REVIEW

OPEN

Impact of inflammasomes on the ocular surface

Gwen S. Stinnett[a], *Chuan-Hui Kuo*[b] *and Santa J. Ono*[c]

Purpose of review
The ocular surface is prone to inflammation due to exposure to environmental irritants and pathogens. Inflammasomes are intracellular, multiprotein complexes that communicate potentially dangerous signals to the immune system. The identification of inflammasomes in various inflammatory ocular surface conditions can aid in the development of therapeutics to treat these chronic inflammatory conditions.

Recent findings
Several inflammasomes have been associated with ocular surface disorders including dry eye disease, keratitis, and allergies. Mechanisms for activation of these inflammasomes with regards to specific disorders have been explored in models to aid in the development of targeted treatments.

Summary
Research efforts continue to characterize the types of inflammasomes and activators of these in inflammatory ocular surface conditions. Various therapies targeting specific inflammasome types or pyroptosis are being tested preclinically to assess effects on decreasing the associated chronic inflammation.

Keywords
cornea, inflammasome, inflammation, ocular surface

INTRODUCTION

As the first point of protection, the integrity of the ocular surface is crucial for clear vision. Maintaining a healthy ocular surface is vital to vision and quality of life. Visual impairment has a profound effect on physical and mental health [1]. Disturbances at the ocular surface, such as infections or inflammation, can affect simple tasks in daily life and contribute to the progression of visual impairment or even blindness if left untreated [2].

The innate immune is the first line of defense against harmful stimuli. Inflammation is the direct result of the activation of the innate immune system, mainly triggered by inflammasomes. Inflammasomes are intracellular, multiprotein complexes leading to activation of caspases and secretion of pro-inflammatory cytokines. This process is integral to maintaining a healthy immune system; however, dysfunction in inflammasome activity leads to chronic inflammation and deleterious health effects.

STRUCTURE AND FUNCTION OF THE OCULAR SURFACE

Healthy vision depends on the outer eye being kept clean and wet. The ocular surface system is composed of the cornea, conjunctiva, the tear film, lacrimal glands, meibomian glands and connective tissue working together to maintain optimal light refraction and protection for the eye [3]. The cornea provides a smooth, refractive and transparent surface for optimal light entrance to the retina; it is avascular and contains few immune-related cells. The conjunctiva maintains a physical barrier to the external environment and contains a large number of immune-related cells [4]. The outermost layer of the eye is covered by the tear film, comprised of lipid layer and a mucin-containing aqueous layer, provides constant lubrication to the cornea and

[a]Department of Ophthalmology and Visual Sciences, Kellogg Eye Center, University of Michigan, Ann Arbor, Michigan, USA, [b]Department of Ophthalmology and Visual Sciences, Faculty of Medicine, Eye Care Centre, The University of British Columbia, Vancouver, BC, Canada and [c]Departments of Ophthalmology & Visual Science, Microbiology & Immunology and Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, Michigan, USA

Correspondence to Gwen S. Stinnett, PhD, University of Michigan Kellogg Eye Center, Department of Ophthalmology and Visual Sciences, 1000 Wall Street, Ann Arbor, MI 48105, USA. Tel: +1 734 763 1246; e-mail: schaferg@med.umich.edu

Curr Opin Allergy Clin Immunol 2024, 24:368–374

DOI:10.1097/ACI.0000000000001004

KEY POINTS

- Inflammation at the ocular surface is associated with many acute and chronic eye disorders.
- Inflammasomes are intracellular multiprotein complexes that are key to the release of pro-inflammatory cytokines from cells.
- Dysfunction in inflammasome activation underlies chronic inflammation associated with ocular surface disorders.
- Identification of specific inflammasome types and mechanism of action can inform the most efficacious treatment.

conjunctiva surface [5]. This protective barrier contains enzymes and immune factors generated and secreted by specialized regions of the epithelium to aid in antimicrobial action. Epithelial cells effectively form a continuous structure at the surface, connecting the individual components as a functional unit and allowing cell communication [3].

INFLAMMATION AT THE OCULAR SURFACE

Ocular surface inflammation accompanies many eye disorders, initiated from a variety of stimuli including environmental agents (allergens, air particulates, pathogens) or diseases (e.g., auto-immune disease, diabetes). Inflammation is associated with activation of the innate immune system involving dendritic cells, macrophages, neutrophils and also fibroblasts and epithelial cells [6]; concomitant molecular and physiological responses add to the complexity [7]. Acute inflammation is protective: it is vital for the clearance of foreign matter or pathogen and the subsequent repair of damaged cells to reduce further damage.

Chronic inflammation can lead to damage to the cornea and impaired or loss of vision. Inflammatory events can occur in specific regions (e.g., allergic conjunctivitis) or other involve multiple ocular surface tissues (dry eye disease, DED). Chronic ocular surface inflammation is considered to be a primary cause of DED progression [8], brought on by tear hyperosmolarity [9]. Amplification of inflammatory responses often has direct effects on tear-film stability, followed by re-activation of inflammatory responses via cytokines and chemokines [10]. Ocular surface inflammatory disorders often result from the "vicious cycle" of inflammation and the involvement of multiple ocular components and cell types [11]. Loss of conjunctival goblet cells and decreased mucin secretion contributes to surface damage [12]. There is a high prevalence of dry-eye disease among autoimmune disorders, which are characterized by systemic inflammation. Sjögren's syndrome is characterized by the presence of activated T-cells in lacrimal glands, cell death and decreased tear production leading to DED [13].

Maintaining cornea clarity despite exposure to potentially inflammatory stimuli is managed through a careful balance of immune responses [14]. An overwhelming immune response to inflammation results in increased expression of dendritic cells in both the peripheral and central cornea compared to noninflammation conditions [15] leading to changes in its transparency and visual impairment.

The conjunctiva is highly immune-responsive; transcriptomics of mouse cornea or conjunctiva revealed diverse immune-related genes and cell types [16,17▪▪], supporting these as immune responsive tissues susceptible to inflammatory responses. Ocular allergies exemplify how the innate immune system progresses to the adaptive immune system invoking inflammatory responses in which pro-inflammatory interleukins and chemokines are secreted [12,18,19]. Seasonal and perennial allergic conjunctivitis (SAC, PAC), and to some extent vernal keratoconjunctivitis (VKC), involve immunoglobulin E (IgE)-mediated release of histamines from mast cells causing acute inflammatory symptoms followed by infiltration of eosinophils, basophils, T cells, neutrophils, and macrophages further prolonging inflammation [20]. Cytokines such as interleukin (IL)-33 are present on ocular surface cells and contribute to chronic inflammation of conjunctivitis and barrier dysfunction [21].

INFLAMMASOMES

Inflammasomes encompass a variety of intracellular protein complexes that form in response to pathogenic-associated molecular patterns (PAMPS) or danger-associated molecular patterns (DAMPS) presented to the cell and culminates in the activation and release of caspases and pro-inflammatory cytokines, particularly IL-1β [22]. Inflammasomes are found predominantly in immune-responsive cells (monocytes and macrophages) but also nonimmune cells, including epithelial cells. They are characterized as canonical (activating caspase-1) or noncanonical [activating caspase-4/5 (human), or -11 (mouse)].

Hallmarks of canonical inflammasomes are activation of pattern-recognition receptors (PRRs) by PAMPs or DAMPs. PRRs include membrane Toll-like receptors and cytoplasmic nucleotide-binding domain and leucine rich repeat containing-like

receptors (NLRs). Once these sensors are activated, the multiprotein inflammasome complex is constructed consisting of oligomers of sensor proteins, enabling the activation of caspase-1 and the activation of pro-inflammatory interleukins (IL-1β, IL-18) and gasdermins. GasderminD (GSDMD) pores in the membrane allow the release of mature IL-1β and IL-18 and often result in a programmed cell death, pyroptosis [23,24]. Noncanonical inflammasomes are directly detect gram-negative bacteria to activate caspase-4/5/11 prior to initiation of pyroptosis [25,26]. The release of cytokines recruits additional inflammatory cells to the damaged site to aid in recovery but also can result in structural instability due to cell loss.

There are several families of inflammasomes with various subtypes however, the most well studied is NLRP3. NLRP3 inflammasome activation is triggered by an array of diverse signals (PAMPS, DAMPS, and intracellular signals) and often depends on two signals for assembly: a priming signal, leading to transcription of NLRP3 through an nuclea factor (NF)-κB pathway, and an NLRP3-specifc activator stimulus [27]. This inflammasome has been associated with many inflammatory diseases outside of the ocular surface [28]; increased expression or activation of inflammasomes, particularly NLRP3, are associated with inflammatory dysfunction. However it is interesting to note the NLRP6 and NLRP12 inflammasomes have been reported the have both negative and positive effects on inflammation [29,30].

INFLAMMASOMES AT THE OCULAR SURFACE

The role of specific inflammasomes has been investigated for several eye disorders. NLRP1, NLRP3, NLRP6, NLRP12, NLRC4, and absent in melanoma 2 (AIM2) inflammasomes have been associated with various anterior eye pathologies [31▪▪] with NLRP3 the most studied (Table 1). NLRP3 has been identified in uveitis [32], keratitis [33], DED [34], ocular allergy [35], and air particulate matter exposure [36,37]. Most studies have been conducted in animal and cell culture models, with few incorporating clinical samples.

Next-generation sequencing (NGS) and transcriptomics have been utilized to identify changes in global gene expression to overcome the limitations related to small clinical samples. Transcriptome analyses of conjunctiva epithelia from VKC patients and controls identified increased NLRP3 expression in disease-active patient conjunctival cells [38,39]. Single cell RNA sequencing of immune cells from corneas of short-term DED or controls revealed increased expression of inflammatory genes including Nlrp3 [17▪▪]. A meta-analysis of NGS studies of Fuchs endothelia corneal dystrophy identified an enrichment of differentially expressed genes categorized as “Inflammasomes” or “NLRP3 Inflammasome” [40]. These studies emphasize the utility of NGS and transcriptomics to inform research questions.

The use of primary corneal or conjunctival epithelial cell lines (HCECs) to model for clinical disease has provided translational evidence for inflammasome activation in various conditions. HCECs subjected to substances or conditions to mimic conditions encountered with dust mite allergy or DED induce NLRP3 activity and pyroptosis in HCECs [35,41]. In addition to a role for the NLRP3 inflammasome, other inflammasomes have been investigated. Bacterial-induced induced keratitis facilitated the expression of noncanonical inflammasome components caspase-4/11 and GSDMD both *in vivo* and *in vitro* [33]. NLRP1 was expressed in primary HCECs and pyroptosis induced using the specific NLRP1 activators Val-boroPro (VbP) and dsRNA [42] but it was not detected in other eye-related primary epithelial cell lines, suggesting a unique role in corneal epithelium. Human

Table 1. Inflammasome types identified in OS disorders

Inflammasome	OS condition	Activators
NLRP1	Sjögren’s syndrome	Val-boroPro, dsDNA
NLRP3	Alkali burn, conjunctivitis, DED, Keratitis, Panuveitis, VKC	Particulates, Allergens, Desiccating stress/ scopolamine, pathogens, fungus
NLRP6	DED, corneal alkali burn	Desiccating stress/sodium hydroxide
NLRP12	DED	Desiccating stress
NLRC4	DED/ Sjögren’s syndrome	Desiccating stress
AIM2	DED/ Sjögren’s syndrome	Pathogens
Caspase4/5/11	Bacterial keratitis	LPS

conjunctiva goblet cells exposed to pathogenic *Staphylococcus aureus* α toxin exhibited NLRP3 inflammasome activation and increased secretion of IL-1β and protective mucin [43]. This effect was not observed in nontoxigenic bacteria.

Much of the research identifying the roles and mechanisms of inflammasome activation in ocular surface disorders is provided by animal models. DED models have identified inflammasome activation or dysfunction in various ocular surface structures including NLRP3, NLRP6, NLRP12, NLRC4, AIM2, and caspase-8 [31▪▪,34,44]. Increased NLRP3 inflammasome activation in conjunctiva, cornea and lacrimal glands of multiple etiologic DED models has been identified. Modeling the dry eye in Sjögren's syndrome, several types of inflammasomes were activated in acute injury to lacrimal glands including NLRP3, AIM2, NLRC4 [45]. Lacrimal gland myoepithelial cells exposed to genomic DNA activated the AIM2 inflammasome leading to cytokine release and cell death [46].

A persistent elevation in NLRP3 and IL-1β protein was observed in a diabetic keratopathy model coincident with radical oxygen species (ROS) generation, which were improved with NLRP3 inhibition [47]. Fungal and bacterial keratitis models appear to signal through the NLRP3 inflammasome as well [48–50]. NLRP3 knockout mice exhibit more severe keratitis symptoms to *Pseudomonas aeruginosa* infection, with reduced caspase-1 and IL-1β, demonstrating a protective effect for the NLRP3 inflammasome in disease severity [48]. Allergens irritate and inflame the conjunctiva and cornea in sensitive individuals. Increased expression of the NLRP3 pathway including NLRP3, caspase-1 and IL-1β was observed in mouse corneal epithelium after exposure to dust mite allergens potentially modulated by IL-33, known to mediate TH-2 immune responses in allergy [35]. Data from our research group confirmed increased NLRP3 and IL-1β expression in conjunctiva of ovalbumin-sensitized mouse conjunctiva (Fig. 1; Kuo CH, personal communication). A hypothetical model for inflammasome activity in relation to ocular allergy response is shown in Fig. 2.

Overall these studies highlight that there are distinct activators of NLRP3 or alternate inflammasomes contributing to the differences observed in protection or facilitation of inflammation in these various models and these may also be cell-type dependent.

MOLECULAR PATHWAYS OF INFLAMMASOMES AFFECTING OCULAR SURFACE INTEGRITY

There are several known mechanisms for activating canonical inflammasomes, including increased ROS, cathepsin release from lysosomes, and K^+ efflux [51,52▪]. The mechanism of activation for the NLRP3 inflammasome has mostly been found to use two signals (priming and activating) however a single activation signal has been observed [43]. The secretion of pro-inflammatory cytokines and mitochondrial DNA signals other immune cells to respond to the site, which in turn increases

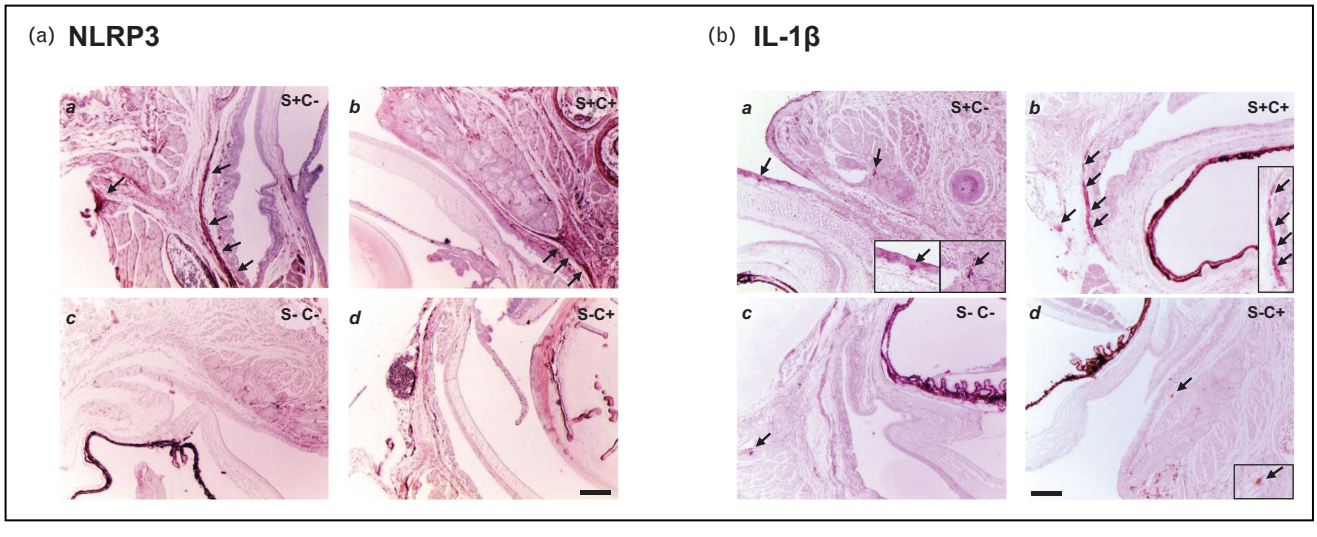

FIGURE 1. NLRP3 and IL-1β protein expression in experimental allergic conjunctivitis. Immunohistochemical expression of NLRP3 (a) and IL-1β (b) in mouse conjunctiva with or without ovalbumin sensitization (S+, S−) before ovalbumin challenge (C+, C−). Corresponding subpanels: *a*: sensitized, no challenge. *b*: sensitized and challenged. *c*: control. *d*: challenge only (no sensitization). NLRP3 staining appears red, as indicated by arrows in (a). IL-1β puncta are indicated by arrows in (b). Scale bar, 10 μM.

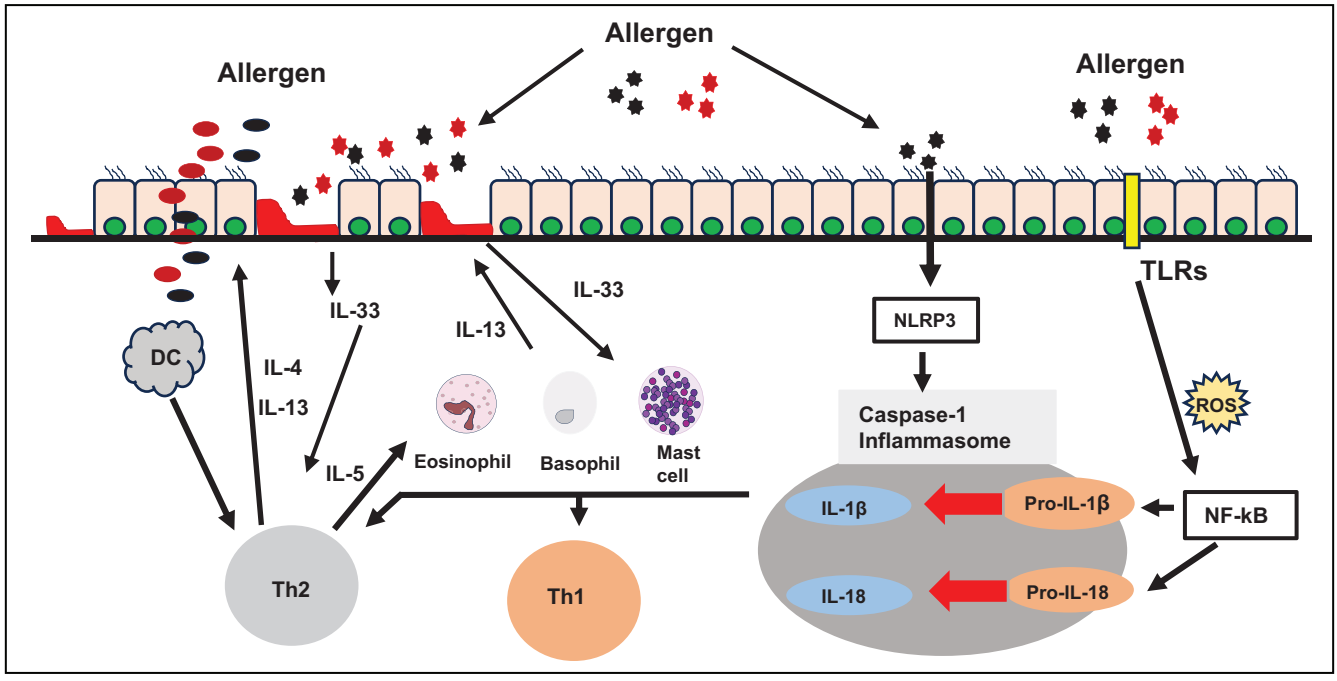

FIGURE 2. Hypothetical role of NLRP3 inflammasome in ocular allergy. When the conjunctiva contacts the allergens, direct activation of the NF-κB pathway serves as a priming signal; DNA damage caused by ROS serves as an activation signal for NLRP3 activation. ROS, which are endogenous danger molecules, also induce inflammasome activation in mast cells, which plays roles both in innate and adaptive responses. IL-33, released from necrotic epithelial cells, induces cytokine production from Th2 cells, including eosinophil, basophil and mast cells and contributes to allergic inflammation in the eye, potentially through modulation of the NLRP3 inflammasome. DC, dendritic cell; IL, interleukin; NF-κB, nuclear factor kappa B; ROS, reactive oxygen species; Th1, T-helper cell type 1; Th2, T-helper cell type 2; TLR, Toll-like receptor.

inflammasome activation and pyroptosis. At the ocular surface, this increase in inflammation can lead to degradation of the tear-film and increased gaps in the conjunctival epithelium leading continued inflammatory responses. In a DED model, although the corneal epithelium appeared intact, fluorescein staining revealed defects perhaps related to loss of tight junctions [53].

Molecular mechanisms underlying inflammasome activation for specific ocular surface disorders continue to be investigated; these can be crucial for identifying novel therapeutic targets for these conditions. Increased ROS, NLRP3 inflammasome activation and pyroptosis were observed in lacrimal glands of Aquaporin5 knockout mice that exhibit signs of DED and inflammation [54] suggest a mechanism for aquaporin5 in pathogenesis of Sjögren's syndrome DED. Small molecule inhibition of NADPH oxidase-2 attenuated alkali burn-induced neovascularization, decreased ROS and IL-1β levels and restored NLRP3/NLRP6 expression balance, implicating a NOX2-NLRP3/NLRP6-IL-1β pathway to damage [55]. In an LPS-induced panuveitis model, infiltration of inflammatory cells, increased NLRP3 and caspase-1 induction were found to signal through a NF-κB mechanism [32]. The diversity of priming signals most likely influences downstream effectors and differences in protective versus pro-inflammatory actions observed in studies.

THERAPEUTIC IMPLICATIONS AND FUTURE DIRECTIONS

Inflammasome pathway-specific therapeutic intervention is an active area of research but there has been limited translational application for ocular surface disorders. Data from studies of inflammasomes in other inflammatory diseases, including the posterior eye, has informed the research for the anterior eye [56,57]. Interventions targeting the NLRP3 inflammasome pathway and pyroptosis revealed promising results in the alleviation of clinical symptoms in animal models. Preclinical animal models use a variety of approaches including: exosomes [58–60]; siRNAs [61]; chemical inhibitors of inflammasomes or pyroptosis [40,62–64] and bioactive compounds used in traditional medicine [41,65]. Current approaches that can be incorporated into research models include gene-editing to target inflammasome-specific genes via siRNA or CRISPR-Cas systems [66], NLRP3-specific

inhibitors [67,68], and nanozymes [69]. In some cases, delivery systems for these therapies have advantages of localized administration but limitations due to safety or pro-inflammatory effects [70,71].

Advances in cell methods and technologies such as the use of organoids are being used in the transition to translational medicine. Recently organoids for lacrimal glands [72–74] and improved conjunctiva organoids [75▪] have been developed. These models should improve research for dry eye and conjunctivitis-related diseases and the role of inflammasomes in disease pathogenesis. Whole genome sequencing is becoming more accessible and should be useful for providing patient-specific treatment especially if combined with individual organoids.

Unlike many ocular surface inflammatory conditions, there are few published studies investigating the role of inflammasomes in ocular allergy, particularly SAC and PAC, despite data showing that Tranilast, used for the treatment of allergic conjunctivitis, directly inhibits NLRP3 [76].

CONCLUSION

An abundance of data supports a role for inflammasomes in pathologies of the anterior eye and the ocular surface. There are promising results from preclinical studies for identifying inflammasome-specific inhibitors and interventions. Identifying precise activators and molecular mechanisms of inflammasomes will assist in our understanding of disease progression and aid in improving therapeutics for inflammatory ocular surface conditions.

Acknowledgements

None.

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ▪ of special interest
- ▪▪ of outstanding interest

1. Court H, McLean G, Guthrie B, *et al.* Visual impairment is associated with physical and mental comorbidities in older adults: a cross-sectional study. BMC Med 2014; 12:181.
2. Burton MJ, Ramke J, Marques AP, *et al.* The Lancet Global Health Commission on Global Eye Health: vision beyond 2020. Lancet Glob Health 2021; 9: e489–e551.
3. Gipson IK. The ocular surface: the challenge to enable and protect vision: the Friedenwald lecture. Invest Ophthalmol Vis Sci 2007; 48:4391–4398; 4390.
4. Bielory L. Allergic and immunologic disorders of the eye. Part I: immunology of the eye. J Allergy Clin Immunol 2000; 106:805–816.
5. Hodges RR, Dartt DA. Tear film mucins: front line defenders of the ocular surface; comparison with airway and gastrointestinal tract mucins. Exp Eye Res 2013; 117:62–78.
6. Iwasaki A, Medzhitov R. Toll-like receptor control of the adaptive immune responses. Nat Immunol 2004; 5:987–995.
7. Netea MG, Balkwill F, Chonchol M, *et al.* A guiding map for inflammation. Nat Immunol 2017; 18:826–831.
8. Pflugfelder SC, de Paiva CS. The pathophysiology of dry eye disease: what we know and future directions for research. Ophthalmology 2017; 124: S4–S13.
9. Research in dry eye: report of the research subcommittee of the international dry eye workshop. Ocul Surf 2007;5:179–93.
10. Rao SK, Mohan R, Gokhale N, *et al.* Inflammation and dry eye disease-where are we? Int J Ophthalmol 2022; 15:820–827.
11. Soifer M, Azar NS, Mousa HM, *et al.* Ocular surface inflammatory disorders (OSID): a collective of systemic etiologies which cause or amplify dry eye syndrome. Front Med 2022; 9:949202.
12. Singh N, Diebold Y, Sahu SK, *et al.* Epithelial barrier dysfunction in ocular allergy. Allergy 2022; 77:1360–1372.
13. Shan H, Liu W, Li Y, *et al.* The autoimmune rheumatic disease related dry eye and its association with retinopathy. Biomolecules 2023; 13:724.
14. Foulsham W, Coco G, Amouzegar A, *et al.* When clarity is crucial: regulating ocular surface immunity. Trends Immunol 2018; 39:288–301.
15. Hamrah P, Liu Y, Zhang Q, *et al.* Alterations in corneal stromal dendritic cell phenotype and distribution in inflammation. Arch Ophthalmol 2003; 121:1132–1140.
16. Alam J, Yazdanpanah G, Ratnapriya R, *et al.* Single-cell transcriptional profiling of murine conjunctival immune cells reveals distinct populations expressing homeostatic and regulatory genes. Mucosal Immunol 2022; 15:620–628.
17. ▪▪ Alam J, Yaman E, Silva GCV, *et al.* Single cell analysis of short-term dry eye induced changes in cornea immune cell populations. Front Med 2024; 11:1362336.

This study used single-cell RNA sequencing to reveal the landscape of immune and inflammatory genes altered in corneal immune cells from a DED mouse model.

18. Kimchi N, Bielory L. The allergic eye: recommendations about pharmacotherapy and recent therapeutic agents. Curr Opin Allergy Clin Immunol 2020; 20:414–420.
19. Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. Cell 2006; 124:783–801.
20. Labib BA, Chigbu DI. Therapeutic targets in allergic conjunctivitis. Pharmaceuticals 2022; 15:547.
21. Hu J, Gao N, Zhang Y, *et al.* IL-33/ST2/IL-9/IL-9R signaling disrupts ocular surface barrier in allergic inflammation. Mucosal Immunol 2020; 13:919–930.
22. Martinon F, Burns K, Tschopp J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. Mol Cell 2002; 10:417–426.
23. Dai Y, Zhou J, Shi C. Inflammasome: structure, biological functions, and therapeutic targets. MedComm 2023; 4:e391.
24. Liu X, Zhang Z, Ruan J, *et al.* Inflammasome-activated gasdermin D causes pyroptosis by forming membrane pores. Nature 2016; 535:153–158.
25. Casson CN, Yu J, Reyes VM, *et al.* Human caspase-4 mediates noncanonical inflammasome activation against gram-negative bacterial pathogens. Proc Natl Acad Sci USA 2015; 112:6688–6693.
26. Shi J, Zhao Y, Wang K, *et al.* Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. Nature 2015; 526:660–665.
27. Krantz M, Eklund D, Särndahl E, *et al.* A detailed molecular network map and model of the NLRP3 inflammasome. Front Immunol 2023; 14:1233680.
28. Abderrazak A, Syrovets T, Couchie D, *et al.* NLRP3 inflammasome: from a danger signal sensor to a regulatory node of oxidative stress and inflammatory diseases. Redox Biol 2015; 4:296–307.
29. Deng Q-C, Deng C-T, Li W-S, *et al.* NLRP12 promotes host resistance against Pseudomonas aeruginosa keratitis inflammatory responses through the negative regulation of NF-κB signaling. Eur Rev Med Pharmacol Sci 2018; 22:8063–8075.
30. Angosto-Bazarra D, Molina-López C, Pelegrín P. Physiological and pathophysiological functions of NLRP6: pro- and anti-inflammatory roles. Commun Biol 2022; 5:524.
31. ▪▪ Mandell JT, de Rivero Vaccari JP, Sabater AL, *et al.* The inflammasome pathway: a key player in ocular surface and anterior segment diseases. Surv Ophthalmol 2023; 68:280–289.

Recent comprehensive review in inflammasomes in ocular surface disease.

32. Chen S, Kong J, Wu S, *et al.* Targeting TBK1 attenuates ocular inflammation in uveitis by antagonizing NF-κB signaling. Clin Immunol Orlando Fla 2023; 246:109210.
33. Xu S, Liu X, Liu X, *et al.* Wedelolactone ameliorates *Pseudomonas aeruginosa*-induced inflammation and corneal injury by suppressing caspase-4/5/11/GSDMD-mediated noncanonical pyroptosis. Exp Eye Res 2021; 211:108750.
34. Zhuang D, Misra SL, Mugisho OO, *et al.* NLRP3 inflammasome as a potential therapeutic target in dry eye disease. Int J Mol Sci 2023; 24:10866.

35. Ran S, Shu Q, Gao X. Dermatophagoides pteronyssinus 1 (DerP1) may trigger NLRP3-mediated corneal epithelial cell pyroptosis by elevating interleukin-33 expression levels. Curr Eye Res 2023; 48:1100–1111.
36. Niu L, Li L, Xing C, *et al.* Airborne particulate matter (PM(2.5)) triggers cornea inflammation and pyroptosis via NLRP3 activation. Ecotoxicol Env Saf 2021; 207:111306.
37. Xiao K, Shang J, Liu Y, *et al.* Effect of NLRP3 repression on NLRP3 inflammasome activation in human corneal epithelial cells with black carbon exposure. Cutan Ocul Toxicol 2022; 41:107–112.
38. Leonardi A, Daull P, Garrigue JS, *et al.* Conjunctival transcriptome analysis reveals the overexpression of multiple pattern recognition receptors in vernal keratoconjunctivitis. Ocul Surf 2021; 19:241–248.
39. Leonardi A, Donato A, Rosani U, *et al.* Endoplasmic reticulum stress and unfolded protein response in vernal keratoconjunctivitis. Invest Ophthalmol Vis Sci 2024; 65:23.
40. Zhang J, Dai Y, Li Y, *et al.* Integrative analysis of gene expression datasets in corneal endothelium samples of Fuchs endothelial corneal dystrophy. Exp Eye Res 2023; 237:109712.
41. Dong Y, Ding Y-Y, Gao W-P. Puerarin alleviates hyperosmotic stress-induced oxidative stress, inflammation, apoptosis and barrier damage of human corneal epithelial cells by targeting SIRT1/NLRP3 signaling. Toxicol In Vitro 2024; 94:105722.
42. Griswold AR, Huang HC, Bachovchin DA. The NLRP1 inflammasome induces pyroptosis in human corneal epithelial cells. Invest Ophthalmol Vis Sci 2022; 63:2.
43. Li D, Hodges R, AukrustNaqvi M, *et al.* Staphylococcus aureus activates NRLP3-dependent IL-1β secretion from human conjunctival goblet cells using α toxin and toll-like receptors 2 and 1. Front Cell Infect Microbiol 2023; 13:1265471.
44. Yu Z, Yazdanpanah G, Alam J, *et al.* Induction of innate inflammatory pathways in the corneal epithelium in the desiccating stress dry eye model. Invest Ophthalmol Vis Sci 2023; 64:8.
45. Delcroix V, Mauduit O, Yang M, *et al.* Lacrimal gland epithelial cells shape immune responses through the modulation of inflammasomes and lipid metabolism. Int J Mol Sci 2023; 24:4309.
46. Yang M, Delcroix V, Lennikov A, *et al.* Genomic DNA activates the AIM2 inflammasome and STING pathways to induce inflammation in lacrimal gland myoepithelial cells. Ocul Surf 2023; 30:263–275.
47. Wan L, Bai X, Zhou Q, *et al.* The advanced glycation end-products (AGEs)/ROS/NLRP3 inflammasome axis contributes to delayed diabetic corneal wound healing and nerve regeneration. Int J Biol Sci 2022; 18:809–825.
48. Ramadan A, Cao Z, Gadjeva M, *et al.* The NLRP3 inflammasome is required for protection against pseudomonas keratitis. Invest Ophthalmol Vis Sci 2023; 64:11.
49. Zhan L, Tian X, Lin J, *et al.* Glabridin inhibits *Aspergillus fumigatus* growth and alleviate inflammation mediated by Dectin-2 and NLRP3 inflammasome. Curr Eye Res 2023; 48:348–356.
50. Lian H, Fang X, Li Q, *et al.* NLRP3 inflammasome-mediated pyroptosis pathway contributes to the pathogenesis of candida albicans keratitis. Front Med 2022; 9:845129.
51. Guo H, Callaway JB, Ting JP-Y. Inflammasomes: mechanism of action, role in disease, and therapeutics. Nat Med 2015; 21:677–687.
52. ■ Barnett KC, Li S, Liang K, *et al.* A 360° view of the inflammasome: mechanisms of activation, cell death, and diseases. Cell 2023; 186:2288–2312.

Current review providing excellent detail on inflammasomes.

53. Chen H, Gan X, Li Y, *et al.* NLRP12- and NLRC4-mediated corneal epithelial pyroptosis is driven by GSDMD cleavage accompanied by IL-33 processing in dry eye. Ocul Surf 2020; 18:783–794.
54. Cao X, Di G, Bai Y, *et al.* Aquaporin5 deficiency aggravates ROS/NLRP3 inflammasome-mediated pyroptosis in the lacrimal glands. Invest Ophthalmol Vis Sci 2023; 64:4.
55. Li Q, Hua X, Li L, *et al.* AIP1 suppresses neovascularization by inhibiting the NOX4-induced NLRP3/NLRP6 imbalance in a murine corneal alkali burn model. Cell Commun Signal 2022; 20:59.
56. Zhang X, Wang Z, Zheng Y, *et al.* Inhibitors of the NLRP3 inflammasome pathway as promising therapeutic candidates for inflammatory diseases (review). Int J Mol Med 2023; 51:35.
57. Zheng X, Wan J, Tan G. The mechanisms of NLRP3 inflammasome/pyroptosis activation and their role in diabetic retinopathy. Front Immunol 2023; 14:1151185.
58. Yu C, Chen P, Xu J, *et al.* hADSCs derived extracellular vesicles inhibit NLRP3 inflammasome activation and dry eye. Sci Rep 2020; 10:14521.
59. Wang S, Hou Y, Li X, *et al.* Comparison of exosomes derived from induced pluripotent stem cells and mesenchymal stem cells as therapeutic nanoparticles for treatment of corneal epithelial defects. Aging 2020; 12: 19546–19562.
60. Harrell CR, Djonov V, Antonijevic A, *et al.* NLRP3 inflammasome as a potentially new therapeutic target of mesenchymal stem cells and their exosomes in the treatment of inflammatory eye diseases. Cells 2023; 12:2327.
61. Liao K, Zeng H, Yang X, *et al.* KCNK5 regulating potassium efflux and inducing pyroptosis in corneal epithelial cells through TNFSF10-mediated autophagy in dry eye. Invest Ophthalmol Vis Sci 2024; 65:34.
62. Huang B, Zhang N, Qiu X, *et al.* Mitochondria-targeted SkQ1 nanoparticles for dry eye disease: inhibiting NLRP3 inflammasome activation by preventing mitochondrial DNA oxidation. J Control Release 2024; 365:1–15.
63. Kim S, Jang YW, Ku Y-A, *et al.* Investigating the anti-inflammatory effects of RCI001 for treating ocular surface diseases: insight into the mechanism of action. Front Immunol 2022; 13:850287.
64. Zhou N, Zhang Y, Jiao Y, *et al.* Discovery of a novel pyroptosis inhibitor acting though modulating glutathionylation to suppress NLRP3-related signal pathway. Int Immunopharmacol 2024; 127:111314.
65. Zhu D, Zheng N, Deng K, *et al.* Aurantio-obtusin alleviates dry eye disease by targeting NF-κB/NLRP3 signaling in rodent models. Biochem Genet 2024; 62:1–14.
66. Choi EH, Suh S, Sears AE, *et al.* Genome editing in the treatment of ocular diseases. Exp Mol Med 2023; 55:1678–1690.
67. Vande Walle L, Lamkanfi M. Drugging the NLRP3 inflammasome: from signalling mechanisms to therapeutic targets. Nat Rev Drug Discov 2024; 23:43–66.
68. Teske KA, Corona C, Wilkinson J, *et al.* Interrogating direct NLRP3 engagement and functional inflammasome inhibition using cellular assays. Cell Chem Biol 2024; 31:349–360; e6.
69. Chu D, Zhao M, Rong S, *et al.* Dual-atom nanozyme eye drops attenuate inflammation and break the vicious cycle in dry eye disease. Nano-Micro Lett 2024; 16:120.
70. Amador C, Shah R, Ghiam S, *et al.* Gene therapy in the anterior eye segment. Curr Gene Ther 2022; 22:104–131.
71. Lv Z, Li S, Zeng G, *et al.* Recent progress of nanomedicine in managing dry eye disease. Adv Ophthalmol Pract Res 2024; 4:23–31.
72. Asal M, Koçak G, Sarı V, *et al.* Development of lacrimal gland organoids from iPSC derived multizonal ocular cells. Front Cell Dev Biol 2022; 10:1058846.
73. Bannier-Hélaouët M, Geurts MH, Korving J *et al.* Establishment, maintenance, differentiation, genetic manipulation, and transplantation of mouse and human lacrimal gland organoids. J Vis Exp JoVE 202;192.
74. Jaffet J, Mohanty A, Veernala I, *et al.* Human lacrimal gland derived mesenchymal stem cells – isolation, propagation, and characterization. Invest Ophthalmol Vis Sci 2023; 64:12.
75. ■ Bannier-Hélaouët M, Korving J, Ma Z, *et al.* Human conjunctiva organoids to study ocular surface homeostasis and disease. Cell Stem Cell 2024; 31:227–243; e12.

Established conjunctival organoid models using human stem cells to enable greater translational experimental design. This system should prove very useful for studying inflammation and inflammasome activity in conjunctivitis disease.

76. Huang Y, Jiang H, Chen Y, *et al.* Tranilast directly targets NLRP3 to treat inflammasome-driven diseases. EMBO Mol Med 2018; 10:e8689.