies in the sera of these patients.

Due to the high prevalence of aging in dry eye, it is difficult to investigate the effects of aging without dry eye in humans. Some studies have identified an increase in inflammatory cytokines that correlates with aging. It has been shown that IL-8, IL-6, TNF-α, and RANTES (CCL5) concentration levels had a positive correlation (increase) with age in older subjects that did not have dry eye (12). At the same time aged tears have decreased levels of specific growth factors, such as insulin-like growth factor type 1 (13). The net result suggests a pro-inflammatory environment.

The immunoglobulin production by the LG also has sex-related differences in mice and human. While IgG and IgM are commonly found at low concentrations in human and rodents tears, IgA is usually found in high levels (77,78). IgA is produced locally in the LG, and its concentration in tears increases with age, more abundantly in males (78,79). The secretion of IgA peaks at four months of age in male rats, decreasing progressively with age, while its concentration in tear film increases with age (9). In female rats the peak of secretion happens around two months, remaining constant throughout life (9). The number of cells containing IgA in the LG reaches its peak at 3 months in both sexes remaining constant throughout life while the number of IgM-producing cells remains constant from birth (9). Bron and colleagues (40) have reported that the amount of IgA and the number of secretory cells of the immune system of the rat eye appear to remain constant with aging although, around the age of 40, infiltration of $CD4^+$ and $CD8^+$ T-cells causes progressive destruction of acinar and ductal tissue (9,40). It is speculated that the presence of IgG and IgM is secondary to plasma transudation or inflammatory processes. In support of this, it has been shown that there is an increase in IgM, which is a serum immunoglobulin, in tears collected from autoimmune mice at the meniscus (79). Using tear washings pooled from female C57BL/6J animals, we also observed an increase in IgM and a decrease in IgA/IgM ratio in tears washings from 24-month old mice (**Table 1**).

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Table 1. Tear parameters in young and aged female C57B/6J mice

Parameter/age	8W	24M	p value
Tear volume* (μL) (n=8)	0.07±0.01	0.15±0.07	0.0005
Body weight (g) (n=8)	20.0±1.12	40.3±6.70	0.01
Tear volume/body weight* (μL/g) (n=8)	0.0034±0.0007	0.0037±0.0010	0.65
Tear IgA† (pg/ml, n=4–8)	1,237±1,279	9,731±4,099	0.0006
Tear IgM† (pg/mL, n=4–8)	3.3±3.0	2,580±2,300	0.007
IgA/IgM ratio† (n=4–8)	325.8±250.8	4.53±2.7	0.03

8W, 8 weeks of age; 24M, 24 months of age.
*One sample equals mean the combined measurements from right and left eye from one mouse; †one sample equals tear washings pooled from the right and left eyes from ten mice (20 eyes = 1 sample; the 8W group had 8 samples while the aged had 4).

Altered lacrimal gland function with aging

Although it is recognized that the aging process promotes significant modifications in LG activity, such as increased numbers of antibodies and changes in lacrimal film production, many mechanisms remain uncertain (9). The tear film is composed of 3 layers: lipids, water, and mucins. Its quantity and composition also depend on other factors, such as drainage and evaporation and, with aging, secretion of proteins such as lipocalin, lysozyme, lactoferrin, and peroxidase decrease (9,60). These proteins can be produced and released by the LG in different ways, such as stored in granules in the apical portion of acinar cells, immediately released into the vessel lumen or attached to a cellular membrane (9,40).

Decreased tear secretion with LG aging is an essential factor for high dry eye levels in the population over 50 years of age (9,61), although it has been reported that a reflex tearing can happen in response to irritative processes on the ocular surface (79). Although tear volume can be used as an indirect measurement for the LG evaluation, studies in rodents have shown contradictory results regarding tear volume with aging (80,81). It has been postulated that increased tear volume measured at the tear meniscus is a sum of tears produced by the LG and secretion by the conjunctival epithelium. We have previously reported a paradoxical increase in tear volume with aging (28), but when we normalized the combined tear volume by body weight (**Table 1**), no such difference was observed. This indicates that tear volume in mice is not a reliable measure of LG function. Alterations on tight junction protein occludin have been reported in the cornea and bulbar conjunctiva of female aged C57BL/6J mice, suggesting a leaky ocular surface epithelium with aging that could explain the paradoxical findings resulting in increased tear volume (69).

Tear composition also changes with aging. In addition to the changes in cytokines as above mentioned, tear peroxidase and epidermal growth factor (EGF) also changes. Peroxidase is an enzyme that converts hydrogen peroxide, which is toxic to organisms, water, and oxygen. It can be found in a wide variety of organisms, like bacteria, plants, and humans, usually located in exocrine secretions, such as milk, saliva, and tears — the principal source of peroxidase. The enzyme activity also changes according to sex and age. In women, the activity of the peroxidase in the lacrimal fluid decreases significantly in the menopause, although it does not show significant changes afterward (82). Some studies have proposed the correlation with estrogen since the level of the hormone also declines. On the other hand, in men, their LG activity decreases only after 80 years of age (64,82). It has been shown that animals aged 8, 12, 24 months have a significant reduction of stimulated secretion of peroxidase in LGs compared to 3-month-old animals (64). EGF is a cytokine secreted by the LG and conjunctival epithelium that regulates proliferation, differentiation, and survival of epithelial cells (79). There are conflicting results about the levels of EGF in tears with aging,

with studies showing either increased or decreased levels. (28,83). However, studies in Sjögren syndrome have shown significantly low EGF concentration levels in tears (84).

Oxidative stress

Oxidative stress and inflammation have been shown to stimulate each other, and both processes have been described in aging (85-89). The ability to metabolize and eliminate toxic substances in the body is reduced as we age, decreasing cellular repair capacity and contributing to cellular degradation (90). An increase in the expression of oxidative stress markers was evidenced in the tear film and ocular surface of patients with dry eye, contributing not only to the severity of the disease but also clarifying its pathogenesis (91,92). Some findings in animal models suggest that an increase in oxidative stress in the LG with aging is directly related to the decrease in its functionality (93). Even in the context of adequate lacrimal film production, the increase in inflammatory mediators in the LG, cornea, and conjunctiva with aging are essential factors in the pathogenesis of ocular surface disease (79). In a study performed in aged mice, oxidative stress and inflammation played an essential role for the dysfunction of the LG by directly acting in the degeneration of autonomic nerves, and these effects were reduced with food restriction (94). Uchino et al. (93) demonstrated that oxidative stress in the LG causes dysfunctions in the tear film, among other alterations (93).

The aging process of the LG can promote the recruitment of lymphocytes and consequently secretion of cytokines, which can cause damage to the efferent nerves and the release of neurotransmitters. This is analogous to the principle of oxidative stress and damage are caused by free radicals, such as ROS. Cells of aerobic organisms, even under normal physiological conditions, develop a chronic state of oxidative stress because of a unbalance of antioxidants and pro-oxidants, as well as mutations in mitochondrial DNA that result in respiratory chain dysfunction and increased production of ROS (40). The oxidative stress occurs when the oxidants present in the body are incapable of neutralizing ROS usually generated by metabolic processes (95).

CONCLUSIONS

Aging is a complex process, and several considerations related to immunological changes should be made for a better understanding of its mechanism. With aging, the immune system shows a dysregulation in its activities, promoting immunosenescence and mainly affecting the adaptive immune response. Although most authors agree that deterioration with aging contributes to the onset of most diseases, such deregulation can also be understood as reflecting adaptive changes to an imperfect situation. The inflammation that occurs with aging involves a controlled and chronic balanced process. The understanding of these changes together is essential for a better approach and intervention in the inflammatory and autoimmunity alterations that accompany the aging of the LG.

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