Ageing and ocular surface immunity
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ABSTRACT
The prevalence of ocular surface immunopathologies is enhanced in the elderly. This increased prevalence has been attributed to age-related dysregulation of innate and adaptive immune system responses. Age-related changes in ocular surface immunity have similar and distinct characteristics to those changes seen in other mucosal tissues. This mini review provides a brief outline of key findings in the field of ocular ageing, draws comparisons with other mucosal tissues and, finally, discusses age-related changes in the context of immunopathogenesis of infectious keratitis and dry eye disease, two of the most common inflammatory disorders of the ocular surface.

INTRODUCTION
With time, the human body loses many homeostatic mechanisms, leading to increased vulnerability to organ dysfunction and ultimately death. Ageing not only leads to body dysfunction per se, but also enhances the susceptibility to foreign invaders such as viruses and bacteria due to dysfunction or dysregulation of the immune system. What molecular and cellular mechanisms underlie ageing? Hallmarks of ageing include genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, dysregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion and altered intercellular communication. For postmitotic cells, such as neurons and muscle cells, these processes lead to a gradual loss of normal structure and function, the so-called ‘chronological ageing’. For continuously dividing cells, like those of the epithelia of the skin or gut, ‘replicative ageing’ further challenges the function of tissues in which these cells reside. Replicative ageing refers to the accumulation of cellular damage, such as telomere shortening and replication-associated DNA mutations, that occurs during the process of cell division. Despite similar trends, interindividual variations in ageing are significant. Precisely what causes the heterogeneity in the rate of progression and the onset of age-related dysfunctions remains largely unknown. Understanding the ageing process, though limited, allows for novel diagnostic and therapeutic interventions. In a number of model organisms, the ageing process was observed to slow down or even arrest temporarily. A number of genes as well as environmental factors (eg, dietary restriction) appear to extend not only the life span, but also maintain health. Rejuvenating the immune system has turned out to be a real possibility, with benefits on both the innate and the adaptive arms of immunity. A decrease in nearly all innate Toll-like receptor (TLR)-induced responses and higher levels of many proinflammatory cytokines are observed in the elderly. The benefits of vaccination to prevent infectious disease are also limited in the elderly, predominantly due to the inability to maintain long-term adaptive immune responses. Age-related deficiencies in maintaining tolerogenic responses and DNA stability cause excessive apoptosis of lymphocytes. This process can add to the severity of certain diseases. For example, in rheumatoid arthritis, increased apoptosis of naive T cells leads to impairment of T cell repertoire; suppressing this phenomenon by rejuvenating the immune system reduces the severity of the disease.

Table 1 summarises age-related changes in the frequencies and functions of immune cells.

Alterations of mucosal immunity by age have been documented both in human and animal models. A reduction in lymphoreticular tissues, antigen-specific IgA antibody responses and lack of oral tolerance induction are three hallmarks of mucosal ageing in the gastrointestinal tract. Peyer’s patches in the gastrointestinal mucosa shrink with age and have reduced frequencies of naive CD4+ T cells and dendritic cells (DCs). In the respiratory system, though nasopharyngeal-associated lymphoreticular tissue remains intact during ageing, age-related immune deficits, known as immunosenescence, have been shown to increase respiratory infections. A different onset of immunosenescence in gastrointestinal, nasopharyngeal and ocular mucosa has been reported, but the general immune response is similar in young and old. For the ocular mucosa, a plausible theory based on the above observation in other tissues is that the rapidity and specificity of the immune response in both the inflammatory and regulatory arms of the immune system are reduced with ageing. As we discuss in the subsequent sections, this reduction may lead to autoimmune disease and increased tissue damage as a result of infection. We also discuss how age-related changes in the frequencies of ocular immune cells and their expression of various cytokines and chemokines are associated with increased autoimmunity.

AGEING AND OCULAR SURFACE IMMUNITY
The tear film, lacrimal glands, corneal and conjunctival epithelia, and meibomian glands ensure the integrity and function of the ocular surface. Studies using human subjects as well as animal models
suggestion that these guardians of the ocular surface integrity are affected in the course of ageing; for example, the lacrimal gland is affected due to exposure to oxidative stress.20–27 Age-related physiological changes of the ocular surface have been reported;23–28 however, the effect of ageing on ocular surface immunity remains poorly understood. Immunological mechanisms play a pivotal role in regulating the ocular surface environment as well. Immune cells directly or via secretion of immunomodulatory factors actively protect the ocular surface.19 Resident corneal antigen-presenting cells,29,30 regulatory T cells31,32 and T helper 1 (Th1) cells33 are among key cellular players in immune homeostasis. Regulatory T cells, for example, suppress autoreactive effector T cells protecting against excessive adaptive immunity. Furthermore, immunomodulatory factors such as vascular endothelial growth factor receptor-3, transforming growth factor-β,34 programmed death-ligand 1, interleukin-1 receptor antagonist and interleukin (IL)-1336 regulate the immune microenvironment of the healthy ocular surface and inhibit immunopathogenic mechanisms.37,38 A breakdown of this immunological balance impairs the function of the eye and the visual system. Immunosenescence is of great importance in a number of ocular surface pathological conditions including infection, autoimmunity and ocular complications of systemic autoimmunity such as graft-versus-host disease (GVHD), systemic lupus erythematosus and Sjögren’s disease. It is known that the incidence of viral, bacterial and fungal keratitis and conjunctivitis is also more severe in elderly HIV-infected patients.49,50 HIV infection itself also seems to influence the ageing process rather than exposure to antiretroviral therapy for prophylaxis or treatment.48 The influence of HIV on accelerated senescence of ocular cells can be complicated by a number of associated pathologies, such as Kaposi sarcoma, a highly vascularised tumour, as well as with HSV keratitis, fungal keratitis (eg, Candida parapsilosis and Candida albicans) and uveitis, which are more severe in elderly HIV-infected patients.49,50

Bacterial keratitis is a serious corneal infectious disease that can result in severe visual disability. Pseudomonas aeruginosa infection in aged mice induces increased tissue damage compared with young mice.51 This difference has been attributed to the persistence of polymorphonuclear neutrophils (PMNs) in the cornea of old mice and contributes to increased corneal tissue destruction. An increased production of chemoattractant macrophage inflammatory protein 2 (MIP-2) likely underlies the altered PMN response in these older animals.51 Neutrophils generate a number of proteases and oxidases that work together to digest the ocular surface and in particular the cornea.52 Overall, alterations of the innate system associated with ageing may increase morbidity caused by bacterial infection, yet the role of ageing in the immunosenescence of adaptive immunity remains elusive.

### Ageing and dry eye disease
Ocular surface autoimmune diseases comprise a diverse spectrum of pathologies and can be classified as ocular specific (eg, dry eye, Mooren’s ulcerative keratitis) or systemic (eg, Sjögren’s syndrome, cicatricial pemphigoid, rheumatoid arthritis, systemic lupus erythematosus).53 A common immune-mediated disorder of the ocular surface is dry eye disease (DED). DED is known to disrupt the integrity of the corneal epithelium, leading to exposure or altered expression of self-antigens, breakdown of corneal immune privilege and a T cell-mediated autoimmune response directed against the ocular surface.19,32,34–36 Based on our current understanding, the principal inducers in the early immune response of DED are antigen-presenting cells, primarily resident ocular surface DCs.19,38 They present ocular surface antigens to T cells in the draining lymph nodes, where naive T cells are primed and expanded as CD4+ Th1 and Th17 effector cells.62–64 Both CD4 T cell subsets have been shown to contribute to the development of ocular surface inflammation in DED.65–74 The observations of (i) increased Th17 cells in the

| Innate | Adaptive |
|---|---|
| Neutrophils | Reduced function including chemotaxis, microbial killing and phagocytosis. |
| Macrophages | Defective chemotaxis, cytokine production and phagocytosis |
| DCs | Change in the balance of plasmacytoid DCs and myeloid DCs |
| Natural killer cells | Decreased proliferation Reduced production of TNF-α, IL-2, IL-12 and IL-2R |
| B cells | Reduced B cell lymphopoiesis; reduced CD4+ T cell help; reduced quantity and quality of antibodies |
| CD4+ T cells | Reduced IL-2 production; dampened co-stimulation |
| CD8+ T cells | Reduced repertoire; increased frequencies of memory cells |
| Tregs | Increased frequencies |

For more information, please see McKay et al47 and references therein. DCs, dendritic cells; IL, interleukin; TNF, tumour necrosis factor.
lymph nodes of DED mice and that (ii) in vivo neutralising of IL-17 restores Treg function and inhibits the induction and progression of DED implicate Th17 cells as a critical CD4 effector cell population mediating DED.\(^{10,31}\) Participation of Th1 in the pathogenesis of DED has been demonstrated as well.\(^{39,45,67–75}\) Increased interferon (IFN)-γ has been found in the conjunctiva of patients with DED and in biopsies taken from patients with Sjögren’s syndrome. In animal models, IFN-γ has been implicated in conjunctival and corneal epithelial apoptosis and loss of goblet cells, and neutralisation of IFN-γ has blocked dry eye induction. Despite involvement of IFN-γ, it is yet to be proven whether Th1 cells are the primary source of IFN-γ in DED since macrophages and other cells can also express IFN-γ. Other immune cell types have also been implicated in the pathogenesis of DED including PMNs that regulate Tregs and Th17 cells.\(^{76}\)

Clinical observations suggest that DED has a higher incidence in the elderly.\(^{77}\) This can be attributed to age-associated decrease in aqueous tear production and age-related alteration of ocular surface immunity.\(^{78}\) Although age is associated with increased inflammation and autoimmunity, very few articles are available on the effects of age on the function of T cell immunity in DED. A recent study in mice investigated age-related changes of the ocular immune system.\(^{18}\) McClellan et al showed increased CD4 T cell infiltration and enhanced expression of IFN-γ and IL-17A in the conjunctiva. Their data suggest that CD4 T cells from aged mice are more pathogenic and capable of inducing DED. However, the exact molecular mechanisms underlying the effects of ageing on DED remain largely unexplored.

Studies on tissues other than the ocular surface may provide insights into how age affects DED. Researchers have previously analysed the effects of ageing on DCs, Th17 and Treg cells. These three cell types are the principal components in DED pathogenesis. It is known that in the course of ageing DCs are functionally impaired in many aspects, including activation, migration and production of cytokines in response to stimuli.\(^{79}\) In contrast to their reduced functions with ageing, an increased reactivity of aged DCs against self-antigens has been observed.\(^{80}\) In addition, DCs from the elderly show enhanced inflammatory cytokine production\(^{31}\) and nuclear factor-κB activation, suggesting that these DCs are in a relatively activated state.\(^{82}\) In addition, frequencies and function of T cells are affected by age. The numbers of murine Tregs increase with age in the spleen and lymph nodes.\(^{83–85}\) In humans, skin Treg numbers are increased in the steady state of aged subjects, and functional changes in Treg may occur with ageing.\(^{86}\) We note that an increase in the number of Tregs does not necessarily imply an increase in Treg function. Indeed, autoimmunity is more common in the elderly and that may be attributed to a reduced Treg function or increased Treg susceptibility to dysfunction under inflammatory conditions as has been seen, for example, in DED.\(^{31}\) In addition, age-related changes of Th17 cells correlate with increased proinflammatory conditions observed in the elderly. More specifically, a correlation has been found between increased Th17 and enhanced IL-1R and IL-1β expression as well as decreased IL-2 and IL-2R expression by naive CD4+ T cells in aged mice.\(^{87,88}\) The expression of IL-17, IL-22 and RORγt is dramatically elevated in Th17 cells, and in particular memory Th17, in aged mice, which may suppress Treg function. The elevated Th17 immune responses, coupled with suppressed Treg function, in the elderly likely contribute to the enhanced development of autoimmune diseases.\(^{89}\) Therefore, a clear understanding of the balance between Th17 and Treg cells is relevant to the mechanism of DED during ageing. This balance may determine the direction to autoimmune pathology versus tolerance. Whether these observations in antigen-presenting cell migration and the Treg/Th17 balance can be extended to the ocular surface and DED or not remains to be explored. We hypothesise that a higher inflammatory reaction of DED in the elderly could be explained by the enhancement of antigen-presenting process, accumulated memory T cells and an imbalance in the number and/or function of Treg and Th17 cells with a high basal level of proinflammatory mediators among the aged.

CONCLUDING REMARKS

In this review, we discussed evidence that indicates the critical effects of ageing on the regulation of innate and adaptive immunity at the ocular surface. Briefly, increased expression of neutrophil chemokines (eg, IL-8) and Toll-like receptors (eg, TLR7) by epithelial cells may contribute to an enhanced innate immune response in the aged ocular surface. In infection, the enhanced persistence of PMNs and their products, such as proteases and oxidases, may damage the ocular surface in the elderly. Aged DCs are functionally impaired in activation, migration and production of cytokines upon stimulation with foreign antigens. However, their reactivity to self-antigens seems to increase with ageing. In regard to adaptive immune cells, the frequencies and activity of Tregs change in the steady state in aged subjects; however, little is known about the dynamics of Tregs in ocular pathological conditions in these subjects. Th17 cells and in particular memory Th17 are dramatically elevated, a phenomenon that coincides with increased chronicity of ocular surface inflammation in the elderly. Future studies are required to study the altered balance between Treg, Th1 and Th17 cells in the elderly and how they enhance the inflammation at the ocular surface. Overall, it seems that in the aged ocular surface response to infections is impaired, resulting in immunodeficiency, whereas increased reactivity to self-antigens results in chronic inflammation and autoimmunity.

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