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Age-related changes in ocular mucosal tolerance: lessons learned from gut and respiratory tract immunity

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Abbreviations: ACAID: anterior chamber-associated immune deviation; APC = antigen-presenting cells; DC = dendritic cell; DTH = delayed-type hypersensitivity; GC = goblet cells; M = months; OVA = ovalbumin

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OUTLINE

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Abstract

The ocular surface is the part of the visual system directly exposed to the environment, and it comprises the cornea, the first refractive tissue layer, and its surrounding structures. The ocular surface has evolved to keep the cornea smooth and wet, a prerequisite for proper sight, and also protected. To this aim, the ocular surface is a bona fide mucosal niche with an immune system capable of fighting dangerous pathogens. However, due to the potentially harmful effects of uncontrolled inflammation, the ocular surface has several mechanisms to keep the immune response in check. Specifically, the ocular surface is maintained inflammation-free and functional by a particular form of peripheral tolerance known as mucosal tolerance, markedly different from the immune privilege of intraocular structures. Remarkably, conjunctival tolerance is akin to the oral and respiratory tolerance mechanisms found in the gut and airways, respectively. And also similarly, this form of immunoregulation in the eye is affected by aging just as it is in the digestive and respiratory tracts. With aging comes an increased prevalence of immune-based ocular surface disorders, which could be related to an age-related impairment of conjunctival tolerance. The purpose of this review is to summarize the present knowledge of ocular mucosal tolerance and how it is affected by the aging process in the light of the current literature on mucosal immunoregulation of the gut and airways.

1. Introduction

Ocular surface disease is a broad term for dry eye and other illnesses affecting the front part of the eye such as Sjögren Syndrome (autoimmune dry eye), meibomian gland dysfunction (a disorder affecting the oil-producing glands located within the eyelids), post-refractive surgery dry eye, age-related dry eye, ocular graft-versus-host-disease, and others. Additional ocular surface disorders of relevance in the elderly are ocular allergy and microbial infections. Dry eye presents with ocular irritation, gritty sensation, and blurred vision. Ocular surface disease is a frequent reason to seek eye care [1], and using trade-off research techniques, severe dry eye has been compared to severe angina in terms of impact on quality of life by affected patients.[1-4] Dry eye is one of the most common eye diseases, with a reported prevalence of 5.5 to 15% worldwide. [5-7] Known risk factors for dry eye disease include aging, contact lens wear, female sex, and autoimmunity.[5, 8-12] Dry eye prevalence increases with every decade in women and men, although it is more prevalent in women.[5] Because it affects more women than men, both sex and gender differences have been implicated[13], although no definitive consensus has been achieved. Both innate and adaptive immunity play a role in dry eye pathogenesis and a vicious circle of inflammation is well established.[14]

The ocular surface is the part of the visual system directly exposed to the environment, and it comprises the cornea, the first refractive tissue layer and the only transparent tissue in the body, and its surrounding structures. The ocular surface has evolved to keep the cornea smooth and wet, a prerequisite for proper sight, and protected. To this aim, the ocular surface is a bona fide mucosal niche with an immune system capable of mounting a strong response to fight dangerous pathogens. Given the potentially harmful effects of uncontrolled inflammation leading to extensive fibrosis and corneal opacification, the ocular surface has several mechanisms to keep the immune response in check to preserve corneal clarity. This regulation is part of what is collectively known as peripheral tolerance because it is how the immune system differentiates self from non-self-antigens and prevents autoimmunity.[15] Specifically, the ocular surface is maintained inflammation-free and functional by a particular form of peripheral tolerance known as mucosal

tolerance[16], which is also at work in the gut and in the airways. Aging of the immune system, or immunosenescence, has been linked to increased frequency of infections, cancer, and autoimmunity in the elderly.[17, 18] For a comprehensive review of how aging affects the specific components of the ocular immune system, see Galletti and de Paiva.[19] Interestingly, a dysregulated immune response underlies many ocular surface disorders that become more prevalent in the elderly[19], suggesting mucosal tolerance in the ocular surface changes with aging. Consistently, immunoregulation in the gut and in the airways changes as we age. Thus, the purpose of this review is to summarize the present knowledge of ocular mucosal tolerance in the context of peripheral immune tolerance of the eye and how it is affected by the aging process. Because the eye is a unique organ that may be intricate to the immunologist unfamiliar with ocular anatomy, we will address peripheral immune tolerance mechanisms in its different sections. But since a considerable body of evidence has emerged from studies of gastrointestinal and respiratory disorders in which age-related immunoregulatory changes play a role, we will first review the literature to learn from other mucosal sites where mucosal tolerance was characterized first and to a greater extent. Our intent is to show differences and similarities in the immunoregulation of these three different mucosal sites through aging. We also want to highlight how age-related perturbations of ocular mucosal tolerance could participate in ocular surface disease development such as dry eye.

2. Aging and mucosal tolerance in the gut and airways

All mucosal sites (gut, airways, ocular surface) are exposed to the environment to a varying extent and thus need to cope with harmless and dangerous antigens of their own. The set of regulatory mechanisms by which the mucosal immune system does not react against the harmless foreign antigens it comes in contact with is known as mucosal tolerance. It is evidently not a passive phenomenon where the immune system "does not see" an antigen but follows a coordinated sequence of events where the antigen "is chosen to be ignored" by the immune system.

2a. Oral tolerance

Because any food is a foreign entity, the gastrointestinal tract has developed ways to cope with this interaction, that is, to tolerate the non-self-antigens that are derived from food protein. This regulation is termed oral tolerance and requires peripherally induced Foxp3$^+$ Tregs.[20] Oral tolerance is crucial for optimal health and modulation of the gut immune system, as shown by mice raised with an elementary diet devoid of dietary antigens: they become more susceptible to developing food allergy upon introduction of a new antigen in the diet than mice fed a conventional diet [21]. Oral tolerance can be evidenced in laboratory animals if they are fed an antigen before parenteral immunization with the same protein. When the antigen is absorbed in the intestines, the absence of inflammation and danger signals indicate to the mucosal immune system that the antigen is not a threat, and tolerance develops.[22] First, an antigen present in the lumen of the gastrointestinal tract needs to be captured by intraepithelial and lamina propria APCs; then, these APCs travel to the lymph nodes guided by the CCR7-CCL19/CCL21 axis, where they induce tolerogenic T cells.[23] Intestinal goblet cells have been shown to deliver antigens to CD103+ lamina propria DCs.[24] These goblet cell-associated passages are critical for oral tolerance as mice devoid of goblet cells do not develop tolerance to dietary antigens.[25] For a thorough discussion of the mechanisms underlying oral tolerance, see [26] and [20].

Factors such as age, dose, frequency, route of delivery, and intestinal microbiome influence oral tolerance and maintenance.[27-30] Either a single high dose or repeated smaller doses and oral administration of antigens are essential for oral tolerance development.[31] Also, intravenous or intraportal delivery of OVA does not induce the same immune response as orally administered OVA, showing that intestinal uptake of the antigen is crucial.[29] Another important factor is age. Several studies have shown that aged mice have decreased oral tolerance.[22, 29, 32-35] Oral OVA administration is sufficient to induce oral tolerance in young (8-week old) mice [22] but middle-aged and elderly (˜15.5 and 19-months old, respectively) mice are refractory.[34, 35] The humoral response to OVA is also impaired in aged mice. In some studies, a progressive decrease in the humoral response was observed in mice aged 9 months or older.[29, 35] In others, a lack of proper antibody levels was seen as

early as 6-8 months of age.[36] Interestingly, 15-month old mice that were orally immunized at an early age showed a comparable humoral response to young mice [37], suggesting that early vaccination is key for preserving proper antibody production in the elderly. Studies have also shown that aged mice (>20 months of age) have an exaggerated cellular and humoral response to orally administered OVA, suggesting that altered immune processes in the elderly might lead to autoimmunity and inflammation.[36] A decrease in DCs and changes in Peyer's patches architecture, seen as early as 6-8 months of age, and T cell dysregulation observed at 24 months have been implicated as mechanisms for disrupted oral tolerance.[36, 38, 39]

Instances when oral tolerance mechanisms fail can have mild clinical consequences, such as urticaria and skin rash, or become life-threatening situations with oral and laryngeal edema, anaphylaxis, and cardiac arrest. Common food allergens are peanuts, nuts, shellfish, and cow milk proteins[40], which trigger IgE production. IgE-mediated food allergies elicit mast cells and basophils that rapidly release histamine and vasoactive factors, leading to the symptoms of hives, angioedema, bronchospasm, and anaphylaxis. Chronic forms of oral tolerance disruption may be accompanied by vomiting, cramping, abdominal pain, and diarrhea. Besides food avoidance, oral immunotherapy (which involves induction of oral tolerance) has emerged as an option for treating certain allergies in children. For example, oral immunotherapy for peanuts entails giving small, escalating doses of peanuts to a child to increase the amount of food without triggering an allergic reaction. [41]

Food allergy is a burdensome health problem. Prevalence of food allergy is highest among children, with reported rates varying between 2% to 26%, depending on the population studied and the method used to define food allergy.[42, 43] High incidence and remission rates and over-reporting characterize this age group.[43] Although food allergies are more frequent in young individuals, they can occur at any age. In a nation-wide USA study[44], serological and clinical prevalence were highest in children 1-5 years (28.1% and 4.3%, respectively) and progressively declined with age, reaching values of 13.0% and 1.3%, respectively, in the 60+ age group. In another cross-sectional study of 109 people in a Hungarian geriatric nursing home (mean age 77 years), specific IgE to food allergens was

detected in 25% of residents and positive skin prick tests with food allergens correlated with chronic alcohol consumption.[45] Contrasting with children, the elderly tend to under-report food allergy symptoms as well as other allergies.[45-53] Despite the decrease in prevalence with age, it is evident that food allergy still represents a significant health issue in the elderly. Furthermore, food allergy in the young arises due to the immaturity of the gut mucosal immune system, and this correlates with animal studies: neonatal mice cannot develop oral tolerance to a fed antigen before 7 days of age.[54] Contrastingly, aged mice lose their ability to develop oral tolerance to newly introduced dietary antigens, as previously detailed.[22, 29, 32-35] Thus, food allergy in the elderly is less frequent but has a different underlying pathophysiology than in children[46]: there is loss of oral tolerance to dietary antigens instead of inability to develop oral tolerance to newly introduced dietary antigens.

Food allergy in the elderly relates to an impaired immune system (immunosenescence) and is compounded by numerous physiological changes, including a deficiency in iron, zinc, and vitamin D.[45-47] Gastric atrophy and anti-acid medication have also been associated with increased food allergy in the elderly, probably because of persistence of intact food allergens due to reduced digestive enzymatic activity.[55] Also, chronic alcohol consumption is linked to gastric atrophy and hypoacidity, pancreatitis, and a direct cytotoxic effect on the gastrointestinal mucosa (leading to inflammation and decreased barrier function), all of which may contribute to the increased association with food allergens in the elderly.[45] Interestingly, in the same study alcohol was not a risk factor for increased skin test positivity for respiratory allergens, suggesting that its effect is locally restricted to oral tolerance mechanisms and not to a generalized potentiation of Th2 responses.[45] However, this topic is controversial and there is inconsistency in several studies[56], warranting further research on the effect of drugs and medications on oral tolerance as we age. At any rate, as evidenced by food allergy presenting in the elderly, failure of oral tolerance in aging is not infrequent and the underlying mechanisms deserve additional study.

Inflammatory bowel disease is another group of disorders in which oral tolerance is affected.[57] Inflammatory bowel disease patients cannot develop oral tolerance to newly fed

antigens[58], exhibit signs of active immunization against common food antigens[59], and display non-tolerant immune responses against gut microbiota.[60] Although food-specific immunity is not involved in inflammatory bowel disease pathogenesis[61], loss of gut mucosal tolerance to food (i.e., oral tolerance) reflects a generalized disruption of the regulatory steps that suppress inflammation towards harmless microbiota antigens in the gut, which is the core pathophysiological mechanism of inflammatory bowel disease.[62] In line with this, restoration of oral tolerance to a food antigen (egg protein) by intravenous administration of antigen-specific Tregs and subsequent feeding of the same antigen (meringue cakes) showed results in a clinical trial involving inflammatory bowel disease patients.[63] Thus, inflammatory bowel disease, despite not having a higher prevalence in the elderly, still constitutes a remarkable example of how dysregulated mucosal tolerance can drive local inflammation in a mucosal site.[62]

Conversely, studies have shown that oral tolerance can be used to prevent or decrease some pathological states. Administration of heat shock proteins in experimental models of atherosclerosis either at the time of initiation or after moderate disease establishment can modify the size of atherosclerotic plaques through increased frequency of $CD4^{+}Foxp3^{+}$ Tregs. Aged (18-months) $ApoE^{-/-}$ mice immunized with mycobacterial heat shock protein 65 and subjected to a high cholesterol diet showed atherosclerosis progression. In contrast, oral administration of mycobacterial heat shock protein 65 before immunization decreased the extension of plaques and increased the frequency of splenic Tregs.[64, 65]

In summary, oral tolerance was the first mucosal tolerance mechanism described[40] and is the one most studied. Several factors affect how the gut immune system handles food-derived and other antigens through the aging process, resulting in decreased or abolished oral tolerance. Thus, aging profoundly impacts oral immunization, food allergy, and other gastrointestinal disease states. Furthermore, because of the systemic influence of the gut immune system[66, 67] and the shared aspects of the mucosal immune responses, some of these observations could also apply to other mucosal sites like the respiratory tract and the ocular surface.

2b. Mucosal tolerance in the airways

Due to the respiratory tract's continuous exposure to airborne antigens, mucosal tolerance is highly relevant as a peripheral tolerance mechanism in the airways.[68] It was first described in 1981[69], about 70 years later than oral tolerance.[40] More recently, its breakdown has been recognized as a key pathophysiological mechanism in allergic airway diseases like allergic rhinitis and asthma.[70] For example, psychological stress and cigarette smoking, two environmental factors linked to asthma severity in patients, directly impair respiratory tolerance in mice.[71, 72] Experimentally, both nasal and bronchial tolerance have been described in animals. Nasal instillation or inhalation of aerosolized antigens can lead to antigen presentation in cervical and peribronchial lymph nodes that drain the nasal cavity and the lower airways, respectively.[73, 74] This is possible because local DCs take up antigen in the mucosal linings and then migrate relying on CCR7 guidance to the draining lymph nodes.[74] The upper and lower airways harbor 4 different DC populations: epithelial $CD103^+$DCs (conventional DC1 or cDC1), stromal $CD11b^+CD24^+CD64^-$ conventional DCs (cDC2), monocyte-derived $CD11b^+CD24^-CD64^+$DCs, and plasmacytoid $B220^+$DCs (pDCs).[75, 76] Of these, cDC2[76] and pDCs[77] seem to be responsible for mucosal tolerance in the lungs.

Lung immune homeostasis depends on a network of interactions between immune cells that include the airway epithelium, macrophages, neutrophils, and tissue-resident lymphocytes, among other cell types [for a thorough review, see [78]]. Contrasting the studies on oral tolerance in young and aged animals (see previous section), no direct assessment of respiratory mucosal tolerance in older individuals or animals has been published. However, many studies have explored the effect of aging on the pulmonary immune response.[78] Aging dysregulates cytokine production in lung epithelial cells favoring pro-inflammatory interleukin-1β and interleukin-6 release[79], and conversely, DCs from aged subjects contribute to airway inflammation by activating bronchial epithelial cells.[80] Of note, aged DCs have higher nuclear factor-κB activity[81], which is inversely associated with their tolerogenic potential.[82] The latter and other observations add to the dysfunction of DCs that comes with age, which favors inflammation and loss of tolerance.[83] Also, the aged lung microenvironment leads to a reduction in tissue-resident alveolar macrophages, which are

better at resolving inflammation after injury.[84] In a comparison of young and aged mice sensitized with ovalbumin (OVA) as an antigen and then challenged with the same antigen in the airways, the older mice developed less airway hyperresponsiveness but more inflammation, eosinophilia, goblet cell hyperplasia, and interferon-γ.[85] Thus, just as is the case for oral tolerance, respiratory tolerance is highly likely to be affected by aging, because many of the mechanisms that underlie the tolerant mucosal immune response are similarly changed by aging in the gut and the airways. However, specific animal studies about the effect of aging on respiratory tolerance are missing.

Allergic airway disease is traditionally associated with young age, but it remains highly prevalent (5-10%) throughout life.[86] In a study of asthmatic patients over 60 years of age, 10% had first developed asthma after their 60th birthday.[87] Asthma in the elderly has distinct clinical features that have been linked to oxidative stress and inflammaging, such as increased neutrophilic infiltration and less atopy.[88] Similar considerations apply to allergic rhinitis in advanced age[89], which are probably associated with an increase in Th2 responses in the elderly.[90] In a Finnish study of 8,000 respondents, incidence of allergic asthma (defined by accompanying allergic rhinitis) decreased with age, but the incidence of non-allergic asthma peaked in adulthood.[91] In another large European multicenter study, occupational exposure was a significant risk factor for the development of new-onset asthma in adults.[92] As in both studies the subjects did not experience asthma during childhood, it is tempting to speculate that changes in respiratory tolerance through aging could be implicated in this phenomenon. In line with this, IgE sensitization to cat allergen (a known aeroallergen) was associated with the development of new-onset asthma in a cohort of aged men (mean age 61) followed for 3 years.[93] Also, in another study of elderly (60+ years) asthma patients, 33% were positive for cat-specific IgE and 53% were positive for at least one indoor allergen, that is, antigens to which they were almost continuously exposed.[94] In the already mentioned study of a Hungarian geriatric home population, 40% of residents were positive for IgE specific for one or more of the 19 respiratory allergens tested, and this trait was associated with smoking.[45] In fact, smoking constitutes a risk factor for IgE sensitization in aged subjects.[95] Tobacco smoke activates several signaling pathways in

bronchial epithelial cells, most prominently the nuclear factor-κB pathway, which triggers a proinflammatory response[96] and is directly involved in mucosal tolerance induction or abrogation in all mucosal surfaces. [97]

Taken all together, the findings summarized above support the notion that aging impairs respiratory tolerance, as it has been firmly established for oral tolerance. Specific studies are needed on the actual extent of respiratory mucosal tolerance loss with aging and how it modulates allergic airway disorders because of the high translational impact.

3. Peripheral immune tolerance in the eye: is it all the same?

Since Medawar's observations that allogeneic skin grafts implanted in the anterior chamber are not rejected [98], it has been clear that the eye controls the immune response within its domains. This feature, shared with the brain and the testes, has been termed immune privilege.[99-102] Unsurprisingly, immune-privileged sites are operationally defined as those where foreign tissue grafts survive indefinitely, contrasting with non-privileged sites where such grafts undergo rapid immune rejection. This property indicates the existence of active regulatory mechanisms that suppress immune responses, explaining how corneal allografts in patients do not require systemic immunosuppression to remain viable. Immune privilege could thus be interpreted as a site-specific form of peripheral immune tolerance[15], i.e., the set of mechanisms through which the immune system differentiates self from non-self-antigens and prevents autoimmunity. In the laboratory, the immune privilege of the eye can be evidenced by a reaction termed anterior chamber-associated immune deviation (ACAID): when an antigen, e.g., OVA, is injected into the anterior chamber of the eye, it sets in motion an immune response that has unique features and a systemic reach. An equivalent reaction seems to occur in ocular varicella-zoster patients.[103] Remarkable progress has been made on the molecular and cellular basis of these observations beyond the scope of this review and to which we will refer collectively as intraocular immunology [see [104]].

Despite the stark anatomical differences, there are many unifying aspects in the immunology of the anterior and posterior segments of the eye[99], the most important of which

is the already mentioned existence of "immune privilege as the result of local tissue barriers and immunosuppressive microenvironments."[105]. A comparable posterior segment equivalent of ACAID has been described as vitreous cavity-associated immune deviation[106], and allogeneic retinal grafts placed in the vitreous cavity or the subretinal space are not rejected.[107] Recently, evidence of immune surveillance in the lens has surfaced.[108] Unfortunately, this abundance of knowledge on intraocular immunology has come with the notion that everything related to the eye also bears immune privilege, which is incorrect and does not apply to the ocular surface (Figure. 1). This is a well-known fact in the clinic since limbal allografts for ocular surface reconstruction, like other solid organ transplants, require HLA typing and systemic immunosuppression.[109] Also, subconjunctival tissue allografts in mice are quickly rejected and lead to immunization.[107]

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placeholder Figure. 1

Although the ocular surface is not immune-privileged, it exhibits another form of peripheral immune tolerance shared with every other mucosal site: mucosal tolerance.[16, 20, 110-112] Mucosal tolerance is critical for immune homeostasis because the mucosal surfaces in the gut, airways, and the eyes serve as barriers to the environment. Therefore, these sites face a dilemma: to cope with commensal microbes, food, and airborne particles while at the same time to attack invading pathogens.[20, 111] Through mucosal tolerance, these organs actively suppress the potential immune response against the myriad harmless antigens to which they are continuously exposed, remaining functional to absorb nutrients, exchange air, or refract light rays. Contrasting with immune privilege, mucosal tolerance can be operationally defined as the active suppression of systemic immunization against a specific antigen if such antigen is administered through a mucosal surface before the immunization.[20]

Mucosal tolerance in the ocular surface can be evidenced by an assay similar to that of ACAID, although the underlying immune mechanisms are different (Table 1).[16, 113] If a

harmless antigen (one that does not elicit an inflammatory response in and of itself) is applied to the ocular surface of mice, it is taken up by antigen-presenting cells (APC) that migrate to the draining cervical lymph nodes, where they present it to naïve T cells.[114] In homeostatic conditions, these APCs do not sense danger-associated signals from the microenvironment along with the antigen, so they have a tolerogenic (anti-inflammatory) program imprinted on them. Thus, when they interact with their cognate naïve T cells, the APC delivers additional signals that induce the T cells to differentiate into regulatory T cells (Treg). Very few antigen-specific Tregs are involved in this response, but they are potent enough to impact immune regulation profoundly. The assay further exploits this aspect to evidence the presence of the Tregs. If these tolerized mice are then injected subcutaneously with the same antigen mixed with an adjuvant that promotes a strong response in naïve animals, the antigen-specific Tregs will suppress the process, leading to poor immunization. Thus, when the tolerized mice are later on challenged by either subcutaneous or intradermal injection of the same antigen alone, instead of a vigorous localized hypersensitivity response that peaks after two days, a small reaction (measured by swelling) develops. This challenge reaction is known as delayed-type hypersensitivity (DTH) and is akin to the purified protein derivative (PPD) skin test for tuberculosis diagnosis.[115] It involves a recall cellular response to an antigen mediated by local uptake by tissue-resident APC and presentation to effector $CD4^+$ T cells that release T helper (Th)1 cytokines, thus amplifying inflammation.[37] In tolerized mice, the previously generated Tregs suppress this reaction, hence the reduced swelling readout. It should be emphasized that the DTH assay used to evidence mucosal tolerance in immunological research is just a tool to assess the presence of either antigen-specific Tregs or effector T cells. For a thorough review of the mechanisms underlying ocular mucosal tolerance, see Galletti *et al.*. [16, 19]

4. Conjunctival tolerance and aging

As in the respiratory and intestinal mucosa, delivery (i.e. instillation) of an antigen to the ocular mucosa leads to tolerance, that is, the generation of Tregs and absence of clinical inflammation signs upon subsequent administration of the antigen. Ocular mucosal (i.e. conjunctival) tolerance was first described in 1994[121] and characterized a few years later[110],

but its role in ocular pathophysiology was only addressed recently.[122-124] Ocular surface Tregs, which underlie mucosal tolerance, have been reviewed elsewhere. [125]

Disruption of ocular mucosal tolerance has been described in several ocular disease models. First, it was observed after topical instillation of benzalkonium chloride.[122] Benzalkonium chloride is a common preservative in eye drops, which are associated with ocular surface toxicity. [126] Mechanistically, loss of mucosal tolerance to an exogenous harmless antigen explains the increased incidence of ocular allergy, secondary dry eye, and discomfort caused by this preservative.[127] Moreover, benzalkonium chloride-induced models of dry eye have been described in mice[128] and rabbits[129], with the accompanying loss of conjunctival goblet cells, corneal epithelial death, and $CD4^+$ T cell activation.[130] Perhaps unsurprisingly, conjunctival tolerance is also affected by a corneal alkali burn[131], as this model is associated with extensive ocular surface damage and epithelial disruption.

Impaired conjunctival tolerance to a harmless antigen was also reported in various models of dry eye,[123, 124, 132-134] an autoimmune disease for which the specific antigens remain uncharacterized but that can be reproduced in naïve mice by adoptive transfer of pathogenic $CD4^+$ T cells.[135-138] In the laboratory, dry eye can be modeled by different methods. First, there are autoimmune animal strains that spontaneously develop eye and lacrimal gland alterations at young age. [139-142] Also, it can be modeled by subjecting young mice to desiccating stress, that is, low humidity conditions with or without cholinergic blockade of lacrimal gland secretion.[143-145] Surgical excision of one or more of the glands that contribute to the tear film in mice also causes ocular surface desiccation and a dry eye phenotype comparable to the others methods.[146] Remarkably, loss of conjunctival tolerance is another unifying feature of all these disease models. Of note, in the induced dry eye models, mucosal tolerance to harmless antigens is impaired not immediately but after three days of desiccating stress, suggesting that there is a threshold of ocular surface damage that must be surpassed before this immunoregulatory mechanism is overcome.[132, 133] Interestingly, middle-aged and elderly wild-type mice spontaneously develop dry eye disease, displaying loss of conjunctival goblet cell density and corneal barrier disruption (hallmarks of dry eye) as early as 9-12 months of age.[147, 148]

The putative antigens targeted by the pathogenic $CD4^+$ T cells that drive the disease in dry eye remain elusive, although some studies have implicated kallikrein proteins.[149-151] In experimental studies, this limitation is usually overcome by introducing a known harmless foreign antigen (e.g., OVA) to the ocular surface as a surrogate ocular surface-derived antigen. In an attempt to understand if changes in conjunctival tolerance also participate in age-related dry eye, we evaluated conjunctival tolerance to OVA in mice of three different ages (2, 9, and 24 months of age, Figure. 2A) following established protocols.[110, 122, 127, 132, 133] Young mice that received OVA eye drops for three consecutive days before immunization displayed less edema in the ears (low DTH), showing that they developed mucosal tolerance to OVA (Figure. 2B). Interestingly, the 9-months-old group did not show a statistical difference in ear thickness when exposed previously to OVA eye drops, that is, did not develop conjunctival tolerance to OVA. The literature shows that loss of oral tolerance to OVA is already present in mice aged 6-8 months.[36] Concordant with previous studies, the elderly group (24 months of age) did not show an adequate response to immunization[18, 152], making the interpretation of the effect of prior topical OVA eye drops difficult in this age group. Furthermore, an increased frequency of $CD4^+Foxp3^+$ cells in ocular draining lymph nodes has been reported, and a numerical increase in these cells might be compensating[153] for a qualitative effect in this age group. Another factor to consider is the DTH readout itself, which is used to evidence antigen-specific memory T cells. However, cutaneous immune responses (as is the case for the DTH) are dependent on adequate antigen presentation by skin APCs, which are also decreased in the aged.[154] Altogether, these results suggest that conjunctival tolerance is impaired by aging, as is also the case for oral tolerance.

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placeholder for Fig. 2

Many changes in the aged ocular surface immune system may favor tolerance disruption [for a complete review, see Galletti and de Paiva [19]]. In the gut, retinoic acid-loaded APCs participate in tolerance induction. Interestingly, a decrease in conjunctival

aldehyde dehydrogenase activity (a critical step in retinoic acid metabolism) in APCs and a higher number of activated APCs is observed in the aged conjunctiva.[155] This is accompanied by an increasingly inflammatory milieu *(*elevated *interleukin-1β, MHC II, interferon-γ,* and *interleukin-12* mRNA transcripts). Aged APCs obtained from ocular draining nodes have an activated phenotype and prime preferentially Th1 cells in antigen-presentation assays *in vitro*.[155] Goblet cells constitute an epithelial cell subpopulation that is highly immunoregulatory in the ocular surface[156] and pivotal in mucosal tolerance induction in the gut.[24, 25] Conjunctival goblet cells also experience age-related changes in humans and mice.[19] Mice deficient in conjunctival goblet cells have defective ocular mucosal tolerance[123, 124] and spontaneously develop dry eye[123, 157], which also suggests a mechanistic association between ocular mucosal tolerance loss and dry eye pathogenesis. Studies of conjunctival tolerance in Sjögren-Syndrome-like mice are lacking in the literature. Aged lacrimal glands also display lymphocytic infiltration.[147, 148, 158] All the aforementioned mechanisms at work in the aged ocular surface have been linked to decreased or altered Treg generation and impaired mucosal tolerance induction in other tissues.[25, 123, 124, 159-161] Thus, it is possible that a combination of a pro-inflammatory milieu, immunosenescence, and age-related changes in APC, goblet cell loss, and altered Tregs influence conjunctival tolerance. Disrupted conjunctival tolerance, in turn, may favor disease onset or progression in the elderly. In line with this, pharmacological inhibition of nuclear factor-κB activity in the ocular surface epithelium restores mucosal tolerance in two dry eye models while improving the disease phenotype, further evidence of a pathophysiological link.[132, 133] Remarkably, 12-14-months-old mice display corneal staining phenotype and respond more slowly to topical corticosteroids when subjected to experimental dry eye using the desiccating stress model.[162, 163]

Thus, a breakdown in ocular mucosal tolerance to harmless antigens seems to be a constant feature in diverse ocular surface disease models (benzalkonium chloride eye drops, desiccating stress, lacrimal gland excision, mice devoid of goblet cells), and remarkably, impairment of this homeostatic mechanism also occurs with aging. In humans, advanced age is associated with an increased prevalence of several ocular surface

disorders, among which dry eye is the most prominent. However, clinical data of ocular surface disorders in the elderly does not always follow clear-cut categories, in part due to symptom overlap between presentations. For instance, allergic conjunctivitis represents a significant fraction (16%) of referrals of elderly patients for allergic disease[47], but it is under-reported or under-recognized because ocular symptoms are considered part of rhinoconjunctivitis.[164] Also, more subtle, chronic allergic reactions in the ocular surface of aged patients may be misinterpreted because of concurrent use of topical eye medications (with preservatives) and/or mistaken for dry eye.[126] Also, patients with allergic rhinoconjunctivitis have increased tear osmolarity[165], a finding implicated in dry eye pathogenesis.[14, 134] Conversely, dry eye patients are more likely to be sensitized to known allergens and report symptoms typically associated with allergic rhinoconjunctivitis.[166] In addition, the diagnosis of "elderly onset Sjögren syndrome," a severe form of dry eye, is controversial, with some groups suggesting that the signs and symptoms are only related to aging of the immune system, while others affirming that is it indeed autoimmunity and should be treated as such.[49-53] At any rate, dysregulated ocular mucosal tolerance underlies the corresponding animal models for all these presentations, including aging, which underscores its pathogenic contribution. Still, much remains to be learned about the pathogenic mechanisms specific to the aged ocular surface and its diseases.

5. Conclusions

The ocular surface immune system is radically different from that of inside the eye globe. Instead of immune privilege, another form of peripheral tolerance is in effect to keep inflammation in check in the outer ocular structures: mucosal tolerance. Conjunctival tolerance is akin to the mucosal tolerance mechanisms found in the gut and airways, and also similarly, this form of immunoregulation in the eye is affected by aging just as it is in the digestive and respiratory tracts. Although the extent of the experimental evidence and clinical data for each location differs greatly, these three mucosal sites reviewed here experience dysregulatory changes with aging that result in loss of mucosal tolerance, a

highly relevant homeostatic function for mucosal health. The best case can be made for the gastrointestinal tract, where there is ample experimental and clinical data supporting the pathophysiological implications of oral tolerance loss in the elderly. In the airways, there is also extensive clinical evidence suggesting disruption of respiratory tolerance in aged subjects and there are several mechanistic studies in animal models that support this notion, but conclusive exploration of mucosal tolerance status in the airways of aged mice is lacking.

The case for the ocular surface, which is the actual purpose of this review, is further complicated by the fact that the putative autoantigens of its most prominent immune disease, dry eye, remain unidentified. There is considerable evidence of mucosal tolerance disruption in several animal models of ocular surface disease, and here we also present new data on how aging affects experimental induction of conjunctival tolerance in mice (Figure 2). As for the gut and the respiratory tract, there are also numerous mechanistic studies on the effect of aging on specific components of the immune response of the ocular mucosa.[19] Perhaps gut immunology, and more specifically inflammatory bowel disease, could serve as a guide to future research into the mechanisms of dry eye in the elderly, given the similarities outlined in this review and elsewhere[16] between the two mucosal sites and immune-based mucosal disorders, respectively. Thus, progress in the eye field could be made by applying current knowledge of age-related changes in other mucosal sites.

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Table 1: Comparison between ocular mucosal tolerance and immune privilege

	Ocular mucosal tolerance	**Ocular immune privilege**
Anatomical location	Ocular surface	Cornea, anterior chamber, vitreous chamber, subretinal space
APCs involved	$CD11c^+$ dendritic cells [114]	$F4/80^+CD11b^+$ macrophages
Route of APC exit from the eye	Lymph (CCR7-dependant chemotaxis) [116, 117]	Blood [104]
Location of antigen presentation	Eye-draining lymph nodes[114]	First thymus [118], then spleen [119]
Mechanism of antigen presentation	*Lymph node:* Tolerogenic $CD11c^+$ DCs present antigen to naïve $CD4^+$ T cells	*Thymus:* $F4/80^+$ macrophages present to NKT cells through CD1d
		Spleen: $F4/80^+$ macrophages transfer antigenic peptides to marginal zone B cells, then B cells present them on MHC I and MHC II to $CD8^+$ and $CD4^+$ T cells. NKT and γδ T cells are required.

Result of antigen presentation	Induction and expansion of antigen-specific $CD4^+Foxp3^+$ Tregs	Induction and expansion of antigen-specific $CD4^+$ $Foxp3^+$Tregs and $CD8^+$ $Foxp3^+CD103^+$ Tregs [120]
Functional result	Systemic suppression of antigen-specific effector T cell responses	Systemic suppression of antigen-specific effector T cell responses

Figure legends

Figure 1. Peripheral tolerance in the eye. The eye globe is delimited by the cornea anteriorly and the sclera posteriorly. Within the eye, the lens separates the anterior chamber (green-filled) from the vitreous cavity (orange-filled). The retina lines the inner surface of the back of the eye globe, and the subretinal space is the virtual space between the neuroretina and the retinal pigment epithelium. All these tissues and structures within the eye globe are regarded as intraocular and have immune privilege, a site-specific form of peripheral tolerance with unique features such as blood-borne antigen-presenting cells (APC) reaching the thymus and spleen. Specific descriptions in the literature of intraocular immune privilege for some intraocular structures are shown in green with their corresponding abbreviations (ACAID: anterior chamber-associated immune deviation, VCAID: vitreous chamber-associated immune deviation, SRAII: subretinal space-associated immune inhibition). By contrast, the ocular surface (pink) is a collective term for the exposed portion of the eye and comprises the cornea, the conjunctiva (the mucosal lining surrounding the cornea that extends to the inner surface of the eyelids), the eyelids, and other tissues and structures not depicted. The ocular surface is regarded as extraocular and, from an immunological viewpoint, it exhibits mucosal tolerance: a peripheral tolerance mechanism common to every mucosal lining that is based on antigen presentation in the lymph nodes and regulatory T cells.

Figure 2. Ocular mucosal tolerance in aged mice. A. Schematic of experimental design in mice of different ages: 2, 9, and 24 months (M). Conjunctival immune tolerance was measured by delayed-type hypersensitivity (DTH) to OVA using the following protocol: OVA eye drops were administered topically for 3 days (d1–3), then mice were immunized (Imm) subcutaneously (s.c.) with OVA + complete Freund's adjuvant on day 8 and finally challenged with the same antigen by intradermal (i.d) ear injection (OVA in the right ear and PBS in the left ear) on day 15. Ear swelling was measured 48 hours later. (B) In vivo DTH (ear swelling) measurements. Results are the difference between the antigen-injected and PBS-injected ears of mice in each group. (n = 5/group, mean ± SEM, Kruskal-Wallis followed

by Dunn's multiple comparisons test). These experiments were approved by the Institutional Animal Care and Use Committees at Baylor College of Medicine.

References

1. Bradley JL, Ozer Stillman I, Pivneva I, Guerin A, Evans AM, Dana R. Dry eye disease ranking among common reasons for seeking eye care in a large US claims database. Clin Ophthalmol 2019; 13:225-32.
2. Schiffman RM, Walt JG, Jacobsen G, Doyle JJ, Lebovics G, Sumner W. Utility assessment among patients with dry eye disease. Ophthalmology 2003; 110:1412-9.
3. Buchholz P, Steeds CS, Stern LS, Wiederkehr DP, Doyle JJ, Katz LM, et al. Utility assessment to measure the impact of dry eye disease. Ocul.Surf. 2006; 4:155-61.
4. Barber L, Khodai O, Croley T, Lievens C, Montaquila S, Ziemanski J, et al. Dry eye symptoms and impact on vision-related function across International Task Force guidelines severity levels in the United States. BMC Ophthalmology 2018; 18:260.
5. Schaumberg DA, Sullivan DA, Buring JE, Dana MR. Prevalence of dry eye syndrome among US women. Am. J. Ophthalmol. 2003; 136:318-26.
6. Schein OD, Tiesch JM, Munoz B, Bandeen-Roche K, West S. Relation between signs and symptoms of dry eye in the elderly. A population-based perspective. Ophthalmology 1997; 104:1395-401.
7. Moss SE, Klein R, Klein BE. Prevalence of and risk factors for dry eye syndrome. Arch.Ophthalmol. 2000; 118:1264-8.
8. Schein OD, Hochberg MC, Munoz B, Tielsch JM, Bandeen-Roche K, Provost T, et al. Dry eye and dry mouth in the elderly: a population-based assessment. Arch Intern Med 1999; 159:1359-63.
9. Terry MA. Dry eye in the elderly. Drugs Aging 2001; 18:101-7.
10. Moss SE KR, Klein BE. Long-term Incidence of Dry Eye in an Older Population. Optom Vis Sci. 2008; 85:668-74. .

11. Farrand KF, Fridman M, Stillman IO, Schaumberg DA. Prevalence of Diagnosed Dry Eye Disease in the United States Among Adults Aged 18 Years and Older. Am J Ophthalmol 2017; 182:90-8.
12. Wang MTM, Muntz A, Lim J, Kim JS, Lacerda L, Arora A, et al. Ageing and the natural history of dry eye disease: A prospective registry-based cross-sectional study. Ocul Surf 2020.
13. Sullivan DA, Rocha EM, Aragona P, Clayton JA, Ding J, Golebiowski B, et al. TFOS DEWS II Sex, Gender, and Hormones Report. Ocul Surf 2017; 15:284-333.
14. Bron AJ, de Paiva CS, Chauhan SK, Bonini S, Gabison EE, Jain S, et al. TFOS DEWS II pathophysiology report. Ocul Surf 2017.
15. Forrester JV, Xu H, Lambe T, Cornall R. Immune privilege or privileged immunity? Mucosal Immunol 2008; 1:372-81.
16. Galletti JG, Guzman M, Giordano MN. Mucosal immune tolerance at the ocular surface in health and disease. Immunology 2017; 150:397-407.
17. Kogut I, Scholz JL, Cancro MP, Cambier JC. B cell maintenance and function in aging. Semin Immunol 2012; 24:342-9.
18. Sasaki S, Sullivan M, Narvaez CF, Holmes TH, Furman D, Zheng NY, et al. Limited efficacy of inactivated influenza vaccine in elderly individuals is associated with decreased production of vaccine-specific antibodies. J Clin Invest 2011; 121:3109-19.
19. Galletti JG, de Paiva CS. The ocular surface immune system through the eyes of aging. Ocul Surf 2021.
20. Pabst O, Mowat AM. Oral tolerance to food protein. Mucosal immunology 2012; 5:232-9.
21. Kim KS, Hong SW, Han D, Yi J, Jung J, Yang BG, et al. Dietary antigens limit mucosal immunity by inducing regulatory T cells in the small intestine. Science 2016; 351:858-63.
22. Melamed D, Friedman A. In vivo tolerization of Th1 lymphocytes following a single feeding with ovalbumin: anergy in the absence of suppression. Eur J Immunol 1994; 24:1974-81.

23. Worbs T, Bode U, Yan S, Hoffmann MW, Hintzen G, Bernhardt G, et al. Oral tolerance originates in the intestinal immune system and relies on antigen carriage by dendritic cells. J Exp Med 2006; 203:519-27.
24. McDole JR, Wheeler LW, McDonald KG, Wang B, Konjufca V, Knoop KA, et al. Goblet cells deliver luminal antigen to CD103+ dendritic cells in the small intestine. Nature 2012; 483:345-9.
25. Kulkarni DH, Gustafsson JK, Knoop KA, McDonald KG, Bidani SS, Davis JE, et al. Goblet cell associated antigen passages support the induction and maintenance of oral tolerance. Mucosal Immunol 2020; 13:271-82.
26. Sricharunrat T, Pumirat P, Leaungwutiwong P. Oral tolerance:Recent advances on mechanisms and potential applications. Asian Pac J Allergy Immunol 2018; 36:207-16.
27. Friedman A. Induction of anergy in Th1 lymphocytes by oral tolerance. Importance of antigen dosage and frequency of feeding. Ann N Y Acad Sci 1996; 778:103-10.
28. Faria AM, Garcia G, Rios MJ, Michalaros CL, Vaz NM. Decrease in susceptibility to oral tolerance induction and occurrence of oral immunization to ovalbumin in 20-38-week-old mice. The effect of interval between oral exposures and rate of antigen intake in the oral immunization. Immunology 1993; 78:147-51.
29. Wakabayashi A, Utsuyama M, Hosoda T, Sato K, Takahashi H, Hirokawa K. Induction of immunological tolerance by oral, but not intravenous and intraportal, administration of ovalbumin and the difference between young and old mice. J Nutr Health Aging 2006; 10:183-91.
30. Moreau MC, Gaboriau-Routhiau V. The absence of gut flora, the doses of antigen ingested and aging affect the long-term peripheral tolerance induced by ovalbumin feeding in mice. Res Immunol 1996; 147:49-59.
31. Friedman A, Weiner HL. Induction of anergy or active suppression following oral tolerance is determined by antigen dosage. Proc Natl Acad Sci U S A 1994; 91:6688-92.

32. Melamed D, Friedman A. Direct evidence for anergy in T lymphocytes tolerized by oral administration of ovalbumin. Eur J Immunol 1993; 23:935-42.
33. Wakabayashi A, Utsuyama M, Hosoda T, Sato K, Hirokawa K. Differential age effect of oral administration of an antigen on antibody response: an induction of tolerance in young mice but enhancement of immune response in old mice. Mech Ageing Dev 1999; 109:191-201.
34. de Faria AM, Ficker SM, Speziali E, Menezes JS, Stransky B, Silva Rodrigues V, et al. Aging affects oral tolerance induction but not its maintenance in mice. Mech Ageing Dev 1998; 102:67-80.
35. Simioni PU, Fernandes LG, Gabriel DL, Tamashiro WM. Effect of aging and oral tolerance on dendritic cell function. Braz J Med Biol Res 2010; 43:68-76.
36. Kato H, Fujihashi K, Kato R, Dohi T, Fujihashi K, Hagiwara Y, et al. Lack of oral tolerance in aging is due to sequential loss of Peyer's patch cell interactions. International Immunology 2003; 15:145-58.
37. Speziali EF, Menezes JS, Santiago AF, Vaz NM, Faria AMC. Lifelong Maintenance of Oral Tolerance and Immunity Profiles in Mice Depends on Early Exposure to Antigen. Scand J Immunol 2018; 87:73-9.
38. Engwerda CR, Fox BS, Handwerger BS. Cytokine production by T lymphocytes from young and aged mice. J Immunol 1996; 156:3621-30.
39. Wakikawa A, Utsuyama M, Wakabayashi A, Kitagawa M, Hirokawa K. Age-related alteration of cytokine production profile by T cell subsets in mice: a flow cytometric study. Exp Gerontol 1999; 34:231-42.
40. Wells HGO, T.B. The Biological Reactions of the Vegetable Proteins I. Anaphylaxis. The Journal of Infectious Diseases 1911; 8:66-124.
41. Jones SM, Burks AW, Dupont C. State of the art on food allergen immunotherapy: oral, sublingual, and epicutaneous. J Allergy Clin Immunol 2014; 133:318-23.
42. Savage J, Johns CB. Food allergy: epidemiology and natural history. Immunol Allergy Clin North Am 2015; 35:45-59.

43. Winberg A, Strinnholm A, Hedman L, West CE, Perzanowski MS, Ronmark E. High incidence and remission of reported food hypersensitivity in Swedish children followed from 8 to 12 years of age - a population based cohort study. Clin Transl Allergy 2014; 4:32.
44. Liu AH, Jaramillo R, Sicherer SH, Wood RA, Bock SA, Burks AW, et al. National prevalence and risk factors for food allergy and relationship to asthma: results from the National Health and Nutrition Examination Survey 2005-2006. J Allergy Clin Immunol 2010; 126:798-806 e13.
45. Bakos N, Schöll I, Szalai K, Kundi M, Untersmayr E, Jensen-Jarolim E. Risk assessment in elderly for sensitization to food and respiratory allergens. Immunol Lett 2006; 107:15-21.
46. Diesner SC, Untersmayr E, Pietschmann P, Jensen-Jarolim E. Food allergy: only a pediatric disease? Gerontology 2011; 57:28-32.
47. Ventura MT, D'Amato A, Giannini M, Carretta A, Tummolo RA, Buquicchio R. Incidence of allergic diseases in an elderly population. Immunopharmacol Immunotoxicol 2010; 32:165-70.
48. Wöhrl S, Stingl G. Underestimation of allergies in elderly patients. Lancet 2004; 363:249.
49. García-Carrasco M, Cervera R, Rosas J, Ramos-Casals M, Morlà RM, Sisó A, et al. Primary Sjögren's syndrome in the elderly: clinical and immunological characteristics. Lupus 1999; 8:20-3.
50. Haga HJ, Jonsson R. The influence of age on disease manifestations and serological characteristics in primary Sjögren's syndrome. Scand J Rheumatol 1999; 28:227-32.
51. Tishler M, Yaron I, Shirazi I, Yaron M. Clinical and immunological characteristics of elderly onset Sjögren's syndrome: a comparison with younger onset disease. J Rheumatol 2001; 28:795-7.
52. Botsios C, Furlan A, Ostuni P, Sfriso P, Andretta M, Ometto F, et al. Elderly onset of primary Sjögren's syndrome: clinical manifestations, serological features and

oral/ocular diagnostic tests. Comparison with adult and young onset of the disease in a cohort of 336 Italian patients. Joint Bone Spine 2011; 78:171-4.
53. Drosos AA, Andonopoulos AP, Costopoulos JS, Papadimitriou CS, Moutsopoulos HM. Prevalence of primary Sjögren's syndrome in an elderly population. Br J Rheumatol 1988; 27:123-7.
54. Strobel S, Ferguson A. Immune responses to fed protein antigens in mice. 3. Systemic tolerance or priming is related to age at which antigen is first encountered. Pediatr Res 1984; 18:588-94.
55. Pali-Scholl I, Jensen-Jarolim E. Anti-acid medication as a risk factor for food allergy. Allergy 2011; 66:469-77.
56. Roh D, Lee DH, Lee SK, Kim SW, Kim SW, Cho JH, et al. Sex difference in IgE sensitization associated with alcohol consumption in the general population. Sci Rep 2019; 9:12131.
57. Kraus TA, Mayer L. Oral tolerance and inflammatory bowel disease. Curr Opin Gastroenterol 2005; 21:692-6.
58. Kraus TA, Toy L, Chan L, Childs J, Cheifetz A, Mayer L. Failure to induce oral tolerance in Crohn's and ulcerative colitis patients: Possible genetic risk. Annals of the New York Academy of Sciences, 2004:225-38.
59. Knoflach P, Park BH, Cunningham R, Weiser MM, Albini B. Serum antibodies to cow's milk proteins in ulcerative colitis and Crohn's disease. Gastroenterology 1987; 92:479-85.
60. Duchmann R, Neurath MF, Meyer zum Buschenfelde KH. Responses to self and non-self intestinal microflora in health and inflammatory bowel disease. Res Immunol 1997; 148:589-94.
61. Witkowski M, Witkowski M, Gagliani N, Huber S. Recipe for IBD: can we use food to control inflammatory bowel disease? Seminars in Immunopathology 2018; 40:145-56.
62. Matsuoka K, Kanai T. The gut microbiota and inflammatory bowel disease. Seminars in immunopathology 2015; 37:47-55.

63. Desreumaux P, Foussat A, Allez M, Beaugerie L, Hébuterne X, Bouhnik Y, et al. Safety and efficacy of antigen-specific regulatory T-cell therapy for patients with refractory Crohn's disease. Gastroenterology 2012; 143:1207-17.e2.
64. Wick C, Onestingel E, Demetz E, Dietrich H, Wick G. Oral Tolerization with Mycobacterial Heat Shock Protein 65 Reduces Chronic Experimental Atherosclerosis in Aged Mice. Gerontology 2018; 64:36-48.
65. van Puijvelde GH, van Es T, van Wanrooij EJ, Habets KL, de Vos P, van der Zee R, et al. Induction of oral tolerance to HSP60 or an HSP60-peptide activates T cell regulation and reduces atherosclerosis. Arterioscler Thromb Vasc Biol 2007; 27:2677-83.
66. Enaud R, Prevel R, Ciarlo E, Beaufils F, Wieërs G, Guery B, et al. The Gut-Lung Axis in Health and Respiratory Diseases: A Place for Inter-Organ and Inter-Kingdom Crosstalks. Front Cell Infect Microbiol 2020; 10:9.
67. Trujillo-Vargas CM, Schaefer L, Alam J, Pflugfelder SC, Britton RA, de Paiva CS. The gut-eye-lacrimal gland-microbiome axis in Sjögren Syndrome. Ocul Surf 2020; 18:335-44.
68. Lowrey JL, Savage ND, Palliser D, Corsin-Jimenez M, Forsyth LM, Hall G, et al. Induction of tolerance via the respiratory mucosa. Int Arch Allergy Immunol 1998; 116:93-102.
69. Holt PG, Batty JE, Turner KJ. Inhibition of specific IgE responses in mice by pre-exposure to inhaled antigen. Immunology 1981; 42:409-17.
70. Chapman TJ, Georas SN. Regulatory tone and mucosal immunity in asthma. Int Immunopharmacol 2014; 23:330-6.
71. Van Hove CL, Moerloose K, Maes T, Joos GF, Tournoy KG. Cigarette smoke enhances Th-2 driven airway inflammation and delays inhalational tolerance. Respir Res 2008; 9:42.
72. Kawano T, Ouchi R, Ishigaki T, Masuda C, Miyasaka T, Ohkawara Y, et al. Increased Susceptibility to Allergic Asthma with the Impairment of Respiratory Tolerance Caused by Psychological Stress. Int Arch Allergy Immunol 2018; 177:1-15.

73. Wolvers DA, Coenen-de Roo CJ, Mebius RE, van der Cammen MJ, Tirion F, Miltenburg AM, et al. Intranasally induced immunological tolerance is determined by characteristics of the draining lymph nodes: studies with OVA and human cartilage gp-39. J Immunol 1999; 162:1994-8.
74. Hintzen G, Ohl L, del Rio ML, Rodriguez-Barbosa JI, Pabst O, Kocks JR, et al. Induction of tolerance to innocuous inhaled antigen relies on a CCR7-dependent dendritic cell-mediated antigen transport to the bronchial lymph node. J Immunol 2006; 177:7346-54.
75. Lee H, Ruane D, Law K, Ho Y, Garg A, Rahman A, et al. Phenotype and function of nasal dendritic cells. Mucosal Immunol 2015; 8:1083-98.
76. Mansouri S, Katikaneni DS, Gogoi H, Pipkin M, Machuca TN, Emtiazjoo AM, et al. Lung IFNAR1(hi) TNFR2(+) cDC2 promotes lung regulatory T cells induction and maintains lung mucosal tolerance at steady state. Mucosal Immunol 2020; 13:595-608.
77. de Heer HJ, Hammad H, Soullié T, Hijdra D, Vos N, Willart MA, et al. Essential role of lung plasmacytoid dendritic cells in preventing asthmatic reactions to harmless inhaled antigen. J Exp Med 2004; 200:89-98.
78. Lloyd CM, Marsland BJ. Lung Homeostasis: Influence of Age, Microbes, and the Immune System. Immunity 2017; 46:549-61.
79. Boe DM, Boule LA, Kovacs EJ. Innate immune responses in the ageing lung. Clin Exp Immunol 2017; 187:16-25.
80. Prakash S, Agrawal S, Vahed H, Ngyuen M, BenMohamed L, Gupta S, et al. Dendritic cells from aged subjects contribute to chronic airway inflammation by activating bronchial epithelial cells under steady state. Mucosal Immunol 2014; 7:1386-94.
81. Agrawal A, Agrawal S, Gupta S. Role of Dendritic Cells in Inflammation and Loss of Tolerance in the Elderly. Frontiers in Immunology 2017; 8.

82. Baratin M, Foray C, Demaria O, Habbeddine M, Pollet E, Maurizio J, et al. Homeostatic NF-kappaB Signaling in Steady-State Migratory Dendritic Cells Regulates Immune Homeostasis and Tolerance. Immunity 2015; 42:627-39.
83. Agrawal A, Tay J, Ton S, Agrawal S, Gupta S. Increased reactivity of dendritic cells from aged subjects to self-antigen, the human DNA. J.Immunol. 2009; 182:1138-45.
84. McQuattie-Pimentel AC, Ren Z, Joshi N, Watanabe S, Stoeger T, Chi M, et al. The lung microenvironment shapes a dysfunctional response of alveolar macrophages in aging. J Clin Invest 2021; 131.
85. Busse PJ, Zhang TF, Srivastava K, Schofield B, Li XM. Effect of ageing on pulmonary inflammation, airway hyperresponsiveness and T and B cell responses in antigen-sensitized and -challenged mice. Clin Exp Allergy 2007; 37:1392-403.
86. Cardona V, Guilarte M, Luengo O, Labrador-Horrillo M, Sala-Cunill A, Garriga T. Allergic diseases in the elderly. Clin Transl Allergy 2011; 1:11.
87. Yanez A, Soria M, De Barayazarra S, Recuero N, Rovira F, Jares E, et al. Clinical characteristics and comorbidities of elderly asthmatics who attend allergy clinics. Asthma Res Pract 2018; 4:5.
88. Boulet LP. Asthma in the elderly patient. Asthma Res Pract 2016; 2:3.
89. Baptist AP, Nyenhuis S. Rhinitis in the Elderly. Immunol Allergy Clin North Am 2016; 36:343-57.
90. Sandmand M, Bruunsgaard H, Kemp K, Andersen-Ranberg K, Pedersen AN, Skinhøj P, et al. Is ageing associated with a shift in the balance between Type 1 and Type 2 cytokines in humans? Clin Exp Immunol 2002; 127:107-14.
91. Pakkasela J, Ilmarinen P, Honkamaki J, Tuomisto LE, Andersen H, Piirila P, et al. Age-specific incidence of allergic and non-allergic asthma. BMC Pulm Med 2020; 20:9.
92. Anto JM, Sunyer J, Basagana X, Garcia-Esteban R, Cerveri I, de Marco R, et al. Risk factors of new-onset asthma in adults: a population-based international cohort study. Allergy 2010; 65:1021-30.

93. Litonjua AA, Sparrow D, Weiss ST, O'Connor GT, Long AA, Ohman JL, Jr. Sensitization to cat allergen is associated with asthma in older men and predicts new-onset airway hyperresponsiveness. The Normative Aging Study. Am J Respir Crit Care Med 1997; 156:23-7.
94. Huss K, Naumann PL, Mason PJ, Nanda JP, Huss RW, Smith CM, et al. Asthma severity, atopic status, allergen exposure and quality of life in elderly persons. Ann Allergy Asthma Immunol 2001; 86:524-30.
95. Raherison C, Nejjari C, Marty ML, Filleul L, Barberger-Gateau P, Dartigues JF, et al. IgE level and Phadiatop in an elderly population from the PAQUID cohort: relationship to respiratory symptoms and smoking. Allergy 2004; 59:940-5.
96. Rom O, Avezov K, Aizenbud D, Reznick AZ. Cigarette smoking and inflammation revisited. Respir Physiol Neurobiol 2013; 187:5-10.
97. Swamy M, Jamora C, Havran W, Hayday A. Epithelial decision makers: in search of the 'epimmunome'. Nat Immunol 2010; 11:656-65.
98. Medawar PB. Immunity to homologous grafted skin; the fate of skin homografts transplanted to the brain, to subcutaneous tissue, and to the anterior chamber of the eye. British journal of experimental pathology 1948; 29:58-69.
99. Niederkorn JY, Larkin DFP. Immune privilege of corneal allografts. Ocular immunology and inflammation 2010; 18:162-71.
100. Niederkorn JY, Stein-Streilein J. History and physiology of immune privilege. Ocular Immunology and Inflammation 2010; 18:19-23.
101. Zhou R, Caspi RR. Ocular immune privilege. F1000 biology reports 2010; 2.
102. Gery I, Caspi RR. Tolerance Induction in Relation to the Eye. Frontiers in Immunology 2018; 9:2304.
103. Kezuka T, Sakai JI, Usui N, Streilein JW, Usui M. Evidence for antigen-specific immune deviation in patients with acute retinal necrosis. 2001. Ocul Immunol Inflamm 2007; 15:241-8.

104. Vendomèle J, Khebizi Q, Fisson S. Cellular and Molecular Mechanisms of Anterior Chamber-Associated Immune Deviation (ACAID): What We Have Learned from Knockout Mice. Front Immunol 2017; 8:1686.
105. Streilein JW. Immune privilege as the result of local tissue barriers and immunosuppressive microenvironments. Current opinion in immunology 1993; 5:428-32.
106. Sonoda KH, Sakamoto T, Qiao H, Hisatomi T, Oshima T, Tsutsumi-Miyahara C, et al. The analysis of systemic tolerance elicited by antigen inoculation into the vitreous cavity: vitreous cavity-associated immune deviation. Immunology 2005; 116:390-9.
107. Jiang LQ, Jorquera M, Streilein JW. Subretinal space and vitreous cavity as immunologically privileged sites for retinal allografts. Invest Ophthalmol Vis Sci 1993; 34:3347-54.
108. DeDreu J, Bowen CJ, Logan CM, Pal-Ghosh S, Parlanti P, Stepp MA, et al. An immune response to the avascular lens following wounding of the cornea involves ciliary zonule fibrils. Faseb j 2020; 34:9316-36.
109. Serna-Ojeda JC, Basu S, Vazirani J, Garfias Y, Sangwan VS. Systemic Immunosuppression for Limbal Allograft and Allogenic Limbal Epithelial Cell Transplantation. Medical hypothesis, discovery & innovation ophthalmology journal 2020; 9:23-32.
110. Egan RM, Yorkey C, Black R, Loh WK, Stevens JL, Storozynsky E, et al. In vivo behavior of peptide-specific T cells during mucosal tolerance induction: antigen introduced through the mucosa of the conjunctiva elicits prolonged antigen-specific T cell priming followed by anergy. J Immunol 2000; 164:4543-50.
111. Macaubas C, DeKruyff RH, Umetsu DT. Respiratory tolerance in the protection against asthma. Current drug targets. Inflammation and allergy 2003; 2:175-86.
112. Steele L, Mayer L, Berin MC. Mucosal immunology of tolerance and allergy in the gastrointestinal tract. Immunologic research 2012; 54:75-82.
113. Niederkorn JY. Role of NKT cells in anterior chamber-associated immune deviation. Expert Rev Clin Immunol 2009; 5:137-44.

114. Dang Z, Kuffová L, Liu L, Forrester JV. Soluble antigen traffics rapidly and selectively from the corneal surface to the eye draining lymph node and activates T cells when codelivered with CpG oligonucleotides. J Leukoc Biol 2014; 95:431-40.
115. Kittel B, Ruehl-Fehlert C, Morawietz G, Klapwijk J, Elwell MR, Lenz B, et al. Revised guides for organ sampling and trimming in rats and mice--Part 2. A joint publication of the RITA and NACAD groups. Exp.Toxicol.Pathol. 2004; 55:413-31.
116. Schlereth S, Lee HS, Khandelwal P, Saban DR. Blocking CCR7 at the ocular surface impairs the pathogenic contribution of dendritic cells in allergic conjunctivitis. Am J Pathol 2012; 180:2351-60.
117. Saban DR. The chemokine receptor CCR7 expressed by dendritic cells: a key player in corneal and ocular surface inflammation. Ocul Surf 2014; 12:87-99.
118. Wang Y, Goldschneider I, Foss D, Wu DY, O'Rourke J, Cone RE. Direct thymic involvement in anterior chamber-associated immune deviation: evidence for a nondeletional mechanism of centrally induced tolerance to extrathymic antigens in adult mice. J Immunol 1997; 158:2150-5.
119. Streilein JW, Niederkorn JY. Induction of anterior chamber-associated immune deviation requires an intact, functional spleen. J Exp Med 1981; 153:1058-67.
120. Jiang L, Yang P, He H, Li B, Lin X, Hou S, et al. Increased expression of Foxp3 in splenic CD8+ T cells from mice with anterior chamber-associated immune deviation. Mol Vis 2007; 13:968-74.
121. Dua HS, Donoso LA, Laibson PR. Conjunctival instillation of retinal antigens induces tolerance Does it invoke mucosal tolerance mediated via conjunctiva associated lymphoid tissues (CALT)? Ocul Immunol Inflamm 1994; 2:29-36.
122. Galletti JG, Gabelloni ML, Morande PE, Sabbione F, Vermeulen ME, Trevani AS, et al. Benzalkonium chloride breaks down conjunctival immunological tolerance in a murine model. Mucosal Immunol 2013; 6:24-34.
123. Ko BY, Xiao Y, Barbosa FL, de Paiva CS, Pflugfelder SC. Goblet cell loss abrogates ocular surface immune tolerance. JCI Insight 2018; 3: 98222.

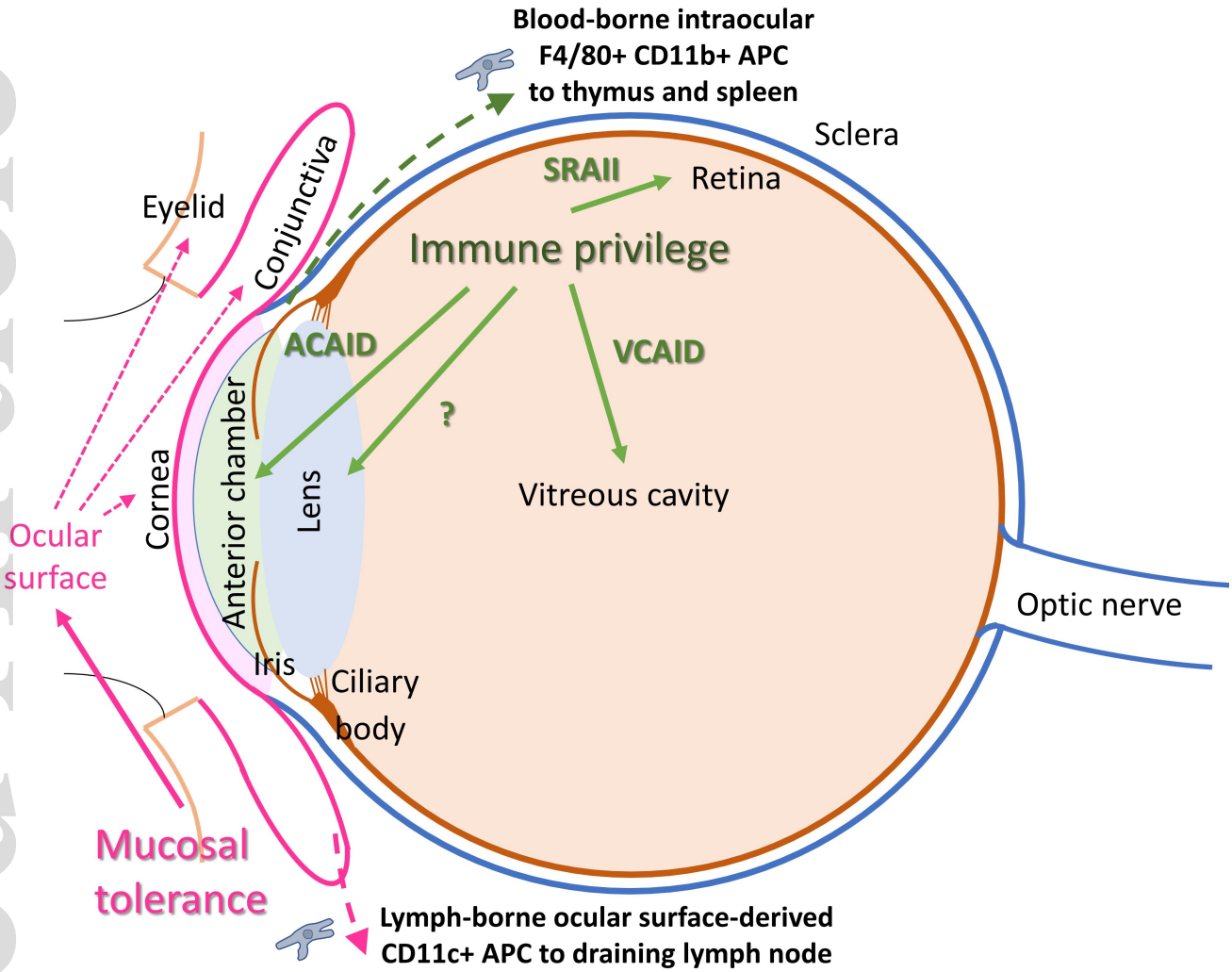
124. Barbosa FL, Xiao Y, Bian F, Coursey TG, Ko BY, Clevers H, et al. Goblet Cells Contribute to Ocular Surface Immune Tolerance-Implications for Dry Eye Disease. Int J Mol Sci 2017; 18:1-13.
125. Foulsham W, Marmalidou A, Amouzegar A, Coco G, Chen Y, Dana R. Review: The function of regulatory T cells at the ocular surface. Ocul Surf 2017; 15:652-9.
126. Baudouin C, Labbé A, Liang H, Pauly A, Brignole-Baudouin F. Preservatives in eyedrops: the good, the bad and the ugly. Prog Retin Eye Res 2010; 29:312-34.
127. Guzman M, Sabbione F, Gabelloni ML, Vanzulli S, Trevani AS, Giordano MN, et al. Restoring conjunctival tolerance by topical nuclear factor-kappaB inhibitors reduces preservative-facilitated allergic conjunctivitis in mice. Invest Ophthalmol Vis Sci 2014; 55:6116-26.
128. Lin Z, Liu X, Zhou T, Wang Y, Bai L, He H, et al. A mouse dry eye model induced by topical administration of benzalkonium chloride. Molecular Vision 2011; 17:257-64.
129. Xiong C, Chen D, Liu J, Liu B, Li N, Zhou Y, et al. A rabbit dry eye model induced by topical medication of a preservative benzalkonium chloride. Investigative Ophthalmology and Visual Science 2008; 49:1850-6.
130. Ouyang W, Wu Y, Lin X, Wang S, Yang Y, Tang L, et al. Role of CD4+ T Helper Cells in the Development of BAC-Induced Dry Eye Syndrome in Mice. Invest Ophthalmol Vis Sci 2021; 62:25.
131. Guzman M, Miglio MS, Zgajnar NR, Colado A, Almejun MB, Keitelman IA, et al. The mucosal surfaces of both eyes are immunologically linked by a neurogenic inflammatory reflex involving TRPV1 and substance P. Mucosal Immunol 2018.
132. Guzman M, Keitelman I, Sabbione F, Trevani AS, Giordano MN, Galletti JG. Mucosal tolerance disruption favors disease progression in an extraorbital lacrimal gland excision model of murine dry eye. Exp Eye Res 2016; 151:19-22.
133. Guzman M, Keitelman I, Sabbione F, Trevani AS, Giordano MN, Galletti JG. Desiccating stress-induced disruption of ocular surface immune tolerance drives dry eye disease. Clin Exp Immunol 2016; 184:248-56.

134. Guzmán M, Miglio M, Keitelman I, Shiromizu CM, Sabbione F, Fuentes F, et al. Transient tear hyperosmolarity disrupts the neuroimmune homeostasis of the ocular surface and facilitates dry eye onset. Immunology 2020.
135. Niederkorn JY, Stern ME, Pflugfelder SC, de Paiva CS, Corrales RM, Gao J, et al. Desiccating Stress Induces T Cell-Mediated Sjogren's Syndrome-Like Lacrimal Keratoconjunctivitis. J. Immunol. 2006; 176:3950-7.
136. de Paiva CS, Volpe EA, Gandhi NB, Zhang X, Zheng X, Pitcher JD, III, et al. Disruption of TGF-beta Signaling Improves Ocular Surface Epithelial Disease in Experimental Autoimmune Keratoconjunctivitis Sicca. PLoS. One. 2011; 6:e29017. Epub 2011 Dec 14.
137. Wang CB, F; Simmons, KT; Zaheer, M; Pflugfelder, SC; de Paiva, CS. . Commensal Bacteria Modulate Ocular Surface Inflammatory Response to Liposaccharide. Invest. Ophthalmol. Vis. Sci. 2017; 58(8):3916.
138. Zaheer M, Wang C, Bian F, Yu Z, Hernandez H, de Souza RG, et al. Protective role of commensal bacteria in Sjogren Syndrome. J Autoimmun 2018; pii: S0896-8411:45-56.
139. Chen FY, Lee A, Ge S, Nathan S, Knox SM, McNamara NA. Aire-deficient mice provide a model of corneal and lacrimal gland neuropathy in Sjogren's syndrome. PLoS One 2017; 12:e0184916.
140. de Paiva CS, Hwang CS, Pitcher JD, III, Pangelinan SB, Rahimy E, Chen W, et al. Age-related T-cell cytokine profile parallels corneal disease severity in Sjogren's syndrome-like keratoconjunctivitis sicca in CD25KO mice. Rheumatology 2010; 49:246-58.
141. Turpie B, Yoshimura T, Gulati A, Rios JD, Dartt DA, Masli S. Sjogren's syndrome-like ocular surface disease in thrombospondin-1 deficient mice. Am. J. Pathol. 2009; 175:1136-47.
142. You IC, Bian F, Volpe EA, de Paiva CS, Pflugfelder SC. Age-related conjunctival disease in the C57BL/6.NOD-Aec1Aec2 Mouse Model of Sjogren Syndrome develops independent of lacrimal dysfunction. Invest Ophthalmol Vis Sci 2015; 56:2224-33.

143. Chauhan SK, El AJ, Ecoiffier T, Goyal S, Zhang Q, Saban DR, et al. Autoimmunity in dry eye is due to resistance of Th17 to Treg suppression. J.Immunol. 2009; 182:1247-52.
144. de Paiva CS, Villarreal AL, Corrales RM, Rahman HT, Chang VY, Farley WJ, et al. Dry Eye-Induced Conjunctival Epithelial Squamous Metaplasia Is Modulated by Interferon-{gamma}. Invest Ophthalmol. Vis. Sci. 2007; 48:2553-60.
145. de Paiva CS, Corrales RM, Villarreal AL, Farley WJ, Li DQ, Stern ME, et al. Corticosteroid and doxycycline suppress MMP-9 and inflammatory cytokine expression, MAPK activation in the corneal epithelium in experimental dry eye. Exp.Eye Res. 2006; 83:526-35.
146. Stevenson W, Chen Y, Lee SM, Lee HS, Hua J, Dohlman T, et al. Extraorbital lacrimal gland excision: a reproducible model of severe aqueous tear-deficient dry eye disease. Cornea 2014; 33:1336-41.
147. Volpe EA, Henriksson JT, Wang C, Barbosa FL, Zaheer M, Zhang X, et al. Interferon-gamma deficiency protects against aging-related goblet cell loss. Oncotarget 2016; 7:64605-6461.
148. McClellan AJ, Volpe EA, Zhang X, Darlington GJ, Li DQ, Pflugfelder SC, et al. Ocular Surface Disease and Dacryoadenitis in Aging C57BL/6 Mice. Am .J. Pathol. 2014; 184:631-43.
149. Jiang G, Ke Y, Sun D, Li H, Ihnen M, Jumblatt MM, et al. A new model of experimental autoimmune keratoconjunctivitis sicca (KCS) induced in Lewis rat by the autoantigen Klk1b22. Invest Ophthalmol Vis Sci 2009; 50:2245-54.
150. Takada K, Takiguchi M, Konno A, Inaba M. Autoimmunity against a tissue kallikrein in IQI/Jic Mice: a model for Sjogren's syndrome. J.Biol.Chem. 2005; 280:3982-8.
151. Stern ME, Schaumburg CS, Siemasko KF, Gao J, Wheeler LA, Grupe DA, et al. Autoantibodies contribute to the immunopathogenesis of experimental dry eye disease. Invest Ophthalmol.Vis.Sci. 2012; 53:2062-75.
152. Roukens AH, Soonawala D, Joosten SA, de Visser AW, Jiang X, Dirksen K, et al. Elderly subjects have a delayed antibody response and prolonged viraemia following

yellow fever vaccination: a prospective controlled cohort study. PLoS One 2011; 6:e27753.
153. Coursey TG, Bian F, Zaheer M, Pflugfelder SC, Volpe EA, de Paiva CS. Age-related spontaneous lacrimal keratoconjunctivitis is accompanied by dysfunctional T regulatory cells. Mucosal Immunol 2017; 10:743-456.
154. Agius E, Lacy KE, Vukmanovic-Stejic M, Jagger AL, Papageorgiou A-P, Hall S, et al. Decreased TNF-alpha synthesis by macrophages restricts cutaneous immunosurveillance by memory CD4+ T cells during aging. The Journal of experimental medicine 2009; 206:1929-40.
155. Bian F, Xiao Y, Barbosa FL, de Souza RG, Hernandez H, Yu Z, et al. Age-associated antigen-presenting cell alterations promote dry-eye inducing Th1 cells. Mucosal Immunology 2019; 12:897-908.
156. Alam J, de Paiva CS, Pflugfelder SC. Immune - Goblet cell interaction in the conjunctiva. Ocul Surf 2020; 18:326-34.
157. Marko CK, Menon BB, Chen G, Whitsett JA, Clevers H, Gipson IK. Spdef null mice lack conjunctival goblet cells and provide a model of dry eye. Am.J.Pathol. 2013; 183:35-48.
158. de Souza RG, Yu Z, Hernandez H, Trujillo-Vargas CM, Lee A, Mauk KE, et al. Modulation of Oxidative Stress and Inflammation in the Aged Lacrimal Gland. Am J Pathol 2020.
159. Manicassamy S, Ravindran R, Deng J, Oluoch H, Denning TL, Kasturi SP, et al. Toll-like receptor 2-dependent induction of vitamin A-metabolizing enzymes in dendritic cells promotes T regulatory responses and inhibits autoimmunity. Nat Med 2009; 15:401-9.
160. Knoop KA, Gustafsson JK, McDonald KG, Kulkarni DH, Kassel R, Newberry RD. Antibiotics promote the sampling of luminal antigens and bacteria via colonic goblet cell associated antigen passages. Gut Microbes 2017:1-12.

161. Knoop KA, McDonald KG, Kulkarni DH, Newberry RD. Antibiotics promote inflammation through the translocation of native commensal colonic bacteria. Gut 2016; 65:1100-9.
162. Steven P, Braun T, Krosser S, Gehlsen U. [Influence of Aging on Severity and Anti-Inflammatory Treatment of Experimental Dry Eye Disease]. Klin Monbl Augenheilkd 2017; 234:662-9.
163. Foulsham W, Mittal SK, Taketani Y, Chen Y, Nakao T, Chauhan SK, et al. Aged mice exhibit severe exacerbations of dry eye disease with an amplified memory Th17 cell response. Am J Pathol 2020.
164. Ventura MT, Scichilone N, Paganelli R, Minciullo PL, Patella V, Bonini M, et al. Allergic diseases in the elderly: biological characteristics and main immunological and non-immunological mechanisms. Clin Mol Allergy 2017; 15:2.
165. Yenigun A, Elbay A, Ozdem A, Bayraktar H, Ozer OF, Dogan R, et al. Dry Eye and Dry Nose Caused by the Effect of Allergic Rhinitis on Tear and Nasal Secretion Osmolarity. Ear Nose Throat J 2020:145561320908480.
166. Yenigun A, Dadaci Z, Sahin GO, Elbay A. Prevalence of allergic rhinitis symptoms and positive skin-prick test results in patients with dry eye. Am J Rhinol Allergy 2016; 30:e26-9.



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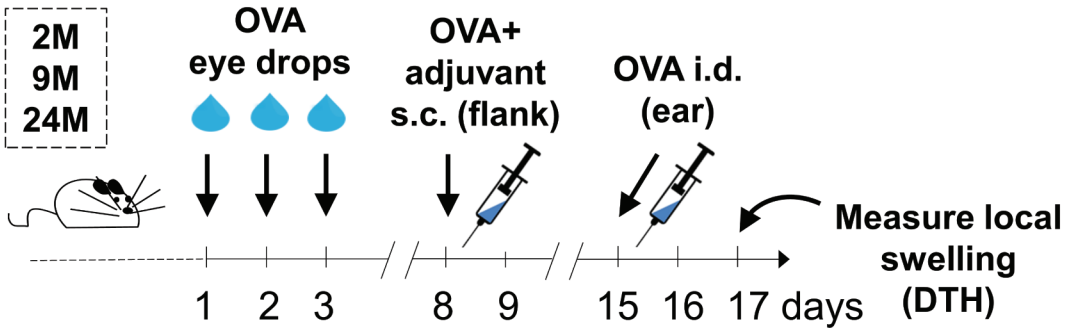

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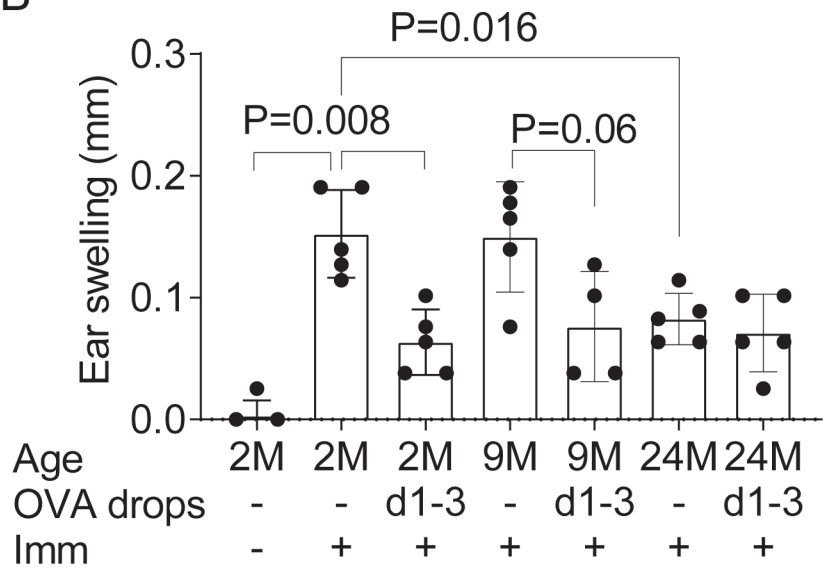

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